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Depth Profile of Bacterial Metabolism and PAH Biodegradation in Bioturbated and Unbioturbated Marine Sediments

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CONTENTS

DEPTH PROFILE OF BACTERIAL METABOLISM AND PAH BIODEGRADATION IN BIOTURBATED AND UNBIOTURBATED MARINE SEDIMENTS

INTRODUCTION

Both polycyclic aromatic hydrocarbons (PAHs) and PAH-degrading bacteria are relatively ubiquitous in estuarine sediments and are commonly found in areas that do not have substantial known sources (Chung and King 2001). Rapid PAH metabolism generally depends on the availability of molecular oxygen to the sedimentary bacteria (Cerniglia 1992, Chung and King 1999, Leahy and Olsen 1997), though recently, PAH mineralization has been coupled with sulfate reduction (Coates et al. 1998, Hayes and Lovely 2002, Zhang and Young 1997, Bedessem et al. 1997) and nitrification (Deni and Penninckx 1999, Bonin et al. 1994, Gilewicz et al. 1991). In unperturbed submerged sediment, heterotrophic bacterial metabolism rapidly depletes oxygen, limiting its availability to the top several millimeters (Rasmussen and Jorgensen 1992).

Processes that physically mix the surface sediment with oxygenated bottom waters can increase the amount of oxygen available to bacteria that are deeper in the sediment. One of these processes involves the activities of benthic macrofauna that excavate and mix large portions of the surface sediment and then increase oxygen transfer by ventilating their burrows (Aller 1988). This bioturbation of the sediment has been linked to dramatic changes in both the composition and the metabolic activity of the associated bacterial assemblage (Hall 1994, Soltwedel and Vopel 2001). Macrofaunal burrows have been shown to harbor unique assemblages of PAH-degrading bacteria that mineralize PAHs more rapidly than those from adjacent nonburrow sediment (Chung and King 1999, 2001, Madsen et al. 1997, Schaffner et al. 1997, Bauer et al. 1988). In a microcosm experiment, Madsen et al. (1997) found that the depth-integrated removal of fluoranthene was twice as high when capitellid worms were present. Bauer et al. (1988) had similar findings with regards to capitellids but involving anthracene degradation by bacteria in sediments. The activity of diverse macrofaunal communities has also been linked to long-term seasonal removal of PAHs and polychlorinated biphenyls (PCBs) using sediment microcosms (Schaffner et al. 1997).

These findings have led several researchers to postulate that the relative composition and abundance of benthic macroorganism communities can influence the rate of PAH degradation by natural bacterial assemblages in marine sediment (Madsen et al. 1997). Chung and King (2001) concluded that the capacity for PAH biodegradation in hydrocarbon-impacted ecosystems depends on the qualities of the naturally occurring bacteria and their responses to environmental parameters rather than on the introduction of new taxa (bioaugmentation) or selective modification of existing ones. The activities of the benthic meio- and macrofauna may create an environment that preferentially selects for PAH-degrading bacteria and may increase the transition zones within the sediment that are important to enhancing depth integrated bacterial metabolism.

We measured rates of heterotrophic bacterial production (leucine incorporation method) and mineralization of naphthalene, phenanthrene, and fluoranthene (^{14}C-radiotracer additions) in sections of sediment cores sampled from two stations in an urbanized waterway feeding San Diego Bay. These stations were initially selected as distinct from each other in bioturbation depth, as determined by

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REMOTS camera analyses (Apitz et al. 2002). The differences were also characterized by pore water analyses of nutrients and electron acceptors and microprobe measurements on replicate cores and published separately (Montgomery et al. 2002a).

MATERIAL AND METHODS

PAH Mineralization

PAH mineralization assays were initiated within three hours of sediment sample collection using a modification of Boyd et al. (1996) and Pohlman et al. (2002). As radiotracers, we used three sentinel PAHs: UL-^{14}C-naphthalene (18.6 mCi mmol^{-1}), 3-^{14}C-fluoranthene (45 mCi mmol^{-1}), and 9-^{14}C-phenanthrene (55 mCi mmol^{-1}) that were purchased from Sigma Chemical. They were added in separate incubations to surface sediment samples (1 mL wet volume) in 100 × 16 mm test tubes to a final concentration of about 0.2 μg g^{-1} (depending on specific activity). Isotope dilution was calculated from the ambient test PAH concentration and was kept under 10%. This step ensured that the system was not overwhelmed with excess PAH. Samples were incubated no longer than 24 h at *in situ* temperature and evolved $^{14}CO_2$ was captured on NaOH-soaked filter papers. H_2SO_4 was added to end incubations and to partition any remaining CO_2 into the headspace of the tube and to the filter paper trap. The filter paper traps containing metabolized $^{14}CO_2$ were removed, radioassayed, and subsequently used to calculate substrate mineralization.

Heterotrophic Bacterial Production

The leucine incorporation method (Kirchman et al. 1985, Kirchman 1993, Smith and Azam 1992) was used to measure bacterial production as adapted by Montgomery et al. (1999). A 0.50 μL aliquot of wet surface sediment from each station was added to 2 mL centrifuge tubes (three experimental and one control) which were precharged with [^{3}H-4,5]-L-leucine (154 mCi mmol^{-1}). The sediment was extracted from the benthic grab sample and added to the 2 mL tube using a 1 mL plastic syringe with the end cut off. One mL of 0.45 μm nom. pore dia. (Acrodisk, Gelman) filtered bottom water (collected <1 m above bottom) was then added to each tube to form a sediment slurry. Samples were incubated for 1 to 2 h at *in situ* temperatures and subsequently processed by the method of Smith and Azam (1992). A constant isotope dilution factor of 2 was used for all samples. This was estimated from actual measurements of sediment dissolved free amino acids (Burdige and Martens 1990) and saturation experiment estimates (Tuominen 1995). One mL syringed samples of wet sediment were dried at 50 ^{o}C and used to covert production values to dry weight. Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam (1989).

Sampling

Replicate gravity cores housed on a multicorer were sampled from two stations in Paleta Creek that feeds the San Diego Bay. Station P17 was sampled on January 16, 2002, and Station P04 was sampled on January 22, 2002. The multicorer was deployed off the research vessel R/V *Ecos* and transferred to the laboratory at ambient temperature within 3 h. Two cores from Station P17 were sectioned and assayed for bacterial production and PAH mineralization while a third replicate core was sectioned for PAH concentration. One core from Station P04 was sectioned and assayed for bacterial production and PAH mineralization while a second replicate core was sectioned for PAH concentration. Slurries for biological assays were made from filtered water overlying the respective cores.

PAH Concentration

Ambient PAH concentrations of 18 semivolatile priority pollutants were determined. First, 10 to 15 g of sediment was dried with diatomaceous earth and then extracted in methanol using accelerated solvent

extraction. The extracts were concentrated under an N_2 stream (Speedvap) and analyzed by gas chromatography/ mass spectrometry (GC/MS) (Fisher et al. 1997). *p*-Terphenyl-d_{14} and 2-fluorobiphenyl were used as surrogate standards, following the method described in Pohlman et al. (2002).

Lignin Concentration

Lignin concentration in sediment samples was measured using an alkaline hydrolysis oxidation method to liberate lignin-derived methoxyphenols (LPs) derived from the parent lignin compound (Table 1). The LPs were subsequently derivatized with 1% BSTFA to silyate exchangeable hydrogen and then analyzed by GC/MS (Goni and Montgomery 2000). We used a J&W Scientific DB-1 column (60 m × 0.32 mm i.d., 0.2 μm film thicknesses) with the following analytical program: 100 °C initial temperature, 4 °C/min temperature ramp, 320 °C final temperature, and final hold of 10 minutes. A splitless, on-column injector with a flow rate of 1.3 mL/min mode was used for the GC. MS spectra of eluted peaks were interpreted using an internal laboratory library we created based on the retention times and m/z values for known standard LPs we purchased from Sigma-Aldrich.

Table 1— Lignin-Derived Phenols Obtained from Cuo-Alkaline Oxidation Used to Identify Source and Reactivity of Terriginous Organic Matter in this Study

Phenolic Group	Phenol suite (code)	Remarks
Vanillyl	vanillin (Vl), acetovanillone (Vn), vanillic acid (Vd)	Synthesized only in vascular plants
Ringyl	syringealdehyde (Sl), acetosyringone (Sn), syringic acid (Sd)	Synthesized only in angiosperms
Cinnamyl	p-coumaric acid (pCd), ferulic acid (Fd)	Synthesized only in non-woody tissues (leaves, needles)

RESULTS

Sediment from Paleta Creek in San Diego Bay is impacted from a variety of historical and modern inputs. Two stations within the creek (P17 and P04) were initially found to have different characteristics in terms of bioturbation depth (Apitz et al. 2002). From both the less bioturbated Station P04 and the more bioturbated Station P17, four replicate cores were taken using a multicore sampling device. Two cores were sectioned (2-3 cm each) and sampled for PAH and lignin concentration, bacterial production, and mineralization of PAH (e.g. naphthalene, phenanthrene, and fluoranthene). In a related study, electron acceptors of two replicate cores were measured by microprobe and then sectioned for measurement of nutrient concentrations in the pore waters (Montgomery et al. 2002a). Based on initial REMOTS camera analyses (Apitz et al. 2002), Station P17 was bioturbated to a depth of 2 to 3 cm and Station P04 was bioturbated to a depth of 12 to 14 cm.

In general, PAH concentration was low compared to many submerged sediments in anthropogenically influenced waterways surveyed by our group (Pohlman et al. 2002, Montgomery et al. 1999, 2002b, Boyd et al. 1999). The highest total PAH concentration was only 3.18 ppm and was found at 8 to 10 cm depth in the core at P17 (Fig. 1). In P04, the highest PAH concentration was found 14 to 17 cm below the surface and was likely the only section below the bioturbation zone though there was reportedly high variability in bioturbation zones even within station replicates, based on REMOTS (Apitz et al. 2002) and microprobe analyses (Montgomery et al. 2002a). The PAH concentrations for all sections were higher in cores from the less bioturbated station, P17, than from P04.

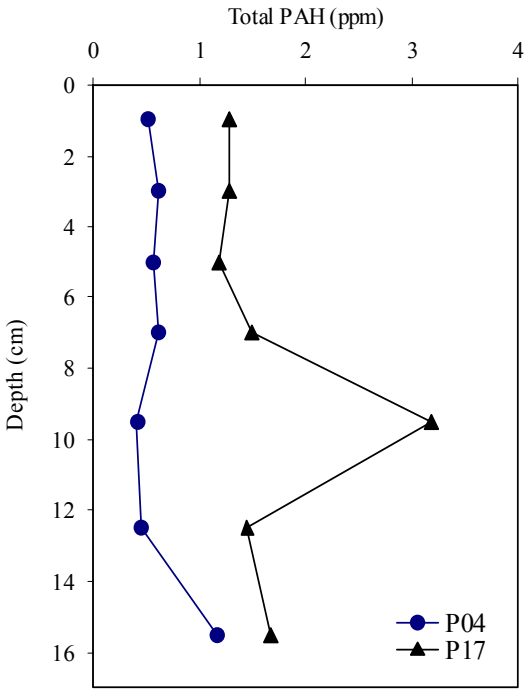

Fig. 1 — Total PAH concentration (ppm) with depth (cm) below the surface water column for cores, P17 and P04

Lignin concentration (Λ_8), the sum of the eight lignin-derived phenols, was prevalent in each core, though our recovery in P17 was poor. In the more bioturbated P04 core, we observed a 60 % decrease in Λ_8 (125 to 52 ppm) from the 0-to-2- to the 2-to-4-cm sections down through the 4-to-6-cm section (Fig. 2). The concentration then increased to approximately 125 ppm downcore from the 6-to-8-cm section. In core P17, lignin concentration was similar at the 0-to-2-cm section and the 4-to-6-cm section (ca. 100 ppm) but decreased to 54 ppm in the 8-to-10-cm section. The error of our lignin measurements was 10%.

Figure 3 shows the lignin phenol indicators of organic matter (OM) source. The ratio of total syringyl to vanillyl phenols (S/V), when greater than 0.6, indicates angiosperm as a plant source. This ratio was less than 0.6 in all samples except for the 4-to-6-cm section at P17, which was 0.71. The ratio of cinnamyl to vanillyl phenols (C/V) can indicate the presence of different types of plant tissue because cinnamyl phenols are only synthesized in nonwoody tissue such as leaves, needles, and grasses; therefore, C/V ratios greater than 0.1 indicate nonwoody tissue. At P17, C/V was slightly greater than 0.1 at 0 to 2 cm (0.11) and at 2 to 4 cm (0.14). All other samples at P17 and P04 had C/V ratios less than 0.1. Finally, the presence of 3,5-dihydroxybenzaldehyde (DHBd), used as a tracer for soil degradation of organic carbon (OC), was low (0.04 to 0.07 mg 100 mg OC^{-1}) at both P17 and P04, though a large value of 0.76 mg 100 mg OC^{-1} was measured at 4 to 6 cm at P17.

The degree to which lignin is degraded in the sediment has been measured using a degradation index (Fig. 4). The ratio of acid to aldehyde for the vanillin family of lignin phenols ($[Ad/Al]_v$) reflects oxidative degradation of aldehyde moieties to acidic moieties in organic matter. Increases in this ratio suggest an increased degradative state. $[Ad/Al]_v$ ratios did increase at P04, mirroring the decrease in lignin concentration seen at the same depths (2 to 6 cm). However, at P17, $[Ad/Al]_v$ decreased in the 4-to-6-cm section from 0.44 to 0.30, though given our 10% error in lignin phenol measurements, this decrease is not statistically significant. The ratio of 3,5-dihydroxybenzoic acid to vanillin (DHBd/V) is

indicative of the relative abundance of sedimentary OM to lignin (Fig. 4). At P17, this ratio was the highest (0.64), more than 10 times the ratios calculated at other depths at P17 and at P04. It is possible that this spurious value might indicate a depositional event.

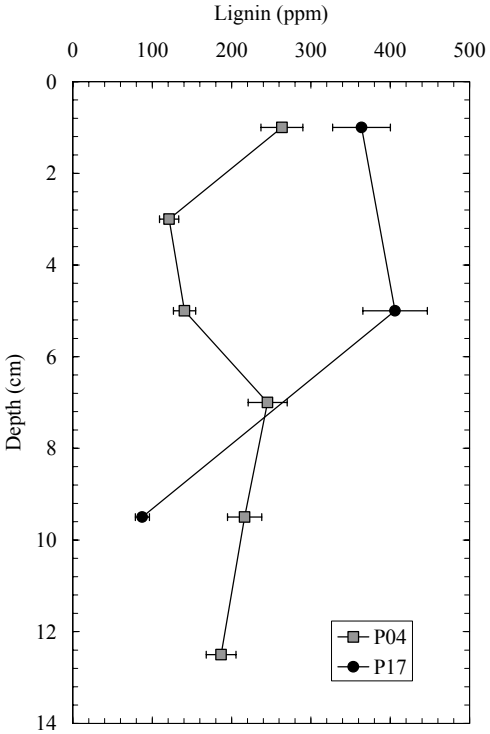

Fig. 2 — Lignin concentration (ppm) with depth (cm) below the surface water column for cores, P17 and P04

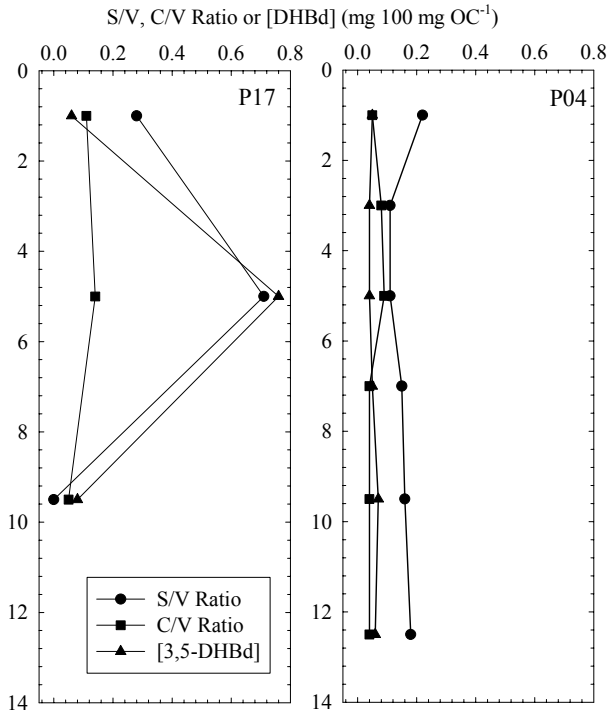

Fig. 3 — S/V and C/V ratios for lignin compounds and DHBd concentration (mg 100 mg organic carbon^{-1}) with depth (cm) below the surface water column for cores, P17 and P04

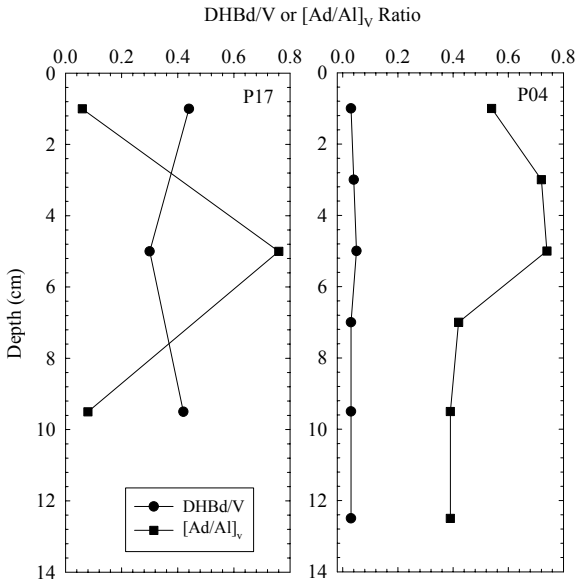

Fig. 4 — Ratios of DHBd/V and $[Ad/Al]_v$ for lignin compounds with depth (cm) below the surface water column for cores, P17 and P04

Heterotrophic bacteria production, using the leucine incorporation assay, was measured on replicate cores from Station P17 (-1B and -2B; Fig. 5) and on one core from Station P04 (-3; Fig. 5). Bacterial production ranged from 11.9 to 297 µg C g^{-1} d^{-1} along the depth profile at P04 and from 6.00 to 198 µg C g^{-1} d^{-1} at P17 and generally decreased with depth at both stations. Production was higher in the two uppermost (0 to 2 and 2 to 4 cm below surface) sections at Station P04 than in the cores from Station P17 but was similar below 4 cm.

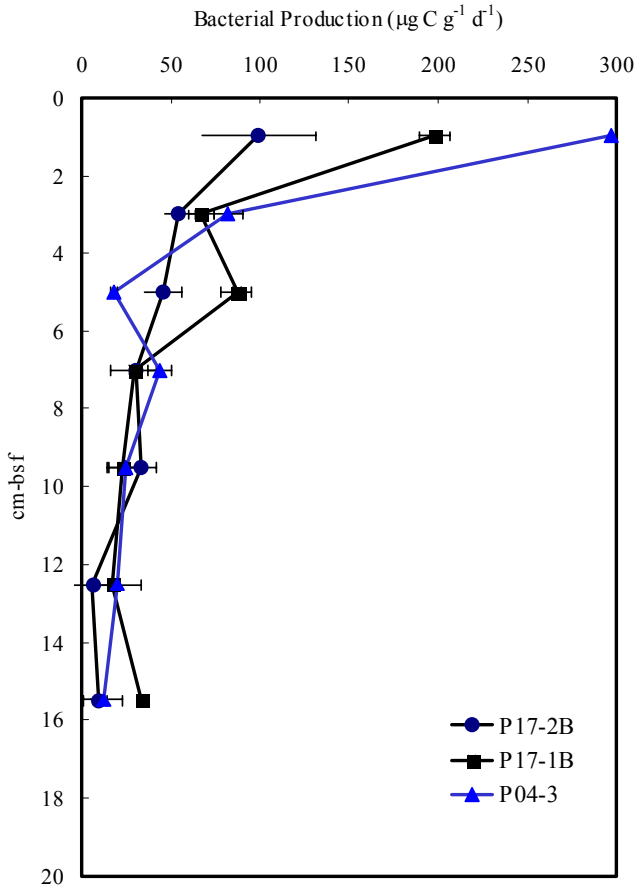

Fig. 5 — Bacterial production (µg C kg^{-1} d^{-1}) with depth (cm) below the surface water column for cores, P04 and replicate cores of P17, -1B and -2B

Bacterial metabolism of PAHs to carbon dioxide was measured using radiotracer additions of ^{14}C-naphthelene, -phenanthrene, and –fluoranthene to sediment slurries mixed with filtered bottom water from the respective station. Naphthalene mineralization ranged from below the detection level of 1×10^{-3} µg kg^{-1} d^{-1} up to 1.06 (± 0.16) µg kg^{-1} d^{-1} in all three cores but most values were not differentiable from background. Only two sections were above the detection limit from both the P04-3 core (2 to 4 and 11 to 14 cm; Fig. 6) and the P17-1B core (0 to 2 cm, 1.06 (± 0.16) µg kg^{-1} d^{-1}; 2 to 4 cm, 0.27 (± 0.04) µg kg^{-1} d^{-1}). Five of the seven sections from the P17-2A core had naphthalene mineralization rates above the detection limit though only three sections appeared to be different (Fig. 6).

Phenanthrene mineralization rates were similar between the P17 cores and were slightly higher in the 0-to-2-cm section (Fig. 7). Rates in the upper two sections (0 to 4 cm) from the P04 core were highest

overall (0 to 2 cm, 3.2 ± 0.44 μg kg^{-1} d^{-1}) with each section higher in P04-3 than in the core from P17-1B (Fig. 8).

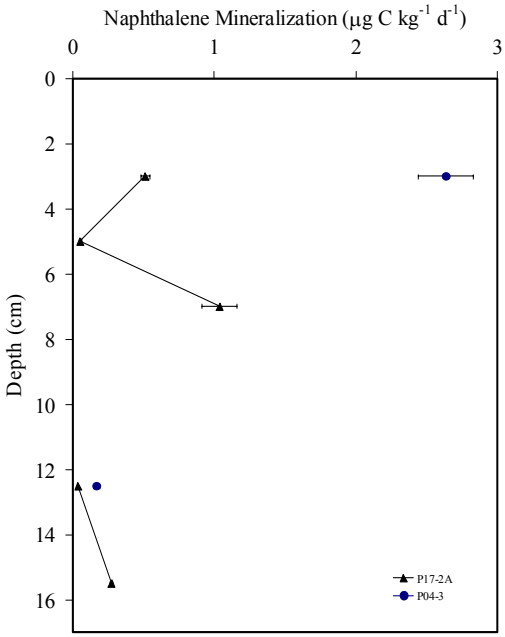

Fig. 6 — Naphthalene mineralization rate (μg C kg^{-1} d^{-1}) with depth (cm) below the surface water column for cores, P17-2A and P04-3

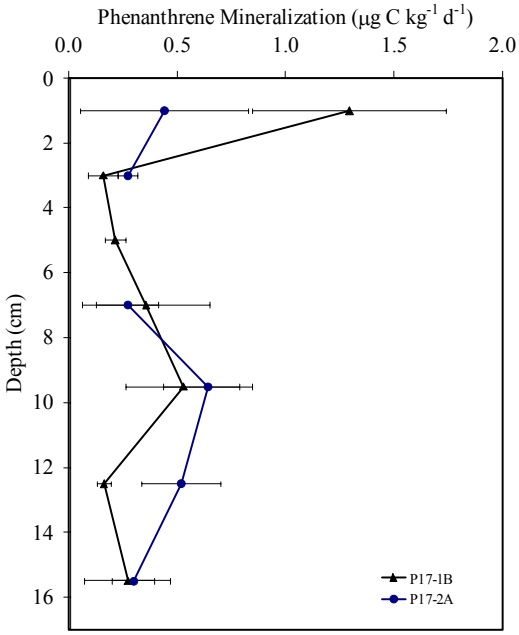

Fig. 7— Phenanthrene mineralization rate (μg C kg^{-1} d^{-1}) with depth (cm) below the surface water column for replicate cores, P17-1B and P17-2A

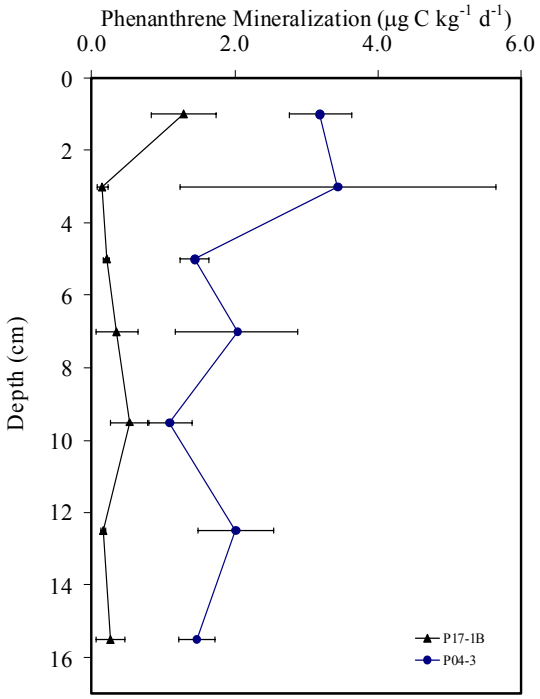

Fig. 8 — Phenanthrene mineralization rate (μg C kg^{-1} d^{-1}) with depth (cm) below the surface water column for cores, P17-1B and P04-3

The average phenanthrene mineralization rate for all sections was about fivefold higher in P04-3 core compared with the P17-1B core (2.1 vs 0.43 μg kg^{-1} d^{-1}). Likewise for fluoranthene mineralization, rates were similar between replicate cores for Station P17 (Fig. 9) but were higher in the P04-3 core than in P17-1B (Fig. 10). Fluoranthene mineralization rates ranged from 0.79 (± 0.49) to 18 (± 17) μg kg^{-1} d^{-1} compared with 0 to 1.1 (± 0.54) μg kg^{-1} d^{-1} at P17.

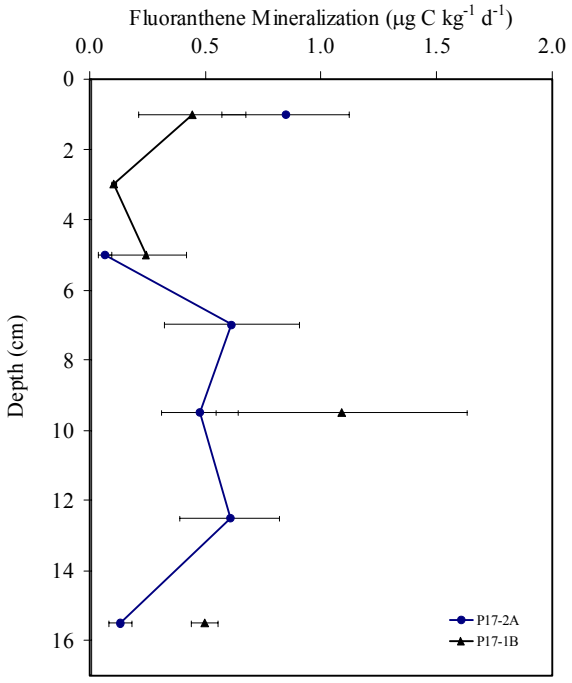

Fig. 9 — Fluoranthene mineralization rate (μg C kg^{-1} d^{-1}) with depth (cm) below the surface water column for replicate cores, P17-1B and P17-2A

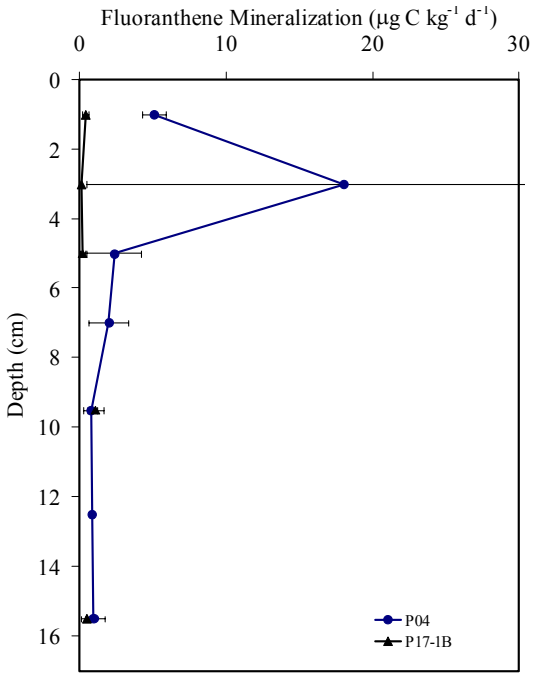

Fig. 10 — Fluoranthene mineralization rate (μg C kg^{-1} d^{-1}) with depth (cm) below the surface water column for cores, P17-1B and P04-3

The turnover rate for phenanthrene and fluoranthene was calculated by dividing the mineralization rate by the ambient concentration of the individual PAH. This value is expressed as the average number of days a PAH molecule would be in the ambient PAH pool assuming the rate of mineralization and PAH flux into the sediment remained constant. Phenanthrene turnover times ranged from 76 to 213 days in the P17-2A core and 39 to 322 in the replicate P17-1B core (Fig. 11) with the average being similar, 130 days for P17-2A and 174 days for P17-1B. The phenanthrene turnover times were about an order of magnitude more rapid in the P04 core, ranging from 8 to 20 days and averaging 13 days (Fig. 12). Fluoranthene turnover times ranged from 193 to 1632 days in the P17-2A core and 236 to 1598 in the replicate P17-1B core (Fig. 13) with the average being very similar, 629 days for P17-2A and 638 days for P17-1B. The fluoranthene turnover times were also an order of magnitude more rapid in the P04 core, ranging from 5 to 91 days and averaging 43 days (Fig. 14). Turnover times could not be calculated for samples where the mineralization rate was below the detection limit. Likewise, turnover times could not be calculated for lignin because of the lack of a suitable radiotracer. However, the ratio of bacterial production to lignin concentration was calculated (Fig. 15).

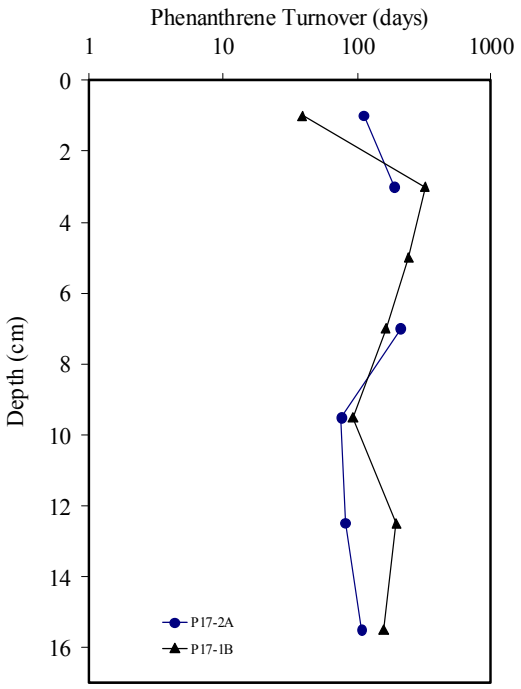

Fig. 11 — Phenanthrene turnover time (days) with depth (cm) below the surface water column for replicate cores, P17-1B and P17-2A

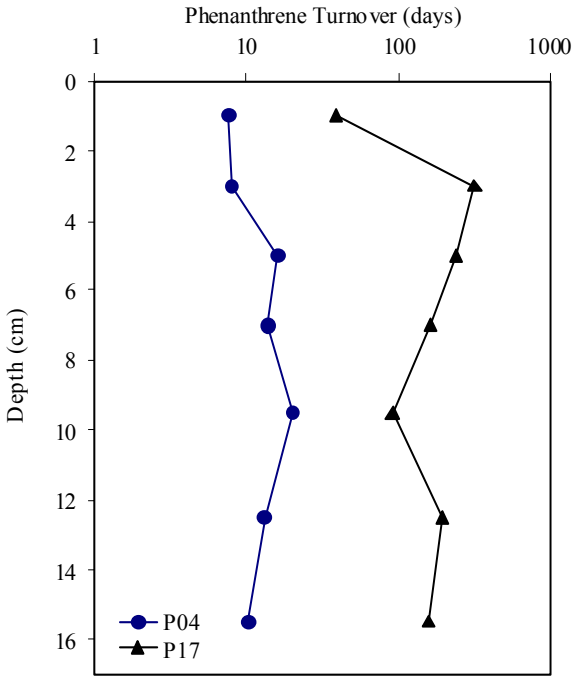

Fig. 12 — Phenanthrene turnover time (days) with depth (cm) below the surface water column for cores, P17 and P04

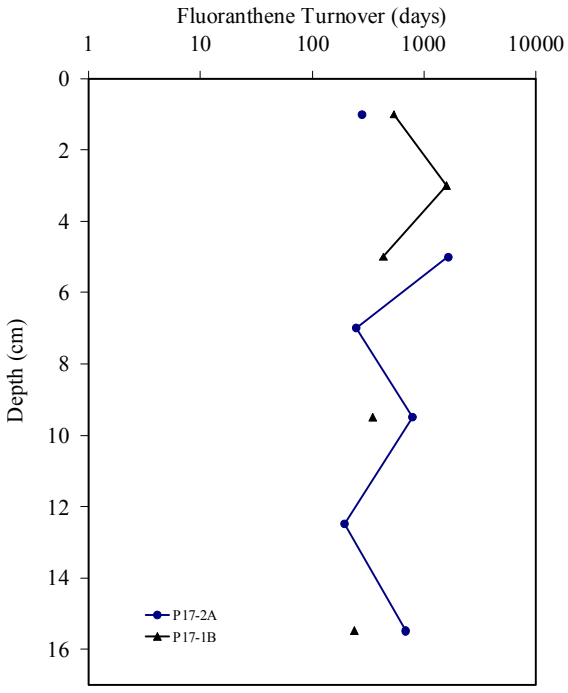

Fig. 13 — Fluoranthene turnover time (days) with depth (cm) below the surface water column for replicate cores, P17-1B and P17-2A

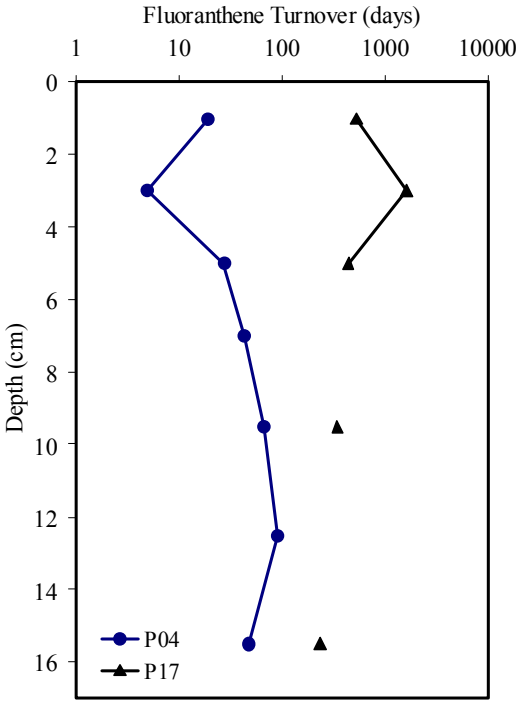

Fig. 14 — Fluoranthene turnover time (days) with depth (cm) below the surface water column for cores, P17 and P04

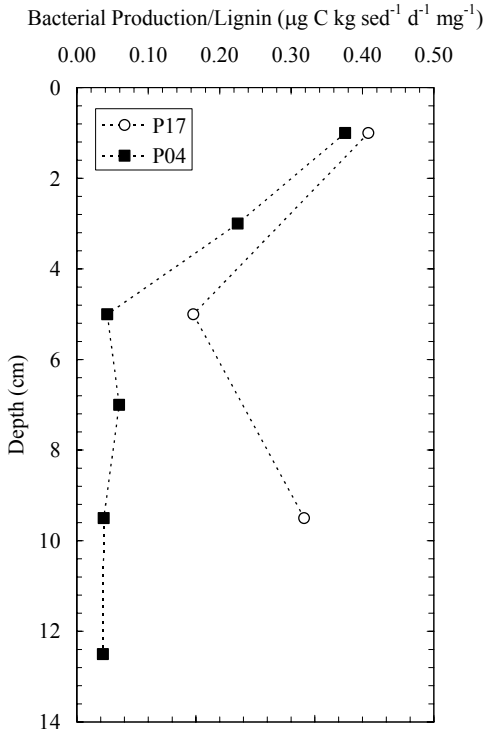

Fig. 15 — Ratio of bacterial production to lignin concentration with depth (cm) below the surface water column for cores, P17 and P04

Sedimentation rate (Apitz et al. 2002) for individual PAHs onto a cm^2 of surface sediment was compared with the mineralization rates for those same PAHs but normalized for the volume of a typical assay (mL = cm^3) (Table 2). The bioturbation depth needed for a cm^2 sediment column to mineralize the amount of PAH depositing onto the cm^2 column is calculated by dividing the sedimentation rate with the mineralization rate for each station (Table 2). With a bioturbation depth of 12 to 15 cm at station P04, but only a 0.63 cm depth needed to biodegrade the amount of fluoranthene depositing on the site, it suggests that there is about 21 μg cm^{-2} yr^{-1} of extra capacity to metabolize fluoranthene (11.5 cm × 1857 ng PAH cm^{-3} yr^{-1}). Conversely, with a bioturbation depth of 2 cm at station P17, but a 12.2 cm depth needed to metabolize the fluoranthene depositing, then there is a deficit capacity of about -1.7 μg cm^{-2} yr^{-1} at this less bioturbated station (-10.2 cm × 162 ng PAH cm^{-3} yr^{-1}).

Table 2 — Sedimentation Rate (Apitz et al. 2002) for Individual PAHs Compared with the Mineralization Rates for those Same PAHs and the Bioturbation Depth Needed to Mineralize the Amount of PAH Depositing onto the Cm^2 Column at Each Site

PAH	Sedimentation (ng PAH cm^{-2} y^{-1})		Mineralization (ng PAH cm^{-3} yr^{-1})		Bioturbation Depth Needed (cm)	
	P04	P17	P04	P17	P04	P17
Naphthalene	27	17	966	190	0.03	0.09
Phenanthrene	626	1139	1169	472	0.54	2.41
Fluoranthene	1171	1972	1857	162	0.63	12.2

DISCUSSION

The presence of active macrofauna and meiofauna can affect the factors known to enhance bacterial PAH biodegradation through numerous mechanisms. Some organisms create burrows and then circulate water through the cavity, which increases the amount of oxygen available for microbial processes as well as the depth of penetration of overlying waters (Madsen et al. 1998). This increased flux of both oxygen and carbon dioxide is a function of the macroorganism abundance (Pelegri and Blackburn 1994). By increasing the surface area in the sediment available to direct contact with the water column, it also increases nutrient transfer and removes accumulated metabolic waste products that limit bacterial metabolism (review by Madsen 1998). The activities of deposit feeders stimulate bacteria metabolism directly by grazing and remineralizing nutrients, or indirectly, by causing changes in aggregate surface area (Holmer et al. 1997).

Macrofauna can also remove PAHs from sediment through direct metabolism (Holmer et al. 1997, Forbes et al. 1996) or by ingesting PAHs at depth and defecating into the overlying water column (Koerting-Walker and Buck 1989), though ingestion has been shown to reduce macrofaunal growth and fecundity (Foss and Forbes 1997). Irrigation of benthic sediments can preferentially remove low molecular weight alkanes and PAHs (Koerting-Walker and Buck 1989) that are known to inhibit bacterial metabolism of higher molecular weight PAHs (Lantz et al. 1997). It is possible that the apparent relationship between benthic microorganisms and PAH-degrading bacteria may not be spurious. The presence of high concentration of oil and the resulting hypoxia (Peterson 1991) are known to be toxic to benthic copepods and other organisms (Carman et al. 2000a,b, Bennett et al. 1999, Carman et al. 1997, Carman and Todaro 1996). By increasing the rate of PAH degradation and reducing accumulation in the sediment, sensitive benthic organisms may actually increase their own growth (Carman et al. 1996).

We found that PAH mineralization was elevated in the bioturbated zones from both stations relative to core subsections from below the bioturbated zone. This is consistent with the hypothesis that the activities of benthic infauna stimulate bacterial metabolism of PAHs. Though PAH mineralization rates were low relative to those found in sediments from other estuarine systems (Montgomery et al. 2002b, Pohlman et al. 2002, Boyd et al. 1999), turnover times in the sediment for phenanthrene and fluoranthene were relatively rapid (39 to 322 d) and similar to those reported by other researchers for three-ring PAHs (16 to 126 d; Shuttleworth and Cerniglia 1995). The low ambient PAH concentrations (1 to 3 ppm) found in all sections from both cores may be too low to select for a bacterial assemblage that will rapidly metabolize PAH. Although low PAH degradation rates are often attributed to low bioavailability (see review by Reid et al. 2000), recent evidence reported by Schwartz and Scow (2001) demonstrates that it may actually be the lack of enzyme induction amongst the PAH-degrading members of the bacterial assemblage that is responsible for low mineralization rates below a threshold PAH concentration. Other researchers have reported this phenomenon for aromatic organics (Zaidi et al. 1988, Roch and Alexander 1997) and, in fact, it is more generally applicable to bacterial carbon metabolism (Button 1985).

Schwartz and Scow (2001) found that PAH-degrading bacteria mineralized phenanthrene more rapidly above 2.5 ppm (8.8×10^{1} μg kg^{-1} d^{-1}) than at a lower ambient concentration of 0.05 ppm (9.5×10^{-2} μg kg^{-1} d^{-1}). Though these values were obtained in a flask studies, they compare very favorably with the rates measured in this study with phenanthrene concentrations of 0.02 to 0.06 ppm, which ranged from 1.6×10^{-1} to 3.5×10^{0} μg kg^{-1} d^{-1}. In other systems, ambient total PAH concentrations above 10 ppm of total PAH correlated with higher PAH mineralization rates as determined with the methods used in this study (Pohlman et al. 2002, Montgomery et al. 1999, 2002b, Langworthy et al. 1998, Boyd et al. 1999) and those used by other researchers (Geiselbrecht et al. 1998, Carman et al. 1995, 1996, Griffiths et al. 1981). Exposure to PAH concentration above the threshold level (which may be species specific) would support natural selection of a PAH-degrading assemblage leading to elevated mineralization rates (Ghiorse et al. 1995).

One explanation for the rapid PAH turnover despite the low ambient PAH concentration could be high flux of PAH from the water column to the sediments within the bioturbation zone. If particles with PAH concentrations above 10 ppm were transported into the benthos, they would locally increase the PAH concentration and elevate the selective pressure for PAH degrading bacteria. High ambient PAH levels might not be measured because of rapid turnover time, but affects of such a PAH flux could be reflected in the composition of the natural bacterial assemblage. Transport of PAHs from particles suspended in the overlying bottom waters into the sediment may involve gravitational settling or activities of the macrobiota themselves. Most research involving the effect of macrofauna on PAH transport has involved their role in resuspended PAH-bound contaminants from the sediments into the water column (Reible and Mohanty 2002, Reible et al. 1996, Ciarelli et al. 1999). However, others have found that certain types of macrofauna trap organic matter and associated PAHs that are suspended in the water column and move them deeper into the sediment (Aller 1988; Holmer et al. 1997). Amphipods transfer PAH-coated particles from the water column to the subsurface through ingestion, encapsulation within a peritrophic membrane and defecation in the subsurface burrows (Lotufo and Landrum 2002). Sediment reworking can also homogenize organic matter concentrations in the bioturbated zone with small meiofauna like capitellids having this effect in the top 10 to 20 mm (Holmer et al. 1997, Madsen et al. 1997) and larger oligochaetes extending down to 10 cm (Cunningham et al. 1999). Reworking of sediments by benthic organisms and the resultant changes in PAH metabolism by bacteria can complicate interpretation of sedimentation and biodegradation rates based on analytical chemistry of the core sections.

In addition, there may be some support of PAH-metabolizing communities by the presence of lignin, which was found in abundance in both cores. Lignin is a polyphenolic molecule having multiple aromatic rings. Therefore, this molecule is structurally similar to PAHs and the enzyme systems used by aquatic microbial communities to metabolize PAHs may cometabolize lignin. Thus, the high rates of PAH turnover that indicate a fast metabolism may be a result of microbial communities subsisting on available terriginous organic matter, rich in lignin, which is likely supplied with sedimentation in the San Diego Bay watershed. Because we are unable to measure lignin turnover in these cores (due to lack of suitable radiolabeled substrate) we may only speculate on this possibility. Nevertheless, several studies have shown a connection between PAH and lignin degradation in laboratory studies (Hammel et al. 1986, Cavalieri and Rogan 1985).

In a related study, PAH and organic matter deposition to the two study stations was measured using sediment trap collections of particles over two weeks subsequent to this study (Apitz et al. 2002). PAH concentrations on the particles collected in these traps were over 40 ppm versus that in the underlying sediment, which was around 1 to 3 ppm (Apitz et al. 2002). In the short term, material in the sediment trap should be similar compositionally to that in the surface sediment unless transported laterally, abiotically changed (e.g., diffusion, resuspension), biodegraded in the bottom boundary layer, or subducted into the sediments and buried or biodegraded. Long term processes involving lateral transport and resuspension are not likely at this site given the low flow and reduced surface water input into this area in San Diego Bay, but they cannot be ruled out. The importance of abiotic diffusion relative to PAH mineralization was measured in this project and will be reported elsewhere (Apitz et al. 2002). Sediment trap material could be trapped in the bottom boundary layer and periodically resuspended from storm events or ship traffic and eventually biodegraded to reduce the PAH concentration from 40 to 1 to 3 ppm before being buried.

It is possible that water column organic matter and associated PAHs deposit at or near the sediment water interface and are then subducted into the bioturbation zone where they are metabolized by PAH-degrading bacteria in the macrofaunal and meiofaunal burrows. There are several lines of evidence collected in this and related studies to support this hypothesis including:

1) rapid PAH turnover times despite low ambient PAH concentration;
2) higher naphthalene, phenanthrene, and fluoranthene mineralization rates in the upper sediments than in the lower sediments;
3) depth of elevated mineralization rates consistent with bioturbation depth estimates from REMOTS analyses (Apitz et al. 2002) of surface sediments from both stations;
4) depth of elevated mineralization rates consistent with bioturbation depth estimates from microprobe and ambient nutrient analyses of replicate cores from both stations (Montgomery et al. 2002a);
5) calculation of PAH deposition rates based on sediment trap data and PAH mineralization rates from the core indicate that the difference in PAH concentration can be accounted for by the bioturbation depths measured for station P04.

Organic geochemical analysis helps to put in perspective the contribution of terriginous OM to microbial production. First, ratios of C/N, which indicate OM source, suggest strong input of terriginous OM to Paleta Creek because C/N ratios were, on average, greater at P17 (16.8) than at P04 (9.2). Redfield et al. (1963) reported a C/N ratio of ca. 7 for marine algae, whereas Ruttenberg and Goni (1997) reported typical terriginous C/N ratios of ≥20 for a number of marine sediments. The mixing of these two end members would produce ranges we observed, yet the higher C/N ratio at P17 suggests more terriginous input than at P04. Deshmukh et al. (2001) have also determined a strong terriginous input to Paleta Creek, from their analysis of the aromatic (phenolic) ring signature measured by ^{13}C-NMR and presence of lignin-derived phenols measured by pyrolysis-gas chromatography/mass spectrometry. Though they did not measure lignin, they suggest that most lignin at Paleta Creek was guaiacyl lignin, which is primarily derived from gymnosperms. Given the strong terriginous OM presence in these sediments, we may then hypothesize that active microbial degradation of lignin is occurring. The increased ratio of acid to aldehyde for the vanillin family of lignin phenols ($[Ad/Al]_v$) reflects an increased degradative state. $[Ad/Al]_v$ ratios increased at P04, which mirrored the lignin concentration decrease at the same depths (2 to 6 cm).

The striking comparison between P17 and P04 is that the depth of bioturbation (and thus oxygenation) of P04 appears to influence the lignin quality and quantity. A hypothesis is that the increased oxygen made available by sediment reworking by macrofauna facilitates lignin degradation by microorganisms. Though bacterial production decreases (Fig. 5), the ratio of bacteria production to lignin concentration shown demonstrates that bacterial production is still high even when lignin concentration is decreasing (Fig. 15). Given that PAH concentrations were well below that which we have measured in other field sites where microbial metabolism is high, an explanation for this phenomenon could be that lignin is supporting microbial metabolism.

In summary, elevated bacterial mineralization of the PAHs, naphthalene, phenanthrene, and fluoranthene were associated with areas of the sediment that appear to be more bioturbated based on analyses using REMOTS (Apitz et al. 2002) and microprobe profiles (Montgomery et al. 2002a). PAH deposition rates determined using sediment trap analyses (Apitz et al. 2002) are consistent with PAH biodegradation rates measured for the top 1 cm at station P04 that was more bioturbated and was consistent with that measured for the top 12 cm in the less bioturbated station, P17. It should be cautioned that though the relationships between bacterial activity and parameters measured on replicate cores appear interpretable, they are not absolute. Because this research involves field work on collected submerged sediment samples, the sampling locations are collected shipboard and so they are approximate. The REMOTS camera analyses demonstrated an extremely high heterogeneity in bioturbation depth over the scale of meters and even within one image (Apitz et al. 2002). Replicate cores used in a preliminary site survey were widely variable in the parameters measured in the microprobe analyses (Montgomery et al. 2002a). Though we have some preliminary indication that terriginous organic matter in the form of lignin may support the high rates of production and PAH metabolism we have measured, we caution that more research is needed to fully document the relationship between lignin, PAH, and microbial activity in

marine sediments. In addition, essentially one time point was evaluated and is being extrapolated to annual PAH transport and degradation. Extrapolation of these measurements to longer time frames and across larger sediment study sites will likely reduce their relevance to describing *in situ* conditions, but this is a limitation of all necessary field work. Confidence in our understanding of PAH transport and biodegradation in marine sediments will come with iteration of these field measurements seasonally and over different ecosystems (Madsen 1998).

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