Bull. Environ. Contam. Toxicol. (1998) 61:637-644

Bulletin of Environmental Contamination and Toxicology

Toxicity of Sediment Associated PAHs to the Estuarine Crustaceans, *Palaemonetes pugio* and *Amphiascus tenuiremis*

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Received: 12 July 1998/Accepted: 5 October 1998

Polycyclic aromatic hydrocarbons (PAHs) are byproducts of anthropogenic and non-anthropogenic decomposition. Anthropogenically produced PAHs have been shown to enter estuarine ecosystems via storm water runoff (eg. Bidleman et al. 1990) as urbanization increases in adjacent upland areas. Over a three year period (1994-1996), monthly assessments of adult *Palaemonetes pugio* (grass shrimp) populations have shown a significant decrease (> 90%) at urbanized sites when compared to the near pristine NOAA/NEERS site at North Inlet, SC (Latitude 33° 20", Longitude 79° 10"). Additional data indicate a similar decline in *P. pugio* densities when agriculturally impacted (non-point source pesticide runoff) sites were compared to the same North Inlet site (Finley et al. in press)

Total PAH levels in both water and sediment at Murrells Inlet have historically been higher than those found in similar pristine estuarine habitats (Sanders 1995; Fulton et al. 1993). Fulton et al. (1993) reported total PAH concentrations in water samples as high as 55 ng/L in Murrells Inlet compared to levels ranging from 0.68 to 1.2 ng/L at the pristine NEERS site at North Inlet, SC. Reported sediment values for Murrells Inlet follow a similar pattern. The average total PAH concentration reported for Murrells Inlet was 518 ng/g dry sediment weight compared to 104 ng/g dry sediment weight average among the North Inlet sites (Sanders 1995).

The goals of this study were to evaluate the potential toxicity to crustaceans of sediment-associated PAHs, and determine if the observed population reductions found in grass shrimp within Murrells Inlet may be related to PAH toxicity. The larval stage of the grass shrimp, *Palaemonetes pugio* was used to assess acute mortality (LC50) while the benthic copepod, *Amphiascus tenuiremis,* was used to evaluate potential PAH sublethal (reproductive) effects. The selection of these two species allows for comparison between a well accepted toxicity test species (*P. pugio*) and an easily cultured and rapidly reproducing crustacean (*A. tenuiremis*) to assess sublethal effects (Chandler and Green 1996). The two crustacean species were exposed to a mixture of PAHs in sediments designed to represent the PAH profile of sediments found in Murrells Inlet.

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MATERIALS AND METHODS

Sediment used for the grass shrimp and copepod tests (~ 12 L) was collected from the pristine North Inlet, SC estuary, returned to the laboratory at Charleston, SC and stored at 4°C. Sediments for grass shrimp tests were pressed through a 1 mm stainless steel sieve and reallocated to glass jars and stored. Copepod sediments were sieved to < 0.125 mm, washed and condensed according to Chandler and Green (1996) and stored. Prior to spiking, the sediment used for the copepod bioassays was reconstituted with test seawater (Chandler and Scott 1991). Dry weight and weight:volume ratios were calculated for determination of spiking volumes. Sediment spiking protocols are described later.

Individual PAHs were obtained from Aldrich Chemical Co. and dissolved in acetone to prepare the stock spiking solution. The PAH contaminant profile of the spiking mixture was determined from existing contaminant data from Murrells Inlet. Data from 30 sites within the Murrells Inlet estuary were compiled (Sanders 1995) and the four most contaminated sites within the MI estuary were used to determine a model PAH mixture. Average concentrations of six high molecular weight (HMW) PAHs containing either 4 or 5 rings (fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene) from the four most contaminated sites comprised the 1X sediment treatment (Table 1). Replicates of 0X (control treatment), 5X, 10X and 100X were also tested. Stocks were quantified by both GC/MS and HPLC and the resulting individual PAH recoveries ranged from 88 - 105%.

Bioassays were performed to assess acute mortality in larval grass shrimp exposed to the PAH mixture. Individual larval compartments (2.5 cm x 2 cm x 2 cm) were made from acrylic and Nitex® mesh (63 µm) (to allow for water circulation) and open on the bottom to allow direct larval association with the sediment. The rationale for compartmentalizing the grass shrimp larvae for the definitive test was to minimize the potential for cannibalism within the test chambers during the extended time of these bioassays. Preliminary tests with the compartment chambers greatly increased control survival over non-isolated larvae, regularly achieving 100% (data not shown). Larval survival for controls (0X) increased from 60% after rangefinder tests to 80% after isolating larvae for these bioassays. Each test chamber consisted of a 1 L crystallizing dish to which 300 mL of sediment and 300 mL of seawater were added. The sediment was spiked with the PAH mixture and stirred for 2 hr to allow for the evaporation of the acetone carrier. An additional 400 mL of seawater were added to each chamber ‘as well as the larval compartments and the treatment chambers were allowed to settle while being gently aerated in an environmental chamber overnight (20°C; 12: 12 hr photoperiod). After 24 hr, larvae were placed into the replicated test chambers and assessed for mortality every 24 hr for 7-d. There were 10 larvae per replicate and each larvae was isolated within the chambers. There were three replicates per treatment. Water quality measurements were

Table 1. Average concentrations from the four highest contaminated sites in Murrells Inlet for the six PAHs used (Sanders 1995) as well as the average nominal concentrations calculated for 1X Sediment Treatment.

Polycyclic Aromatic Hydrocarbon	Field Concentration (ng/g dry weight)	1X Sediment Treatment (ng PAH / g dry sediment weight, Nominal)	
		Grass Shrimp Test	Copepod Test
Fluoranthene	178	178	179
Pyrene	236	235	236
Benzo(a)Anthracene	199	198	204
Chrysene	178	176	177
Benzo(b)Fluoranthene	169	167	200
Benzo(a)Pyrene	210	210	215
Total PAH	1170	1164	1211

taken every 24 hr [salinity (20‰), dissolved oxygen (>75% saturation), pH (7.8-8.2) and temperature (25 °C)]. Two drops/larvae of concentrated *Artemia* sp. (newly hatched) were added daily into each compartment. Sediments for the copepod bioassays were spiked with the PAH mixture in acetone and mixed on a magnetic stir plate for 48 hr. Copepod test chambers (150 mL beakers) were cleaned and filled with sterile-filtered (<0.2 µm) artificial seawater. To these chambers, 10 g wet weight of test sediment were extruded onto the bottoms. Twenty-five barren females and 25 male copepods were added to each test chamber and were then connected to a recirculating seawater culture system (1 mL/min exchange rate) (Chandler and Green 1996). Loss of copepods into the recirculating system was prevented with a 45 µm mesh trap over each beaker. On days 3, 6, 9 and 12, 2 mL of heat shocked phytoplankton detritus (~1 x 10^7 cells of mixed species *Phaeodactylum tricornatum, Isochrysis galbana* (T-iso), and *Dunaliella tertiolecta*) were fed to each test chamber. Phytoplankton densities were monitored using a multichannel Coulter Counter. After 14-d, all males, females, egg masses and offspring were counted and analyzed. Each test was run in quadruplicate in an environmental chamber to control temperature and light cycles (20°C; 12:12 hr photoperiod). Temperature (20°C salinity (28‰), dissolved oxygen (>75% saturation) and pH (7.8-8.2) were also measured daily.

Probit analysis of the *P. pugio* mortality data was used to obtain 96 and 168 hr LC_{50} estimates. Pairwise comparisons for mortality data in grass shrimp and copepods were performed using ANOVA (Student-Newman-Keuls method) with significance at $p \leq 0.05$ (SigmaStat, Jandel Scientific). Sublethal reproductive endpoints for *A. tenuiremis* count data were log10 (x+1) transformed and analyzed using ANOVA (Dunnett's Multiple Comparison tests) with significance at p 10.05 (SAS 1988). Count data was analyzed for trends using the Cochran-Armitage trend test for counts with a significance at α=0.05 (Piegorsh and Bailer 1997).

RESULTS AND DISCUSSION

The results of the 96 hr and 168 hr bioassays are shown in Figure 1A. The calculated LC_{50}s for *P. pugio* were 8.20 X (5.08-13.20) (9542 ng PAH / g dry sediment) for 96 hr and 5.55 X (3.19-9.40) (6464 ng PAH/g dry sediment) for 168 hr. Copepod survival of parental males and females (Figure 1B), as well as numbers of females successfully producing clutches (Figure 2), were not significantly different among 0X, 1X, and 5X sediment treatments. However, in the 10X PAH treatment (12110 ng/g sediment), female survival was significantly ($p<0.05$) reduced by 24% when compared to controls. After 14 days, 76.2% of the females were gravid in 0X sediments; 63.9% in 1X sediments; 58.7% in 5X sediments; and 60.9% in 10X sediments. Mean clutch size (Figure 2) was significantly reduced in the 1X PAH treatment (83.7% of controls), but the 5X and 10X treatments had no effect on mean clutch size. A significant dose-response trend was observed for PAH effects on copepod nauplii per female but not for clutch size or copepodite production. Control sediments produced the largest number of nauplii per surviving female (8.6; Figure 2). The 10X PAH treatment produced the fewest (2.8 per surviving female). The subsequent copepodite stage was also affected by PAH exposure, but was by far the most variable endpoint tested (Figure 2). Normalized to the number of surviving females at the end of 14 days, these values become: 0.67 copepodites/female at 0X; 0.31 copepodites/female at 1X; 0.28 copepodites/female at 5X; and 0.11 copepodites/female at 10X. When these data are placed in the context of potential reproduction (i.e., the sum of the total number of eggs, nauplii and copepodites divided by number of surviving females), versus realized reproduction (reproduction actually accomplished by day 14 of exposure; nauplii + copepodites / surviving females), the same significant decreasing reproductive dose-responses remain evident (Figure 2).

A comparison of the grass shrimp LC_{50}s to published sediment quality criteria indicates that the results fall between Long et al.'s (1995) ERL (1700 µg/kg dry) and ERM (9600 µg/kg dry) for high molecular weight PAHs (HMW PAH). The ERL and ERM for total PAHs is 4022 µg/g dry weight and 44792 ng/g dry weight, respectively. According to Long et al. (1995) the ERL is the 10th percentile of toxicity response and the range below the ERL is defined as the range of concentrations where effects would be minimal or rarely observed. The PEL (Probable Effect Level) is defined as the lower limit of the range of contaminant concentrations that are usually (or always) associated with adverse

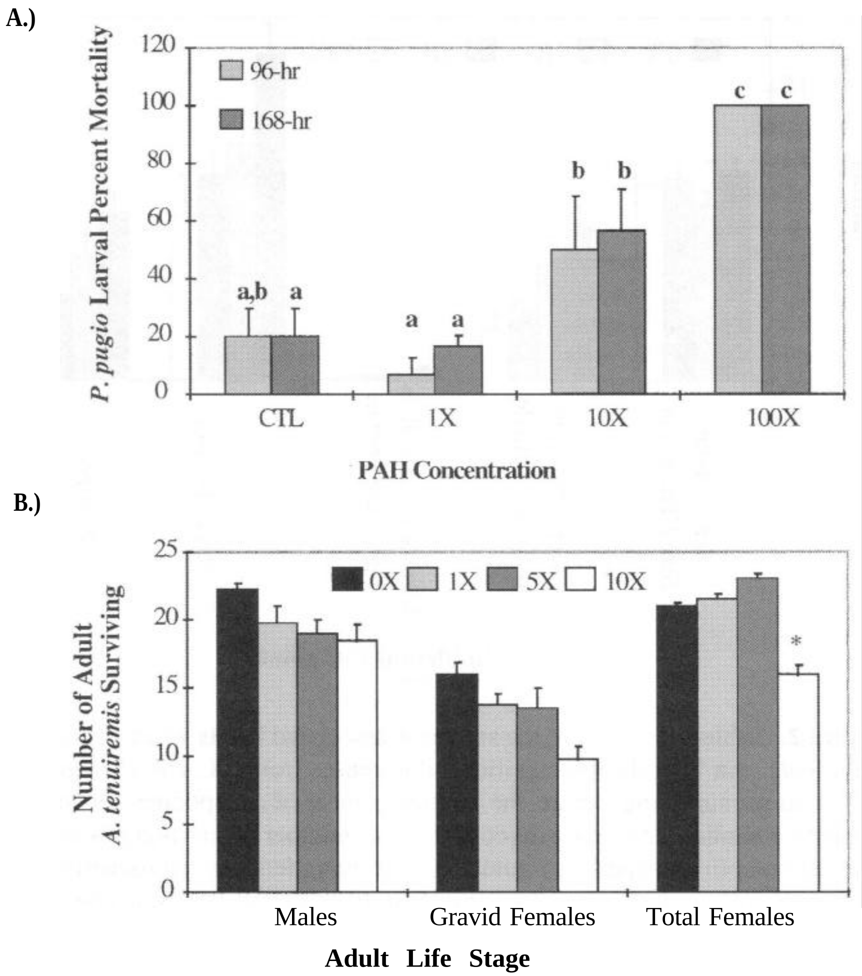

Figure 1. A.) Larval grass shrimp mean mortality (± standard error) after 96 hr and 168 hr of exposure to the sediment-associated PAH mixture. All pairwise comparisons were performed using a one-way ANOVA plus Student-Newman-Keuls method (p 10.05) (SigmaStat, Jandel Scientific). Letters (a,b,c) indicate statistical differences between treatments at 96 hr and 168 hr. B.) The mean number (± standard error) of adult copepods surviving 14-d exposure to the sediment-associated PAH mixture. The initial numbers of males and non-gravid females were 25 per replicate. An '*' indicates significant differences from 0X ($p \leq 0.05$).

biological effects (MacDonald 1994). The 96 hr LC50 for *P. pugio* exceeds the PEL of 6676 ng/g dry weight for HMW PAHs and the LC50 for 168 hr is less than the PEL. While the lethal effects of the PAH mixture fall above the lower

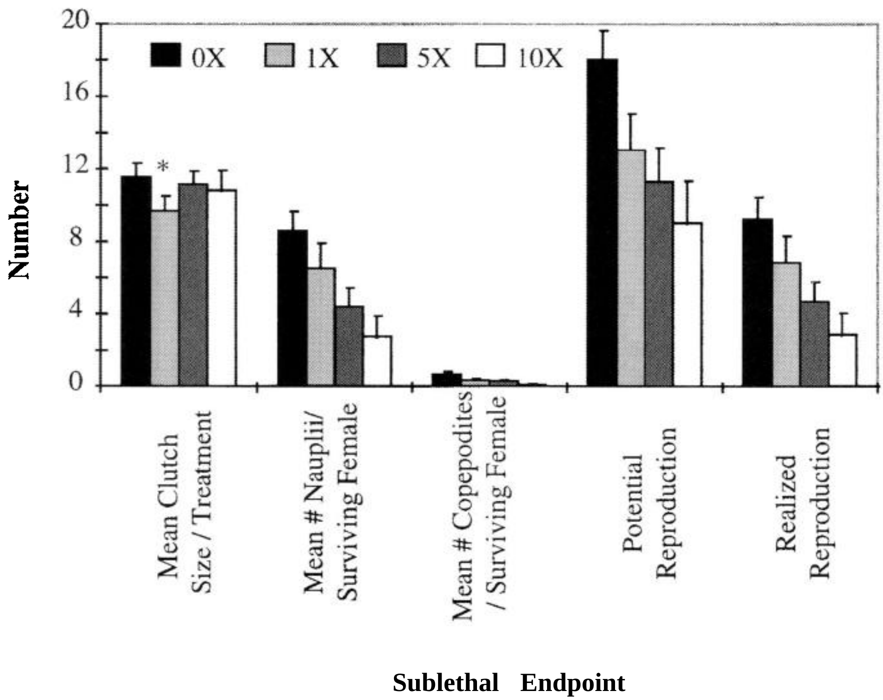

Figure 2. Sublethal effects of the sediment-associated PAHs when exposed to *A. tenuiremis.* An '*' indicates significant differences from 0X. The average number of nauplii per surviving female, the average number of copepodites per surviving and both potential (eggs+nauplii+copepodites / number of surviving females) and realized (nauplii+copepodites / number of surviving females) reproductive output per female followed a distinct dose:response. All data shows the average (± standard error), and were analyzed by Dunnett's Multiple Comparison Test (p ≤10.05). Trend tests using the Cochran-Armitage trend test for counts showed significant trends were evident in mean nauplii per female, realized reproduction and potential reproduction.

limits of published sediment quality guidelines, sublethal effects were seen at concentrations between 1X and 10X. The consistent dose:response trends apparent in the number of nauplii and copepodites per surviving female, as well as the calculated potential effects on reproduction, indicates that PAHs possibly effect reproduction at levelswhich are normally thought to be non-effects levels.

The copepod results were in general agreement with other reports from the literature where reproductively active copepods were exposed to a single PAH. Lutofo and Fleeger (1997) found that reproduction was negatively impacted in meiobenthic copepods *Schizopera knabeni* and *Nitocra lacustris* exposed to sediment-associated phenanthrene. Decreases in offspring production, egg hatching and an increase in larval and embryonic developmental time were observed at concentrations as low as 22 µg/g dry sediment weight. Additional data for sediment-associated fluoranthene was also reported for the meiobenthic copepod *S. lacustris.* At concentrations as low as 38 µg/g dry sediment weight, there were decreases in nauplii and copepodite production reaching 50%. There was no corresponding effect on clutch size at levels up to 2000 µg/g sediment (Lutofo 1997). The consistency of these trends strongly suggest that longer exposure periods of two or more generations would generate dramatic differences between 0X and 10X exposure concentrations in our tests. Even the 1X and 5X concentration range, which appears to indicate the threshold exposure level in these 14 day tests, may yield significantly negative impacts on population growth over longer exposures periods similar to field conditions.

Our data tends to indicate that grass shrimp are more acutely sensitive to the PAH mixture, but sublethal reproductive effects in copepods may be evident at concentrations less than the grass shrimp LC50 (96 hr = 8.20X or 168 hr = 5.55X). For these tests, it appears that a mixture of PAHs can inhibit or decrease the reproductive output of adult female copepods in a dose dependent manner, even though there is generally not an impact on the number of fertilized eggs produced (clutch size). The mechanism of action for this decline could be a lethal response in nauplii and copepodites, or a developmental effect, inhibiting the survival of the offspring from incubation to larvae. In all treatments, the realized output was at least 50% less than the potential output. This reduction in potential reproductive output increased in a dose dependent manner as there was a -75% decrease in realized output at 10X.

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