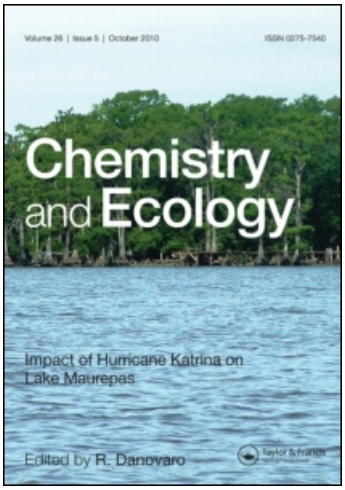

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Comparison of Solid-Phase and Pore-Water Approaches for Assessing the Quality of Marine and Estuarine Sediments

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COMPARISON OF SOLID-PHASE AND PORE-WATER APPROACHES FOR ASSESSING THE QUALITY OF MARINE AND ESTUARINE SEDIMENTS

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As part of our continuing evaluation of the pore-water approach for assessing sediment quality, we made a series of side-by-side comparisons between the standard 10-day amphipod whole sediment test with the corophiid *Grandidierella japonica* and a suite of tests using pore water extracted from the same sediments. The pore-water tests evaluated were the sea urchin (*Arbacia punctulata*) sperm cell test and morphological development assay, the life-cycle test with the polychaete *Dinophilus gyrociliatus*, and acute exposures of red drum (*Sciaenops ocellatus*) embryo-larval stages. Sediment and surface microlayer samples were collected from contaminated sites. Whole-sediment, pore-water, and surface microlayer toxicity tests were performed. Pore-water toxicity tests were considerably more sensitive than the whole-sediment amphipod test, which is currently the most sensitive toxicity test now recommended for determining the acceptability of dredged material for open ocean disposal.

KEY WORDS: Toxicity test, sediments, pore waters, ocean disposal, amphipod, sea urchin, polychaete

INTRODUCTION

The Marine Protection Research and Sanctuaries Act (MPRSA) of 1972 (16 U.S.C. 14311434, 33 U.S.C. 1401–1444; 86 Stat. 1052) stipulates that any proposed dumping of dredged material into ocean waters must be evaluated through the use of criteria published by the Environmental Protection Agency (EPA). Since 1977, guidance for these evaluations has been provided by a manual jointly developed by EPA and the US Army Corps of Engineers (CE) (EPA/AC, 1977), commonly called the "green book". A revised manual (EPA/CE, 1991) is currently under final review and will replace the old green book.

The biological effects tests required in the old green book (acute mortality tests with macroinvertebrates) are insensitive to even highly contaminated sediments (Rogerson *et al.*, 1985). The only substantial change in the biological effects section in the revised green book is the recommendation to include a benthic infaunal amphipod species. While benthic amphipods are generally more sensitive than the hard clams and sand worms used previously, one of the recommended amphipod species, *Ampelisca abdita,* is commonly found in great abundance in relatively contaminated areas (Long and Buchman, 1989). The most commonly used amphipod test species, *Rhepoxynius abronius,* is sensitive to a high proportion of fine-grained sediments (DeWitt *et al.*, 1988), which could confound the interpretation of test results.

Recently, several different toxicity tests were evaluated for assessing the quality of marine and estuarine sediments (Long *et al.*, 1990). One test involves the use of sediment pore (interstitial) water (Carr, 1988; Carr *et al.*, 1989). The equilibrium-

partitioning theory predicts that pore water is the controlling exposure medium in the toxicity of sediments to infaunal organisms (Adams *et al.*, 1985; DiToro, 1990). The primary purpose of the present study was to compare the relative sensitivity among a solid-phase test and a suite of pore-water toxicity tests. In addition, surface microlayer samples were also collected at each site for comparison with the sediment toxicity tests in order to determine if there was a correlation between sediment and surface microlayer toxicity in the vicinity of dredging operations.

MATERIALS AND METHODS

Sample Collection

Sediment samples were collected at three sites in Lavaca Bay adjacent to the Alcoa facility at Point Comfort, Texas (Figure 1). The fishery has been closed in this area for several years because of elevated levels of mercury in the edible tissues of several species of fish. A chlor-alkali facility discharged large quantities of mercury into the bay during the 1940s and 1950s. The extent and degree of the contamination of the sediment with mercury is thoroughly documented (Holmes, 1986). The Turning Basin (TB) site had not been dredged previously whereas the Ship Channel (SC) site had been dredged routinely. A suction dredging operation, located approximately 500 metres east of the Turning Basin site, was in progress when the samples were collected, with an obvious sediment plume visible to the west of the dredge. A recent study also detected high concentrations of polynuclear aromatic hydrocarbons (PAHs) in the vicinity of the West Island (WI) site; a creosote plant presumably discharged material into Lavaca Bay (GERG, 1990).

Sediments were collected with a hand-held coring device constructed from 10.2 cm polyvinyl chloride pipe and equipped with a valve for maintaining hydrostatic pressure during withdrawal of the sediment sample. About fifteen cores (5 to 7 cm deep) were homogenized in a polyethylene container with a stainless steel spoon. Sub-samples of the homogenized sediment were transferred to Ziploc® bags and placed on ice for transport to the laboratory where they were held at 4°C. The following day, the sediments were either prepared for the solid-phase test or the pore water was extracted from them. Mercury concentrations in the sediment were determined by atomic absorption spectrophotometry at the US Fish and Wildlife Service Pautuxet Analytical Control Facility. Sediment grain size was determined by the buoyoucous hydrometer method (ASTM, 1963).

Surface microlayer samples were collected at each site with a modification of the plate sampler described by Hardy *et al.* (1985). Our microlayer sampler was equipped with a plate and squeegees constructed of hydrophobic Teflon® instead of a glass plate with rubber squeegees. The microlayer sample, scraped from the surface of the Teflon® plate by the squeegees, passed through a Teflon® funnel into an acid washed (I-Chem®) amber glass sample bottle. Samples were held on ice in the field and frozen immediately (within 8 hours of collection) upon return to the laboratory. The toxicity of microlayer and pore-water samples is not affected by freezing (Hardy *et al.*, 1987; Carr *et al.*, 1989).

Solid-Phase Test

The amphipod species used in the solid-phase tests was the corophiid *Grandidierella japonica* (ASTM, 1990). Parental animals were obtained from a commercial supplier

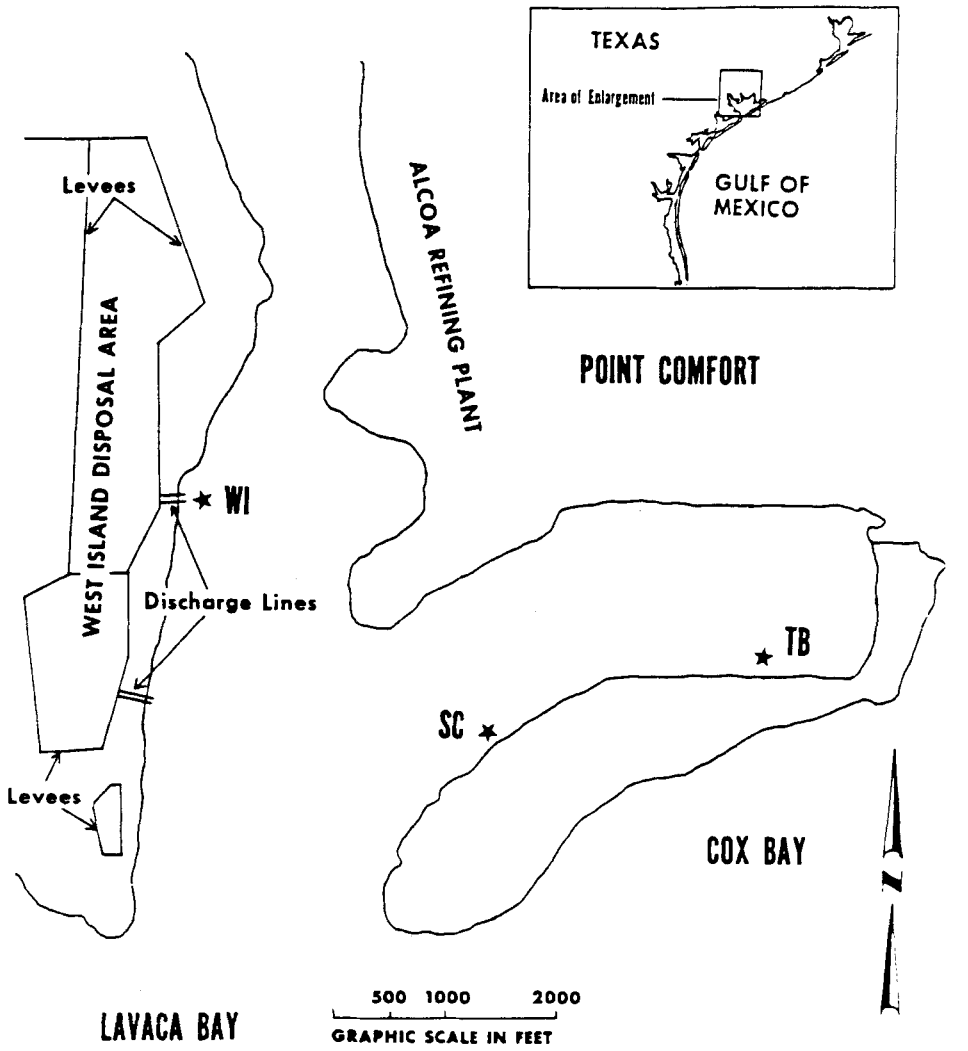

Figure 1 Location of sampling sites in Lavaca Bay, Texas.

(Reish Marine Studes, Inc., Alamitos, CA) and raised through at least one generation in our laboratory before they were used in testing. The amphipods were cultured in 38-l aquaria equipped with corner filters and a 2 to 3 cm layer of fine washed sand. The cultures were maintained under static-renewal conditions at 20 ± 1°C and 30 ± 1‰ salinity and fed a mixture of ground rabbit pellets and dried cereal leaves (Sigma Chem. Co, St. Louis, MO). Test animals were removed from the aquaria with a fine mesh aquarium net, and non-gravid animals of the same size (4 ± 1 mm total length) were selected for testing.

The solid-phase test was conducted according to established procedures (Swartz *et al.*, 1985; ASTM, 1990). Exposures were conducted in 2 l glass beakers (2 cm of

sediment with 1500 ml of overlying sea water) covered with watch glass lids (5 replicates/treatment) and supplied with gentle aeration under static conditions in a water bath. The reference sediment was the same sandy substrate in which the animals had been cultured. A measured aliquot of ground rabbit pellet and dried cereal leaves suspension was added to each exposure chamber at the beginning of the experiment; preliminary experiments indicated that survival of controls was reduced without the addition of food (unpublished data). Water quality measurements were made on days 1, 3, 6 and at the termination of the 10-day test. At the end of the 10-day test, animals were retrieved by washing the sediment through a fine mesh aquarium net and sorting through the retained material. Missing adult amphipods were assumed to be dead and newly released juveniles were also enumerated.

Pore-Water Extractions

Pore-water was extracted from the sediments on the day after collection with a Teflon® squeeze extraction device described previously (Carr *et al.*, 1989). Instead of a 0.7 μm GF/F glass fibre filter (Whatman, Inc., Clifton, NJ) that had been employed previously, an 8 μm nylon filter (Spectrum Med. Inc., Los Angeles, CA) was used. The results of recent studies indicate that certain dissolved hydrophobic contaminants may be adsorbed to GF/F glass fibre filters (Word *et al.*, 1987). A comparison of pore-water extraction procedures suggested that fewer soluble contaminants are adsorbed to nylon filters than the GF/F glass fibre filters (Carr and Chapman, in review). Pore-water samples extracted from a particular site were combined and sub-samples were transferred to acid washed amber glass bottles with Teflon®-lined lids (I-Chem®) and frozen until just prior to testing. Pore-water also was extracted from a reference site in Corpus Christi Bay and processed as described for the Lavaca Bay sediments.

The day before the test, a sub-sample of pore-water from each treatment was thawed at room temperature and water quality characteristics (salinity, dissolved oxygen, pH and ammonia) were measured. The salinity was adjusted to either 25, 30 or 35‰, depending on the test conditions (see the following section), by the addition of Milli-Q deionized water or hypersaline brine (produced by slow evaporation of natural sea water). Because the salinity of the Lavaca Bay pore-water samples was 30 ± 1‰, the dilution factor was similar for all samples for a particular test and never exceeded 20% for any test. All samples in this study were above 80% dissolved oxygen saturation initially. The samples were then held refrigerated (4°C) overnight and gradually returned to the test temperature (20°C, except for the red drum test which was conducted at 25°C) before the start of the test.

Pore-Water and Microlayer Toxicity Tests

Two different tests were conducted with the gametes and embryo-larval stages of the sea urchin *Arbacia punctulata*. Adult sea urchins were collected from the ship channel jetties in Port Aransas, Texas. The sperm cell test followed the standard EPA method (Weber *et al.*, 1988) with several modifications. Sperm were exposed to the test treatments for 30 min (at 20 ± 1°C and 30 ± 1‰ salinity) and followed with the addition of a predetermined number of eggs. After a one hour incubation period, the test was terminated by the addition of 10% buffered formalin and the percentage of fertilized eggs was determined. In the EPA procedure, the sperm density is estimated spectrophotometrically or by direct counting with the aid

of a haemocytometer. We have found that sperm collected dry (i.e., not allowed to contact sea water), and held on ice, remains viable for at least 8 hours, which allows a pretest to be conducted beforehand. We run tests routinely on a matrix of gametes from at least two females and two males with at least three different sperm dilutions per male. This pretest allows us to preselect a sperm/egg combination and ratio that optimizes the sensitivity of the test rather than testing sperm from four different males simultaneously in the actual test, as recommended in the EPA procedure (Weber *et al.*, 1988).

The morphological development assay with *Arbacia punctulata* (Oshida *et al.*, 1981; Pagano *et al.*, 1982) was executed in conjunction with the sperm cell test. The same sperm/egg ratio determined to be optimum in the pretest was also used in the morphological development test. The eggs were added first followed immediately by the sperm. The embryos were exposed to the test treatments for 48 hr at 20°C at which time the test was terminated by the addition of 10% buffered formalin. Aliquots from each of the five replicates/treatment were examined microscopically to determine the percentage of embryos that had developed normally.

The life-cycle test with the polychaete *Dinophilus gyrociliatus (Carr et al.*, 1986, 1989) was also conducted with pore water. This test was run under static conditions at 20°C and 25‰ salinity for seven days. In addition to survival, reproductive effects based on the number of eggs deposited at the termination of the test was also used as an end point.

Acute toxicity tests were also conducted with embryo-larval stages of red drum, *Sciaenops ocellatus,* following ASTM (1988) procedures. Red drum embryos were obtained from Dr. Connie Arnold at the University of Texas Marine Science Institute in Port Aransas, Texas. The embryos were collected from the spawning tanks within 8 hours of fertilization and the exposures were commenced as soon as possible thereafter. The exposures were conducted in stender dishes at 25°C at the salinity of the spawning tanks (35 ± 2‰) with 5 embryos in 10 ml/replicate with 5 replicates/treatment. Survival and hatching success were monitored over the two-day exposure. Exposure media from the 5 replicates/treatment were pooled at the termination of the test for water quality measurements.

Surface microlayer samples were also tested for toxicity with the two sea urchin assays and the acute test with embryo-larval stages of red drum. Water quality parameters were adjusted to the test conditions (as stated previously) before the start of the test, if necessary. Ammonia determinations were made with an Orion electrode and dissolved oxygen measured with a YSI meter. Differences among treatments for the various tests were determined by analysis of variance and Duncan's multiple comparison procedures with the aid of SAS (SAS Institute Inc., 1985).

RESULTS

Sediment grain size, moisture content, and mercury concentration were different among sites (Table 1). Because the test was started with non-gravid animals of the same age class and reproduction occurred in all treatment replicates, reproduction could be used as an end point. There were no significant differences in survival or reproduction of *Grandidierella japonica* between the reference and experimental treatments (Figure 2). Water quality characteristics at the termination of this static test were similar among treatments (Table 2).

Table 1 Sediment Characteristics at the Test Sites, Larvaca Bay, Texas.

Site	*% Moisture*	*% Sand*	*% Silt*	*% Clay*	*Hg (μg/g dry)*
Reference	ND*	98	0	2	ND
Ship Channel	38.5	33	20	47	1.1
Turning Basin	34.0	26	32	42	0.07
West Island	57.1	15	30	55	0.15

* ND = not determined

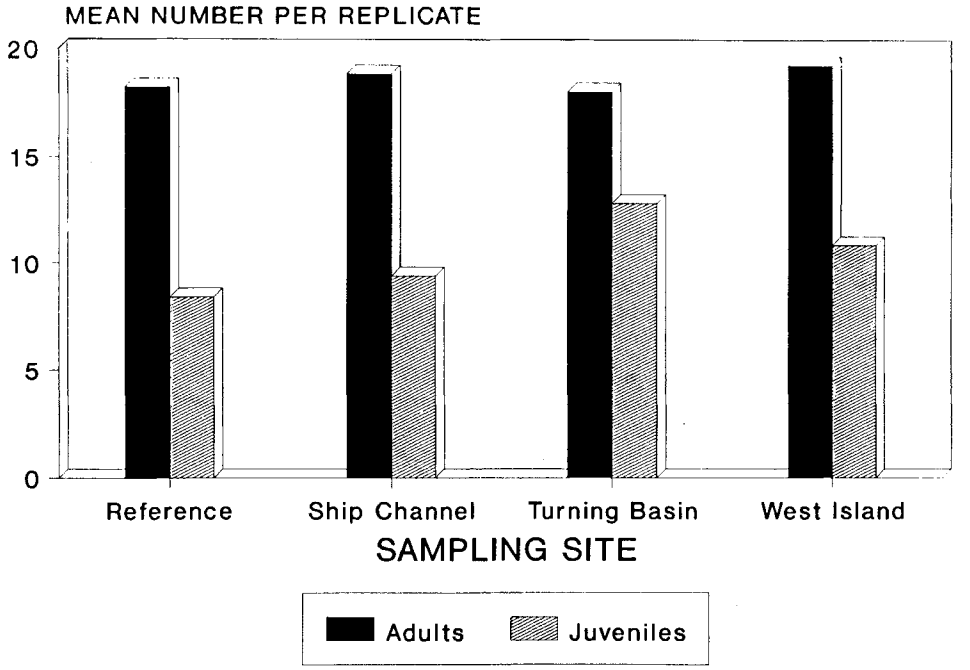

Figure 2 Survival and reproduction of the amphipod, *Grandidierella japonica*, exposed to sediments from Lavaca Bay. There were no significant differences among treatments.

Table 2 Water quality characteristics at the test sites in Lavaca Bay, Texas, at the end of the 10-day static solid-phase test with the amphipod *Grandidierella japonica*.

Treatment	*Temperature* (°C)	*Salinity (‰)*	*Dissolved Oxygen* ($mg\,l^{-1} \pm SD$)	*pH* (± SD)	NH_3 (as N) ($mg\,l^{-1} \pm SD$)
Reference	20.5	30	7.1 ± 0.4	8.1 ± 0.2	50.2 ± 19.5
Ship Channel	20.5	31	6.0 ± 0.7	8.2 ± 0.1	8.2 ± 2.4
Turning Basin	20.5	31	6.9 ± 0.1	8.1 ± 0.1	6.0 ± 2.3
West Island	20.5	30	6.1 ± 0.1	7.8 ± 0.1	151 ± 30.5

Highly significant differences were observed between the reference and all Lavaca Bay sites for both the pore water and microlayer samples in the sea urchin sperm cell test (Figure 3). Pore waters from the Turning Basin and West Island sites were particularly toxic and this result was corroborated by the sea urchin morphological development assay (Figure 4) in which little or no fertilization was observed for these samples, indicating a strong spermicidal effect. The water quality parameters for the pore-water samples for the sea urchin tests are shown in Table 3. Because the salinity for these samples was within the acceptable range for these tests (i.e., 30 ± 1‰), no adjustments were made on these samples prior to testing.

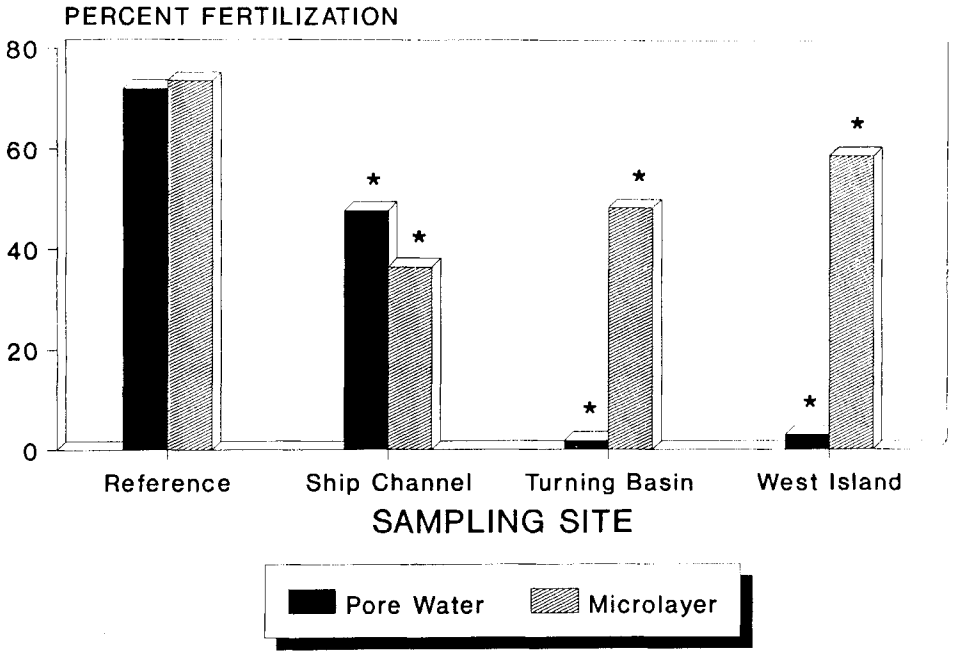

Figure 3 Sea urchin (*Arbacia punctulata*) sperm cell test results for pore-water and microlayer samples from Lavaca Bay, Texas. Asterisk indicates treatments significantly different ($p \leq 0.01$) from the reference.

A highly significant decrease in reproduction was observed in the sediment pore-water life-cycle test with the polychaete *Dinophilus gyrociliatus* for all of the Lavaca Bay sites as compared with pore water from the reference site (Figure 5). The West Island pore-water sample was again observed to be highly toxic with 100% mortality observed after four days of exposure in this test. Water quality characteristics were similar for the pooled samples from each treatment at the termination of this static 7-day test except for ammonia (Table 4), which was higher in reference (179 $\mu g\ l^{-1}$) and West Island (180 $\mu g\ l^{-1}$) pore water than at the other two sites. These ammonia concentrations are well below the level that causes a detectable response in this test (Carr *et al.*, 1989).

The 24-hour acute toxicity test with red drum embryo-larval stages indicated that the West Island and Turning Basin sediment pore-water samples (Ship Channel pore water was not tested) were highly toxic after a 24-hour exposure (Figure 6). Under

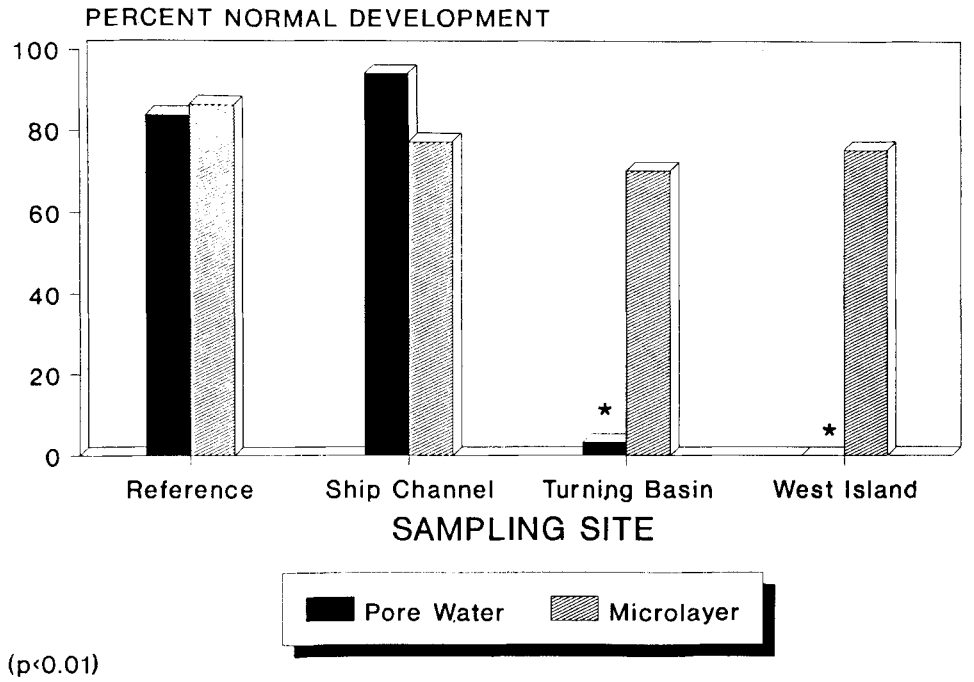

Figure 4 Sea urchin morphological development test results for sediment pore-water and microlayer samples from Lavaca Bay. Asterisk indicates treatments significantly different ($p \leq 0.01$) from the reference.

Table 3 Water quality parameters for the pore-water samples from sites in Lavaca Bay, Texas prior to conducting the sea urchin tests with *Arbacia punctulata*.

Treatment	*Temperature* (°C)	Salinity (‰)	Dissolved Oxygen ($\mu g\,l^{-1}$)	pH	NH_3 (as N) ($\mu g\,l^{-1}$)
Reference	20	30	7.4	7.40	19
Ship Channel	20	30	7.3	8.31	157
Turning Basin	20	30	6.8	8.53	93
West Island	20	29	6.0	7.83	134

the conditions used in this test, hatching is normally complete after 18–20 hours. Microlayer samples from sites in Lavaca Bay did not exhibit any significant toxicity in this test.

DISCUSSION

The sensitivity of *Grandidierella japonica* to contaminated sediments has been shown to be comparable to the more commonly employed marine benthic amphipod test species *Rhepoxynius abronius* and *Ampelisca abdita* (Word *et al.*, 1989). Studies conducted in our laboratory (unpublished data) and elsewhere (Nipper *et al.*, 1989)

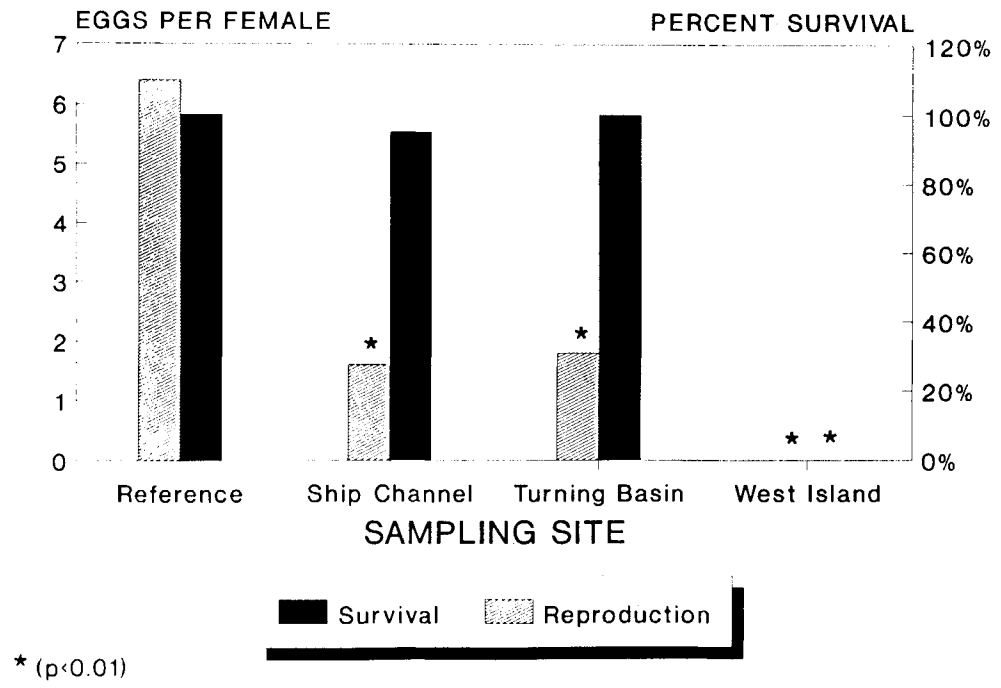

Figure 5 Reproduction and survival of the polychaete, *Dinophilus gyrociliatus*, exposed to sediment pore-water from Lavaca Bay. Asterisk indicates treatments significantly different (p μ 0.01) from the reference.

Table 4 Water quality parameters at the test sites in Lavaca Bay, Texas at the end of the seven-day static sediment pore-water test with the polychaete *Dinophilus gyrociliatus*.

Treatment	*Temperature* (°C)	Salinity (‰)	Dissolved Oxygen ($mg l^{-1}$)	pH	NH_3(as N) ($\mu g l^{-1}$)
Reference	20.5	20	7.3	8.19	179
Ship Channel	20.5	28	7.3	8.44	135
Turning Basin	20.5	26	7.5	8.14	62
West Island	20.5	28	7.5	8.12	180

indicate that *Grandidierella japonica*, unlike *Rhepoxynius abronius*, is relatively insensitive to grain size effects. The 10-day solid-phase amphipod test included in this study is representative of the most sensitive test that will be recommended in the revised green book (EPA/CE, 1991) for assessing the quality, and ultimately the disposal method and location, of dredged material in US coastal waters. No significant effects on survival or reproduction were observed in the solid-phase tests with *G. japonica* for any of the sediments from Lavaca Bay. However, highly significant effects were observed in four different species exposed to sediment pore-water from these same sediments. Microlayer samples from all three Lavaca Bay sites also exhibited toxicity in the sea urchin sperm cell test, which seems to be the

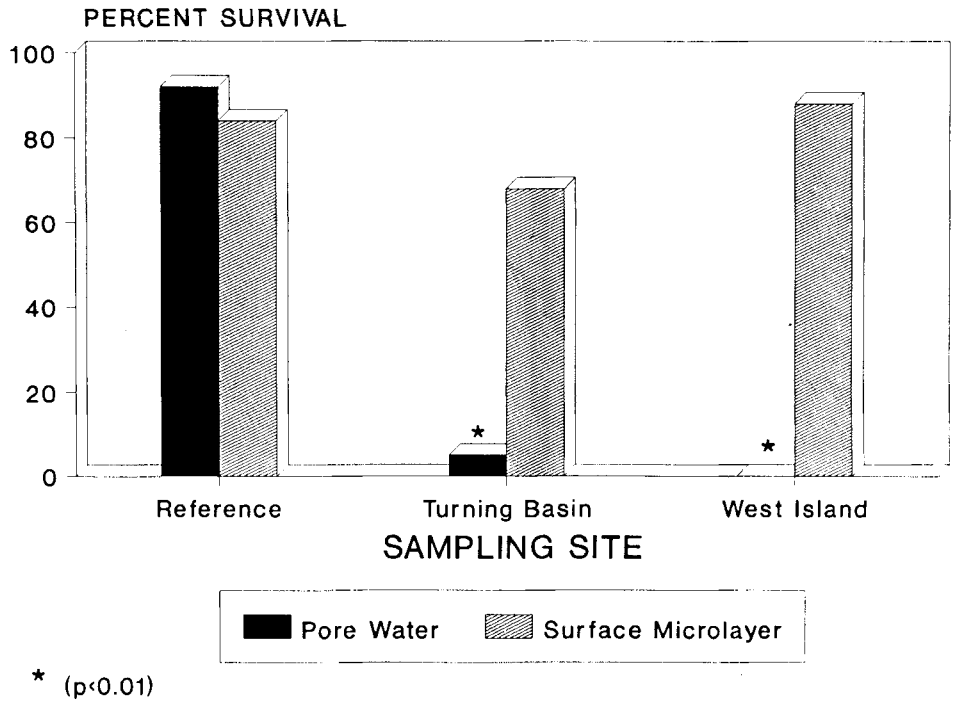

Figure 6 Survival of red drum (*Sciaenops ocellata*) larvae exposed to sediment pore-water and surface microlayer samples from Lavaca Bay. Asterisk indicates treatments significantly different ($p \leq 0.01$) from the reference.

most sensitive of the four pore-water tests evaluated. There appears to be a direct relationship between the toxicity of the surface microlayer samples and their proximity to the dredging operation that was in progress at the time the samples were collected (Figure 1 and 3).

There was no correlation between the total mercury content of the bulk sediments and the observed toxicity. The mercury concentration of sediment from the Ship Channel site was about one order of magnitude higher than the mercury concentration from the Turning Basin and West Island sites. However, several of the pore-water tests (Figure 4–6) revealed that the latter two sites were considerably more toxic than the Ship Channel site.

Historically, the majority of dredged material samples that have been tested for toxicity with solid-phase tests passed the regulatory criteria for ocean disposal. Although inclusion of sensitive benthic infaunal amphipods as required test species is an improvement over the species used routinely in the past, these tests may not adequately protect sensitive species and life stages that may be impacted by current dredged material discharge practices. We believe that the use of more sensitive tests and test species will provide the information necessary to make informed decisions about the disposal of dredged material in oceanic and near coastal environments.

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