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Short-Term Effects of Polynuclear Aromatic Hydrocarbons on Sea-Surface Microlayer Phytoneuston

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Polynuclear aromatic hydrocarbons (PAHs) are of ecological concern because they are the most widespread class of chemical environmental contaminant, having an estimated annual discharge into aquatic ecosystems of 230,000 metric tons (Neff 1979). These pollutants are of special interest to biologists because of their localization in rivers, estuaries, and coastal waters where they quickly become adsorbed by organic and inorganic particulate matter and are readily accumulated by aquatic biota. Since these chemical compounds are lipophilic, they are incorporated into both plant and animal tissue, purportedly by passive diffusion and/or active metabolism. The toxic nature of PAHs is of current concern. The Environmental Protection Agency has identified 16 different PAHs as priority pollutants because of their potentially harmful effects to the environment and their known carcinogenic and mutagenic capabilities.

PAHs can enter aquatic environments via several pathways, including that of biogenic organic exudates, fossil fuel emissions and spills, industrial and domestic wastewater discharges, urban runoff and atmospheric fallout (Neff 1979). In most instances there is a direct relationship between PAH concentrations in the aquatic environment and the degree of industrialization in the immediate vicinity.

The Puget Sound area, in the state of Washington, is characterized by an increasing population reflected by the growth of Seattle and Tacoma and their respective industrial-based economies. Previous work on the sea-surface microlayer of Puget Sound has shown that contaminant metals and organics are present at some sites at concentrations from 10 to more than 1000 times greater than in underlying seawater (Hardy 1982; Hardy et al. 1985).

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Most prior work examining the effect of PAHs on algae has been confined to laboratory investigations using freshwater taxa grown in unialgal culture under optimal conditions (Bastian and Toetz 1982; Batterson et al. 1978; Giddings 1979). Since the sea-surface microlayer may contain an inordinately high concentration of PAHs relative to the water column, we decided to investigate the short-term effect of these hydrophobic contaminants on phytoneuston community dynamics under controlled _in situ_ conditions during the summer season when bloom conditions can prevail in areas of Puget Sound.

MATERIALS AND METHODS

Three separate experiments were conducted at different times. The first experiment analyzed the effect of 1 mg/L fluoranthene on the rate of growth of phytoneuston during a 96-h period of time. The second experiment examined the effect of 1 mg/L fluoranthene on phytoneuston growth rate immediately following a rainstorm event. The third experiment compared the effects of two concentrations (1 mg/L, 10 mg/L) of fluoranthene and benzanthracene on the growth of phytoneuston during a 144-h period of time.

Phytoneuston growth rate is dependent on both recruitment and retention of organisms from the water column below as well as the formation and retention of new organisms by reproduction. The growth rate of phytoneuston populations was determined by the equation:

$$k = \frac{\log_e N - \log_e N_o}{t}$$

where k = exponential growth rate
N = number or organisms
t = time interval

All investigations were performed out-of-doors in a Fiberglas tank with a capacity of 1,065 L and a slow flow-through of seawater. The tank was fitted with inlet and outlet plumbing to eliminate surface layer skimming and provided with a constant flow of unfiltered seawater from 10 m below the surface of Puget Sound from Sequim Bay at a rate of 34 L min^{-1}. After several days a microlayer film formed on the surface water within the tank. A previous study has shown that tank microlayers do not differ significantly from the natural phytoneuston community of Puget Sound (Hardy and Valett 1981).

After development of the microlayer, a 1.22-m diameter Plexiglas chamber with six circular portals, each with a diameter of 30.5 cm, was placed on the water surface of the tank. This device served to segregate six microlayer subcommunities, each with a surface area of 730 cm^2, yet permitted contact with the water column below and the atmosphere above.

Two crystalline PAHs, fluoranthene and benzanthracene, were each first dissolved in an organic carrier of methylene chloride. This solution was mixed with a commercial brand of mineral oil and heated to 41°C to evaporate the organic carrier. By using the formula for the volume of a cylinder, $V = \pi r^2 h$, it was determined that a volume of 3.6 mL of mineral oil-PAH solution was needed to provide a microlayer depth of 50 µm in each portal. In the first and second experiments, of the six portals available, two served as untreated controls. Unaugmented mineral oil was added to two of the portals and 1 mg/L fluoranthene dissolved in mineral oil was added to the final two portals. Hence, this experimental design allowed for duplicate treatments. In the third experiment only one portal served as an untreated control. In addition, a single portal contained unaugmented mineral oil. Portals three and four contained 1 mg/L and 10 mg/L fluoranthene, respectively. Portals five and six contained 1 mg/L and 10 mg/L benzanthracene, respectively.

Since PAHs have a strong affinity for lipids, it is likely that the PAHs remained within the hydrophobic microlayer in proximity of the phytoneuston. All mineral oil inoculations required the addition of several drops of the surfactant Tween 80 to reduce surface tension and to allow the oil to spread evenly over the water surface.

Phytoneuston from each subcommunity were collected at 24-h intervals, using the glass plate method which involved vertically immersing a 7.62 x 2.54-cm glass microscope slide into each portal and withdrawing it, thereby allowing the surface film to adhere to the glass. Glass plates are efficient collectors of surface-active materials, including microalgae (Carlson 1982). A single immersion of the glass microscope slide consistently sampled 0.1 mL of microlayer. Sampling variability was determined by dipping 10 replicate glass slides in rapid succession into the tank. The coefficient of variation for total abundance was 17%. Organisms were washed off the slide with a mild detergent-filtered seawater mixture, preserved in Lugol's iodine solution and placed in a settling chamber. Enumeration was performed with an inverted microscope at 400x magnification, according to the counting techniques outlined in Standard Methods (American Public Health Association 1975).

RESULTS AND DISCUSSION

Concentrations of 1 mg/L of the PAH fluoranthene in mineral oil had a definite impact on the growth rate of phytoneuston during the initial 48-h exposure period (Figure 1). It appears that fluoranthene either retarded reproduction and/or contributed to the mortality of established microalgae communities during short-term exposure. A relatively low growth rate of phytoneuston that occurred in the unaugmented mineral oil can perhaps be attributed

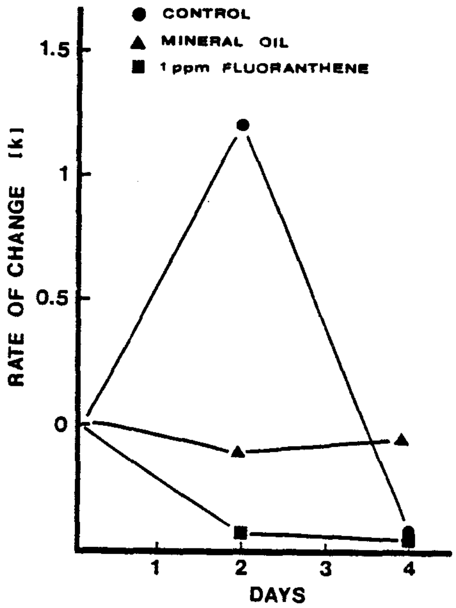

Figure 1. Rate of change in overall phytoneuston abundance.

to PAH impurities present in the liquid paraffin. A more plausible explanation, however, is that the viscous mineral oil acted as a physical barrier that delayed the dispersion of newly formed daughter cells. Even though the surfactant Tween 80 reduced the surface tension to allow the oil to spread, the viscosity may have been high enough to hamper dispersion. After four days the untreated control and 1 mg/L fluoranthene-treated phytoneuston communities showed nearly identical growth rates. Apparently the microflora was able to adapt to the PAH concentration and grow unimpeded to equal the rate of growth of the control community, which slowed after a very rapid initial increase.

A study by Bastian and Toetz (1982) found complete inhibition of growth after 14 days with 0.417 mg/L fluoranthene under laboratory conditions using a freshwater blue-green alga taxon. The interpretation of these results is interesting in the case of the Puget Sound microlayer, since fluoranthene concentrations as high as 0.388 mg/L have been measured in Puget Sound waters (Hardy, personal communication). This may lead one to conclude that some areas of Puget Sound, such as Commencement Bay, would be completely devoid of microalgae because of PAH contamination. This apparently is not the situation. A precursory analysis of samples from the urban bays of Puget Sound have indicated the presence of extensive microlayer communities. There are several explanations for this paradox. One must understand the unique flora that comprises the microlayer. The algae living in this habitat are capable of adapting to widely fluctuating environmental extremes of salinity, light intensity, and temperature, depending on the season, which in this maritime temperate latitude (48° N) ranges from dry, clear and hot in summer to moist, overcast and cool in winter. An analysis of the species composition, which was monitored throughout the experiment, indicated that several species of the diatom Nitzschia and the microflagellate Exuviaella marina comprised the major portion of the flora. Ecological data pertaining to Nitzschia and many microflagellates indicate that these genera have the inherent ability to adapt to environmental extremes and are very pollution tolerant (Palmer 1969). This is probably the major reason why the microlayer community is dominated by these taxa. Undoubtedly, past extreme environmental conditions affecting the microlayer community have selected for these hardy, cosmopolitan taxa and caused the elimination of others through the process of evolution.

Another probable cause of ubiquity of microlayer phytoneuston, even in areas of high PAH concentration, is the ability of these organisms to adapt relatively quickly to environmental contaminants (Stockner and Antia 1976). Our results indicate that after only 2 days of inhibition, the natural phytoneuston community virtually grew unimpeded, regardless of a fluoranthene concentration of 1 mg/L. It has been postulated that in some instances PAH can be utilized as an organic substrate to enhance the growth of microalgae (Kauss and Hutchinson 1975; Soto et al. 1975). We therefore feel it imperative to use diverse assemblages of organisms to measure the effect of pollution rather than unialgal cultures, since results will be more representative of true conditions. It would be unwise to extend the results of a single taxon bioassay to the diverse assemblage of organisms comprising an ecosystem. Other researchers, using several taxa and a variety of PAHs or crude oil in bioasay experiments, have also emphasized this fact (Winters et al. 1976).

Upon the completion of our first in situ experiment, we were fortunate to experience a rainstorm event resulting in precipitation of 1.57 cm. We decided to utilize this opportunity to investigate the recruitment potential of the microlayer community from the natural seed source in the presence of the residual 1 mg/L fluoranthene. No additional fluoranthene was added to the portals as, even after the rainstorm, a visible slick was still quite evident. Phytoneuston populations increased dramatically within the untreated control portals after severe depletion by rainfall, but experienced a relatively slow initial recruitment and growth rate within the PAH-treated portals during the first 48 h following the rain, even though conditions were adequate for rapid growth (Figure 2). After 48 h, the recruitment rate, growth rate, and presumably retention, increased dramatically in the presence of 1 mg/L fluoranthene. The general pattern of inhibition during the first 48 h of exposure agrees with our first experiment. Prior studies have found similar results of rapid recovery of growth after relatively long exposure to PAH in unialgal culture (Bastian and Toetz 1982; Kauss and Hutchinson

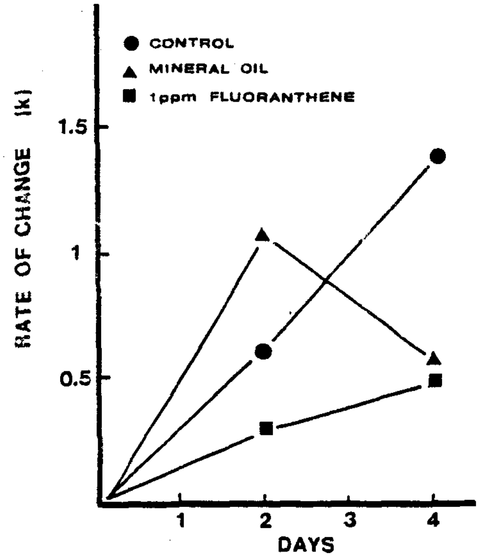

Figure 2. Rate of change in overall phytoneuston abundance immediately following rainstorm event.

1975). It has been documented that algae can fully recover from initial exposure to certain chemicals (Soto et al. 1975).

In our third experiment we had the opportunity to follow a population bloom of the dinoflagellate Exuviaella marina. In this instance we observed the taxon's growth using different concentrations of two PAHs, fluoranthene and benzanthracene. Our experiment was terminated after 144 h, which coincided with a declining growth rate. The population growth curves did not exhibit the typical 48-h lag period in the PAH-treated portals. Some factor(s) very favorable for growth must have suppressed or overridden the initial inhibitory effect noticed in the previous two experiments. The tremendous rate of growth of this microflagellate was tracked, unimpeded, in both concentrations of fluoranthene and benzanthracene (1 and 10 mg/L). No differences were found in the rates of growth in the untreated control, in the unaugmented mineral oil or in the two concentrations of the PAHs.

From our experiments it appears that the phytoneustonic component of the sea-surface microlayer has an inherent adaptive capacity which, under normal conditions, is effectuated after a relatively short exposure period of two days. When conditions, however, are highly favorable for growth, i.e., a bloom situation, PAH toxicants in concentrations up to 10 mg/L do not appear to deter exponential growth of certain taxa. This genetically inherent, adaptive capacity of microalgae to survive and reproduce, unimpeded, in the presence of pollutants, may indeed explain their ubiquitous distribution even in areas with relatively high concentrations of PAH.

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