

## The Effect of Mercury and PCBs on Organisms from Lower Trophic Levels of a Georgia Salt Marsh

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**Abstract.** We examined several indicators of salt marsh function, focusing on primary producers, microbes, and grass shrimp, at a Superfund site (LCP) contaminated with mercury and polychlorinated biphenyls (PCBs) and a reference site (Cross-River) in Georgia. Primary production of *Spartina alterniflora* was assessed by measuring peroxidase activity (POD), glutathione concentration (tGSH), photosynthesis ( $A_{net}$ ), and transpiration (E). Microbial populations were assessed by measuring living-fungal standing crop (as ergosterol) and Microtox®. Grass shrimp (*Palaemonetes pugio*) reproductive potential was determined by measuring individual egg mass, average egg area, brood size, and brood mass of gravid females. Comparison of the sites suggested that *P. pugio* reproduction was affected at the LCP site, but we were unable to document clear negative effects on other organisms we investigated. Due to natural environmental gradients, the Cross-River site may not have been a perfect control for the LCP site. Therefore, data from just the LCP site were reanalyzed using multiple regression. Fungal biomass was related to methylmercury concentrations, but the direction of the relationship differed between wholly dead shoots (positive) and partially dead shoots (negative). *S. alterniflora* POD was positively related to methylmercury concentrations. *S. alterniflora*  $A_{net}$  and E were negatively related to elevation and salinity, respectively. Despite high levels of contamination at the LCP site, our results provided only suggestive evidence for impacts on organisms at lower trophic levels.

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Saltmarsh wetlands serve as areas of transition from upland to estuarine and ultimately pelagic systems and provide a multitude of important ecological, biogeochemical, and hydrological

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functions (Mitsch and Gosselink 1993; Vernberg 1993). High levels of primary production in southeastern U.S. salt marshes support a vigorous detrital-based food web (Montague and Wiegert 1990; Vernberg 1993; Newell and Porter 1999). Additionally, plant-derived energy can be exported from a salt marsh as dissolved organic carbon (Moran and Hodson 1994), nutrients (Turner 1993), and consumer biomass (Odum and Heald 1972; Montague and Wiegert 1990). Despite high levels of persistent contaminants in coastal and estuarine sediments (Valette-Silver 1993), few studies have addressed how contaminants affect wetland processes important in interactions with surrounding ecosystems (Klopatek 1988; Catallo 1993). The direct and indirect dependence of estuarine species on the detrital-based food web of the salt marsh suggests the need for improved assessment and monitoring of possible pollutant effects on lower trophic levels.

The food web of southeastern U.S. saltmarshes has been well described. Primary productivity by higher plants (mostly *Spartina alterniflora*) is among the highest in the world (Pezeshki and DeLaune 1991). Approximately 56% of standing-decaying *S. alterniflora* leaf biomass is converted to fungal biomass (Newell *et al.* 1996; Newell and Porter 1999). Fungal degradation of decaying *S. alterniflora* shoots and shredding by invertebrates facilitates deposition on the marsh surface and availability to detritivores. Sediment bacterial biomass is comparable to that of fungal biomass, indicating that bacteria are a major component of benthic microbial productivity (Newell and Porter 1999). *S. alterniflora* detritus and associated microbiota provide the energetic base for saltmarsh food webs, which support a variety of meiofaunal species (Peterson *et al.* 1985; Montague and Wiegert 1990). The grass shrimp, *Palaemonetes pugio*, contributes to the decomposition of detritus (Welsh 1975) and consumes both detritus and associated microbes and meiofaunal species (Welsh 1975; Posey and Hines 1991; Walters *et al.* 1996). *P. pugio* serves as an important energetic link between resident saltmarsh organisms and the estuarine and pelagic fish species of commercial and recreational importance (Nixon and Oviatt 1973; Kneib 1997).

A number of studies have characterized the individual responses of *S. alterniflora* (Mrozek *et al.* 1983; Pezeshki and DeLaune 1993), fungi (Hicks and Newell 1984; Purkayastha *et al.* 1994; Newell and Wall 1998), bacteria (Capone *et al.* 1983; Winger *et al.* 1993), or shrimp (Cunningham and Grosch 1978;

Buikema *et al.* 1980; Kraus and Weis 1988) to various chemical pollutants. Few studies, however, have attempted an integrated assessment of responses to pollutants of the suite of organisms that constitute the base of the detrital food web. To examine the health of the detrital food web in a contaminated salt marsh, we assessed the status of primary producers, fungal decomposers, bacterial populations, and grass shrimp reproduction. We worked at the LCP Superfund site in Brunswick, GA, USA. Since 1919, this site has hosted several industrial ventures, including an oil refinery, a paint and varnish company, and a chlor-alkali plant (Sprenger *et al.* 1997). As a result of these activities the adjacent salt marsh was heavily contaminated with polychlorinated biphenyls (PCBs, primarily Aroclor 1268) and mercury (Kannan *et al.* 1997; Sprenger *et al.* 1997; Maruya and Lee 1998; Newell and Wall 1998; Horne *et al.* 1999). We took two approaches to addressing pollutant impacts at this site. First, we compared the LCP site to an adjacent reference site. Second, to avoid potential problems associated with site-matching, we correlated response variables with pollutant levels and abiotic factors within the LCP site.

## Materials and Methods

### Site Selection and Sediment Samples

Ten stations along two transects were selected at both the US EPA LCP site and a reference site on the Turtle River (hereafter, Cross-River) in Brunswick, GA, USA (31° 12'N, 81° 30'W) in June 1997. Transects were chosen to encompass high and low marsh elevations at both sites and high and low chemical concentrations at the LCP site. Relative elevation of plots within each marsh were measured to the nearest mm using a total station laser transit. Five sediment cores were collected on June 16, 1997, from each individual station (center and 1 m north, south, east, and west) and divided lengthwise into thirds. Thirds from each core at each station were combined and homogenized and the composite samples were used for Microtox® and chemical analysis. All manipulations of sediments were done under an N<sub>2</sub> atmosphere.

### Biochemistry

The youngest leaves were removed from five *S. alterniflora* plants at each station on September 24, 1997, and placed on dry ice. Half of the youngest leaf, split vertically, was weighed and placed at -80°C pending peroxidase (POD) analysis. The other half was homogenized in 10 volumes of 6% metaphosphoric acid (with 1 mM EDTA), centrifuged at 20,000 g for 10 min at 4°C and the supernatant stored at 0-4°C until tGSH analysis. In each case, values from replicate plants were averaged to yield a single value/station.

POD analysis was conducted following Byl *et al.* (1994) with minor modifications. Leaves were chopped, homogenized in 10 volumes cold 0.5 M CaCl<sub>2</sub> and centrifuged at 2,000 g for 10 min at 4°C. The supernatant was removed and the pellet resuspended in 10 volumes of 0.5 M CaCl<sub>2</sub> and recentrifuged. Supernatants were combined and centrifuged a third time to clarify the solution. Combined supernatants were diluted fourfold with 0.5 M CaCl<sub>2</sub> and analyzed in triplicate on a 96-well spectrophotometer for 2 min at 510 nm. The following reagents comprised the reaction mixture: reagent A, 0.01 M MES (2-[N-morpholino] ethanesulfonic acid) buffer pH 5.5 with 1.5 mM H<sub>2</sub>O<sub>2</sub>; reagent B, 2.5 mM 4-aminoantipyrine and 0.17 M phenol in water. The reaction solution consisted of 10 µl sample, 75 µl reagent

A, and 70 µl reagent B, mixed in that order. Standard curves were generated using horseradish peroxidase (crude preparation, Sigma Chemical Company) and activity normalized to tissue fresh weights.

Total glutathione (tGSH) was determined using a modification of the glutathione reductase enzymatic recycling method of Griffith (1980). Samples were harvested and homogenized as described above. The reaction solutions consisted of the following: Reagent 1, 0.3 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in 150 mM sodium phosphate buffer (15 mM EDTA, 0.4% BSA, pH 7.2); Reagent 2, 1.5 units ml<sup>-1</sup> glutathione reductase (Sigma Chemical Company, St. Louis, MO) in 50 mM imidazole buffer (1 mM EDTA, 0.02% BSA, pH 7.2). Samples were diluted 1:10 in 5% Na<sub>2</sub>HPO<sub>4</sub> (pH 7.2) immediately before use. The reaction mixture consisted of 40 µl of reagent 1, 32 µl of reagent 2, and 32 µl of diluted sample. Reaction mixtures were initiated with 8 µl of a 9 mM NADPH solution in 50 mM imidazole buffer (1 mM EDTA, 0.02% BSA, pH 7.2), at 25°C and monitored, in triplicate, at 412 nm for 2 min on a 96-well spectrophotometer.

### Gas Exchange Measurements and Plant Biomass

Gas exchange of *S. alterniflora* was measured with 12 cm<sup>3</sup> leaf chamber attached to an ADC Corp. LCA4 open gas exchange system using a portable light source (1.5 mmol m<sup>-2</sup>s<sup>-1</sup>). Gas exchange was measured on September 24, 1997, during midday at low tide under ambient temperature and humidity conditions. Gas exchange was measured on the third fully expanded leaf from the top of 4-5 plants/station and averaged to yield a single value/station. *S. alterniflora* stem density (number of stems > 10 cm tall) and height (tallest plant) were measured in three 0.25 × 0.25 m quadrants adjacent to each station, and averaged to yield a single value/station.

### Fungal Biomass and Microtox

Mean fungal biomass at each site was reported earlier (Newell and Wall 1998); here we reanalyze the data for patterns within the LCP site. Original descriptions of methods for ergosterol as an index of living fungal mass can be found in Newell (1993).

Separate subsamples of composited sediments from each station were analyzed for porewater and solid phase toxicity using Microtox®. Pore water was extracted by centrifuging the sediment at 6,000 g for 15 min at 10°C. Pore water was transferred into 20-ml scintillation vials and stored at 0-4°C until analysis. The salinity of each sample was determined using a refractometer. The pore water volume was measured and adjusted to a pH of 7 using 0.5 M NaOH. Samples were immediately analyzed for 15 min with a Microbics M500 Toxicity Analyzer following instructions for the "Basic Test" (Microbics Corporation 1992). This test measures the luminescence of the bacteria (*Vibrio fischeri*) in the presence of a dilution series of the experimental sample. The EC<sub>50</sub> is expressed as the percent of the original sample that reduces bacterial luminescence by 50% after 15 min. Whole sediments were analyzed following the solid phase test (SPT) procedures (Microbics Corporation 1992). Sample (7 g) was suspended in 35 ml of a 3.5% NaCl solution, serially diluted as 1.5-ml subsamples, and cooled to 15°C. Cooled samples were mixed with 20 µl of reconstituted *V. fischeri* for 20 min before being filtered with a column filter. An aliquot of the filtrate was removed (0.5 ml) and allowed to stand for 10 min before bioluminescence was measured. EC<sub>50</sub> (solid phase) values were generated from the resultant dose-response curve and normalized to sediment dry weights.

Microtox® SPT can be affected by the silt and clay content of sediments, as high silt-clay contents can preferentially adsorb the test bacteria, causing reduction in light emission not due to toxicity (Ringwood *et al.* 1997). Therefore, the clay-silt content of the samples was

**Table 1.** Comparison of LCP and Cross-River (CR) sites

Organism/Matrix	Measured Variable	Means LCP ( $\pm$ SD); CR ( $\pm$ SD)	Result	p Value
Porewater	salinity (ppt)	28.5 (1.9); 29.2 (2.0)	LCP = CR	> 0.05
Sediment	PCBs ( $\mu\text{g g}^{-1}$ DW)	46.0 (52.7); 0.32 (0.07)	LCP > CR	0.0002
	total mercury ( $\mu\text{g g}^{-1}$ DW)	18.4 (21.9); 0.49 (0.08)	LCP > CR	0.0002
	methylmercury ( $\text{ng g}^{-1}$ DW)	64.9 (48.1); 10.1 (3.6)	LCP > CR	0.0001
	% fines (clay + silt)	82.2 (8.9); 77.1 (11.9)	LCP = CR	> 0.05
<i>S. alterniflora</i>	stem density (number $0.0625 \text{ m}^{-2}$ )	8.5 (1.9); 11.8 (4.9)	LCP = CR	> 0.05
	canopy height (cm)	118.1 (23.1); 84.3 (21.5)	LCP > CR	0.0032
	peroxidase (units $\text{g}^{-1}$ FW)	8.8 (1.3); 10.4 (1.2)	LCP < CR	0.0093
	total glutathione (pmol $\text{mg}^{-1}$ FW)	54.4 (21.9); 58.8 (20.0)	LCP = CR	> 0.05
	photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	13.9 (4.6); 10.6 (2.5)	LCP = CR	> 0.05
	transpiration ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	1.26 (0.34); 0.57 (0.25)	LCP > CR	0.0001
Fungi	biomass (mg ergosterol $\text{g}^{-1}$ organic matter of wholly dead shoots)	0.89 (0.15); 0.68 (0.10)	LCP > CR	0.0085
	biomass (mg ergosterol $\text{g}^{-1}$ organic matter of partially dead shoots)	0.63 (0.18); 0.42 (0.12)	LCP > CR	0.0098
<i>V. fischeri</i>	EC <sub>50</sub> (porewater)	23.7 (7.1); 17.2 (4.2)	LCP > CR	0.0241
	EC <sub>50</sub> (solid phase)	0.38 (0.24); 0.34 (0.17)	LCP = CR	> 0.05
<i>P. pugio</i>	length (mm)	35.2 (1.4); 32.7 (2.8)	LCP > CR	0.0003
	female mass (g)	0.087 (0.015); 0.065 (0.018)	LCP > CR	0.0001
	brood size (# of eggs)	302.7 (71.4); 289.0 (76.1)	LCP = CR	> 0.05
	brood mass (mg)	16.3 (4.4); 16.3 (4.5)	LCP = CR	> 0.05
	individual egg mass (mg)	0.054 (0.007); 0.056 (0.004)	LCP = CR	> 0.05
	average egg area ( $\text{mm}^{-2}$ )	0.37 (0.07); 0.34 (0.03)	LCP = CR	> 0.05

determined by the standard pipet method (Klemm *et al.* 1993; Strobel *et al.* 1995).

### Grass Shrimp

Daggerblade grass shrimp (*P. pugio*) were collected by dip net at three stations (> 20 m apart) along tidal creeks at both sites (LCP and Cross-River). Samples were pooled and shrimp preserved in 10% formalin (3.6% formaldehyde in estuarine water). Shrimp length was measured from the tip of the rostrum to the end of the telson. Adult shrimp (18–40 mm) were separated by sex and the proportion of ovigerous females recorded. Brood size was expressed as both egg/embryo number and dry mass. Tracings of 25 eggs/embryos from each brood were made with the aid of a camera attached to a stereomicroscope set at 50 $\times$ , and measured. Since the eggs were often irregularly shaped, their sizes were reported as cross-sectional area ( $\text{mm}^2$ ). Broods and females were dried separately at 60°C and weighed.

### Chemical Analysis

PCB analytical procedures (after Kannan *et al.* 1997) are described in Newell and Wall (1998). Procedural blanks, PCB-spiked beach sand, and a standard reference marine sediment (SRM1941a; NIST) were analyzed in conjunction with marsh sediments. Blanks did not contain any target PCBs above the detection limit ( $\sim 1 \text{ ng/g}$  dry weight). Dibromooctafluorobiphenyl (added as a recovery surrogate) and spiked PCB recoveries exceeded 80% in all samples. The mean recovery of PCBs in SRM1941a with certified concentrations exceeded 90%.

Total mercury was determined following Smith (1993) and Newell and Wall (1998). The detection limit for the method is approximately 5 ng/g. Methylmercury was determined using a combination of two methods (Horvat *et al.* 1993; Liang *et al.* 1994). Methylmercury was determined by aqueous phase ethylation, cryogenic trapping, and chromatographic desorption followed by detection with cold vapor atomic

fluorescence. Distillation was used to separate methylmercury from other interfering species as described by Horvat *et al.* (1993).

### Statistical Analysis

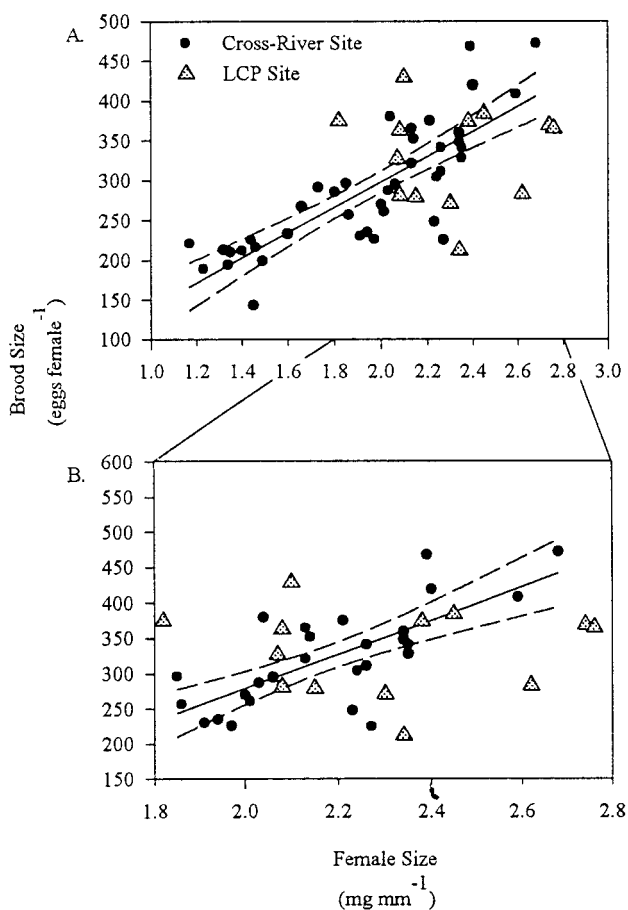
Comparisons between the LCP and Cross-River sites were made with Student's *t* tests, or, if variances were unequal, with Wilcoxon's rank sums tests. Linear regression was used to compare the relationship between female shrimp size, expressed as a ratio of weight to length ( $\text{mg mm}^{-1}$ ) and brood size (eggs female $^{-1}$ ) or brood mass (mg) from both sites.

To determine if response variables were dependent on chemical concentrations and/or environmental factors within the LCP site, LCP data were analyzed using stepwise multiple regression (criterion for entry and removal of independent variables was a *p* value of  $\leq 0.15$ ). Heteroscedasticity was assessed by inspection of residuals versus predicted values and by testing whether the first and second moments were correctly specified (SAS Institute Inc. 1990). The influence of individual points was assessed based on the studentized residual error. Only a single  $A_{\text{net}}$  measurement (studentized residual error > 6) was considered to be an outlier and removed.

## Results

### Site Comparison

Concentrations of PCBs, total mercury, and methylmercury in sediments were higher at the LCP than the CR site (Table 1), but concentrations at CR were higher than those at pristine sites on Sapelo Island (Newell and Wall 1998). LCP sediments contained a wide range of total mercury (2.3–70.6  $\mu\text{g/g}$  dry weight), methylmercury (18.6–190.0 ng/g dry weight), and



**Fig. 1.** Relationship between the size ( $\text{mg mm}^{-1}$ ) and brood size (no. of eggs female<sup>-1</sup>) of gravid *Paleomonetes pugio* collected from the LCP and Cross River sites. A: Graph of all animals collected from LCP and Cross-River sites. Regression line (solid) and 95% confidence intervals (dashed) for Cross-River site (adjusted  $R^2 = 0.66$ ;  $p < 0.0001$ ). B: Similar analysis using data restricted to the range of sizes of *P. pugio* collected at the LCP site (adjusted  $R^2 = 0.55$ ;  $p = 0.014$ ). No significant relationship was found for animals collected from the LCP site

total PCBs (5.5–156.0  $\mu\text{g/g}$  dry weight). Porewater salinities and % fines (% clay + silt) did not differ between sites. The range of station elevations within the LCP and CR sites was 0.34 m and 0.18 m, respectively.

No significant differences in *S. alterniflora* stem density, tGSH concentrations, or photosynthesis rates were seen between the LCP and CR sites (Table 1). *S. alterniflora* canopy height and transpiration were significantly higher at the LCP site, and POD activity was significantly higher at the CR site. Fungal biomass was significantly greater at the LCP site. Microtox<sup>®</sup> porewater  $\text{EC}_{50}$  values were higher (= less toxicity) at the LCP site, and solid phase  $\text{EC}_{50}$  values indicated no significant differences between LCP and CR sites.

Equal sampling effort at the LCP and CR sites yielded 13 and 43 gravid female *P. pugio* respectively. The length and mass of female *P. pugio* were greater at the LCP site; however, no differences were seen in brood size or mass or in individual egg mass or area (Table 1). Female size ( $\text{mg mm}^{-1}$ ) was positively related to brood size (eggs female<sup>-1</sup>) at the CR site

(adjusted  $R^2 = 0.66$ ,  $p < 0.0001$ , Figure 1A). A similar positive relationship (adj.  $R^2 = 0.51$ ,  $p < 0.0001$ ) was observed with the CR data set restricted to the female size range observed at LCP; however, no relationship was observed with the LCP data set (Figure 1B). A similar analysis using brood mass (mg) yielded qualitatively identical results (complete CR data set: adj.  $R^2 = 0.70$ ,  $p < 0.0001$ ; restricted data set: adj.  $R^2 = 0.47$ ,  $p < 0.0001$ ; LCP data set: no significant relationship).

#### Within LCP Site Multiple-Regression Analysis

Multiple regressions of response variables against sediment characteristics and pollutant levels indicated that both were important for particular response variables. Of the pollutants, methylmercury entered into five of eight regression models whereas total mercury and PCBs entered into only one model each (Table 2).

The multiple regression model that best explained *S. alterniflora* POD activity included methylmercury and total mercury (Table 2). Methylmercury, which explained most of the variance, had a positive relationship with POD, whereas total mercury had a negative relationship with POD. A linear regression between POD and methylmercury alone was also significant (Figure 2A). No significant model was generated for glutathione concentration (Table 2).

The multiple regression model for photosynthesis was driven by a negative relationship with elevation and a weaker positive relationship with PCBs (Table 2). A linear regression between photosynthesis and elevation alone was also significant (Figure 2B). Transpiration was negatively related to salinity (Table 2, Figure 2C).

The multiple regression model for fungal biomass in wholly dead shoots included methylmercury and elevation, with most of the variance explained by a positive relationship with methylmercury (Table 2). Fungal biomass in partially dead shoots was negatively related to methylmercury (Table 2, Figure 2D).

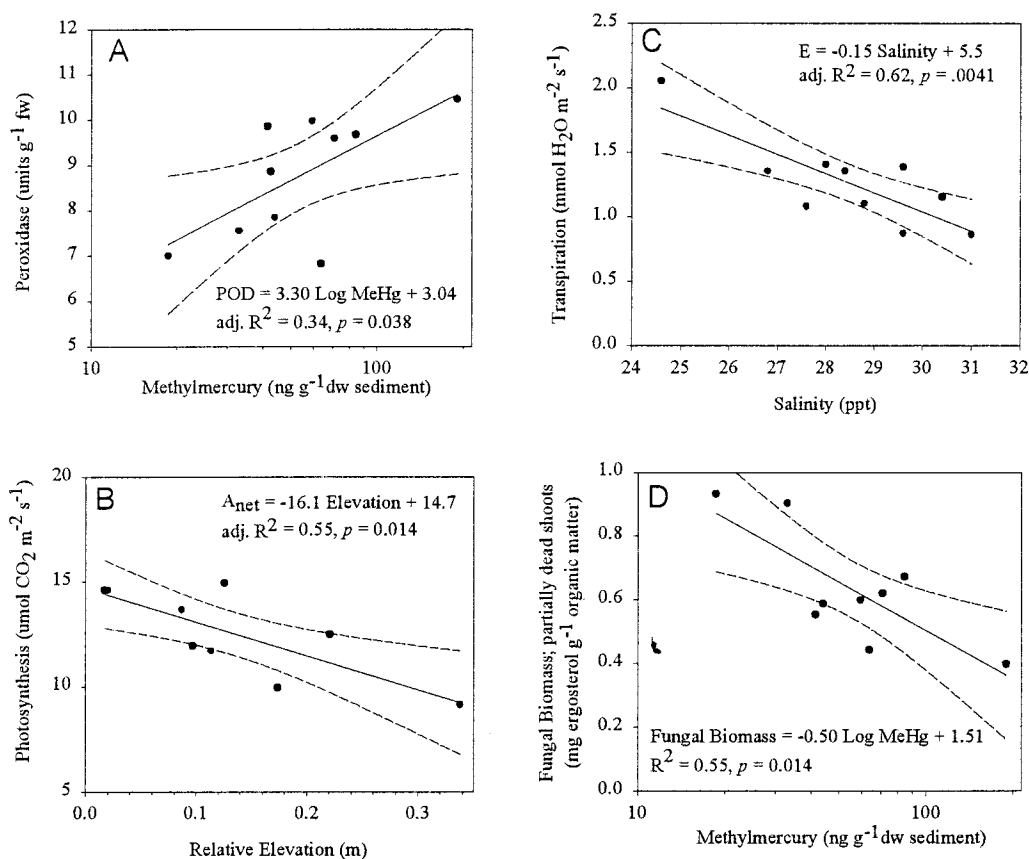
Microtox<sup>®</sup>  $\text{EC}_{50}$  (porewater) values were positively related to % fines (Table 2). The model for Microtox<sup>®</sup>  $\text{EC}_{50}$  (solid phase) included methylmercury and % fines, with most of the variance explained by a positive relationship with methylmercury (Table 2).

## Discussion

Despite high contaminant levels, we observed few biological differences between the LCP and CR sites, with the exception of a possible alteration in *P. pugio* reproduction. To sidestep potential problems due to physical differences between the sites, we also related responses from within the LCP site to contaminant concentrations using multiple regression. Plausible relationships between methylmercury and both *S. alterniflora* POD and living-fungal biomass were suggested with this approach. We also detected plausible relationships between *S. alterniflora* gas exchange and marsh elevation and salinity, but not between gas exchange and pollutant levels.

**Table 2.** Results of multiple regression analyses with data from the LCP site

Dependent Variable	Multiple Regression Results	Contributing Independent Variable(s)	Relationship	Partial R <sup>2</sup>	p Value
Peroxidase	adj. R <sup>2</sup> = 0.57 p = 0.021, n = 10	Log methylmercury	(+)	0.44	0.008
Total glutathione	adj. R <sup>2</sup> = 0.22 p = 0.096, n = 10	Log mercury Log methylmercury	(-) (-)	0.23	0.060
Photosynthesis	adj. R <sup>2</sup> = 0.89 p = 0.0005, n = 9	Relative elevation	(-)	0.60	0.0004
Transpiration	adj. R <sup>2</sup> = 0.62 p = 0.0041, n = 10	Log PCBs Salinity	(+) (-)	0.32	0.003
Fungal biomass (wholly dead shoots)	adj. R <sup>2</sup> = 0.58 p = 0.049, n = 9	Log methylmercury	(+)	0.47	0.050
Fungal biomass (partially dead shoots)	adj. R <sup>2</sup> = 0.55 p = 0.014, n = 9	Elevation Log methylmercury	(-) (-)	0.23	0.106
Microtox® (porewater)	adj. R <sup>2</sup> = 0.44 p = 0.032, n = 10	%Fines	(+)		
Microtox® (solid phase)	adj. R <sup>2</sup> = 0.72 p = 0.0047, n = 10	Log methylmercury %Fines	(+) (-)	0.53 0.26	0.018 0.024



**Fig. 2.** Relationships (LCP site data only) between A: *S. alterniflora* peroxidase activity and sediment methylmercury; B: *S. alterniflora* photosynthetic rates and relative station elevation; C: *S. alterniflora* transpiration rates and porewater salinities; and D: fungal biomass from partially dead *S. alterniflora* shoots and sediment methylmercury. The regression lines (solid) and 95% confidence interval (dashed lines) are shown

### Effects of Sediment Characteristics

Sediment characteristics affected our measurements of gas exchange and bacterial activity. The plant canopy was taller at

LCP than at CR, suggesting that site elevations were not perfectly matched, because *S. alterniflora* height increases at lower elevations due to effects of drainage on sediment chemistry (Howes *et al.* 1986). Within the LCP site, the influence of

sediment variables was evident in the negative relationships between photosynthesis and elevation and between transpiration and porewater salinity (similar relationships have been described elsewhere, e.g., Longstreth and Strain 1977; Drake 1989; Hwang and Morris 1994). Assays with *V. fischeri* confirmed previous reports that results are affected by particle size (Ringwood *et al.* 1997). Although the relationships with % fines were in opposite directions for the porewater and solid phase assays, and therefore difficult to interpret, they suggest that Microtox® analyses should be interpreted with caution when comparing sites that may differ in sediment size distributions.

The influence of sediment characteristics on response variables has important implications for our ability to detect impacts of pollutants in salt marsh systems. Salt marshes are characterized by strong gradients in edaphic characteristics across elevation and along the estuary. Since these edaphic gradients can strongly affect measured indicators of marsh function, and often might co-vary with the distribution of pollutants (e.g., at the LCP site most of the mercury was present in the high marsh near where it was originally dumped), it is essential that the edaphic gradients within a site be measured and taken into account. Similarly, the strong influence of edaphic factors on response variables necessitates that reference sites match impact sites very closely in all important respects other than the presence of pollutants. As we found, such precise site-matching is often difficult to accomplish. In many cases the best solution may be to measure edaphic variables of likely importance and attempt to remove their influence through multiple regression approaches.

### Effects of Pollutants

The most dramatic difference observed between the two sites was the apparent disruption of the linear relationship between the size of gravid *P. pugio* and the number of eggs and brood mass. Shrimp mass-brood size relationships (Wood 1967) have also been shown to be susceptible to disruption on exposure to an artificial refinery mixture (ARM) in the laboratory (Buikema *et al.* 1980). Results with *P. pugio* similar to ours were independently documented by Lee *et al.* (personal communication) at the LCP site. Since *P. pugio* are marsh residents (Kneib 1997), and since the sites were separated by a 0.6-km-wide tidal channel (low tide), movement of shrimp between LCP and CR was probably minimal, if not zero.

No adverse influences of PCBs on *S. alterniflora* from the LCP site were detected, despite reports that low levels of PCBs decrease *S. alterniflora* growth and biomass (Mrozek *et al.* 1983). Similarly, mercury, as mercuric chloride, has been shown to decrease gas exchange and increase peroxidase activity and glutathione levels in *S. alterniflora* (Wall *et al.* unpublished), but this was not evident at the LCP site. However, high methylmercury levels at LCP were associated with increased peroxidase activity. The effect of methylmercury on *S. alterniflora* enzyme systems is largely unknown, but because POD activities were higher at the CR site than the LCP, it is not clear whether the results presented in Figure 2A are indicative of physiological stress. Nevertheless, the utility of POD as an indicator of methylmercury stress in *S. alterniflora* deserves further attention.

Fungal biomass was related to methylmercury concentrations, but the direction of the relationship differed between wholly and partially dead shoots (positive and negative, respectively). Since fungal biomass was over 30% higher in both shoot types at the LCP site versus the CR site, there is little evidence overall that methylmercury negatively affected decomposer fungi (Newell and Wall 1998).

Bioluminescence of *V. fischeri* was positively related to methylmercury. Using natural bacterial communities in sediments collected from an *S. alterniflora*-dominated salt marsh, an overall stimulation of CO<sub>2</sub> and CH<sub>4</sub> production was observed following the separate addition of HgCl<sub>2</sub>, PbCl<sub>2</sub>, and FeCl<sub>2</sub>; however, the addition of methylmercury inhibited CH<sub>4</sub> and CO<sub>2</sub> production (Capone *et al.* 1983). All metals were added at concentrations (1,000 ppm dry weight sediment) that far exceed those observed at the LCP site, and thus the potential for stimulation of bacterial populations at lower concentrations of methylmercury is not known.

### Conclusions

Despite high levels of contaminants at the LCP site, we observed only subtle indications of toxicity to lower trophic levels. Salt marsh organisms, which are adapted to environments with high levels of physical stress, particularly from salinity and sulfides, may be preadapted to tolerate anthropogenic pollutants. Moreover, the low redox levels and high levels of sulfides and organic carbon, generally present in reduced wetland sediments, may act to sequester and limit the bioavailability of pollutants in these marsh systems (Catallo 1993). The effect of physicochemical parameters on toxicity of both inorganic and organic stressors is complex (Hamelink *et al.* 1994). Salinity, hardness, pH, redox potential, as well as particles and their coatings, *i.e.*, hydrous oxides, sulfides, clays, and natural organic matter, all contribute to alterations in pollutant toxicity. Compounding this ambiguity in prediction of toxic interactions is the lack of knowledge concerning kinetics of pollutant transport to and into biological membranes in these environments. Added to the possibility of organism adaptation to high levels of physical stress, mentioned above, it may not be surprising that only subtle toxic effects were observed. Our results were similar to those of Horne *et al.* (1999) who reported that meiofaunal densities at the LCP site were not different from those at a reference site, although meiofaunal trophic structure did change. Modest impacts of pollutants on lower trophic levels in saltmarsh systems may facilitate transfer of pollutants to higher trophic levels where they can bioaccumulate, have greater effects on saltmarsh organisms like shrimp and fish (Maruya and Lee 1998; Horne *et al.* 1999; Smith and Weis 1997), and pose a threat to humans that consume top predators.

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