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Development of an Estuarine Sediment Burrowing Bioassay for Shipboard Use

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Estuarine sediments, particularly mud-type, are notorious accumulators of pollutants (Sinex 1971). Estuarine sediment sampling cruises have found high concentrations of xenobiotics in some areas but not all sediment contaminants are bioavailable or bioeffective. The use of sediment bioassays is recommended (Chapman et al. 1987), although there can be considerable difference in results (Long 1989).

An oyster pediveliger larva bioassay showed significant decrease in sediment toxicity within days after sample collection (Phelps and Warner in press). Current thinking is to conduct bioassays on freshly collected, not stored, sediments (Swartz et al. 1985; Chapman 1988). A rapid estuarine sediment bioassay based on the burrowing speed of the commercial euryhaline soft-shell clam, Mya arenaria, was extensively tested in the laboratory (Phelps 1989). With estuarine sampling cruises of several days to a week, this rapid bioassay on shipboard with freshly collected sediments might be useful in determining sites with in situ toxicity where additional synoptic sampling should be made for biological and chemical components of the Sediment Quality Triad, such as community structure and chemical contaminants.

Because it was not known whether the M. arenaria laboratory bioassay test could be adapted to shipboard where there is vibration and varying conditions such as sample euryhalinity, it was decided to take this test to sea. This report describes modifications of the bioassay resulting from attempted use on a week-long EPA cruise. This cruise collected and analyzed sediments from the Chesapeake and Delaware Bays and ocean dump sites, with salinities ranging from 2 to 32 ppt.

MATERIALS AND METHODS

The clam burrowing bioassay for estuarine sediment used young (1-2 cm) M. arenaria obtained from a natural set in the flowing seawater system of the Virginia Institute of Marine Sciences Center at Wachapreague, VA. The clams were shipped to

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the Chesapeake Biological Laboratory (CBL) of the University of Maryland at Solomons, MD near the middle of the Chesapeake Bay. Clams were stored at CBL up to three months in sediment at 10°C and ambient salinity (13 ppt), and kept similarly in a cooler aboard ship. Before a bioassay the clams were placed at ambient temperature (18-22°C) for 12 hr, which does not cause undue mortality. The bioassay was conducted as previously described, by placing 20 clams on a one-liter sediment sample in a plastic box with 13.5 sq. cm surface area and 5 cm of water, noting the number of clams completely burrowed at increasing intervals, and calculating the estimated time for 50% of clams to burrow (ET50) by Logit analysis (Phelps 1989). Up to five sediment samples were tested simultaneously against a CBL control (sandy) sediment. A sediment was considered significantly different if the bioassay ET50 95% F.L. exceeded the simultaneous control sediment bioassay ET50 95% F.L. Clams that had been used in bioassays causing burrowing inhibition were discarded as possibly impaired (Phelps et al. 1985).

Sediment samples were collected on the EPA OSV Antelope MAREST cruise May 17-23, 1985, starting from Annapolis, MD, collecting down the Chesapeake Bay, at ocean sites off Virginia and Maryland, up the Delaware Bay, through the Delaware Ship Canal and back to Annapolis, MD (EPA 1985a, 1985b). One liter of surface sediment collected with a Smith-McIntyre grab was bioassayed using water from sampling sites. Additional surface sediment samples were stored in plastic bags at 4°C.

Immediately upon return, May 24-27, control sediment bioassays were conducted at the Chesapeake Biological Laboratory (CBL) comparing the effects of changes in sediment and water salinity. For bioassays comparing sediments at different salinities the bioassay water was the same as the clam ambient salinity, 13 ppt. CBL control (sandy) sediment was adjusted to 3, 8, 13, 28 and 32 ppt by rinsing three times (10:1 v:v sediment:water) with dilutions of artificial sea water (Instant Ocean). The standing water was poured off and water at 13 ppt carefully layered over the sediment for the bioassay. There was no obvious mixing of overlay water into the sediment. Although this procedure may have set up conditions to alter pore water chemistry, the adjusted sediments were compared by simultaneous bioassay within an hour of preparation and the changed pore-water salinity was probably the primary difference.

To compare the effect on the bioassay of changing the water salinity, the same technique was applied but with CBL control sediment at 13 ppt being overlaid with a different salinity water: 3, 8, 13, 18, 22 and 32 ppt (Instant Ocean). The bioassay clams were acclimated from 6 to 48 hr at ambient temperature (18 - 20°C) in 3, 8, 13, 18, 22 and 32 ppt water before being introduced to the 13 ppt test sediment overlaid with 3, 8, 13, 18, 22 and 32 ppt water. As a result of these laboratory tests, all the cruise sediments were immediately retested (May 25-27) with overlaid bioassay water at 13 ppt and clams at 13 ppt. Five

of the 11 sediments causing inhibition of burrowing were retested with the bioassay over a three-day period (May 25-28). There was enough of one sample (LB6) to use stored material twice.

Sediments of the Chesapeake Bay can be classified into three types with distinct communities: fine sand, mud, and muddy-sand transition (Lippson 1973). M. arenaria burrowing speed was compared in the three sediment types from control sites in the Chesapeake Bay, and a coarse building sand to simulate ocean sand.

RESULTS AND DISCUSSION

At the beginning of the EPA cruise starting from Annapolis, bioassays on CBL control sediment with water from the sampling sites were similar to laboratory controls, but at higher salinities the burrowing speeds slowed significantly. As the cause of the burrowing inhibition was unknown, the shipboard bioassays were postponed until tests on the effects of changes in sediment and water salinities could be run in the laboratory.

Laboratory comparison of clam burrowing speeds into different sediment types showed no significant difference between fine sand, coarse sand and muddy-sand, and only marginally slower speed in mud sediment (Table 1). This is consistant with the finding of M. arenaria as a numerically dominant species in all three types of Chesapeake Bay sediment communities (Mountford et al. 1977).

Laboratory bioassays with different sediment salinities but using water at the clam ambient salinity (13 ppt) found slight but significant increase in burrowing speed only at 32 ppt sediment salinity (Fig. 1). Bioassays conducted with water salinity different from original clam ambient salinity (and ambient sediment salinity) found large changes in burrowing speed, especially at lower water salinities, and even after 48 hours of clam acclimation at the new salinity (Fig. 2).

When the collected cruise sediment samples were re-tested in the laboratory with overlaid water at 13 ppt, 11 out of 39 sediments (28%) showed significant inhibition of clam burrowing speed (Table 2). EPA sediment analysis was available for 4 of the 11 samples (EPA 1985c) (Table 3). There was no correlation of burrowing speed inhibition with the peak value of any of the sediment chemical or physical constituents. Possibly the factors affecting clam burrowing were not being measured. Copper spiking of marine sediment found clam burrowing inhibition related only to changes in sediment pore-water copper, not total sorbed copper

Table 1. M. arenaria burrowing speed and sediment type.

	Mud	Muddy sand	Fine sand	Coarse sand
ET50 (hr)	1.17	0.25	1.01	1.00
(F.L.)	(.69 - 1.95)	(.10 - .63)	(.56 - 1.12)	(.63 - 1.58)

Table 2. Sediment sample collection data and ET50 (hrs).

Site	EPA No.	Date	Sal. (ppt)	Sed. type	ET50 ship (95% F.L.)	ET50 laboratory (95% F.L)
Chesapeake Bay Samples						
4.1W	008	5/17	13	M	1.8 (1.4-2.3)	1.1 (.84-1.4)
4.3E	009	5/17	18	M		0.71 (.53-.94)
4.3C	010	5/17	16	M		1.26 (.76-2.1)
4.3W	011	5/17	13	M		0.65 (.55-.76)
5.1C	012	5/17	18	MS		0.66 (.56-.76)
5.1W	013	5/17	16	FS		0.39 (.34-.46)
5.3E	014	5/17	17	M		0.86 (.71-1.04)
5.3C	015	5/17	20	M		0.61 (.51-.74)
5.3W	016	5/17	17	M		0.84 (.71-1.0)
6.3C	017	5/17	26	-	0.95 (.69-1.3)	3.0 (2.5-3.5)*
7.2	018	5/17	28	FS		0.60 (.47-1.3)
7.2E	019	5/17	26	FS	0.49 (.39-.63)	1.13 (.85-1.51)
7.4N	020	5/19	-	-		0.64 (.53-.78)
7.4	021	5/19	33	FS	0.43 (.33-.55)	0.43 (.33-.55)
LB5	023	5/19	32	MS		0.96 (.81-1.1)*
LB6	024	5/19	32	M	4.7 (3.2-6.8)*	7.4 (5.4-10.0)*
Ocean Samples						
A010	--	5/19	33	FS		0.41 (.34-.50)
NB1	025	5/19	34	FS	0.72 (.57-.91)	0.95 (.85-1.12)
DN2	--	5/19	32	FS	0.46 (.38-.55)	0.78 (.65-.92)
206	--	5/20	32	CS	0.75 (.55-1.06)	1.2 (1.0-1.5)
Delaware Bay Samples						
DB2E	026	5/22	30	FS	0.49 (.40-.60)	0.65 (.59-.76)
DB2C	027	5/22	30	-	1.5 (1.2-2.3)*	0.96 (.76-1.2)
DB2W	028	5/22	29	-	0.85 (.71-1.0)	0.53 (.41-.69)
DB3E	029	5/22	23	-	0.82 (.63-1.0)	0.60 (.46-.81)
DB3C	030	5/22	24	-	0.51 (.42-.63)	0.78 (.69-.88)
DB3W	031	5/22	27	-	0.84 (.69-1.0)	1.4 (1.2-1.7)*
DB4E	--	5/22	16	MS		1.2 (1.2-1.4)*
DB4W	--	5/22	17	CS		1.1 (.84-1.6)*
DB5E	--	5/22	9	C		1.4 (1.2-1.8)*
DB5C	--	5/22	9	C		0.80 (.66-.97)
DB5W	--	5/22	7	C		3.5 (2.5-5.0)*
DB6E	--	5/22	2	M		2.1 (1.7-2.6)*
DB6W	--	5/22	2	-		5.6 (4.2-7.4)*
Mid-Chesapeake Bay Samples						
MCB2.1	--	5/22	1	M		2.5 (1.9-3.3)*
MCB2.	--	5/22	3	M		1.2 (.035-1.47)
MCB3.2C	--	5/22	19	M		0.43 (.36-.51)
MCB3.2W	--	5/22	13	M		0.59 (.50-.70)
MCB3.3E	--	5/22	-	M		0.20 (.10-.38)
MCB3.3C	--	5/22	-	M		0.24 (.19-.32)

*ET50 significantly different from simultaneous control
M=mud; MS=muddy sand; FS=fine sand; CS=coarse sand; C=clay

Table 3. Chemical and physical analyses of bioassayed sediment samples.

Site	EPA No.	% Solids	Cd ppm	Cr ppm	Cu ppm	Pb ppm	Ni ppm	Zn ppm
Chesapeake Bay Samples								
4.1W	008	3.7	54	485	362	540	460	2840
4.3E	009	43.9	4.4	20	14	11	15	85
4.3C	010	23.6	8.5	52	34	41	39	164
4.3W	011	62.7	3.2	32	13	18	35	106
5.1C	012	64.1	3.1	42	26	28	36	134
5.1W	013	53.0	3.8	15	11	15	10	62
5.3E	014	85.1	2.4	2	2	3	7	5
5.3C	015	26.2	7.6	49	27	27	31	148
5.3W	016	33.3	6.0	36	25	25	32	107
6.3C	017*	62.5	3.2	26	11	14	20	65
7.2	018	73.2	2.7	11	3	4	8	37
7.2E	019	81.2	2.5	7	2	3	7	31
7.4N	020	84.4	2.4	6	2	3	7	19
7.4	021	83.5	2.4	5	2	4	12	22
LB5	023*	56.2	3.6	24	13	12	23	63
LB6	024*	32.7	6.1	49	22	28	44	163
Ocean Samples								
NB1	025	93	2.1	2	2	3	6	4
Delaware Bay Samples								
DB2E	026	84.4	2.4	9	2	6	10	31
DB2C	027	83.8	2.4	9	3	6	10	43
DB2W	028	86.7	2.3	5	2	3	6	15
DB3E	029	79.6	2.5	8	4	8	7	40
DB3C	030	81.3	2.5	6	2	6	7	35
DB3W	031*	84.5	2.4	8	2	6	8	44

* ET50 values significantly different from simultaneous controls

(Phelps et al. 1985). Other investigators have reported estuarine biota are sensitive primarily to ionic and unsorbed chemical species (Sunda and Guillard 1976). If future studies hope to relate sediment chemical analysis to effects on living organisms sediment pore water may need to be included. This bioassay also may be more sensitive to sediment constituents that can change rapidly under estuarine conditions than long-term chemical contamination.

All of the retested sediment samples showed loss of burrowing inhibition by the third day of testing (fifth day post-cruise) (Fig. 3). The loss of sediment burrowing inhibition was similar to laboratory studies with fresh copper-spiked sediment showing a sharp decrease in burrowing inhibition within three days associated with decrease in sediment pore water copper (Phelps et al. 1985). A similar short-term loss of toxicity was found in stored Baltimore Harbor sediments with a Crassostrea gigas larval bioassay (Phelps and Warner 1990).

The controls of this euryhaline shipboard clam burrowing bioassay for estuarine sediments were similar to laboratory bioassays and not significantly affected by test conditions aboard ship (motion, etc.), except for changes in salinity of the test water. The test clams could be held at 10°C if placed at ambient temperature (18-22°C) for 12 hr before the bioassay. From these results it is apparent this burrowing bioassay could be applied to fresh estuarine sediment samples on shipboard as long as the clams and the overlying test water were at the same salinity. The water for the bioassay should be adjusted to the same salinity as that from where the clams originated.

The post-cruise testing confirmed the importance of conducting this bioassay within five days of sediment collection. Perhaps the generally negative results for the clam burrowing bioassay reported by Chapman et al. (1987) were due to the up to one week storage of sediments before testing. This clam burrowing bioassay is compact and can be used on shipboard to rapidly screen euryhaline sediments collected during a cruise, indicating the immediate need for more sampling to determine the cause of the burrowing inhibition. This rapid bioassay could also be used in the laboratory with experiments on sediment fluxing of toxicants. As estuarine conditions greatly affect chemical bioavailability, this bioassay would be helpful in determining the conditions of significant toxicant release from sediment.

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