

## Polychlorinated Biphenyl- and Mercury-Associated Alterations on Benthic Invertebrate Community Structure in a Contaminated Salt Marsh in Southeast Georgia

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**Abstract.** The community structure of a benthic macroinvertebrate assemblage in a contaminated salt marsh was evaluated as part of an ecological characterization of a former chloralkali production facility in Georgia. Sample locations were chosen based on a gradient of the primary contaminants of concern, total mercury and polychlorinated biphenyls (PCBs), primarily Aroclor 1268. Sediment concentrations of Aroclor 1268 ranged from 2.3 to 150 mg/kg dry weight, while mercury concentrations ranged from 15 to 170 mg/kg dry weight in the study area. Mercury and PCBs were determined to be co-located in the sediments. Total organic carbon composition of the sediments was negatively associated with PCB and mercury concentrations. A total of 29 benthic taxa was identified in 49 samples; replicate samples were taken at each of five sampling locations. Mean infaunal density across all sampling locations was estimated at approximately 61,000 to 234,000 organisms m<sup>-2</sup>. Overall, polychaetes comprised 57% of the infaunal community with *Manayunkia aestuarina* as the dominant species. Oligochaetes, nematodes, crustacea, insects, and gastropods comprised 23.0, 18.0, 1.0, 0.7, and 0.2% of the overall benthic community, respectively. Density estimates of individual species between sampling locations showed no consistent patterns in response to pollutants. However, an analysis of higher taxonomic levels revealed some general trends. In uncontaminated areas, the benthic community was dominated by nematodes and oligochaetes, whereas moderate to highly contaminated areas were dominated by polychaetes and a smaller percentage of oligochaetes and nematodes. A trophic analysis of the same data set revealed that the community shifted from an evenly distributed percentage of surface and subsurface feeders in the uncontaminated areas to a community dominated by surface feeders in the more contaminated locations. Carnivores comprised from 0.13 to 0.90% of the trophic structure, with the percentage of carnivores generally decreasing with increasing contamination. Mercury and PCBs were bioaccumulating in representative marsh benthic invertebrates, presenting a poten-

tial source of contaminants to marsh consumers. Tissue PCB and tissue mercury concentrations were positively related to sediment PCB and mercury concentrations, respectively. A standard 14-day toxicity test using the amphipod *Leptocheirus plumulosus* showed no acute toxicity across the sampling locations.

Coastal salt marshes are often affected by pollutants due to their proximity to heavy industries, industrial complexes, and urban areas. Because of the diversity of industries that are often in such areas, a single marsh may be contaminated by a variety of related or unrelated chemical compounds. The site currently under evaluation is in southeastern Georgia (Figure 1) and had historically been subjected to wastes from petroleum refining, paint and solvent processing, and chlor-alkali production. Primary site contaminants were determined to be mercury and PCBs, presumably as by-products of chlor-alkali production. Polycyclic aromatic hydrocarbons (PAHs) and lead were also found at the site at low concentrations, but were not a focus of the current study. Contaminants were released from the process area of the facility into a neighboring salt marsh from two distinct outfall locations. Due to tidal influence, the contaminants were thoroughly distributed throughout large sections of the marsh. An evaluation of benthic invertebrate community health was determined to be a key component in assessing the ecological damage potentially resulting from the introduction of such pollutants into the salt marsh ecosystem.

Because of their life-history characteristics, benthic organisms are suitable for evaluating environmental change and the impacts of environmental contaminants. These general characteristics include a sedentary lifestyle that reflects local sediment conditions, life spans that integrate contaminant impact over time, habitation in the sediment and water interface where contaminants accumulate, and differential levels of tolerance to contaminants among different species represented in the community (Dauer *et al.* 1993). The use of benthic macrofauna in the evaluation and monitoring of contaminated areas can facilitate the determination of spatial and temporal distributions

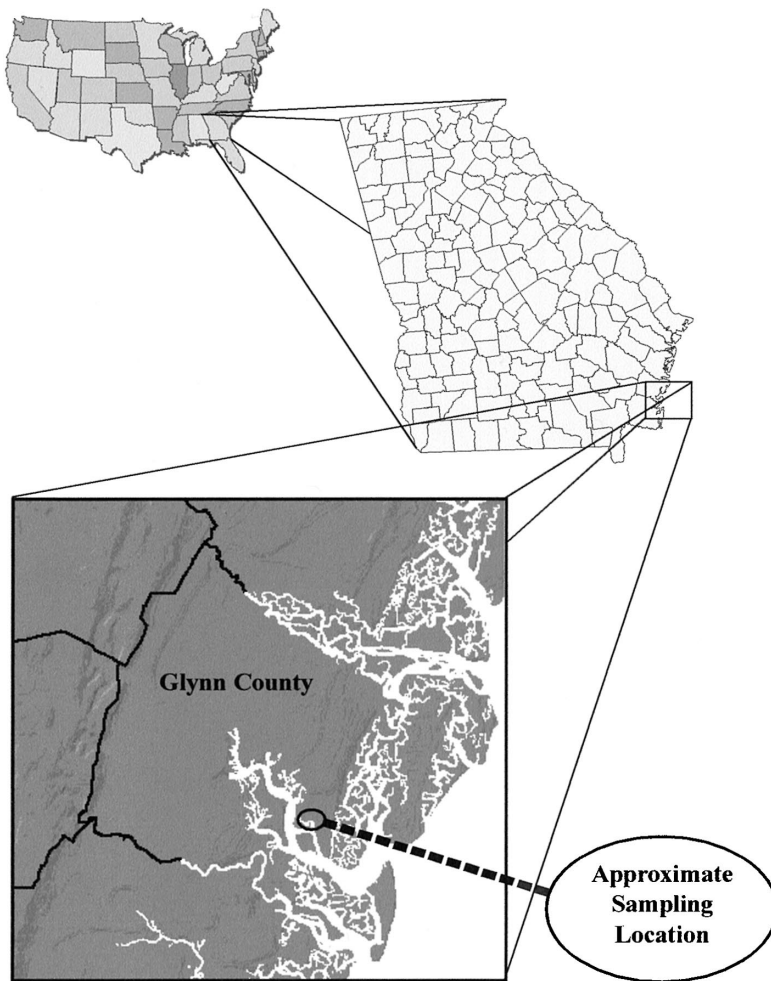


Fig. 1. General location of study area

of contaminants, as well as the magnitude of contaminant impacts (Bilyard 1987). Estimates of species richness used to characterize community structure sometimes show patterns of low diversity for habitats classified as stressed. In addition, community composition changes from dominance by long-lived equilibrium species in relatively unstressed situations to dominance by short-lived opportunistic species in relatively stressed situations (Dauer 1993). *In situ* benthic studies provide direct information of the impacts on the ecosystem of interest and allow examination of contaminant effects on local indigenous species (Bilyard 1987).

Benthic macroinvertebrates are also important indicators of potential impacts to higher trophic levels because of their place in the food web. Salt marsh benthos form the foundation of the estuarine food web, and as such they are an important link for energy flow between primary production in the marsh and upper trophic level species in nearby estuarine waters. Food webs may be altered or shifted in areas where the benthic community is injured due to the presence of contaminants, thereby impacting consumer-level organisms. For the most part, however, higher organisms can migrate to other feeding areas when food becomes limiting. The primary effects in such a case would center on the interruption of ecosystem processes such as energy flow or nutrient cycling. Important food chain concerns manifest when the benthic community appears healthy, at least in terms of density and diversity (and therefore food availabil-

ity), but contaminants are bioaccumulative and biomagnified. In this case, benthic forage provides a dose of a potentially toxic contaminant to consumer organisms, thereby potentially causing a cascading effect up the food chain. Because salt marsh systems are important nursery areas and provide habitat for organisms that are sources of food for higher trophic levels, biomagnification potential of site contaminants is of primary concern.

Based on these issues, we posed the following questions: (1) Is the structure of the benthic community altered along a contamination gradient of PCBs (primarily Aroclor 1268 and weathered forms) and mercury that is representative for the site? (2) Is the trophic structure of the benthic community altered along the identified contamination gradient? (3) Are sediments sampled along this gradient acutely toxic to surrogate benthic species in toxicity tests? (4) Are PCBs and mercury accumulating in the tissues of benthic invertebrates in the marsh, thereby providing a direct link between organisms foraging in the marsh and potentially toxic contaminants?

## Materials and Methods

### *Sediment Sampling*

Sediment was collected using decontaminated stainless steel trowels or a 10-cm hand bucket auger. The bucket auger was used only when

sediment was collected under water (*i.e.*, during an incoming or outgoing tide). Plant material was excluded from the samples by pushing aside the current annual growth of vegetation and root mat. Because of the size of the area impacted by contaminants, sediment contaminant screening was conducted to establish a concentration gradient on which to base the benthic sampling regime (Ferraro *et al.* 1991). Sediment was screened for metal contamination using a Spectrace<sup>®</sup> 9000 field-portable x-ray fluorescence (XRF) analyzer and for PCBs using an Ohmicron Rapid Prep<sup>®</sup> immunoassay kit. Screening was performed by collecting 200 g of sediment, which was transferred directly to resealable plastic bags for XRF and immunoassay analyses.

Sediment was collected throughout the study area to locate a biologically significant contamination gradient. To meet the partial mortality assumptions of the toxicity tests that were to be conducted, most of the sampling effort concentrated in areas assumed to be intermediate in contamination. Sediment sampling locations from the drainages and tributaries within the marsh were collected in pairs. At low tide, one sample was collected from the bottom of the tributary, and one was collected from the surface of the marsh immediately adjacent to the tributary. A total of 50 locations were sampled for field screening on the basis of previous analytical results, proximity to potential source areas (such as the historic outfall), local topography, drainage patterns, and habitat. Areas on the marsh surface were selected on the basis of proximity to a particular source area on the site and were sampled to characterize areas likely to accumulate contaminants. The latter included depressions that were likely to contain pooled water during ebb tide.

The results of the field screening and existing data were evaluated and used to select four on-site locations for additional benthic sampling and evaluation. The site locations selected were adjacent to the historic outfall (Location 4), in a tributary draining the outfall lagoon area (Location 3), in a tributary draining the marsh approximately 50 m west of the lagoon area (Location 2), and in a tributary draining the marsh approximately 330 m west of the lagoon area (Location 1). An off-site reference location was also selected.

Sediment for laboratory analysis was then collected from each of the five areas into a 20-L plastic bucket until a volume sufficient to fulfill analytical requirements was obtained. Approximately 12 L of sediment was required for toxicity testing and analysis for metals, polychlorinated biphenyls (PCBs), total organic carbon (TOC), and grain size. Each sample was homogenized, and aliquots were transferred into separate sample containers for subsequent chemical analysis and toxicity testing. Sediment was stored at 4°C prior to further processing.

### *Benthic Macroinvertebrate Sampling*

Benthic macrofauna were sampled to determine impacts to the community structure in the four on-site marsh sampling locations relative to a reference marsh sampling location. In this investigation, macrofauna were defined as those organisms that passed through a 2-mm sieve and were retained on a 0.25-mm sieve. These organisms usually account for approximately 90% of the biomass and 70% of the numbers in an estuarine salt marsh.

Macrofauna were collected from the reference location and each of the four on-site sediment sampling locations identified by field screening. A total of 10 replicates was collected from a 1-m<sup>2</sup> portion of the intertidal marsh using a 3-cm-diameter acetate core. One replicate sample from the reference area was lost, which decreased the number of replicates in the reference area to nine. Samples were collected by pushing the core approximately 5 cm into the sediment. The core was rotated to shear off the sediment at the bottom, and the sleeve was slowly withdrawn from the sediment. If the sediment did not remain in the acetate sleeve, a plastic cap or gloved hand was placed on the top of the sleeve. The sleeve was then pulled out of the sediment while the suction held the core in place. The sediment was removed from the

acetate sleeve and placed into the sample container. Since chemical analyses were not performed on the samples collected in this manner, the acetate sleeve was wiped free of residue from the previous sample and reused between replicates.

The samples were held in coolers on wet ice prior to field sorting. To separate the organisms from the sediment, a portion of the core was placed on a 2-mm sieve and rinsed with water. To minimize the volume of water used and expedite the separation of the organisms from the sediment and debris, a fine stream of water from a sprayer was used. All organisms, sediment tubes, and debris retained by the sieve were preserved in a 10% buffered formalin solution. Macroinfauna were classified to the lowest possible taxa, sorted, and counted. Each identified taxon was further characterized by feeding strategy, that is, as surface feeders, subsurface feeders, or carnivores (Sacco *et al.* 1994).

### *Toxicity Testing*

The toxicity of sediments sampled from the same locations as the benthic survey was evaluated using a modification of the standard 10-day acute toxicity test using the amphipod *Leptocheirus plumulosus*. Modifications to the test design included an extension of the exposure period to 14 days and the inclusion of behavioral observations. *Leptocheirus* is often used as a surrogate benthic invertebrate in estuarine benthic macroinvertebrate toxicity assays (Schlekat *et al.* 1992; US EPA/US ACE 1994). Test endpoints included behavioral abnormalities, survival, and sediment avoidance.

### *Tissue Concentrations*

Fiddler crabs (*Uca* sp.) and marsh periwinkle (*Littorina* sp.) were collected from several of the same locations as those used for the benthic macroinvertebrate and toxicity assay sampling to gain estimates of representative PCB and mercury body burdens and bioaccumulation potential within the marsh system. Marsh periwinkles were collected at the reference location and at location 2. Fiddler crabs were collected at the reference location and locations 1, 2, and 4. Sampled organisms from each location were depurated for 24 h and then placed in 1-L glass jars on ice prior to analysis. Organisms were pooled from each location into replicates to provide sufficient mass for analysis.

Tissues were frozen at -10°C for 2 h. Each sample was then weighed to the nearest 0.1 g and sectioned into small pieces for homogenization. The tissue sample was then added to a dry, decontaminated stainless steel blender head, covered with dry ice, and blended at high speed for 2 min. After homogenization, the blender head was removed from the blender motor, and the sample was transferred to a clean 250-ml analytical-quality glass jar. A lid was then loosely placed on the sample jar, and the jar was placed in a freezer at -10°C overnight, which allowed sublimation of the dry ice.

For PCB analysis, a 10-g aliquot of tissue homogenate was dried with anhydrous sodium sulfate and then Soxhler-extracted into 250 ml of methylene chloride solvent. The methylene chloride extract was then put through a gel permeation chromatography cleanup process to separate analytes from lipids. The resultant clean solution was then transferred to a TurboVap II<sup>®</sup> system for concentration to 1 ml final extract volume. Sample analysis on the concentrated extract was conducted on a Hewlett Packard<sup>®</sup> 5890A Series II Gas Chromatograph (GC) equipped with an electron capture detector (ECD). Results from the GC analysis were compared with those of a set of reference standards.

For total mercury analysis, a 0.5-g aliquot of the tissue homogenate was transferred to a precleaned digestion vessel. Five milliliters of HNO<sub>3</sub> was then added to the homogenate, and the digestion vials were slowly heated for a period of time sufficient for complete digestion of the tissue sample. The digested homogenate was then transferred to a



100-ml volumetric flask and diluted to volume with deionized water. The sample was then analyzed for total mercury using a Perkin-Elmer<sup>®</sup> Atomic Absorption Spectrophotometer, adapted for the cold vapor technique that is recommended for mercury analysis.

#### Data Analysis—Benthic Macroinvertebrate Survey

Raw density numbers from each replicate sample for each taxonomic group were used to produce a density estimate ( $\#/m^{-2}$ ) of the mean number of oligochaetes, polychaetes, crustacea, gastropods, nematodes, and insects present at each sampling location. The mean number for each major taxonomic group in a given sample was divided by the estimate of the mean number of total invertebrates in each sample to produce a percent community composition index. For example, if the estimated mean oligochaete density was 28,000 oligochaetes  $m^{-2}$  in one reference sample replicate, and the total invertebrate mean density was estimated to be approximately 61,000 invertebrates  $m^{-2}$  in that sample, the percent community composition index of oligochaetes would then be calculated at approximately 46% for that particular sample. Percent community composition estimates were generated for all of the replicate samples taken at each of the five sampling locations. Once these indices were calculated, multiple regression was used to identify potential predictors of community composition from the PCB, mercury, and TOC concentrations measured in each of the marsh samples. Initially, a main effects regression model (*i.e.*, inclusive of PCB, Hg, and TOC concentration) was fit to the percent community composition data. With that as a starting point, and if at least one of the three variables produced a significant F value, simpler and simpler models were fit through an iterative process (backward selection) to obtain the model that best described the data. To fulfill the assumption of normality for the multiple regression model, it was necessary to transform the percent community composition index. Numerous transformations were applied, but the one providing the best results from the Shapiro-Wilk normality test was a simple square root transformation.

Similarly, the data were then divided by feeding strategy following the methods presented in Sacco *et al.* (1994). Organisms were characterized as surface feeders (surface-deposit and suspension feeding organisms), subsurface deposit feeders, or carnivores. As above, percent community composition indices were calculated; however, in this instance, the data were categorized by trophic structure rather than taxonomic group. Once these indices were calculated, multiple regression was used to identify potential predictors of trophic structure composition from the PCB, mercury, and TOC concentrations measured in each of the marsh samples. Initially, a main effects regression model (*i.e.*, inclusive of PCB, Hg, and TOC concentration) was fit to the trophic composition data. With that as a starting point, and if at least one of the three variables produced a significant F value, simpler and simpler models were fit through an iterative process (backward selection) to obtain the model that best described the data. Data transformations were not necessary in the analysis of the trophic structure composition data.

#### Data Analysis—Toxicity Testing

Survival differences in the *L. plumulosus* toxicity test between sediment taken from each of the five sampling locations were initially compared with a one-way analysis of variance, independent of sediment contaminant concentration information. When contaminant concentration data became available, based on the gradient approach used, Pearson correlation analysis was used to identify possible relationships between organism survival in the toxicity tests and contaminant concentrations present in the sediment. Survival data were arcsin square root transformed prior to analysis. Survival data were checked for normality with a Shapiro-Wilk test and for homoscedasticity of error variances with Levene's test prior to further analysis.

#### Data Analysis—Tissue Concentrations

Potential changes in fiddler crab body burden of mercury and PCBs across the sampling locations were measured by simple linear regression. Sediment mercury and PCB concentrations were used as predictors of mercury and PCB body burden.

Because marsh periwinkles were only found at two of the sampling locations, the regression approach taken above could not be used. Potential differences in periwinkle tissue concentrations were determined by a one-way analysis of variance. Tissue contaminant data was checked for normality with a Shapiro-Wilk test and for homoscedasticity of error variances with Levene's test prior to further analysis.

## Results

#### Sediment Chemistry

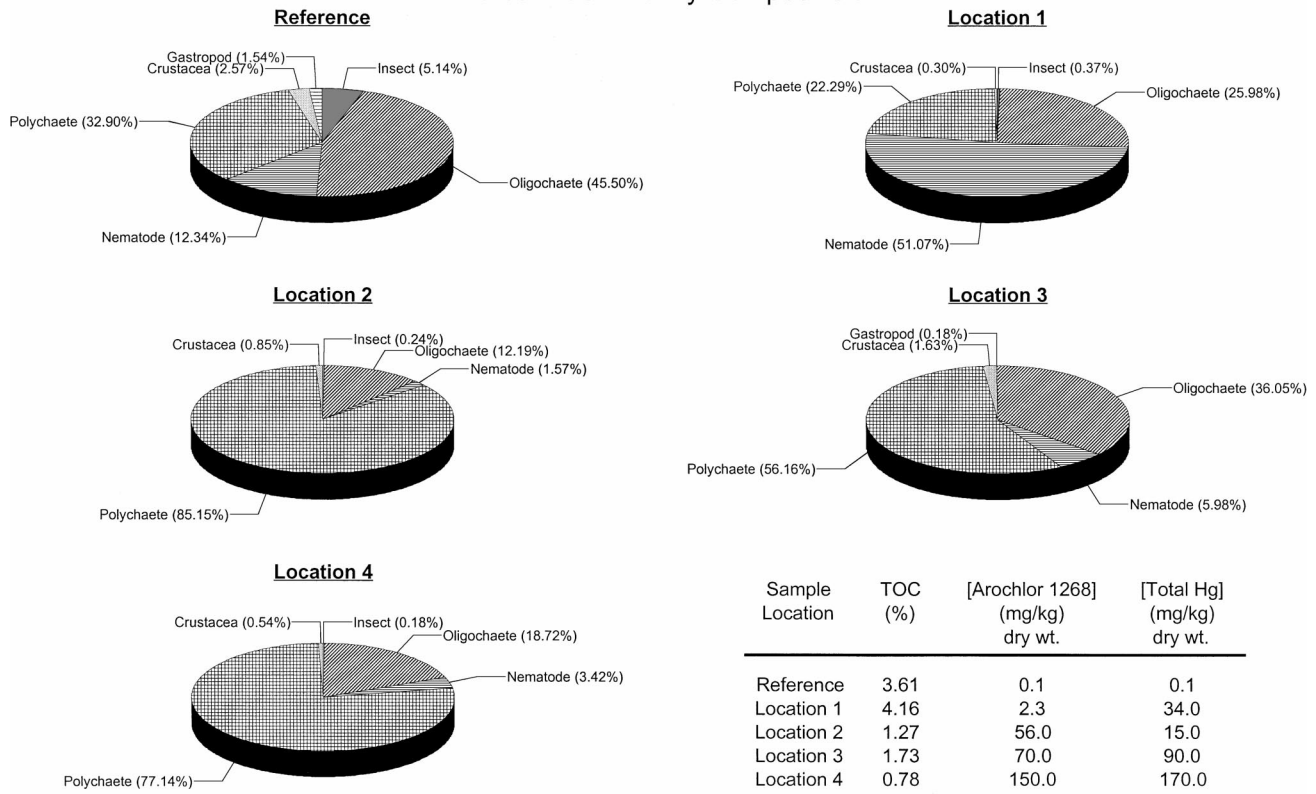
Reference sediments contained low levels of mercury and PCBs, whereas sediments collected from the study site displayed elevated levels of PCBs and mercury (Figure 2, inset table). A concentration gradient was obtained with PCB (Aroclor 1268) concentrations ranging from 2.3 mg/kg to 150 mg/kg dry weight and mercury concentrations ranging from 15 mg/kg to 170 mg/kg dry weight (Figure 2, inset table). Two notable trends were found in the sediment chemistry data. The first relationship was that mercury and PCB contamination were co-located in the marsh, *i.e.*, mercury concentrations were highest in the areas that PCB concentrations were highest ( $r = 0.905$ ;  $p < 0.05$ ). The second relationship was that sediment TOC levels were negatively associated with both PCB and mercury concentrations ( $r = -0.289$ ;  $-0.404$ ;  $p < 0.05$ ). Grain size was similar across the sampling locations, however, the reference station showed a slightly higher percentage of silt than did the on-site samples (Table 1).

#### Benthic Infauna

Differences in the mean densities of individual species were not attributable to the presence of contamination along the sample gradient. The mean density of total oligochaetes ranged from approximately 28,000 to 50,000 individuals  $m^{-2}$  (Table 2). Mean polychaete density ranged from approximately 20,000 to 200,000 individuals  $m^{-2}$  (Table 2). *Manayunkia aestuarina* accounted for 54.5 to 94.5% of all observed polychaetes across the five sampling locations. Mean nematode density ranged from approximately 2,700 to 98,000 individuals  $m^{-2}$  (Table 2). The mean number of crustacea observed in the samples ranged from approximately 400 to 2,000 individuals  $m^{-2}$  (Table 2). Mean insect density ranged from 0 to approximately 3,100 individuals  $m^{-2}$  (Table 2). Mean gastropod density ranged from 0 to 900 individuals  $m^{-2}$  (Table 2). Total mean invertebrate density ranged from approximately 61,000 to 235,000 individuals  $m^{-2}$  (Table 2).

Oligochaete percent community composition was positively associated with increasing TOC content ( $T = 3.26$ ;  $p < 0.005$ ) and negatively associated with increasing mercury concentration ( $T = 2.26$ ;  $p < 0.05$ ) (model  $r^2 = 36.1\%$ ;  $F = 13.0$ ;  $p < 0.001$ ) (Figure 2). Since such a large number of juvenile oligochaetes was found in some of the samples, a model that

### Benthic Invertebrate Percent Community Composition



**Fig. 2.** Taxonomic composition of the benthic invertebrate community along PCB and mercury contamination gradients

**Table 1.** Grain size distribution in sediment samples (results reported as percentage by mass)

Classification	Particle Diameter (mm)	Sampling Location				
		Reference	Location 1	Location 2	Location 3	Location 4
Gravel	4.75–76.2	0.0	0.0	0.0	0.0	0.0
Coarse sand	2.00–4.74	0.0	0.0	0.0	0.0	0.0
Medium sand	0.425–1.99	0.4	1.0	0.4	0.2	0.1
Fine sand	0.075–0.424	5.4	6.8	3.8	2.9	2.6
Silt	0.005–0.074	52.6	24.7	21.7	34.4	39.4
Clay	0.001–0.004	34.5	58.6	55.8	49.4	48.5
Colloids	<0.001	7.2	8.8	18.3	13.0	9.4

included the effects of the three variables on the percent community composition of juvenile oligochaetes was also fit. Subsequent iterations of the regression analysis as above determined that juvenile oligochaetes were present in higher numbers in locations where the sediment TOC was higher ( $r^2 = 22.5\%$ ;  $F = 13.62$ ;  $p = 0.001$ ). Polychaete percent community composition was positively associated with increasing mercury ( $T = 6.47$ ;  $p < 0.001$ ) and PCB ( $T = -4.44$ ;  $p < 0.001$ ) concentrations (model  $r^2 = 60.7\%$ ;  $F = 35.0$ ;  $p < 0.001$ ) (Figure 2). Nematode percent community composition was negatively associated with increasing PCB ( $T = 6.49$ ;  $p < 0.001$ ) and mercury ( $T = -8.68$ ;  $p < 0.001$ ) concentration and TOC ( $T = -4.12$ ;  $p < 0.001$ ) content (model  $r^2 = 65.3\%$ ;  $F = 28.21$ ;  $p < 0.001$ ) (Figure 2). The percent community composition of crustacea was not related to measured PCB, mercury, or TOC concentrations. The significance level of the

effect due to TOC was slightly higher than the critical level ( $p = 0.06$ ) (Figure 2). Insect percent community composition was negatively associated with increasing mercury concentrations ( $r^2 = 20.1\%$ ;  $F = 11.8$ ;  $p = 0.001$ ) (Figure 2). Percent gastropod community composition was positively associated with increasing TOC concentration ( $r^2 = 12.3\%$ ;  $F = 6.6$ ;  $p = 0.01$ ) (Figure 2).

The percentage of subsurface feeders was positively associated with increasing TOC concentration ( $r^2 = 14.7\%$ ;  $F = 8.13$ ;  $p < 0.01$ ) (Figure 3). The percentage of surface feeders was positively associated with both increasing PCB ( $T = 8.84$ ;  $p < 0.001$ ) and mercury ( $T = -6.44$ ;  $p < 0.001$ ) concentrations (model  $r^2 = 66.6\%$ ;  $F = 45.79$ ;  $p < 0.001$ ) (Figure 3). There were no significant relationships identified between the percentage carnivore composition and TOC, PCB, or mercury concentrations (Figure 3).

**Table 2.** Estimated densities (number/m<sup>2</sup>) of benthic organisms sampled (numbers are calculated by extrapolating the mean replicate cores [diameter of the core = 3 cm] to a square meter area)

Group	Organism	Feeding	Reference		Location 1		Location 2		Location 3		Location 4		
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Oligochaete	Oligochaete A	Sub	7,702	4,698	13,723	17,029	12,166	6,228	8,064	4,167	3,537	5,304	
	Oligochaete B	Sub	6,445	5,751	5,234	6,996	11,601	7,145	2,829	5,418	6,932	13,412	
	Oligochaete C	Sub	0	0	0	0	0	0	4,810	11,163	0	0	
	Oligochaete D	Sub	157	472	0	0	707	1,375	566	1,193	1,273	3,557	
	Oligochaete juvenile	Sub	13,518	8,403	30,840	19,770	4,103	4,966	11,883	17,520	2,987	2,688	
	Total oligochaete		27,822		49,797		28,577		28,153		14,729		
Polychaete	<i>M. aestuarina</i>	Surf	18,705	15,939	26,596	25,146	188,580	103,944	8,771	11,489	56,729	49,694	
	<i>S. benedicti</i>	Surf	0	0	6,083	5,337	1,132	1,116	23,908	27,395	566	731	
	<i>Capitella</i> sp	Sub	157	472	0	0	283	895	283	596	1,698	3,114	
	Capitellid A	Sub	314	624	6,649	13,238	1,273	1,939	9,054	11,419	707	1,202	
	Capitellidae other	Sub	943	1,582	2,688	3,430	8,205	8,244	849	2,232	566	1,789	
	<i>Nereidae</i> sp	Surf	0	0	566	1,367	141	447	849	989	424	683	
	Syllidae	Carn	0	0	141	447	0	0	0	0	0	0	
	Orbiniidae	Sub	0	0	0	0	0	0	141	447	0	0	
		Total polychaete		20,120		42,724		199,614		43,856		60,691	
Nematoda	Nematode	???	7,545	7,845	97,897	61,527	3,678	5,131	4,669	5,379	2,688	3,089	
Crustacea	<i>Uca</i> sp	Surf	0	0	0	0	990	955	141	447	141	447	
	<i>Sesarma</i> sp	Surf	0	0	0	0	0	0	0	0	283	596	
	Gammaridae	Surf	472	1,000	283	596	424	683	141	447	0	0	
	<i>Cyathura polita</i>	Surf	786	746	0	0	424	955	990	955	0	0	
	<i>Leptocheilia</i> sp	Surf	0	0	141	447	0	0	0	0	0	0	
	Harpacticoid copopod	?	157	472	141	447	0	0	0	0	0	0	
	Crab larva	Surf	157	472	0	0	141	447	0	0	0	0	
		Total crustacea		1,572		566		1,981		1,273		424	
	Insecta	Collembola	Surf	1,729	2,810	141	447	141	447	0	0	0	0
Dolichopodidae		Carn	314	624	424	683	283	596	0	0	141	447	
Tabanidae		Carn	157	472	0	0	0	0	0	0	0	0	
Ceratopogonidae		Surf	157	472	0	0	0	0	0	0	0	0	
Diptera other		???	786	1,028	141	447	141	447	0	0	0	0	
		Total insecta		3,144		707		566		0		141	
Gastropoda	Gastropoda	Surf	943	1,415	0	0	0	0	141	447	0	0	
Other	Rhynchocoela	Carn	0	0	141	447	0	0	141	447	0	0	
	Acarina	???	157	472	283	596	0	0	0	0	141	447	
	Total other		157		424		0		141		141		
	Total mean density		61,304		192,116		234,416		78,233		78,815		

### Toxicity Tests

The 14-day acute solid phase *L. plumulosus* toxicity assay indicated no observed behavioral differences between animals exposed to the reference sediments and the contaminated test sediments. In addition, no difference ( $p > 0.05$ ) in the survival was detected (Table 3). Reference survival was 78%, whereas survival in organisms exposed to site samples ranged from 63 to 92%. There were no significant Pearson correlation relationships between observed mortality and contaminant concentrations ( $p > 0.05$ ).

### Body Burden

Fiddler crab mercury concentrations were significantly elevated at location 4 with a mean concentration of 2.6 mg/kg dry weight. Location 2 crabs had a mean mercury concentration of 2.0 mg/kg dry weight, whereas the next location down gradient (location 1) had a mean mercury concentration of 0.7 mg/kg dry weight. Mean mercury concentration in fiddler crabs was found to be 0.06 mg/kg dry weight at the reference location. Fiddler crab mercury burdens were positively associated with increas-

ing sediment mercury concentration among locations ( $r^2 = 43.4\%$ ;  $F = 19.16$ ;  $p < 0.001$ ).

Mercury concentrations in *Littorina* were elevated at the sampling location near the historic outfall. Location 2 concentrations (mean tissue concentration = 33.1 mg/kg dry weight) were significantly elevated when compared to the reference location (mean tissue concentration = 0.6 mg/kg dry weight) ( $F = 102.5$ ;  $p < 0.001$ ).

Aroclor 1268 sediment concentrations were found to be positively related with tissue PCB burdens in *Uca* sp. ( $r^2 = 80.2\%$ ;  $F = 101.52$ ;  $p < 0.001$ ). Concentrations of PCBs in the crabs were highest near the historic outfall location (mean 43 mg/kg dry weight) and became lower with distance from the outfall (location 2 mean tissue PCB concentration = 40 mg/kg dry weight, location 1 mean tissue PCB concentration = 4.9 mg/kg dry weight). The reference location crabs had a mean tissue PCB concentration of 0.08 mg/kg dry weight (see Table 4).

Aroclor 1268 concentrations in *Littorina* sp. were found to be elevated near the historic outfall, with location 2 snails showing a mean concentration of 4.2 mg/kg dry weight; the reference location snails showed a PCB 1268 concentration of 0.05 mg/kg dry weight ( $F = 103.13$ ;  $p < 0.001$ ).

### Benthic Invertebrate Trophic Composition

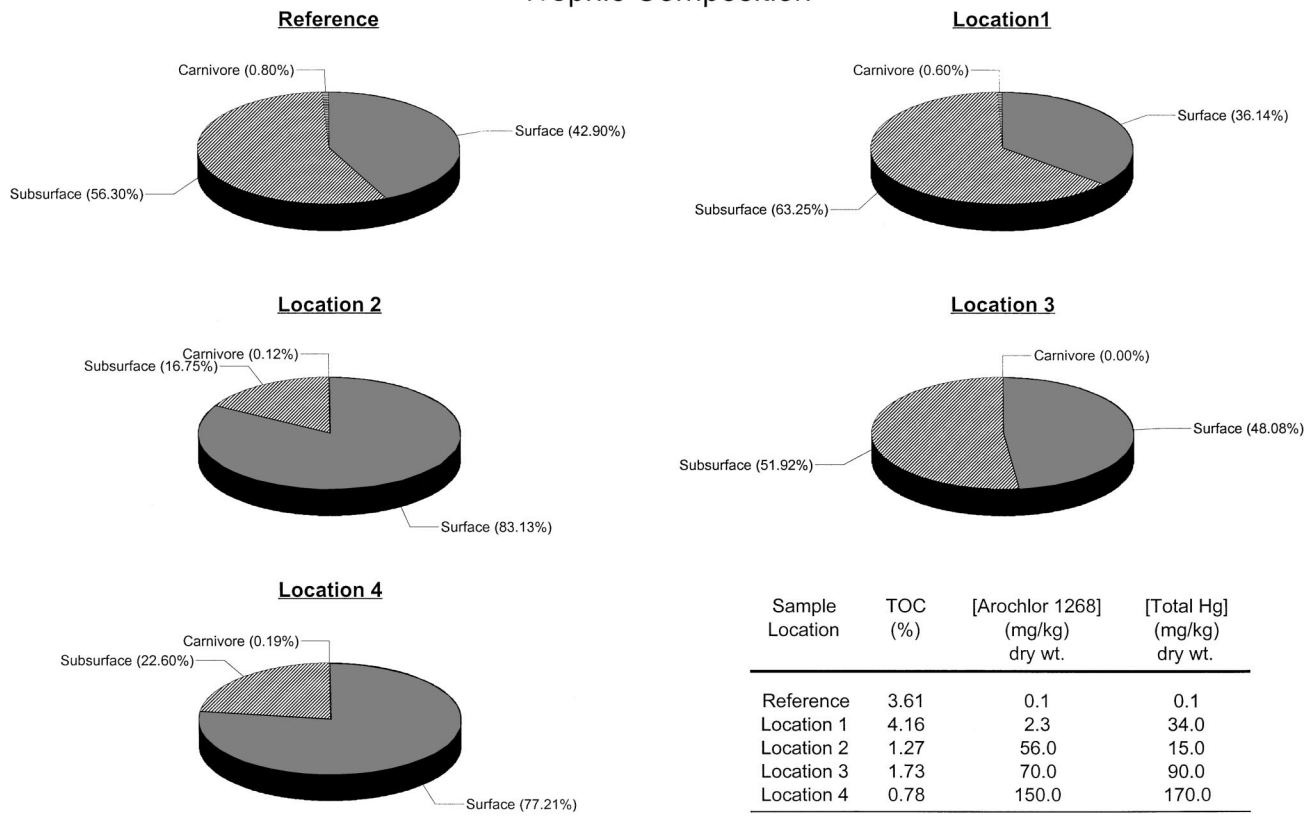


Fig. 3. Trophic composition of the benthic invertebrate community along PCB and mercury contamination gradients

Table 3. *Leptocheirus plumulosus* toxicity test results

Location	% Survival	% St. Dev.	Behavioral Abnormalities
Reference	78	8	no
Location 1	92	3	no
Location 2	83	12	no
Location 3	68	14	no
Location 4	63	21	no

## Discussion

### Benthic Infauna

Significant shifts in benthic community taxonomic composition were observed along the PCB and Hg contamination gradient present in the marsh study site. Oligochaetes tended to comprise a greater proportion of the community in the samples taken in the relatively uncontaminated locations. Conversely, the percentage of the community consisting of polychaetes tended to be higher in the more highly contaminated PCB and mercury areas. The proportion of nematodes comprising the infaunal benthic community decreased precipitously with increasing PCB and mercury concentrations. These trends may reflect an overall difference in the tolerance of these groups to environmental disturbance. It also indicates that as a class, oligochaetes and nematodes are equilibrium taxa, while providing additional

support to the idea that many polychaetes are early colonizers that are able to survive in polluted or otherwise disturbed habitats. Equilibrium species are relatively long-lived and tend to dominate the community in undisturbed, unstressed habitats, whereas opportunistic species are relatively short-lived and dominate disturbed or stressed habitats (Dauer 1993). Differential survival among species is the primary mechanism leading to shifts in community dominance and diversity (Scott 1989). It is recognized that toxic effects are generally expected to occur at the species level. However, given that sensitivity to environmental change is similar among closely related species, results presented for higher taxonomic groups also have value.

Historical data on salt marsh communities provides us with additional evidence for these observations. Lists of pollution-tolerant and -sensitive taxa vary depending on location, and that variation may be due to life-history differences of the organisms (Seitz and Schaffner 1995). The polychaete *Streblospio benedicti* was only found at the contaminated locations and was found at high densities. *S. benedicti* as well as *Manayunkia aestuarina* are considered to be early colonizers in the succession of benthic communities. The imbalance of the community in favor of these early successional species may indicate that the communities sampled in this study were not in equilibrium (Grassle and Grassle 1976; Gaston and Young 1992). The polychaete *Capitella capitata* and other members of the family Capitellidae are considered to be relatively tolerant of pollution, based on a study of Commencement Bay (Becker *et al.* 1990). In the study marsh, acute pollutant stresses may have resulted in an exclusion or decline of sensitive groups from the community



**Table 4.** Whole body tissue concentrations

Location	<i>Uca</i>				<i>Littorina</i>			
	Hg		Aroclor 1268		Hg		Aroclor 1268	
	(mg/kg) dry weight	SD	(mg/kg) dry weight	SD	(mg/kg) dry weight	SD	(mg/kg) dry weight	SD
Reference	0.06	0.01	0.08	0.02	0.6	0.2	0.05	0.001
Location 1	0.7	0.07	4.9	0.7	—	—	—	—
Location 2	2.0	0.8	40.0	14.9	33.1	7.8	4.2	1.0
Location 3	2.6	0.9	43.0	13.0	—	—	—	—
Location 4	—	—	—	—	—	—	—	—

(Bilyard 1987). Conversely, chronic pollutants, although less dramatic in their effects, can result in decreased recruitment, growth, and fecundity or induce other physiological changes that may ultimately result in changes in the community (Bilyard 1987). In any case, trends in opportunistic species and equilibrium species composition may be the best indicators when analyzing community structure trends in disturbed environments (Dauer and Alden 1995).

In terms of trophic structure, the study marsh demonstrated a poor mix of macrobenthic trophic groups (Gaston and Nasci 1988) as indicated by the dominance of surface feeders (surface-deposit and suspension feeding organisms), and subsurface deposit feeders (Figure 3). This investigation found that the proportion of the community comprised of surface feeders was positively related to increased PCB and mercury concentrations, *i.e.*, surface feeders dominated the community where contaminant levels were at their highest. Subsurface feeders were more commonly observed in the less contaminated locations. Because our trophic classification was based on that of Sacco *et al.* (1994), we were unable to compare our results directly with similar studies of contaminated estuaries that used slightly different classification schemes. However, several general trends found in this investigation appear to be consistent with other findings. Gaston and Young (1992) found evidence that surface-dwelling benthic species were more prominent in areas of higher contamination in a Louisiana marsh. Their results suggest that the dominance of surface feeders in contaminated areas may indicate potential negative effects of contaminants on subsurface benthic organisms. They concluded that the dominance of surface and shallow subsurface foragers may be due to surficial foragers being less susceptible or less exposed to sediment contaminants than subsurface foragers (Gaston and Young 1992).

A Commencement Bay study of a contaminant mixture suggested that the presence of toxic chemicals is unlikely to favor any particular species over another, but would result in a continuous decline in abundance and biomass of all species with increasing concentration (Becker *et al.* 1990). In this study, abundance of individual species did not appear to be affected by the presence of contamination, but the disposition of larger taxonomic and trophic groups did appear to be affected by site contaminants.

In another study, low faunal densities of pollution tolerant species (*C. capitata* and *Nereis glandicincta*) were found in areas where organic matter concentrations approached 3.5% (Raman and Ganapati 1983). Two locations from the study site exhibited TOC levels above 3.5% (location 1 and Reference), but faunal densities appeared to be unaffected in comparison to

the remaining locations with lower TOC values. Therefore, organic enrichment does not appear to play a role in the densities of pollution-tolerant species at the study site.

At least two other factors have been identified in the literature that may confound results in a benthic community survey like the one conducted here. In silt-clay sediments, responses to disturbances during the colonizing phase involve a shift from subsurface deposit feeders to those inhabiting and feeding on surface sediments and suspensions (Scott 1989). This response is not always observed in other grain size distributions. Sediments collected in our benthic analysis were primarily composed of a silt-clay distribution; therefore, the grain size distribution alone could not account for the shifts in composition that were observed at the study site. Our analysis showed no apparent trends in community composition as related to grain size.

A second potential confounding factor is sediment instability. Since macrofauna depend on spatial partitioning to maintain diversity, their diversity can be greatly affected by sediment instability (Warwick *et al.* 1990). This could not have been the case at the southeastern Georgia site because all of the sediment samples were similar in texture, vegetative cover, salinity regime, and tidal influence. No trends or differences in spatial partitioning were either expected or observed.

#### Toxicity Test

The toxicity testing found no discernible toxicity to a surrogate benthic organism, *L. plumulosus*. This result lessens concerns over acute toxicity to the benthic community on the marsh, but does not dismiss the strong evidence identified for chronic community-level and food chain-level effects.

#### Body Burdens

The presence of high levels of PCBs and mercury in the study marsh is a severe ecological problem due to their persistence in the environment, bioaccumulation potential, and toxicity. Fiddler crabs and marsh periwinkles, and probably other benthic invertebrates, accumulate PCBs (Nimmo *et al.* 1971; Clark *et al.* 1986) and mercury (Breteler *et al.* 1981) from contaminated sediment and transfer them to higher trophic levels. Birds and mammals that are exposed to PCBs show abnormal growth, decreased reproductive success, altered metabolism, and aberrant behavior (Eisler 1986). Exposure to mercury is known to



result in decreased growth, developmental anomalies, decreased reproductive success, and neurological impairment in birds and mammals (Eisler 1987).

### Conclusions

Based on the results of this study, it can be concluded that shifts in community composition and trophic structure are observed in the study marsh, and that those shifts appear to increase with increasing PCB and mercury loading. Sediments in the marsh do not appear to be acutely toxic to a surrogate benthic species in standardized toxicity tests, but PCBs and mercury are bioaccumulating in the marsh food chain. This is of primary concern because endangered species, such as the wood stork, and migratory birds are commonly observed foraging in the study marsh.

### References

- Becker DS, Bilyard GR, Ginn TC (1990) Comparisons between sediment bioassays and alterations of benthic macroinvertebrate assemblages at a marine Superfund site: Commencement Bay, Washington. *Environ Toxicol Chem* 9:669–685
- Bilyard GR (1987) The value of benthic infauna in marine pollution monitoring studies. *Mar Pollut Bull* 18:581–585
- Breteler RJ, Valiela I, Teal JM (1981) Bioavailability of mercury in several Northeastern U.S. *Spartina* ecosystems. *Estuar Coast and Shelf Sci* 12:155–166
- Clark JR, Patrick JM, Moore JC, Forester J (1986) Accumulation of sediment-bound PCBs by fiddler crabs. *Bull Environ Contam Toxicol* 36:571–578
- Dauer DM (1993) Biological criteria, environmental health and estuarine macrobenthic community structure. *Mar Poll Bull* 26:249–257
- Dauer DM, Alden RW III (1995) Long-term trends in the macrobenthos and water quality of the lower Chesapeake Bay (1985–1991). *Mar Pollut Bull* 30:840–848
- Dauer DM, Luckenbach MW, Rodi AJ Jr (1993) Abundance biomass comparison (ABC method): effects of an estuarine gradient, anoxic/hypoxic events and contaminated sediments. *Mar Biol* 116:507–518
- Eisler RE (1986) Polychlorinated biphenyl hazards to fish, wildlife, and invertebrates: synoptic review. U.S. Fish Wildl Serv Biol Rep 85(1.7), 72 pp
- Eisler RE (1987) Mercury hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wildl Serv Biol Rep 85(1.10), 90 pp
- Ferraro SP, Swartz RC, Cole FA, Schults DW (1991) Temporal changes in the benthos along a pollution gradient: discriminating the effects of natural phenomena from sewage-industrial wastewater effects. *Est Coast Shelf Sci* 33:383–407
- Gaston GR, Nasci JC (1988) Trophic structure of macrobenthic communities in the Calcasieu Estuary, Louisiana. *Estuaries* 11:201–211
- Gaston GR, Young JC (1992) Effects of contaminants of macrobenthic communities in the upper Calcasieu Estuary, Louisiana. *Bull Environ Contam Toxicol* 49:922–928
- Grassle JF, Grassle JP (1976) Sibling species in the marine pollution indicator *Capitella* (Polychaeta). *Science* 192:567–569
- Nimmo DR, Wilson PD, Blackman RR, Wilson AJ (1971) Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. *Nature* 231:50–52
- Raman AV, Ganapati PN (1983) Pollution effects on ecobiology of benthic polychaetes in Visakhapatnam Harbor (Bay of Bengal). *Mar Pollut Bull* 14:46–52
- Sacco JN, Seneca ED, Wentworth TR (1994) Infaunal community development of artificially established salt marshes in North Carolina. *Estuaries* 17(2):489–500
- Schlekat CE, McGee BE, Reinharz E (1992) Testing sediment toxicity in Chesapeake Bay using the amphipod *Leptocheirus plumulosus*: an evaluation. *Environ Toxicol Chem* 11:225–236
- Scott KJ (1989) Effects of contaminated sediments on marine benthic biota and communities. In: Corell A (ed) Committee on contaminated marine sediments. National Academy Press, Washington, DC, pp 132–154
- Seitz RD, Schaffner LC (1995) Population ecology and secondary production of the polychaete *Loimia medusa* (Terebellidae). *Mar Biol* 121:701–711
- US EPA/US ACE (1994) Evaluation of dredged material proposed for discharge in waters of the U.S. Testing manual. EPA-823-B-94-002
- Warwick RM, Platt HM, Clarke KR, Agard J, Gobin J (1990) Analysis of macrobenthic and meiobenthic community structure in relation to pollution and disturbance in Hamilton Harbour, Bermuda. *J Exp Mar Biol Ecol* 138:119–142