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Seasonal Biotransformation of Naphthalene, Phenanthrene, and Benzo[*a*]pyrene in Surficial Estuarine Sediments

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Transformation rates of naphthalene, phenanthrene, and benzo[*a*]pyrene in oxidized surficial sediments of a polluted urban estuary, Boston Harbor, Mass., were determined over a period of 15 months. Three sites characterized by muddy sediments were selected to represent a >300-fold range of ambient polycyclic aromatic hydrocarbon (PAH) concentration. Transformation rates were determined by a trace-level radiolabel PAH assay which accounted for PAH mineralization, the formation of polar metabolites, residue, and recovered parental PAHs in sediment slurries. Transformation rates of the model PAHs increased with increasing ambient PAH concentrations. However, turnover times for a given PAH were similar at all sites. The turnover times were as follows: naphthalene, 13.2 to 20.1 days; phenanthrene, 7.9 to 19.8 days, and benzo[*a*]pyrene, 53.7 to 82.3 days. At specific sites, rates were significantly affected by salinity, occasionally affected by temperature, but not affected by pH over the course of the study. Seasonal patterns of mineralization were observed for each of the PAHs at all sites. The timing of seasonal maxima of PAH mineralization varied from site to site. Seasonal potential heterotrophic activities as measured by acetate and glutamate mineralization rates did not always coincide with PAH mineralization maxima and minima, suggesting that the two processes are uncoupled in estuarine sediments.

Polycyclic aromatic hydrocarbons (PAHs) belong to a class of environmentally persistent compounds which are ubiquitous in aquatic and terrestrial ecosystems. Owing to their hydrophobic properties, most PAHs dissolve only sparingly in water and are taken up readily by suspended particles which are coated in a complex matrix of organic matter in aquatic environments (24). As a result of particle settlement, sediments tend to be the major sink for PAHs in streams, lakes, estuaries, and oceans. Concentrations of PAHs in marine sediments may range from less than 100 ng/g of sediment in abyssal plains to more than 100,000 ng/g in urban estuaries (21, 31, 42). An understanding of the fate of PAHs in coastal environments is important, because high PAH levels in coastal ecosystems may pose a threat to human public health and the well-being of marine biota. The toxic, mutagenic, and carcinogenic properties of PAHs are well known (2, 41).

PAHs are degraded in the marine environment by photooxidation and chemical oxidation, but biological transformation is probably the prevailing route of PAH loss in sediments. Biodegradative mechanisms, both procaryotic and eucaryotic, require the presence of bimolecular oxygen to initiate enzymatic attack on the PAH rings (14). However, many bacteria, but not eucaryotes, can utilize low-molecular-weight PAHs as a sole carbon and energy source, converting them into simple substrates of central metabolic pathways (8, 9). Thus, bacterial degradation represents a potential route for the ultimate elimination of PAHs from sediments.

Despite the abundance of information about PAH degradation by pure cultures and enrichments, accurate rates of biodegradation and the fates of PAHs in natural sediments have been difficult to ascertain. However, some environmental factors which may affect PAH degradation in sediments have been identified. The limited diffusion of O_2 into organic-rich sediments appears to restrict PAH biodegradation to the well-oxygenated surficial sediments (4, 10). Other factors which affect PAH degradation include temperature, nutrients, and PAH structure (1). The activities of benthic worms may also stimulate PAH degradation in sediments (6, 13). For the low-molecular-weight PAHs such as naphthalene, the turnover rates in oxygenic sediments may be as low as a few hours (19). Higher-molecular-weight PAHs, though, are quite recalcitrant and may persist indefinitely (7, 17, 19, 25).

The objective of this investigation was to estimate the biodegradation rates of three model PAHs (naphthalene, phenanthrene, and benzo[*a*]pyrene) in oxidized surficial sediments of a polluted estuary, Boston Harbor, Mass. The model compounds are two-, three-, and five-ring PAHs, ranging in molecular mass from 104 to 252 daltons. The solubilities of the model PAHs span a wide range: ca. 240 μmol/liter for naphthalene (11), 2 to 6 μmol/liter for phenanthrene (11, 38), and 0.002 to 0.006 μmol/liter for benzo[*a*]pyrene (38). The resistance to biological attack also spans a wide range, from the relatively biodegradable naphthalene to the recalcitrant benzo[*a*]pyrene (17, 19). A radiolabel assay in sediment slurries was used to obtain seasonal biodegradation rates for the model PAHs over a period of 15 months. The rates were used to calculate average yearly transformation rates and turnover times. The results suggest that rates of PAH biodegradation are not directly related to temperature effects, but demonstrate site-dependent seasonal peaks. Possible environmental factors affecting PAH biodegradation in estuarine environments are discussed.

MATERIALS AND METHODS

Sampling. Three sites in Boston Harbor (Fig. 1) were chosen to represent a wide range of PAH contamination. Boston Harbor is a glacially carved, tidally dominated estuary, typical of many bays in New England. The harbor and its 31 associated islands cover an area of 114 km^2 with 190 km of shoreline. All reaches of the harbor and its tributary streams are polluted (Federal Water Pollution Control Federation, Northeast Water Quality Management Center, Department of Interior, Washington, D.C., 1967), and at least

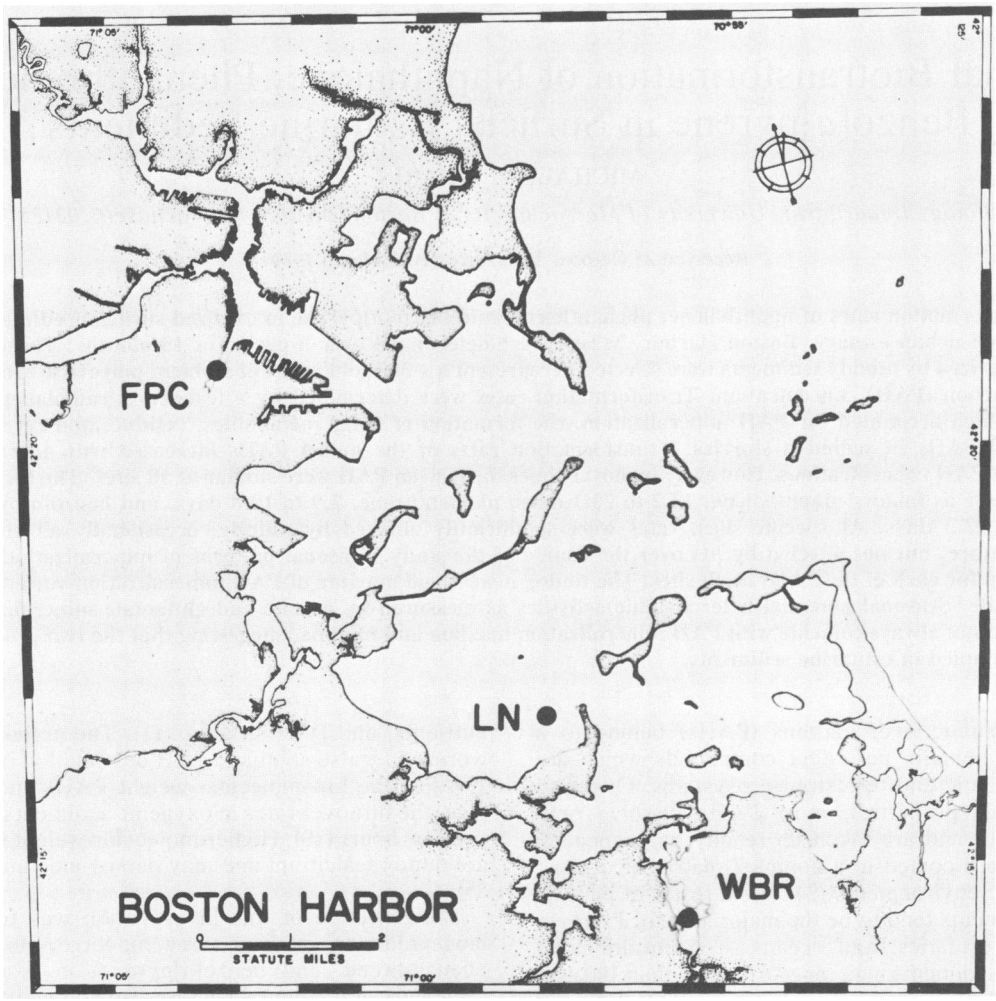

FIG. 1. Map of Boston Harbor showing the sampling sites.

one-third of the harbor is grossly polluted by municipal and industrial sewage, with deposits of decayed organic matter and oil residues covering much of the bottom (M. G. Fitzgerald, WHOI-80-38, Woods Hole Oceanographic Institute, Woods Hole, Mass., 1980). Freshwater influx to the harbor is low, and the entire harbor is flushed in 42 days, predominantly by diurnal tides. Aside from two main shipping channels, the mean depth of the harbor is 3 m or less at mean low water. Two of the sampling sites were at the mouths of tributaries of the Harbor. Fort Point Channel (FPC) is an inner-city channel characterized by little freshwater input, mostly from combined storm sewers and industrial runoff, and extremely high PAH levels (Table 1). Weymouth Back River (WBR) is a relatively undisturbed tidal creek, with relatively low PAH levels (Table 1). A third site, Lower Neck (LN) in Quincy Bay, was characterized by PAH levels intermediate between those at the first two sites. All three sites were composed of muds consisting of fine silt clays. PAH concentrations at the three sites are reported elsewhere (31), and the concentrations of naphthalene, phenanthrene, and benzo[*a*]pyrene are given in Table 1.

Sediment samples were taken in triplicate at each site with an Eckman dredge (Wildco, Saginaw, Mich.). The top 2 to 3 mm of sediment from each grab was subsampled with a

TABLE 1. PAH concentrations in surficial sediments of the Boston Harbor sites

Site	PAH concn (ng/g of dry sediment)			
	Naphthalene	Phenanthrene	Benzo[*a*]pyrene	Total[a]
WBR	367 ± 503	228 ± 164	198 ± 14	2,324 ± 627
LN	144 ± 63	665 ± 178	3,382 ± 1,657	9,414 ± 1,506
FPC	43,628 ± 38,828	63,683 ± 38,829	94,984 ± 4,749	718,364 ± 186,775

[a] Total, Total for 14 PAHs: naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzanthracene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, dibenzanthracene, indenoperylene, and benzo[*g,h,i*]perylene.

TABLE 2. Range of physical and chemical characteristics of the Boston Harbor sampling sites during the sampling period (April 1983 through June 1984)

Site	Depth[a] (m)	Temp (°C)	Salinity (‰)	pH	TOC[b] (mg/g)
WBR	4.0	0.6–22.2	22.2–28.5	7.8–8.1	19.3
LN	4.9	3.2–21.2	19.5–29.5	7.8–8.2	28.6
FPC	2.4	1.5–19.8	26.2–33.2	7.2–8.1	96.1

[a] Depth of site at mean low water.
[b] TOC, Total organic carbon (in milligrams per gram of dry sediment) was analyzed for only one sampling date (17 July 1984).

TABLE 3. Mass balance of [^{14}C]PAH experiments

Radiolabeled PAH	Treatment	% Radioactivity recovered[a] (± SD)
Naphthalene	Experimental	33.9 ± 16.7
	Control	44.3 ± 71.1
Phenanthrene	Experimental	75.6 ± 18.0
	Control	95.9 ± 3.7
Benzo[*a*]pyrene	Experimental	97.7 ± 7.7
	Control	95.5 ± 6.8

[a] Radioactivity recovered as sum of parent compound, CO_2, polar, and residue fractions.

sterile wooden spatula, and the subsamples were placed in glass bottles, kept at ambient water temperature in chest coolers, and returned to the laboratory for further processing. Salinity and conductivity (measured with an RS5-3 salinometer [Beckman Instruments, Inc., Fullerton, Calif.]), sediment temperature, and pH were recorded at each site and on each sampling date. Total organic carbon was determined on a CHN analyzer by the Darling Marine Laboratory, Walpole, Maine. Physical and chemical characteristics of the sites are given in Table 2.

Transformation rates. Techniques for determining PAH transformation rates were based on the protocol of Schwall and Herbes (28). Surficial sediment samples were diluted 1:10 (wt/wt) in estuarine water and homogenized with a magnetic stirrer. The water was obtained from the water column overlying the sampling site and filtered through a 0.2-μm-pore-size membrane (Nuclepore Corp., Pleasanton, Calif.) prior to mixing. Aliquots of the sediment slurry (10 ml) were transferred via sterile wide-mouth serological pipettes to triplicate sterile 25-ml Erlenmeyer flasks fitted with Teflon-lined serum caps and glass center wells. Portions (0.1 ml) of radiolabeled PAHs dissolved in acetone were added to each incubation flask at a final concentration of 20 ng/g of wet slurry, a concentration below the ambient PAH concentrations. Thus, there were three replicate flasks per radiolabeled PAH, one for each triplicate sediment grab at a site. Flasks were incubated at in situ temperatures in the dark without shaking for 60 h for phenanthrene and naphthalene additions or 5 days for benzo[*a*]pyrene addition. At these incubation times, transformation rates were linear with time (data not shown) for the respective PAHs. Less than 10% of the parent radiolabeled PAHs were transformed. Triplicate frozen controls, one set for each site and each PAH, were included. These controls gave the same results as did occasional controls killed by autoclaving or Formalin (final concentration, 2%) addition. Controls typically showed 5% or less of the activity in experimental flasks.

Incubations were stopped by injecting 0.3 ml of 10% trichloroacetic acid through the Teflon-lined serum cap. Radiolabeled CO_2 was trapped in the glass center wells by the addition of 0.3 ml of 2 N KOH to the wells. Flasks were shaken for 30 min at 110 rpm, and the KOH was transferred into vials containing 10 ml of Aquasol (Du Pont, NEN Research Products, Boston, Mass.) for liquid scintillation counting. The sediment slurry was extracted three times with 50 ml of benzene-methanol (2:1, vol/vol) in a 125-ml separatory funnel shaken for 30 min. The organic fractions were pooled and condensed by rotoevaporation. Aliquots of the organic fraction were chromatographed on thin-layer chromatography silica GF sheets (Eastman Kodak Co., Rochester, N.Y.) by being developed with benzene as the mobile phase. Spots resolved on the chromatograms with an R_f of 0.65 were identified as residual, nontransformed PAHs and placed in 10 ml of Omnifluor (Fisher Scientific Co., Pittsburgh, Pa.) for scintillation counting. The identity of the spots was confirmed by comigration on thin-layer chromatography with authentic PAH standards. The remaining radioactive material on the chromatogram (R_f, 0 to 0.1) was designated as polar metabolites and also placed in the scintillation cocktail for counting. The residue, which should include any nonextractable cell-bound and sediment-bound radioactivity, was air dried and wet-combusted with 200 μl of perchloric acid and 400 μl of H_2O_2 at 75°C for 2 h. Aquasol-2 scintillation cocktail (Du Pont Co., Wilmington, Del.) was added to the combusted residue prior to counting. The unextractable nature of the radiolabel in the residue was confirmed by exhaustive Soxhlet extractions with methanol, methanol-benzene, and benzene (31), which resulted in only a further 5% recovery. Counts were performed on a Tricarb liquid scintillation counter (no. 3330; Packard Instrument Co., Inc., Rockville, Md.). Quenching was determined by the internal-standards method, with [^{14}C]benzene for the Omnifluor cocktails and [^{14}C]glutamate for the Aquasol-2 cocktails.

The average recovery of radiolabeled PAHs and transformed PAHs is shown in Table 3. The low recovery and high variability of recovery of the radiolabel in the experimental flasks dosed with [^{14}C]naphthalene was probably due to loss of the volatile parent, naphthalene, during rotoevaporation and thin-layer chromatography. The less volatile phenanthrene and benzo[*a*]pyrene resulted in average recoveries greater than 75 and 95%, respectively.

Calculation of transformation rates, seasonally adjusted rates, and turnover time. Transformation rates were calculated by using the assumption that added radioactive PAHs were completely mixed and equilibrated with ambient sediment PAHs in the slurries. The transformation rate, R, was calculated as follows: $R = r(C_a + C_r)/C_r$, where r is the rate of [^{14}C]PAH transformation corrected for sterile controls, C_a is the ambient concentration of PAHs in sediment, and C_r is the concentration of added [^{14}C]PAH. Because [^{14}C]PAH was added in trace amounts compared with known ambient PAH concentrations, R represents a good estimate of actual biodegradation rates in the sediment slurries.

Averaged transformation rates were determined for each site to take into account seasonal variations. These seasonally adjusted transformation rates were calculated by multiplying the transformation rate at a given date by a weighted factor. The sum of weighted factors is 1.0, and so each weighted factor is the percentage of time during the year that was represented by the sample.

Turnover time (T_t) was calculated by dividing the incubation time by the percentage of PAHs transformed during that incubation time. T_t represents the time required for the entire pool of PAHs in a given sample to be transformed, but

does not take into account physical factors such as deposition. Since transformation rates are typically dependent on the PAH concentration it is assumed that the rate of transformation at a given site is equal to the rate of PAH input; that is, there is a steady state between PAH loss and gain. T_r is also useful for comparison with results from other ecosystems and locales as determined by different investigators.

Glutamate and acetate mineralization. Incubation flasks were prepared as for PAH biodegradation assays, except that plastic center wells and rubber septa without Teflon linings were used. Radiolabeled substrate concentrations were 130 and 30 pg/ml of slurry for glutamate and acetate, respectively. Incubations were carried out in the dark at in situ temperatures for 1 h. Replicate controls consisted of sediment slurries killed with 0.5 ml of *m*-cresol. Incubations were terminated by injecting 0.3 ml of 2 M sulfuric acid through the closed rubber septum. $^{14}CO_2$ was collected in the center wells by the addition of 2 ml of 2-ethoxyethanol–ethanolamine (1:1, vol/vol). Flasks were shaken for 30 min at 110 rpm, and the center wells were removed and placed into 10 ml of Aquasol. Radioactivity was counted as described above, and quenching was determined by using the channels ratio method. Concentrations of glutamate and acetate in sediment were not determined; therefore, the rates of $^{14}CO_2$ formation are expressed as relative mineralization rates, in contrast to the PAH mineralization rates.

Chemicals. [9-^{14}C]phenanthrene was obtained from Amersham Co., Arlington Heights, Ill., and [1-^{14}C]naphthalene and [7-^{14}C]benzo[*a*]pyrene were supplied by Pathfinder Co., St. Louis, Mo. Naphthalene, phenanthrene, and benzo[*a*]pyrene had specific activities of 6.12, 19.3, and 8.56 mCi/mmol, respectively. All radiolabeled PAHs were purified prior to use by Florisil (Fisher) column chromatography to a purity greater than 98% as determined by thin-layer chromatography analysis.

L-[U-^{14}C]glutamate and [U-^{14}C]acetate were obtained from Research Products International Corp., Mount Pleasant, Ill. The specific activities were 1.4 and 1.1 mCi/mg, respectively. All solvents were distilled in glass (Burdick and Jackson, Muskegon, Mich.).

Statistical analyses. Correlations, analysis of variance, and linear regressions were carried out on an IBM-PC/XT microcomputer with the STATPACK statistical package.

RESULTS

Transformation rates of model PAHs. Transformation rates of naphthalene, phenanthrene, and benzo[*a*]pyrene were measured between 29 April 1983 and 17 June 1984 at three Boston Harbor sites (Fig. 2). The magnitude of the biodegradation rates was directly related to the ambient PAH concentrations at each site. PAH transformation rates never exceeded 4 ng/h per g of sediment at the WBR site, the sediment least heavily contaminated with PAHs. On the other hand, phenanthrene transformation was as high as 372 ng/h per g of sediment at the FPC site, the sediment most heavily contaminated with PAHs.

In general, phenanthrene transformation rates were greater than or equal to rates for naphthalene at any given site. At the WBR site, where naphthalene concentrations were 1.6 times higher than phenanthrene concentrations, phenanthrene and naphthalene were transformed at similar rates. Similarly, at the FPC site, where phenanthrene levels were 1.5 times higher than naphthalene levels, naphthalene and phenanthrene transformation rates were similar, although their maxima did not coincide at a given date. At the

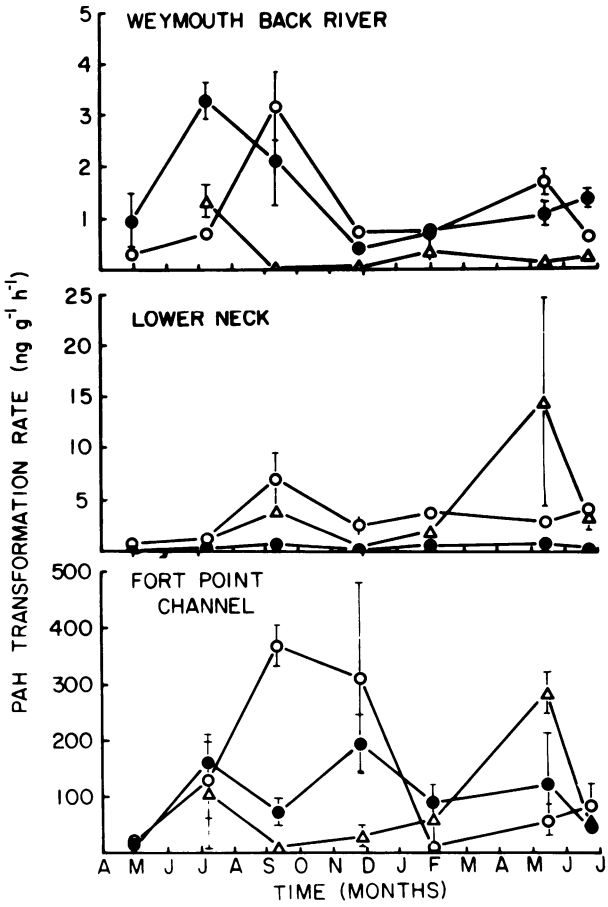

FIG. 2. PAH transformation rates at the three sampling sites in Boston Harbor. Symbols: ●, naphthalene transformation; ○, phenanthrene transformation; △, benzo[*a*]pyrene transformation. Symbol error bars show standard errors of the mean based on triplicate samples.

LN site, the ambient phenanthrene concentration was 4.6 times higher than that of naphthalene, and the resulting naphthalene transformation rates, never exceeding 1 ng/h per g of sediment, were significantly lower than the phenanthrene and benzo[*a*]pyrene rates. Benzo[*a*]pyrene transformation proceeded at a rate significantly lower than the rates for phenanthrene and naphthalene in most cases, even though benzo[*a*]pyrene concentrations were considerably higher than naphthalene and phenanthrene concentrations at the LN and FPC sites. However, there were two occasions, June 1984 at the LN and FPC sites, when benzo[*a*]pyrene transformation rates exceeded the rates for naphthalene and phenanthrene.

Clearly, seasonal patterns of total PAH biodegradation varied from site to site. Transformation rates for all three PAHs increased in the spring (March to July) from annual minima, which occurred from December to March (Fig. 2). At the WBR and LN sites, transformation rates decreased to the lowest seasonal rates by late November and remained at this base-line rate through February. However, this pattern was not due entirely to temperature. Both temperature and salinity, but not pH, had significant effects on transformation rates at the two sites as revealed by analysis of variance (data not shown). Further site-by-site analysis by a linear

TABLE 4. Linear regression analysis of seasonal temperature and salinity with PAH transformation rates

PAH and site	Temp[a]			Salinity		
	r^b	m	b	r^b	m	b
Naphthalene						
WBR	0.610[c]	0.109	0.101	0.082		
LN	0.013			0.523[c]	0.040	−0.823
FPC	0.066			−0.080		
Phenanthrene						
WBR	0.553[c]	0.037	0.081	0.689[e]	0.249	−6.052
LN	0.037			0.532[c]	0.372	−7.550
FPC	0.246			0.562[d]	6.887	−2.330
Benzo[a]pyrene						
WBR	0.227			−0.104		
LN	−0.058			−0.125		
FPC	0.044			−0.496[c]	−5.294	1.482

[a] Abbreviations: r, simple correlation coefficient; m, slope of the line; b, y intercept.
[b] Twenty degrees of freedom (df) for naphthalene and phenanthrene regressions and 17 df for benzo[a]pyrene regression.
[c] Significant correlation, $0.01 < P < 0.05$.
[d] Significant correlation, $0.001 < P < 0.01$.
[e] Significant correlation, $P < 0.001$.

regression method (Table 4) indicates that temperature significantly explained the variation of PAH transformation in only two cases (naphthalene and phenanthrene rates at the WBR site). Variation in salinity, however, explained the variation of the PAH transformation rate in five of nine cases. Other regression models were also tested; however, linear regression best fit the data.

The average recovery of radiolabeled PAHs in the three transformed fractions (i.e., CO_2, polar metabolites, and sediment residue) is shown in Table 5. With increasing molecular weight or ring size, from naphthalene to benzo[a]pyrene, the proportion of transformed PAH recovered as $^{14}CO_2$ decreased. Only 10% of transformed benzo[a]pyrene was recovered as $^{14}CO_2$, whereas the majority was in the form of polar metabolites. Monitoring only the $^{14}CO_2$ fraction would have resulted in a severe underestimate of the extent of transformation of all the PAHs examined.

Seasonal mineralization rates of PAH. Seasonal mineralization rates of acetate, glutamate, naphthalene, phenanthrene, and benzo[a]pyrene are shown by site in Fig. 3 to 5. Acetate and glutamate mineralization rates were used as a relative measure of gross metabolic activity for comparison with the PAH-mineralizing activities in the same sediments. Seasonal rates of acetate and glutamate mineralization were similar, both in magnitude and in seasonal pattern of maxima and minima, for all sites. Seasonal activity peaked in the spring (May) at all three sites, coincident with the rise in water temperature. The rates returned to base-line levels through the rest of the year.

TABLE 5. Percentage of transformed PAHs in CO_2, polar metabolite, and residual fractions

Fraction	% of radiolabel in fraction[a] for:		
	Naphthalene	Phenanthrene	Benzo[a]pyrene
CO_2	41.0	39.1	11.0
Polar	33.2	19.1	56.9
Residue	25.8	41.8	32.1
Total	100	100	100

[a] Percentage in each fraction calculated by averaging all seasonal data at all three of the sites.

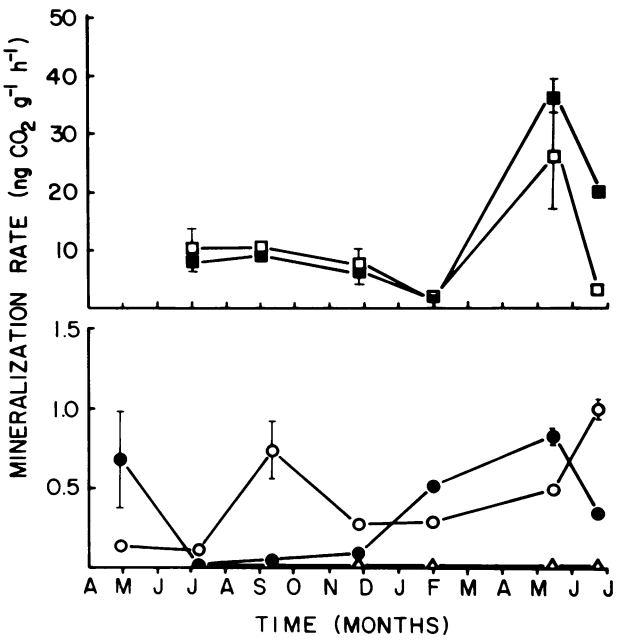

FIG. 3. PAH, glutamate, and acetate mineralization rates at the WBR site. Symbols: ●, naphthalene; ○, phenanthrene; △, benzo[a]pyrene; ■, glutamate; □, acetate. Symbol error bars show standard errors of the mean based on triplicate samples.

The seasonal pattern of PAH mineralization, however, is distinctly different from the pattern of mineralization of acetate and glutamate. Benzo[a]pyrene was mineralized only at the limits of detection, with two exceptions, once at

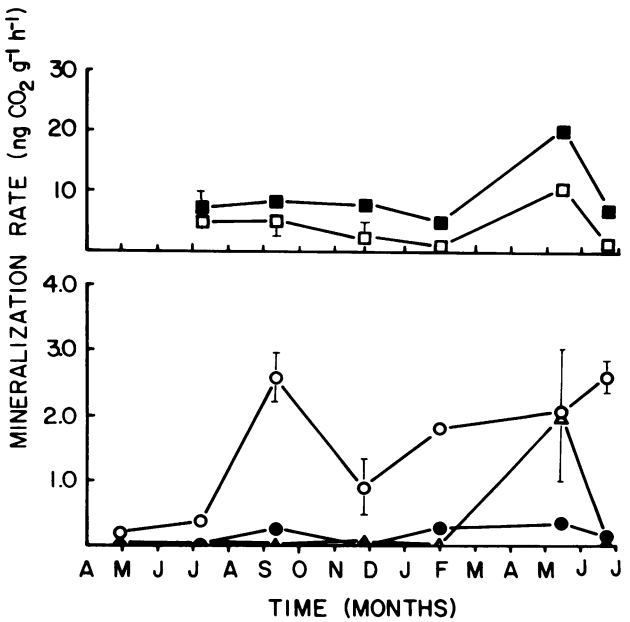

FIG. 4. PAH, glutamate, and acetate mineralization rates at the LN site. Symbols: ●, naphthalene; ○, phenanthrene; △, benzo[a]pyrene; ■, glutamate; □, acetate. Symbol error bars show standard errors of the mean based on triplicate samples.

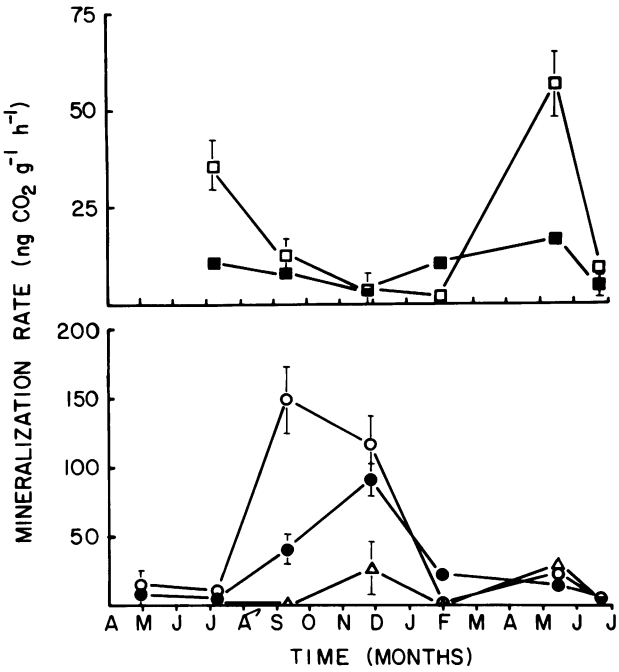

FIG. 5. PAH, glutamate, and acetate mineralization rates at the FPC site. Symbols: ●, naphthalene; ○, phenanthrene; △, benzo[*a*]pyrene; ■, glutamate; □, acetate. Symbol error bars show standard errors of the mean based on triplicate samples.

the LN site and once at the FPC site. Phenanthrene and naphthalene mineralization rates followed similar trends at the WBR site, with peaks in the spring and a phenanthrene mineralization peak as well in the fall. The same pattern was observed at the LN site, except that phenanthrene was mineralized at a consistently higher rate than naphthalene was, probably owing to the higher concentration of naphthalene than phenanthrene at the LN site (Table 1). The seasonal pattern of mineralization of naphthalene and phenanthrene at the highly contaminated FPC site was unlike the pattern at the WBR and LN sites. Rates increased dramatically in early fall (September) and remained high through December. There was a second, much less pronounced peak of activity in the early spring.

As with transformation rates, both temperature and salinity but not pH had significant effects on mineralization rates at the three sites as revealed by analysis of variance (data not shown). Further site-by-site analysis by a linear regression method (Table 6) indicated that temperature did not explain the variation of PAH mineralization, and in only one case, for glutamate mineralization at the WBR site, did temperature relate significantly with mineralization rates. Variation in salinity, however, explained the variation of mineralization rates of acetate, naphthalene, and phenanthrene at every site. Benzo[*a*]pyrene mineralization did not regress significantly with salinity, perhaps because mineralization alone severely underestimates total benzo[*a*]pyrene transformation (Table 5).

Turnover time (T_t) of PAH. Although average transformation rates varied almost 3 orders of magnitude for a given PAH at different sites in Boston Harbor sediments, the T_t of a given PAH was fairly constant among the three sites (Table 7). The shortest turnover times were for phenanthrene, ranging from 7.9 to 19.8 days, and the longest turnover times were for benzo[*a*]pyrene, ranging from 53.7 to 82.3 days.

TABLE 6. Linear regression analysis of seasonal temperature and salinity with PAH, acetate, and glutamate mineralization rates

Hydrocarbon and site	Temp			Salinity		
	r^b	*m*	*b*	r^b	*m*	*b*
Acetate						
WBR	0.266			−0.706[c]	−3.771	118.1
LN	0.318			−0.762[c]	−1.502	47.5
FPC	0.460			−0.681[c]	−7.550	236.0
Glutamate						
WBR	0.710[c]	−0.304	1.721	−0.454		
LN	0.134			−0.661[c]	−1.989	67.4
FPC	0.342			0.409		
Naphthalene						
WBR	−0.302			−0.620[c]	−0.094	3.08
LN	−0.305			0.470[c]	0.017	−0.360
FPC	−0.293			0.462	12.149	−34.7
Phenanthrene						
WBR	0.375			0.654[d]	0.078	−1.826
LN	−0.068			0.481[c]	0.138	−0.360
FPC	0.080			0.707[d]	32.678	−962.3
Benzo[*a*]pyrene						
WBR	−0.035			−0.392		
LN	−0.108			−0.184		
FPC	−0.228			−0.083		

[a] Abbreviations: *r*, simple correlation coefficient; *m*, slope of the line; *b*, y intercept.
[b] Twenty df for naphthalene and phenanthrene regressions and 17 df for acetate, glutamate, and benzo[*a*]pyrene regressions.
[c] Significant correlation, $0.01 < P < 0.05$.
[d] Significant correlation, $P < 0.001$.

Naphthalene turnover times, between 13.2 and 20.1 days, were essentially within the range for phenanthrene. These data indicate that biotransformation rates are dependent on PAH concentrations, as would be expected of an enzymatic activity. Furthermore, benzo[*a*]pyrene is more resistant to transformation than naphthalene and phenanthrene are.

DISCUSSION

Seasonal studies are fundamental to the understanding of the ecological factors that affect complex processes in na-

TABLE 7. Seasonally adjusted PAH biodegradation rates in Boston Harbor sediments

PAH and site	Biodegradation rate[a] (ng/h per g)	Turnover rate (days)
Naphthalene		
WBR	1.16	13.2
LN	0.30	20.1
FPC	94.9	19.2
Phenanthrene		
WBR	1.20	7.9
LN	3.29	8.4
FPC	134	19.8
Benzo[*a*]pyrene		
WBR	0.15	53.7
LN	1.72	82.3
FPC	49.5	80.0

TABLE 8. Survey of estimated turnover times of selected PAHs in waters, sediments, and soils

PAH and environment	Temp (°C)	T_t (days)	Reference
Naphthalene			
Estuarine water	13	500	23
Estuarine water	24	30–79	23
Estuarine water	10	1–30	26
Seawater	24	330	23
Seawater	12	15–800	22
Estuarine sediment	25	21	4
Estuarine sediment		287	25
Estuarine sediment[a]	22	34	17
Estuarine sediment[a]	30	15–20	16
Estuarine sediment[a]	2–22	13–20	This paper
Stream sediment	12	>42	19
Stream sediment[a]	12	0.3	19
Reservoir sediment	22	62	17
Reservoir sediment	22	45	17
Phenanthrene			
Estuarine sediment[a]	22	56	17
Estuarine sediment[a]	2–22	8–20	This paper
Reservoir sediment	22	252	17
Reservoir sediment	22	112	17
Sludge-treated soil	20	282	7
Benzo[a]pyrene			
Estuarine water	10	2,000–9,000	26
Estuarine sediment[a]	22	>2,800	17
Estuarine sediment[a]	2–22	54–82	This paper
Stream sediment	12	>20,800	19
Stream sediment[a]	12	>1,250	19
Reservoir sediment	22	>4,200	17
Sludge-treated soil	20	>2,900	7

[a] PAH-contaminated environments.

ture; however, a scarcity of field studies, coupled with methodological difficulties, has hampered the collection of accurate PAH degradation rates. First, PAHs are found in diverse habitats, yet studies to assess the degradation rates in nature are few. Second, the environmental parameters that affect rates vary with time, but environmental parameters such as seasonal fluctuations are rarely taken into account. Third, individual PAHs often occur in trace concentrations, making realistic rate determination analytically difficult. Most researchers dose their experimental systems with high concentrations of PAH for analytical ease, making extrapolation of rates to the environment unreliable. Fourth, the methodology is arduous and not amenable to large replication. Lastly, the rates are often so low that incubation times as long as months are used to detect degradation. Long incubations can lead to changes in the microbial community structure and activity (34). In this investigation, I have surmounted some of these difficulties to obtain seasonal degradation rates for naphthalene, phenanthrene, and benzo[a]pyrene in estuarine sediments.

With the large variation in methodology from one report to another in mind, I have estimated and summarized turnover times (T_t) of naphthalene, phenanthrene, and benzo[a]pyrene which have been reported in the scientific literature (Table 8). Several conclusions can be derived from the available information. First, the turnover times for individual PAHs may vary greatly. For example, naphthalene turnover times range from 0.3 to 800 days. Differences in methodology and the type of environment explain, in large part, the variation in turnover times. As expected, T_t tends to be shorter in sediments than in the water column. This is probably a consequence of two factors: (i) higher naphthalene concentrations in sediment than in the water column, and (ii) much larger numbers of PAH-degrading bacteria in the sediments than in the water column (30). Also, the turnover times tend to be shorter in hydrocarbon-contaminated sediments than in sediments not highly contaminated with PAHs. Again, this is most probably a function of the enrichment of bacteria capable of PAH degradation (1) or induction of catabolic pathways by extant PAH transformers.

A second conclusion that can be drawn from Table 8 and the results of this study is the general increase in T_t with increasing molecular weight of the PAH. The field-derived data bolster the conclusions gleaned from pure-culture and enrichment-culture experiments. The higher-molecular-weight PAHs such as the five-ring benzo[a]pyrene have turnover times approaching the order of years, even in surficial sediments. In accordance with previous observations (28), results from Boston Harbor sediments show that the amount of PAH mineralized as a percentage of total transformation decreases with increasing molecular weight. The major fraction of transformed PAH was the polar metabolite fraction. This suggests that the high-molecular-weight PAHs are only partially degraded in sediments. Under in situ conditions, these compounds may be buried in anoxic sediments before they are significantly degraded; therefore, actual turnover times may be much longer than estimated here. Without oxygen, PAHs may persist indefinitely in anaerobic sediments and soils (4, 10).

My calculated turnover times for naphthalene and phenanthrene are within the range of reports for estuarine sediments (4, 16, 17, 22, 23), and they are generally much shorter than the turnover times of naphthalene and phenanthrene in freshwater sediments and soil. Salinity itself may affect PAH-particle interactions, since the solubilities of naphthalene, phenanthrene, and benzo[a]pyrene decrease with increasing salinity (11, 38). The high pH of marine sediments may result in lower PAH adsorption to particles (39), leading to a more bioavailable form of the PAHs (35). The pH in the Boston Harbor sites did not vary enough to affect PAH transformation rates. Unfortunately, comparative studies involving the use of similar methodologies are too few for strong conclusions to be drawn.

In this investigation, salinity often had a significant effect on PAH transformation rates, but the effect was site and compound specific (Tables 4 and 6). Phenanthrene transformation and mineralization rates correlated positively with salinity at all sites. Naphthalene mineralization rates also correlated positively with salinity at two sites, but correlated negatively with salinity at the WBR site. Benzo[a]pyrene rates did not correlate with salinity, except for a negative correlation of benzo[a]pyrene transformation with salinity at the FPC site. The effect of salinity on PAH transformation is unclear, but salinity can affect PAH solubility (11, 38), and some PAH degraders are obligate marine organisms (37). PAH degraders have been repeatedly isolated from marine environments (12, 15, 20, 32), and PAH-degrading consortia may be adapted to the salinity regimes of an estuary (R. P. Kerr and D. G. Capone, Mar. Environ. Res., in press). In subsequent experiments along a salinity transect in Boston Harbor (Shiaris, submitted for publication), phenanthrene mineralization by sediment slurries was significantly affected when the sodium chloride concentration in the water phase was altered. Salinity may have a differential effect on the naphthalene and phenanthrene pathways or exclusive de-

graders, since the compounds are probably metabolized by separate pathways (20). The saline stimulation of phenanthrene degradation rates is in contrast to the effect of salinity on the degradation rates of other hydrocarbons (36) and aromatic compounds (3, 33). In the work described in references 3 and 33, however, the environments were probably not preexposed to high concentrations of the compounds compared with the concentrations in Boston Harbor.

Turnover times for benzo[*a*]pyrene in Boston Harbor sediments were shorter than those given in previous reports (Table 8), which include results for estuarine sediments. Benzo[*a*]pyrene transformation rates are often underestimated because metabolites and residue are not accounted for in the experimental design and only a minor portion of benzo[*a*]pyrene transformation results in CO_2 accumulation. Also, Boston Harbor has a history of chronic PAH contamination, which may allow for the long-term adaptation of versatile PAH degraders in these sediments (5). On the other hand, the radiolabeled benzo[*a*]pyrene may be in a more bioavailable form than ambient benzo[*a*]pyrene (35), resulting in a significant overestimation of the transformation rates.

Seasonal patterns of PAH mineralization indicated that PAH mineralization activity at the FPC site was uncoupled from microbial heterotrophic activity; the latter was measured by monitoring acetate and glutamate mineralization. At the WBR site, which was relatively low in organic carbon and ambient PAH levels, the PAH mineralization maxima were coincident with the maxima for heterotrophic activity in the spring to early summer. A similar trend was observed at the LN site, with a second maximum for phenanthrene mineralization in the early fall. In contrast, at the FPC site, which was rich in organic carbon and PAHs, PAH mineralization maxima occurred in the late summer to early fall, following the heterotrophic activity maximum in the spring.

It is tempting to suggest a basis for the uncoupling of PAH-mineralizing activity from heterotrophic activity at the FPC site. Heitkamp et al. (18) provided compelling evidence that bacteria are the predominant agents of naphthalene degradation in estuarine sediments and that bacterial PAH-mineralizing pathways are under inducible control (29, 40). Availability and seasonal variation of simple carbohydrates, lipids, proteins, and oligopolymers in the organic-rich sediments may lead to diminished numbers of PAH degraders by simple competition. Alternatively, the phenomenon of diauxie may play a role in suppressing PAH transformation in the presence of abundant carbon sources. The FPC site, in particular, receives an abundance of sewage. As bacterial activity increases with temperature in spring, PAH degradation may be slow until alternative carbon sources are depleted. Interestingly, Reber and Kaiser (27) found that glucose stimulated the degradation of aromatics by strains of *Pseudomonas putida*. The conflicting evidence indicates a need to examine more closely the effect of organic substrates and concentrations on the degradation of PAHs in the environment. Meiofaunal activities and succession are additional factors that may affect the onset of PAH degradation in estuarine sediments during the warm months. Benthic worms, by means of sediment-mixing activities, can stimulate PAH degradation (13), perhaps by increasing oxygen and nutrient concentrations in the sediments. An unexplored possibility is that benthic fauna act more directly and in concert with bacteria to degrade PAHs (23a).

Low transformation rates during the winter and early spring are probably controlled by low sediment temperatures and the resulting suppression of all bacterial activity. This is also consistent with the evidence that temperature was significantly correlated to PAH degradation but that on a site-by-site basis, temperature did not often explain the variation in PAH degradation.

From this study and previous work, it is clear that the transformation of PAHs in sediments is under the control of complex biological and environmental factors. In oxidized surficial sediments, salinity, season, temperature, and ambient PAH concentration play a major role in the rates of PAH transformation. Marked differences in rates from site to site suggest that the quality and quantity of available carbon sources, nutrients (1), or meiofaunal activity (6, 13) are also master variables of PAH degradation in oxidized surficial sediments. Further experiments to focus on the effect of these factors under realistic environmental conditions should yield meaningful information on the fate of PAHs in aquatic ecosystems.

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