

Report

# Assessing Photoinduced Toxicity of Polycyclic Aromatic Hydrocarbons in an Urbanized Estuary

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ABSTRACT. Increases in contaminants associated with urban sprawl are a particular concern in the rapidly developing coastal areas of the southeastern United States. Polycyclic aromatic hydrocarbons (PAHs) are contaminants associated with vehicle emissions and runoff from impervious surfaces. Increased vehicular traffic and more impervious surfaces lead to an increased loading of PAHs into coastal estuarine systems. The phototoxic effect of PAH-contaminated sediments on a sediment-dwelling meiobenthic copepod, *Amphiascus tenuiremis*, was estimated in Murrells Inlet, a small, high-salinity estuary with moderate urbanization located in Georgetown and Horry Counties, South Carolina, USA. Field-determined solar ultraviolet radiation (UV) and UV extinction coefficients were incorporated into laboratory toxicity experiments, and a model was developed to predict areas of specific hazard to *A. tenuiremis* in the estuary. The model incorporated laboratory toxicity data, UV extinction coefficients, and historical sediment chemistry and bathymetric data within a spatial model of sedimentary areas of the estuary. The model predicted that approximately 8–16% of the total creek habitat suitable for meiobenthic copepods is at risk to photoinduced PAH toxicity. This area is in the northern, more developed part of Murrells Inlet.

#### INTRODUCTION

Urban sprawl and unplanned development are pervasive problems throughout the United States, causing rapid increases in population, roadways, and traffic as well as decreases in rural farmland and areas of undeveloped land (Mitchell 2001). Rapid population increases projected in the southeastern coastal United States (Allen and Lu 2000, Vernberg and Vernberg 2001) are of particular concern because of the related increases in development pressure on estuarine and coastal water resources. The populations of the U.S. coastal areas of South Carolina and Georgia are expected to increase by as much as 100% by 2026 (LU-CES 2000). These increases in population will lead to the development of more urbanization and sprawl. The associated increases in vehicular traffic and runoff from impervious surfaces such as paved roads are likely to both increase runoff flows and alter storm water composition, adding to the pressure on estuarine systems.

Estuaries are an economically valuable and highly

productive coastal resource, and they serve many useful purposes (Vernberg et al. 1992, Rathbun 1998, Porter et al. 1996). These ecosystems have obvious aesthetic value, but they also provide habitat for commercially and ecologically significant shellfish, fish, and crustacean species (Rathbun 1998). Nearshore and estuarine fish comprise 50% of the world's harvest (Kennish 1992), and the majority of these species depend on meiobenthic copepods for food during the early stages of life (Coull 1990). As coastal areas become increasingly populated, the ecological function and integrity of estuarine areas are threatened.

More than 50% of the population of the United States is estimated to live and work in coastal counties that comprise only 10% of the country's landmass (Vernberg et al. 1992). As a result of this anthropogenic pressure, estuarine areas over the past century have served as a repository for a variety of industrial and municipal wastes, dredged materials, and sewage sludge. These loadings have led to widespread contamination by heavy metals, pesticides, petroleum hydrocarbons, nutrients,

and pathogens (Kennish 1992). Understanding and monitoring the impacts of contaminant loading may help managers make decisions to preserve the ecological function of coastal areas (Kennish 1992, Vernberg et al. 1992, Rathbun 1998).

One widespread class of urban contaminants is polycyclic aromatic hydrocarbons (PAHs). These are generated mainly during the combustion of fossil fuels (Neff 1979, Huang et al. 1993). PAHs bind readily to particulate surfaces and are relatively stable in their ground states. These contaminants are persistent in substrates such as marsh sediment, particularly when the sediment is characterized by low oxygen and high organic carbon contents (Arfsten et al. 1996).

Traditionally. testing the toxicity of PAHcontaminated sediments has been conducted in the absence of UV to avoid photodegradation of intact PAHs. The result is an overall underestimation of PAH toxicity in environmental risk assessments (Arfsten et al. 1996). When modifying water quality criteria, the U.S. Environmental Protection Agency (EPA) recognized that photoinduced PAH toxicity poses an additional risk to aquatic organisms, yet the modification of current sediment quality criteria has not received comparable attention (Ankley et al. 1995). Benthic organisms may be at greatest risk for the phototoxic effects of PAH-contaminated sediments because their ecology places them in close association with these sediments (Monson et al. 1995). Few studies have examined the phototoxic effects of PAHcontaminated sediments on benthic organisms.

Research has shown that photodegradation of PAHs is directly proportional to the intensity of incident solar radiation (Wortham et al. 1993). Literathy et al. (1990) investigated the fluorescence of PAHs in Kuwaiti crude oil with respect to seawater absorption, suspended sediments, and solar radiation levels. Fluoresence is used to characterize the photolysis of PAHs. The authors found that the photolysis of PAHs may occur prior to the photoexcitation and subsequent photoenhanced toxic activities of these contaminants. They showed that, in August, maximum PAH photolysis occurred at midday with radiation wavelengths of 330-350 nm in the UV-A range, with only a 6% decrease in photolysis at a depth of 1 m. In marine waters, the rate of photochemical reactions involving PAHs is governed by many factors, including UV radiation intensity and the specific absorption wavelengths for individual PAHs (Literathy et al. 1990).

Because of the projected population increases around Murrells Inlet, concurrent increases in the amount of area developed, the expansion of impervious surfaces, and greater vehicular traffic, PAH contamination in the Murrells Inlet estuary is expected to increase. This study evaluates an ecological hazard associated with the PAH contamination that results from increased development. The specific objective of this study is to identify geographic areas within the Murrells Inlet estuary that could be considered hazardous to A. tenuiremis because of photoinduced toxicity in PAHs. Hazard criteria were created based upon laboratory phototoxicity bioassays, habitat considerations. environmental solar UV intensity, and PAH contamination levels.

## **METHODS**

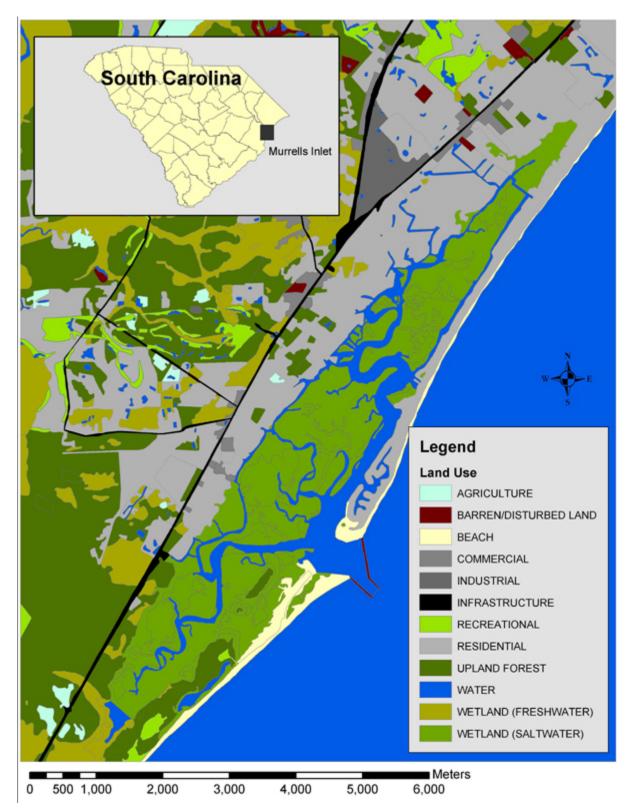
## Study areas and test organism

Murrells Inlet estuary (MIE) is a high-salinity, 26.7 km<sup>2</sup> estuary containing urbanized uplands, marshland, and water. It is located approximately 122 km northeast of Charleston, South Carolina, USA (Fig. 1). Murrells Inlet is characterized by semidiurnal tides with a mean range of 1.37 m. The area has been experiencing high rates of residential and commercial development to satisfy the demands of a growing coastal population (Porter et al. 1997).

Although PAH levels vary throughout Murrells Inlet, they are highest in locations adjacent to developed areas, such as marinas and highway overpasses (USES Annual Report 1994; Fig. 2). PAH concentrations were measured in Murrells Inlet by Fortner et al. (1996). The mean total PAH concentration in Murrells Inlet is 518 ng/g. The PAHs measured were phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene. These PAHs excite in the UV-B spectrum and emit in the UV-A and visible spectra (Futoma 1981).

North Inlet estuary (NIE) was used as the control site in toxicity tests. North Inlet is located 70 km northeast of Charleston, South Carolina, USA, and approximately 32 km south of Murrells Inlet (Fig. 1). It comprises 80 km<sup>2</sup>of undeveloped barrier islands, marshland, and upland forest, and it exhibits consistently low total PAH concentrations (Fortner et al. 1996; Table 1).

Fig. 1. Murrells Inlet land use.



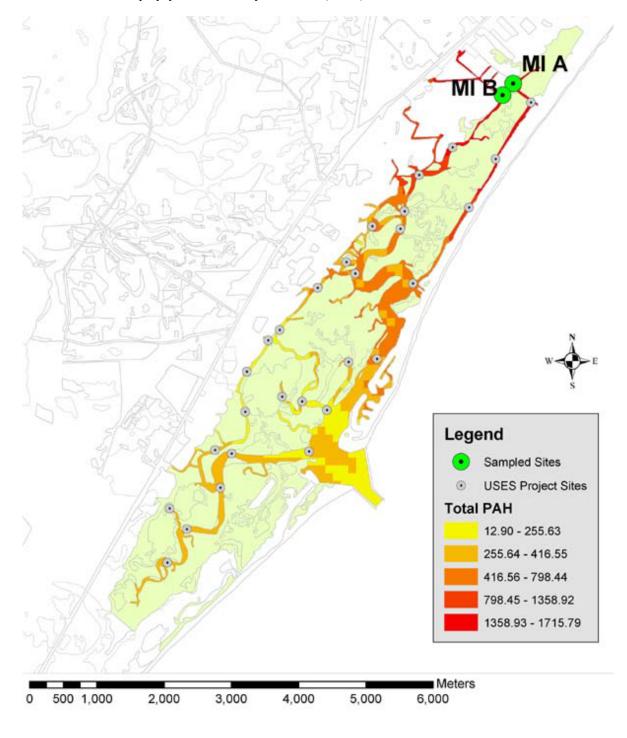


Fig. 2. Estimates of levels of polycyclic aromatic hydrocarbons (PAHs) in sediments from Murrells Inlet.

A. tenuiremis is an amphi-Atlantic, sedimentingesting, benthic, harpacticoid copepod that has been used successfully in several field and laboratory-spiked sediment toxicity studies (Chandler and Green 1996, Wirth et al. 1998). It is an easily cultured,

sexually dimorphic, infaunal copepod with holobenthic larvae. Its offspring occur in easily distinguishable clutches and juvenile stages for posttest, life-stage enumeration (Chandler and Green 1996).

**Table 1.** Summary of individual polycyclic aromatic hydrocarbons (PAH) in sediments from two Murrells Inlet Estuary sites (MIE A and MIE B) for the October 1998 (Fall) and May 1999 (Spring) toxicity tests (ng/g dry weight). North Inlet Estuary (NIE) control site data is from Fortner et al. 1996.

РАН	1996	Fall		Spring		
	NIE	MIE A	MIE B	MIE A	MIE B	
Anthracene	2	92.5	17.6	55.4	85.1	
Fluoranthene	14	208	107	190	178	
Pyrene	19	161	84.1	139	130	
Benzo(a)anthracene	11	110	49.3	79.8	66.4	
Chrysene	11	219	92.9	115	116	
Benzo(b)fluoranthene	14	217	122	106	126	
Benzo(k)fluoranthene	26	84.8	41.5	92.3	90.6	
Benzo(a)pyrene	17	109	46.2	79.8	87.4	
Benzo(g,h,i)perylene	16	73.9	35.8	50.4	52.5	
Naphthalene		32	208	21	42.2	
1-Methylnaphthalene		15.2	116	< 7.38	13.3	
2-Methylnaphthalene		25.3	203	13.6	27.6	
Biphenyl		< 16	33.8	< 11.6	< 18.2	
2,6 Dimethylnaphthalene		11.1	90.1	< 6.87	11.5	
Acenaphthene		< 16.4	30.3	< 11.9	< 18.6	
Acenaphthylene		9.39	31.8	< 3.1	< 4.85	
2,3,5 Trimethylnaphthalene		< 4.73	9.15	< 3.44	< 5.37	
Fluorene		9.78	8.56	12.1	17.9	
1-Methylphenanthrene	•••	< 9.39	< 9.03	< 6.82	< 10.7	
Perylene		40.2	18	25.2	35.9	
Ideno(1,2,3-cd)pyrene	•••	86.9	37.6	62.3	63.9	
Dibenz(a,h)anthracene		16.7	9.97	13.1	13.2	
Total PAH	139	1568.3	1193	1179.1	1288.5	

# **Sediment PAH analyses**

Triplicate sediment samples from the NIE control site and from two Murrells Inlet sites (MIE A and MIE B; Fig. 2) were analyzed for PAHs at the National Oceanic and Atmospheric Administration Center for Coastal Environmental Health and Biomolecular Research Laboratory in Charleston, South Carolina, USA (Fortner et al. 1996). The procedure for measuring organic contaminants in sediments was modified from Krahn et al. (1988), Sanders (1995),

and Schantz et al. (1997). The following PAHs were tested: anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)-fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, naphthalene, methylnaphthalene, 2-methylnaphthalene, biphenyl 2,6 dimethylnaphthalene, acenaphthene, acenaphthylene, trimethylnaphthalene, fluorene, methylphenanthrene, perylene, ideno(1,2,3-cd)pyrene, and dibenz(a,h)anthracene (Table 1).

**Table 2.** Summary of semivariance analyses for all kriged continuous surfaces. PAH stands for polycyclic aromatic hydrocarbons. K<sub>e</sub> stands for UV extinction coefficients.

Layer	Model	Lag distance	Lag interval	Range	Semivariance R <sup>2</sup>	Number of neighbors	Search radius
October 1998 K <sub>e</sub>	Linear	8000	1075	1209	0.5	16	4000
May 1999 K <sub>e</sub>	Linear	8000	708	4251	0.79	16	4000
August 1999 K <sub>e</sub>	Linear	8000	1100	1094	0.46	16	4000
Silt-clay	Exponential	8000	800	1677	0.91	16	4000
Total PAH			800	9687	0.83	16	4000

## Phototoxicity test design

Laboratory reproduction bioassays were used to determine whether toxicity to *A. tenuiremis* was greater with co-exposure to PAH-contaminated sediments from Murrells Inlet and simulated UV compared to the same sediments that have not been irradiated with UV, i.e., UV-shaded. Copepod chronic reproductive bioassays were conducted at known UV intensities and four site-specific concentrations of sediment contaminants. These data were combined with field UV intensity data to determine whether the UV levels measured in the laboratory were consistent with those in the natural habitat of our organism. The phototoxicity test design was modified from the benthic copepod sediment toxicity test developed by Chandler and Green (1996).

NIE control and MIE contaminated sediments were press-sieved on 63 µm stainless steel sieves prior to testing to remove large particulates and meiofauna. Sediment tests were replicated nine times: tests of UV-exposed contaminated sediments were replicated five times, and tests of UV-shaded contaminated sediments were replicated four times. All test glassware was acid-washed and rinsed with 1:1 acetone-hexane to neutralize glass surfaces and minimize adsorption of sediment PAHs to the glass substrate. Test chambers were made from 50 mL glass beakers with 2.5 cm holes blown in them. The holes were covered with 63 µm mesh to allow seawater to flow through the test chambers. For each sediment type, 10 mL of sediment

was covered with 25 mL of filtered, artificial seawater (ASW), and 30 gravid *A. tenuiremis* per replicate were harvested from stock sediment cultures. These were loaded into the 50 mL glass beakers.

A total of 30 individual test beakers were placed under constant ASW flow in an environmental chamber held constant at  $30 \pm 1$  ppt salinity,  $20 \pm 1$ °C, and  $7.5 \pm 0.1$ pH. Two 1 m long, 40-watt, 340-nm UV bulbs (Q-Panel Lab Products, Cleveland, Ohio) were placed above test chambers as a UV source on a 16:8 h light:dark cycle (L:D). Full-spectrum fluorescent light bulbs were placed 15 cm below the test chambers to provide a non-UV source on a 16:8 h L:D. UV intensity was measured every three days. Each beaker received 21 x 106 cells/mL phytoplankton food every three days. An IL1400-3987 photometer along with submersible SUL-005 and SED-240 detectors (International Light Inc., Newburyport, Massachusetts, USA) measured UV-A and UV-B, respectively. Non-UV control shields were made of 500 mL amber polypropylene bottles with the bottom 2 cm removed so that they could be placed over the test beakers to block the passage of UV to the UV-shaded control chambers.

UV extinction in seawater was measured prior to each toxicity test to determine the UV-A and UV-B exposures at the sediment-water interface. Extinction coefficients for UV-A and UV-B were derived using a 2 L rectangular container filled with ASW from the flow-through system used in the toxicity tests. The

container was placed beneath the UV bulbs in the location later occupied by the toxicity test setup. UV-A and UV-B were measured at the air-water interface and at 1 cm increments down to the container bottom. Extinction coefficients for UV-A and UV-B were calculated for the 1 cm depth (the average depth to the sediment-water interface) using the following formula (Kirk 1994):

$$I_{\rm d} = I_{\rm o} e^{-K_{\rm e}^{(d)}} \tag{1}$$

where  $K_e = \text{UV}$  extinction coefficient, d = depth,  $I_d = \text{UV}$  intensity at depth, and  $I_o = \text{initial UV}$  intensity.

A. tenuiremis was cultured for 14 d in these sediments while exposed to UV or not exposed to UV, i.e., UV-shaded. At test end, surviving copepods in the gravid, naupliar, copepedite, and adult life stages were sieved onto a 53 μm sieve, checked for dead individuals, preserved in 5% formalin and rose bengal, and enumerated for sex, life stage, and clutch size.

## Statistical analyses

All toxicity test data were analyzed using Microsoft Excel, SAS, and MINITAB software. Standard assumptions of normality and independence of observations were evaluated and found to be valid. Multiple regression techniques were performed on data from replicate experiments to determine which of the following independent variables were significant predictors of toxicity end points (P > 0.95): season, presence/absence of UV shade, sediment from the contaminated (MIE) or control (NIE) sites, and their interaction. Once predictors for end points were established, estimates of means for UV and UVshaded treatments were compared using ANOVA in SAS. SAS LSMEANS was used for pairwise comparisons among UV-exposed and UV-shaded treatments ( $\alpha = 0.05$ ). This resulted in an actual individual comparison significance value of 0.006 based on nine predetermined comparisons. Toxic end points included adult mortality, percentage of females gravid at test end, mean clutch size, and realized offspring production. Based on Chandler and Green (1996), production was determined by the following formula:

## Hazard model development

To qualify as a hazard area for *A. tenuiremis* in Murrells Inlet, locations were required to meet three criteria: (1) they needed to have PAH levels equal to the minimum observed to cause toxicity to *A. tenuiremis* in the laboratory, (2) they had to have UV levels equal to the minimum observed to cause toxicity to *A. tenuiremis* in the laboratory, and (3) they had to be designated as habitat for *A. tenuiremis*.

Areas of Murrells Inlet that had PAH and UV levels above the minimum observed to be toxic to *A. tenuiremis* in the laboratory were identified using continuous surface estimates of PAH and UV levels. These surface estimates were derived using various GIS techniques and statistical analyses, including kriging, from PAH measurements taken from 30 sample sites (Fortner et al. 1996) and field-measured UV levels in the estuary.

Site-specific total PAH values, extinction coefficients. and monthly average surface UV intensity values for each of the 30 sample sites along with corresponding latitudinal and longitudinal coordinates were entered into the GS+ geostatistical software (Gamma Design Software, Plainwell, Michigan, USA). Semivariance analyses were conducted before proceeding with spatial interpolation of each parameter. The kriging option in the GS+ package was used to develop continuous surfaces of UV at the air-water interface, UV extinction coefficients, and total PAH levels in sediment. After kriging, each data layer was imported into Arcview (Environmental Systems Research Institute, Redlands, California, USA). Maps of all continuous surface data were generated and converted to grids that divided spatial attributes into discrete numeric units. These grids were used in algebraic algorithms in ARC/GRID (Environmental Systems Research Institute, Redlands, California, USA).

A bathymetric model of Murrells Inlet was generated using a differential global positional system and a depth finder to obtain real-time depth measurements. The result is an extensive spatial model of mean low-water depth, i.e., average depth at low tide, throughout the estuary. Summary statistics were obtained using the MINITAB statistical package. Measurements of UV intensity from band A, i.e., wavelengths of 320–400 nm, were obtained during concurrences of low tide and 12:00 noon  $\pm 2$  h in an effort to characterize potential high-hazard, midday UV conditions. The

UV-B band, i.e., wavelengths of 280–320 nm, was not measured in the field because of tide and time limitations that precluded the measurement of both bands of UV. Measurements were obtained with the SUL-005 detector at the sediment-water interface along a shore-to-depth transect to a depth at which UV was approximately 1% of that measured at the surface (UV-A  $\approx 25~\mu\text{W/cm}^2$ ). UV-A extinction coefficients were determined from plots of light intensity vs. depth (Kirk 1994). Ten sites were measured monthly, and all 30 sample sites were measured during October 1998, May 1999, and August 1999. UV extinction coefficients ( $K_e$ ) for each site and month were determined from natural logs of linear plots of UV-A values vs. depth (Kirk 1994):

$$K_e = \ln(I_o/I_d)/z \tag{3}$$

where  $K_e = UV$  extinction coefficient, z = depth,  $I_o = UV$  intensity at surface, and  $I_d = UV$  intensity at depth.

U.S. National Wetlands Inventory (NWI) data for Murrells Inlet were used to identify areas that were classified as appropriate intertidal and subtidal habitat for *A. tenuiremis*. These areas extended from the tidal creek fringe dominated by *Spartina alterniflora* (smooth cordgrass) to the low intertidal mudflat.

To generate the final hazard model, cells in the PAH hazard grid, the UV hazard grid, and the habitat grids were assigned values and multiplied across grids. UV hazard grid cells for each month were assigned a value of 2 if their UV levels were equal to or greater than the minimum level toxic to A. tenuiremis at 1 cm depth in the laboratory. All other cells were assigned a value of 1. Similarly, PAH hazard grid cells with PAH levels equal to or greater than the minimum laboratory level to contribute to photoenhanced toxicity for A. tenuiremis were assigned a value of 2. Each UV hazard grid was multiplied by the PAH hazard grid, resulting in a composite grid of hazard for the estuary. Hazard grids were then multiplied by the suitable habitat grid. The habitat grid cells were coded 2 if the NWI classification corresponded to one of the following habitat types: Estuarine Subtidal Unconsolidated Bottom, Estuarine Intertidal Unconsolidated Shore, Regularly Flooded, Estuarine Subtidal Unconsolidated Bottom, or Estuarine Intertidal Unconsolidated Shore, Irregularly Flooded. The resulting grid represents areas that meet all three criteria noted above as potentially hazardous to A.

tenuiremis in Murrells Inlet.

#### **RESULTS**

Key results for copepod survival, UV intensity, and hazard modeling are presented below; supplemental results, discussion, and conclusions are shown in Appendix 1.

# Copepod survival

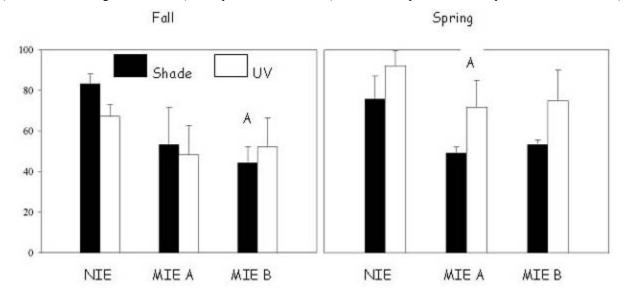
In the October 1998 experiment, no significant UV-related mortality was observed for adult females in sediments from the contaminated sites at the Murrells Inlet estuary (MIE) or the control sites at the North Inlet estuary (NIE). However, in the October 1998 toxicity test, the UV-shaded treatment with the control sediments yielded significantly higher survival than UV-shaded treatment of contaminated sediments (P = 0.0009). During the May 1999 experiment, copepod adult female survival was significantly higher in UV-exposed sediments from the control site.

Survival was significantly lower in UV-exposed contaminated sediments from MIE A than in UV-exposed sediments from the control site (P = 0.0035). The presence of UV, sediment contamination, and season were all significant predictors of survival in both experiments (ANOVA; P < 0.05). A summary of copepod survival is shown in Fig. 3.

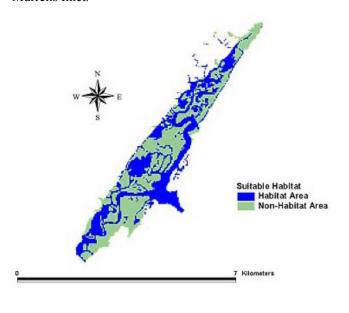
## **UV** intensity

Suitable *Amphiascus tenuiremis* habitat in Murrells Inlet was mapped using the U.S. National Wetlands Inventory data (Fig. 4). UV intensities for the months of October 1998, May 1999, and August 1999 were developed for A. tenuiremis habitat on the bottom, i.e., the sediment-water interface. In May 1999, the maximum derived UV intensity at the sediment-water interface was 2344 (μW/cm<sup>2</sup>, and 63% of total suitable organism habitat in the estuary showed UV intensities at the bottom equal to or greater than the minimum observed to cause toxicity to A. tenuiremis in the laboratory. In August 1999, the maximum UV intensity at the bottom was 2143 µW/cm<sup>2</sup>, with 63% of suitable habitat areas meeting or exceeding the toxicity minimum in the laboratory. In October 1998, the maximum bottom UV was 1635 µW/cm<sup>2</sup>, and 62% of areas in the creek beds showed UV intensities equal to or greater than the laboratory-determined UV minimum. Seasonal results are shown in Figs. 5–7.

**Fig. 3.** Adult *Amphiascus tenuiremis* female copepod survival in the October 1998 (Fall) and May 1999 (Spring) toxicity tests. 'A' indicates female survival is significantly reduced in contaminated sediment from one of the two Murrells Inlet Estuary sites (MIE A and MIE B) site relative to that of copepods in the celean sediment from the North Inlet Estuary site (NIE) under the same light conditions (UV exposed or UV shaded). Error bars represent one sample standard deviation (SD).



**Fig. 4.** Suitable habitat for *Amphiascus tenuiremis* based on the U.S. National Wetlands Inventory classification of Murrells Inlet.



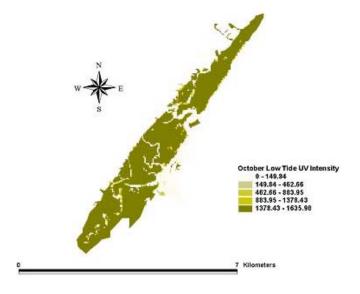
Distribution patterns of UV intensity on the bottom were found to be similar for all three months. Deeper areas near the mouth of the inlet were characterized by little or no UV penetration. Shallower parts of the northern and southern inland areas saw the highest UV penetration at the bottom. The inland areas of the extreme northern and southern portions of the estuary received enough UV at the bottom to reach the laboratory minimum for causing toxicity to A. tenuiremis. This suggests that the northern, urbanized portion of the estuary creek bed, with its high total PAH levels, could be susceptible to photoenhanced toxicity. For some parts of the inland estuarine areas, depths were 0 m at low tide, and UV on the creek bed was equal the initial UV intensity. UV intensity on the bottom appeared consistent across the months of May, August, and October despite seasonal trends in UV extinction levels. This may be because trends in UV levels at the surface may counteract seasonal trends in extinction.

#### Hazard models

Geographic areas that could be considered hazardous because of phototoxicity to *A. tenuiremis* within the Murrells Inlet estuary were mapped for low and mid tide during the months of October 1998, May 1999, and August 1999 (Figs. 8–13). For all three months, these hazardous areas were located during low tide in the urbanized extreme northern portion of the estuary, mainly on the inland side. In May 1999, 929,416 m<sup>2</sup> or

approximately 16% of the suitable *A. tenuiremis* habitat was susceptible to phototoxic effects during low tide. In the creek bed, susceptible areas were either exposed during low tide or less than 0.5 m deep. For August 1999, also during low tide, 16% (935,072 m²) of suitable habitat was susceptible. In October 1998, 16% of suitable habitat was susceptible (929,416 m²) at low tide. At mid tide, the areas susceptible to phototoxic effect were reduced by half for all three months. Approximately 503,355 m² or 8.5% of suitable habitat was susceptible in May 1999, 8.7% or 509,011 m² in August 1999, and 8.4% (495,814 m²) in October 1998. These areas were also in the northern urbanized portion of the estuary. No areas were susceptible as a phototoxicity hazard to *A. tenuiremis* during high tide.

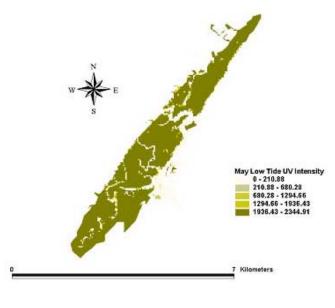
**Fig. 5.** Intensity of ultraviolet light in the UV-A range  $(\mu W/cm^2)$  on the bottom (the sediment-water interface) during low tide for October 1998.



Creek beds considered hazardous to *A. tenuiremis* because of phototoxicity during low and mid tide were located in the northern portions of the estuary in all three months. The amount of area susceptible to photoxic effects during mid tide was significantly reduced relative to those at low tide. These results suggest that depth has implications for our hazard model. Most of the area considered susceptible at low tide for all months was less than 0.32 m deep, and the total area did not change substantially during these months. This is contrary to what was expected, because neither surface UV intensity nor UV extinction was consistent throughout the year. Surface UV intensity was highest during the summer

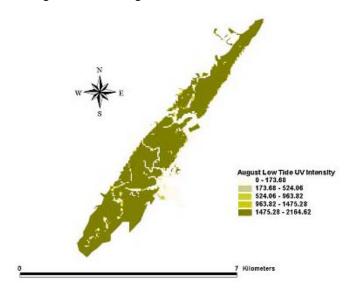
months, when UV extinction levels were also high. The converse was true of the winter months. Peaks in UV extinction during the summer could be caused by sediment resuspension and phytoplankton population dynamics (see above). The result of these seasonal trends is a counteraction that causes little or no seasonal change in the UV intensity reaching the bottom where *A. tenuiremis* lives.

**Fig. 6.** Intensity of ultraviolet light in the UV-A range  $(\mu W/cm^2)$  at the bottom (the sediment-water interface) during low tide for May 1999.

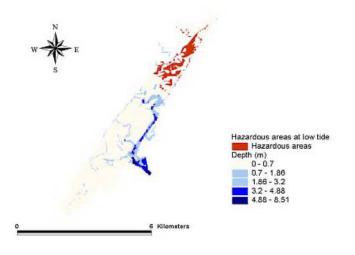


The area in which phototoxicity poses the greatest hazard to A. tenuiremis in the northern portion of Murrells Inlet is characterized by more residential and industrial development than other parts of the inlet. All susceptible areas were within 600 m of urbanized sections, suggesting that urbanization may be linked to the potential for photoenhanced toxicity. These areas also had the highest levels of total polycyclic aromatic hydrocarbons (PAHs). Initially, these areas were expected to be hazardous because of their PAH content, but high extinction levels were ultimately expected to reduce if not eliminate this hazard. This expectation was strengthened by the findings of Skjemstad et al. (1993), who showed that PAHs associated with sediments high in silt clay content were protected from photo-oxidation by UV. The results of the present study support these findings. These areas are only likely to be susceptible to photoenhanced toxicity when the water is very shallow, allowing UV to penetrate to the PAH-contaminated bottom sediments. Over all, the hazard to *A. tenuiremis* from the photoenhanced toxicity of Murrells Inlet sediments exists for only approximately 16% of the habitat suitable for the copepod, regardless of the season. Approximately 8% of the estuary is considered hazardous to *A. tenuiremis* during mid tide.

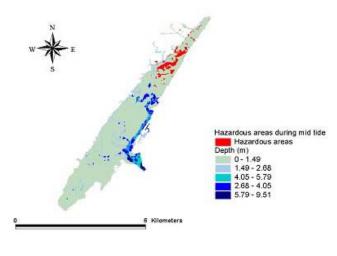
**Fig. 7.** Intensity of ultraviolet light in the UV-A range  $(\mu W/cm^2)$  at the bottom (the sediment-water interface) during low tide for August 1999.



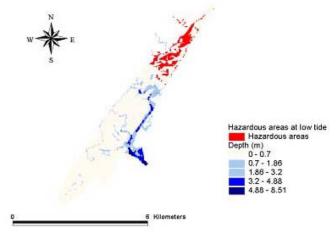
**Fig. 8.** The October 1998 low tide hazard for *Amphiascus tenuiremis* from photoinduced toxicity of polycyclic aromatic hydrocarbons.



**Fig. 9.** The October 1998 mid tide hazard for *Amphiascus tenuiremis* from photoinduced toxicity of polycyclic aromatic hydrocarbons.



**Fig. 10.** The May 1999 low tide hazard for *Amphiascus tenuiremis* from photoinduced toxicity of polycyclic aromatic hydrocarbons.

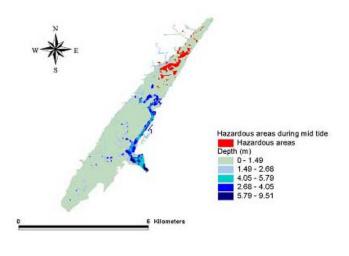


#### **DISCUSSION**

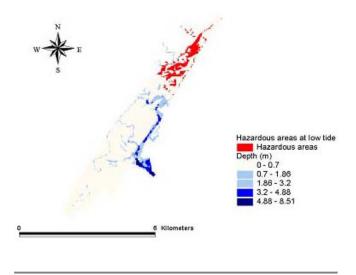
These results clearly show that development-related contaminants have an impact on the ecology of an urbanized estuary. Within the Murrells Inlet estuary (MIE), the areas most susceptible to enhanced phototoxicity from polycyclic aromatic hydrocarbons (PAHs) were located in the northern, urbanized portions of the estuary. Because PAHs result from fossil fuel combustion, the appearance of these chemicals in

Murrells Inlet is assumed to be related to storm water drainage. Increases in PAH contamination from storm water runoff are expected to continue in the Murrells Inlet estuary and other estuaries in the southeastern United States as development pressure expands areas of urbanization and traffic. Increases in PAH contamination, in turn, will lead to increased toxicity to key organisms.

**Fig. 11.** The May 1999 mid tide hazard for *Amphiascus tenuiremis* from photoinduced toxicity of polycyclic aromatic hydrocarbons.



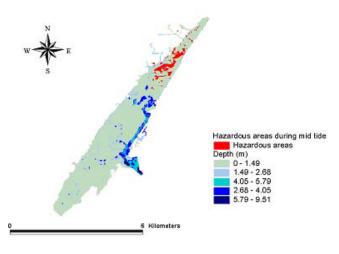
**Fig. 12.** The August 1999 low tide hazard for *Amphiascus tenuiremis* from photoinduced toxicity of polycyclic aromatic hydrocarbons.



Previous work on benthic macrofauna suggests that toxicity in UV-exposed sediment in the Murrells Inlet

estuary is higher than toxicity in North Inlet sediment (control). The highest toxicity was observed in the sediments characterized by highest PAH levels. Experiments conducted without UV indicate that PAH-contaminated sediments from Murrells Inlet were more toxic to *A. tenuiremis* than North Inlet sediments (Kovatch et al. 1999, Wirth et al. 1998). Results from the present study suggest that toxicity of PAH-contaminated sediments from Murrells Inlet is enhanced with co-exposure to UV. In the October 1998 experiments, realized offspring production and clutch size were both affected by PAH-contaminated MIE sediments with UV exposure relative to the same sediments without UV exposure.

**Fig. 13.** The August 1999 mid tide hazard for *Amphiascus tenuiremis* from photoinduced toxicity of polycyclic aromatic hydrocarbons.



Interestingly, the phototoxic response did not vary between MIE sites despite differences in the amount of PAH contamination. This suggests that there is no relationship between the amount of PAH and the phototoxicity in these sediments. One explanation for this may be that, although PAH is more toxic in the presence of UV, the phototoxic response measured in these experiments was caused by the presence of another contaminant in these field-collected sediments (see Ankley 1994). Another possibility is that differences in PAH-related phototoxicity cannot be measured at these PAH levels.

In this study, co-exposure of meiobenthic copepods to

UV and PAH-contaminated sediments resulted in increased mortality, enhanced reproductive toxicity, and reduced clutch sizes. However, toxic responses of A. tenuiremis to PAH and UV co-exposure were end point- and season-specific. The fact that season was a significant variable in all the regression models provides evidence that seasonal differences in sediment toxicity can be important. These differences are likely manifest as changes in the biogeochemical properties of the sediments and in the relative composition of sediment mixtures, e.g., labile and refractory organic carbon, grain size, etc. Additional experiments to identify the toxic effects of individual PAHs should include sediments spiked in a manner that promotes the identification and evaluation of additive toxicity to mimic the predominant PAHs in this estuary. See Appendix 1 for continued discussion.

This study provides some insight into the potential ecological hazards associated with PAH contamination resulting from increased development pressure. Population increases accompanied by more development, impervious surfaces, and vehicular traffic, i.e., sprawl, in the Murrell's Inlet area suggest that PAH contamination in the Murrells Inlet estuary is likely to increase as well. The aesthetic, economic, and ecologic value of estuaries demands that the impacts of these kinds of anthropogenic activities be more precisely evaluated before the function and integrity of estuarine systems are further compromised.

# **CONCLUSIONS**

Integration is the key to a successful environmental impact assessment and systems analysis (Fedra 1994). Managers should therefore make use of available computer technologies that enhance the integration process (O'Regan 1996) and provide visually oriented environmental models to support their decisions (Giertsen 1994). In the present study, laboratory data were integrated with field data and historical data on polycyclic aromatic hydrocarbons (PAHs) from Murrells Inlet to establish a spatial model of the potential hazard from the photoenhanced toxicity of PAHs in Murrells Inlet estuary, even if the small number of site samples processed in laboratory bioassays limits the comprehensiveness of these hazard maps. This study may provide general information about the minimum level of PAH contamination to induce a phototoxic response from A. tenuiremis.

Based upon laboratory observations of toxicity at specific UV levels and PAH concentrations and historical site-specific PAH level data, predictions were made of the potential for photoinduced toxicity at locations in the estuary that are characterized by levels similar to or greater than those levels tested in the laboratory. Predictions at specific sites not tested in the laboratory were also made. These further predictions were based upon historical data on the distributions and concentrations of PAHs throughout the Murrells Inlet estuary. The end result is a series of maps that represent the hazard presented by photoinduced toxicity during three different seasons of the year. See Appendix 1 for additional conclusions.

Research that integrates laboratory and spatial modeling techniques can provide important descriptive data for setting sediment-quality criteria and for developing science-based coastal regulations and monitoring. In this case, hazards to A. tenuiremis associated with increased PAH contamination in an urbanized estuary were demonstrated through the integration of laboratory and spatial modeling techniques. Although the model was conservative, the effects of development-related PAH toxicity on a key species, A. tenuiremis, were clear. The Murrells Inlet estuary is typical of other developing or urbanized estuaries in the southeastern United States. Other estuaries experiencing high growth and the resultant increases in impervious surfaces and vehicular traffic are likely to exhibit similar effects.

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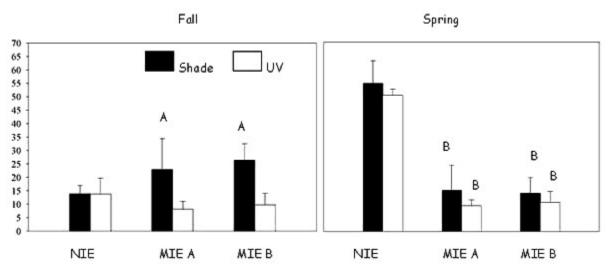
#### **APPENDIX 1**

# **Supplemental results**

# **Realized offspring production**

During the October 1998 experiment, realized production of offspring for *Amphiascus tenuiremis* was significantly less in UV-exposed contaminated sediment in the two Murrell Inlet estuary sites, i.e., MIE A and MIE B, than for the same sediment without UV exposure (P < 0.001; Fig. A1.1). In the May 1999 experiment, realized reproductive success in UV-exposed contaminated sediment was significantly lower than UV-exposed clean sediment from the North Inlet control site (P < 0.0001). Meanwhile, realized offspring production in the UV-shaded contaminated sediment from MIE A and MIE B was also significantly lower than it was in the UV-shaded clean sediment from the North Inlet (P < 0.0001). In the October 1998 experiment, reproductive output by the copepods was greater for those in UV-shaded contaminated sediment in Murrell Inlet than it was for those in UV-shaded clean sediment in the North Inlet. Realized offspring production in the clean North Inlet sediment was more than three times greater in the spring than it was in the fall. Sediment contamination, presence of UV, and season were all significant predictors of *A. tenuiremis* reproductive success in both October 1998 and May 1999 (ANOVA, P < 0.05).

**Figure A1.1.** Adult *Amphiascus tenuiremis* realized offspring production in the fall (October 1998) and spring (May 1999) toxicity experiments. 'A' indicates offspring production is significantly higher for copepods in UV-shaded contaminated sediment than for copepods in the same sediment exposed to UV. 'B' indicates offspring production is significantly reduced for contaminated sediments in the Murells Inlet Estuary sites (MIE A and MIE B) relative to clean sediments from the North Inlet Estuary (NIE) under the same light exposure. Error bars represent one sample standard deviation.

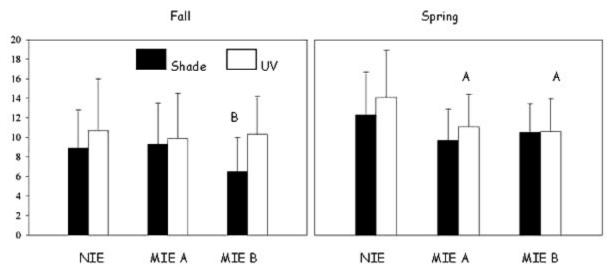


## **Copepod clutch size**

In the October 1998 experiment, average A. tenuiremis clutch size in UV-exposed contaminated sediment at MIE B was significantly higher than the same sediment without UV exposure (P = 0.0001; Fig. A1.2). UV-exposed contaminated sediment from both MIE A and MIE B in the May 1999 experiment had significantly lower clutch

sizes than UV-exposed sediment from the control site (P < 0.0001). No significant clutch-size effects were observed for sediments not exposed to UV from any of the sites during the May 1999 experiment. Sediment contamination and presence of UV were significant predictors of egg clutch size in both bioassays (ANOVA, P < 0.05).

**Figure A1.2.** Adult *Amphiascus tenuiremis* clutch size in the fall (October 1998) and spring (May 1999) toxicity experiments. 'A' indicates clutch size is significantly higher for copepods in UV-shaded contaminated sediment than for copepods in the same sediment exposed to UV. 'B' indicates clutch size is significantly reduced for the Murells Inlet Estuary sites (MIE A and MIE B) relative to the North Inlet Esturay (NIE) under the same light exposure. Error bars represent one sample standard deviation.



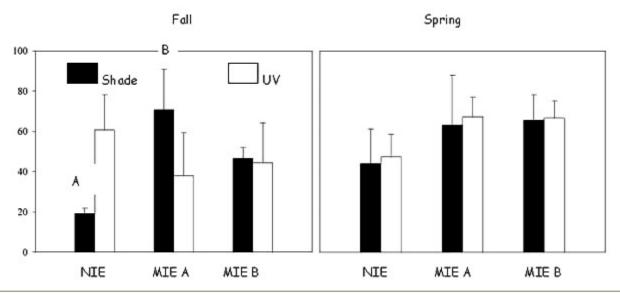
# Analyses of polycyclic aromatic hydrocarbons

Of all the polycyclic aromatic hydrocarbon (PAH) congeners, chrysene, benzo(b)fluoranthene, naphthalene, 2-methylnaphthalene, and pyrene were the most abundant. In October 1998, PAH levels at Murrells Inlet were 1568 and 1193 ng/g for the MIE A and B sites, respectively. At MIE B, the most abundant PAHs were naphthalene (208 ng/g), 2-methylnaphthalene (203 ng/g), and fluoranthene (107 ng/g). The highest individual PAHs at MIE A were chrysene (219 ng/g), benzo(b)fluoranthene (217 ng/g), and fluoranthene (208 ng/g). In May 1999, Murrells Inlet PAH levels were 1179 and 1289 ng/g at MIE A and B, respectively. Predominant PAHs at MIE A were fluoranthene (190 ng/g), pyrene (139 ng/g), and chrysene (115 ng/g). At MIE B, fluoranthene (178 ng/g), pyrene (130 ng/g), and benzo(b)fluoranthene (126 ng/g) were the most abundant. In the October 1998 and May 1999 experiments, fluoranthene levels were abundant at both sites. In October 1998, MIE B naphthalene and 2-methylnaphthalene levels were six to eight times those of MIE A (32 and 25.3 ng/g, respectively), even though the total PAH level at MIE A was the highest. On the other hand, in October 1998, chrysene and benzo(b)fluoranthene levels at MIE A were approximately twice those of MIE B (92.9 and 122 ng/g, respectively). MIE A had the highest total PAH concentration in October 1998, but this level was reduced ~ 400 ng/g in the spring. A summary of results can be found in Table 1 in the text.

# Percent gravid females

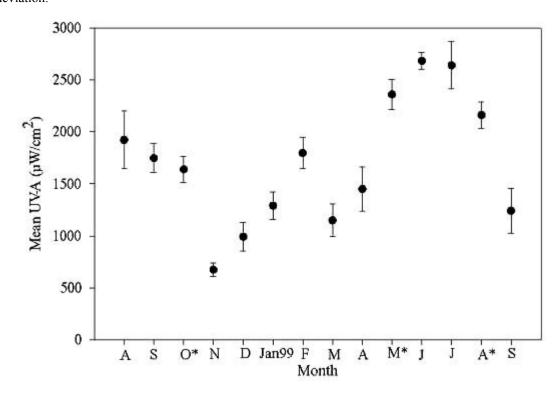
In October 1998, the percentage of females that were gravid at the end of the test was significantly reduced by coexposure to UV and PAH-contaminated sediment at MIE A relative to the same sediment without UV exposure (P = 0.0035; Fig. A1.3). The percentage of gravid females was also significantly higher for UV-shaded contaminated sediment at MIE A than for the UV-shaded sediment from the control site (P < 0.0001). UV exposure of control site sediments had a significant positive effect on the percentage of egg-carrying females when compared to UV-shaded sediment from the same site (P = 0.0004). Contamination and UV exposure were significant predictors of the percentage of females that were gravid at the end of both tests (ANOVA, P < 0.05).

**Figure A1.3.** Percentage of *Amphiascus tenuiremis* gravid females in the October 1998 (fall) and May 1999 (spring) toxicity test. 'A' indicates the percentage of gravid females is significantly reduced in UV-shaded sediment relative to the same sediment exposed to UV. 'B' indicates the percentage of gravid females is significantly higher in UV-shaded sediment than in the same sediment exposed to UV. MIE = Murrells Inlet estuary; NIE = North Inlet estuary. Error bars represent one sample standard deviation.



## **Surface UV intensity**

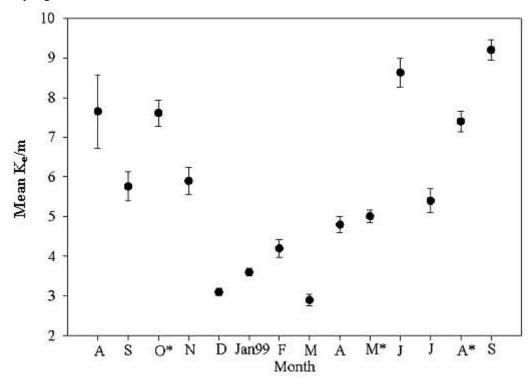
Results of semivariance analyses are provided in Table 2 in the main text. Average UV intensities varied throughout the year (Fig. A1.4), a result that reflects the findings of Literathy et al. (1990). The minimum initial UV intensity occurred in November 1998, when average initial UV was  $674.96 \pm 67.8 \, \mu \text{W/cm}^2$  (mean  $\pm$  SE). Other low months were the winter months of December 1998 and January 1999, with levels of  $993 \pm 136 \, \mu \text{W/cm}^2$  and  $1291 \pm 133 \, \mu \text{W/cm}^2$ , respectively. September 1999 was also relatively low when compared with other months, at  $1241 \pm 217 \, \mu \text{W/cm}^2$ . In contrast, surface UV measured in June of 1999 was as high as  $2682.60 \pm 80.5 \, \mu \text{W/cm}^2$ . Other summer months, i.e., May, July, and August, were consistently high as well, with UV-A levels of  $2360 \pm 147 \, \mu \text{W/cm}^2$ ,  $2639 \pm 225 \, \mu \text{W/cm}^2$ , and  $2161 \pm 124 \, \mu \text{W/cm}^2$ , respectively. The results of the UV sampling were not surprising, because lower UV levels were observed during winter months, and the highest levels were measured in the spring and summer, as observed by Literathy et al. (1990). Complete UV samplings of all sites occurred during October 1998, May 1999, and August 1999. These months represent three unique seasons and were therefore used in the grid modeling.



**Figure A1.4.** Average initial intensity of ultraviolet light in the UV-A range by month. Error bars represent one sample standard deviation.

#### **Extinction coefficients**

Extinction coefficients varied considerably during the year (Fig. A1.5). Trends in extinction levels increased in the fall of 1998, dropped off considerably during winter and spring months, and rose again in the summer and fall of 1999. The highest extinction coefficients, i.e., lowest UV levels penetrating the water column, were observed during the summer and fall months of August, September, and October of 1998 and June, August, and September of 1999, with  $K_e$  values of  $7.65 \pm 0.923/m$  (mean  $\pm$  SE),  $5.76 \pm 0.37/m$ ,  $7.61 \pm 0.33/m$ ,  $8.63 \pm 0.36/m$ ,  $7.41 \pm 0.26/m$ , and  $9.16 \pm 0.26/m$ , respectively. The lowest extinction levels occurred in December 1998 and January, February, and March of 1999, with  $K_e$  values of  $3.11 \pm 0.09/m$ ,  $3.55 \pm 0.1/m$ ,  $4.22 \pm 0.22/m$ , and  $2.92 \pm 0.14/m$ , respectively. Average extinction coefficients at MIE A and MIE B ranged from a minimum of  $3.75 \pm 0.15/m$  to a maximum  $12.75 \pm 1.47/m$  (Fig. 13 in main text). The lowest average  $K_e$  was observed close to the mouth of the inlet, and the highest was calculated at a site in the extreme northern portion of the estuary, adjacent to the landward side of the estuary. However, it is important to note that  $K_e$  values at the different sites varied greatly throughout the year. For example, at one site,  $K_e$  was  $6.34 \pm 0.37/m$ , but had a range of 2.03-22.35/m. This type of variation occurred at one-third of all the sites measured.



**Figure A1.5.** Average extinction coefficients for all months. Error bars represent one sample standard deviation. An asterisk (\*) indicates sampling at all 30 sites.

Extinction coefficients varied considerably over space and time. The highest values and lowest UV penetration were concentrated in the shallow inland areas of the estuary, particularly in the extreme northern and southern portions of the inlet. Lower values and the highest UV penetration were focused near the mouth of the inlet and in nearby deeper portions of MIE. Silt-clay content may help to explain distribution of  $K_e$ . The areas characterized by higher silt-clay, and thus higher carbon, contents were also found to have higher extinction coefficients. Lower silt-clay, and thus higher sand, content was observed in the deeper parts of the inlet, close to the mouth. This suggests that sediments with high silt and clay contents, which have a tendency to become resuspended by activities such as boating and wave action, will scatter incident light, resulting in less UV penetration through the water column, as in Ireland et al. (1996). Because of a decrease in observed anthropogenic activities during the winter months, resuspension of particulate matter is less likely, resulting in lower extinction coefficients and higher UV penetration to the creek bed.

When silt-clay content was used to predict  $K_e$  in a linear regression model, this variable was found to be significant (P < 0.0001) in the regression. These results suggest that  $K_e$  may be explained and/or predicted in terms of silt-clay content. It may be possible to develop a model to calculate  $K_e$  if the silt-clay content of bottom sediments is known by measuring incident UV intensity. It was anticipated that, even though the highest levels of PAHs were found in the northern, urbanized portion of the estuary, the high UV extinction occurring there may effectively minimize any potential for hazard, thus providing a protective effect in these more contaminated areas.

Seasonal trends in extinction coefficients may be explained, in part, by phytoplankton cycles in the water column. Phytoplankton population densities are often highest during the month of September in Murrells Inlet (D. L. White, *personal communication*) and in other estuarine areas (Smayda 1957, Patten et al. 1963, Mallin et al. 1991). Peaks in phytoplankton populations have also been observed during the month of June (Carpenter 1971). These peaks in phytoplankton in the water column coincide with peaks in extinction levels observed during the current study. UV-A and UV-B seawater extinction coefficients (see *Methods*) were  $4.88 \pm 0.3$ /m and  $6.9 \pm 1.1$ /m (mean  $\pm$  SE), respectively, at the sediment-water interface. Using these coefficients in the Kirk (1994) equation,

actual *A. tenuiremis* UV-A and UV-B exposures were  $581.17 \pm 5 \,\mu\text{W/cm}^2$  and  $76.91 \pm 2.25 \,\mu\text{W/cm}^2$ , respectively. These values are approximately 32% of fall (October 1998) field exposures. Spring mean sediment-water interface UVA and UV-B exposures were  $583.06 \pm 4.83 \,\mu\text{W/cm}^2$  and  $77.62 \pm 1.12 \,\mu\text{W/cm}^2$ . Laboratory test values are approximately 25% of spring field exposures based upon May 1999 field measurements. Water quality parameters for the fall and spring toxicity tests were within acceptable limits (American Society for Testing and Materials 1980).

The spring toxicity tests indicated that co-exposure of *A. tenuiremis* to UV and PAH-contaminated sediments caused significant mortality (30% on average). These results are consistent with studies using *Rhepoxynius abronius* (Swartz et al. 1990, Boese et al. 1998), *Lumbriculus variegates* (Ankley et al. 1994, Monson et al. 1995), *Hyallela azteca* (Ankley et al. 1994), and *Daphnia magna* (Holst and Giesy 1989). However, in the fall, co-exposure of *A. tenuiremis* to UV and PAH-contaminated sediments did not appear to cause significant lethal toxicity at the UV and somewhat higher total PAH levels tested here. These findings are consistent with a study involving the midge *Chironomus tentans* (Ankley et al. 1994), *H. azteca*, and *L. variegates* and similar predominant PAH congeners, i.e., fluoranthene, pyrene, and chrysene. When exposed for 10 days to PAH-contaminated sediments with UV, *C. tentans* survival was unaffected, but *H. azteca* and *L. variegates* suffered more than 60% mortality.

Increased copepod reproductive toxicity occurred during the October 1998 experiment in UV-exposed contaminated sediments relative to non-UV exposures of the same sediments. Similarly, reproductive toxicity was seen in the branchiopod *Daphnia magna* when exposed to low levels of UV (31–117 µW/cm² UVA) and 190–880 µg/L anthracene solutions (Holst and Giesy 1989). Reproductive toxicity also occurred during the May 1999 experiment, but these results were similar for contaminated sediments exposed to UV or not exposed to UV. This indicates that reproductive toxicity was not enhanced by UV exposure. In May 1999, realized offspring production in control sediments exposed to UV and not exposed to UV was more than three times greater than that measured in October 1998. UV-related clutch size effects were not consistent across experiments. In the October 1998 experiment, UV exposure actually enhanced egg clutch size at one contaminated sediment site. In May 1999, UV-exposed contaminated sediments resulted in significantly smaller clutch sizes than the UV-exposed control sediments, suggesting a UV effect. Significant differences in clutch size were not observed among contaminated sediments that were not exposed to UV, indicating that toxicity tests conducted in the absence of UV light may not measure potential reproductive toxicity in the environment.

UV exposure had an effect at both the control and the PAH-contaminated sites during the October 1998 experiment. The percentage of egg-carrying females was significantly greater in the UV-exposed control sediment, whereas the UV-exposed contaminated sediment showed significant increased toxicity relative to the same sediment without UV exposure. These results suggest that UV alone is not toxic to *A. tenuiremis*. Rather, it appears to enhance reproduction. However, when UV exposure is combined with PAH contamination, enhanced reproductive toxicity can occur.

A model to predict the 10-d LC50 (lethal concentration, 50%) phototoxicity of specific PAH congeners was developed by Swartz et al. (1990). This model generally characterizes the PAHs with lower solubilities as the most phototoxic. According to the model, among the most phototoxic PAH congeners, arranged from greatest to least phototoxic potential, are anthracene (the most phototoxic), chrysene (extremely phototoxic), benzo(a)pyrene, and benzo(b)fluoranthene. All of these congeners were detected in Murrells Inlet sediments, with chrysene and benzo(b)fluoranthene common in our test sediments. Copepod reproduction in all PAH-contaminated sediment treatments was low, whereas chrysene and benzo(b)fluoranthene were consistently high (92.9–219 ng/g) relative to other congeners. The present study may extend the predictability of the Swartz model to copepod reproductive endpoints.

# **Supplemental conclusions**

Without limiting exposure to specific contaminants, i.e., spiking sediment with known levels of specific

chemicals, it is impossible to attribute toxicity to a particular class of contaminants. However, because contaminated sediments in the field are composed of a complex mixture of chemicals nearly impossible to duplicate in the laboratory, it is necessary to use field sediments in toxicity tests. Newsted and Giesy (1987) developed a method for predicting the photoinduced toxicity of PAH mixtures to another zooplankton, *Daphnia magna*, based on PAH structure and absorption properties as well as organism transmittance. A similar study could be designed for our organism using the PAHs comprising our total PAH level. Another predictive model developed by Swartz et al. (1990) explained the toxicity of field PAH mixtures by considering factors such as toxic units, PAH structure, additivity, and the 10-d LC50 determination. Developing a study such as this one using Murrells Inlet sediments and incorporating the UV effect would help predict photoinduced toxicity in Murrells Inlet.

The previously noted *C. tentans* survival after UV exposure may be attributed to a more efficient system for dealing with oxidative stress caused by photoactivated PAHs or an exoskeleton that reduces UV penetration to assimilated PAHs. These possibilities should be explored with respect to *A. tenuiremis*. It would be beneficial to conduct a study co-exposing *A. tenuiremis* to UV and water-only PAH solutions to investigate whether these fine sediments refract UV. The tendency of *A. tenuiremis* to burrow may result in the reduction of UV-activated bioaccumulated PAHs. Thus, organism ecology and structure should be considered when evaluating PAH phototoxicity. In addition, the potentially different mixture effects on mortality should be explored further. Presently, neither total PAH concentration nor individual concentration differences can easily explain the lack of consistency in fall and spring PAH lethality.

In the future, the hazard model will be improved by additional laboratory experiments using a range of sites in the Murrells Inlet estuary with varying UV intensities. By obtaining results from a variety of sites, a more complete model could be generated with a broader range of PAH levels. It would also be beneficial to investigate the length of co-exposure to PAHs and UV as a potential hazard factor. In addition, replicate UV measurements at sites, including determination of UV-B, would more effectively characterize UV intensities during the course of the day, potentially supporting a more realistic hazard model.

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