Microbial Ecology

Dissolved Oxygen Saturation Controls PAH Biodegradation in Freshwater Estuary Sediments

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are common contaminants in terrestrial and aquatic environments and can represent a significant constituent of the carbon pool in coastal sediments. We report here the results of an 18-month seasonal study of PAH biodegradation and heterotrophic bacterial production and their controlling biogeochemical factors from 186 sediment samples taken in a tidally influenced freshwater estuary. For each sampling event, measurements were averaged from 25–45 stations covering ~250 km^2. There was a clear relationship between bacterial production and ambient temperature, but none between production and bottom water dissolved oxygen (DO) % saturation or PAH concentrations. In contrast with other studies, we found no effect of temperature on the biodegradation of naphthalene, phenanthrene, or fluoranthene. PAH mineralization correlated with bottom water DO saturation above 70% ($r^2 > 0.99$). These results suggest that the proportional utilization of PAH carbon to natural organic carbon is as much as three orders of magnitude higher during cooler months, when water temperatures are lower and DO % saturation is higher. Infusion of cooler, well-oxygenated water to the water column overlying contaminated sediments during the summer months may stimulate PAH metabolism preferentially over non-PAH organic matter.

Introduction

Natural and anthropogenic processes are responsible for introducing PAHs into the environment [1, 3, 27, 28, 37]. Harbors, marinas, and wastewater treatment and industrial facilities in urban watersheds are entry points for PAHs and other contaminants which sorb to particles and eventually accumulate in sediments [16, 21, 34, 35], resulting in contamination of ~10% of U.S. river, lake, and coastal sediments [58]. If extrapolated to global sediment, PAH may potentially represent a carbon pool of 0.01×10^{15} g (using lowest estimates of coastal sediment deposition [23]). Cleanup costs for affected sediments in the United States alone are estimated in the billions to trillions of dollars [49, 56]. Current and future urban development will likely increase the PAH loading in coastal and estuarine waterways [44, 60].

Following deposition, particle-bound PAHs may be removed by processes such as physical resuspension [33], bioturbation [48], porewater partitioning [38, 40, 41], ingestion or sorption by animals [59], or biodegradation [9, 10]. The environmental factors that regulate rates of *in situ* PAH biodegradation are poorly understood and difficult to model [2, 43]. Approaches to assess bioremediation potential have included both laboratory studies (to predict *in situ* rates) and field analysis (to measure processes *in situ*). These have produced disparate results, indicating that laboratory studies may not be sufficient to determine environmental rates of bioremediation [7].

For carbon-based contaminants, such as PAHs or petroleum, the primary goal of bioremediation efforts is to stimulate natural or introduced microbial populations to utilize more carbon, thereby degrading more contaminant. Efficacy testing usually involves nonspecific measures of bacterial metabolism such as total respiration (CO_2 evolution), bacterial biomass, or O_2 utilization [43]. Ideally, the optimal bioremediation approach would preferentially stimulate PAH carbon utilization relative to other forms of carbon. In this manner, costly remediation efforts, such as nutrient or cometabolyte

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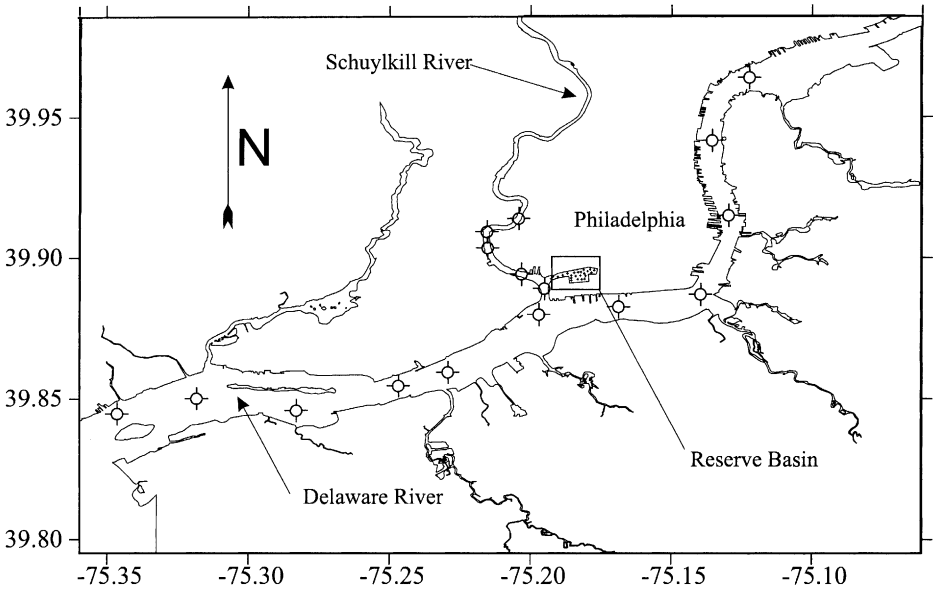

Figure 1. Study site.

additions, would be minimized. Knowledge of factors controlling biogeochemical cycles, particularly carbon flow through the microbial assemblage, is a critical first step in the ultimate design and implementation of any bioremediation strategy.

Two major factors affecting sediment microbial activities are ambient temperature and DO concentration. Studies in the Delaware Bay and other East Coast estuaries have shown water temperature correlates with both heterotrophic bacterial productivity (total carbon demand) [13, 17, 20, 25, 50] and contaminant biodegradation [6, 9, 26, 52]. DO has also been shown to affect the microbially mediated turnover of organic matter in estuarine sediments [62]. Low DO has been shown to limit microbial utilization of aromatic organic contaminants in uncontaminated sediments [6, 9, 14]; however, recent studies suggest PAH mineralization may occur at a rapid rate under anoxic conditions [12, 36, 47]. In previous studies in the Delaware and Schuylkill Rivers the sediment surface and nepheloid layer (flocculent material above the sediment surface) were found to be dynamic with significant transport and deposition of particles and particle-associated PAH [46]. Thus it was hypothesized that surface sediment should be roughly in equilibrium with the overlying water and that characteristics of the bottom water should affect microbial communities living in the uppermost surface sediments. In studying the surface sediment using short-term, field carbon-cycling techniques, a better understanding of the factors limiting PAH biodegradation could be obtained. A large temporal and spatial sampling program covering a 250-km^2 portion of the Delaware and Schuylkill Rivers near Philadelphia, PA (USA), was undertaken primarily to determine the effect of temperature and DO, but also others parameters such as sediment organic carbon content, on sediment microbial activities and PAH biodegradation.

Methods

Study Site and Sample Collection. This study was conducted in a tidally influenced freshwater region of the Delaware Bay near Philadelphia, PA (USA) (Fig. 1). A series of stations was established in the industrialized region of the Delaware and Schuylkill Rivers, where environmental impacts have been well documented [39, 54, 61]. The large cluster of refineries located on the banks of the Schuylkill River is a suspected current source for hydrocarbon contaminants in the sample area. Samples were also taken within the Philadelphia Naval Complex Reserve Basin (PNCRB). Cruise dates were 17–19 December 1997, 18–20 June 1998, 16–18 September 1998, 14–16 December 1998, and 3–5 May 1999. Sediment samples were collected using a Petite Ponar within the PNCRB from an *Avon* inflatable and with a Smith-Mack grab from R/V *Cape Henlopen* in the Delaware and Schuylkill Rivers. Up to 46 stations were sampled during each event. Water samples (for use in bioassays) were collected with a 2.5-L Nansen-type bottle in the PNCRB and a 30-L Niskin bottle in the Delaware and Schuylkill Rivers.

Bacterial Production. Bacterial production was measured using a modification of the leucine incorporation method [31]. A 0.50-μL surface sediment subsample 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 (1 mCi $mmol^{-1}$).

One mL of 0.22-μm filtered bottom water (collected <1 m above bottom) was then added to each tube to form a sediment slurry. Samples were incubated for 1–2 h at *in situ* temperatures and subsequently processed by the method of Smith and Azam [53]. A Beckman LS6500 scintillation counter was used for analysis. A constant isotope dilution factor of 1000 was used for all samples. This was estimated from actual measurements of sediment-dissolved free amino acids [8] and saturation experiment estimates [55]. The effects of atmospheric oxygen in the incubation tube headspace were assumed to be negligible by calculating the incubation tube diameter, assuming an oxygen diffusion coefficient of 2×10^{-5} cm^2 s^{-1}, and estimating the "depth" of oxygen penetration (<0.1 mm). Recent evidence suggests that even production data in anaerobic samples are not affected when using the ^{3}H-leucine centrifugation method [5].

PAH Mineralization. PAH mineralization was measured using ^{14}C-labeled substrates in a manner similar to previous studies [15, 45]. Briefly, [UL-^{14}C] naphthalene (18.6 mCi $mmol^{-1}$, >98% purity by HPLC), [3-^{14}C] fluoranthene (45 mCi $mmol^{-1}$, >98% purity by HPLC), or [9-^{14}C] phenanthrene (47 mCi $mmol^{-1}$, >98% purity by HPLC) was added to surface (1–5 mm) sediment samples (1 mL wet weight) in 100 × 16 mm test tubes to a final concentration of ~500 ng g^{-1} (depending on specific activity). Three replicates were prepared with one control. Then, 0.5 mL of 0.22-μm filtered bottom water was added to each tube to make a sediment slurry. Sediment slurries were created in order to facilitate diffusion of labeled substrates throughout the sediment. Isotope dilution was calculated from the ambient test PAH concentration (determined by GC/MS—see below). Samples were incubated for <24 h at *in situ* temperature and evolved $^{14}CO_2$ was captured on NaOH-soaked filter papers suspended in the headspace. One mL 2 N H_2SO_4 was added to end incubations and to force any remaining CO_2 into headspace of the tube to the filter paper trap. This was done at the beginning of each incubation for the control tubes. Filter papers containing metabolic $^{14}CO_2$ were removed and radioassayed on a Beckman LS6500 scintillation counter. The resultant disintegrations per minute (dpm) values were used to calculate substrate mineralization. Because it is not possible to predict the stochiometric relationships between $^{14}CO_2$ molecules liberated and molecules of parent PAH degraded, mineralization rates were calculated directly from $^{14}CO_2$ recovery and the specific activity of the initial ^{14}C-labeled substrate on a per carbon basis. $^{14}CO_2$ from control incubations (acidified at $t = 0$) were subtracted from the average of experimental triplicates. Controls generally yielded on the order of 100–300 dpm. Oxygen diffusion was calculated as with production measurements. In addition to facilitating the dispersion of added substrates in the incubation tubes, sediment slurries were used so that an overlying water could lens could provide additional protection against oxygen diffusion into the sediment during the course of incubation. Oxygen penetration was calculated to be <2 mm into the sediment slurry (~25 mm).

Ambient PAH Concentrations. Ambient PAH concentrations were determined by drying of 10–15 g of wet sediment with diatamaceous earth, accelerated solvent extraction of dried samples, and GC/MS analysis of the extracts [19]. *p*-Terphenyl-d_{14} and 2-fluorobiphenyl were used as surrogate standards. Matrix spike duplicates were prepared, extracted, and analyzed from one sample per sampling event. A series of calibrations standards (Supelco) were run after every 12 unknown samples. A Hewlett-Packard 6890 GC coupled to a 5973 MS was used for all PAH analyses. A 60-m 5% phenyl 95% methyl (SPB-5) 0.250-μm ID capillary column was used. Samples were injected by means of an autosampler into a splitless mode inlet maintained at 250°C. Pressure pulse programming was used to increase inlet pressure from 16 to 25 psi prior to the septum purge at 2 min into the run. Overall column flow was 1.0 mL min^{-1}. Helium was the carrier gas. The initial column temperature was 40°C. The temperature was ramped as follows: (1) at 1 min run-time, ramp 4.0°C min^{-1} to 180°C, hold 5 min; (2) ramp 4.0°C min^{-1} to 220, hold 5 min; (3) ramp 4.0°C min^{-1} to 280°C, hold 5 min; (4) ramp 4.0°C min^{-1} to 300°C, hold 10 min. The distal column end will empty directly in the source area of the 5973 MS. The solvent delay was 7.0 min and the MS was set to scan from 35 to 300 amu. The MS was tuned with perfluorotributylamine (PFTBA). The MS temperature was set to 106°C; the source was set to 230°C.

Total Organic Carbon. Total organic carbon (TOC) was measured in sediments by standard methods [57] using a Carlo Erba Model 1108 Elemental Analyzer equipped with Eager 300 software. Data are presented in percent carbon on a dry weight basis. Sediment samples from the May 1999 sampling were compromised during storage (cooler shipped to wrong address and allowed to warm) and were not analyzed for TOC.

Temperature and Dissolved Oxygen. Temperature, salinity, and DO were measured with a DataSonde IV in the Reserve Basin and with a SeaBird CTD in the Delaware and Schuylkill Rivers. For interseasonal comparisons, DO was converted from mg L^{-1} to % saturation (corrects for temperature-dependent oxygen solubility) using Seabird Seasave software. All sensors were calibrated prior to use.

Statistical Analysis. Statistical analyses were conducted using GraphPad® Instat and Microsoft® Excel software. Because samples were not taken in a completely random grid (for instance, samples within the PNCRB were taken very close to one another), a coefficient of skewness was calculated for each averaged variable. The concern was that samples very close to one another geographically would bias the data set toward a mean that was nonrepresentative. Data sets with coefficients greater than +1.0 are indicative of an abnormal distribution around the mean value. In order to determine if skewness in these data sets would change the conclusions of this work, entire distribution means were compared to data averaged from the second quartile of the distibutions with a coefficient of skewness >1.

Results

PAH Concentrations. Individual PAH concentrations were averaged for all stations from each sampling event (Table 1). Individual values ranged from nondetected to 8.2, 21.2, and 11.7 μg g^{-1} for naphthalene, phenanthrene, and fluoranthane, respectively (PAHs used to assay mineralization). Highest sediment concentrations for PAHs were found in Schuylkill River, Reserve Basin, and Station 9 (close to an outfall) sediments. QA/QC procedures for PAH analyses were conducted in accordance with standard EPA methods as referenced in the Methods section [19]. QA/QC values for only one sampling (June 1998) are presented (Table 2); however, each sampling event yielded similar results.

Ambient Conditions. Seasonal temperature and DO measurements were averaged for all stations from each sampling event (Table 3). Average temperatures ranged from 5.07 to 24.7°C. Average DO % saturations were highest in December 1997 and May 1999. TOC varied by approximately two orders of magnitude (~0.1% to over 10%). Highest % carbon values were found in the Schuylkill River and Reserve Basin samples.

Microbial Activities. Average bacterial production was lowest in December 1997 and highest in June 1998 with individual measurements ranging from 1.5 μg C g^{-1} d^{-1} (Dec 1997) to 16 mg C g^{-1} d^{-1} (June 1998). Average mineralization rates for PAHs varied considerably, but were consistently highest in May 1999. Mineralization rates for individual stations ranged from nondetected (with each substrate) to 20, 25, and 94 ng g^{-1} h^{-1} (naphthalene, phenanthrene, and fluoranthane, respectively). The highest rates were observed in May 1999. Mineralization rates displayed the most skew, and although average values were lower in the second quartile, seasonal trends were consistent with watershed averages (Table 3).

Table 1. Average sediment PAH concentrations (μg g dry wt^{-1}) for each sampling event

	Dec97		*Jun98*		*Sep98*		*Dec98*		*May99*	
PAH	*Mean ± SE*	*Range*	*Mean ± SE*	*Range*	*Mean ± SE*	*Range*	*Mean ± SE*	*Range*	*Mean ± SE*	*Range*
Naphthalene	1.14 ± 0.33	ND-8.24	0.45 ± 0.12	ND-1.83	0.67 ± 0.34	ND-2.75	0.32 ± 0.12	ND-1.67	0.48 ± 0.23	ND-3.77
Acenaphthalene	0.56 ± 0.22	ND-2.43	0.81 ± 0.21	ND-2.42	0.79 ± 0.23	ND-2.15	0.24 ± 0.07	ND-0.86	0.30 ± 0.10	ND-1.01
Acenaphthene	0.57 ± 0.28	ND-6.25	0.49 ± 0.16	ND-2.38	0.62 ± 0.28	ND-2.49	0.19 ± 0.11	ND-0.97	0.26 ± 0.05	ND-0.49
Fluorene	0.92 ± 0.37	ND-4.29	0.95 ± 0.37	ND-3.13	0.63 ± 0.21	ND-1.65	0.38 ± 0.10	ND-0.81	0.36 ± 0.11	ND-0.86
Phenanthrene	4.18 ± 1.67	0.13–15.5	4.20 ± 2.20	ND-21.3	1.28 ± 0.47	ND-4.88	1.60 ± 0.56	ND-4.52	1.11 ± 0.39	0.15–3.25
Anthracene	1.79 ± 0.79	0.12–6.99	1.78 ± 0.78	ND-7.93	0.80 ± 0.24	ND-2.29	1.02 ± 0.47	ND-8.34	0.52 ± 0.15	0.10–1.39
Fluroanthene	2.56 ± 1.04	0.11–9.47	1.50 ± 0.50	ND-5.87	1.67 ± 0.53	ND-7.45	1.72 ± 0.67	ND-7.29	1.94 ± 0.68	0.33–5.92
Pyrene	2.52 ± 1.0	0.14–9.46	1.49 ± 0.51	ND-5.84	1.56 ± 0.56	ND-7.83	1.75 ± 0.64	ND-5.52	1.83 ± 0.61	0.32–5.63
Benzo[*a*]anthracene	1.30 ± 0.62	0.12–6.83	1.16 ± 0.34	ND-3.20	1.12 ± 0.39	ND-3.64	0.82 ± 0.12	ND-1.96	1.19 ± 0.25	ND-2.21
Chrysene	1.59 ± 0.75	0.14–7.58	1.31 ± 0.39	ND-3.68	1.34 ± 0.46	ND-4.35	1.04 ± 0.23	ND-1.95	1.32 ± 0.31	ND-2.51
Benzo[*b*]fluoranthene	0.88 ± 0.47	ND-7.58	1.04 ± 0.28	ND-1.86	1.04 ± 0.31	ND-2.75	0.60 ± 0.17	ND-1.30	0.98 ± 0.30	ND-2.19
Benzo[*k*]fluoranthene	1.17 ± 0.67	ND-4.33	0.90 ± 0.25	ND-2.34	0.82 ± 0.25	ND-2.37	0.55 ± 0.15	ND-0.92	0.75 ± 0.23	ND-1.38
Benzo[*a*]pyrene	0.82 ± 0.32	ND-3.26	1.31 ± 0.34	ND-2.87	1.30 ± 0.41	ND-3.27	0.82 ± 0.17	ND-1.95	0.95 ± 0.30	ND-2.43
Indeno[1,2,3-*cd*]pyrene	0.64 ± 0.33	ND-3.86	0.16 ± 0.13	ND-1.77	0.28 ± 0.17	ND-2.52	0.83 ± 0.57	ND-5.41	0.25 ± 0.20	ND-2.13
Dibenzo[*a,h*]anthracene	0.32 ± 0.14	ND-0.99	ND	ND	ND	ND	ND	ND	0.042 ± 0.015	ND-0.45
Benzo[*g,h,i*]perylene	0.72 ± 0.36	ND-4.39	0.14 ± 0.14	ND-2.16	0.03 ± 0.28	ND-0.40	0.97 ± 0.41	ND-2.92	0.27 ± 0.18	ND-2.35

ND: Nondetected.

Table 2. Typical QA/QC parameters for PAH analyses

PAH	*MSD* ($\mu g\ g^{-1}$)	*MSD2* ($\mu g\ g^{-1}$)	*Average* ($\mu g\ g^{-1}$)	*RPD* (%)	*Actual* ($\mu g\ g^{-1}$)	*Percent recovery* (%)
Naphthalene	27.2	27.3	27.3	0.60	27.5	99
Acenaphthalene	23.8	23.9	23.8	0.29	23.6	101
Acenaphthene	21.8	21.9	21.8	0.60	21.5	102
Fluorene	19.5	19.8	19.7	1.63	19.3	102
Phenanthrene	21.0	21.4	21.2	2.08	20.8	102
Anthracene	11.7	11.9	11.8	1.87	11.5	103
Fluroanthene	21.8	20.2	21.0	7.38	22.2	94.9
Pyrene	22.0	20.3	21.1	8.19	22.2	95.1
Benzo[*a*]anthracene	21.0	19.5	20.2	7.70	20.4	99.2
Chrysene	21.8	20.0	20.9	8.90	21.1	99.1
Benzo[*b*]fluoranthene	16.6	15.2	15.9	8.56	15.0	106
Benzo[*k*]fluoranthene	15.3	13.9	14.6	9.81	14.2	103
Benzo[*a*]pyrene	15.4	14.5	14.9	6.10	13.8	108
Indeno[1,2,3-*cd*]pyrene	29.8	30.3	30.1	1.76	28.4	106
Dibenzo[*a,h*]anthracene	16.3	16.7	16.5	2.24	15.4	107
Benzo[*g,h,i*]perylene	17.3	17.6	17.4	2.07	16.9	103

MSD: Matrix spike duplicate; RPD: relative percent difference. Actual is certified standard.

Table 3. Average DO saturation, bottom water temperature, bacterial production, and PAH mineralization for each sampling event (mean ± standard error)

Sampling	*Dissolved oxygen (% saturation)*	*n*	*Temperature (°C)*	*n*	*Bacterial production ($\mu g\ C\ g^{-1}\ d^{-1}$) (skew-adjusted)*	*n*	*Naphthalene mineralization ($ng\ C\ g^{-1}\ h^{-1}$) (skew-adjusted)*	*n*	*Phenanthrene mineralization ($ng\ C\ g^{-1}\ h^{-1}$) (skew-adjusted)*	*n*	*Fluoranthene mineralization ($ng\ C\ g^{-1}\ h^{-1}$) (skew-adjusted)*	*n*
December 1997	79.6 ± 0.54	46	5.07 ± 0.019	46	4.53 ± 0.62 (*3.41*)	42	0.486 ± 0.067 (*0.355*)	45	1.68 ± 0.39 (*1.01*)	45	1.07 ± 0.33 (*0.542*)	44
June 1998	66.5 ± 1.8	43	21.0 ± 0.12	43	34.7 ± 2.9	38	$7.51 \times 10^{-3} \pm 9.6 \times 10^{-4}$ (5.18×10^{-3})	38	1.52 ± 0.69 (*0.301*)	38	0.731 ± 0.24 (*0.257*)	37
September 1998	37.1 ± 2.8	44	24.7 ± 0.021	44	23.3 ± 2.2	39	0.237 ± 0.033 (*0.151*)	45	1.21 ± 0.31 (*0.455*)	45	0.158 ± 0.036 (8.56×10^{-3})	45
December 1998	75.1 ± 2.7	34	9.69 ± 0.048	34	14.3 ± 2.1 (*12.4*)	30	0.168 ± 0.034 (*0.105*)	31	1.01 ± 0.42 (*0.535*)	31	0.196 ± 0.061 (*0.165*)	28
May 1999	88.9 ± 1.3	29	14.4 ± 0.1 (*14.2*)	29	10.5 ± 0.87	27	4.82 ± 0.81 (*1.81*)	27	3.59 ± 0.73 (*3.26*)	27	15.3 ± 3.8 (*11.8*)	27

Skew adjusted values (in parentheses) are second quartile averages of data sets whose coefficient of skewness was >1.0.

PAH turnover times were calculated by dividing the total utilization (assuming a conservative metabolic efficiency of 50% [24, 52]) into the ambient concentration (Table 4). These values estimate the time required for all of a given PAH to be degraded given linear utilization (and no further input). Furthermore, a turnover time assumes as the concentration of ambient PAH decreases, PAH degraders maintain the same rate of PAH degradation. Neither assumption is ideal, as seasonal environmental changes are known to affect microbial activities and general enzyme kinetics clearly show a concentration–activity relationship. Findings for the May 1999 sampling were consistent with those in a recent survey of literature values [52], where similar tracer techniques were used. Turnover times from earlier months sampled during this study were longer than many turnover times surveyed from the literature [6, 26, 29, 47, 52]. Methods in this study differ from related studies in that they rely on shorter incubation times of <24 h (which reduces adaptation by the microbial community to "new" artificial conditions), tracer (below ambient) additions of ^{14}C-labeled compounds (which should not stimulate natural microbial communities), and direct conversion of specific activity to amount of PAH degraded (on a per carbon basis rather than a percent ^{14}C recovery). Additionally, this study site had almost no variation in salinity, which may affect PAH biodegradation [51].

Discussion

Factors Affecting Heterotrophic Production. Average heterotrophic production did not correlate with sediment PAH concentrations ($r^2 < 0.1$) or with sediment total organic carbon (data not presented). However, there was a weak negative relationship between average bottom DO % saturation and average heterotrophic production ($r^2 = 0.32$). Not surprisingly, there was a positive correlation between bottom water temperature and average heterotrophic production ($r^2 = 0.80$) (Fig. 2). These results agree well with other investigations in the Delaware River and other watersheds in the U.S. mid-Atlantic region [18, 22, 25, 32, 50].

Factors Affecting PAH Mineralization. In contrast with bacterial production and most other studies of aromatic compound biodegradation [4, 6, 30, 42], there was no correlation between average temperature and average individual PAH utilization ($r^2 < 0.01$). PAH mineralization also showed no correlation with PAH concentration (not shown) or sediment total organic carbon (Table 5). One other study using comparable tracer methods found little correlation between PAH mineralization and temperature at some sites [52], but

Table 4. PAH turnover times and percent of heterotrophic production accounted for by individual PAH utilization (mean ± standard error)

Sampling	*Naphthalene turnover time (days)*	*n*	*Phenanthrene turnover time (days)*	*n*	*Fluoranthene turnover time (days)*	*n*	*Production from naphthalene utilization (%)*	*n*	*Production from phenanthrene utilization (%)*	*n*	*Production from fluoranthene utilization (%)*	*n*
December 1997	100 ± 23.5	45	237 ± 50.8	44	450 ± 132	44	0.26 ± 0.090	45	0.89 ± 0.21	44	0.57 ± 0.22	45
June 1998	3430 ± 510	29	430 ± 90.0	35	410 ± 121	35	$5.2 \times 10^{-4} \pm 1.2 \times 10^{-4}$	35	0.046 ± 0.0051	37	0.051 ± 0.017	37
September 1998	130 ± 20.1	27	128 ± 44.7	38	462 ± 81.6	39	$0.024 \pm 4.9 \times 10^{-3}$	42	0.032 ± 0.016	42	0.016 ± 0.0035	42
December 1998	150 ± 42.8	25	314 ± 88.2	26	1210 ± 400	24	$6.67 \times 10^{-4} \pm 2.1 \times 10^{-4}$	29	0.16 ± 0.058	29	0.033 ± 0.010	25
May 1999	5.32 ± 1.24	22	17.0 ± 5.90	24	24.6 ± 14.2	24	1.1 ± 0.27	23	0.82 ± 0.16	23	3.5 ± 2.8	23

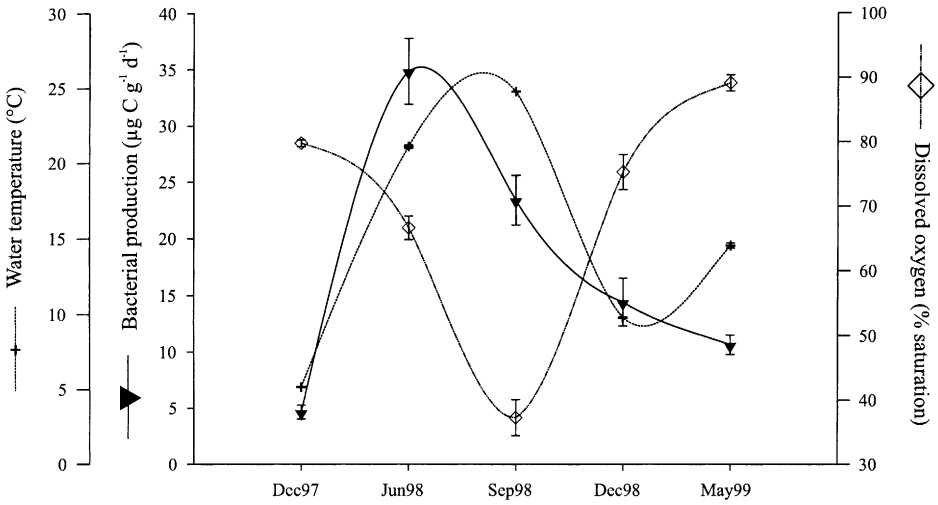

Figure 2. Average bacterial production (▼), bottom water temperature (+), and dissolved oxygen saturation (◇) from December 1997 to May 1999.

not in all cases. When average PAH mineralization from this study was related to average bottom DO % saturation using nonlinear regression analysis, an exponential increase in PAH mineralization with increasing DO % saturation above ~70% was observed (Fig. 3). A semilog plot of average bottom water temperature and average PAH mineralization gave r^2 values >0.99 for all PAHs tested. Below 70% DO saturation, mineralization of PAHs was relatively low and unvaried. This relationship held true for all three PAHs tested. These results contrast with laboratory incubations (14–20 days) using natural sediments [6] in which DO % saturation below ~40% inhibited PAH mineralization. Skew-adjusted (second-quartile average) PAH mineralization was also modeled using exponential regression; r^2 values were >0.95 (Fig. 3, inset).

Methodological differences most likely account for the disparity seen between turnover times calculated from the tracer methods used in this study and long-term hydrocarbon utilization studies conducted under anaerobic conditions in the laboratory [11, 12, 47]. The experiments in this study were conducted with only surface sediments where microbial populations would have fewer tendencies to adapt for anaerobic conditions. Tracer techniques measure the instantaneous rates of biodegradation which can then be related to the ambient pool of substrate [15], whereas long-term laboratory incubations (needed to assay appreciable mineralization of PAH) measure the potential for the microbial community to degrade contaminants. In instances where historical contamination is the prime source of hydrocarbons in sediments, degradation rates calculated from long-term laboratory incubations might approximate the *in situ* degradation rate. In complex, human-affected environments, continual deposition of PAH and other hydrocarbon contaminants makes the calculation of turnover times much more problematic [46].

Importance of PAH as a Carbon Source. PAHs compete with other (usually more labile) organic matter in natural biogeochemical cycles. Thus attempts to stimulate biodegradation of PAHs may also stimulate the utilization of non-PAH carbon or other natural organic matter (NOM), increasing the use of limiting factors such as nutrients and DO. Utilization of NOM preferentially to PAH would tend to enrich PAH content in sediment, particularly if PAH concentrations are normalized to sediment OM. Sediment OM concentrations (w/w) varied greatly from 0.1 to >10% (data not shown) and had only modest correlation with total PAH concentrations (r^2 = 0.66). To determine the importance of PAH as a carbon source to natural sediment bacteria, the percentage of heterotrophic bacterial production accounted for by individual PAH biodegradation was calculated

Table 5. Goodness of fit from regression analysis of sediment TOC against sediment bacterial production, naphthalene mineralization, phenanthrene mineralization, or fluoranthane mineralization

Date	*Bacterial production* (r^2; *P, n*)	*Naphthalene mineralization* (r^2; *P, n*)	*Phenanthrene mineralization* (r^2; *P, n*)	*Fluoranthane mineralization* (r^2; *P, n*)
December 1997	<0.01; 0.87, 44	<0.01; 0.74, 44	0.025; 0.30, 43	0.085; 0.054, 43
June 1998	0.02; 0.27, 18	0.23; 0.056, 15	0.076; 0.33, 13	0.23; 0.08, 13
September 1998	<0.01; 0.47, 39	0.029; 0.27, 42	0.012; 0.47, 42	0.11; 0.032, 42
December 1998	<0.01; 0.40, 24	0.018; 0.52, 24	0.047; 0.29, 24	<0.01; 0.70, 21

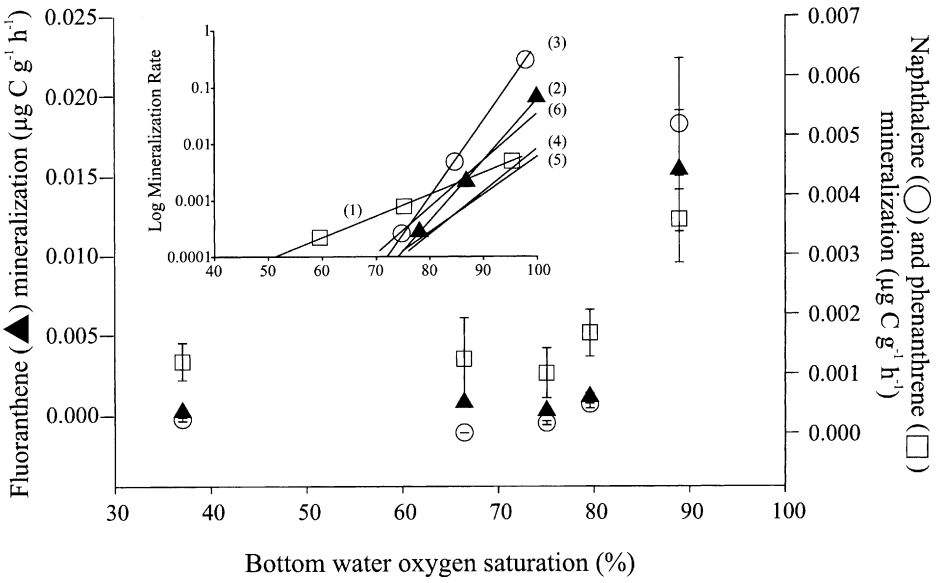

Figure 3. Average naphthalene (○), phenanthrene (□), and fluoranthene (▲) mineralization as a function of % DO saturation. Inset graph shows DO % saturation related to the log of mineralization rate equations. (1) naphthalene: $y = 2 \times 10^{-14}e^{0.3103x}$; $r^2 = 0.99$; (2) phenanthrene: $y = 1 \times 10^{-6}e^{0.0901x}$; $r^2 = 0.99$; (3) fluoranthene: $y = 1 \times 10^{-12}e^{0.2486x}$; $r^2 = 1.0$; (4) skew-adjusted (see text) naphthalene: $y = 1.9 \times 10^{-10}e^{0.1733x}$; $r^2 = 0.99$; (5) skew-adjusted phenanthrene: $y = 9.5 \times 10^{-10}e^{0.1542x}$; $r^2 = 0.96$; (6) skew-adjusted fluoranthene: $y = 1.4 \times 10^{-10}e^{0.191x}$; $r^2 = 0.98$.

(Table 4). Production in this sense represents the total carbon demand of the microbial assemblage, and we make the assumption that this production is fueled primarily by NOM. Therefore the ratio of PAH utilization to production essentially is the ratio of PAH utilization to NOM utilization. This relative utilization of PAH:NOM was highest during December 1997, December 1998, and May 1999. Higher relative utilization of PAH carbon to NOM is the ultimate goal of hydrocarbon bioremediation efforts. Therefore, in seasons where the proportion of PAH utilization to heterotrophic production is higher, more intrinsic remediation may be expected to occur. However, in no case was more than 4% of the carbon demand of the microbial consortium supplied by any individual PAH.

Turnover times have been used to estimate the time of removal for contaminants (and natural organic matter). Overall, turnover times indicated that PAHs are likely to accumulate and persist during several seasons, especially in early summer. This is important as PAHs tend to persist much longer in impacted environments than can usually be predicted by calculated turnover times. Although fine-scale temporal resolution is lacking in this study (5 days sampled out of 500), if turnover times are averaged over the course of the year only phenanthrene appears to be degraded fast enough to prevent sediment accumulation (225 days). A previous analysis of PAH concentrations on suspended particles within the watershed indicated current deposition and/or resuspension likely occurs [46]. Mass balance calculations of sediment transport (using turbidity measurements) in and out of the PNCRB during tidal cycles also indicated net deposition of PAH-containing particles [46]. Turnover times can only approximate environmental persistence given no additional input. It appears that continuing deposition of PAH-containing particles allows net accumulation of PAHs in sediments in the watershed.

Results from this study indicate DO saturation in waters overlying sediments is a prime factor regulating surface sediment PAH biodegradation in this freshwater tidal estuary. In contrast with other studies, we found no apparent relationship between ambient temperature and PAH biodegradation. It may be that when DO is not limiting and water temperature is low (depressing total heterotrophic production), PAH-degrading assemblages have a selective advantage in hydrocarbon-contaminated sediments. This has implications for remediation strategies because effective contaminant utilization may occur in cooler months, where previously it has been assumed there is little biodegradation. We may predict that without other growth limitations, bioremediation should occur at a significant rate throughout the year. Results from this study indicate that bottom water DO saturation above ~70% is critical for appreciable PAH biodegradation in surface sediments. A potential year-round remediation strategy for PAH-impacted sediments, on a limited-area approach, might be to introduce cool, well-oxygenated water to regions overlying surface sediments. This strategy would limit temperature-related total heterotrophic bacterial production, but preferentially stimulate PAH biodegradation.

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