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Effects of Phenanthrene on Primary Production of Phytoplankton in Two New Jersey Estuaries

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Contamination with petroleum hydrocarbons such as polycyclic aromatic hydrocarbons (PAH), both as acute oil spills and as more chronic contamination from oil byproducts, fossil fuel combustion, aerosols and surface runoff, are an increasingly common threat to the health of coastal waters. PAHs have been found to be toxic to many organisms. PAHs are common in the environment, particularly in heavily industrialized areas. They are poorly soluble in water and therefore, tend to adsorb to organic matter and are taken up and accumulated in phytoplankton and other organisms.

A number of studies have been conducted to evaluate the effects of petroleum hydrocarbons on primary production (Carman *et al.* 1997; McConkey *et al.* 1997; Siron *et al.* 1993; Riznyk *et al.* 1987; Bate and Crafford 1985; Harrison *et al.* 1986). Effects seem to vary depending on the composition of the algal community studied, the geographic region, the time frame of the experiment, and the contaminant chosen. Some of these studies have indicated a toxic effect of hydrocarbons, whereas others have indicated an enhancement of primary production. Few studies have looked at the effects of individual PAHs on natural phytoplankton communities.

The present experiments were performed using natural phytoplankton assemblages and a single PAH contaminant, phenanthrene. Phenanthrene was chosen because it is commonly found in petroleum products and is one of the PAHs listed on the EPA's Priority Pollutant list. It is present at one of our study sites (Piles Creek) at water concentrations of 80 to 800 ng/L *(L.R. McGuinness, unpublished data).*

The study was conducted at two sites in New Jersey: Piles Creek and the Mullica River. The goal of this study was to examine the effects of phenanthrene on natural phytoplankton assemblages from two contrasting sites, one contaminated (Piles Creek), one pristine (the Mullica River). It has been shown that microbial populations can become acclimated to PAHs (Leahy and Colwell 1990). We therefore hypothesized that phenanthrene would be less toxic to phytoplankton collected from Piles Creek than to phytoplankton collected from the Mullica

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River. Previous studies have attributed an apparent stimulatory effect of petroleum hydrocarbons on primary production to inhibitory effects on predators (Carman *et al.* 1997; Oviatt *et al.* 1982). In this study we attempted to isolate the phytoplankton from large predators as well as minimize bacterial interactions. We used the rate of primary production as an indicator of the effects of phenanthrene on the natural phytoplankton communities under laboratory conditions.

MATERIALS AND METHODS

The laboratory experiments were performed using water obtained from two estuaries: Piles Creek and the Mullica River. Piles Creek is a tributary of the Arthur Kill located in Linden, NJ, and represented a site contaminated with high levels of PAHs. The Mullica River, an estuary located in southeastern NJ, whose watershed lies within protected federal and state land, represented a relatively pristine site. Samples were obtained on flooding tides near high tide between June and August 1995. The temperature, salinity, and pH of water collected at the study sites during the experimental period are presented in Table 1.

Table 1. Field conditions and biomass specific gross primary productivity (see text for calculations) based on control treatments only.

	pH	Temp (°C)	Salinity (ppt)	Chl *a* (mg/m^3)	Biomass specific production (µmoles O_2/mg chl *a*/hr)
Experiment 1					
Piles Creek	7.38	20	22	24.37	95.61
Mullica River (18 hr)	7.09	19	22	6.29	187.60
Experiment 2					
Piles Creek		26	27	29.10	54.30
Mullica River		25	22	1.70	132.94

Bucket samples were drawn from surface waters and combined in 20-L carboys. The collection of water and travel to the laboratory required approximately 2-3 hr and experiments were begun immediately upon return to the laboratory.

Water from each site was filtered through a 114 pm nylon mesh (Nitex™) to remove large zooplankton. This procedure did not substantially alter the concentration of phytoplankton, or presumably, the small heterotrophs. The filtrate was then apportioned into 300 mL Biological Oxygen Demand (BOD) bottles. Half of these bottles were covered in black tape and aluminum foil (dark bottles). The remaining bottles were left uncovered (light bottles).

Phenanthrene (98% purity, Aldrich) in acetone was made up in stock solutions such that the additions of 20 µL to an incubation bottle produced final

concentrations of 0, 0.1, 1.0, and 10 ppm (mg/L). The bottles comprised the following treatments: initial (no acetone or phenanthrene amendment, fixed upon completion of all additions), control (no amendment), acetone control (20 µL carrier solvent only), and 0.1, 1.0, and 10 ppm (final concentration) phenanthrene amendments. Bottles were incubated under constant light at 20°C. Incubations were performed in duplicate or triplicate. Chlorophyll a content (phytoplankton biomass) was determined on initial water samples according to Parsons et al. (1984) with a fluorometer (Turner Designs, Sunnyvale, CA, model 10-AU) calibrated by spectrophotometric analysis of chlorophyll a standards (Sigma, St. Louis, MO)

After incubation, the bottles were fixed with Winkler reagents and analysis was performed by automated Winkler titration (Friederich *et al.* 1991). Photosynthesis and respiration rates were calculated using the differences in oxygen concentrations between final and initial samples, divided by the incubation time. These differences ranged from 0 to 46 µ*M*. Two experiments were conducted.

Experiment 1: This experiment was conducted in order to determine whether the addition of phenanthrene to natural waters induced a change in phytoplankton photosynthesis or microbial respiration. Water samples from each site were incubated under fluorescent lights (29-50 einst/m^2/d, in a 20°C controlled temperature room. Light intensities were measured using a Li-Cor LI- 1000 DataLogger and SPQA 1456 Spherical Sensor.

Dissolved oxygen was measured initially and after incubation times of 16.5-18 hr for Piles Creek water and 18 and 36 hr for Mullica River water. Incubation times began upon addition of phenanthrene and ended upon fixation of the bottles.

Experiment 2: The second experiment was designed to study the effects of phenanthrene on the phytoplankton communities themselves by minimizing the initial biomass of the microheterotrophs as well as to remove confounding effects of different types and amounts of dissolved substances that could react with the phenanthrene. Large zooplankton were removed as described above. An inoculum of each natural phytoplankton community was prepared by concentrating 10 L of the 114 µm-filtered estuarine water on a 10 µm Nitex screen. Final volumes of the Piles Creek and Mullica River water concentrates were 400 mL and 450 mL, respectively. Phytoplankton communities collected from both study sites were incubated in uncontaminated seawater obtained from the Institute of Marine and Coastal Sciences (IMCS). IMCS obtains seawater at high tide off the coast of Cape May, New Jersey, holds it in a settlement tank for a minimum of one week and passes it through a sand filter. Previous experimentation indicated that phytoplankton from the two field sites would survive and grow in this experimental system. The seawater (0.2 pm filtered) was adjusted to the salinity of each study site with deionized water and to a final volume of approximately 10 L.

Treatments were prepared as previously described for Experiment 1, in triplicate. Incubations were conducted in a 20°C room under 29-50 einst/m^2/d fluorescent lighting for 24 to 26 hr.

RESULTS AND DISCUSSION

The phenanthrene concentrations used in these experiments were much higher than would be encountered in the environment (the 10 ppm concentration is greater than the solubility concentration). In experiment 1, net photosynthesis (oxygen production in light bottles) in Piles Creek water was significantly enhanced in the 0.1 and 1.0 ppm phenanthrene treatments relative to acetone controls ($p<0.01$ and $p<0.05$, respectively; Fig. 1A), based upon two-way ANOVA and Tukey-Kramer multiple comparison tests (Sokal and Rohlf 1981; Gagnon *et al.* 1989). The greatest rate of photosynthesis occurred in the 0.1 ppm phenanthrene addition (more than twice that of the control treatments). The photosynthetic rates were comparable between the control and acetone control treatments, indicating no significant effect of the acetone carrier. Respiration rates were similar in all treatments.

The highest photosynthetic rate in the Piles Creek water was approximately three times greater than in Mullica River water (Figs. 1A,B). Initial chlorophyll concentrations were 3-4 times higher in Piles Creek water than in Mullica River water, while the biomass specific productivity rate [gross photosynthesis (0, produced in light bottles plus O_2 consumed in dark bottles) per unit chlorophyll *a* per hr] of the Mullica River was twice that of Piles Creek (Table 1). In Mullica River treatments, photosynthesis at the end of 18 hr was greater in each of the PAH additions than in the either of the control treatments (Fig. 1B), with the greatest enhancement (more than 3-fold) in the 0.1 ppm treatment ($p<0.01$). By the end of 36 hr, photosynthesis in Mullica River water was further enhanced in the two highest phenanthrene concentrations with the greatest increase seen in the 1.0 ppm treatment (Fig. 1C). By 36 hr the respiration rates in all of the treatments had decreased by about 30% from what they had been at 18 hr, and the 0.1 and 1.0 ppm phenanthrene treatments had respiration rates significantly lower than the acetone control.

The gross Photosynthesis/Respiration (P/R) ratios of the Piles Creek water were all >2 (>3 for 0.1 and 1.0 ppm phenanthrene treatments), indicating greater rates of oxygen production than consumption. In the Mullica River water at the end of 18 hr the P/R ratios of the controls were about 1, while the P/R ratios of all the phenanthrene treatments were approximately 1.6. By 36 hr the P/R ratios of controls were still about 1, while the phenanthrene treatments had all increased to 2 or greater.

In experiment 2, photosynthesis was much greater in the Piles Creek water inoculum than in the Mullica River water. There was a much larger quantity of

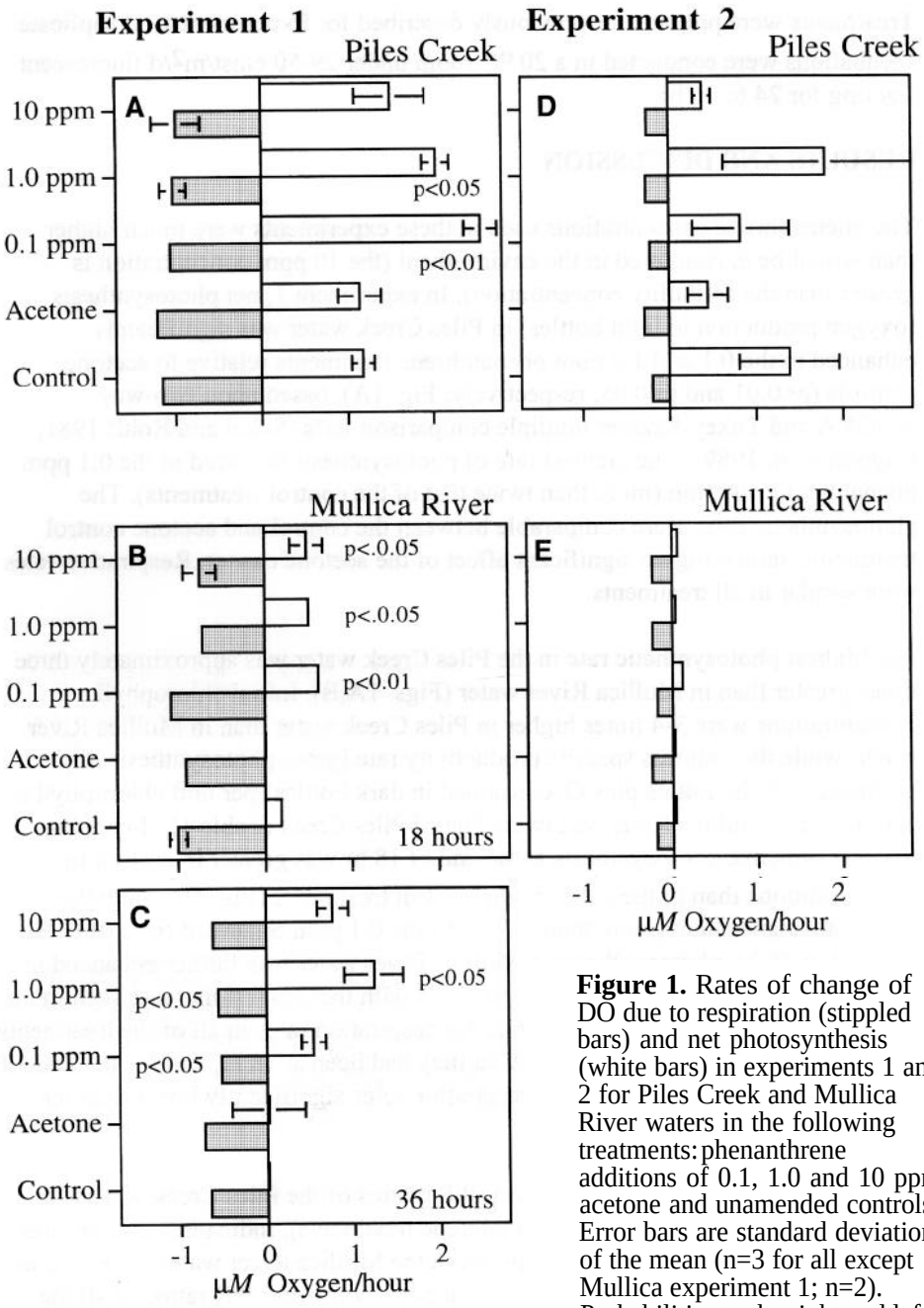

Figure 1. Rates of change of DO due to respiration (stippled bars) and net photosynthesis (white bars) in experiments 1 and 2 for Piles Creek and Mullica River waters in the following treatments: phenanthrene additions of 0.1, 1.0 and 10 ppm, acetone and unamended controls. Error bars are standard deviations of the mean (n=3 for all except Mullica experiment 1; n=2). Probabilities to the right and left of bars refer to differences from acetone controls. Unless specifically designated, differences were not significant. Error bars are indicated on all diagrams.

phytoplankton in the Piles Creek inoculum (29.1 mg/m^3 chlorophyll) than in the Mullica River inoculum (1.7 mg/m^3 chlorophyll), however, as in experiment 1, the biomass specific production rate of the Mullica River water was greater than that of the Piles Creek water (Table 1). The photosynthetic rate of the Piles Creek phytoplankton was approximately 30% higher in the 1.0 ppm addition treatment than the unamended control and three times that of the acetone control, but the differences among treatments were not statistically significant (Fig. 1D). There was more variability in the Piles Creek water measurements of oxygen production in experiment 2 than in any other water. The acetone control in the Piles Creek water suggested an inhibitory effect on primary production relative to unamended controls, but it was not statistically significant. Mullica River water showed a slight enhancement of oxygen production in the phenanthrene treatments, but there were no statistically significant differences between any treatments (Fig. 1E). Respiration in the dark bottles of both waters was consistent among phenanthrene additions and controls, was similar between Mullica River water and Piles Creek water and was lower than respiration rates in experiment 1, where no attempt was made to remove microheterotrophs which are expected to dominate planktonic respiration (Griffith *et al.* 1990) (Fig. lD,E).

The P/R ratios ranged from 2.6 to 8 for Piles Creek water due to high phytoplankton biomass. In the Mullica water the P/R ratios were approximately 1 for all except the 0.1 ppm treatment which had a P/R of 2.

In both experiments and waters, photosynthesis was enhanced at the lowest levels of phenanthrene addition. The apparent enhancement of photosynthesis by the addition of phenanthrene in our study contrasts with other studies that have shown that production may be inhibited by the presence of fuel oils. Bate and Crafford (1985) found that the photosynthetic rates, as measured by oxygen production or carbon fixed, of five phytoplankton species were inhibited by the water soluble fraction of used lubricating oil. Other studies have shown inhibition of production of natural assemblages of phytoplankton by diesel fuel and fuel oils (Siron *et al.* 1993). The toxic effect has been attributed to the light aromatic constituents of these mixtures, which would include phenanthrene.

Other researchers have reported a stimulatory effect of diesel fuel and fuel oils on algal communities. Oviatt et al. (1982) found that there was an enhancement of production and biomass with the addition of fuel oil. They attributed this increase to a decrease in grazing pressures. Cat-man et al. (1997) saw a similar stimulator-y effect on benthic microalgae from diesel fuel additions. Lower rates of consumption of sedimentary microalgae by meiofauna as well as decreases in abundance of copepods indicated a decrease in grazing pressure on benthic microalgae. Harrison et al. (1986) found an enhanced production correlated to a shift in species composition in phytoplankton exposed to crude oil, with diatoms being more sensitive than other taxa. The short time scale of our experiments precludes a population shift. The waters in experiment 1 were initially filtered to

remove large predators but we did not monitor zooplankton during the experiment. The enhancement of photosynthesis seen in the phenanthrene treatments of experiment 1 could be attributed to an inhibition of grazers < 114 pm. In experiment 2, where the water was filtered to remove the >114 µm organisms as well as to minimize <10 µm organisms, there was no significant enhancement of oxygen production attributable to phenanthrene additions although Piles Creek had a qualitative trend similar to experiment 1. This could indicate that if the response of the phytoplankton in experiment 1 was due to decreases in predation, the actions of the <10 µm organisms had a significant impact on oxygen production. The identity of these organisms is unknown.

Few studies have examined the effects of specific PAHs on phytoplankton. Studies of the effects of naphthalene on the freshwater flagellate *Chlamydomonas angulosa* showed an immediate decline in cell numbers and production rates (Soto et al. 1975). Riznyk et al. (1987) reported an initial inhibitory effect on phytoneuston populations contaminated with fluoranthene in mesocosms, but the production rates quickly returned to similar rates as controls.

An alternative explanation for enhanced photosynthesis in response to phenanthrene additions is that the phenanthrene may stimulate a process in the photosynthetic apparatus. It has been found that quinones can uncouple the energy transduction in chloroplasts (Miyoshi and Fujita 1987). The end result of this would be to increase the oxygen production of chloroplasts. The predominant photoproduct of phenanthrene is 9,10 phenanthrenequinone (McConkey et al. 1997). We did not measure the concentrations of the photoproducts of phenanthrene in these experiments, however the results of our study suggest that further research into this pathway may be warranted.

The Mullica River and Piles Creek systems have markedly different plankton communities, as indicated by chlorophyll concentrations, P/R ratios and biomass specific primary production rates. Although we expected that phenanthrene would be toxic to phytoplankton and that Piles Creek phytoplankton (having had previous exposure to the PAH) would be better adapted to its presence, the studies described here indicate that photosynthesis was enhanced in Piles Creek and Mullica River experiments with the addition of phenanthrene, as much as three times greater than control levels. However, respiratory rates were relatively unaffected by the addition of phenanthrene. The similar response of two different communities to phenanthrene additions may suggest a common mechanism of action of this compound on both planktonic communities, whether it is a decrease in grazing pressure or stimulation of photosynthesis. Further research into PAH toxicity is needed with an emphasis on an understanding of the mechanism of action in phytoplankton.

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