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Estuarine Sediment Bioassay with Oyster Pediveliger Larvae (*Crassostrea gigas*)

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Estuaries are rich ecosystems serving as breeding and nursery grounds for a number of economically important fish and shellfish species. Estuaries receive pollutants and toxicants from many sources and these chemicals become trapped and concentrated in sediments. Conventional bulk analysis of chemicals in sediments does not indicate bioavailability or toxicity, which may be more closely related to sediment pore-water concentration (Phelps et al 1985; Swartz et al 1985). In estuaries, salinity is an important variable for determining bioavailability of toxics (Sunda and Guillard 1976).

There are several standard bioassays for toxicants in water but few for sediment (Chapman et al 1987). Among current sediment bioassays are the utilization of marine sediment-dwelling amphipods (Swartz et al 1979), and responses of organisms to sediment slurries or elutriates (Tsai et al 1979). There is need of a bioassay for solid sediment which is suitable for a wide range of salinities, using an organism commonly found in estuaries such as the oyster. The earliest and most sensitive life history phase of the oyster that comes in contact with sediment is the pediveliger stage which settles and undergoes metamorphosis to the oyster spat. Failure of metamorphosis may be an even more sensitive indicator of the toxicity of the setting surface than survival (Crisp and Austin 1960). The pediveliger larva of the Pacific oyster, Crassostrea gigas, was explored as a sediment bioassay organism because of nearly year round commercial availability, and common use in previous work with culture and toxicity testing (Coon et al 1986). One comparison bioassay was also made with the native Chesapeake Bay species, Crassostrea virginica which may become more readily available in the future.

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MATERIALS AND METHODS

Several thousand eyed pediveliger Crassostrea gigas larvae were obtained weekly by Federal Express from the Coast Oyster Company, Quilcene, Washington. The larvae were approximately the same age although some batches were more ready to set than others. The oyster larvae were maintained in 2.7-L glass jars with aerated artificial seawater (ASW). An antibiotic mixture of neomycin, streptomycin and penicillin (Sigma, St. Louis, Missouri) was added at 1:100 to all solutions. Larvae were fed a mixture of live Monocrysis and Isocrysis algae. The water was changed every 2 d by filtering the larvae from the holding jars (149 um Nytex screen). In some cases a heavy protozoan infestation caused excessive mortality and the best remedy was daily filtering of larvae with vigorous rinsing before returning to fresh ASW.

The mud-type control sediment was obtained from the upper 3 cm of an undisturbed sediment grab (Van Veen) at 11 m depth, from an unpolluted site near the western side of the Chesapeake Bay (Buoy 64). Control sediment collected 2 June 1987 (17 ppt salinity) was stored at 4 C; control sediment obtained 18 Sept. 1987 (19 ppt salinity) was stored at 4°C and 0°C (frozen). Presumably toxic sediment was obtained from Baltimore Harbor on 14 Nov. 1987 (10 ppt salinity) with a Ponar grab and stored at 4 C and 0 C (frozen).

For the bioassay, actively swimming larvae were placed in ASW containing 0.0001 M epinephrine (Sigma, St. Louis, Missouri) to induce metamorphosis (Coon et al 1986). A stock solution of 0.01 M epinephrine in 0.05 N HCl was freshly prepared for each test series and diluted 1:90 with ASW at the desired salinity for the bioassay. Different treatments were tried: initially larvae were treated for 2 hr then rinsed before placing on sediment. Subsequently, the rinsing step was eliminated as epinephrine did not increase mortality, and finally the larvae were treated for 20 hr and those responding to the epinephrine, i.e. settling, were hand selected.

Sediments were tested in the 2.75-mL wells of 24 well Falcon (Lincoln Park, New Jersey) tissue culture plates, which were held at room conditions for the bioassay, 21°C. Approximately 1.0-mL of mud-type sediment was pressed through 149 um Nytex screen into a well and 1.0-mL ASW layered on top. About 30 larvae were pipetted into each well at the sediment-water interface. Usually two wells were set up for each experiment and the results were pooled. Wells of control sediment and ASW were run for each experimental batch of larvae and set of experiments.

At 96 hr the well contents were withdrawn using an automatic pipette and filtered with 149 um Nytex screen. At 96 hr both mortality and metamorphosis were more readily distinguishable than earlier. The larvae retained by the screen were backwashed into a

petri dish with fresh ASW, counted, and separated by microscopy into living, dead, and metamorphosed. Live larvae were distinguished by cilia movement, movement and clarity of internal organs, and transparency. Dead larvae were often grey and opaque with no evidence of internal organization or movement. Some dead larvae showed retraction or partial decomposition of tissues and some had invasion by protozoa. Metamorphosis was determined by the transition from rounded veliger to a flat shape with new shell growth.

The difficulty of live-dead determination on low toxicity sediments led to attempting neutral red dye staining of the live larvae by the method of Crippen and Perrier (1974). The fixation phase was unsatisfactory so the technique was modified by adding a few drops of 1/1000 neutral red in distilled water to the container with sieved larvae for a final concentration of 1/10,000 neutral red in ASW. After 3 hr the live larvae had been stained and were counted, and the moribund larvae that had been invaded by bacteria or protozoa had distinctive patterns of clumping of stain and could be eliminated.

The salinity tolerance of larvae was tested with several ASW salinities between 1 and 34 ppt (Gilson refractometer). Sediment salinity was adjusted by stirring with diluted ASW for 10 min (10:1 water:sediment) and centrifuging for 2 min.

The pH of fresh control sediment was adjusted by stirring for 10 min (10:1 water: sediment) with 32 ppt ASW at pH 4, 5, 6, 7 or 8 (0.1 N HCl addition), and centrifuging.

Sediment was spiked with copper to make a toxic condition (Cairns et al 1984; Phelps et al 1985). A 1000 mg/L Cu stock solution (Fisher Scientific Atomic Absorption Standard, Baltimore, Maryland) was diluted with ASW and mixed vigorously with wet sediment (10:1 water:sediment) for 10 min. The sediment slurry was centrifuged and rinsed twice with ASW using the same procedure with 1 min mixing. Copper concentration of sorbing solutions ranged from 80 - 800 ppm. The final concentration of sorbed copper in sediment was not determined. Effects of aging copper-enriched sediment were explored with 600 mg/L copper-spiked sediment prepared 8 and 3 d before comparison with freshly spiked sediment.

One batch of Crassostrea virginica pediveliger larvae was available. Bioassays of copper-enriched sediment (200 ppm) were conducted at both 30 and 15 ppt salinity. The C. virginica bioassays used natural filtered seawater.

RESULTS AND DISCUSSION

The fact that C. gigas pediveliger larvae treated with epinephrine settled and survived for 96 hr on sediment in tissue culture plates, and could be recovered by filtration, indicated

they could be an appropriate test organism for a sediment bioassay. Mortality in ASW alone, 2.8%, was statistically the same as in the presence of control sediment, 6.4% (n = 11), which is less than the 10% proposed as a standard for bioassays (Chapman et al 1987).

Only salinities below 5.2 ppt had increased control mortality. However, below 23.5 ppt salinity there was no metamorphosis (Fig. 1). Initially, the percent metamorphosis at 30 - 34 ppt salinity was highly variable. With 20 hr epinephrine pretreatment and hand selection of settling larvae, metamorphosis statistics improved: in ASW metamorphosis was 38%, s.d. 21.4; and on control sediment, 37%, s.d. 23.2 (n = 7). The failure of C. gigas larvae to show metamorphosis below 23 ppt salinity indicated they may not be suitable for this bioassay at lower salinities. C. virginica larvae have been reported to have better survival than C. gigas at lower salinities (Davis 1958). In the course of these experiments it was noted that the epinephrine could be inactivated by higher temperatures, and one shipment arrived inactive.

Mortality on control sediment stored at 4° C or 0° C (frozen) increased significantly to 60% after 2 mon storage, along with a decrease in sediment pH (Table 1, Fig. 2). However, experimental lowering of pH only caused 9% mortality (Fig. 3). Additional toxics may have been mobilized from the control sediment by the combination of storage and lowered pH. It would appear advisable to use control sediment for only 1 mon after collection.

Table 1. Effects of storage time and temperature on sediment toxicity and pH.

Bioassay Date	Storage (d)	(C)	pH	Bioassay no.	Mort.	Meta.
Control Sediment						
9/18	7	4	8.0	53	3 (6%)	0 (0%)
9/18	60	4	6.5	55	12 (22%)	0 (0%)
9/18	60	0	7.7	53	11 (21%)	6 (11%)
6/2	115	4	6.0	52	25 (48%)	0 (0%)
Baltimore Harbor Sediment						
11/14	0		8.0	55	55 (100%)	0 (0%)
11/21	7	4	7.8	61	27 (44%)	0 (0%)
11/21	7	0		43	5 (12%)	0 (0%)
Copper-Enriched Sediment (600 mg/L)						
	0			58	49 (84%)	0 (0%)
	3	4		52	46 (88%)	0 (0%)
	8	4		47	21 (45%)	1 (2%)

Baltimore Harbor sediment was 100% toxic on the day of collection but had a significant decrease in toxicity when bioassayed after storage for 1 wk at 4° C, and an even greater loss of toxicity when held at 0° C (frozen) (Table 1, Fig. 2). Using a burrowing bioassay, a similar decrease in toxicity of Chesapeake Bay sediments has been noted after 5 d storage (Phelps unpublished MS).

The stored clay-type Baltimore Harbor sediment had loss of odor and alteration of the colloidal structure. In addition, freezing may change the relative concentrations of toxics in the solid phase and pore water of sediments.

The copper enrichment of sediment was a successful treatment. Copper-spiked sediment caused increasing larval mortality with an LD50 = 313 mg/L of Cu in the sorbing solution (Fig. 4). The true sediment-copper LD50 for C. gigas larvae was not determined as the copper on sediment and in pore water was not measured. As with Baltimore Harbor toxic sediment, the copper-spiked sediment showed decreased toxicity upon storage for a few days (Table 1). Copper-spiked marine sediment has been shown to lose effects on clam burrowing behavior after 2 d, associated with decreased copper in pore water (Phelps et al 1985). It appears that diagenesis of sediment-sorbed estuarine toxics may occur rapidly when sediment is stored and it is recommended that bioassays be conducted with fresh sediment (Maleug et al 1986).

For the set of experiments with C. virginica, the pediveliger larvae had average control mortality in ASW and control sediment of 10%. The average control metamorphosis was 3.5%, much lower than C. gigas. On copper-spiked sediment (200 mg/L), C. virginica larvae had 25% mortality and no metamorphosis, which was similar to the results of 21% mortality and no metamorphosis with C. gigas larvae. The one available batch of C. virginica pediveliger larvae did not appear all in the same stage of development.

Interestingly, no metamorphosis was observed on any of the copper-enriched sediments (Fig. 4). Metamorphosis failure is a valid bioindicator of general sediment toxicity, but in general the metamorphosis bioassay had much more variability than the toxicity bioassay. Larvae that have not fully developed to the eyed pediveliger state do not respond to epinephrine and this competency factor varied considerably from batch to batch. With competent larvae the success of epinephrine-induced metamorphosis has been reported at greater than 90% (Coon et al 1986).

Advantages of the C. gigas larva estuarine sediment bioassay are:
1. It is rapid (96 h).
2. It is easy to set up and takes little space.

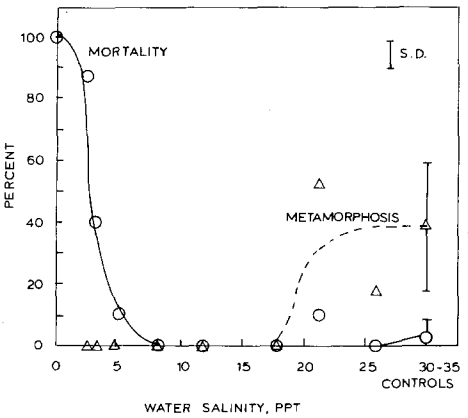

Figure 1. Salinity, and C. gigas larval survival and metamorphosis.

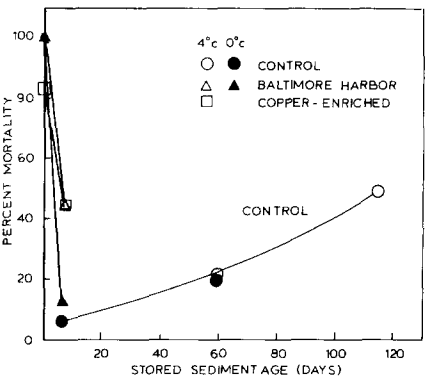

Figure 2. Changes in sediment toxicity with storage.

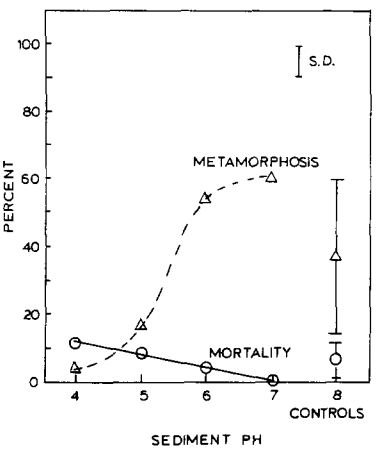

Figure 3. Effect of sediment pH on C. gigas larval

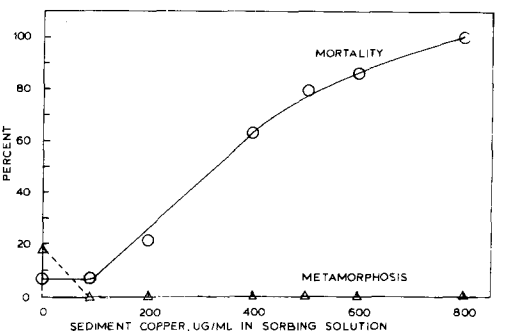

Figure 4. C. gigas larval mortality and metamorphosis on copper-enriched sediment.

3. The bioassay organism is sensitive, euryhaline, commercially readily available almost year round, and requires minimal laboratory support.
4. The bioassay simulates real-world sediment effects on oyster larvae.
5. The bioassay is suitable for solid-phase sediment testing.
6. The metamorphosis bioassay appears very sensitive, though not euryhaline.
7. The bioassay organism appears to have sensitivity similar to the larvae of another commercial oyster species.
8. Although only sediment less than 149 um is tested, this fraction contains the greatest concentrations of sorbed toxics.

Some preliminary recommendations for the bioassay:
1. Sediment and seawater controls must be run simultaneously with bioassays.
2. Larval competency should be examined after 20 hr epinephrine pretreatment.
3. For the metamorphosis bioassay, larvae need to be hand-selected after epinephrine pretreatment.
4. Epinephrine shipments should be tested for effectiveness upon arrival and stored dessicated at 4 C.
5. Sediments should be tested within 3 d of collection.
6. Control sediment should not be stored over 1 mon.

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