In: Microbial Ecology Research Trends
Editor: Thijs Van Dijk, pp. 1-38
ISBN: 978-1-60456-179-1

Chapter I

Evaluating PAH Biodegradation Relative to Total Bacterial Carbon Demand in Coastal Ecosystems: Are PAHs Truly Recalcitrant?

Thomas J. Boyd, David C. Smith, Jude K. Apple, Leila J. Hamdan, Christopher L. Osburn and Michael T. Montgomery

Abstract

Various techniques have been used to evaluate microbial metabolic activity in natural environments. In recent years, tracer additions of radiolabeled substrates coupled with short term, environmentally-relevant incubation experiments have become standard for assessing overall microbial growth rates as well as utilization rates for specific components of the bioavailable carbon pool. Using these techniques, microbial activities have been determined over a wide range of spatial and temporal scales in a variety of estuarine settings, providing valuable insight into carbon cycling in these dynamic systems. In this paper, we focus on patterns of microbial carbon consumption in estuarine sediments and relate these to the utilization of polyaromatic hydrocarbons (PAHs) as a recalcitrant, yet microbially available component of the carbon pool. Drawing on extensive field studies and those published in the literature, we relate microbial metabolic activities to the utilization of specific carbon species, discuss the partitioning of carbon between bacterial production and respiration (utilization efficiency), address the substrate-activity relationship for utilization of recalcitrant organic carbon and identify seasonal effects on the utilization of natural and anthropogenic carbon pools. These studies elucidate the advantages, disadvantages and caveats of relating microbial consumption of bulk labile organic carbon to the utilization of specific components and improve our ability to predict the fate of organic contaminants in estuarine systems.

I. Introduction

Estuaries are dynamics environments with considerable physical, chemical and biological forcing factors, as freshwater mixes into the sea. Their complex nature makes understanding biogeochemical cycles at both spatial and temporal scales particularly challenging. The heterogeneity of the sources and types of organic matter entering estuarine systems makes predicting microbial utilization of individual structural components difficult at best. Given the natural complexity, it is difficult to predict the residence time and biodegradability of organic contaminants introduced into estuaries from human activities. As human impact on coastal systems continues to increase (*e.g.* Small and Nicholls 2003), it will be ever more imperative to better our understanding of contaminant turnover in sensitive estuarine systems.

The ability of microbes to degrade organic contaminants such as PAHs has been known for decades [*e.g.* Sisler and ZoBell 1947] and an excellent general review of environmental factors controlling marine hydrocarbon biodegradation is provided by Atlas [1981]. In theory, organic contaminants should represent "just another" substrate available for microbial growth and respiration. Most persistent organic compounds bear some chemical resemblance to natural compounds, thus allowing at least limited biodegradation. And, as been demonstrated empirically, most organic compounds are biodegradable provided favorable environmental conditions. Hence, considerable effort has been made in relating chemical structure to degradability for a number of organic contaminant classes. For cultured microorganisms, structure-activity relationships have been developed. Initially, converting laboratory or theoretical structure-activity relationships to field settings proved difficult. *Wolfe et al.* [1980] found a reasonable correlation between second order biodegradation rate constants in natural water samples and second order alkaline hydrolysis rates for phthalates and several pesticides in distilled water. Although not the first such study to link structure to biodegradability, Wolfe *et al*. [1980] used natural water samples, thus attempting to include the effects of physicochemical forcing factors on biodegradation in the field. Research in structure-activity relationships has refined models and generated computer algorithms to predict not only biodegradation potential, but also environmental risk and potential degradation pathways [Boethling *et al.* 1989, Kompare 1998, Dearden 2002, Jaworska *et al.* 2003, Hou *et al.* 2003]. Despite these gains, Wammer and Peters [2005] showed a very narrow range of biodegradation rates based solely on structure (i.e. without environmental forcing factors such as bioavailability), indicating that environmental forcing factors are a critical component in understanding biodegradation rates.

At the molecular level, chemical structure dictates whether a given enzyme system can catabolize an organic molecule. The family of enzymes expressed in response to PAHs often fall into one of two broad categories of multi-enzymes (upper and lower degradation pathways) [*c.f.* Dagley 1987]. A strain that is expressing genes responsible for naphthalene degradation is also typically expressing genes useful for degrading other lower molecular weight aromatic hydrocarbons. Thus subtle differences in chemical structure would be of little importance in determining hydrocarbon metabolism rates unless the compound was so novel as not to be recognized by any of the enzymes of either the upper or lower pathway. Chemical structure can also be important in metabolic efficiency as PAHs that are more thermodynamically stable may require more cellular energy to metabolize and give the

degrading strain a disadvantage when competing for nutrients and oxygen with natural organic matter (NOM) degraders.

A factor dictating biodegradation is contaminant concentration. It is widely accepted that a threshold concentration exists under which biodegradation is not metabolically favorable [Boethling and Alexander 1979a]. The threshold concentration is believed to either represent a level where no net energy can be obtained from the substrate or a level insufficient to stimulate the synthesis and deployment of catabolic enzymes [Wiggins *et al.* 1987, Aelion *et al.* 1987, Wiggins and Alexander 1988]. Exceeding the threshold concentration, one might expect a greater rate of biodegradation [Boethling and Alexander 1979b, Alexander 1985]. Pre-exposure of populations to low and high levels of contaminants may therefore impact subsequent biodegradation [Aelion *et al.* 1989]. Hwang et al. [1989] suggested that different enzyme systems may be responsible for contaminant biodegradation at different concentrations and that enzymes with different kinetics are expressed depending on contaminant concentration and pre-exposure. Adaptation appears to be only one aspect of the story as contaminant-adapted cells eventually loose the ability to degrade compounds at lower concentrations [Roch and Alexander 1997]. Because various enzyme systems working at an array of saturation values are generally responsible for contaminant biodegradation in soils, water and sediments, kinetics necessarily depend on pre-exposure and contaminant concentrations [Scow *et al.* 1986, Wiggins *et al.* 1987, Wiggins and Alexander 1988, Scow *et al.* 1989].

Another parameter impacting biodegradation of organic contaminants in soils and sediments is contaminant bioavailability. Contaminants aged in soil and sediments become less extractable and amenable to biodegradation the longer the aging process continues. Hatzinger and Alexander [1995] demonstrated decreased nitrophenol and phenanthrene biodegradation after extensive sediment or soil aging. Mineralization of contaminants generally decreased as a function of the aging time, indicating a decrease of bioavailability with increased aging [Hatzinger and Alexander 1995]. While the exact means of bioavailability loss for every contaminant in every soil and sediment type is unknown, many explanations exist. A recent review has proposed several general “cases” to conceptualize sorption in soils and sediments [Luthy *et al.* 1997]. In this conceptual model, different geosorbents were evaluated in terms of sorption kinetics, activation energy, heat of sorption, competitive binding sites, steric effects, and solvent extractability. While conceptual models have helped define general processes impacting bioavailability, Chung and Alexander [1998] were unable to definitively model either solvent extractability or bioavailability despite using 16 different soil types. There is evidence that metal-organic complexes may be partially responsible for sediment-PAH sorption [Eschenbach *et al.* 1998]. This phenomenon would be especially applicable to estuarine sediments as estuarine mixing greatly impacts the ionic composition of sedimentary material. Reichenberg and Mayer [2006] suggest two different aspects control bioavailability; the accessible quantity – that being available (or which can become available), and the chemical activity – the ability of the “local” environment to yield the contaminant to resident microorganisms. In soils and sediments, this may of less importance when OM utilization may be dominated by surface-associated strains. A common conceptual model is presented by Johnsen *et al.* [2005 – Figure 3]. In this model, microbes must come in contact with the diffusion boundary dictated by particle sequestration and the

particle-to-water diffusion coefficient of the sorbed contaminant. If microbes actively attach to particles or in fact to the PAH molecules as shown by Wick et al [2002], the diffusion boundary layer may be minimal, giving attached bacteria a selective advantage for biodegradation. If not, biodegradation through intermediary dissolution into the aqueous phase would be assumed for free-living bacteria [Reid *et al.* 2000, Johnsen *et al.* 2005, Johnsen and Karlson 2007].

While geosorbents have received much of the experimental and modeling attention, the role of black carbon (soot) and colloids on contaminant bioavailability have also been assessed. Black carbon constitutes approximately 4% of the organic carbon in soils, and about 9% in sediments [Cornelissen and Gustafsson 2005]. It is believed that hydrophobic contaminants are more strongly bound to black carbon than mineral-based geosrobents [Jonker *et al.* 2005]. In marine systems, soot also appears to be a dominant sorbent for PAH [Gustafsson and Gschwend 1997, Lohmann *et al.* 2005]. In estuarine porewaters, colloids effectively sorbed 25-52% of added PAHs within roughly one day [Chin and Gschwend 1992]. Surprisingly few direct studies have targeted the impact of colloidal sorption with PAH biodegradation. Meredith et al [1998] observed no change in PAH biodegradation based after addition of colloidal humic acid as a sorbent. In contrast, Vacca et al [2005] reported enhanced biodegradation of PAH sorbed to humic acid colloids, postulating that bacteria were able to utilize PAH without previous partition from the colloidal to aqueous phase. One can imagine competing forces in natural systems, with some facilitating and others hindering the biodegradation of hydrophobic organic contaminants. Empirical data on the persistence of "degradable" concentrations of PAH clearly demonstrate that bioavailability has a large impact on the fate of contaminants in soils and sediments [*c.f.* Johnsen and Karlson, 2007, Johnsen et al., 2005, Reid et al., 2000].

Given that hydrocarbons represent an excellent source of energy, routinely made use of by humans, it should come as no surprise that hydrocarbon contaminants also represent a potential source of energy and carbon for microorganisms. Although bacteria in nature seldom grow as they do in laboratory cultures, it is obvious that given ample organic material and nutrients, copiotrophic bacteria will grow and reproduce rather than utilize the organic material for energy alone. In nature, the ability of bacteria to find both ample substrate and nutrients is rare and most likely episodic. Microorganisms generally live in a state between growth and starvation [*c.f.* Kjelleberg et al., 1987]. Large-scale hydrocarbon contamination represents a "feast" in terms of carbon and energy to impacted environments (if toxicity effects are minimal). Without nutrients, principally nitrogen (for proteins and nucleic acids) and phosphorous (for ATP and nucleic acids), organisms are unable to significantly increase biomass, thus biodegradation is limited. Microorganisms typically try to preferentially increase biomass over utilizing carbon for maintenance energy. Cornelissen and Sijm [1996] reported that while 47-83% of available energy is generally utilized for growth, aromatic hydrocarbon biodegradation (toluene and PAHs) showed much lower growth efficiencies (9-26% used for growth). Boyd et al. [2001] observed relatively high growth efficiencies for benzene (average 66%) and toluene utilization (average 59%) in groundwater under anaerobic conditions. In this study, no correlation was found between benzene or toluene utilization and *in situ* nutrients (NO_3^-, NH_4^+, PO_4^{3+}). Similarly, Guerin and Jones [1989],

found no correlation between PAH biodegradation and nutrient concentrations in estuarine sediments.

While it stands to reason that nutrient additions should stimulate biodegradation of hydrocarbons, demonstrating it in the field has been problematic. Paris and Rogers [1986] found virtually no phenol biodegradation enhancement following nutrient enrichment. Jones and Alexander [1988] however observed a shortened acclimation period for nitrophenol utilization when nutrients were abundant. Acclimation, not only to substrates, but perhaps also to elevated nutrient concentrations, appears to have a significant impact on altering the microbial assemblage, stimulating "degraders" to become a more prominent percentage of the total population. In attempting to stimulate organic contaminant degradation by nutrient addition, Steffensen and Alexander [1995] showed that altering an environment to stimulate contaminant biodegradation may serve to stimulate "other" organic matter biodegradation, not biodegradation of the contaminant of interest. This phenomenon was observed in a gasoline-contaminated groundwater site where a groundwater circulation system designed to introduce atmospheric oxygen to anaerobic groundwater greatly stimulated overall bacterial production, but virtually eliminated benzene and toluene utilization which had been occurring anaerobically [Boyd *et al.* 2001]. In terms of stimulating biodegradation, numerous attempts have been made to augment contaminated sites with nutrients in an attempt to enhance microbial catabolic activity. Abundant successful applications in microcosms and mesocosms have been described, but definitive field demonstrations are few [Venosa *et al.* 1996, Macnaughton *et al.* 1999, Venosa *et al.* 2002, Röling *et al.* 2004, Jiménez *et al.* 2006]. Crude petroleum biodegradation appears overall to be stimulated by nutrient additions [Venosa *et al.* 1996, Wrenn *et al.* 1997], PAH and aromatic compound degradation appears less so [Garcia-Blanco *et al.* 2007]. While, biodegradation kinetics may not be altered, addition of fertilizer has been shown to change the microbial community composition [*c.f.* Ogino et al. 2001]. As we discuss in the following section, changes in community composition may be an important precursor to specific organic contaminant degradation.

While nutrient additions have shown limited effectiveness in stimulated contaminant biodegradation, the addition of specific hydrocarbon-degrading bacteria (bioaugmentation) has been even more problematic. For the most part, *in situ* microbial consortia generally out compete added microbes [*c.f.* Chapelle, 1999]. As mentioned above, it appears that "degraders" become a more active component of the assemblage after environments are loaded with organic contaminants, but it has been difficult to correlate an increase in degrader abundance or overall bacterial abundance to enhanced contaminant utilization [*e.g.* Hickman and Novak, 1989]. In general, bioaugmentation using specific microbial strains has shown promise for chlorinated solvent bioremediation [Ellis *et al.* 2000, Morrill *et al.* 2005, Da Silva *et al.* 2006] and some successes have been documented in microcosms [Jouanneau *et al.* 2005, Yu *et al.* 2005] and engineered remediation systems [Trably *et al.* 2003, Domde *et al.* 2007]. While added organisms have been detected in contaminated environments after bioaugmentation [Mishra *et al.* 2001], *in situ* bioaugmentation for PAH bioremediation appears to be a reasonably ineffective treatment [Johnsen *et al.* 2007].

One underlying theme common to soil hydrocarbon plumes and aquatic sediments is the utilization of molecular oxygen to a point where contaminants tend to remain only in localized anaerobic zones. For instance, in groundwater, the majority of the plume may be

anaerobic and high in contaminants, while the edges are low in contaminant and are still oxic. In sediments, this generally occurs below some depth where organic carbon utilization exceeds oxygen diffusion. While anaerobic PAH degradation has been documented under sulfate-reducing [Coates *et al.* 1996, Coates *et al.* 1997] and nitrate-reducing environments [McNally *et al.* 1998], a general consensus is that anaerobic biodegradation is extremely slow under natural conditions [*e.g.* Quantin *et al.* 2005]. Dissolved oxygen appears to be a major factor driving PAH biodegradation in estuarine sediments and aquifers [Bauer and Capone 1985, Brubaker and Stroo 1992, Montgomery *et al.* 2002, Boyd *et al.* 2005a].

While there are many trends have been observed regarding general hydrocarbon and specifically PAH biodegradation control, a comprehensive study covering multiple environments and describing the impact of multiple forcing factors does not exist. In general PAH are typically considered an "exception" rather than just another component of the carbon pool available to microorganisms as a source of carbon and energy. This chapter reports on a series of studies completed from 1997 through 2004 in seven different estuarine systems. In these studies, PAH biodegradation was measured during short-term tracer incubations along with concurrently-measured bacterial production, temperature, dissolved oxygen and other environmental forcing factors. We begin the discussion of our findings with sections on the what is known regarding factors controlling natural organic matter (NOM) biodegradation in estuarine systems and the methods used to assess overall microbial activities and specific carbon (*i.e.* contaminant) biodegradation. We then discuss PAH relative to NOM utilization and what is known of the interplay between utilization of labile and refractory organic matter. The results of our studies along with others that highlight underlying principles of PAH biodegradation in estuarine systems are then described. Finally, a conceptual framework for assessing biodegradation is proposed. It is hoped that this cross-system approach to assessing PAH biodegradation in estuarine sediments leads to an improved fundamental understanding of both PAH and perhaps other recalcitrant organic matter biodegradation.

II. Assessing Microbial Activities

The first careful measurement of PAH utilization at environmentally realistic levels in estuarine sediments was performed by Herbes and Schwall [1978]. This study was particularly relevant as they measured not only PAH mineralization, but also its incorporation into cellular biomass and soluble metabolic intermediates. In order to account for variations in ambient concentration of PAH, radiolabeled PAHs were added at various concentrations so that saturation kinetics could be determined. The authors also measured the utilization of radiolabeled PAH over time in order to investigate degradation kinetics. In subsequent studies, ^{14}C-labeled substrates were used in short-term incubations to assess spatial (salinity through and estuary) and temporal (seasonal) variation in aromatic hydrocarbon biodegradation [Pfaender and Bartholomew 1982, Bartholomew and Pfaender 1983] These studies were significant for two reasons. First, as in Herbes and Schwall's studies, both respiration and incorporation were measured allowing calculation of degradation efficiency (although they were not reported), and second, the authors assumed added radiotracers were

in excess of any ambient contaminant. A significant methodological unknown when analyzing radiolabeled substrate mineralization is the efficiency of biodegradation. If one has a minimal number of samples, cellular incorporation can be readily assayed [*e.g.* Herbes and Schwall 1978]. By "digitizing" graphs in this paper, growth efficiencies were calculated as ~38% for naphthalene and ~78% for anthracene utilization. Typically, mineralization (i.e. conversion to CO_2) is the only measured parameter in ^{14}C-substrate addition studies. Clearly, unknown growth efficiencies would introduce errors in substrate utilization estimates.

In addition to efficiency considerations, the studies of Shiaris [1989a, 1989b] highlight another difficulty with assessing contaminant degradation using ^{14}C-labled substrates: the impact of isotopic dilution. Tracer-based biodegradation studies generally rely on one of two assumptions: 1) the radiotracer is added at a concentration far exceeding the ambient concentration, or 2) the radiotracer is added at a concentration that is negligible compared to the ambient concentration. In the first scenario, the microbial assemblage is presented with a "new" substrate to degrade and therefore must synthesize the metabolic capability to do so. This is partially the cause for lags observed when measuring biodegradation rates [*e.g.* Shimp and Pfaender 1987, Aelion *et al.* 1987]. Assumption two is preferable in terms of understanding the *in situ* rate of substrate biodegradation. The idea in this scenario is that organisms will be minimally impacted by a tracer addition of substrate (*i.e.* < 10% of the ambient pool), and will continue their metabolism without up-regulating protein synthesis to degrade the contaminant of interest. Common practice is also to end the incubation in as short a time as possible so that bottle effects are minimized (*i.e.* organisms do not utilize energy and carbon synthesizing proteins strictly to deal with a new environment). A compication for assumption two is that one must measure the ambient concentration of the same compound added as a radiotracer. This allow recalculation of specific activity based on the total pool of substrate in question. The importance of calculating isotopic dilution has been reported for ecological studies [*e.g.* King and Berman 1984], but is largely disregarded in the PAH biodegradation literature. It is a critical component in performing experiments with isotopic tracers as it can dramatically impact the final rate measurements. In addition, because measurements are generally conducted with contaminated groundwater, soils or sediments, ambient concentrations almost certainly impact the analysis. For instance, if one examines the experiment of Boethling and Alexander [1979] in which radiolabeled substrates were added to stream water at concentrations ranging over six orders of magnitude, the impact of an appreciable ambient contaminant load would change the results markedly. The rate of CO_2 production from ^{14}C-labeled 2,4-D ranged 6 orders of magnitude. If exposed stream water were used for the experiments and had an ambient concentration of 2.2 μg mL^{-1}, recalculating the rates using isotopic dilution would only give two orders of magnitude variation in respiration rates. The work of Shiaris [1989a, 1989b], provided detailed methods for calculating and using isotopic dilution in reporting PAH mineralization rates.

Another critical experimental unknown when measuring ^{14}C-labeled substrate mineralization is the stoichiometry of the contaminant biodegradation process. For PAHs this may be lead to considerable uncertainty as some compounds are available as uniformly labeled (UL), for instance naphthalene, while others are labeled only on one of the ring positions, like fluoranthene-3-^{14}C. So, for instance, if one adds 10 molecules of UL-^{14}C-naphthalene to an incubation, then recovers 10 molecules of $^{14}CO_2$, it is unknown if ten

naphthalene molecules were partially degraded, one molecule was completely degraded to CO_2, or some intermediate number of naphthalenes were mineralized. In this scenario, over 90% error in degradation rate estimates is possible. Although this seems unmanageable, there are several phenomena that might mitigate experimental error. There are only several major pathways that have been observed for breakdown of aromatic compounds [*c.f.* Dagley 1987]. If one were to allow biodegradation to occur for only a brief period, one might expect buildup of "known" degradation intermediates as certain pathway steps require more activation energy than others leading to transient buildup of certain intermediates. Short incubation times fit perfectly with tracer study theory, wherein a negligible amount of ^{14}C-labeled substrate is added relative to the ambient concentration. If done properly, a short incubation time coupled with a small number of labeled substrate molecules relative to ambient molecules, one might expect to drive the stoichiometry towards each $^{14}CO_2$ coming from one UL substrate molecule. For this reason, our biodegradation studies assume one molecule of ^{14}C-labeled naphthalene is degraded for each $^{14}CO_2$ molecule recovered [*c.f.* Boyd *et al*. 2005].

Short-term tracer incubations are however susceptible to another uncertainty. When tracer substrate is added to soils or sediments, one cannot be certain there is instantaneous homogenous distribution of isotopic tracer with the ambient pool of substrate. Tracer theory relies on equilibrium mixing of added and substrate. Radiolabeled substrates are generally added in solvent diluent. The impact of solvent addition has been studied extensively [*c.f.* Herbes and Schwall 1978, Bauer and Capone 1985] and can be minimized to have no statistical effect. However, considerable effort has been given to understanding the "aging" of ^{14}C-labeled substrates in sediment slurries [*c.f.* Aptiz et al. 1999]. Reid *et al* [1998] found that the most rapid equilibrium for added radiolabeled PAH tracers occurred when soils or sediments were dried before mixing. While this might allow more homogenous distribution of added isotope, it is impractical for performing rapid microbiological assays given the deleterious effect it would have on natural sediment microorganisms. For lower molecular weight PAHs (up to three rings), sorption and desorption to sediment slurries was found to be linear and rapid and was concluded to have little impact on biodegradation rate measurements [Chandra *et al.* 1996]. For studies presented here, ^{14}C-labeld substrates were added to polystyrene tubes after minimal dilution in solvent. The solvent was then allowed to evaporate prior to inoculation with sediment slurry. PAH sorption to the incubation vessel was assayed and averaged >90% remaining after aqueous rinses.

In previous work by the current authors and others a strong relationship between availability of dissolved oxygen (DO) and PAH biodegradation has been noted [see Introduction section]. Because oxygen comprises ~21% of the atmosphere, it can become a contaminating presence when performing biodegradation studies to determine *in situ* rates. One difficulty in performing incubations is an accurate knowledge of sediment DO content of collected sediments prior to incubation. Without this information, it is impossible to acclimate the incubation headspace to the appropriate oxygen partial pressure. Incubations reported here were performed in 100 mL culture tubes using surficial sediment material. One milliliter of filtered natural water collected immediately above the sediment surface was added to make sediment slurries. We calculated that oxygen introduced to the tube during sample processing would likely penetrate only ~2 mm into the overlay water used to make

the slurries [Boyd *et al.* 2005a] and thus not significantly influence the results. Bastviken and Tranvik [2001] conducted a study to determine if atmospheric oxygen could bias estimates of bacterial production in aerobic and anaerobic waters. They concluded it was possible to accurately measure leucine incorporation in anoxic samples without using an anaerobic hood to transfer and incubate samples.

Given the uncertainties inherent in measuring PAH biodegradation, it seems of utmost importance to perform experiments with consistent methodology. For the reasons discussed, it is appropriate to design experiments such that true tracer levels of PAH are added to determine *in situ* rates, rather than determine "potential" by adding PAH to pristine sediment or water. This seems intuitive as the driver to measure PAH utilization rates is almost universally related to detectable levels in the environment. Although the uncertainties in methodologies may be considerable, one might predict that consistent application of a particular experimental protocol should provide largely systematic error such that cross-season or cross-system comparisons prove valid even if actual rates are not completely accurate (Table 1).

III. Estuarine Microbial Activities

Bacteria dominate the biomass of estuarine microbial communities and mediate almost every ecologically relevant biogeochemical process [Day *et al.* 1989]. Understanding environmental factors that regulate the microbial metabolism are fundamental to understanding natural and contaminant carbon cycling in estuarine ecosystems. Manipulative experiments and field studies alike have identified significant effects of temperature [Apple *et al.* 2006], dissolved organic carbon concentrations (DOC) [Shiah and Ducklow 1994a], inorganic nutrients [Smith and Kemp 2003], nutrient stoichiometry [Goldman *et al.* 1987], salinity [del Giorgio and Bouvier 2002], and organic substrate source and quality [Revilla *et al.* 2000, Amon *et al.* 2001] on microbial carbon metabolism. These various factors differentially influence pathways of carbon metabolism.

Temperature and Salinity

Temperature and salinity are two of the most important factors influencing biotic and abiotic processes in estuarine ecosystems, with temperature exhibiting a universal effect on the range and magnitude of biological processes [Malone *et al.* 1988, Smith and Kemp 1995, Lomas *et al.* 2002, Apple *et al.* 2006] and salinity being a fundamental force shaping biogeochemical processes, species distribution and water-column chemistry [Day *et al.* 1989]. Recent analysis of data from over 50 estuarine systems corroborates the primacy of temperature and salinity in shaping the variability in microbial processes and water column chemistry [Apple *et al.* 2007].

Table 1. Locations, substrates, mineralization rates and turnover times for estuarine sediments

		Mineralization Rate (μg C kg^{-1} d^{-1})0					Turnover Time (d)				
Estuary	**Substrate**	**Stations**	**Average**	**Median**	**Range**		**Stations**	**Average**	**Median**	**Range**	
					low	**high**				**low**	**high**
Charleston, SC	Naphthalene	157	31.7	2.29	0	567	41	218	73	3.7	3680
	Phenanthrene	185	30.9	0.41	0	935	118	2586	526	1.1	28716
	Fluoranthene	185	191	0.34	0	16730	107	1500	517	0.6	16347
Chesapeake Bay, VA	Naphthalene	66	6.5	2.07	0	45	32	191	8.1	0.07	2498
	Phenanthrene	65	3.9	2.96	0	92	54	1470	431	0.32	22388
	Fluoranthene	62	18.4	1.08	0	854	49	723	195	5.6	7264
Narragansett Bay, RI	Naphthalene	15	1.51	1.1	0.13	0.48	0	NA	NA	NA	NA
	Phenanthrene	12	0.31	0.22	0	0.92	12	225	122	5.1	756
	Fluoranthene	13	1.36	1.13	0	2.87	13	102	41	5	412
Pearl Harbor, HI	Naphthalene	37	93.3	1.26	0	603	35	1229	702	34	6950
	Phenanthrene	37	74.1	1.56	0	895	35	14590	918	17	125495
	Fluoranthene	37	168	1.54	0	2911	30	2164	565	8	34125
San Francisco Bay, CA	Naphthalene	24	1.23	0.5	0	8.8	3	8.3	3.9	1	20
	Phenanthrene	17	11.8	4.1	0	91.4	11	8	1.47	0.17	62.4
	Fluoranthene	20	0.65	0.2	0	5.4	16	342	184	3.2	2088
San Diego Bay, CA	Naphthalene	22	2.5	0.91	0	10.9	0	NA	NA	NA	NA
	Phenanthrene	22	1.3	0.4	0	8.6	20	1006	185	8	8342
	Fluoranthene	22	4.8	2.97	0	42	20	1922	796	20	11592
Delaware Bay, PA	Naphthalene	198	2.2	0.36	0	48.5	164	1470	131	0.01	21000
	Phenanthrene	196	4.3	1.50	0	60	185	440	125	2.2	4220
	Fluoranthene	185	6.1	0.41	0	223	183	973	367	0.19	15600

Table 2. Concentration of labile and refractory DOC in Chesapeake Bay and Delaware Bay in µg C L^{-1}. L1 refers to DOC utilized by bacterioplankton during aerobic respiration in less than 5 days. L2 refers to DOC utilized by bacterioplankton during aerobic respiration in less than 20 days. ROM refers to refractory DOM requiring greater than 20 days to be oxidized

Date	Location	Depth (m)	Lat	Long	DOM (total)	L1-DOM	L2-DOM	ROM (DOC -(L1+L2))	% Labile (L1)	% Labile (L2)	% Labile (Sum L1+L2)
Sep-03	Tidal Fresh Chesapeake Bay	1.0	39.4531	-76.0222	2950	346	539	2064	12	18	30
Sep-03	Tidal Fresh Chesapeake Bay	3.0	39.4531	-76.0222		318	397				
Sep-03	Tidal Fresh Chesapeake Bay	6.0	39.4531	-76.0222		363	433				
Sep-03	Mesohaline Chesapeake Bay	1.0	37.8345	-76.1815	2750	253	423	2074	9	15	25
Sep-03	Mesohaline Chesapeake Bay	12.0	37.8345	-76.1815		148	309				
Sep-03	Mesohaline Chesapeake Bay	17.0	37.8345	-76.1815		131	376				
Sep-03	Polyhaline Chesapeake Bay	1.5	36.9602	-75.9970	1880	206	341	1333	11	18	29
Sep-03	Mesohaline Delaware Bay	1.0	39.2387	-75.3157		250	19				
Sep-03	Mesohaline Delaware Bay	5.0	39.2387	-75.3157		374	502				
Sep-03	Mesohaline Delaware Bay	10.0	39.2387	-75.3157		421	361				
Mar-04	Oligohaline Delaware Bay	2.0	39.5014	-75.5580	2500	794	1096	610	32	44	76
Mar-04	Mesohaline Delaware Bay	2.0	39.3824	-75.4779	2000	646	892	463	32	45	77
Mar-04	Mesohaline Delaware Bay	5.9	39.3824	-75.4779		933	1288				
Mar-04	Mesohaline Delaware Bay	10.4	39.3824	-75.4779		495	684				
Mar-04	Tidal Fresh Chesapeake Bay	1.9	39.4403	-75.9961	1790	566	782	442	32	44	75
Mar-04	Tidal Fresh Chesapeake Bay	5.0	39.4403	-75.9961		698	964				
Mar-04	Mesohaline Chesapeake Bay	1.9	37.8712	-76.1579	2140	617	851	672	29	40	69
Mar-04	Mesohaline Chesapeake Bay	16.5	37.8712	-76.1579		570	787				
Mar-04	Mesohaline Chesapeake Bay	26.5	37.8712	-76.1579		505	697				
Mar-04	Mesohaline Potomac River	1.9	38.1715	-76.5875	1810	635	878	297	35	48	84
Mar-04	Mesohaline Potomac River	8.0	38.1715	-76.5875		572	790				
Mar-04	Mesohaline Potomac River	14.2	38.1715	-76.5875		539	744				
Mar-04	Polyhaline Chesapeake Bay	2.0	37.2324	-76.1295	1888	446	616	826	24	33	56
Mar-04	Polyhaline Chesapeake Bay	4.0	37.2324	-76.1295		271	378				
Mar-04	Polyhaline Chesapeake Bay	9.0	37.2324	-76.1295		403	556				
									24	34	58

*L1 and L2 calculated from 5 and 20 day BOD data respectively assuming a ratio of xxx

Temperature plays a fundamental role in regulating the activity and growth of all microorganisms [Rose 1967, Madigan *et al.* 2003]. The effect of temperature on cellular processes in non-psychrophylic cultured bacteria has been well documented, with a general consensus that metabolic rates approximately doubles for each 10ºC increase in temperature [Morita 1974]. The temperature effects on natural bacterioplankton communities in temperate and sub-tropical estuaries are not as well understood but have been the subject of numerous studies [Hoch and Kirchman 1993, Sampou and Kemp 1994, Shiah and Ducklow 1994a, Pomeroy *et al.* 1995, Felip *et al.* 1996, Raymond and Bauer 2000, Staroscik and Smith 2004], the overwhelming majority of which have focused on the temperature dependence of bacterial growth rates and production (BP) alone.

In general, these studies share two fundamental conclusions. First, that the temperature dependency of bacterial growth and production is stronger at lower temperatures, and second, that temperature effects are often modulated by other environmental conditions, namely the availability of inorganic nutrients and the quality and quantity of organic matter substrates available for growth. Three studies of seasonal PAH biodegradation in estuarine sediments showed found no direct correlation between the former and *in situ* temperature [Shiaris 1989, Montgomery *et al.* 2002, Boyd *et al.* 2005a]. The rate of PAH delivery to the sediments may vary during the season, providing a constant flux [*c.f.* Arzayus *et al*. 2001, Pohlman *et al*. 2002]. Accumulation may be impacted not only by PAH degraders, but also by lower activities of benthic invertebrates that rework sediments allowing DO and nutrient influx and removal of wastes [Schaffner *et al.* 1997].

Estuarine circulation creates an environment where dramatic changes in salinity may occur on relatively small spatial and temporal scales. In this regard, changes in salinity have the potential to influence microbial metabolic processes, especially for microorganisms faced with the need to maintain internal osmotic state. del Giorgio and Bouvier [2002] documented increased mortality, decreased cell growth and single-cell activity in the estuarine turbidity maxima, attributing this to physiological stress associated with changing ionic strength. Salinity may also have an direct effect on phylogenetic composition of resident bacterial communities [Barcina *et al.* 1997], which in turn influences microbial metabolic processes. For example, shifts in both phylogenetic composition and the abundance of highly-active cells have been documented along salinity gradients in a wide range of estuaries [del Giorgio and Scarborough 1995, Crump *et al.* 1999, Pinhassi *et al.* 1999, Bouvier and del Giorgio 2002, Cottrell *et al.* 2006] and have been linked to the variability and magnitude of community-level metabolic processes [Yokokawa *et al.* 2004]. Concurrent changes in metabolism and salinity may also be related to changes in resource availability, as the supply and quality of organic matter varies dramatically along the estuarine gradient. In their study of York River estuary, Schultz and Ducklow [2000] observed distinct differences in the type of DOM used by bacterioplankton at different salinity ranges, while Schultz et al. [2003] report a strong correlation between bacterial metabolism and salinity, with highest values of specific growth at the freshwater endmember. Unlike the effect of temperature, the role of salinity in the regulation of microbial metabolism represents the effect of a complex combination of underlying mechanisms that are not altogether understood. In the only experiments we know of to assess salinity impacts, Shiaris [1989] found no relationship between *in situ* salinity and PAH utilization in Boston Harbor.

Dissolved Oxygen Concentrations

Microbial degradation of organic matter occurs most effectively in the presence of oxygen, thus the availability of dissolved oxygen (DO) plays an extremely important role in the longevity and cycling of organic matter in aquatic systems. Organic matter loading to estuarine ecosystems typically exceeds the input of oxygen that would be necessary to convert it all into CO_2, resulting in the accumulation of unconsumed organic matter, particularly in the sediments. Researchers have found that the oxygen demand of 1ml of sediment in the Georgia coastal zone during summer was equivalent to the total amount of dissolved oxygen in almost 1 L of overlying water [Day *et al.* 1989]. Oxygen consumption in the water column alone is lower, yet still substantial and at rates typical of estuarine systems can drive the water column hypoxic (i.e. < 2mg L^{-1}) in 5-7 days [Jonas 1997]. The availability of dissolved oxygen is also determined to a large extent by ambient water temperatures, as temperature regulates the solubility of oxygen in water and mediates the magnitude of the very metabolic processes (discussed above) that consume oxygen. Sediment oxygen consumption (SOC) rates generally increase with increasing temperature until bottom water concentrations of dissolved oxygen fall below 2.5 mg L^{-1}, at which point SOC rates become oxygen limited [Cowan and Boynton 1996]. Likewise, dissolved oxygen concentrations tend to be higher in colder months when solubility is higher and pelagic and benthic respiration is lower [Hopkinson, Jr. and Smith 2005]. In this regard, temperature and oxygen interact to regulate the relationship between oxygen availability and microbial metabolism. The primary mechanism is the requirement of oxygen as a terminal electron acceptor in aerobic respiration, with temperature modulating the availability of oxygen directly through the effect on solubility and indirectly via stimulation of oxygen-consuming microbial metabolic processes. As briefly discussed in the introduction, DO appears to have a profound effect on PAH biodegradation in estuarine sediments – seemingly to the exclusion of most other environmental forcing factors [Bauer and Capone 1985, Hurst *et al.* 1996, Boyd *et al.* 2005a].

Types and Lability of NOM in Estuarine Environments

The term “labile organic matter” is often applied to OM utilized for bacterial re-growth in 1-2 weeks. Thus LOM represents the net lability of the highly-labile pool (L1) and that of the intermediate lability (L2) pool [Søndergaard and Middelboe 1995, del Giorgio and Davis 2003]. Although it is tempting to routinely combine the L1 and L2 pools, first order organic matter decay rates (k) for these substrate pools are often significantly different. Hendrickson, et al. [2007] demonstrated that k values range from 0.094 to 0.001 day^{-1} depending on the source of the organic mater. del Giorgio and Davis [2003] identified similar differences in the shape of slopes describing the consumption of organic matter, with evidence of rapid consumption of the highly-labile fraction relative to that of intermediate lability. However, following the consumption of DOM over longer periods, (i.e. 7-10d) across a wide range of estuaries reveals higher proportion of semi-labile DOM and underscores the importance of this DOM in carbon cycling in estuarine systems. Thus, the

most labile substrates in aquatic systems appear to originate from autochthonous sources, specifically phytoplankton [Münster and Chróst 1990, Canuel and Martens 1993, Søndergaard and Middelboe 1995, Connolly and Coffin 1995], and exhibits the highest *k* value of all classes of organic matter routinely observed in estuarine systems [Hendrickson *et al.* 2007]. However, microbial carbon consumption is supplemented by a pool of semi-labile DOM, especially in estuarine systems.

The relative lability of the OM pool exhibits seasonal patterns which are linked to kinetics of supply of allochthonous and autochonous sources [Hamdan and Jonas 2006, Hendrickson *et al.* 2007]. In the Chesapeake Bay and Delaware Bay, during Fall of 2003, on average, the water column L1 pool accounted for 9 to 12% of the total DOM pool (Table 2). Conversely, during spring 2004, the water column L1 pool accounted for 24-34% of total DOM indicating that the supply of autochonous DOM resulting from phytoplankton primary production greatly influenced the lability of the bulk pool. A similar pattern was observed in the mesohaline Potomac River, where the concentration of LDOM was often highest during early spring and late summer in surface waters in conjunction with a bimodal chlorophyll a maximum [Hamdan and Jonas 2006].

Seasonal cycles in sediment LOM concentration and composition have also been observed in Cape Lookout Bight, NC [Canuel and Martens 1993], and San Francisco Bay [Lesen 2006] which reflect autochonous production and supply of LOM to the water column. Deposition of particulate matter to sediments resulting from phytoplankton primary production is the dominant source of LOM in many locations [Zimmerman and Canuel 2001, Sobczak *et al.* 2002, Stepanauskas *et al.* 2005]. In Cape Lookout Bight LOM accounts for approximately 21% of the total sediment OM pool, higher than the average observed in aquatic systems, and results primarily from phytoplankton-derived particulate organic matter. Organic matter derived from terrestrial sources is a minor component of sediment LOM in this location [Canuel and Martens 1993, Sobczak *et al.* 2002, Stepanauskas *et al.* 2005]. A study of San Francisco Bay by Sobczak et al. [2002] revealed that LDOM contributes significantly to the LOM pool and at times equals or exceeds LPOM. Unlike most locations, data from San Francisco indicate that LDOM in sediments results from deposition and hydrolysis of LPOM but also from direct import of LDOM from the water column. This indicates that LDOM import is anomalous in this location compared to other estuaries, however, in general, DOM import to estuarine sediments is rarely studied.

Pulses of LOM or phytoplankton biomass in the water column result in a time lagged (depending on hydraulic residence) depositional event of LOM to sediments. The deposition of LOM may have a significant and predictable impact on mineralization rates of OM as well as contaminants in estuarine sediments. Differential bacterial utilization of OM types impact the resulting composition of the bulk OM in sediments. The rapid uptake of the L1 and L2 fraction will result in an increasingly refractory OM pool [Canuel and Martens 1993, Hendrickson *et al.* 2007] and thus results in a complex mixture of increasingly humic material. Microbial utilization of the remaining (bulk) material is poorly understood due to limited knowledge of chemical structure and substrate utilization rates of the R fraction of OM [Münster 1993, Hendrickson *et al.* 2007]. The presence of high concentrations of ROM in marine and estuarine sediments will enhance sediment affinity for hydrophobic anthropogenic substrates such as PAHs [Xing 1997, Lueking *et al.* 2000, Granberg *et al.*

2005, Parrish *et al.* 2005]. However, an increase in the bulk organic matter content (L1, L2 and R) may decrease the magnitude of sorption of PAHs to sediment particles and thus render PAHs more bioavailable.

Influence of LOM on Mineralization of PAHs

In sediments with elevated concentration of labile substrates, relative to OM poor locations, the utilization of refractory compounds, such as PAH may be reduced due to selective utilization of more labile compounds[Hendrickson *et al.* 2007]. However, some studies indicate that the uptake of recalcitrant OM may actually be enhanced by the presence of organic LOM or other organic compounds [Turnewitsch *et al.* 2007]. Some studies have investigated the impact of OM additions on the bioavailability (to bacteria) of specific PAHs (e.g., naphthalene, fluoranthene, and pyrene). In one study, non-PAH contaminated marine sediments were amended with a 2% increase in total organic carbon resulting from terrestrial lignin (R source) and lysed microalgae (*Tetraselmis* sp.) (LOM source), the latter of which was high in fatty acids and amino acids (L1 and L2) [Granberg *et al.* 2005]. To the same samples, 30 μg g^{-1} pyrene was added to determine the effect of OM additions on pyrene bioavailability. In surface (oxic) sediments amended with LOM, microbial pyrene mineralization (measured as $^{14}CO_2$ evolution) was 31% higher than observed in unamended sediments and 55% higher than observed in R amended sediments. The addition of LOM may have enhanced mineralization rates due to the addition of both carbon and nitrogen, the latter which may have been the a limiting nutrient. However, the addition of ROM resulted in lower mineralization rates compared to both the LOM-amended and control treatments. Bonding of pyrene to lignin leading to lower bioavailability was used to explain this finding. Xing [1997] likewise observed that sorption of naphthalene to soil organic matter increased as the aromaticity of the soil increased, and relativity lability of the OM pool decreased.

Selck et al. [2005] conducted a experiment to determine the relative bioavailability of fluoranthene after sediments were amended with ROM (terrestrial lignin) and LOM (*Tetraselmis* sp. Lysate). After 45 days of exposure, fluoranthene contaminated sediments amended with LOM had an 80% decline in total organic carbon indicating increased mineralization rates of both fluoranthene as well as bulk OM compared to unamended controls. Conversely, organic carbon content moderately increased in ROM treated sediments. After 45 days of exposure, the lowest concentrations of fluoranthene and highest concentrations of daughter products of fluoranthene were detected in sediments enriched with LOM. Interestingly, in the presence of LOM, the toxicity of fluoranthene to *Amphiura filiformis* (brittle star) increased, likely due to the production of daughter products during microbial degradation.

The studies described above indicate that bacterial mineralization of PAHs in sediments containing LOM may occur more rapidly than in sediments enriched with ROM due to both physicochemical and biological interactions between substrate, contaminant and sediment [Xing 1997, Lueking *et al.* 2000, Granberg *et al.* 2005, Selck *et al.* 2005]. A survey of estuarine sediments in Delaware Bay near Philadelphia, PA showed no correlation between bulk sediment DOM and PAH mineralization (naphthalene, phenanthrene and fluoranthene)

[Boyd *et al.* 2005a]. However, sediment OM was not fractionated to determine the proportion of labile and recalcitrant material. Such information would greatly enhance understanding of PAH mineralization in natural estuarine sediments, specifically after seasonal depositional events of autochonous OM.

The period that LOM concentrations in the water column and sediments in temperate estuaries are highest (late spring to early summer) [Canuel and Martens 1993, Canuel and Zimmerman 1999, Zimmerman and Canuel 2001, Stepanauskas *et al.* 2005] coincides with annual maximum increases in ambient sediment temperature and the onset of seasonal water column hypoxia in some estuaries [Shiah and Ducklow 1994b, Hamdan and Jonas 2006]. Some reports indicate that temperature increases have a minor impact on PAH mineralization rates, and instead the availability of molecular oxygen has the strongest impact on regulating PAH mineralization [Boyd *et al.* 2005b]. The data above indicates that seasonal patterns in PAH mineralization may also be attributable to increased bioavailability of PAHs due to physical-chemical interactions with fresh LOM or stimulation of microbial communities of PAH degraders due to the presence of fresh LOM.

IV. PAH Biodegradation in Estuaries

Ambient PAH concentration is the net result of PAH flux into the media minus chemical and biological removal. In bioremediation studies, PAH concentrations have been measured in sediments over time and if the concentration of a given compound changes little, then it is assumed that the PAH is poorly biodegraded or recalcitrant. However, this determination cannot truly be made in the absence of knowledge of the amount of flux or biodegradation rate. Although flux is difficult to measure in natural systems, there are two reports comparing PAH flux to biodegradation rates in coastal sediments [Pohlman *et al.* 2002, Chadwick *et al.* 2006]. Chadwick *et al.* [2006] concluded that PAH flux to the sediments of San Diego Bay (sediment trap study) was largely negated by PAH biodegradation (radiotracer additions) in bioturbated surface sediments. Other transport processes (e.g. groundwater seepage, diffusion) were several orders of less significant in affecting ambient PAH concentration. Pohlman *et al.* [2002] came to a similar conclusion when examining seasonal PAH transport on particles into the Naval Reserve Basin in Philadelphia though they found that PAH metabolism was a larger component of total heterotrophic organic matter degradation during the winter months (higher oxygen levels).

In six of the seven ecosystems studied (Table 1), average naphthalene mineralization rates were less rapid than either those for phenanthrene or fluoranthene or both. A similar phenomenon has also been reported by Rockne and Strand [2001]. Though we did not find a linear relationship between PAH concentration and mineralization rate, we did find the same general trends of higher mineralization rates being associated with sediments with higher ambient PAH concentrations (Figures 1-7). This trend is more evident is ecosystems with a greater range of ambient PAH concentrations (1 to 10,000 $\mu g\ g^{-1}$), such as Pearl Harbor, HI (Figure 1). PAH flux may be more related to ambient PAH concentration in sediment at the extremes, that is, relatively pristine environments with little anthropogenic impacts would likely have both low ambient PAH concentrations (below 1 $\mu g\ g^{-1}$) and low flux (and

likewise low mineralization). Heavily impacted ecosystems (PAH concentration > 100 µg g^{-1}) might be expected to have such high flux that the microbial system is overwhelmed and a high ambient PAH concentration may be maintained despite relatively high mineralization rates. In sediments in most anthropogenically influenced ecosystems (PAH concentration = 1-100 µg g^{-1}), the PAH inputs could be episodic and so PAH flux and ambient concentration would be more uncoupled.

To further investigate the relationship between ambient PAH concentration and PAH biodegradation, we took data from published reports of flask studies of bioremediation treatments. Because PAH mixtures used in the studies typically have different concentrations of the various PAH compounds, the initial concentration of the individual PAH compound was regressed with the amount of that compound that had biodegraded over the course of the treatment.

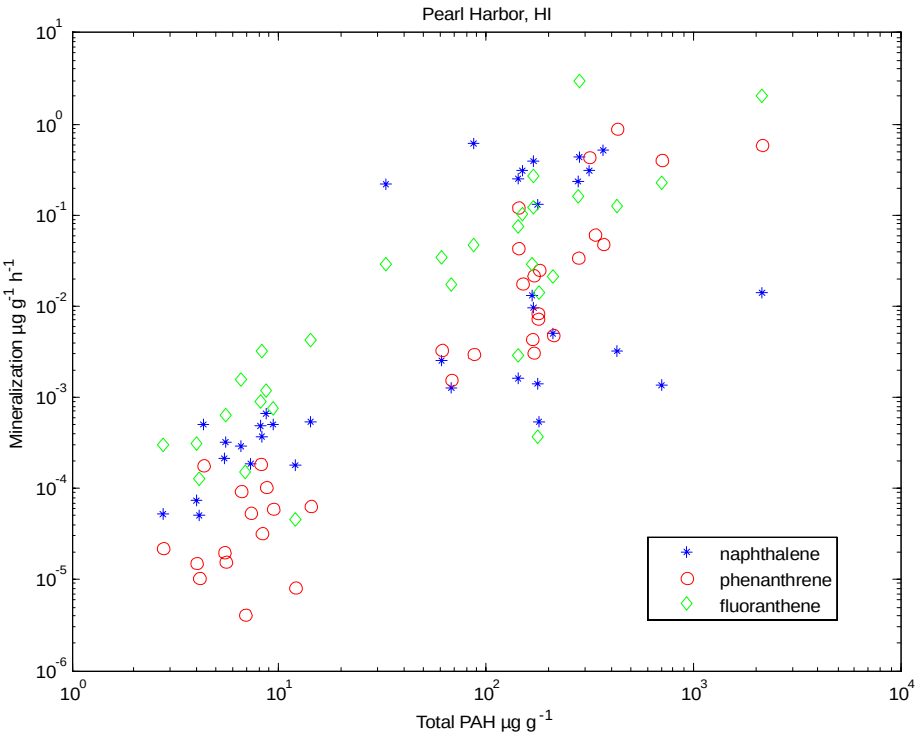

Figure 1. log Total PAH related to log mineralization of naphthalene, phenanthrene and fluoranthene, Pearl Harbor, HI.

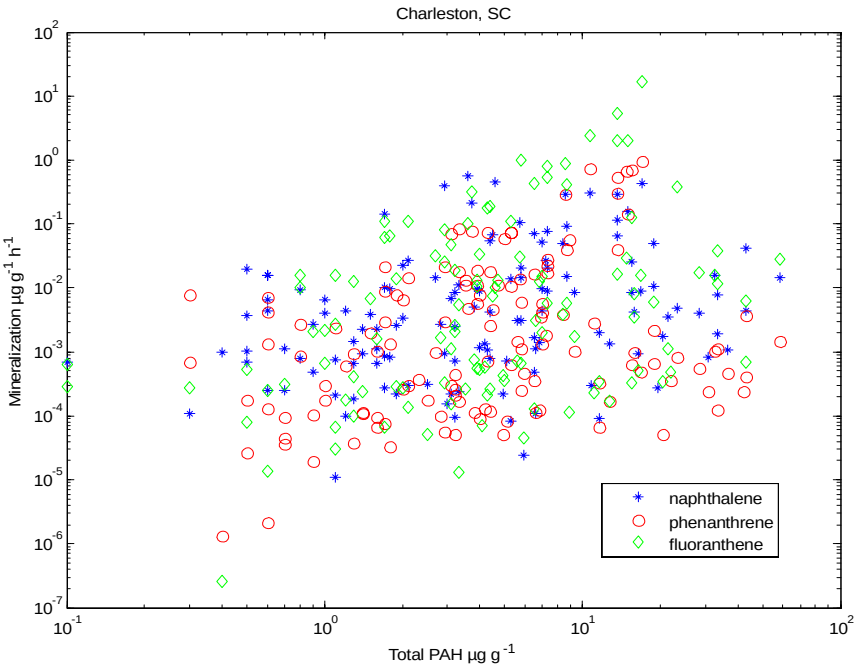

Figure 2. log Total PAH related to log mineralization of naphthalene, phenanthrene and fluoranthene, Charleston Harbor, SC.

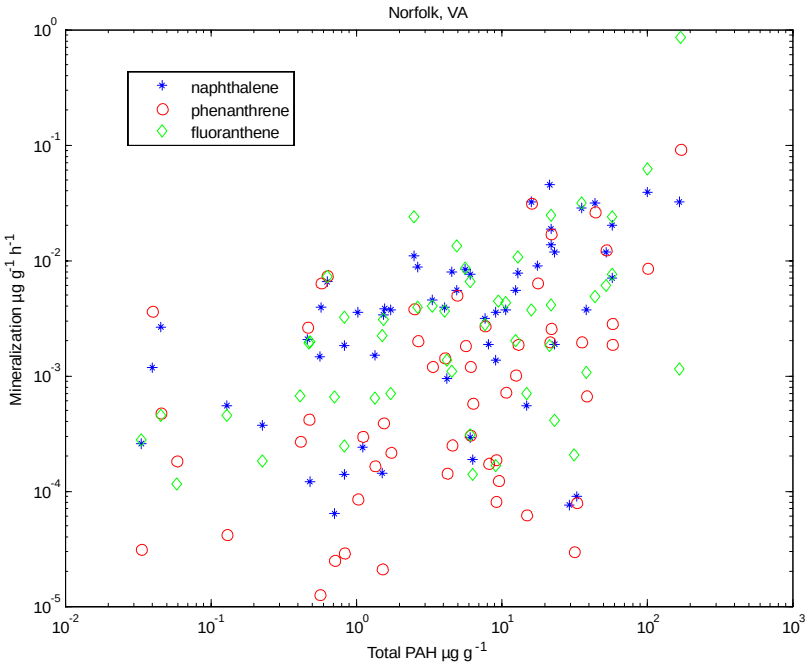

Figure 3. log Total PAH related to log mineralization of naphthalene, phenanthrene and fluoranthene, Norfolk Harbor, VA.

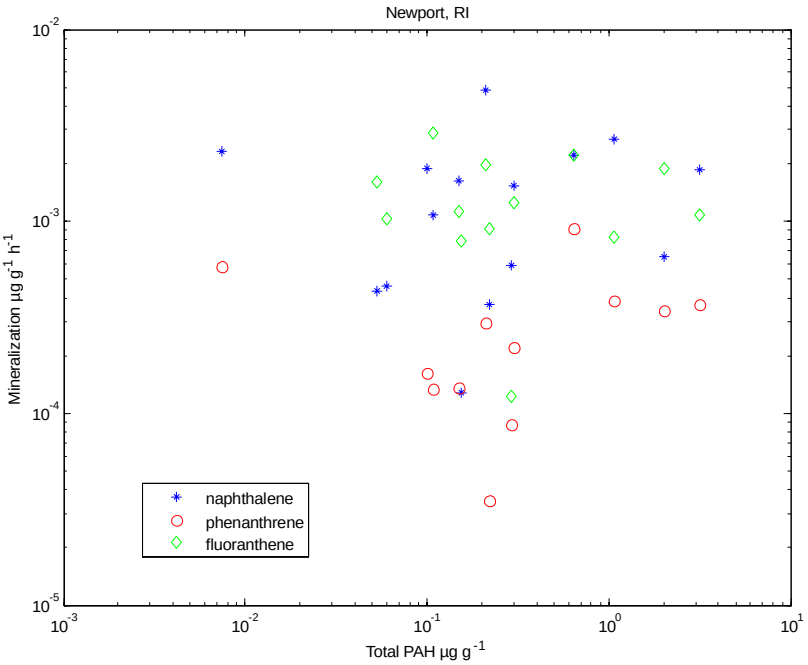

Figure 4. log Total PAH related to log mineralization of naphthalene, phenanthrene and fluoranthene, Newport Harbor, RI.

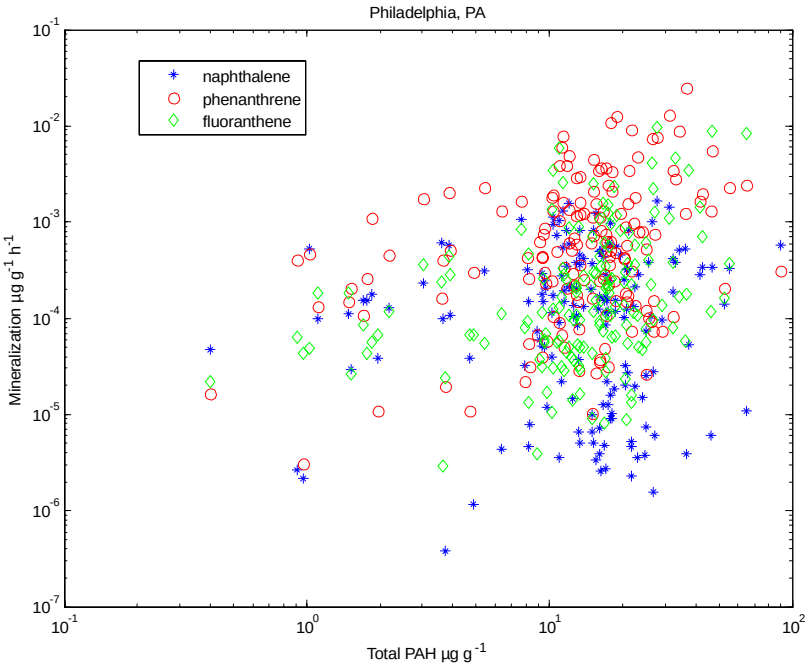

Figure 5. log Total PAH related to log mineralization of naphthalene, phenanthrene and fluoranthene, Delaware Bay and Schuylkill River, Philadelphia, PA.

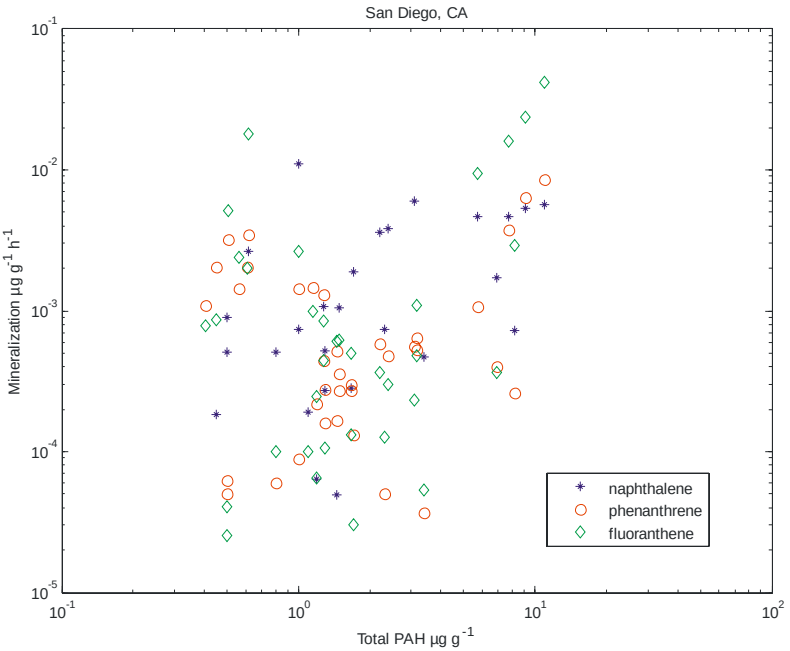

Figure 6. log Total PAH related to log mineralization of naphthalene, phenanthrene and fluoranthene, San Diego Bay, CA.

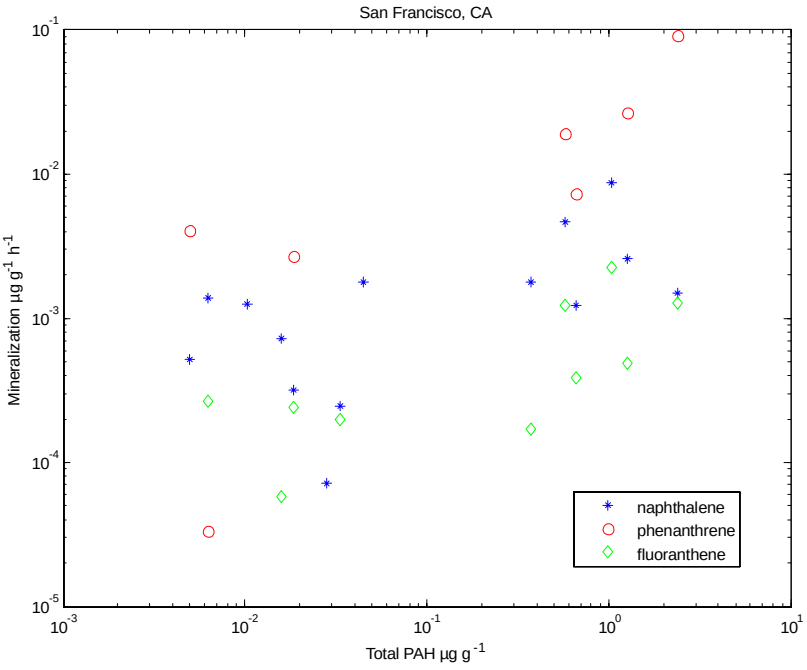

Figure 7. log Total PAH related to log mineralization of naphthalene, phenanthrene and fluoranthene, San Francisco Bay, CA.

If chemical structure of the individual compound was more important to biodegradation than initial concentration, then there would be a non-linear relationship between initial concentration and amount biodegraded. Data calculated from Talley *et al.* [2002], Lantz *et al.* [1997], and Ghosh *et al.* [2003] showed linear relationships (r^2 = 0.96, 0.98, 0.93, respectively) between initial PAH concentration and the amount of the individual PAH that was biodegraded (Figure 8). This suggests that in these bioremediation treatments, chemical structure was not a determinant of whether a compound was recalcitrant to biodegradation.

The relationship was even stronger (r^2 = 1.0) in Ko *et al.* [1995] data comparing the concentration of individual PAHs in sediment trap samples with those that eventually make it through the biodegradation process in the water column and nepheloid layer into the sediment (Figure 8). This relationship even held when comparing unbranched phenanthrene (C0) to branched isomers (C1-C5) (r^2 = 0.99, Figure 8) [Dutta and Harayama 2000]. Ratio changes among parent and branched isomers of PAHs are often given as evidence of biodegradation or weathering of the hydrocarbons. Interestingly, the C6 and C7 isomers were biodegraded much less than was predicted based on their initial concentration suggesting that only the C0:C6 or C0:C7 may be useful as a relative measure of biodegradation.

Impact of Temperature and Salinity

While salinity may play a part in controlling NOM utilization, there was no evidence for salinity control on sediment production or PAH mineralization in this study (r^2 < 0.20, P < 0.05).

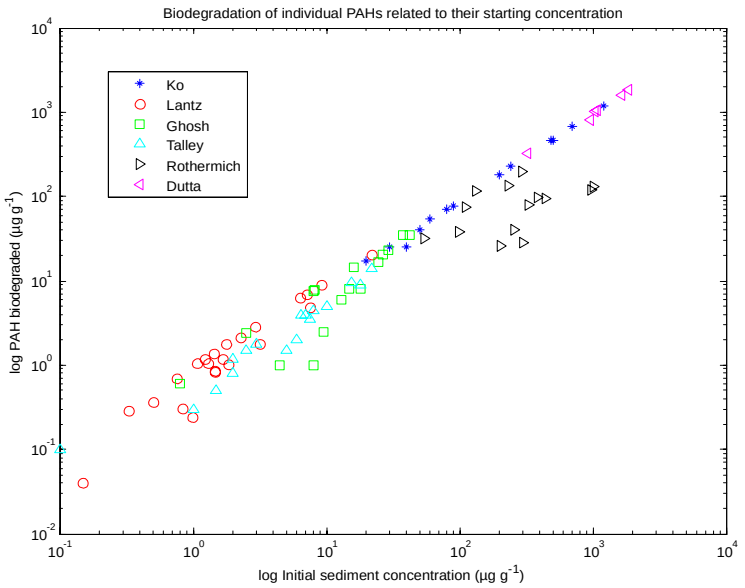

Figure 8. Initial concentration related to degraded amount with 2-5 ring PAHs spiked into sediment. Linear regressions: $y = 0.60x - 0.43$; $r^2 = 0.97$, [Talley *et al.* 2001]; $y = 0.94x - 0.25$; $r^2 = 0.98$, [Lantz *et al.* 1997]; $y = 0.88x - 2..7$; $r^2 = 0.93$, [Ghosh *et al.* 2003]; $y = 0.98x - 9.1$; $r^2 = 1.0$, [Ko *et al.* 1995]; $y = 1.0x - 37$; $r^2 = 0.99$, [Dutta and Harayama 2000].

Data for each site were subjected to linear regression both in terms of raw data and as seasonal averages where available [see Boyd *et al.* 2005 for methods]. Our results are similar to those of Shiaris [1989a]. Salinity, being a conservative tracer, should indicate what proportion of oceanic to estuarine end-member resides in a given parcel of water. Though OM lability varies between freshwater, estuarine and seawater end-members, sediment PAH mineralization and microbial activities (bacterial production, [Smith and Azam 1992])could not be related to mixing processes. In estuarine systems exposed to tidal salinity fluctuations, microorganisms should be less likely to undergo metabolic shifts due to salnity changes. Early reports suggested changes in salnity might be responsible for flocculation of particles and eventual settlement [Sholkovitz 1976], thus perhaps providing additional substrate to the sediments when mixing is intense. It is now widely accepted that estuarine mixing is less universal in dictating particle deposition in most of an estuary [Fisher *et al.* 1998]. Organisms would thus be less likely to ramp up their metabolism when sensing higher ionic strength. Finally, chronic deposition of particle-associated PAHs might likely obscure any fine-scale salinity or temperature relation to microbial PAH degradation in

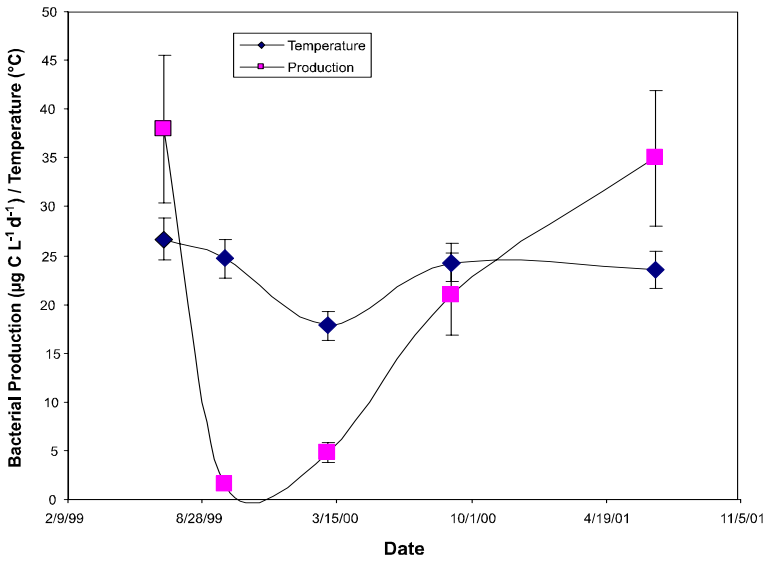

Figure 9. Average temperature and bacterial production in an fuel-impacted tidally-influenced groundwater well (Norfolk, VA).

As with salinity, we were unable to correlate any microbial activity index with temperature ($r^2 < 0.38$, $P < 0.05$). This was somewhat surprising for bacterial production, which is known to vary seasonally with temperature. In order to gain a higher resolution, we sampled a groundwater well which was contaminated with fuel hydrocarbons (significant levels of PAHs) and known to be tidally influenced. The well was located at the Norfolk Naval Station in VA (USA). Sampling was performed every two hours for two days. The well was so sampled five times over a two year period. Over the entire sampling period bacterial production was found to loosely track seasonal temperature shifts (Figure 9). Similar temperature effects were not seen with naphthalene, phenanthrene or fluoranthene mineralization. PAH mineralization was highest in March, May and October, representing

cooler months. As with sediments in Delaware Bay near Philadelphia, we hypothesize higher temperatures stimulate overall heterotrophic production which is fueled by more labile carbon substrates negating a direct relationship between production and PAH mineralization [Boyd *et al.* 2005a].

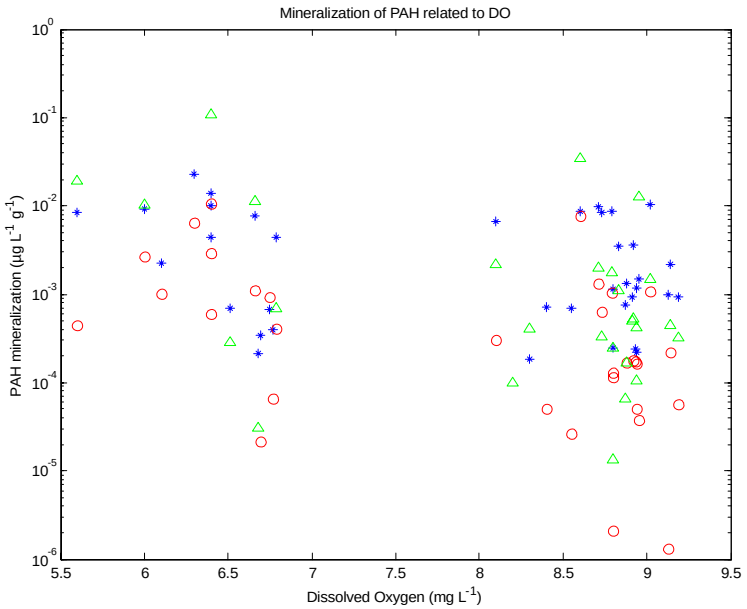

Figure 10. Relationship between sediment PAH mineralization rate and bottom water DO in Charleston Harbor estuary.

Impact of Dissolved Oxygen

PAHs are known to be degraded under anoxic conditions, however much of the burden to the sediments comes from chronic deposition of particle-associated PAHs [Ko and Baker 1995, Arzayus *et al.* 2001, Pohlman *et al.* 2002, Countway *et al.* 2003, Ko *et al.* 2003]. We are aware of only one study which examined the influence of multiple forcing factors on PAH biodegradation in estuarine sediments which encompassed all temperate seasons [Pohlman *et al.* 2002, Boyd *et al.* 2005a]. In this study of the Delaware Bay and Schuylkill River, a positive relationship was observed for naphthalene, phenanthrene and fluoranthene mineralization and ambient concentration, although linear regressions were not significant (Figure 5). The influence of temperature and DO were evaluated and it was found that DO was the primal forcing factor dictating mineralization rate in surface sediments. The overall conclusion was that during cooler months, when overall heterotrophic activity is suppressed, relatively cool, highly oxygenated water allows for maximum PAH biodegradation rates. At these times of year, deposition is low, so PAH mineralization can approximate PAH settling. During warmer months, when high overall production utilizes DO toward hypoxia in surface sediments, PAH mineralization is low and relatively high deposition rates increase net PAH accumulation in surface sediments.

Most of the other studies described in this chapter spanned several seasons, but only in Charleston, SC and Chesapeake Bay (Norfolk) sites were seasonal data available. In the case of Charleston, DO was not available from shipboard sensors, however for several of the field campaigns, representative numbers were available from South Carolina Department of Health and Environmental Control's (www.scdhec.net) monitoring network. PAH mineralization was regressed against DO, but we found no linear relationship as in Delaware Bay (Figure 10). While this was somewhat surprising, it is clear from the data that DO never approached levels of hypoxia during any of our samplings. In the Delaware Bay, two summer seasons showed considerable hypoxia and we observed a significant "inflection" in the relationship between PAH mineralization and DO at about 70% saturation. Because the Delaware Bay work spanned DO down to ~30 % saturation where little PAH mineralization occurred, it is clear that at this site DO plays a major role in facilitating PAH biodegradation.

Unlike the Delaware Bay work, DO did not correlate with PAH biodegradation or bacterial production in Chesapeake Bay sediments (Norfolk). Much like relationships between PAH concentration and degradation, correlations were not significant, but a general trend showing higher rates coincident with higher DO was evident (Figure 11 as an example). Seasonal measurements of temperature, salinity, DO, and PAH mineralization were averaged for the seven seasonal samplings in Norfolk. Unlike the Delaware Bay samples, correlations were insignificant for all potential forcing factors ($r^2 < 0.25$, $P < 0.05$). Data from the higher resolution sampling at the groundwater well showed a clearer trend toward higher DO relating to higher PAH mineralization (Figure 12). Correlation coefficients for all three substrates were higher, but still not significant ($r^2 < 0.45$, $P < 0.05$). While the relationship was strong in the Delaware Bay samples, it cannot be stated that a statistically significant relationship exists between DO and PAH mineralization a t the other sites included in this study.

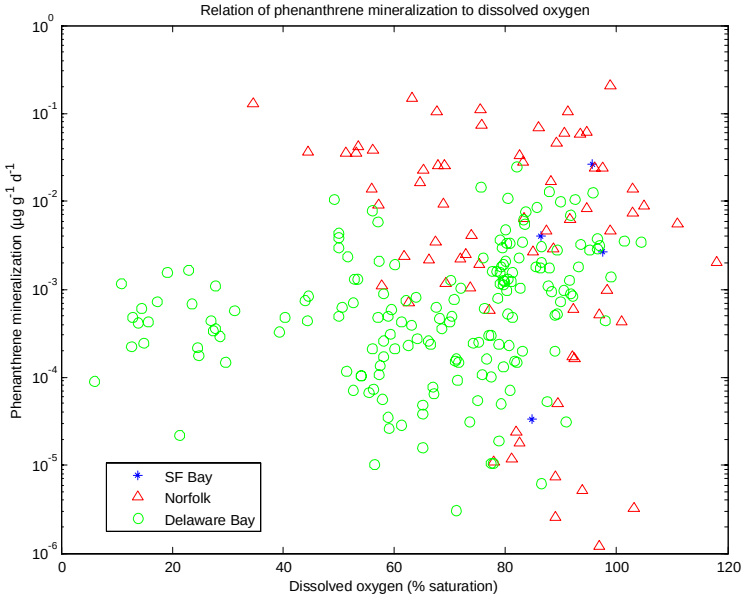

Figure 11. Relationship between sediment phenanthrene mineralization rate and bottom water DO in labeled estuary.

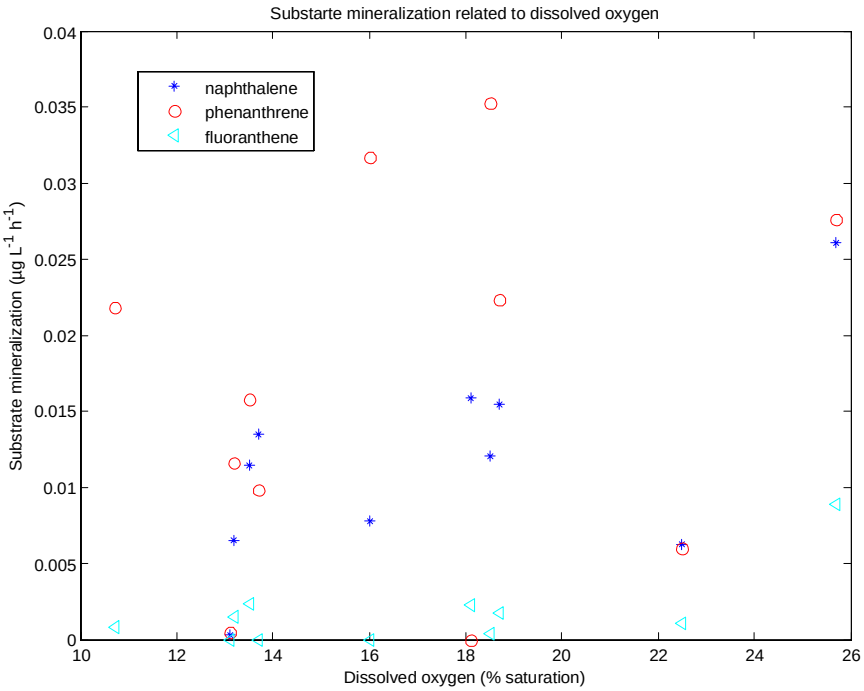

Figure 12. PAH mineralization related to DO over tidal cycles in fuel-contaminated groundwater.

Impact of Recalcitrant DOM

In order to determine the impact of recalcitrant OM on sediment bacterial production and PAH mineralization, lignin phenols were measured for selected sites [Goñi and Montgomery 2000]. If lignin behaves as a proxy for ROM in estuaries (as a component of humic material), it's sedimentary content should have either a positive (by stimulating degraders to become a larger proportion of the overall microbial assemblage) or negative (by competing with PAHs for microbial attack) impact on PAH biodegradation. Previous studies have linked the addition of labile OM with increases in PAH biodegradation, while addition of ROM hinders PAH biodegradation. We have found no correlation with overall heterotrophic production (which should be a proxy for availability of LOM) and PAH mineralization in any of our data. In natural systems, LOM should out-compete PAHs for available limiting nutrients and DO when the microbial assemblage is active (i.e. during warmer periods in temperate estuaries). As with others, we have observed coherence between ambient temperature and bacterial production [*c.f.* Boyd *et al.* 2005, Figure 14], although statistical regressions have generally been insignificant.

In San Francisco Bay and Charleston Harbor sediments, there was a very loose negative relationship between PAH mineralization and total lignin content for naphthalene and fluoranthene mineralization (Figure 13) while no relationship was observed for phenanthrene mineralization. In Norfolk Harbor sediments, we observed no or a very loose positive relationship between total lignin content and sediment PAH mineralization (Figures 14). In all cases, it was not possible to correlate sediment PAH mineralization with sediment lignin

content (r^2 < 0.25, P < 0.05). The presence of lignin in sediments likely provides an environmental stimulus to harbor if not deploy the enzymes for aromatic hydrocarbon degradation. We hypothesized that a concentration-dependent biodegradation response similar to what is observed with total PAH content would also be observed with lignin. At this point, it is not possible to assess the impact of *in situ* ROM on PAH biodegradation in the environment, though ROM additions appear to inhibit PAH utilization in laboratory experiments.

V. Conclusion

In this study we have relied on large datasets to visualize trends in PAH mineralization and some of the environmental forcing factors which might impact its rate. PAHs represent a refractory energy and carbon source that is degradable by estuarine sediment microbes (and likely particle-associated or attached water column bacteria). PAH were degraded in every site presented here, making biodegradation potential a ubiquitous phenomenon. If broad specificity enzyme systems are expressed in response to sediment PAHs, it is likely that PAH structure has less control over PAH biodegradation rate as *in situ* PAH concentration. Ample concentration is likely necessary to stimulate biodegradation. Two to five ring PAHs generally showed a linear response to biodegradation based on their starting concentration(s). Sediment PAH concentration appears to be the major factor found to control PAH biodegradation. Higher concentrations stimulate microorganisms to switch from utilizing labile substrates to PAHs.

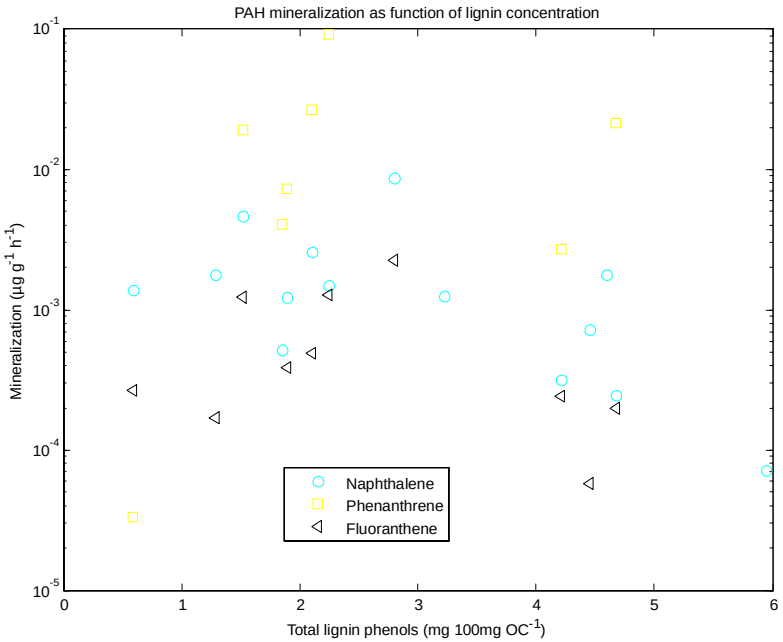

Figure 13. Relationship between total sediment lignin phenols and PAH mineralization, San Francisco Bay.

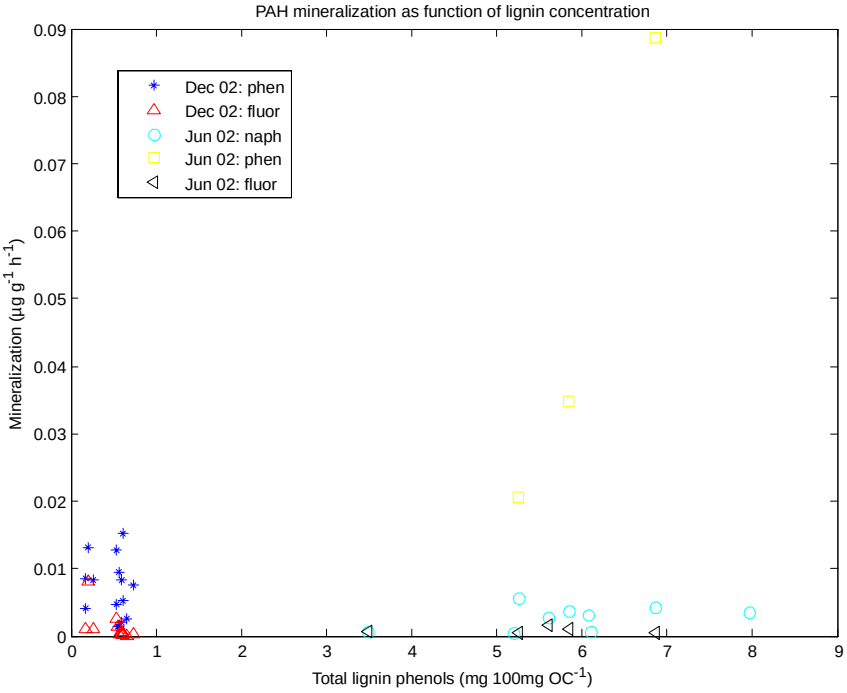

Figure 14. Relationship between total sediment lignin phenols and PAH mineralization, Norfolk Harbor, Chesapeake Bay.

PAH biodegradation in surface sediments was also found to be influenced by DO availability. In cooler months, when DO is relatively high, but overall heterotrophic production is temperature depressed, PAH mineralization may be highest. This observation was demonstrated markedly in Delaware Bay [Boyd *et al.* 2005a] and to a lesser degree in a tidally-influenced fuel-impacted groundwater plume. However, DO was not determined to be a major controlling factor for PAH biodegradation in Chesapeake Bay sediments near Norfolk, VA. Temperature and salinity were not determined to be controlling factors in PAH biodegradation, although temperature may control overall heterotrophic activity in temperate estuaries. Recalcitrant organic matter represented by lignin might impact PAH biodegradation by both up-regulating the synthesis of catabolic enzymes and competing with PAH for those enzymes. We observed both positive and negative relationships between lignin content and PAH mineralization. A needed component for exploring sediment PAH degradation is a more thorough understanding of ROM and PAH fluxes. All of our studies have focused on industrial waterways where field sampling represents only a snapshot in time. Mass balance estimates suggest relatively high particle-bound PAH fluxes to the sediments [Arzayus *et al.* 2001, Pohlman *et al.* 2002, Countway *et al.* 2003]. Regardless of which factors control rates, biodegradation must account for much of the difference between total PAH deposition and ultimate PAH burial in estuarine sediments.

Acknowledgements

The opinions and assertions contained herein are not to be construed as official or reflecting the views of the US Navy or the Federal Government at large. Mention of tradenames, or commercial products does not constitute endorsement or recommendation for use. The authors would like to thank the Captains and Crews of the *R/V Cape Henlopen*, *R/V Pt. Sur*, *R/V Barnes*, *R/V Cape Hatteras* and the *R/V Ecos* for logistical support for this work.

References

Aelion, C. M., D. C. Dobbins, and F. K. Pfaender. 1989. Adaptation of aquifer microbial communities to the biodegradation of xenobiotic compounds: Influence of substrate concentration and preexposure. *Environmental Toxicology and Chemistry* 8:75-86.

Aelion, C. M., C. M. Swindoll, and F. K. Pfaender. 1987. Adaptation to and Biodegradation of Xenobiotic Compounds by Microbial Communities from a Pristine Aquifer. *Applied and Environmental Microbiology* 53:2212-2217.

Alexander, M. 1985. Biodegradation of organic chemicals. *Environmental Science and Technology* 19:106-111.

Amon, R., H. P. Fitznar, and R. Benner. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnology and Oceanography* 46:287-297.

Apitz, S. E., E. Arias, S. A. Clawson, e. W. Lin, R. J. Melcher, and B. B. Hemmingsen. 1999. The development of a sterile, PAH-spiked, aged marine sediment for biodegradation experiments: chemical results. *Organic Geochemistry* 30:891-900.

Apple J.K., Boyd T.J. and Smith E.M. Temperature, salinity and the coherence of BP and chlorophyll-a in estuarine systems. *Journal of Coastal Research*. (submitted).

Apple, J. K., P. A. del Giorgio, and W. M. Kemp. 2006. Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. *Aquatic Microbial Ecology* 43:243-254.

Arzayus, K. M., R. M. Dickhut, and E. A. Canuel. 2001. Fate of atmospherically deposited polycyclic aromatic hydrocarbons (PAHs) in Chesapeake Bay. *Environmental Science and Technology* 35:2178-2183.

Atlas, R. M. 1981. Microbial degradation of petroleum hydrocarbons: An environmental perspective. *Microbiological Reviews* 45:180-209.

Barcina, I., P. LeBaron, and J. Vives-Rego. 1997. Survival of allochthonous bacteria in aquatic systems: a biological approach. *FEMS Microbiology Ecology* 23:1-9.

Bartholomew, G. W., and F. K. Pfaender. 1983. Influence of spatial and temporal variations on organic pollutant biodegradation rates in an estuarine environment. *Applied and Environmental Microbiology* 45:103-109.

Bastviken, D., and L. Tranvik. 2001. The leucine incorporation method estimates bacterial growth equally well in both oxic and anoxic lake waters. *Applied and Environmental Microbiology* 67:2916-2921.

Bauer, J. E., and D. G. Capone. 1985. Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intertidal marine sediments. *Applied and Environmental Microbiology* 50:81-90.

Boethling, R. S., and M. Alexander. 1979a. Effect of concentration of organic chemicals on their biodegradation by natural microbial communities. *Applied and Environmental Microbiology* 37:1211-1216.

Boethling, R. S., and M. Alexander. 1979b. Microbial degradation of organic compounds at trace levels. *Environmental Science and Technology* 13:989-991.

Boethling, R. S., B. Gregg, R. Frederick, N. W. Gabel, S. E. Campbell, and A. Sabljic. 1989. Expert systems survey on biodegradation of xenobiotic chemicals. *Ecotoxicology and Environmental Safety* 18:252-267.

Bouvier, T. C., and P. A. del Giorgio. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnology and Oceanography* 47:453-470.

Boyd, T. J., M. T. Montgomery, B. J. Spargo, D. C. Smith, R. B. Coffin, C. A. Kelley, and J. G. Mueller. 2001. Effects of oxygenation on hydrocarbon biodegradation in a hypoxic environment. *Bioremediation Journal* 5:145-157.

Boyd, T. J., M. T. Montgomery, J. K. Steele, J. W. Pohlman, S. R. Reatherford, B. J. Spargo, and D. C. Smith. 2005a. Dissolved oxygen saturation controls PAH biodegradation in freshwater estuary sediments. *Microbial Ecology* 49:226-235.

Boyd, T. J., D. M. Wolgast, I. Rivera-Duarte, O. Holm-Hansen, C. D. Hewes, A. Zirino, and D. B. Chadwick. 2005b. Effects of dissolved and complexed copper on heterotrophic bacterial production in San Diego Bay. *Microbial Ecology* 49:353-366.

Brubaker, G. R., and H. F. Stroo. 1992. *In situ* bioremediation of aquifers containing polyaromatic hydrocarbons. *J.Hazard.Mater.* 32:163-177.

Canuel, E. A., and C. S. Martens. 1993. Seasonal-Variations in the Sources and Alteration of Organic-Matter Associated with Recently-Deposited Sediments. *Organic Geochemistry* 20:563-577.

Canuel, E. A., and A. R. Zimmerman. 1999. Composition of particulate organic matter in the southern Chesapeake Bay: *Sources and reactivity. Estuaries* 22:980-994.

Chadwick D.B., Kirtay V., Blake A.C., Arias E., Davidson B., Guerrero J., Apitz S.E., Germano J., Maa J., Smith C., Paulsen R., Montgomery M.T., Ziebis W., Gieskes J. and Groves J. (2006) Pathway Ranking for In-place Sediment Management (CU1209): Final Site II Report - Pearl Harbor. CU1209, 1-857.

Chandra, S. D., C. H. Ward, and J. B. Hughes. 1996. Biodegradation of sorbed fluorene in sediment slurries. Hazard.Waste Hazard.Mater. 13:375-385.

Chapelle, F. H. 1999. Bioremediation of petroleum hydrocarbon-contaminated ground water: The perspectives of history and hydrology. *Ground water* 37:122-132.

Chin, Y.-P., and P. M. Gschwend. 1992. Partitioning of polycyclic aromatic hydrocarbons to marine porewater organic colloids. *Environmental Science and Technology* 26:1621-1626.

Chung, N., and M. Alexander. 1998. Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils. *Environmental Science and Technology* 32:855-860.

Coates, J. D., R. T. Anderson, and D. R. Lovley. 1996. Oxidation of Polycyclic Aromatic Hydrocarbons under Sulfate-Reducing Conditions. *Applied and Environmental Microbiology* 62:1099-1101.

Coates, J. D., J. Woodward, J. Allen, P. Philp, and D. R. Lovley. 1997. Anaerobic Degradation of Polycyclic Aromatic Hydrocarbons and Alkanes in Petroleum-Contaminated Marine Harbor Sediments. *Environmental Science and Technology* 63:3589-3593.

Connolly, J. P., and R. B. Coffin. 1995. Model of carbon cycling in planktonic food webs. *Journal of Environmental Engineering* 121:682-690.

Cornelissen, G., and Ö. Gustafsson. 2005. Importance of unburned coal carbon, black carbon, and amorphous organic carbon to phenanthrene sorption in sediments. *Environmental Science and Technology* 39:764-769.

Cornelissen, G., and D. T. H. M. Sijm. 1996. An energy budget model for the biodegradation and cometabolism of organic substances. *Chemosphere* 33:817-830.

Cottrell, M. T., R. R. Malmstrom, V. Hill, A. E. Parker, and D. L. Kirchman. 2006. The metabolic balance between autotrophy and heterotrophy in the western Arctic Ocean. Deep-Sea Research Part I: *Oceanographic Research Papers* 53:1831-1844.

Countway, R. E., R. M. Dickhut, and E. A. Canuel. 2003. Polycyclic aromatic hydrocarbon (PAH) distributions and associations with organic matter in surface waters of the York River, VA Estuary. *Organic Geochemistry* 34:209-224.

Cowan, J. L. W., and W. R. Boynton. 1996. Sediment-water oxygen and nutrient exchanges along the longitudinal axis of Chesapeake Bay: Seasonal patterns, controlling factors and ecological significance. *Estuaries* 19:562-580.

Crump, B. C., E. V. Armbrust, and J. A. Baross. 1999. Phylogenetic Analysis of Particle-Attached and Free-Living Bacterial Communities in the Columbia River, Its Estuary, and the Adjacent Coastal Ocean. *Applied and Environmental Microbiology* 65:3192-3204.

Da Silva, M. L. B., R. C. Daprato, D. E. Gomez, J. B. Hughes, C. H. Ward, and P. J. J. Alvarez. 2006. Comparison of bioaugmentation and biostimulation for the enhancement of dense nonaqueous phase liquid source zone bioremediation. *Water Environment Research* 78:2456-2465.

Dagley, S. 1987. Lessons from biodegradation. Annual Reviews of Microbiology 41:1-23.

Day J. W. J., C. A. S. Hall, W. M. Kemp, and A. Yanez-Arancibia. 1989. Estuarine Ecology. John Wiley and Sons, New York.

Dearden, J. C. 2002. Prediction of environmental toxicity and fate using quantitative structure-activity relationships (QSARs). *Journal of the Brazilian Chemical Society* 13:754-762.

del Giorgio, P. A., and T. C. Bouvier. 2002. Linking the physiologic and phylogenetic successions in free- living bacterial communities along an estuarine salinity gradient. *Limnology and Oceanography* 47:471-486.

del Giorgio P. A., and J. Davis. 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems. Elsevier Science, San Diego.

del Giorgio, P. A., and G. Scarborough. 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton:

implications for estimates of bacterial growth and production rates. *Journal of Plankton Research* 17:1905-1924.

Domde, P., A. Kapley, and H. J. Purohit. 2007. Impact of bioaugmentation with a consortium of bacteria on the remediation of wastewater-containing hydrocarbons. *Environmental Science and Pollution Research* 14:7-11.

Dutta, T. K., and S. Harayama. 2000. Fate of crude oil by the combination of photooxidation and biodegradation. *Environmental Science and Technology* 34:1500-1505.

Ellis, D. E., E. J. Lutz, J. M. Odam, R. J. Buchanan, C. L. Bartlett, M. D. Lee, M. R. Harkness, and K. A. DeWeerd. 2000. Bioaugmentation for accelerated *in situ* anaerobic bioremediation. *Environmental Science and Technology* 34:2254-2260.

Eschenbach, A., R. Wienverg, and B. Mahro. 1998. Fate and stability of nonextractable residues of [^{14}C]PAH in contaminated soils under environmental stress conditions. *Environmental Science and Technology* 32:2585-2590.

Felip, M., M. L. Pace, and J. J. Cole. 1996. Regulation of planktonic bacterial growth rates: The effects of temperature and resources. Microbial Ecology 31:15-28.

Fisher, T. R., J. D. Hagy, and E. Rochelle-Newall. 1998. Dissolved and particulate organic carbon in Chesapeake Bay. *Estuaries* 21:215-229.

Garcia-Blanco, S., A. D. Venosa, M. T. Suidan, K. Lee, S. Cobanli, and J. R. Haines. 2007. Biostimulation for the treatment of an oil-contaminated coastal salt marsh. *Biodegradation* 18:1-15.

Ghosh, U., J. R. Zimmerman, and R. G. Luthy. 2003. PCB and PAH speciation among particle types in contaminated harbor sediments and effects on PAH bioavailability. *Environmental Science and Technology* 37:2209-2217.

Goldman, J. C., D. A. Caron, and M. R. Dennet. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by C:N ratio. *Limnology and Oceanography* 32:1239-1252.

Goñi, M. A., and S. Montgomery. 2000. Alkaline CuO oxidation with a microwave digestion system: Lignin analyses of geochemical samples. *Analytical Chemistry* 72:3116-3121.

Granberg, M. E., R. Hansen, and H. Selck. 2005. Relative importance of macrofaunal burrows for the microbial mineralization of pyrene in marine sediments: impact of macrofaunal species and organic matter quality. *Marine Ecology-Progress Series* 288:59-74.

Guerin, W. F., and G. E. Jones. 1989. Estuarine ecology of phenanthrene-degrading bacteria. *Estuarine Coastal and Shelf Science* 29:115-130.

Gustafsson, Ö., and P. M. Gschwend. 1997. Soot as a strong partition medium for polycyclic aromatic hydrocarbons in aquatic systems. *Molecular Markers in Environmental Geochemistry* 671:365-381.

Hamdan, L. J., and R. B. Jonas. 2006. Seasonal and interannual dynamics of free-living bacterioplankton and microbially labile organic carbon along the salinity gradient of the Potomac River. *Estuaries and Coasts* 29:40-53.

Hatzinger, P. B., and M. Alexander. 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environmental Science and Technology* 29:537-545.

Hendrickson, J., N. Trahan, E. Gordon, and Y. Ouyang. 2007. Estimating relevance of organic carbon, nitrogen, and phosphorus loads to a blackwater river estuary. *Journal of the American Water Resources Association* 43:264-279.

Herbes, S. E., and L. R. Schwall. 1978. Microbial transformation of polycyclic aromatic hydrocarbons in pristine and petroleum-contaminated sediments. *Applied and Environmental Microbiology* 35:306-316.

Hickman, G. T., and J. T. Novak. 1989. Relationship between subsurface biodegradation rates and microbial density. *Environmental Science and Technology* 23:525-532.

Hoch, M. P., and D. L. Kirchman. 1993. Seasonal and interannual variability in bacterial production and biomass in a temperate estuary. Marine Ecology - Progress Series 98:283-295.

Hopkinson C. S., Jr., and E. M. Smith. 2005. Estuarine respiration: an overview of benthic, pelagic and whole system respiration. Oxford University Press, Oxford.

Hou, B. K., L. P. Wackett, and L. B. M. Ellis. 2003. Microbial pathway prediction: A functional group approach. *Journal of Chemical Information and Computer Sciences* 43:1051-1057.

Hurst, C. J., R. C. Sims, J. L. Sims, D. L. Sorensen, J. E. McLean, and S. Huling. 1996. Polycyclic aromatic hydrocarbon biodegradation as a function of oxygen tension in contaminated soil. *J.Hazard.Mater*. 51:193-208.

Jaworska, J. S., R. S. Boethling, and P. H. Howard. 2003. Recent developments in broadly applicable structure-biodegradability relationships. *Environmental Toxicology and Chemistry* 22:1710-1723.

Jiménez, N., M. Viñas, J. Sabaté, S. Díez, J. M. Bayona, A. M. Solanas, and J. Albaiges. 2006. The *Prestige* oil spill. 2. Enhanced biodegradation of a heavy fuel oil under field conditions by the use of an oleophilic fertilizer. *Environmental Science and Technology* 40:2578-2585.

Johnsen,A.R., and U.Karlson 2007. Diffuse PAH contamination of surface soils: environmental occurrence, bioavailability, and microbial degradation. *Applied Microbiology and Biotechnology in press*.

Johnsen, A. R., S. Schmidt, T. K. Hybholt, S. Henriksen, C. S. Jacobsen, and O. Andersen. 2007. Strong impact on the polycyclic aromatic hydrocarbon (PAH)-degrading community of a PAH-polluted soil but marginal effect on PAH degradation when priming with bioremediated soil dominated by mycobacteria. *Applied and Environmental Microbiology* 73:1474-1480.

Johnsen, A. R., l. Y. Wick, and H. Harms. 2005. Principles of microbial PAH-degradation in soil. Environmental Pollution 133:71-84.

Jonas, R. B. 1997. Bacteria, dissolved organics and oxygen consumption in salinity stratified Chesapeake Bay, an anoxia paradigm. *Am.Zool.* 37:612-620.

Jonker, M. T. O., S. B. Hawthorne, and A. A. Koelmans. 2005. Extremely Slowly Desorbing Polycyclic Aromatic Hydrocarbons from Soot and Soot-like Materials: Evidence by Supercritical Fluid Extraction. *Environmental Science and Technology* 39:7889-7895.

Jouanneau, Y., J. C. Willison, C. Meyer, S. Krivobok, N. Chevron, G. Blake, and J. L. Besombes. 2005. Stimulation of pyrene mineralization in freshwater sediments by

bacterial and plant bioaugmentation. *Environmental Science and Technology* 39:5729-5735.

King, G. M., and T. Berman. 1984. Potential effects of isotopic dilution on apparent respiration in ^{14}C heterotrophy experiments. Marine Ecology - Progress Series 19:175-180.

Kjelleberg, S., M. Hermansson, P. Marden, and G. W. Jones. 1987. The transient phase between growth and nongrowth of heterotrophic bacteria, with emphasis on the marine environment. *Annual Reviews of Microbiology* 41:25-49.

Ko, F.-C., and J. E. Baker. 1995. Partitioning of hydrophobic organic contaminants to resuspended sediments and plankton in the mesohaline Chesapeake Bay. *Mar.Chem*. 49:171-188.

Ko, F.-C., L. P. Sanford, and J. E. Baker. 2003. Internal recycling of particle reactive organic chemicals in the Chesapeake Bay water column. *Marine Chemistry* 81:163-176.

Kompare, B. 1998. Estimating environmental pollution by xenobiotic chemicals using QSAR (QSBR) models based on artificial intelligence. *Water Science and Technology* 37:9-18.

Lantz, S. E., M. T. Montgomery, W. W. Schultz, P. H. Pritchard, B. J. Spargo, and J. G. Mueller. 1997. Constituents of an organic wood preservative that inhibit the fluoranthene-degrading activity of *Sphingomonas paucimobilis* strain EPA505. Environmental Science and Technology 31:3573-3580.

Lesen, A. E. 2006. Sediment organic matter composition and dynamics in San Francisco Bay, California, USA: Seasonal variation and interactions between water column chlorophyll and the benthos. *Estuarine Coastal and Shelf Science* 66:501-512.

Lohmann, R., J. K. MacFarlane, and P. M. Gschwend. 2005. Importance of black carbon to sorption of native PAHs, PCBs, and PCDDs in Boston and New York, Harbor sediments. *Environmental Science and Technology* 39:141-148.

Lomas, M. W., P. M. Glibert, F.-K. Shiah, and E. M. Smith. 2002. Microbial processes and temperature in Chesapeake Bay: current relationships and potential impacts of regional warming. *Global Change Biology* 8:51-70.

Lueking, A. D., W. L. Huang, S. Soderstrom-Schwarz, M. S. Kim, and W. J. Weber. 2000. Relationship of soil organic matter characteristics to organic contaminant sequestration and bioavailability. *Journal of Environmental Quality* 29:317-323.

Luthy, R. G., G. R. Aiken, M. L. Brusseau, S. D. Cunningham, P. M. Gschwend, J. J. Pignatello, M. Reinhard, S. J. Traina, W. J. Weber Jr, and J. C. Westall. 1997. Sequestration of hydrophobic organic contaminants by geosorbents. *Environmental Science and Technology* 31:3341-3347.

Macnaughton, S. J., J. R. Stephen, A. D. Venosa, G. A. Davis, Y.-J. Chang, and D. C. White. 1999. Microbial Population Changes during Bioremediation of an Experimental Oil Spill. *Applied and Environmental Microbiology* 65:3566-3574.

Madigan M. T., J. M. Martinko, and J. Parker. 2003. Brock Biology of Microorganisms., 10th edition. Prentice Hall, Upper Saddle River, NJ.

Malone, T. C., L. H. Crocker, S. E. Pike, and B. W. Wendler. 1988. Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. *Marine Ecology Progress Series* 48:235-249.

McNally, D. L., J. R. Mihelcic, and D. R. Lueking. 1998. Biodegradation of three- and four-ring polycyclic aromatic hydrocarbons under aerobic and denitrifying conditions. *Environmental Science and Technology* 32:2633-2639.

Meredith, C. E., and M. Radosevich. 1998. Bacterial degradation of homo- and heterocyclic aromatic compounds in the presence of soluble/colloidal humic acid. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes 33:17-36.

Mishra, S., J. Jyot, R. C. Kuhad, and B. Lal. 2001. Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge-contaminated soil. *Applied and Environmental Microbiology* 67:1675-1681.

Montgomery, M. T., C. L. Osburn, T. J. Boyd, S. Reatherford, and D. C. Smith. 2002. Bacterial degradation of polycyclic aromatic hydrocarbons in surface sediments of coastal ecosystems. *Soil and Sediment Contamination* 11:1021.

Morita R. Y. 1974. Temperature Effects on Marine Microorganisms. University Park Press, Baltimore, MD.

Morrill, P. L., G. Lacrampe-Couloume, G. F. Slater, B. E. Sleep, E. A. Edwards, M. L. McMaster, D. W. Major, and B. Sherwood Lollar. 2005. Quantifying chlorinated ethene degradation during reductive dechlorination at Kelly AFB using stable carbon isotopes. *Journal of Contaminant Hydrology* 76:279-293.

Münster, U. 1993. Concentrations and fluxes of organic-carbon substrates in the aquatic environment. Antonie Van Leeuwenhoek 63:243-274.

Münster U., and R. J. Chróst. 1990. Origin, composition, and microbial utilization of dissolved organic matter. Springer-Verlag, New York.

Ogino, A., H. Koshikawa, T. Nakahara, and H. Uchiyama. 2001. Succession of microbial communities during a biostimulation process as evaluated by DGGE and clone library analyses. J.Appl.Microbiol. 91:625-635.

Paris, D. F., and J. E. Rogers. 1986. Kinetic concepts for measuring microbial rate constants: Effects of nutrients on rate constants. *Applied and Environmental Microbiology* 51:221-225.

Parrish, Z. D., M. K. Banks, and A. P. Schwab. 2005. Assessment of contaminant lability during phytoremediation of polycyclic aromatic hydrocarbon impacted soil. *Environmental Pollution* 137:187-197.

Pfaender, F. K., and G. W. Bartholomew. 1982. Measurement of aquatic biodegradation rates by determing heterotrophic uptake of radiolabeled pollutants. *Applied and Environmental Microbiology* 44:159-164.

Pinhassi, J., F. Azam, U. L. Zweifel, R. A. Long, J. Martinez, U. L. Zweifel, and Å. Hagström. 1999. Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquatic Microbial Ecology* 17:13-26.

Pohlman, J. W., R. B. Coffin, C. S. Mitchell, M. T. Montgomery, B. J. Spargo, J. K. Steele, and T. J. Boyd. 2002. Transport, deposition and biodegradation of particle bound polycyclic aromatic hydrocarbons in a tidal basin of an industrial watershed. *Environ.Monit.Assess*. 75:155-167.

Pomeroy, L. R., J. E. Sheldon, W. M. Sheldon, and F. Peters. 1995. Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. *Marine Ecology Progress Series* 117:259-268.

Quantin, C., E. J. Joner, J. M. Portal, and J. Berthelin. 2005. PAH dissipation in a contaminated river sediment under oxic and anoxic conditions. *Environmental Pollution* 134:315-322.

Raymond, P. A., and J. E. Bauer. 2000. Bacterial consumption of DOC during transport through a temperate estuary. *Aquatic Microbial Ecology* 22:1-12.

Reid, B. J., K. C. Jones, and K. T. Semple. 2000. Bioavailability of persistent organic pollutants in soils and sediments - A perspective on mechanisms, consequences and assessment. *Environmental Pollution* 108:103-112.

Reid, B. J., G. L. Northcott, K. C. Jones, and K. T. Semple. 1998. Evaluation of spiking procedures for the introduction of poorly water soluble contaminants into soil. *Environmental Science and Technology* 32:3224-3227.

Revilla, M., A. Iriarte, I. Madariaga, and E. Orive. 2000. Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. *Estuarine Coastal and Shelf Science* 50:297-313.

Roch, F., and M. Alexander. 1997. Inability of bacteria to degrade low concentrations of toluene in water. *Environmental Toxicology and Chemistry* 16:1377-1383.

Rockne, K. J., and S. E. Strand. 2001. Anaerobic biodegradation of naphthalene, phenanthrene, and biphenyl by a denitrifying enrichment culture. *Water Research* 35:291-299.

Röling, W. F. M., M. G. Milner, D. M. Jones, F. Fratepietro, R. P. J. Swannell, F. Daniel, and I. M. Head. 2004. Bacterial Community Dynamics and Hydrocarbon Degradation during a Field-Scale Evaluation of Bioremediation on a Mudflat Beach Contaminated with Buried Oil. *Applied and Environmental Microbiology* 70:2603-2613.

Rose A. H. 1967. Thermobiology. Academic Press Inc., London, UK.

Sampou, P., and W. M. Kemp. 1994. Factors regulating plankton community respiration in Chesapeake Bay. *Marine Ecology Progress Series* 110:249-258.

Schaffner, L. C., R. M. Dickhut, S. Mitra, P. W. Lay, and C. Brouwer-Riel. 1997. Effects of physical chemistry and bioturbation by estuarine macrofauna on the transport of hydrophobic organic contaminants in the benthos. *Environmental Science and Technology* 31:3120-3125.

Schultz Jr, G. E., and H. W. Ducklow. 2000. Changes in bacterioplankton metabolic capabilities along a salinity gradient in the York River estuary, Virginia, USA. *Aquatic Microbial Ecology* 22:163-174.

Schultz Jr, G. E., E. D. White III, and H. W. Ducklow. 2003. Bacterioplankton dynamics in the York River estuary: primary influence of temperature and freshwater inputs. *Aquatic Microbial Ecology* 30:135-148.

Scow, K. M., S. K. Schmidt, and M. Alexander. 1989. Kinetics of biodegradation of mixtures of substrates in soil. *Soil Biol.Biochem.* 21:703-708.

Scow, K. M., S. Simkins, and M. Alexander. 1986. Kinetics of mineralization of organic compounds at low concentrations in soil. *Applied and Environmental Microbiology* 51:1028-1035.

Selck, H., M. E. Granberg, and V. E. Forbes. 2005. Impact of sediment organic matter quality on the fate and effects of fluoranthene in the infaunal brittle star Amphiura filiformis. *Marine Environmental Research* 59:19-45.

Shiah, F.-K., and H. W. Ducklow. 1994a. Temperature and substrate regulation of bacterial abundance, production and specific growth-rate in Chesapeake Bay, USA. *Marine Ecology - Progress Series* 103:297-308.

Shiah, F.-K., and H. W. Ducklow. 1994b. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. *Limnology and Oceanography* 39:1243-1258.

Shiaris, M. P. 1989. Phenanthrene mineralization along a natural salinity gradient in an urban estuary, boston-harbor, massachusetts. *Microbial Ecology* 18:135-146.

Shiaris, M. P. 1989. Seasonal biotransformation of naphthalene, phenanthrene, and benzo[A]Pyrene in surficial estuarine sediments. *Applied and Environmental Microbiology* 55:1391-1399.

Shimp, R. J., and F. K. Pfaender. 1987. Effect of adaptation to phenol on biodegradation of monosubstituted phenols by aquatic microbial communities. *Applied and Environmental Microbiology* 53:1496-1499.

Sholkovitz, E. R. 1976. Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater. Geochimica et Cosmochimica Acta 40:831-845.

Sisler, F. D., and C. E. Zobell. 1947. Microbial utilization of carcinogenic hydrocarbons. *Science* 106:521-522.

Small, C., and R. J. Nicholls. 2003. A Global Analysis of Human Settlement in Coastal Zones. *Journal of Coastal Research* 19:584-599.

Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. *Mar.Microb.Food Webs* 6:107-114.

Smith, E. M., and W. M. Kemp. 1995. Seasonal and regional variations in plankton community production and respiration for Chesapeake Bay. *Marine Ecology - Progress Series* 116:217-231.

Smith, E. M., and W. M. Kemp. 2003. Planktonic and bacterial respiration along an estuarine gradient: responses to carbon and nutrient enrichment. *Aquatic Microbial Ecology* 30:251-261.

Sobczak, W. V., J. E. Cloern, A. D. Jassby, and A. B. Muller-Solger. 2002. Bioavailability of organic matter in a highly disturbed estuary: The role of detrital and algal resources. *Proc.Natl.Acad.Sci.U.S.A.* 99:8101-8105.

Søndergaard, M., and M. Middelboe. 1995. A cross-system analysis of labile dissolved organic-carbon. *Marine Ecology - Progress Series* 118:283-294.

Staroscik, A. M., and D. C. Smith. 2004. Seasonal patterns in bacterioplankton abundance and production in Narragansett Bay, Rhode Island, USA. *Aquatic Microbial Ecology* 35:275-282.

Steffensen, W. S., and M. Alexander. 1995. Role of competition for inorganic nutrients in the biodegradation of mixtures of substrates. *Applied and Environmental Microbiology* 61:2859-2862.

Stepanauskas, R., M. A. Moran, B. A. Bergamaschi, and J. T. Hollibaugh. 2005. Sources, bioavailability, and photoreactivity of dissolved organic carbon in the Sacramento-San Joaquin River Delta. *Biogeochemistry* 74:131-149.

Talley, J. W., U. Ghosh, S. G. Tucker, J. S. Furey, and R. G. Luthy. 2002. Particle-scale understanding of the bioavailability of PAHs in sediment. *Environmental Science and Technology* 36:477-483.

Trably, E., D. Patureau, and J. P. Delgenes. 2003. Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge. *Water Science and Technology* 48:53-60.

Turnewitsch, R., B. Domeyer, and G. Graf. 2007. Experimental evidence for an effect of early-diagenetic interaction between labile and refractory marine sedimentary organic matter on nitrogen dynamics. *Journal of Sea Research* 57:270-280.

Vacca, D. J., W. F. Bleam, and W. J. Hickey. 2005. Isolation of Soil Bacteria Adapted To Degrade Humic Acid-Sorbed Phenanthrene. *Applied and Environmental Microbiology* 71:3797-3805.

Venosa, A. D., M. T. Suidan, K. Lee, S. E. Cobanli, S. Garcia-Blanco, and J. R. Haines. 2002. Bioremediation of oil-contaminated coastal freshwater and saltwater wetlands. Pages 139-148 *in*.

Venosa, A. D., M. T. Suidan, B. A. Wrenn, K. L. Strohmeier, J. R. Haines, B. L. Eberhart, D. King, and E. Holder. 1996. Bioremediation of an Experimental Oil Spill on the Shoreline of Delaware Bay. *Environmental Science and Technology* 30:1764-1775.

Wammer, K. H., and C. A. Peters. 2005. Polycyclic aromatic hydrocarbon biodegradation rates: A structure-based study. *Environmental Science and Technology* 39:2571-2578.

Wick, L., A. De Munain, D. Springael, and H. Harms. 2002. Responses of Mycobacterium sp. LB501T to the low bioavailability of solid anthracene. *Applied Microbiology and Biotechnology* 58:378-385.

Wiggins, B. A., and M. Alexander. 1988. Role of chemical concentration and second carbon sources in acclimation of microbial communities for biodegradation. *Applied and Environmental Microbiology* 54:2803-2807.

Wiggins, B. A., S. H. Jones, and M. Alexander. 1987. Explanations for the acclimation period preceding the mineralization of organic chemicals in aquatic environments. *Applied and Environmental Microbiology* 53:791-796.

Wolfe, N. L., D. F. Paris, W. C. Steen, and G. L. Baughman. 1980. Correlation of microbial degradation rates with chemical structure. Environmental *Science and Technology* 14:1143-1144.

Wrenn, B. A., M. T. Suidan, K. L. Strohmeier, B. L. Eberhart, G. J. Wilson, and A. D. Venosa. 1997. Nutrient transport during bioremediation of contaminated beaches: Evaluation with lithium as a conservative tracer. *Water Research* 31:515-524.

Xing, B. 1997. The effect of the quality of soil organic matter on sorption of naphthalene. *Chemosphere* 35:633-642.

Yokokawa, T., T. Nagata, M. T. Cottrell, and D. L. Kirchman. 2004. Growth rate of the major phylogenetic bacterial groups in the Delaware estuary. *Limnology and Oceanography* 49:1620-1629.

Yu, K. S. H., A. H. Y. Wong, K. W. Y. Yau, Y. S. Wong, and N. F. Y. Tam. 2005. Natural attenuation, biostimulation and bioaugmentation on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments. *Marine Pollution Bulletin* 51:1071-1077.

Zimmerman, A. R., and E. A. Canuel. 2001. Bulk organic matter and lipid biomarker composition of Chesapeake Bay surficial sediments as indicators of environmental processes. *Estuarine Coastal and Shelf Science* 53:319-341.