

Report to the  
MASSACHUSETTS BAYS PROGRAM

**BIOAVAILABILITY AND BIOTRANSFORMATION OF  
POLYCYCLIC AROMATIC HYDROCARBONS IN BENTHIC  
ENVIRONMENTS OF COASTAL MASSACHUSETTS**

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## MASSACHUSETTS BAYS PROGRAM

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### FOREWORD

The roots of the Massachusetts Bays Program extend back to 1982, when the City of Quincy filed suit against the Metropolitan District Commission and the Boston Water and Sewer Commission over the chronic pollution of Boston Harbor, Quincy Bay, and adjacent waters. Outdated and poorly maintained sewage treatment plants on Deer Island and Nut Island were being overwhelmed daily by sewage from the forty-three communities in the Metropolitan Boston area. Untreated and partially treated sewage were spilling into Boston Harbor.

Litigation over the pollution of Boston Harbor culminated in 1985 when the United States Attorney filed suit on behalf of the Environmental Protection Agency against the Commonwealth of Massachusetts for violations of the Federal Clean Water Act. The settlement of this suit resulted, in 1988, in the creation of the Massachusetts Water Resources Authority, the agency currently overseeing a multi-billion dollar project to repair and upgrade Metropolitan Boston's sewage treatment system. In addition, the settlement resulted in the establishment of the Massachusetts Environmental Trust - an environmental philanthropy dedicated to improving the Commonwealth's coastal and marine resources. \$2 million in settlement proceeds were administered by the Trust to support projects dedicated to the restoration and protection of Boston Harbor and Massachusetts Bay.

The Trust provided \$1.6 million to establish the Massachusetts Bays Program, a collaborative effort of public officials, civic organizations, business leaders, and environmental groups to work towards improved coastal water quality. The funding was used to support both a program of public education and a scientific research program focusing on the sources, fate, transport and effects of contaminants in the Massachusetts and Cape Cod Bays ecosystem. To maximize the efficiency of limited research funding, the sponsored research program was developed in coordination with research funded by the MWRA, the United States Geological Survey, and the Massachusetts Institute of Technology Sea Grant Program. The study described in this report addresses the bioavailability of polycyclic aromatic hydrocarbons in the sediments of Boston Harbor and less contaminated areas of Massachusetts Bay to benthic organisms, as well as the potential for food chain transfer to commercially important species. This information is helping to meet the Massachusetts Bays Program goal of producing an area-wide management plan for water quality enhancement and protection. In addition, the study has provided a basis for identifying data gaps to be addressed through additional research.

In April, 1990, following a formal process of nomination, the Massachusetts Bays Program became part of the National Estuary Program. The additional funding provided as part of this joint program of the Environmental Protection Agency and the Commonwealth of Massachusetts is being used to continue a coordinated program of research in the Massachusetts Bays ecosystem, as well as supporting the development of a comprehensive conservation and management plan for the coastal and marine resources of Massachusetts and Cape Cod Bays.

The information in this document has been subject to Massachusetts Bays Program peer and administrative review and has been accepted for publication as a Massachusetts Bays Program document. The contents of this document do not necessarily reflect the views and policies of the Management Conference.

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## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) comprise a ubiquitous class of organic contaminants distributed world-wide through the use of fossil fuels for energy. Due to their hydrophobic properties (May *et al.*, 1978), once introduced to the aquatic environment PAH sorb readily to suspended particles (Karickhoff *et al.* 1979; Means, 1980). As a result of particle settlement, sediments tend to be the major sink for PAH in streams, lakes, estuaries, and oceans (La Flamme and Hites, 1978). Sediment concentrations of PAH in Boston Harbor are among the highest reported in the United States (Boehm, 1984; Shiaris and Jambard-Sweet 1986, MacDonald, 1991). PAH can be both toxic and mutagenic (Richards and Jackson, 1982). Therefore an understanding of the fate of PAH in coastal sediments and Boston Harbor sediments in particular is important because of their potential threat to the well being of marine biota and human consumers. Transfer of PAH to marine biota and the human food chain and disturbance of ecological systems are dependent on the availability and persistence of these contaminants within sediments and transport within benthic ecosystems.

Unlike some other persistent organic contaminants, PAH are degraded in the marine environment by photooxidation, chemical oxidation, and biological transformation by prokaryotic and eukaryotic organisms. Biotransformation is of major importance as it may influence both bioavailability and loss of PAH from the sediments. Biodegradative mechanisms, both prokaryotic and eukaryotic, require the presence of bimolecular oxygen to initiate enzymatic attack on the PAH rings (Gibson, 1968). Many bacteria, but not eukaryotes, have the ability to utilize low molecular weight PAH as a sole carbon and energy source, converting PAH into simple substrates of central metabolic pathways (Cerniglia, 1981, 1984). Thus, bacterial degradation represents the only route for the complete breakdown of PAH within sediments.

Despite the abundance of information on PAH degradation by pure cultures and enrichments, accurate rates of biodegradation and the fates of PAH in natural sediments have been difficult to ascertain. Evidence of microbial oxidation and degradation of PAH in benthic systems has been demonstrated in a number of cases (Lee *et al.* 1986 among others). Once formed, the polar metabolites of PAH with three or more rings can persist for periods of weeks to months (Hinga *et al.* 1987; McElroy *et al.* 1987). A number of factors have been shown to influence PAH degradation in sediments. The limited diffusion of O<sub>2</sub> into organic rich sediments appears to restrict PAH biodegradation to the well oxygenated surficial sediments (Bauer and Capone 1988; DeLaune *et al.* 1981). Other factors that affect PAH degradation include temperature, nutrients, and PAH structure (Atlas 1981). The activities of benthic worms may also stimulate PAH degradation in sediments through bioturbation, bioirrigation and metabolism (McElroy 1985a; Bauer *et al.* 1988; Gardner *et al.* 1987). For the low molecular weight PAH such as naphthalene, the turnover times (average residence times) in oxygenic sediments may be as low as hours (Herbes and Schwall 1978). Larger PAH are more resistant to degradation, particularly in anoxic sediments, may persist indefinitely (Bossert *et al.* 1984; Heitkamp and Cerniglia 1987; Herbes and Schwall 1978; Pruell and Quinn 1985).

These summaries of PAH analyses on field collected organisms concur with data from laboratory investigations of PAH bioaccumulation and metabolism in demonstrating that routine chemical analysis only gives an accurate picture of PAH body burden in organisms with very limited abilities to metabolize PAH. Furthermore, although we are beginning to get a picture of the ability of some marine organisms to metabolize PAH, the fate and availability of PAH metabolites in the environment and the potential influence of other biogeochemical factors on these processes remain unresolved, yet clearly important questions.

### **Specific Objectives**

The overall objectives of this project were to:

- 1) address the bioavailability of PAH in sediments from Boston Harbor and less contaminated areas of Massachusetts Bay to benthic organisms; and
- 2) address the role of metabolism in bioavailability of PAH, PAH persistence in surficial sediments, PAH flux to the water column, and PAH potential for food chain transfer to commercially important species.

Specific questions to be addressed include:

- 1) Does equilibrium partitioning theory adequately explain bioaccumulation of these two model PAH from sediments of varying organic carbon concentration and high and low contaminant burdens in coastal Massachusetts?
- 2) Do macrofauna stimulate removal and/or metabolism of these two model PAH in this system?
- 3) How does bioaccumulation differ between two common infaunal organisms important in aquatic food chains, one which is a deposit feeder capable of PAH metabolism, and one which primarily filter feeds and has limited ability to metabolize PAH?
- 4) What is the potential for removal and degradation of PAH from the sediment types compared in these experiments?
- 5) Is PAH degradation coupled to overall microbial activity in these systems?
- 6) In what form are PAH removed from these systems, and is this influence by contaminant exposure history and organic carbon content?

This information is essential to determination of the long term fate of PAH in sediments, PAH impact on marine resources and their transport through marine food chains to human consumers. This mechanistic approach will yield information that can help provide more realistic models of PAH fates and effects which should eventually be integrated with physical and chemical models of contaminant distribution and fate to be generated by other studies funded by the Massachusetts Bays Program (MBP) and Massachusetts Water Resources Authority (MWRA).

## METHODS

### Scientific Approach

To better understand the environmental factors controlling PAH fate in coastal sediments and to obtain more realistic estimates of biodegradation rates and removal of PAH from coastal systems, we examined the effect of sediment organic matter concentration on the biodegradation and release of two model PAH: phenanthrene (PHN) and benzo[a]anthracene (BA) in benthic microcosms. These two specific PAH were chosen as models of the lower molecular weight ( $\leq 3$  rings), more easily degraded PAH, and the higher molecular weight ( $\geq 4$  rings), more recalcitrant PAH. In addition to organic carbon content, we also compared sediments from relatively contaminated sites in Boston Harbor, MA, and those from relatively uncontaminated sites in Southeastern Massachusetts waters near Cape Cod. In conjunction with studies on the fate and metabolism of benzo[a]anthracene and phenanthrene, we followed both aerobic and anaerobic microbial activity. To our knowledge, no comparable effort has been made to quantify both PAH metabolism and general microbial activity in the same system. These processes were assessed in the presence and absence of two representative infaunal organisms, the deposit feeding polychaete *Scolecopelides (=Marenzelleria) viridis* and the suspension feeding bivalve *Mya arenaria*. Both are dominant members of the intertidal infaunal community in both contaminated and relatively clean environments, and serve as food sources for larger species consumed by humans.

Experiments were conducted in 4.7 L polycarbonate benthic chambers (sediment-water microcosms) depicted in Figure 1. Chambers were maintained in a constant temperature chamber at 14°C under red light to minimize photodegradation. Each chamber contained a 10 cm deep sediment reservoir and a water column mixed continuously by a magnetic stir bar suspended from the chamber top. A constant flow of air was passed through a coil of vermicelli sized silicon tubing near the top of the water column and seawater was pumped slowly (2-3 ml/min) through the chambers to maintain adequate water quality. Each experiment was divided into 3 segments as described below.

- 1) Equilibration: Sediment was added to each chamber, flow initiated and conditions were allowed to stabilize with respect to microbial activity.
- 2) A 1 cm layer of radiolabeled surface sediment was added to each chamber and microbial activity and PAH degradation and flux were assessed.
- 3) A group of worms and clams were added to each chamber to determine the effect of their presence on microbial activity and PAH degradation and flux. At the end of the experiment PAH accumulation and metabolism in macrofauna was assessed.

Five experiments were conducted. The first experiment was undertaken to refine our experimental protocols, and determine appropriate sediment types, organisms, and incubation times for each portion of the experiment. Due to the changing conditions and procedures

occurring throughout the preliminary experiment, the results are not presented in this report. Only the results of the complete replicated experiments (#II-V) are reported here. Throughout each experiment measurements were taken as described in Figure 2. With the exception of the preliminary experiment (I), each experiment was conducted on one sediment type, radiolabeled with either BA or PHN. Specific details on sampling and methodology are presented in the following section. Eight chambers were run simultaneously for each experiment (4 with benzanthracene and 4 with phenanthrene), providing 4 replicate chambers during phase 1 and 2, and 2 replicate chambers during phase 3 of each experiment.

### **Specific Methods**

#### **Organisms**

Small (2-3.5 g wet weight) *M. arenaria* were collected from intertidal flats on Gull Point in the Germantown section of Quincy, MA, using a clam rake. *S. viridis* were collected from intertidal flats on Wollaston Beach in Quincy using the same methods. Locations of the sampling sites are given in Table 1 and Figure 3. Worms were removed from sediment particles individually and identified under the dissecting scope. Organisms were kept in fresh aerated seawater at 11-14 °C at the University of Massachusetts for up to several days before being transported to Woods Hole for use in an experiment. Each chamber received 3 clams and 15-20 worms. The total wet weight of worms added was approximately 2.5 g.

Analysis of benzanthracene and phenanthrene content of these organisms (see specific methods below) indicated concentrations of these individual PAH to be below the limit of detection for this analysis (200 ng/g wet weight).

#### **Sediment Preparation**

Sediments were collected from ship by a Van Veen grab. At the time of collection, the top 1 cm of the sediment in the grab was removed and held separately for labeling with radioisotope. The remainder of the top 10 cm in the grab was saved to form the unlabeled sediment reservoir in each chamber. Sediment samples were kept on ice until returned to the laboratory where they were sieved to 1 mm to remove macrofauna and held in the dark at 4 °C until used. Separating the top cm from the subsurface sediments was undertaken to minimize alteration of the chemical structure of the sediment *in situ* in the benthic chambers. Locations of sediment collection sites are given in Table 1 and Figure 3.

At the time of collection, samples of each sediment type were archived for analysis of organic carbon and PAH content.

#### **Sediment Organic Carbon and Nitrogen Content**

Sediment particulate organic carbon and nitrogen content, and molar elemental C/N ratio were determined by CHN analysis. Sediment samples were dried at 60°C and 1 g subsamples of dried material transferred to acid-cleaned 7 ml glass vials. Each sample was ground to a fine powder with a glass rod and acidified to remove inorganic carbon. The latter was removed by adding 5 ml of 5N H<sub>3</sub>PO<sub>4</sub> and 1 drop of concentrated HCl to each sample and mixing

completely. The acidic supernatant was removed by centrifugation, and the sediment washed several times with distilled water to remove residual acid. Samples were redried at 60°C, pulverized with a glass rod and analyzed for organic carbon and nitrogen on a Perkin Elmer Model 2400 CHN elemental analyzer.

### **Sediment Radiolabeling**

Prior to beginning the second phase of each experiment surficial sediments (top 1 cm collected) were radiolabeled with either  $^{14}\text{C}$ -PHN or  $^{14}\text{C}$ -BA. Both isotopes were checked for purity by TLC (see below) and found to be  $\geq 98\%$  pure prior to use in an experiment. Briefly, a solution of both radiolabeled and unlabeled BA or PHN (5uCi/uMol) in acetone was coated around the walls of a gallon glass jar and the solvent allowed to evaporate under nitrogen. A sediment slurry was then placed in the container and the isotope and sediment allowed to equilibrate with constant mixing for 16 hours. The labeling solution was removed by centrifugation and the sediments resuspended in clean seawater. Subsamples were taken to determine total radioactivity added prior to transfer of sediments to Woods Hole for placement in the chambers.

### **Dissolved Oxygen**

Dissolved oxygen concentration in the water column of each chamber was determined with a Yellow Springs Instruments (YSI) Model 54A oxygen/temperature meter calibrated using 100% O<sub>2</sub>-saturated seawater. Oxygen uptake in each chamber was determined by measuring the decrease in oxygen concentration in the overlying water over time (typically 9-12 hours) when air and seawater flow into the chambers had been interrupted.

### **Sediment Redox Potential and pH**

Sediment redox potentials were measured using a platinum electrode (Howes et al., 1981), and pH was determined using a combination pH electrode submerged in the sediments. Both measurements were made at 1 cm intervals from the surface to the bottom of the sediment reservoir.

### **Sediment Coring**

Sediment cores of 6 cm<sup>2</sup> surface area were obtained utilizing a piston coring technique developed by Howes designed to minimize sediment compression. Prior to removal of the core from the reservoir, thin-walled liners (1/32") were inserted around the cores to hold the cavity open until glass rods could be inserted to fill the void immediately after the core was removed to prevent alteration of the sediment surface area or subsurface hydrography of the chambers. After glass rod insertion the liner was carefully removed. Separate cores were taken for analysis of: 1) porewater nutrients, 2) sulfate reduction, and 3) PAH content, metabolism and mineralization.

## Porewater Nutrient Analyses

Sediment cores were extruded and sectioned into four, 2 cm intervals. Each 2 cm section was placed into a tared acid-cleaned conical centrifuge tube and weighed. 15 ml of 2N KCl in distilled water was added to each tube and the tubes were sealed, mixed well, and placed at 4°C overnight. Each tube was then centrifuged and the supernatant removed and filtered through a 0.2 µm Supor-200 filter (Gelman Sciences, Inc.). The centrifuge tubes containing the sediment pellet were placed at 60°C until the pellet was dry and the tube and pellet were then weighed. The filtered supernatants were analyzed for ammonium (Scheiner 1976) and phosphate (Murphy and Riley 1962) as described below. In addition, the porewater samples were analyzed for inorganic sulfate by the method of Tabatabai (1974a,b). All analyses were corrected for dilution of the porewater by the added KCl. A KCl blank was also run for each analysis.

## Sediment Sulfate Reduction Measurements

After removal from the chamber,  $^{35}\text{S}$  (50 µl) was injected into the sediment at 2 cm intervals through silicone plugs in the plastic cores. Each core was then incubated for 24 hrs at 15°C. Activity was terminated by placing the cores at -20°C. Zero time cores were frozen to establish an isotope background. Due to the excessive time required to complete the rest of the analysis, only the top 2 cm section on the cores were analyzed.

$^{35}\text{S}$ -reduced sulfur was recovered by the active distillation of core sections according to the procedure of Howes et al. (1984). Reduced  $^{35}\text{S}$ -sulfur was fractionated into acid volatile sulfur (AVS) containing  $^{35}\text{S}$  hydrogen sulfide and  $^{35}\text{S}$  ferrous sulfide, and chromium reducible sulfur (CRS) containing  $^{35}\text{S}$ -pyrite and  $^{35}\text{S}$  elemental sulfur.

$^{35}\text{S}$ -AVS was analyzed by the acidification of the sediments under anaerobic conditions with HCl to pH of 1.0 and the collection of released hydrogen  $^{35}\text{S}$ -sulfide in traps containing an excess of zinc chloride and zinc hydroxide. The sediments remaining were then treated with reduced acidic chromium chloride under anaerobic conditions to convert  $^{35}\text{S}$ -CRS into hydrogen  $^{35}\text{S}$ -sulfide. The latter was then trapped in fresh traps as described above.

Subsamples from each trap were chemically analyzed for hydrogen sulfide according to a modification of the technique of Cline (1969) in order to provide a measure of the AVS and CRS pools in the sediments. Trap contents were analyzed for zinc  $^{35}\text{S}$  by mixing with Aquasol II (Dupont/New England Nuclear Co.) and counting on a liquid scintillation counter ("Tri-Carb" 4000 series liquid scintillation counter, United Technologies/Packard).

## Water Column Nutrient Flux

Sixty ml water column samples were obtained by syringe and filtered through 0.2 µm Gelman "acrodisc" sterile filters into acid-cleaned 60 cc Nalgene bottles and stored at -20°C until analyzed for nitrate, ammonium and phosphate content. Nitrate was analyzed on a Lachat Instruments "Quik-Chem" automated ion analyzer utilizing cadmium reduction/azo dye chemistry (Wood et al. 1967).

Ammonium was detected using the indophenol method based on development of a deep blue color when ammonia reacts with phenol and alkaline hypochlorite (Scheiner 1976). Phosphate was analyzed by the ascorbic acid/molybdenum blue technique described by Murphy and Riley (1962).

Flux of nutrients to the water column was determined by measuring the rise in nutrient concentrations in samples of overlying water over time (typically 9-12 hours) when air and seawater flow into the chambers had been interrupted.

### **Determination of Radioactivity**

PAH concentrations and production of PAH metabolites was calculated from radioactivity recovered using the specific activity of PAH added. Liquid samples were quantified by liquid scintillation counting (LSC) using a Packard Tri-Carb 2200CA liquid scintillation counter with IBM Model 30 PS/2 computer and dot matrix printer with automatic quench correction and Ultima Gold (Packard Inst.), a non-solvent based cocktail, as the scintillant. Radioactivity in solid samples was determined by complete combustion in a Harvey Sample Oxidizer Model OX-600. The oxidizer combusts the sample entirely to CO<sub>2</sub> which is trapped in base and then subjected to LSC. Samples were counted to 5% accuracy or for 60 minutes each.

### **Collection of Macrofauna**

Specimens of *S. viridis* and *M. arenaria* were removed from sediment at the end of the experiment by rinsing the sediment from each chamber onto a 1 mm sieve and gently agitating the sieve in a tub of seawater. Clams and worms were placed in petri dishes with a small amount of clean sediment and allowed to purge themselves overnight. The next morning organisms were removed from clean sediment and wet weights determined prior to freezing. Organisms were stored at -20°C until analyzed for total radioactivity and metabolite production.

### **Radioactivity in Sediment Samples**

The top 2 cm of sediment cores was extruded onto solvent rinsed aluminum foil and wet weight of the core top determined. Sediment were then be diluted and homogenized to a total volume of 25 ml with seawater. With constant agitation subsamples were taken with glass pipette for determination of total radioactivity, radioactive CO<sub>2</sub>, and the presence of polar metabolites.

### **Sediment and Tissue Combustion**

Total radioactivity was determined in up to 0.5 ml of sediment homogenate or up to 0.3 g of wet tissue by complete combustion at 900 ° F using a Harvey sample oxidizer using cocktail and methods supplied by the manufacturer. Duplicate standards of <sup>14</sup>C-mannitol were run with each set of samples, and a blank was run every 4 samples.

## **Radioactive CO<sub>2</sub> Determination**

A 10 ml subsample of sediment homogenate was placed in a 25 ml flask fitted with a teflon-lined stopper and a glass well containing a filter paper saturated with 2 N KOH. After the flask was sealed the homogenate was acidified to a pH < 3 by injection of 0.3 ml of 10% trichloroacetic acid (TCA). After the acidified slurry was shaken overnight, the base-saturated filter paper traps and an 0.5 ml distilled water rinse of the glass well was subjected to LSC after being held for 48 h to reduce chemiluminescence. Background corrections were made using filters from control flasks incubated at the same time.

## **Extraction of Sediment and Animal Tissues for Parent PAH and Polar Metabolites**

Five mls of sediment homogenate were acidified with 0.3 ml 10% TCA, vortexed and allowed to stand for 30 min. The homogenate was then extracted sequentially twice with 2 ml each ethyl acetate and twice with 2 ml methylene chloride. At each step the sample was vortexed for 15 seconds with fresh solvent and the extract removed by centrifugation. All extracts were combined, and excess water removed by the addition of anhydrous sodium sulfate. Volume was reduced under a gentle stream of nitrogen at 45 °C to just dryness and the sample brought up to 60 ul in acetone. Ten ul subsamples were taken in duplicate for determination of radioactivity in the extract and for determination of the percentage of parent compound versus polar metabolites using thin layer chromatography. The water phase remaining after extraction was counted for radioactivity and the extracted residue combusted to determine unextractable radioactivity.

A similar procedure was used for tissue samples with the following modifications. Tissues were minced with scissors prior to addition of 3 ml of ethyl acetate and 0.3 ml 10% TCA at the same time. Tissues were then homogenized in a "Vertishear" tissumizer. After the first extraction the procedure followed that described above for sediments.

## **Thin Layer Chromatography**

Ten ul aliquots of sediment or organism extracts were spotted in duplicate on silica gel plates (60A fluorescent, 5 X 250 cm, 250 um, Whatman Ltd., Maidstone, England) and run for 20 minutes in a mixture of hexane:toluene (7:3). Under these conditions, polar metabolites remain at the origin and the parent PAH migrates up the plate approximately 10 cm. The exact location of parent compound was confirmed by co-migration of authentic PAH standards as well as visualization by UV light. After drying a uniform spot of silica gel was removed from each sample lane corresponding to the origin and location of parent PAH by scrapping with a razor blade. Silica gel was counted in "Omniflour" (Fisher Scientific) cocktail, which was found to give better counting efficiencies for silica than "Ultima Gold."

## **Water Column Samples for Determination of Flux of PAH and Metabolites and Radioactive CO<sub>2</sub>**

During the second and third phase of the experiment effluent from each chamber was passed through a 0.45 Whatman, GFC glass fiber filter and an XAD-4 resin column to collect PAH and metabolites. Sample collection had to be discontinued for several days after sediment

cores were collected, due to elevated suspended sediment load, and when flow was interrupted for respiration measurements. Seventeen to 45 L samples of effluent water were passed through XAD for analysis.

XAD columns were prepared by placing 4 grams of XAD in a 10 ml glass syringe fitted with a polyester gauze filter plug at the bottom. The columns were rinsed with 5 volumes of methanol, then 10 volumes of clean seawater prior to being placed on-line in the system. After collection, samples were eluted from the XAD by 3, 5 ml rinses with acetone. The eluate was allowed to reduce its volume in the hood overnight. The next day it was acidified with 0.3 ml 10% TCA and extracted as described above for sediments.

At several points during each experiment, 250 ml samples of chamber effluent water were collected after having passed through the XAD resins. These were kept on ice and analyzed for  $^{14}\text{CO}_2$  as described above for sediment  $^{14}\text{CO}_2$  with the following modifications. A 500 ml flask with 2 glass KOH wells each containing 2N KOH saturated filters and 0.75 ml 10% TCA was used to collect the sample. Each filter was counted separately, and the background corrected sum of both used to determine total water column  $^{14}\text{CO}_2$ .

### **PAH Analysis**

Measurements of endogenous levels of BA and PHN were made using techniques described in Shiaris (1986) using a Hewlett-Packard 1090 high pressure liquid chromatograph equipped with a 20 mm 3 $\mu\text{m}$  C<sub>18</sub> reverse phase column and a 1040A diode array spectrophotometric detector.

### **Statistical Methods**

Statistical differences among parameters in each experiment were determined by one way ANOVA with comparison of means by Tukey's protected T-test for measurements of O<sub>2</sub> flux, sulfate reduction and nutrient flux. Differences among REDOX profiles within each experiment and for pre-animal conditions in all experiments were determined by Scheffe's multiple range test (Zar, 1984). These tests were run using the GB-STAT software package from Dynamic Microsystems, Inc.

All variables measuring the fate of radiolabel were tested to determine whether or not they were normally distributed using a Shapiro-Wilks test for skewness. Variables demonstrating significant departures from normality were transformed prior to conducting a repeated measures, univariate ANOVA. Differences between individual means were compared using the Tukey-Kramer multiple range test. The SUPER ANOVA software package from Abacus Corporation was used to conduct these analyses.

Error bars were not shown in the figures presented in most cases because of the decreasing number of replicates available throughout each experiment as additional experimental treatment conditions were imposed. All statistical comparisons were based on the tests described above using a p value of  $\leq 0.05$  as significant.

## RESULTS AND DISCUSSION

### Microbial Activity and General Biogeochemical Environment

Microbial activity was assessed by following whole chamber oxygen consumption, nutrient flux to the water column, and profiles of redox, pH, sulfate reduction and pore water nutrients in the sediment. Within each experiment these measurements were made prior to the addition of PAH, and before and after the addition of macrofauna. As expected, sediments collected from different locations from the relatively contaminated environment of Boston Harbor, Fort Point Channel (FPC) and Spectacle Island (SI), and those from the relatively uncontaminated sites in the Cape Cod region, (Buzzards Bay Station R (BB) and Little Pond (LP), provided a wide range of biogeochemical environments within the different experiments. Sediment oxygen demand in each experiment was used as an approximation of the rate of carbon mineralization with each experimental system. Ranking each site for overall microbial activity on the basis of mean oxygen flux measured before the addition of macrofauna to the system (Table 3), it can be seen that in order of decreasing oxygen demand, the sediments can be ranked as follows: Little Pond > Spectacle Island > Fort Point Channel > Buzzards Bay.

In general, sediment oxygen demand increased with sediment organic carbon content and with the addition of macrofauna to sediment chambers (Figure 4, Table 3). Although in general, sediment respiration rates have not been observed to be closely related to sediment total organic content, the close agreement between these parameters in the experiments presented here is most likely attributable to the experimental design in which only surface sediments, where the labile fraction of the total organic matter pool is greatest, were used. Sediment O<sub>2</sub> flux in experiments with sediments from Spectacle Island is somewhat elevated in comparison to measurements made in experiments with sediments from Fort Point Channel, even though the latter site has a higher total organic carbon content. Temperature control of experiments with sediments from Spectacle Island was disrupted for one week during Hurricane Bob (August 1991) and the elevations in O<sub>2</sub> flux may be related to elevations in incubation temperatures.

### Sediment Oxidation State

Redox profiles measured in sediment chambers before the addition of macrofauna indicated significant differences in the biogeochemical environment among the various experiments and are consistent with observations made on total organic carbon content, sediment oxygen demand and sulfate reduction (Figure 5; Tables 2-4). Of the four sediment types used, sediments from the Buzzards Bay site revealed redox profiles that were moderately oxidizing from surface to bottom (80 mm); these redox profiles were significantly different from those measured in the other three experiments. The redox profiles of sediments from Little Pond and Spectacle Island were not significantly different from one another but were significantly different from measurements made in sediment from Fort Point Channel. Sediments from Little Pond, the site with the highest total organic carbon content, exhibited the steepest gradient in redox conditions with the Redox Discontinuity Layer (RDL) occurring in the upper 15 mm of the sediment. Sediments from Spectacle Island also revealed a highly reducing environment with an RDL value similar to that observed in sediments from Little Pond. Sediments from Fort Point Channel were intermediate in the depth of the RDL observed (35 mm) between sediments from Buzzards Bay and the other two sites. These trends in redox profiles and depth of the

RDL are generally consistent with measurements of total organic carbon content of the different sediment samples and measurements of sediment oxygen demand. Experiments with sediments from Spectacle Island deviate from this trend to some extent, presumably due to the higher incubation temperature that occurred during Hurricane Bob.

The addition of macrofauna and/or PAH resulted in changes in redox profiles and the RDL in all sediments except those from Spectacle Island (Figures 6-9). Sediments from Buzzards Bay were moderately oxidized under all experimental conditions with slight shifts in the upper 50 mm occurring with the addition of macrofauna and phenanthrene or benzantracene. With sediments from Little Pond, the addition of phenanthrene (with and without macrofauna) had a significant effect on increasing the depth of the RDL but no significant differences were observed with the addition of benzantracene. No differences in redox profiles or RDL were observed with sediments from Spectacle Island. In Fort Point Channel sediments, the addition of macrofauna had a significant effect on increasing the RDL.

### **Sulfate Reduction**

Sulfate reduction rates were measured for subsamples from the upper 20 mm of sediment cores taken from each chamber in each experiment. Differences in sulfate reduction rates were observed with sediment type, the addition of phenanthrene, and in some instances, the addition of macrofauna (Table 3; Figure 10). Sulfate reduction rates in the upper 20 mm were not tightly correlated with trends in sediment organic carbon content, oxygen demand or redox profiles from the different experiments. The addition of phenanthrene to sediment chambers (with and without macrofauna) had the most pronounced effects on sulfate reduction rates in sediments with the exception of those from Buzzards Bay (Table 3).

Interestingly, for all experiments, except those using Buzzards Bay sediments, the addition of phenanthrene to sediment chambers resulted in a 4- to 15-fold increase in sulfate reduction rates in comparison to values measured in sediment chambers with benzantracene. These data suggest that the addition of phenanthrene to sediment chambers resulted in enhanced microbial activity in the upper 20 mm of the sediment core. No comparable stimulation of sediment oxygen demand was observed in chambers receiving phenanthrene versus those receiving benzantracene. These data indicate that phenanthrene was available as an additional carbon source, primarily for anaerobic microorganisms in these experiments. This is, to our knowledge, the first record of similar stimulation of anaerobic microbial activity due to the presence of a low molecular weight PAH. The absence of this effect in the Buzzards Bay sediments is probably due to the generally oxidized nature of these sediments in the chambers.

### **Porewater Nutrients and Nutrient Efflux**

The availability of dissolved nitrogen and phosphorous may be contributing factors to overall microbial activity in marine sediments. Sediments from each of the four sites varied in the concentrations of porewater ammonia and phosphate (Figures 11 and 12). The highest porewater ammonia and phosphate levels were measured in sediment samples from Spectacle Island and Little Pond, respectively. The addition of macrofauna altered the distribution of porewater nutrients to some extent through bioirrigation of the sediments but the differences were not significant (data not shown).

Efflux of nutrients from sediments in each of the experiments are presented in Table 5. The addition of macrofauna resulted in significant increases in the flux of ammonia from all sediments except those from Spectacle Island and benzantracene-dosed sediments from Fort Point Channel with animals. Although in the latter cases mean values were higher than those made in sediment chambers without macrofauna, there was also high variability in the measurements. The efflux of other nutrients (i.e., nitrate and phosphate) were also generally higher in chambers with macrofauna although in some instances high variability in individual measurements resulted in no statistical differences. Alterations in nutrient flux associated with the addition of macrofauna to the sediment chambers in each experiment are consistent with observations of increased bioturbation and bioirrigation by macrofauna.

### **Fate of Radiolabeled PAH in Surface Sediments**

Mean values for the concentration of added benzantracene (BA) and phenanthrene (PHN) in the top 2 cm of sediment in cores collected two weeks after spiking, (just before the addition of macrofauna), and in cores collected 4 weeks after spiking, (just prior to collection of macrofauna) for all experiments are shown in Tables 6 and 7 respectively. On a weight/molar basis, no significant reductions were observed over time, or with the addition of macrofauna. Significant differences were observed however, among concentrations of added PAH in the different sediment types utilized. The average concentration of added benzantracene (2.0 nmol/gww) was slightly larger than that of added phenanthrene (1.8 nmol/gww). The average concentration of both PAH was lowest in Buzzards Bay sediments (1.2 nmol/gww) and highest in Fort Point Channel sediments (2.4 nmol/gww). On a dry weight basis/molar basis, average concentrations of PAH were highest in Little Pond and Fort Point Channel sediments and lowest in Buzzards Bay sediments.

The experimental plan called for the preparation of equimolar, wet weight concentrations for both PAH in each sediment type. The relatively small deviations observed were probably due to differences in the water content of sediment slurries at the time of labeling and uneven distribution of labeled sediment in the experimental chambers. Variability between cores taken from replicate chambers was relatively large in some cases (coefficients of variation ranged up to 43%). Due to the relatively large sample size needed to measure both parent PAH and metabolites in sediments and the need to collect separate cores for measurement of pore water nutrients and sulfate reduction, only one core for radioisotope measurements could be taken from each chamber at each sampling period. Duplicate cores collected at the end of some experiments showed coefficients of variation of a similar magnitude within an individual chamber (data not shown).

These chambers were run as open systems. Although samples of each component were taken as frequently as possible, we were only able to obtain snap shots of the distribution and radiolabeled PAH and metabolic breakdown products at various time points after experimental manipulation of the chambers. Considering the limited sampling possible and the variability observed in sediment concentrations of added PAH, we felt that calculation of a mass balance would be inappropriate. Rather than taking a mass balance approach, we have looked at the fate of PAH and metabolites in these systems from the standpoint of concentrations appearing in various reservoirs and the rate of removal from the sediment reservoir.

Also shown in Tables 6 and 7 is the percentage of radioactivity extracted from sediment samples that remained as unmetabolized parent compound. In all cases greater than 93% and more commonly greater than 95% of extractable radioisotope was unmetabolized.

Although no build-up of polar metabolites was observed in sediment extracts, significant differences were observed over time between the two PAH added, and between different sediment types in the levels of PAH completely mineralized to CO<sub>2</sub> in surface sediments. Expressed as the percentage of total added BA in the sediment sample converted to CO<sub>2</sub>, the two sediments from Cape Cod (Buzzards Bay and Little Pond), demonstrated a very low and similar (.027 and .023 % respectively) degree of mineralization. The percentage of total added BA converted to CO<sub>2</sub> in the two Boston Harbor sediments (Spectacle Island and Fort Point Channel) was an order of magnitude higher.

With the exception of the Fort Point Channel sediment, the degree of mineralization observed for phenanthrene was 30 to 200 times greater than that seen for benzanthracene. The greatest difference was observed in the Buzzards Bay sediment. In contrast, the degree of phenanthrene mineralization observed in the Fort Point Channel sediment was only 3 times that observed for benzanthracene.

No consistent trends were observed between chambers with and without macrofauna. Only for benzanthracene in Buzzards Bay and Little Pond sediments, was more <sup>14</sup>CO<sub>2</sub> observed in chambers with macrofauna. This stimulation of BA mineralization by macrofauna was not observed in Boston Harbor sediments, nor was stimulation of phenanthrene mineralization observed in any of the four sediment types. These results are contrary to previous studies using larger polychaetes where stimulation of microbial degradation was observed (Bauer *et al.* 1988). The degree of mineralization decreased with time for phenanthrene in Spectacle Island sediments. This pattern was not evident in the other sediment types.

Also shown in Tables 6 and 7 is the percentage of unextractable radioactivity remaining in sediments. This was calculated from the difference between total radioactivity as determined by complete combustion, and that which could be extracted into organic solvents. With the exception of the Little Pond sediments, a greater percentage of radioactivity remained unextractable from BA than from PHN. For either isotope, unextractable radioactivity tended to be higher in the sediments with lower organic carbon concentrations.

### **Radioactivity at Depth in the Sediment Reservoir**

In the Buzzards Bay sediment experiment, the core bottoms (2-10 cm depth) were analyzed for total radioactivity. As shown in Table 8, only a small amount of radioactivity was found in the remainder of the core. Furthermore, no indication of increased radioactivity at depth was observed in chambers with macrofauna. Radioactivity below the top 2 cm accounted for an average of only 7% of the total recovered from the core. Due to the much lower levels of radioactivity at depth and manpower constraints, sediment bottoms were not analyzed in subsequent experiments.

## Ambient Concentrations of Benzanthracene and Phenanthrene in Sediment

Presented in Table 9 are the ambient concentrations of benzanthracene and phenanthrene in the sediments used in these experiments determined by HPLC chromatography expressed on a wet weight, dry weight and lipid weight basis. As expected concentrations of both PAH in Fort Point Channel sediments were very high (50,000 to 90,000 ng/g dry weight for benzanthracene and phenanthrene respectively). However, levels particularly of benzanthracene were surprisingly low for Spectacle Island, for both PAH surprisingly high for Buzzards Bay and extraordinarily high for Little Pond.

There are no known sources, other than atmospheric deposition and run-off, of PAH to Little Pond. Unlike the other sediments used in these experiments, Little Pond sediment contained significant amounts of fine detrital material. It is possible that this plant-derived material may have contributed to the estimate of ambient PAH concentration. Although these measurements were made using a diode array detector which matches the spectra of each eluting peak against standard reference material, other material co-eluting with phenanthrene or benzanthracene may have contributed to the signal strength. Without confirmation by gas chromatography with mass spectral detection, it is impossible to say.

Sediment from Spectacle Island was used in these experiments because sediments from this site had relatively low organic carbon content and was one of the sites used by Gschwend et al. in their MWRA study of sediment/water exchange of contaminants. Previous measurements of PAH concentrations at this site by Shiaris and Jambard-Sweet (1986) indicated measurable levels of both benzanthracene and phenanthrene (669 and 251 ng/g dry weight respectively). However, subsequent measurements conducted as part of the Gschwend *et al.* study indicated contaminant levels at Spectacle Island to be highly variable over small spatial scales although reported values in the top 6 cm still were appreciable (263 and 571 ng/g dry weight for benzanthracene and phenanthrene) (McGroddy, 1993). McGroddy's values for these two PAH in Fort Point Channel sediments were also much lower (around 2000 ng/g dry weight for each PAH) than that observed in this study, or previously observed by Shiaris and Jambard-Sweet.

As discussed above, the technique used for PAH analysis in this current study is not necessarily as selective for individual PAH as conventional GC-mass spectral analysis. In addition, only single samples were analyzed here, nor was standard reference material available, so we have no quantitative measure of either the variability or the accuracy in our measurement. These factors and the apparent inconsistency in our data with respect to historical data and that recently obtained from some of the same sites by McGroddy limits the strength of any interpretations of the data on ambient PAH concentrations presented here.

Although classification of the Boston Harbor and Cape Cod sediments based on measured concentrations of benzanthracene and phenanthrene (high vs. low) was not as successful as anticipated, overall, on the basis of historical data (MacDonald, 1991), we feel confident in assuming that microorganisms from the Boston Harbor sites were exposed to elevated concentrations of PAH compared with microorganisms from at least the Buzzards Bay, and probably the Little Pond site.

## Flux of PAH-derived Material to the Water Column

Shown in Table 10 are data summarizing the flux of added PAH and their metabolites to the water column collected on XAD traps during the period before (weeks 1 & 2) and after (weeks 3 & 4) addition of macrofauna to the chambers in each experiment. Overall, significant differences were observed between the two PAH, sediment type, and time. Flux of PAH-derived radioactivity from Fort Point Channel sediments exceeded that observed in all other experiments by 10 to 140 times. In order to observe patterns in the flux of PAH-derived radioactivity from the sediment it was necessary to plot values for Fort Point Channel off-scale in Figure 13. These values for flux are based only on added PAH, if we had assumed that ambient levels of PAH behaved similarly to added PAH, flux from the Fort Point Channel site would have been greater still by a factor of more than 1000.

For both PAH, flux from Fort Point Channel sediments was 3 to 6-fold greater during the first two weeks after isotope addition, than during the remaining two weeks of the experiment. Although less pronounced, this tendency was observed in the other sediment types as well.

Also shown in Table 10 is the overall percentage of radioactivity released from the sediment that was still unmetabolized PAH. The category "overall percent parent" refers to the product of the proportion of radioactivity extractable from XAD times the percentage of extractable radioactivity present as parent compound. In some samples a significant proportion of material eluting from XAD was in an aqueous fraction. Material in the aqueous fraction probably represents conjugated metabolites resistant to acid hydrolysis. As can be seen in the graphs on Figure 14, which are plotted on the same scale, despite the fact that phenanthrene is more susceptible to complete degradation by microbial activity, particularly in experiments with sediments from Cape Cod, phenanthrene-derived material in the water column appeared to be less metabolized than that of benzantracene-derived material under similar circumstances.

In comparison of sediment types, material sorbing off Fort Point Channel sediments almost always contained the lowest percent of parent PAH (2-9% of total). The percent of parent compound observed in material sorbing of the other sediment types was 10 to 20 greater than that observed in material emanating from Fort Point Channel sediments. The significantly increased flux of total PAH-derived material and the degree of metabolism observed indicate that removal of PAH from the Fort Point Channel Sediments was clearly accelerated over that observed in the other three sediment types. These observations fit with those made previously by Chin and Gschwend (1992) and McGroddy (1993) noting significantly increased levels of PAH-bound colloids and PAH in the porewaters of Fort Point Channel sediments as compared to other sites in Boston Harbor.

## Flux of Radiolabeled CO<sub>2</sub> to the Water Column

Discrete water samples were collected at weekly intervals during each experiment except the one using Spectacle Island sediments, where sampling was disrupted by Hurricane Bob, for analysis of <sup>14</sup>CO<sub>2</sub> flux to the water column. From these measurements flux of added PAH mineralized to CO<sub>2</sub> was calculated. Significant differences were observed over time, between

the two PAH tested, and among the sediment types assessed. The presence or absence of macrofauna had no significant effect. As can be seen in Figure 15, only in experiments with Buzzards Bay sediments was a strong temporal trend in flux of added PAH mineralized to CO<sub>2</sub> observed, and then only for chambers with phenanthrene. In this experiment, flux of added phenanthrene mineralized to CO<sub>2</sub> appeared to decrease exponentially with time. As was seen in the sediment samples, mineralization of benzantracene was minor in comparison to that of phenanthrene.

### Turnover of Benzantracene and Phenanthrene in Surface Sediments

Presented in Table 11 are turnover times calculated from the amount of PAH mineralized to CO<sub>2</sub> on a whole chamber basis. This calculation assumes that added PAH mimics the behavior of *in situ* burdens of PAH and that the system is at steady state, and therefore takes into account both the added and ambient concentrations of PAH in the sediment. Although some of the assumptions of this calculation (a steady-state system) are not necessarily met in the experiments described here, it was calculated as a means to compare degradation between experimental treatments, not as a true reflection of how long it would take the PAH in these sediments to be degraded.

As can be seen in Table 11, significant differences were observed between the turnover times calculated for the two PAH and for the different sediment types. The presence of macrofauna had no significant effect on turnover. Phenanthrene was calculated to be removed 1.5 to 24 times faster than benzantracene. On average, the turnover times for benzantracene in the Boston Harbor sediments was shorter than for the less contaminated sites. Turnover times for phenanthrene were exceptionally small for the Buzzards Bay sediments.

The patterns observed for PAH mineralization appear to be completely independent of organic carbon content of the sediments and overall microbial activity as discussed above. It is probable that prior adaptation of the microbial community to conditions of high contaminant loading in the Boston Harbor sediments was responsible for the greater ability of the microbes from these sediments to oxidize benzantracene.

Turnover times of phenanthrene are similar to those reported previously in Boston Harbor by Shiaris, (1989) but shorter than values reported by Heitkamp and Cerniglia (1987). Turnover times of benzantracene are similar to that reported by Herbes and Schwall (1978) for an oil-contaminated stream, but substantially less than that reported for sediments from an uncontaminated site.

### Bioaccumulation into Macrofauna

Two abundant near shore benthic macrofauna were chosen for this experiment, *M. arenaria* a suspension feeding bivalve, and *S. viridis*, a surface deposit-feeding polychaete. Levels of naturally incurred residues of both benznathracene and phenanthrene were below the limits of detection employed in these experiments (200 ng/gdw). Presented in Tables 12 and 13 are the data on bioaccumulation of added PAH by these two species. Significant differences were observed in accumulated PAH-derived material with respect to species, sediment type, and PAH. Overall, *S. viridis* accumulated PAH to a much greater extent than *M. arenaria*, and

benzanthracene-derived material was accumulated to a greater extent than phenanthrene-derived material. Although we had no direct measure of activity or health of the organisms during the experiment, similar amounts of fecal pellets and siphon tubes were observed between chambers and between experiments, and no dead organisms were recovered at the end of any experiment.

In an attempt to normalize for factors driving bioaccumulation such as the concentration of PAH in the sediment, the organic carbon content of the sediment and the lipid content of the organism, accumulation factors were calculated ( $AF = (\text{pmol/g lipid organism}) / (\text{pmol/g organic carbon sediment})$ ) (Tables 12 and 13). Normalization had only a minor effect on the magnitude of differences observed. Clearly, more than organic carbon content is controlling bioaccumulation from these particular sediments into these two organisms. These data are in agreement with values previously reported by others (McElroy and Means 1987; Lake *et al.* 1990; Capuzzo *et al.* (1992).

### Metabolism by Macrofauna

Levels of radioactivity accumulated in *M. arenaria* were too low to adequately support metabolite analysis, so none were attempted. However, based on previous work on PAH metabolism in bivalves (Stegeman, 1985) it is unlikely that significant levels of metabolites would have been observed in *M. arenaria*.

Extracts of worm tissues were found to contain 51 to 97% parent compound. Significant differences in the percentage of PAH-derived material remaining unmetabolized in the extract was observed between sediment type. No significant differences were observed between metabolism of phenanthrene and benzanthracene. The degree of PAH metabolism observed in worms exposed to Buzzards Bay sediments was significantly greater than that observed in other sediments which were not significantly different from each other.

Despite addition of acid to cleave water soluble, conjugated metabolites back into organic soluble, polar metabolites, a sizable portion of radioactivity remained in the aqueous extract. Assuming material in the aqueous extract was some form of water soluble metabolite, this fraction was combined with the percentage of metabolites in the organic extract to produce a more complete estimate of the true percentage of unmetabolized PAH remaining in the tissue. This combined estimate, termed Overall Fraction as Parent, is also presented in Tables 12 and 13. Expressed in this way, there were no significant differences between the degree of metabolism observed between benzanthracene and phenanthrene, or between the four sediment types.

The degree of metabolism observed in worm tissue does not appear to correlate with flux of PAH derived material from the sediment reservoir, nor with the level of microbial degradation observed.

## SUMMARY AND MANAGEMENT CONSIDERATIONS

Although preliminary in nature, the work described above represents a first attempt to simultaneously quantify the link between activity of the general microbial community and the fate of PAH in surface sediments of a wide range of sediment types. It was also, to our knowledge, the first study to quantify flux of parent PAH as well as metabolites and degradation products (CO<sub>2</sub>) from a relatively intact sediment reservoir. Although there is always the question of how well added PAH mimic the behavior of naturally incurred PAH, at present, there is no other way to obtain information on metabolites. Furthermore, analyses conducted by McGroddy (1993) as part of the Gschwend et al. MWRA study indicate that only a fraction of the naturally incurred PAH residues (those derived directly from petroleum products) are readily available for free exchange with pore water, and by inference, uptake by infaunal organisms.

The work completed provided a quantitative assessment of:

- 1) sedimentary routes of exposure for two representative low and high molecular weight PAH and their metabolic products to higher organisms including the soft-shell clam, *M. arenaria*, and the polychaete, *S. viridis*;
- 2) levels of these PAH and metabolites likely to be found in the diet of commercially important species such as lobster and winter flounder;
- 3) microbial degradation of these PAH in four sediment types representative of the range of fine-grained sediments seen in Boston Harbor and less contaminated regions of Massachusetts Bay;
- 4) flux of these PAH and their metabolites out of a wide range of sediment types in the presence and absence of infaunal organisms.
- 5) the coupling of microbial carbon metabolism with that of PAH metabolism.

These data clearly show that metabolism and removal of PAH from surficial sediment is influenced by microorganisms. They also show, that at least for phenanthrene, the presence of PAH can stimulate anaerobic microbial activity. Susceptibility of an individual PAH to prokaryotic degradation differs from its susceptibility to metabolism by eukaryotes. Organic carbon content of the sediment does not appear to be the primary controlling factor for accumulation or metabolism by macrofauna or metabolism and degradation by microbes. Other factors such as the presence of other contaminants, or prior exposure history of resident microbes may be more important than sediment organic matter in controlling the rate of microbial PAH degradation and metabolism and uptake of PAH by benthic macrofauna. Under these experimental conditions, removal of PAH from the sediment appears to be primarily mediated by microbial activity. Most, and in some cases almost all material being removed from the sediment reservoir, was no longer parent PAH. This is consistent with the low level

of metabolites found in surface sediment samples. Estimates of PAH flux from harbor sediments should consider the contribution of such processes to adequately assess the fate of PAH in Massachusetts Bay.

The deposit-feeding polychaete worm *S. viridis* accumulated more phenanthrene and benzantracene than the suspension feeding clam *M. arenaria*. In addition, worms rapidly metabolized PAH. Metabolites accounted for more than 50% of the total body burdens of PAH measured. Sediment type did not have a significant influence on the degree of metabolism observed in accumulated PAH. Although significant differences were observed between total accumulated PAH-derived material among the four sediment types, these differences were not correlated with sediment organic carbon concentration or general level of contaminant burden.

PAH contamination of marine biota poses risks to both ecological systems and human health. Although there are no Food and Drug Administration guidelines for PAH levels in seafood, there is growing concern that the presence of PAH in tissues of marine biota may pose significant human health risks because of the carcinogenic and mutagenic nature of individual PAH. The extent of the problem in Boston Harbor and Massachusetts Bay has not been quantified but the relatively high ranking of Boston Harbor with respect to PAH sediment contamination nation-wide (MacDonald 1991) certainly warrants careful investigation of the relative ecological and human health risks associated with this high level of PAH contamination. Although general trends in PAH contamination have been defined (e.g., higher concentrations of total PAH in the inner harbor of Boston, lesser concentrations with distance from the inner harbor; see review by Cahill and Imbalzano 1991), critical information on the behavior and effects of individual compounds is lacking. If harbor sediments continue to be a major source of PAH to the Massachusetts Bay ecosystem, even with the improvement in water quality from the reduction of point source PAH contamination (Menzie-Cura & Associates, Inc. 1991), the potential risks to both marine biota and the human consumer must be defined.

To better understand the fate and potential effects of PAH in Boston Harbor and Massachusetts Bay, the following research program should be undertaken:

1. Define the sources of contamination for specific PAH - what is the relative contribution of pyrogenic and petrogenic point and non-point sources to loading of individual compounds? An inventory of every compound is not feasible but an assessment of compounds representative of different compound classes such as benzantracene or benzo[a]pyrene for the higher molecular weight PAH and phenanthrene for the more biodegradable PAH should be possible.
2. Determine the persistence, degradation rates, and biogeochemical cycling of specific PAH in a wide range of sediments from the inner harbor and Massachusetts Bay. In addition to sediment organic carbon content, analysis of pore water colloidal material should be conducted to better characterize sorptive capacity of the sedimentary environment. How does the flux of specific compounds, sediment and pore water concentrations, and the body burdens of resident organisms vary with site?

3. Determine the tissue concentrations of individual PAH in representative species of fish and shellfish at the same sites where sediment PAH concentrations are measured. Representative species should be chosen with regard to their mode and location of feeding, their importance in either the human or aquatic food chain, and their ability to metabolize PAH.

4. On a population basis, using similar species during seasons with limited migrations, define patterns of contaminant exposure and the relationship between exposure and changes in physiological condition or other parameters of biological change. The results of these studies should be used in the determination of sediment quality criteria.

Such a program would lead to a better understanding of the causal relationship between input of specific PAH and the relative ecological and human health risks associated with such inputs.

Future studies should focus on the behavior of specific PAH compounds in harbor sediments and the unique aspects of highly contaminated sites with respect to PAH flux, degradation and bioavailability. The high flux of partially metabolized PAH from Fort Pt. Channel demonstrated in this study suggests that microbial populations at this site may have been pre-adapted to high contaminant concentrations, allowing for rapid biodegradation. Sediments from this site have also been observed by Chin and Gschwend (1992) to have relatively high sorption coefficients for the PAH pyrene, larger than would be predicted based on simple partitioning. Chin and Gschwend suggested that colloidal material at Fort Point Channel may serve to enrich the concentration of higher molecular weight organics such as PAH in pore waters where they would be available for remobilization by bioirrigation. McGroddy (1993) hypothesized that the enriched concentrations of PAH in pore waters of Fort Point Channel were due to a petrogenic source of PAH at this site. The interaction of geochemical and microbial processes in influencing PAH availability and trophic transfer needs to be addressed.

Specific management issues that must be addressed, especially in consideration of the ecological and human health risks associated with PAH contamination, are the development of PAH guidelines for benthic habitats. These should include consideration of guidelines for the disposal of PAH-contaminated dredged materials, further development of interim sediment criteria, and the determination of concentrations of PAH and/or PAH-metabolites in commercially important resource species.

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Table 1

Sampling Locations

Site	Sample Type	Latitude	Longitude
Wollaston Beach Quincy	<i>Scolecopelides viridis</i>	42o17.03"N	71o01.12"W
Gull Point Germantown Quincy	<i>Mya arenaria</i>	42o15.26"N	70o57.52"W
Buzzards Bay Station R	Sediment Exps.1&2	41o29.27"N	70o53.34"W
Little Pond Falmouth	Sediment Exp. 3	Not Determined	
Spectacle I. Boston Harbor	Sediment Exp. 4	42o19.77"N	70o59.57"W
Ft. Point Channel Boston Harbor	Sediment Exp. 5	42o21.37"N	71o02.68"W

Table 2. Carbon and Nitrogen Content of Sediments at Each Sampling Site

Site	%C	%N	Total C umole/cm <sup>3</sup>	Total N umole/cm <sup>3</sup>
Buzzards Bay	1.73 (0.01)	0.22 (0.00)	742 (6.1)	79 (1.3)
Little Pond	8.89 (0.40)	0.98 (0.04)	1312 (58.5)	124 (4.5)
Spectacle Island	2.31 (0.08)	0.24 (0.01)	931 (34.2)	83 (4.9)
Ft. Point Channel	4.70 (0.03)	0.38 (0.04)	1043 (7.1)	71 (7.4)

All values are mean values of three replicates ( $\pm$  1 S.E.)

Table 3. Sediment Oxygen Demand and Sulfate Reduction Rates for All Experiments

Experiment	Treatment	O <sub>2</sub> Flux	O <sub>2</sub> Flux umole/cm <sup>2</sup> /d	SO <sub>4</sub> Reduction umole/cm <sup>2</sup> /d
II - BB	Pre-animals		0.62 (0.03)	7.2 (1.0)
	BA-No Animals		0.77 (0.03)	5.2 (0.3)
	PH-No Animals		0.71 (0.06)	5.3 (1.4)
	BA-Animals		1.36 (0.12)**	6.5 (0.5)
	PH-Animals		1.49 (0.17)**	8.1 (0.6)**
III - LP	Pre-animals		1.25 (0.04)	16.7 (5.2)
	BA-No Animals		1.35 (0.15)	46.9 (13.6)
	PH-No Animals		1.29 (0.13)	413.7 (90.1)**
	BA-Animals		3.45 (0.37)**	57.7 (9.8)
	PH-Animals		2.29 (0.26)**	356.1 (109.8)*
IV - SI	Pre-animals		1.06 (0.07)	66.4 (5.0)
	BA-No Animals		1.18 (0.14)	40.6 (6.9)
	PH-No Animals		1.34 (0.15)	160.4 (18.1)**
	BA-Animals		1.57 (0.33)	74.1 (38.8)**
	PH-Animals		1.72 (0.38)	267.4 (57.4)**
V - FPC	Pre-animals		0.81 (0.16)	37.4 (5.6)
	BA-No Animals		1.03 (0.14)	11.3 (2.8)
	PH-No Animals		0.85 (0.18)	194.1 (53.9)*
	BA-Animals		1.81 (0.06)**	13.2 (2.7)
	PH-Animals		1.94 (0.11)**	169.5 (45.0)*

All values (with the exception of pre-animal values) are mean values of four replicates measured during weeks 3 and 4 of each experiment ( $\pm$  1 S.E.); pre-animal values are mean values of eight replicates measured during week 2 of each experiment; \* p<0.05, \*\* p<0.01. Comparing chambers with and without animals

Table 4. Scheffe Comparisons of REDOX Profiles from All Experiments

	II	III	IV	V
II	0	42.73 <sup>**</sup>	42.16 <sup>**</sup>	18.76 <sup>**</sup>
III	42.73 <sup>**</sup>	0	0.002	4.86 <sup>*</sup>
IV	42.16 <sup>**</sup>	0.002	0	4.67 <sup>*</sup>
V	18.76 <sup>**</sup>	4.86 <sup>*</sup>	4.67 <sup>*</sup>	0

Comparisons by one-way ANOVA for REDOX profiles measured before the addition of animals to sediment chambers; \* p<0.05, \*\* p<0.01.

Table 5. Nutrient Flux for All Experiments

Experiment	Treatment	NH <sub>4</sub> <sup>+</sup>	PO <sub>4</sub> <sup>-</sup> umole/cm <sup>2</sup> /d	NO <sub>3</sub> <sup>-</sup>
II - BB	BA-No Animals	0.00 ( - )	0.002 (0.001)	0.02 ( - )
	PH-No Animals	0.00 ( - )	0.001 (0.001)	0.02 ( - )
	BA-Animals	0.09 (0.02)	0.006 (0.002)	0.07 (0.00)
	PH-Animals	0.09 (0.02)	0.010 (0.002)	0.10 (0.01)
III - LP	BA-No Animals	0.06 (0.02)	0.005 (0.002)	0.08 (0.02)
	PH-No Animals	0.06 (0.03)	0.006 (0.003)	0.05 (0.02)
	BA-Animals	0.35 (0.12)	0.048 (0.040)	0.18 (0.05)
	PH-Animals	0.28 (0.02)	0.021 (0.010)	0.15 (0.01)
IV - SI	BA-No Animals	0.02 (0.01)	0.014 (0.003)	0.14 (0.02)
	PH-No Animals	0.01 ( - )	0.012 (0.002)	0.12 (0.02)
	BA-Animals	0.12 (0.06)	0.017 (0.010)	0.12 (0.01)
	PH-Animals	0.15 (0.09)	0.020 (0.008)	0.12 (0.02)
V - FPC	BA-No Animals	0.04 (0.01)	0.005 (0.002)	1.03 (0.14)
	PH-No Animals	0.04 (0.01)	0.007 (0.003)	0.85 (0.18)
	BA-Animals	0.10 (0.05)	0.023 (0.007)	1.81 (0.06)
	PH-Animals	0.22 (0.03)	0.025 (0.005)	1.94 (0.11)

All values are mean values of four replicates measured during weeks 3 and 4 of each experiment ( $\pm 1$  S.E.).

Table 6

## Summary of Radioactivity Recovered from Surface Sediment Samples

Benzenanthracene		All Values as Mean (std)					
Sediment	Week	Organisms	nmol/gww	nmol/gdw	% Ext. as Parent	% of Total as CO <sub>2</sub>	% Unext.
Buzzard's Bay	2	-	0.99 (.33)	1.84	97.58 (0.4)	0.04 (.02)	18.8 (.7)
	4	+	1.80 (.51)	2.97	95.10 (2.0)	0.08 (.02)	22.7 (2.9)
	4	-	2.11 (.32)	3.91	95.76 (1.9)	0.08 (.01)	27.2 (2.7)
Little Pond	2	-	2.90 (1.22)	10.73	95.70 (0.9)	0.02 (.003)	11.04 (1.08)
	4	+	2.09 (.37)	7.74	94.90 (0.8)	0.05 (.02)	9.3 (.34)
	4	-	3.09 (.43)	11.44	94.20 (0.5)	0.04 (.001)	9.5 (.15)
Spectacle Island	2	-	2.31 (1.00)	4.82	99.90 (0.4)	0.40 (.14)	18.0 (3.9)
	4	+	2.03 (.58)	4.23	98.10 (.1)	0.09 (.003)	20.0 (.2)
	4	-	2.15 (.05)	4.49	98.40 (.1)	0.10 (.02)	19.2 (0.8)
Fort Pt. Channel	2	-	1.29 (.59)	4.15	95.40 (1.8)	0.33 (.13)	8.9 (5.8)
	4	+	2.48 (.10)	7.93	98.30 (0.3)	0.10 (NA)	10.8 (3.4)
	4	-	2.48 (.07)	7.93	98.40 (0.1)	0.18 (.04)	10.1 (0)

For Week 2 N=4. For Week 4 N=2.

NA=not available

Table 7

## Summary of Radioactivity Recovered from Surface Sediment Samples

Phenanthrene		Organisms	All Values as Mean (std)				
Sediment	Week		nmol/gww	nmol/gdw	% Ext. as Parent	% of Total as CO <sub>2</sub>	%Unext.
Buzzard's Bay	2	-	1.10 (.37)	2.04	97.40 (1.3)	5.40 (1.9)	28.7 (8.9)
	4	+	0.98 (.11)	1.82	94.90 (1.5)	3.30 (1.5)	21.8 (9.7)
	4	-	0.67 (.04)	1.23	98.40 (1.3)	7.40 (4.7)	32 (8.9)
Little Pond	2	-	1.71 (.47)	6.33	97.50 (1)	0.89 (.058)	9.14 (1.09)
	4	+	1.79 (.50)	6.63	93.40 (4)	0.84 (.032)	5.94 (.02)
	4	-	1.99 (.14)	7.35	98.30 (.1)	0.59 (.019)	6.11 (.52)
Spectacle Island	2	-	1.88 (.44)	3.50	98.80 (.2)	12.40 (4)	27.8 (3.8)
	4	+	1.44 (.22)	3.00	98.70 (.3)	2.18 (.08)	35.1 (5.9)
	4	-	1.89 (.59)	3.94	99.10 (.1)	2.87 (.35)	32.7 (3.5)
Fort Pt. Channel	2	-	2.78 (.58)	8.90	98.70 (.3)	0.84 (.084)	19.2 (4.3)
	4	+	3.34 (.42)	10.78	99.50 (0.1)	1.02 (.113)	24.8 (4.4)
	4	-	2.90 (.44)	9.34	99.40 (.0)	1.20 (.12)	23.9 (5.1)

For Week 2 N=4. For Week 4 N=2.

Table 8

Buzzard's Bay Sediment  
 Percent of Total Activity in Sediment Bottoms  
 Week 4, After Animal Addition to Ch. 1-4

Ch. #	PAH	+/- Animals	Tot. DPM Bottom	Tot. DPM Surface	% Tot. in Bottom
1	BA	+	5528	103750	5.06
3	BA	+	7709	190658	3.89
4	BA	-	5890	168397	3.38
5	BA	-	13729	228325	5.67
2	PHN	+	8915	81771	9.83
4	PHN	+	5974	104498	5.41
6	PHN	-	9953	56968	14.87
8	PHN	-	7038	71032	9.01

**Table 9**

**Ambient PAH Concentrations**

Sediment Source	% Dry Wt.	ng/gdw BA	ng/gdw PHN	ng/gww BA	ng/gww PHN
Buzzard's Bay	54	1007	882	544	467
Little Pond	27	12096	15800	3266	4266
Spectacle Is.	48	2100	<6	1008	<3
Fort Pt. Ch.	31	45679	89998	14161	27899

Table 10

Summary of Radioactivity from Water Column Collected on XAD

Week	Organisms	Sediment Type							
		Buzzards Bay		Little Pond		Spectacle Is.		Fort Pt. Ch.	
		nmol/ m2-day	%Parent Overall	nmol/ m2-day	%Parent Overall	nmol/ m2-day	%Paren Overall	nmol/ m2-day	%Parent Overall
Benanthracene									
1-2	- Mean	3.6	24.2	6.6	15.5	13.2	5.3	1815.9	9.3
1-2	- std.	0.5	6.9	0.7	0.7	1.3	1.2	262.5	1.3
3-4	+ Mean	4.0	24.2	3.8	21.9	2.9	29.0	313.3	1.9
3-4	+ std.	0.6	2.0	0.7	3.9	0.5	11.4	47.6	0.3
3-4	- Mean	2.8	21.9	2.8	12.4	3.5	8.2	313.5	1.9
3-4	- std.	0.2	0.3	0.1	3.0	1.3	5.3	33.7	0.2
Phenanthrene									
1-2	- Mean	4.9	56.2	7.0	41.7	6.2	7.0	1119.1	5.7
1-2	- std.	2.6	15.4	1.3	3.9	0.9	3.5	81.1	0.4
3-4	+ Mean	2.5	49.6	4.4	50.4	2.8	47.2	323.6	1.9
3-4	+ std.	0.5	4.7	0.1	0.8	0.4	9.0	64.2	0.4
3-4	- Mean	1.1	33.7	2.7	46.5	2.9	23.7	146.5	0.9
3-4	- std.	0.1	2.2	0.2	6.5			8.3	0.0

Weeks 1-2 N=4. Weeks 3-4 N=2

Table 11

Sediment	Week	Organism	Turnover Time in Days		Mean (sd)	
			BA	PHN	BA	PHN
Buzzard's Bay	2	-	736	18.9	678.0	11.6
			240	7.66	366.0	5.0
			1126	9.24		
	4	+	608	10.7		
			28.1	41.3	48.2	31.4
			70.9	21.4		
4	-	40.3	14.6	53.0	12.9	
		65.8	11.2			
Little Pond	2	-	1506	46.8	1061.0	44.0
			1271	37.9	(391)	(4.3)
			750	43.9		
			715	47.3		
	4	+	548	47.5	538.0	51.6
			527	55.6		
	4	-	697	62.6	736.0	59.0
			774	55.3		
Spectacle Island	2	-	127	3.03	122.5	4.6
			137	6.56	(25.8)	(1.62)
			141	5.33		
			84.8	3.57		
	4	+	475	12.5	399.0	16.6
			323	20.7		
	4	-	270	20.8	333.0	17.6
			396	14.3		
Fort Pt. Channel	2	-	74.9	39	104.0	44.3
			53.1	37	(98.5)	(7.9)
			38.1	54.4		
			250	46.7		
	4	+	537	36.3	402.0	41.0
			267	45.6		
	4	-	107	39.2	130.0	35.0
			152	30.6		

For Week 2 N=4. For Week 4 N=2

Table 12

## Bioaccumulation of Added Benzanthracene

Exp	Organism nmol/gww	Organism nmol/gdw	AF	%Parent in Ext.	Overall %Parent	Parent Only nmol/gww
<i>Scolecopides viridis</i>						
BB	6.92	32.5	2.24	0.54	0.29	2.02
BB	6.79	31.9	2.20	0.72	0.41	2.80
LP	6.08	29.1	2.89	0.82	0.32	1.96
LP	5.48	25.7	2.55	0.83	0.49	2.69
SI	0.97	4.6	0.26	0.74	0.42	0.41
SI	2.29	10.8	0.61	0.86	0.64	1.48
FPC	2.97	14.0	1.16	0.95	0.35	1.05
FPC	1.79	8.4	0.70	0.93	0.40	0.71
<i>Mya arenaria</i>						
BB	0.193	1.427	0.062			
BB	0.146	1.084	0.047			
LP	0.189	1.398	0.088			
LP	0.272	2.012	0.126			
SI	0.178	1.316	0.047			
SI	0.153	1.131	0.040			
FPC	0.088	0.653	0.034			
FPC	0.096	0.710	0.037			

AF = (nmol/glw organism) / (nmol/goc sediment)

AF calculated assuming 2% lipid in organisms

Table 13

## Bioaccumulation of Added Phenanthrene

Exp	Organism nmol/gww	Organism nmol/gdw	AF	Fraction in Ext.	Overall % Parent	Parent Only nmol/gww
<i>Scolecopides viridis</i>						
BB	1.23	5.78	0.40	0.51	0.20	0.25
BB	1.48	6.93	0.48	0.55	0.14	0.20
LP	3.25	15.26	1.51	0.93	0.48	1.55
LP	5.14	24.12	2.39	0.85	0.34	1.75
SI	1.60	7.51	0.42	0.97	0.32	0.51
SI	1.97	9.27	0.52	0.98	0.60	1.18
FPC	1.81	8.52	0.71	0.84	0.29	0.52
FPC	1.44	6.75	0.56	0.86	0.31	0.44
<i>Mya arenaria</i>						
BB	0.09	0.65	0.028			
BB	0.06	0.42	0.018			
LP	0.20	1.45	0.091			
LP	0.22	1.63	0.103			
SI	0.26	1.96	0.070			
SI	0.31	2.30	0.082			
FPC	0.09	0.66	0.035			
FPC	0.09	0.68	0.036			

AF = (nmol/glw organism) / (nmol/goc sediment)

AF calculated assuming 2% lipid in organisms

## FIGURE LEGENDS

1. Experimental Chambers: Schematic diagram of an experimental chamber.
2. Experimental Sampling Scheme: Outline of how the experiment design and sampling plan was laid out.
3. Sampling Locations: Map showing locations of sediment and organism collections sites.
4. Sediment Oxygen Demand: Average sediment oxygen demand as measured by  $O_2$  flux to the water column for all experiments plotted against organic carbon content of the sediment.
5. Summary Redox Profile: Average redox profiles expressed as Eh as a function of sediment depth observed in each sediment type.
6. Redox Profiles - Buzzards Bay: Average redox profiles expressed as Eh as a function of sediment depth. Pre-animals refers to profiles observed before the addition of macrofauna (phase 1 and 2 of the experiment). /No animals refers to profiles observed during phase 3 of the experiment in chambers that did not receive macrofauna. /Animals refers to profiles observed during phase 3 of the experiment in chambers that did receive macrofauna.
7. Redox Profiles - Little Pond: Average redox profiles expressed as Eh as a function of sediment depth. Pre-animals refers to profiles observed before the addition of macrofauna (phase 1 and 2 of the experiment). /No animals refers to profiles observed during phase 3 of the experiment in chambers that did not receive macrofauna. /Animals refers to profiles observed during phase 3 of the experiment in chambers that did receive macrofauna.
8. Redox Profiles - Spectacle Island: Average redox profiles expressed as Eh as a function of sediment depth. Pre-animals refers to profiles observed before the addition of macrofauna (phase 1 and 2 of the experiment). /No animals refers to profiles observed during phase 3 of the experiment in chambers that did not receive macrofauna. /Animals refers to profiles observed during phase 3 of the experiment in chambers that did receive macrofauna.
9. Redox Profiles - Fort Point Channel: Average redox profiles expressed as Eh as a function of sediment depth. Pre-animals refers to profiles observed before the addition of macrofauna (phase 1 and 2 of the experiment). /No animals refers to profiles observed during phase 3 of the experiment in chambers that did not receive macrofauna. /Animals refers to profiles observed during phase 3 of the experiment in chambers that did receive macrofauna.
10. Sulfate Reduction Rates: Average sulfate reduction rates measured in sediment cores expressed as  $\mu\text{mol S}/\text{cm}^2\text{-day}$  for all experiments expressed as a function of sediment organic carbon content.

11. Porewater Ammonia: Average profiles of total ammonia expressed as  $\mu\text{mol}/\text{cm}^3$  as a function of depth for each sediment type.

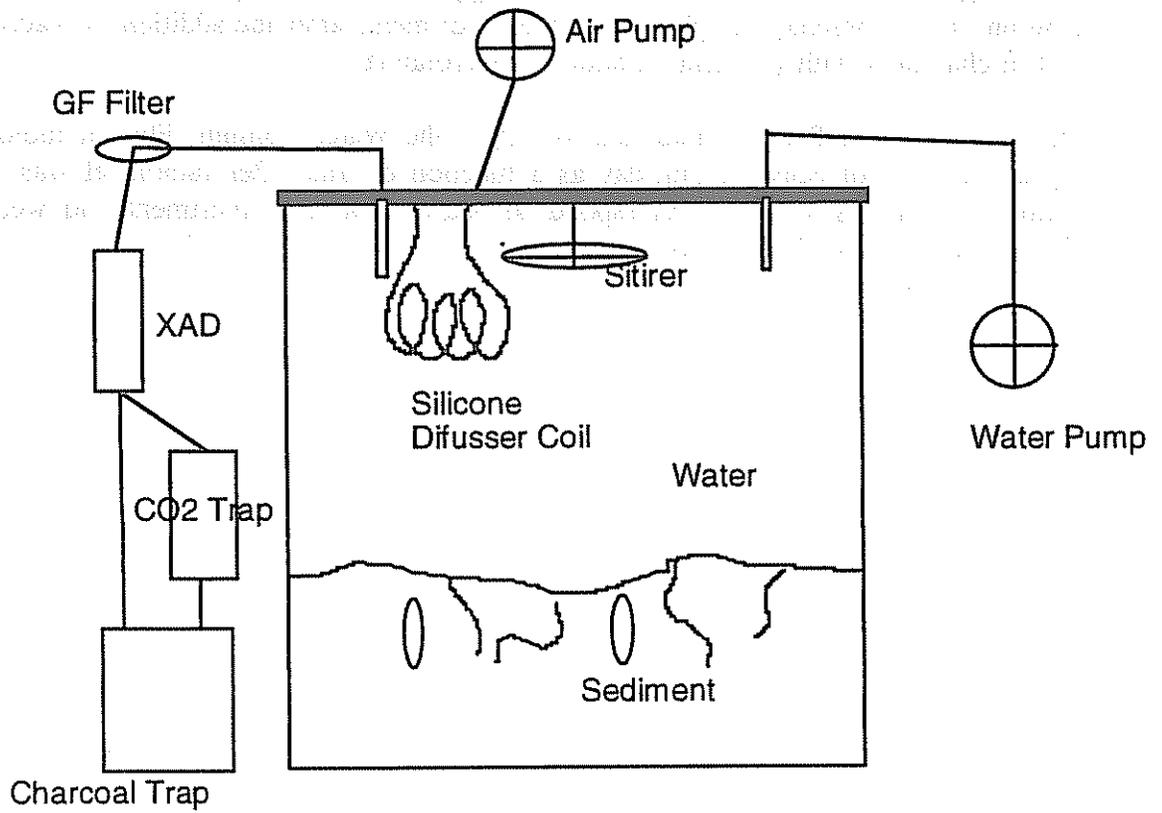
12. Porewater Phosphate: Average profiles of total phosphate expressed as  $\mu\text{mol}/\text{cm}^3$  as a function of depth for each sediment type.

13. Flux of Added PAH to the Water Column Collected on XAD: Flux of added PAH expressed as  $\text{nmol}/\text{m}^2\text{-day}$  during phase 2 of the experiment (Wks 1-2), before the addition of macrofauna, and phase 3 of the experiment, after the addition of macrofauna, (Wks 2-3) in chambers with (+) and without (-) macrofauna.

14. Percentage of Material Collected on XAD Remaining as Unmetabolized Benzanthracene or Phenanthrene: The percentage of material in the water column collected on the XAD resin remaining as unmetabolized parent PAH during phase 2 of the experiment (Wks 1-2), before the addition of macrofauna, and phase 3 of the experiment, after the addition of macrofauna, (Wks 2-3) in chambers with (+) and without (-) macrofauna.

15. Flux of Added PAH Mineralized to  $\text{CO}_2$  to the Water Column: Flux of radiolabeled  $\text{CO}_2$  expressed as  $\text{nmol converted}/\text{m}^2\text{-day}$  as a function of time after radiolabel was added to the chambers. Weeks 1 and 2 correspond to phase 2 of the experiment and weeks 3 and 4 correspond to phase 3 of the experiment.

Figure 1



**Figure 2**

**Experimental Sampling Scheme**

**Phase 1 - Add sediment to chamber and equilibrate, N=8**

Week 1:     A. Sediment O<sub>2</sub> consumption  
              B. Water column nutrients

Week 2:     Repeat A-B and  
              C. Sediment redox profiles  
              D. Porewater nutrients  
              E. Sediment sulfate reduction

**Phase 2 - Add radiolabeled sediment to surface, N=4**

Week 3:     Repeat A-B and  
              F. Water column <sup>14</sup>CO<sub>2</sub>

Week 4:     Repeat A-F and  
              G. Water column <sup>14</sup>C (total and % parent)  
              H. Sediment <sup>14</sup>C (total and % parent)

**Phase 3 - Add macrofauna to half of the chambers, N=2**

Week 5:     Repeat A-B and F

Week 6:     Repeat A-H and  
              I. Macrofauna <sup>14</sup>C (total and % parent)

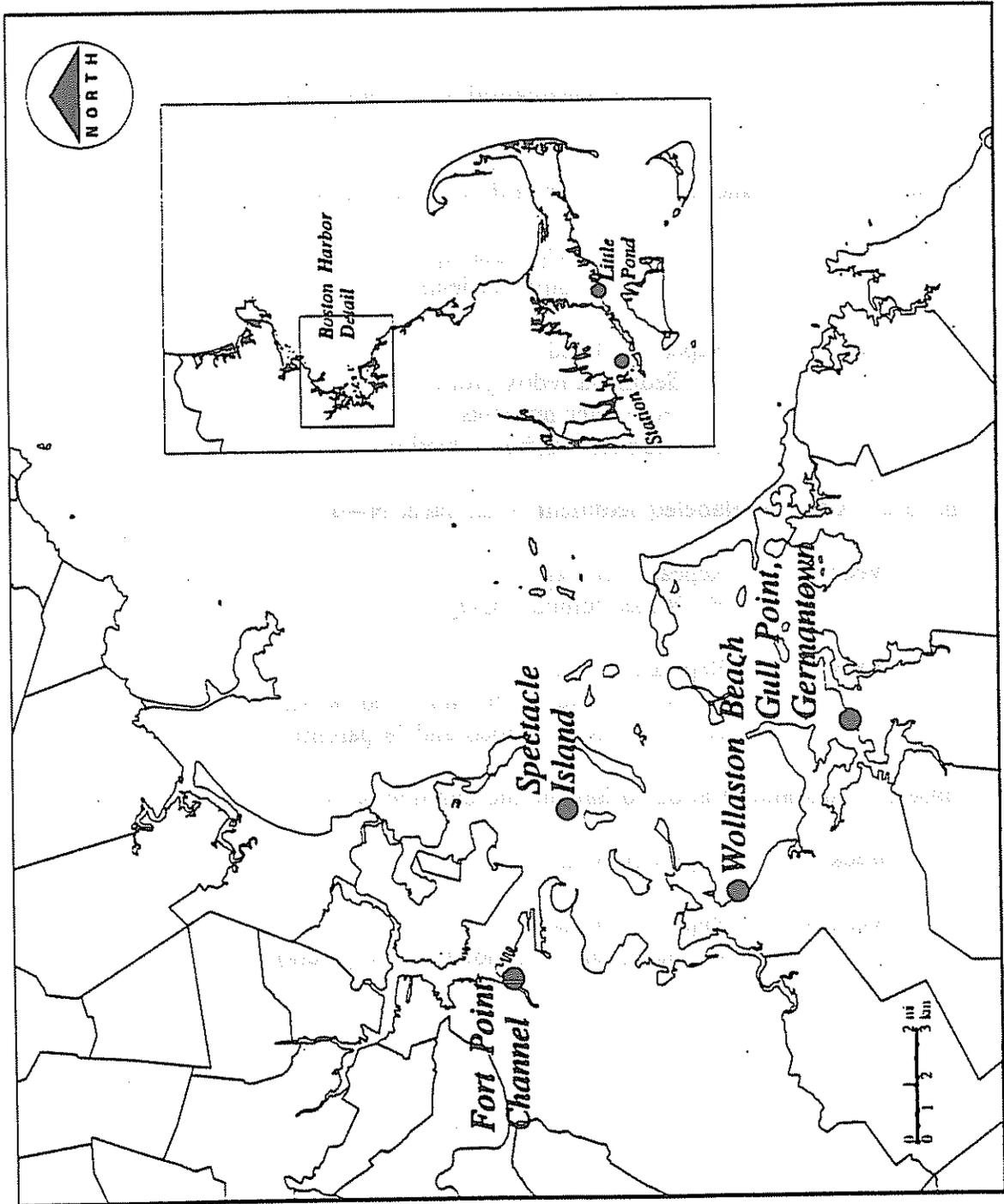
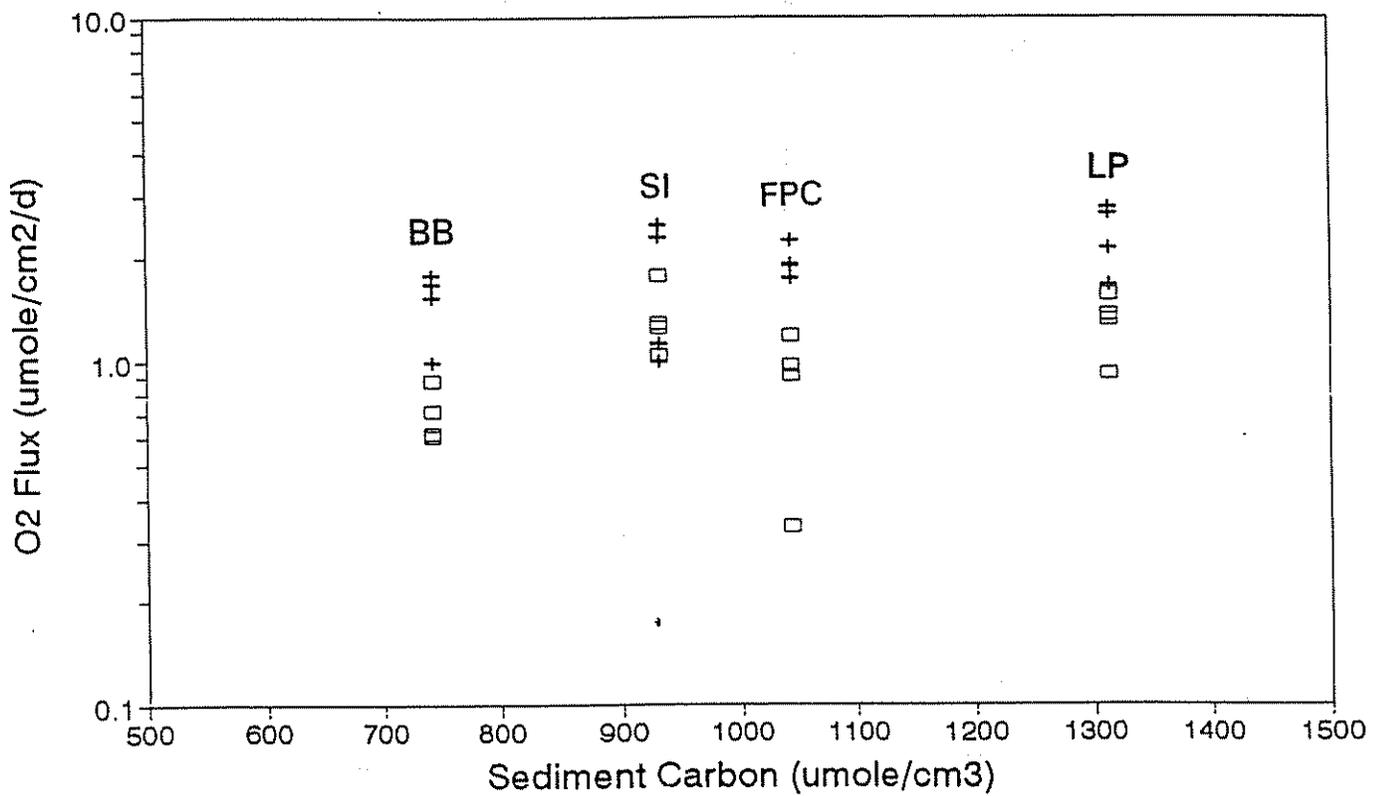
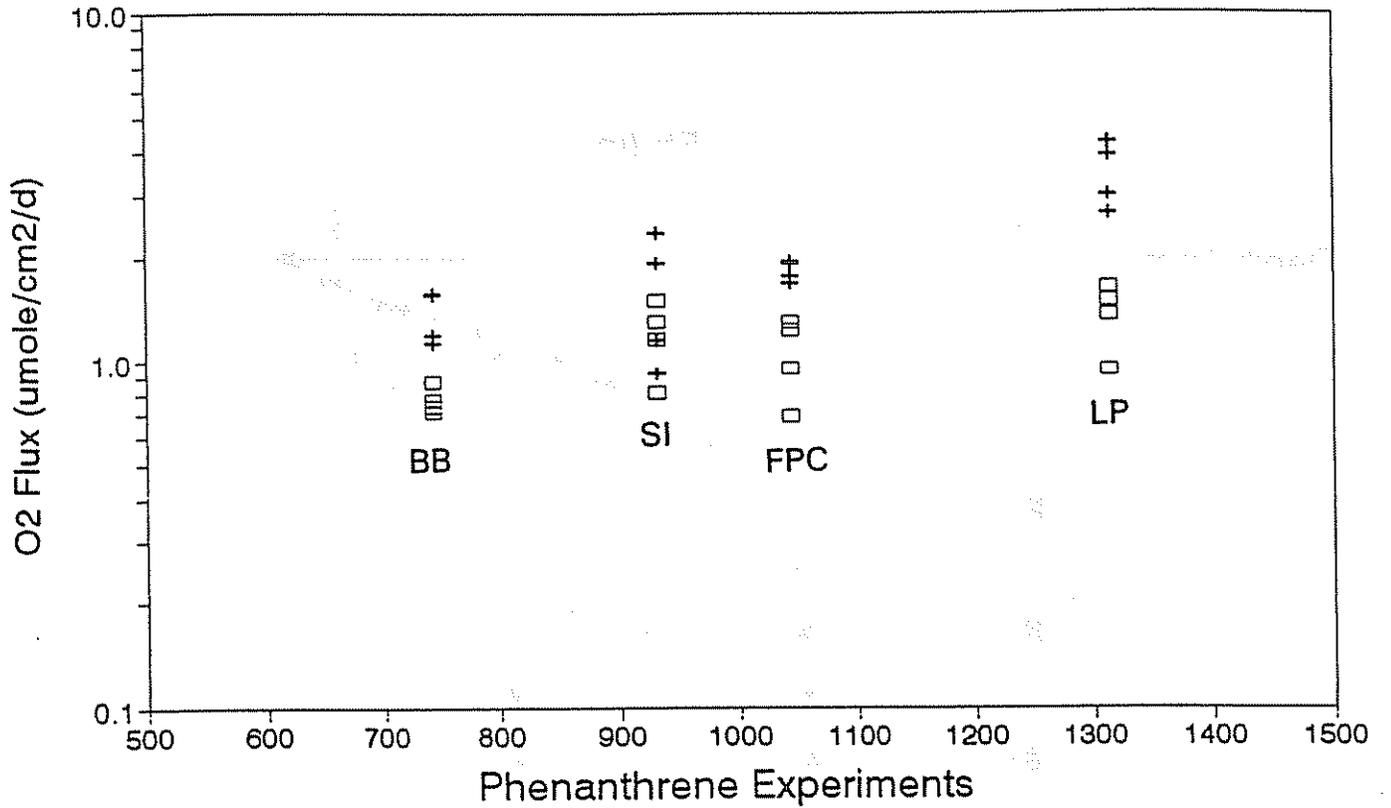


Figure 3. Location of Sample Sites

Figure 4

# Sediment Oxygen Demand Benzanthracene Experiments



□ No Animals + Animals

Figure 5

# Summary of REDOX Profiles All Experiments

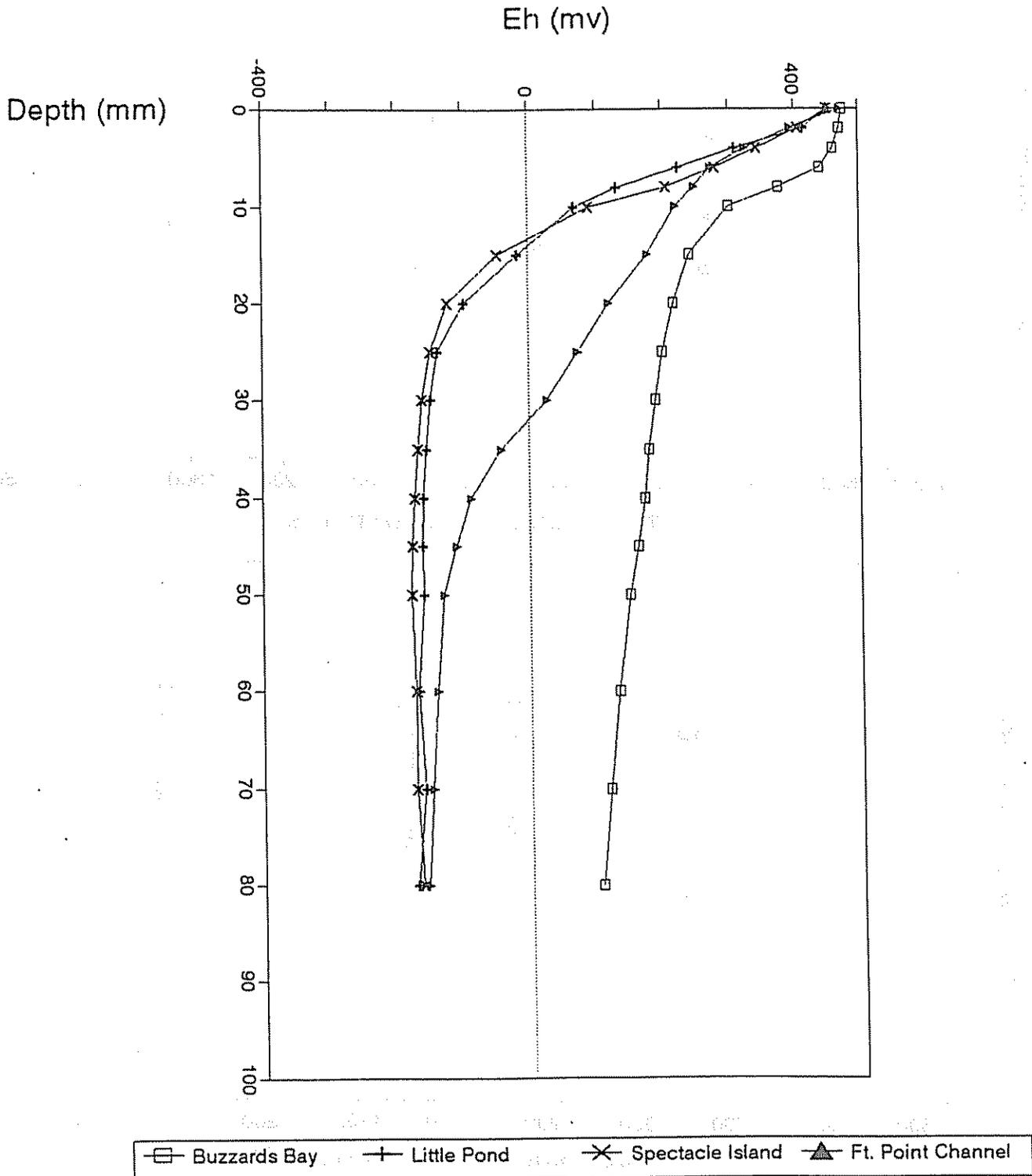


Figure 6

# REDOX Profiles Buzzards Bay

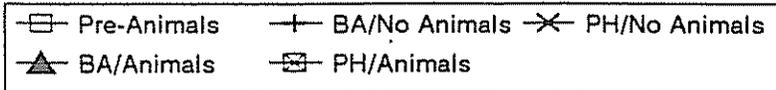
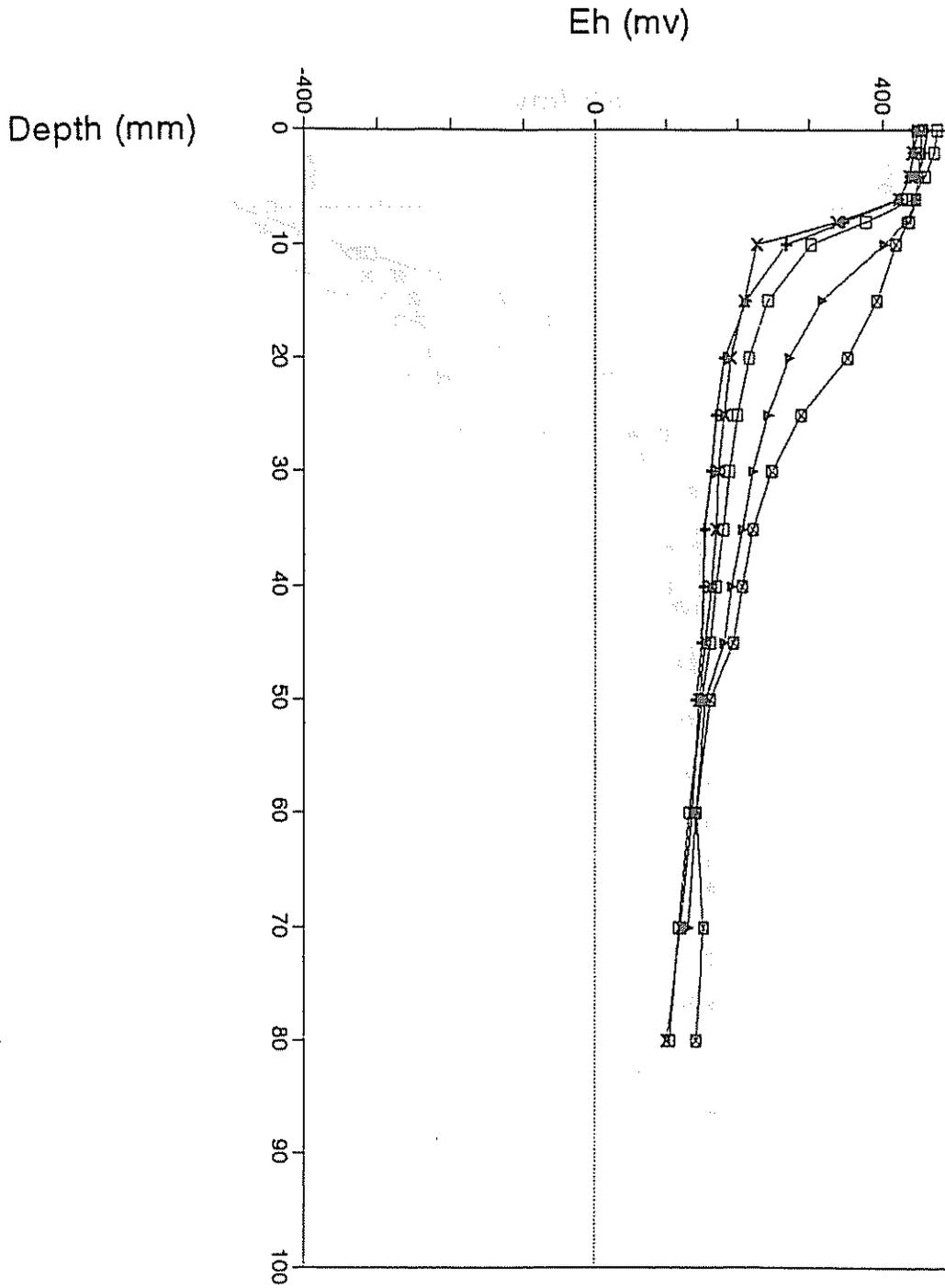


Figure 7

# REDOX Profiles Little Pond

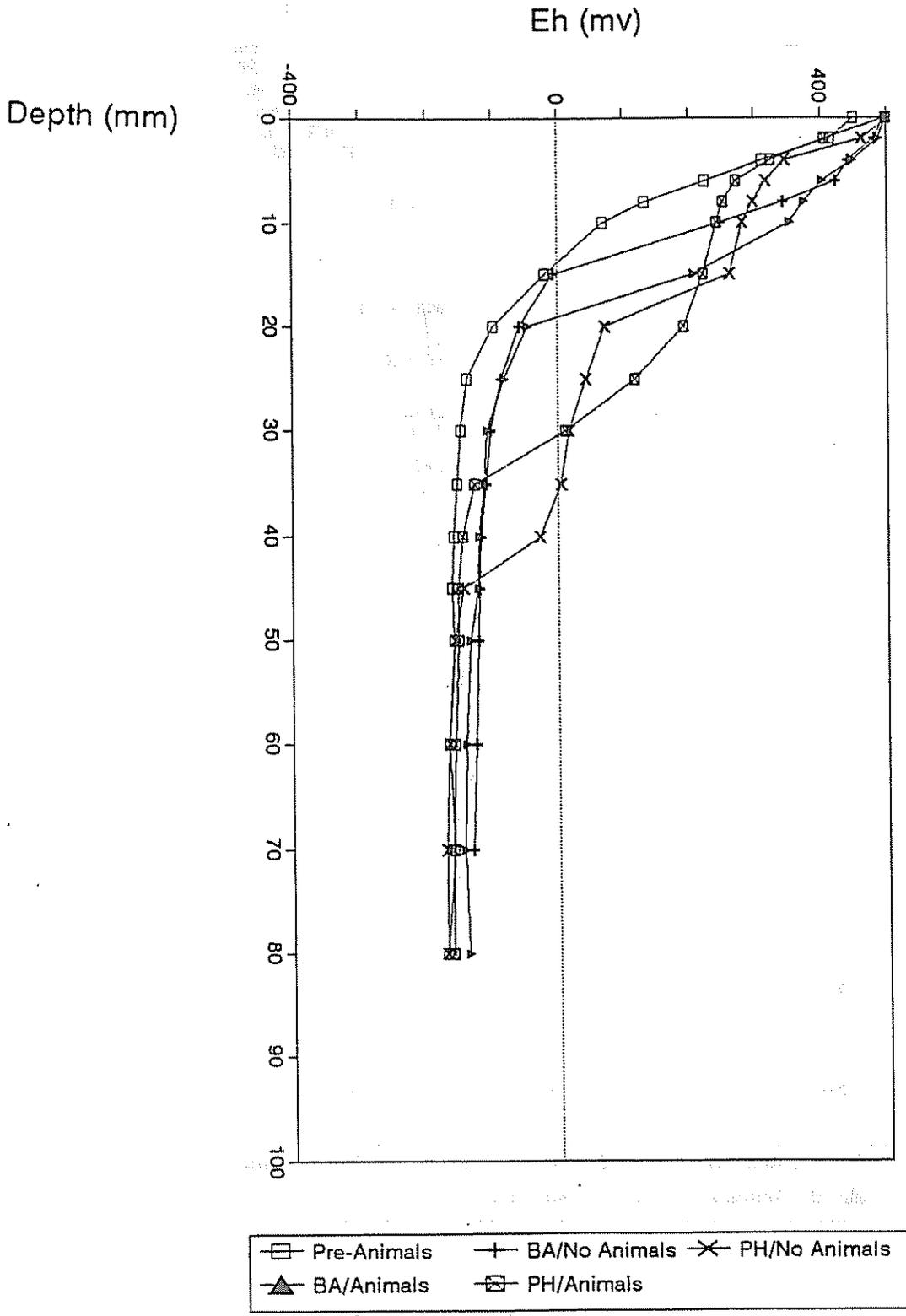


Figure 8

# REDOX Profiles Spectacle Island

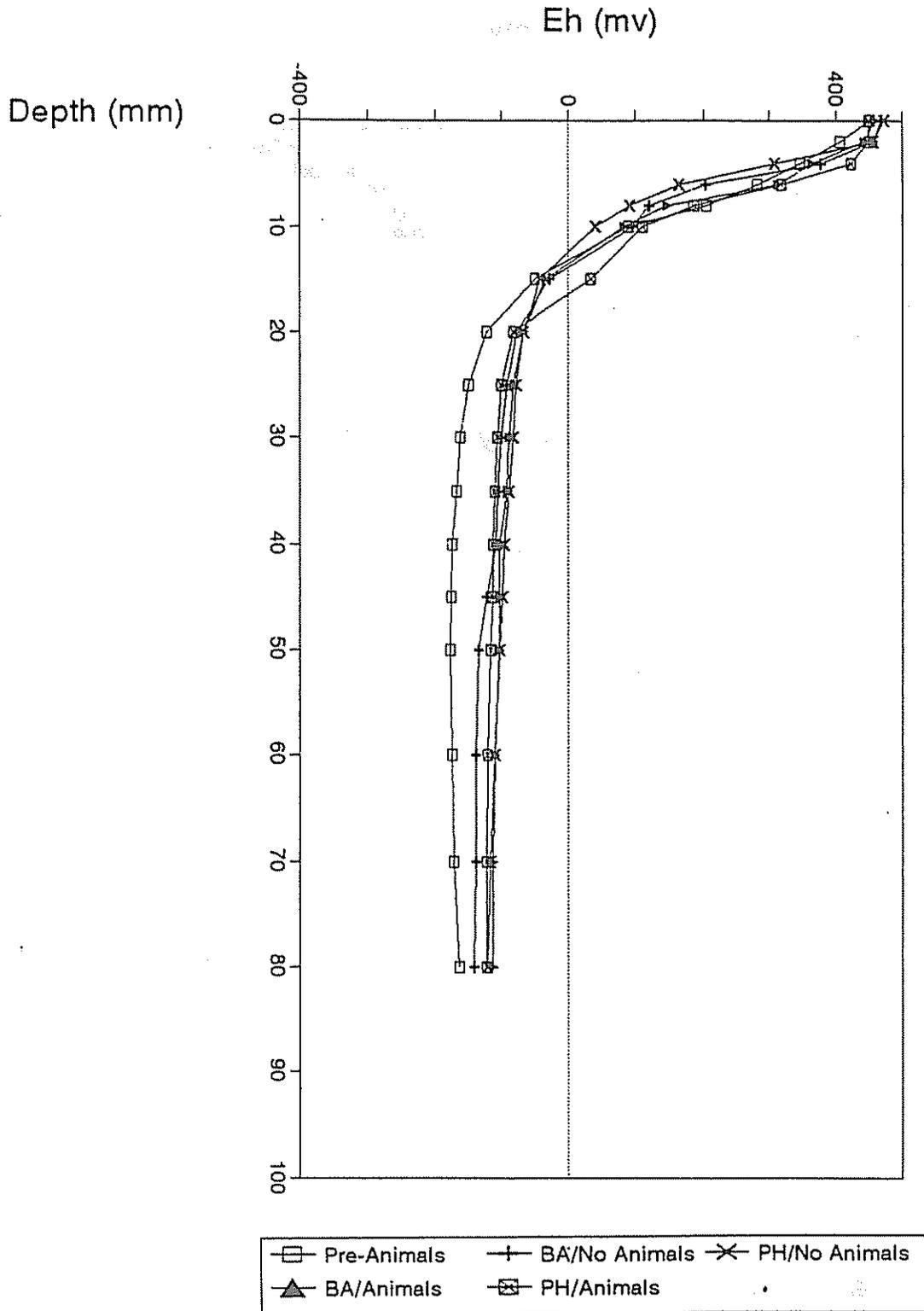


Figure 9

# REDOX Profiles Ft. Point Channel

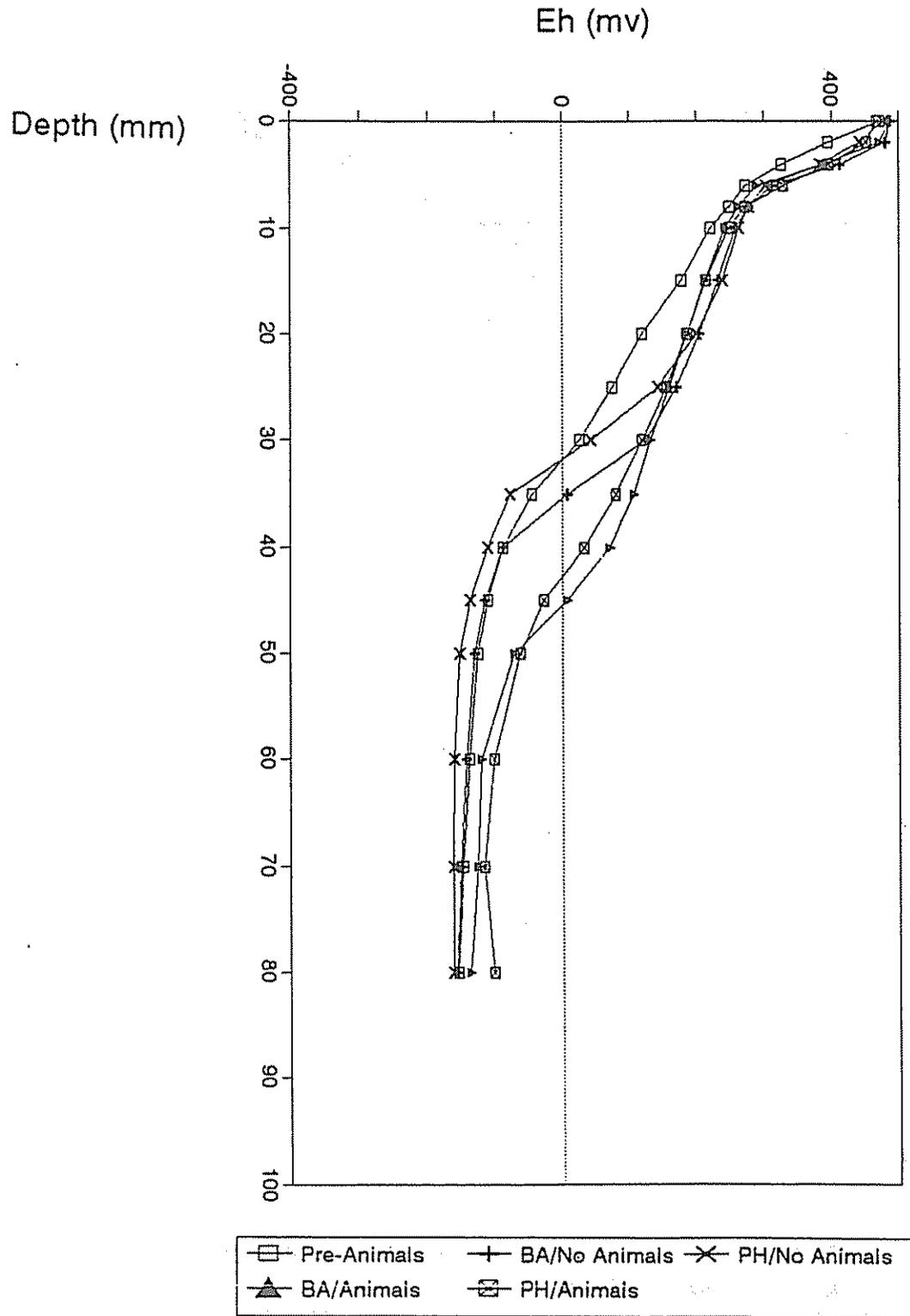
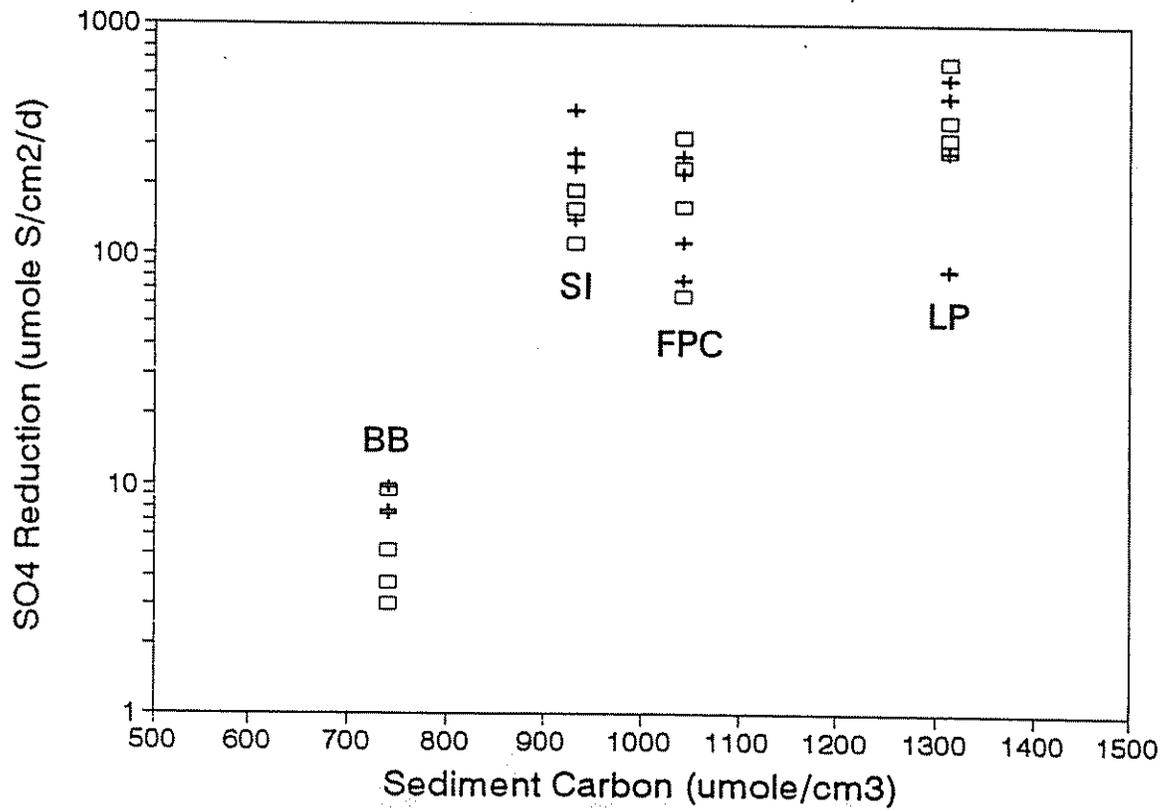
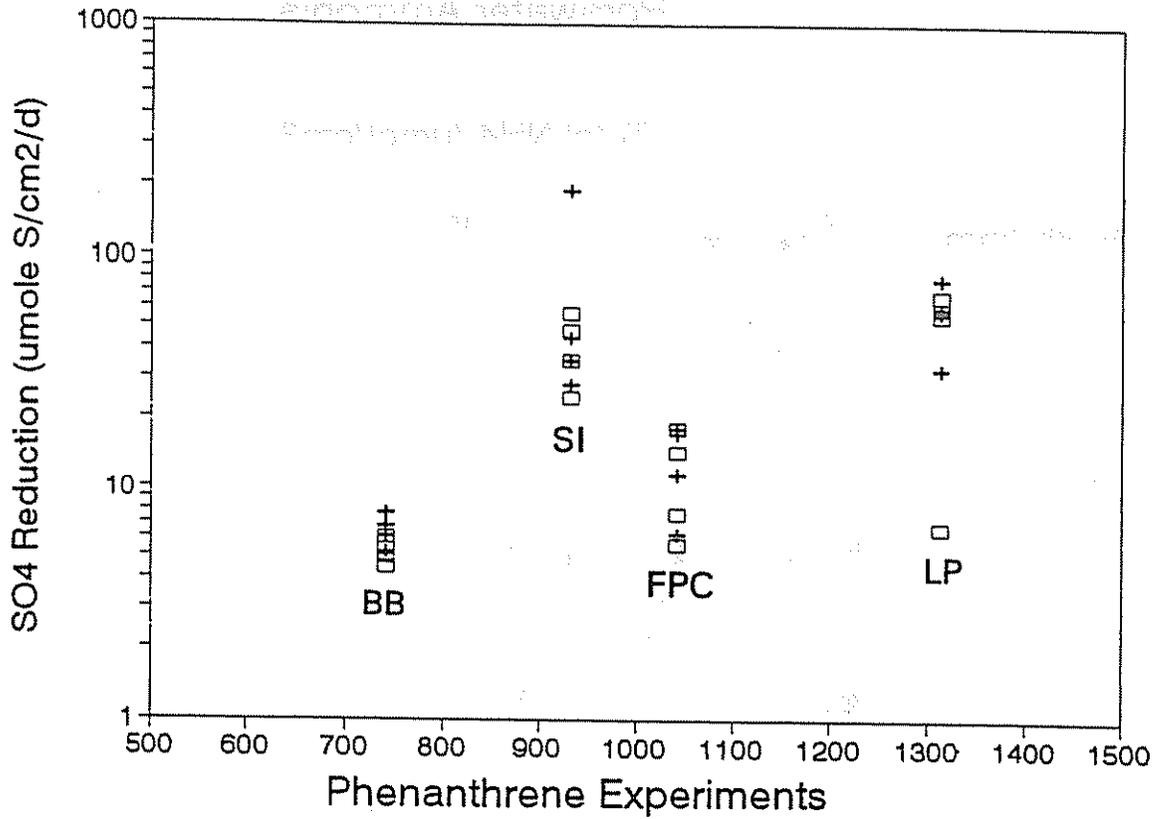


Figure 10

# Sulfate Reduction Rates Benzenanthracene Experiments



□ No Animals    + With Animals

Figure 11

# Comparison of Sediment Sources Porewater Ammonia

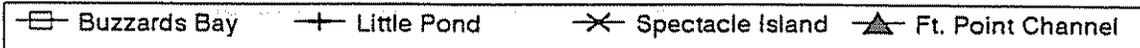
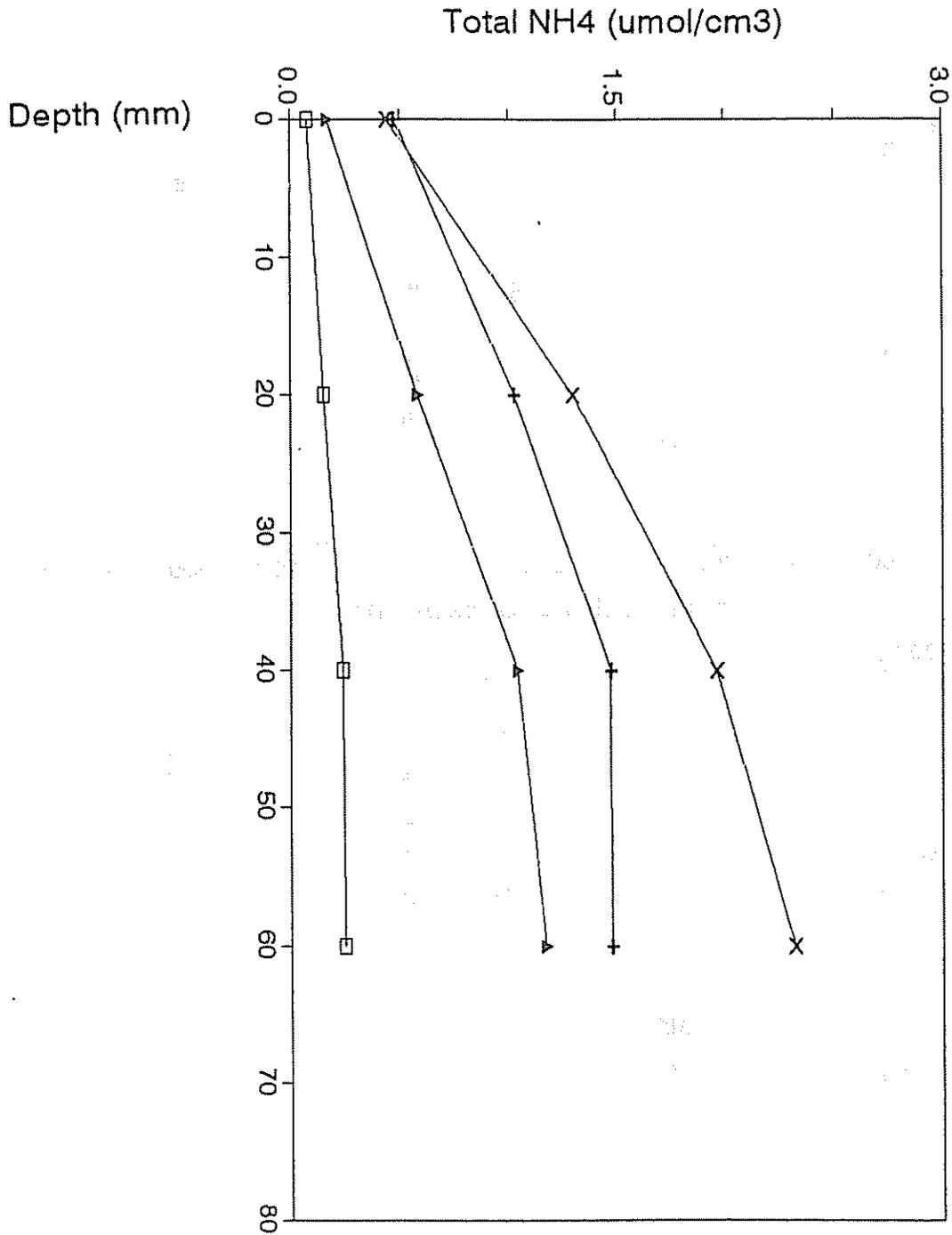


Figure 12

# Comparison of Sediment Sources Porewater Phosphate

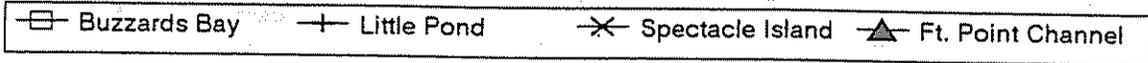
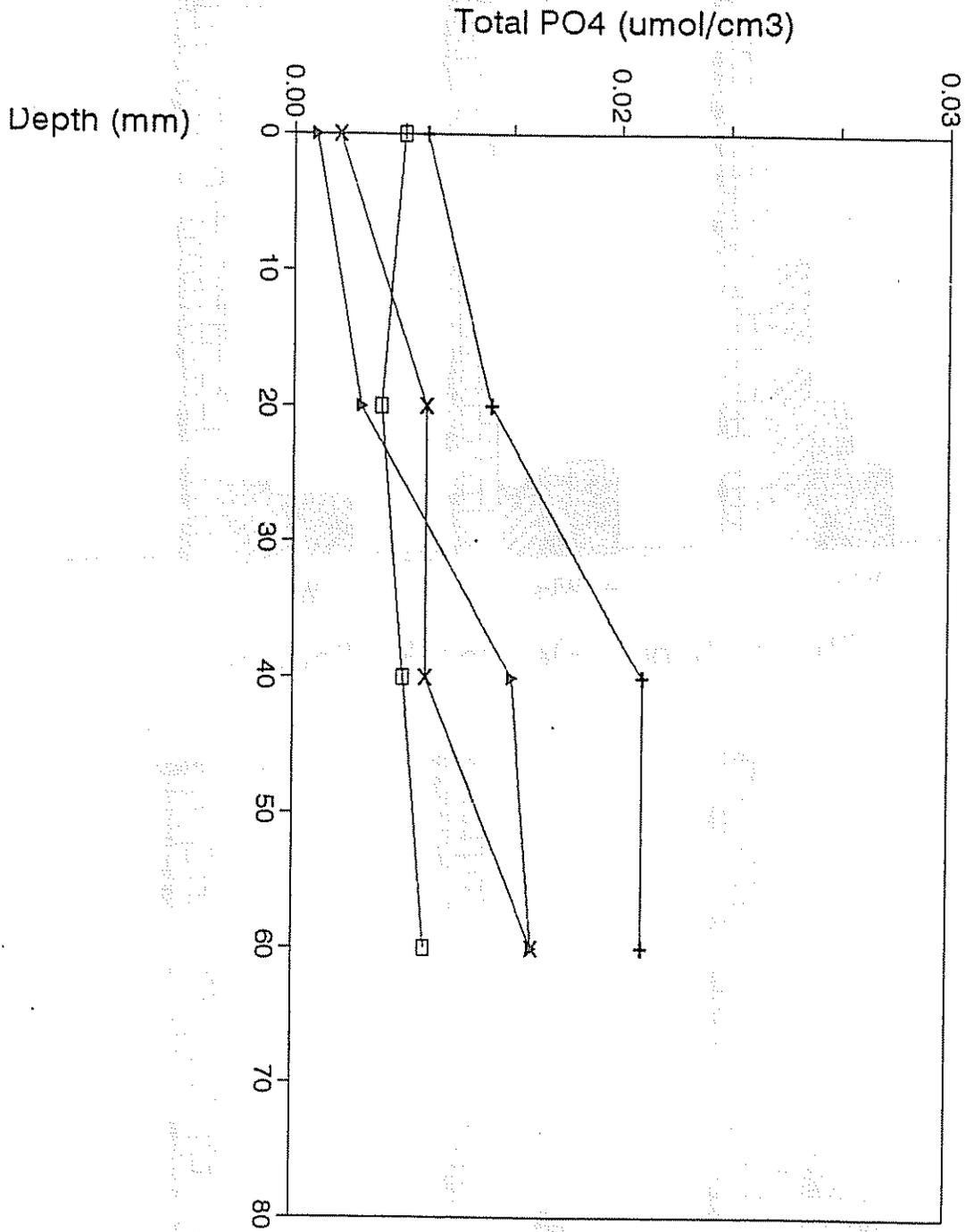
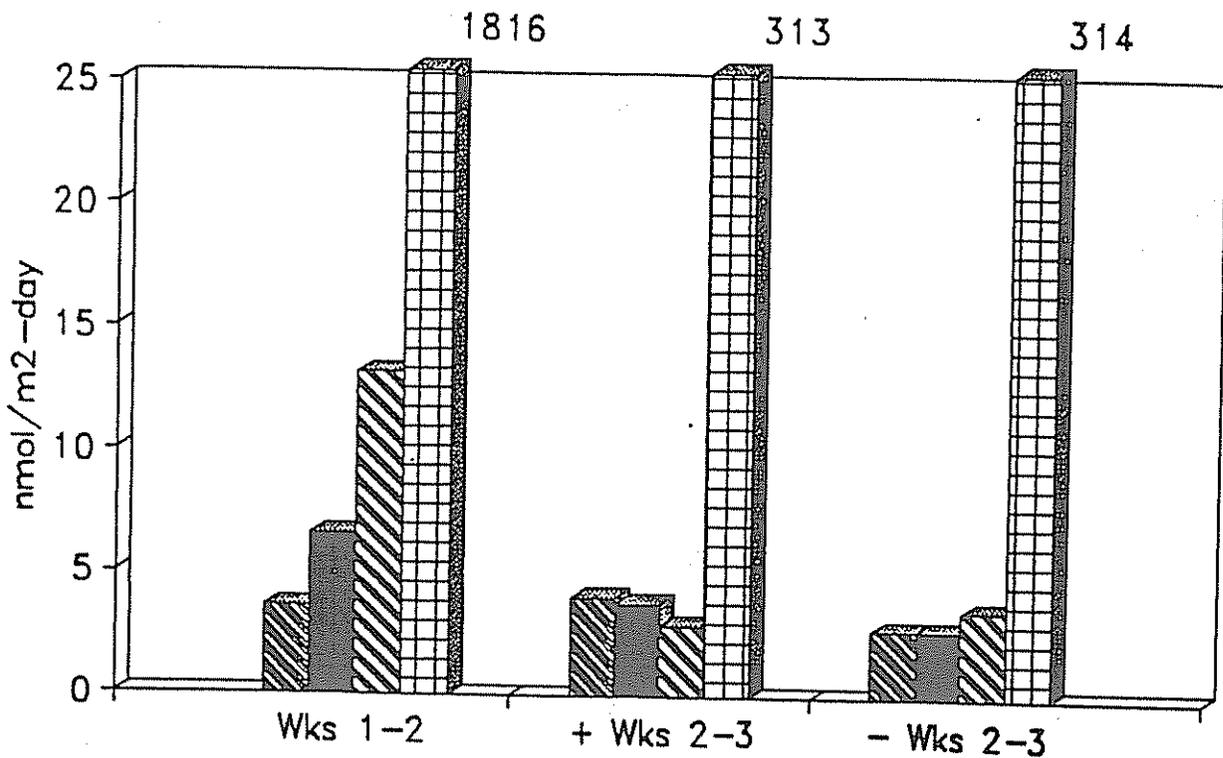
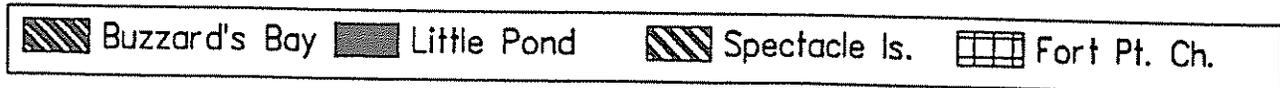
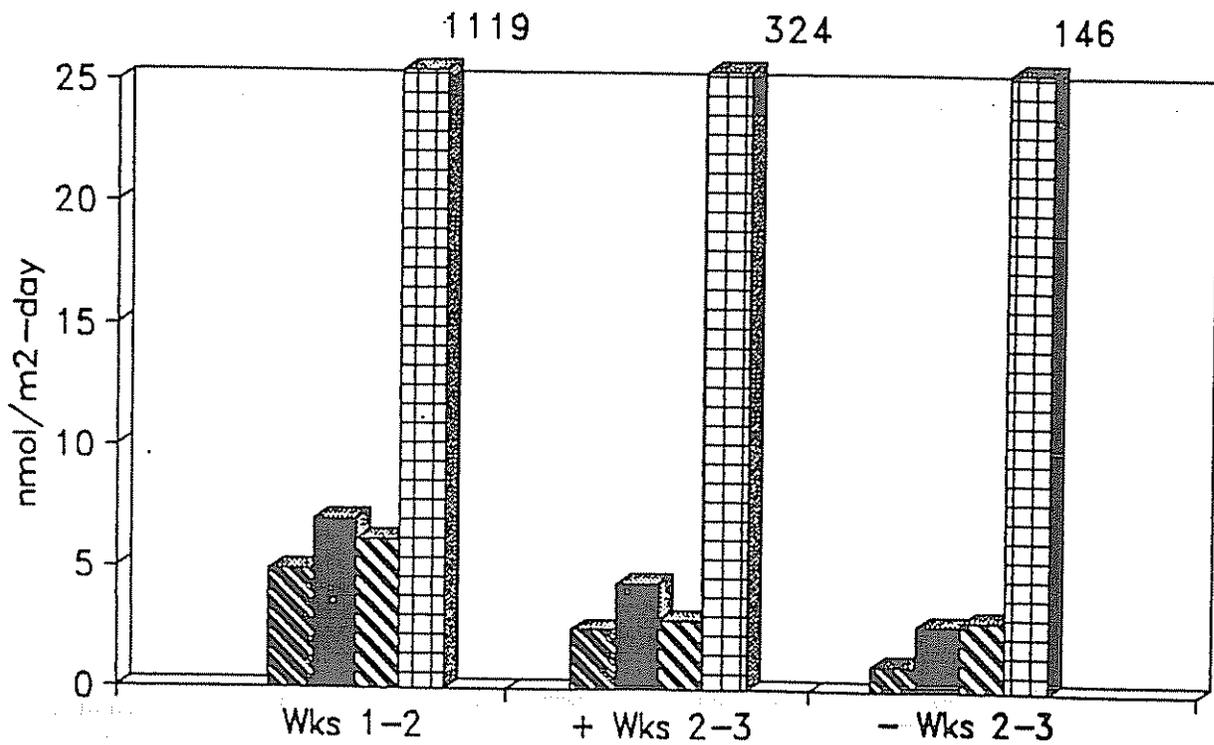


Figure 13

# Flux from Sediment Benanthracene-Derived Radioactivity

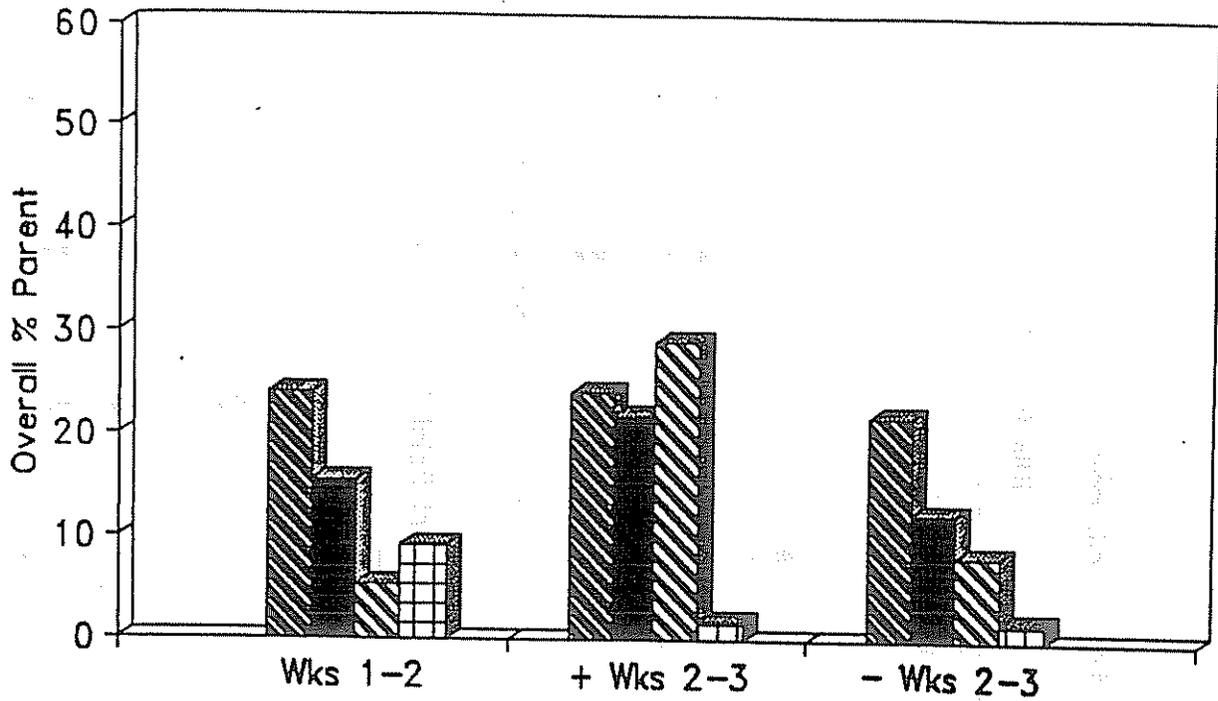


# Phenanthrene-Derived Radioactivity

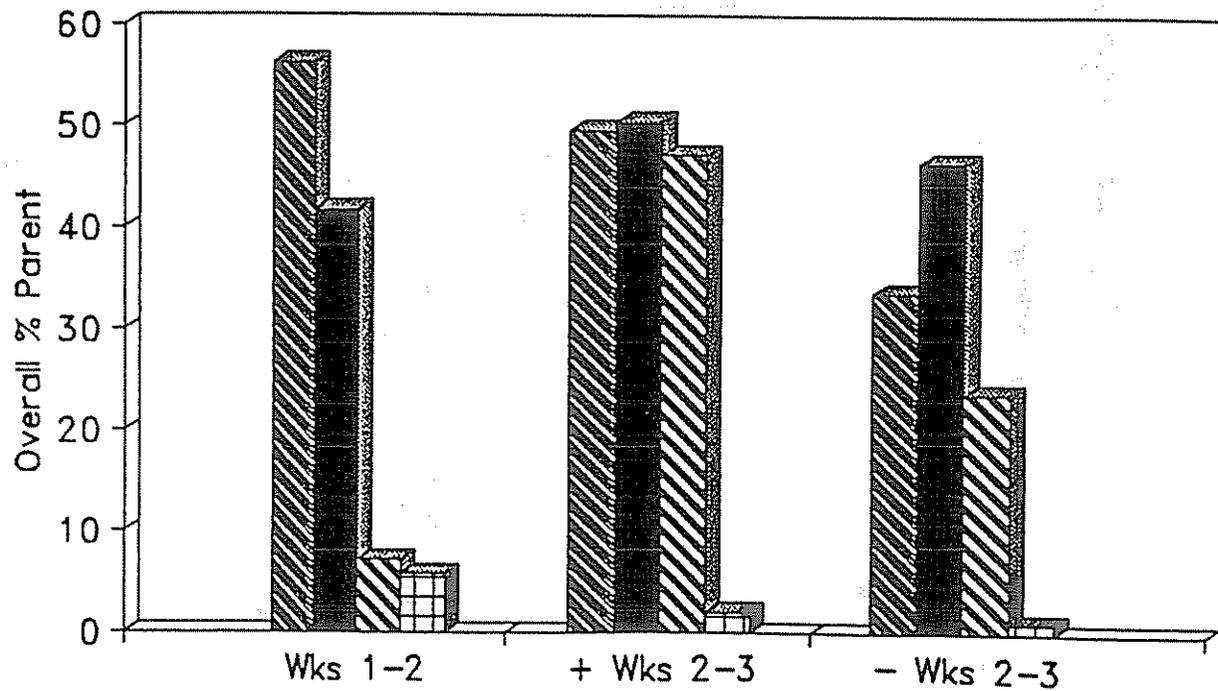


# Flux from Sediment

## Percent Remaining as Benzanthracene



## Percent Remaining as Phenanthrene



Buzzard's Bay
  Little Pond
  Spectacle Is.
  Fort Pt. Ch.

# Flux of $^{14}\text{CO}_2$ to Water Column

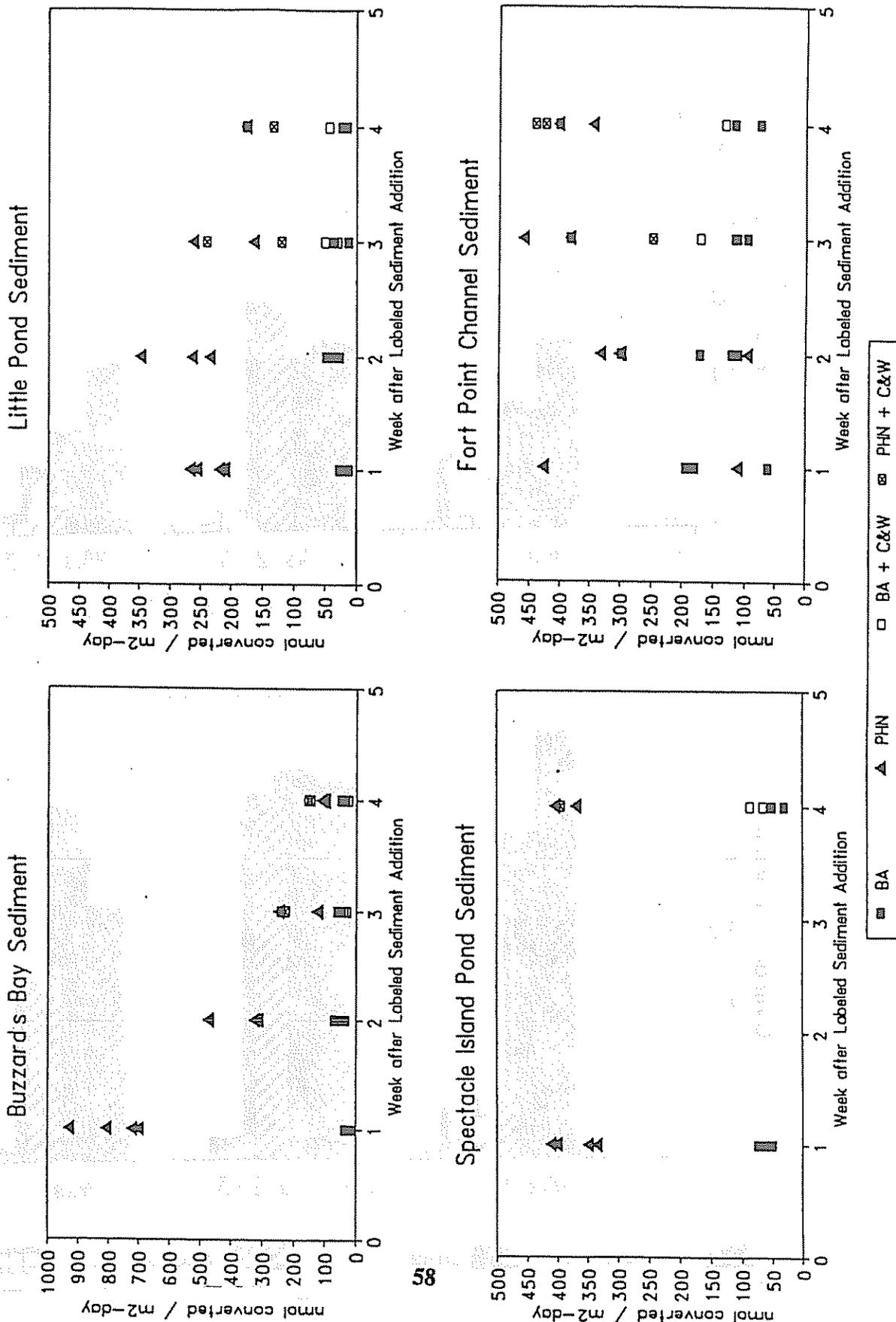


Figure 15