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Clam Burrowing Bioassay for Estuarine Sediment

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Pollutants entering estuaries generally sorb to suspended material and become transported to sediments, where they may build up to considerable levels. Sorption of a pollutant decreases its concentration in water and toxicity to neritic life (Sunda and Guillard 1976), but once accumulated in sediments little may be known of its effects on benthos. Sediment bioassays are needed to detect the bioactive phases of sediment-sorbed pollutants, and these may be most closely related to sediment pore water concentrations than other, conventionally measured, sediment chemical components (Phelps et al. 1985).

A marine sediment bioassay based on clam burrowing behavior has been employed on the West Coast (Chapman et al. 1987). Behavioral modification is an important indicator of environmental stress (Olla et al. 1980) and may directly affect survival. For example, Pearson et al. (1981) have shown that oiled sediment slows burrowing time in the marine clam Protothaca staminea, leading to increased predation by the Dungeness crab. However, no corresponding behavioral bioassay has been developed for clam species inhabiting east coast estuaries.

The principal objective of this research was to develop a rapid sediment bioassay for east coast estuaries based on the burrowing speed of a native euryhaline clam species. The young of the commercial soft-shell clam, Mya arenaria, appeared suited for this bioassay and is easily obtained as it settles in high numbers in flowing seawater systems in the spring and fall, or can be collected by estuarine surface dredge.

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MATERIALS AND METHODS

Approximately 1000 young Mya arenaria, 17 - 25 mm, were obtained on 7/20/84 from the Wachapreague Laboratory of the Virginia Institute of Marine Science. The clams had set naturally that spring in the flowing seawater system of the laboratory so they were approximately the same size and age. Clams were transferred to the Chesapeake Biological Laboratory (CBL) of the University of Maryland at Solomons MD on the western shore of the mid Chesapeake Bay and held in shallow trays of sediment in a flowing seawater system. Although there was a considerable salinity difference between Wachapreague (34 ppt) and Solomons (9 ppt), no mortality resulted. Initially, young Mulinia lateralis, the coot clam, were collected by anchor dredge near Solomons and tested for the burrowing bioassay. However, M. lateralis had high mortality over the holding period, showed no growth, and had much greater control variablility; thus this clam species was eliminated from consideration for the bioassay.

The clams were held in shallow trays of fine sand sediment collected from an intertidal natural clam site at Scientist Cliffs, MD, 16 km north of Solomons, MD. Although symptoms of sediment anoxia (darkening) developed in the holding trays, there was less than 3% clam mortality over the seven month holding period. Fresh sediment was collected several times from the same site for the bioassays and had no symptoms of anoxia during experiments.

The burrowing bioassay was conducted by placing 20 clams in a regular array on one liter of sediment in a plastic box on a flowing seawater table. (Later studies have shown the bioassay can also be conducted with static water; Phelps, in review). The sediment depth was 4 - 6 cm, at least twice the maximum clam length. Starting with five-minute intervals, the number of clams that had achieved burrowing initiation, i.e., positioned vertically prior to digging, was recorded. Initiation of burrowing was chosen as the behavioral endpoint rather than complete burial because Mulinia lateralis was part of the initial study and this clam does not bury completely. Up to six bioassays were run simultaneously and each set of bioassays included a control. If at all possible, experiments comparing different treatments were tested with a simultaneous burrowing bioassay. This was to reduce the effects of external variables on clam behavior, which is considered essential for behavioral studies in general (Olla et al. 1980).

Data analysis by microcomputer LOGIT gave ET50, elapsed time in hours for 50% of the population to initiate

burrowing, with 95% FL (fiducial limits) (Finney 1981). A change in burrowing speed (ET50) was considered significant if the 95% FL did not overlap the control 95% FL.

Previous studies of a clam burrowing bioassay indicated the sediment surface area of the bioassay could be reduced without affecting burrowing speed (Phelps et al. 1983). To find a minimum sediment surface area, bioassays were conducted in commercially available plastic boxes ranging from 180 to 625 sq. cm. surface area. Subsequent bioassays used 20 clams on 180 sq. cm. surface area, corresponding to pint-size freezer boxes.

Significant slowing of burrowing speed, e.g. weakening, has been reported for clams held out of sediment (Pearson pers. comm.). To test for such weakening, clams were taken out of sediment for 24, 12, 6, and 2 hours before comparing burrowing speeds with a control.

Young M. arenaria held in trays of sediment at 10 ° C for several months do not grow, and show low mortality. Such storage would be useful to conduct bioassays over an extended period. The effect of short-term cold storage on burrowing speed was explored by placing clams at 10 ° C for one week and then removing at 24, 12, 6, and 2 hours before a simultaneous bioassay with a control.

Many marine species show endogenous tidal activity rhythms. M. arenaria was examined for tidal (endogenous) changes in burrowing speed by conducting bioassays at 4-hour intervals over a 12-hour period.

To examine the possibility of using a single set of clams several times for control bioassays, one set of clams was tested for fatiguing by repeatedly bioassaying at one hour intervals over a nine hour period. However, clams that had been used in bioassays with toxic sediment were never re-used due to possible impairment.

Over the six months of experiments from to August 1984 to February 1985, water temperature in the flow-through seawater system ranged from 26.5 to 4 ° C, and salinity from 9 to 16 ppt. Control bioassays were conducted several times over the experimental period. At the same time, the lengths of thirty clams were measured for growth.

In December, two size classes differing by almost one cm were selected from the original clam population, and an additional size class was obtained from

the fall set of very small clams in the flowing seawater system of CBL. The burrowing speeds of the three clam size classes were compared with a simultaneous bioassay.

Copper-spiked marine sediment has been used as an effective negative control in studies of clam burrowing bioassays (Phelps et al. 1983, 1985). Copper-spiked estuarine sediment was prepared by mixing freshly collected sandy sediment with estuarine water (5:1, water:sediment) containing 0.1, 1, 10 and 20 ug Cu/ml ($Cu(NO_3)_2$, Fisher Atomic Absorption Reagent). The slurry was stirred for 10.0 minutes, centrifuged to remove spiking solution, and rinsed twice with estuarine water by the same procedure stirring for only one minute. No attempt was made to retain the very small amount of fine sediment. Sediment-sorbed copper was measured by extracting 10.0 gm dried sediment with 10.0 ml HNO_3 (Fisher, Reagent Grade) at 80° C for five hours, diluting to 20 ml with deionized water and analyzing for copper by flame atomic absorption spectroscopy (Perkin Elmer 404 Atomic Absorption Spectrophotometer). The four levels of copper-enriched sediment were prepared simultaneously and bioassayed within two hours with a control, to avoid the rapid changes in pore-water copper that have been reported to occur (Phelps et al. 1985).

RESULTS AND DISCUSSION

There was no significant change in the burrowing speed of clams with reduction in surface area available for burrowing (Table 1).

Table 1. Sediment surface area and clam burrowing speed (ET50).

Sed. (sq cm)	180	263	308	435	625
ET50 (h)	.59	.46	.26	.44	.39
95% F.L.	.42-.81	.30-.71	.12-.59	.34-.56	.30-.49

The highest clam density of 9 sq. cm. sediment/cm. clam length was similar to their greatest reported natural density, 9.1 sq. cm. sediment/cm. clam length (Dow and Wallace 1950). This density appeared suitable for the bioassay.

Significant clam weakening, e.g., significant increase in ET50, ocurred when clams were out of sediment for over 12 hours (Fig. 1). Bioassay clams should be stored in sediment.

Clams taken from storage at 10 ° C to a temperature of 22 C were slowed significantly for up to 24 hours (Fig. 1). Clams can be held at 10 ° C., but should be warmed for at least 24 hours before being used.

M. arenaria showed no significant changes in burrowing speed when assayed at four-hour intervals over a 12-hour tidal cycle. This burrowing bioassay may be conducted irrespective of tidal cycle.

Clams bioassayed repeatedly at one-hour intervals only had significant slowing of burrowing, i.e. fatiguing, after five bioassays (Fig. 2). A single set of young clams therefore could be used repeatedly for control bioassays.

Over the six-month holding period on the sea water table at CBL the length of the M. arenaria population increased significantly, by an average of 9 mm (Fig. 3). There was a linear relationship between clam length and the log of burrowing speed (ET50) (Fig. 4):

log ET50 (h) = 0.036 LENGTH (mm) - 1.27 (corr. = .9997)

Clams for simultaneous bioassays should have less than one cm difference in length.

The M. arenaria clam-burrowing data reported by Pfitzenmeyer and Drobeck (1967) was compared with calculated speeds by the above equation (Table 2). Lower calculated ET50 may be due to the different behavioral endpoints: initiation of clam burrowing (this study) and complete clam burial (Pfitzenmeyer and Drobeck 1967).

Table 2. Mya arenaria size data from Pfitzenmeyer and Drobeck (1967) with calculated burrowing speeds

Size (mm)	Avg. (mm)	Measured ET50 (h) (95% F.L.)	Calculated ET50 (h)
35 - 50	43	2.6 (2.23-2.95)	1.9
50 - 65	58	5.3 (4.67-6.12)	6.6
65 - 75	70	18.2 (15.1-21.9)	17.8

Over the six-month holding period the control bioassays showed no changes in burrowing speed correlating with increasing or decreasing salinity (Fig. 3). The highest water temperature (26.5 ° C) was close to the lethal limit for M. arenaria which is a northern clam species. However, no changes in burrowing speed were noted at the higher water temperature, but at water temperatures below 13 ° C the burrowing speed was significantly

reduced (Fig. 3):

log ET50 (h) = −0.06 TEMP (°C)+ 0.49

Pfitzenmeyer and Drobeck (1967) also reported decreased burrowing speed at lower water temperatures. When their data were used to calculate the relationship between burrowing speed and water temperature, with adjustment for their larger average clam size (above), their equation was:

log ET50 (h) = −0.052 TEMP (°C) + 0.36

This is quite similar to the previous equation that was derived from the present study, and may be considered a general equation for M. arenaria burrowing speed at water temperatures below 13 °C.

The uptake of copper by fresh estuarine sandy sediment was linear with the concentration of copper added to the estuarine water (Table 3):

SED = 2.5 WATER − 2.7 (corr. = .967)

SED is concentration of sorbed copper on sediment (ug/gm) and WATER is concentration of copper in solution (ug/cc).

Copper spiking of sediment did not affect burrowing speed up to 13.2 µg Cu/gm sediment, but at 51.4 µg Cu/gm sediment the clams had significant delay in burrowing (Table 3). This indicates that clam burrowing speed can be affected by a sediment-sorbed metal. Normal burrowing speed has been shown to be important for clam survival.

Table 3. Effect of estuarine sediment copper enrichment on Mya arenaria burrowing speed.

Water Cu (µg/ml)	Sediment Cu (µg/gm)	Burrowing Speed ET50 (h)
Control	0.08	0.34
0.1	<0.05	0.62
1.0	0.2	0.38
10.0	13.2	0.40
20.0	51.4	1.21

In summary, young M. arenaria clams appeared suitable for a rapid burrowing bioassay for estuarine sediment by having an average control burrowing speed (ET50) of 0.45 hr. Up to six bioassays, including a control, can be conducted simultaneously. This clam burrowing bioassay can be conducted in a small space (pint freezer box size) with about one liter of sediment, at any time of day, and at any water temperature from 13 to 27° C. The bioassay should be conducted with clams approximately the same size. The bioassay has been shown to be

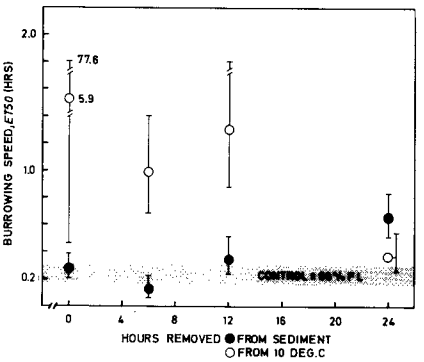

Figure 1. Effect of removal from sediment and removal from 10 deg. C on burrowing speed.

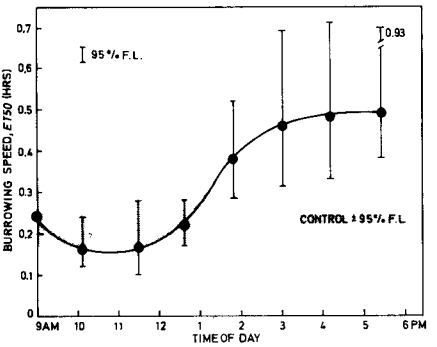

Figure 2. Effect of rapid repeated bioassays on burrowing speed.

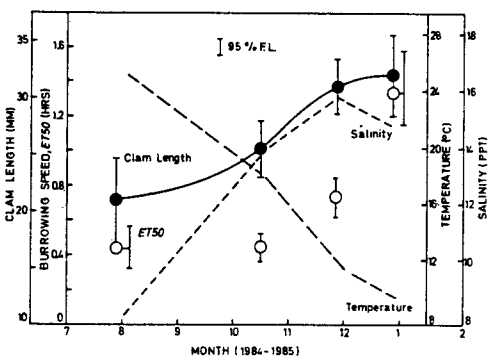

Figure 3. Mya arenaria growth, water temperature, salinity, and control burrowing speeds, in the laboratory (Aug.-Jan.).

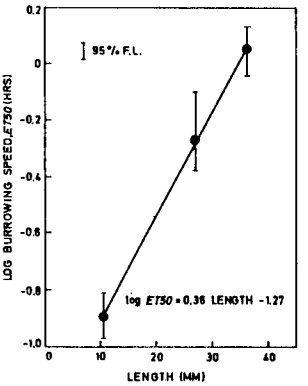

Figure 4. Mya arenaria size and burrowing speed.

responsive to sediment-sorbed copper. The control population burrowing speeds (ET50) had low variability, and the clams showed good growth and survival when held in a flowing seawater system for seven months. Young clams adapted readily to different salinities and could be stored in sediment at 10° C if warmed for 24 hours before using in a bioassay. Mya arenaria appears a promising species for a rapid sediment bioassay suitable for the estuaries of the East Coast, that receive intensive sediment pollutant loading.

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