# NOTE

# Biogenic bromophenols as negative recruitment cues

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ABSTRACT· Infauna greatly modify the sediments in which they live. One form of modification occurs via the organisms' secretions. In a number of families of Polychaeta and Hemichordata, the worms' secretions include halogenated aromatic compounds such as bromophenols. To determine how the presence of such compounds affects juvenile infauna, we directly added synthetic bromophenols to natural sediments at field concentrations and observed the responses of recently settled juveniles of 2 bivalve and 1 polychaete species. In nature the bromophenols are secreted into sediments by a capitellid polychaete. A significant percentage of the bivalve juveniles (50 and 67%) did not burrow into the sediments experimentally contaminated with bromophenols, while all the bivalve juveniles burrowed into the control sediments. The arenicolid polychaete juveniles burrowed into the experimentally contaminated sediments, but the rate of burrowing of these juveniles was significantly slower in the contaminated sediments than in the control sediments. The addition of bromophenols to sediments, therefore, had a significantly negative effect on acceptance of the sediment by recently settled juveniles of all 3 species. These results are consistent with the concept of such secretions determining the composition of assemblages in areas with biogenic producers of haloaromatic compounds.

KEY WORDS: Recruitment · Polychaete · Bivalve · Settlement · Cue · Bromophenol · Rejection  Haloaromatic

Sedimentary environments are habitats intimately modified by their inhabitants' burrowing, feeding, defecating, and secreting. As a result the fundamental structure of the sediment is closely linked to the type of biota which predominate in a given habitat (Rhoads & Young 1970, Brenchley 1982). Compounds released from organisms inhabiting sediments can affect the response of new recruits to the habitat. Such compounds can have a positive or a negative effect on recruits (positive: Highsmith 1982; negative: Woodin et al. 1993). In 1993 we demonstrated that nereid polychaetes reacted negatively to sediments previously occupied by a terebellid polychaete, *Thelepus crispus.*

T. crispus contains large concentrations of bromobenzyl alcohol which is found in the sediments around its tubes (Woodin et al. 1993) and we strongly suggested, but did not demonstrate directly, that the reactions of the nereids to the sediments occupied by *T. crispus* were due to the presence of this compound in the sediment. Five families of polychaetes are now known to contain such brominated aromatic compounds as well as a number of species of Hemichordata and 1 species of Phoronida (Faulkner 1995). Several of these species are extremely common. The terebellid *Lanice conchicolega*, for example, occurs in enormous dense patches in the subtidal to intertidal of the North Sea (Buhr 1976, Weber & Ernst 1978, Goerke & Weber 1991), while hemichordates such as *Balanoglossus* occur commonly throughout southeastern USA estuaries (Peterson & Peterson 1979). Sediments associated with these organisms appear to be contaminated with brominated aromatic compounds (King 1986, Woodin et al. 1987, Steward et al. 1992). Thus, reactions of recruits to these compounds could act to determine the composition of the community. To test this idea we explicitly contaminated sediments with compounds known to be released by a capitellid polychaete, *Notomastus lobatus*, and tested the responses of recently settled juveniles belonging to 3 infaunal species to control and contaminated sediments.

**Materials and methods. *Background information on infauna:*** As in our 1993 paper, we used recently settled juveniles of infaunal bivalves and polychaetes rather than settling larvae to avoid the potentially serious problem of differentiation between competent larvae and those of similar size and appearance but not yet competent (Bachelet et al. 1992). Sediment-dwelling juveniles of both bivalves and polychaetes can crawl away as well as waft away from a site (Roe 1975, Sigurdsson et al. 1976, Sastry 1979); thus, rejection of a site may occur at the juvenile as well as the larval stage.

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The juveniles used were less than 1 wk post initiation of metamorphosis and were an arenicolid polychaete, *Arenicola cristata*, a venerid bivalve, *Mercenaria mercenaria*, and a matrid bivalve, *Mulinia lateralis*. *A. cristata* and *M. mercenaria* were cultured as described in Woodin et al. (1995), while *M. lateralis* were collected as new spat from the field. (No *M. lateralis* were present in extensive collections the week prior.) At 6 setigers the larvae of *A. cristata* build tubes and are juveniles; given sediments, they will burrow and show rejection behavior. Individuals used in the experiments had a mean length of 683 μm. Mean sizes of *M. mercenaria* and *M. lateralis* were approximately 242 and 374 μm respectively. Recently settled spat of both these species do not penetrate the sediments and burrow; rather they nestle into the substratum to approximately half the length of the shell, position themselves with the hinge down, and gape slightly (Carriker 1961, Luckenbach 1984). In some types of sediments the spat fail to show this nestling behavior, instead remaining on the surface, typically resting on one valve or sometimes actively crawling across the sediment. Similarly, juveniles of *A. cristata* actively reject some sediments by either initiating a crawling sequence or by standing with the anterior two-thirds of the body arched vertically off the sediment surface. In flowing water such juveniles of both polychaetes and bivalves are easily eroded (Woodin et al. 1995). Sediments eliciting such behaviors were classified as unacceptable.

**Behavioral observations:** Glass dishes with a 1.5 to 2 cm layer of test sediment on the bottom, covered by a layer of seawater 2 cm deep, were used for observations. Individual juveniles were gently pipetted into the water column, and allowed to drift onto the sediment surface. Individuals which contacted the sediment surface forcefully, landed on a sand grain larger than their length, or, for the worms, landed in any position other than their ventral surface were not used in the analyses. Timing of responses to the sediments began upon the contact of the individual with the sediment surface. Using a dissecting microscope, individuals were monitored continuously from their arrival on the sediment surface until they disappeared below the surface (polychaetes) or nestled in among the surface sediments and began to feed (bivalves), or until expiration of the designated observation period per individual, 5 min for *Mercenaria mercenaria* and *Mulinia lateralis* and 6 min for *Arenicola cristata*. The length of the observation period was set to a minimum of 10× the mean time individuals took to initiate burrowing in preliminary observations of behavior in unmanipulated sediments. Data collected included number of individuals burrowing or nestling and length of time from initial contact with the sediment surface until initiation of burrowing or nestling.

**Experimental sediments:** Experimental sediments were collected in the field and immediately transported to the laboratory where they were maintained under seawater with aeration. In the field, the top 5 mm of tidally exposed sediments were collected with a paint scraper. Areas of disturbance such as fecal mounds, burrow scrapings, tubes, and feeding traces were avoided. The sediment collection sites were medium to fine grained sandflats at Oyster Landing, North Inlet, South Carolina, USA (33° 20' N, 79° 12' W; median grain size 0.38 mm, silt-clay 0%) and on the landward side of Pawleys Island, South Carolina (33° 24' N, 79° 8' W, median grain size 0.39 mm, silt-clay 0.05%). In the experiments with juveniles of *Mulinia lateralis* and *Mercenaria mercenaria*, a minimum of 2 replicate dishes per treatment were used with 5 juveniles added individually to different quadrants of the sediment within a dish. In the *Arenicola cristata* experiments, new dishes of sediment were used with each juvenile. In all cases controls were alternated with experimental sediments to ensure that the juveniles were still responsive.

The field collected sediments were mixed on a shaker overnight with 1 seawater change and then split into 2 halves, 1 designated control and 1 experimental. Experimental sediments were contaminated with 4-bromophenol, 2,4-dibromophenol, and 2,4,6-tribromophenol in concentrations mimicking those found in sediments within the *Notomastus lobatus* bed (Steward et al. 1992). Appropriate amounts of bromophenols were added to 1 ml HPLC-grade methanol and then mixed into the sediment by shaking on a shaker at 300 rpm at 4°C for 60 min. Control sediments had 1 ml methanol added and were also placed on the shaker for 60 min. Following shaking, the excess seawater was removed and the sediment was placed into the observation dish and new seawater added to a depth of 2 cm. Samples were taken from each sediment batch and analyzed by gas chromotography for concentrations of bromophenols (see Steward et al. 1992 for extraction and detection methods). Ranges of bromophenol concentrations in the experimental sediments were 122 to 240 ng $g^{-1}$ 4-bromophenol, 5 to 19 ng $g^{-1}$ 2,4-dibromophenol, and 3 to 14 ng $g^{-1}$ 2,4,6-tribromophenol. As reported previously by Steward et al. (1992) for field sediments, the control sediments, which were field collected, contained some bromophenols but at much lower concentrations (17 to 69 ng $g^{-1}$ 4-bromophenol, <1 to 2 ng $g^{-1}$ 2,4-dibromophenol, and 1 to 9 ng $g^{-1}$ 2,4,6-tribromophenol).

The data were analyzed in 2 ways. First, the number of individuals which failed to initiate burrowing or nestling within the observation period was compared across treatments by a 1-way Fisher's Exact Test. Second, for the experiments with *Mercenaria mercenaria*

Table 1 *Arenicola cristata, Mercenaria mercenaria, Mulinia lateralis.* Percentages of recently settled juveniles that did not burrow into control (Control sed.) or bromophenol contaminated sediments (Exper. sed.). Significance values are for 1-way Fisher's Exact Tests. ns: $p > 0.05$. N: total number of juveniles observed per treatment. P: polychaete; B: bivalve

| Species | N | Control sed. | Exper. sed. | Significance |
|---|---|---|---|---|
| *A. cristata* (P) | 30 | 0 | 13 | ns |
| *M. mercenaria* (B) | 20 | 0 | 50 | $p < 0.05$ |
| *M. lateralis* (B) | 18 | 0 | 67 | $p < 0.01$ |

and *Arenicola cristata*, the mean times to initiate burrowing by burrowing individuals within a dish were compared across treatments by a Kruskal-Wallis test.

**Results.** For both bivalve species a significant number failed to nestle into the sediments with bromophenols added (Table 1) 50 and 67% of the *Mercenaria mercenaria* and *Mulinia lateralis*, respectively, failed to nestle into the sediments with bromophenols, while 100% of both species did nestle into control sediments. In addition, for those spat which accepted the sediment, times to initiation of nestling were much slower for *M. mercenaria* in contaminated sediments (Table 2).

The majority of the *Arenicola cristata* juveniles burrowed into both the control and contaminated sediments (Table 1). A comparison of burrowing times indicated, however, that time to burrowing was twice as long in the bromophenol contaminated sediments as in the control sediments and this difference was significant (Table 2).

**Discussion.** Our previous results with nereid polychaete juveniles suggested that recruitment was significantly affected by biogenic contamination of sediments with haloaromatic compounds. In both laboratory and field experiments, significantly more juveniles remained within uncontaminated than contaminated sediments (Woodin et al. 1993). These results paralleled our laboratory behavioral observations where times to initiation of burrowing were significantly longer in contaminated sediments than in control sediments (71 vs 21 s) and where 25% of the nereid juveniles on contaminated sediments failed to burrow while all burrowed into the control sediments (Woodin & Marinelli 1991). In all of these previous experiments we contaminated the sediments by either collecting them in the field adjacent to the tubes of the terebellid polychaete *Thelepus crispus* or by adding *T. crispus* to sediments in the laboratory. *T. crispus* makes a 3, 5-dibromo-4-hydroxy benzyl alcohol and we extracted significant amounts

from the biogenically contaminated sediments but not from the controls (Woodin et al. 1993). Unfortunately, we were not able to add this compound directly to the sediments without also adding *T. crispus*. In the behavioral experiments reported here we added directly the commercially available, haloaromatic compounds produced by the capitellid polychaete *Notomastus lobatus* (Chen et al. 1991).

As expected from our results with biogenically contaminated sediments, times to initiation of burrowing or nestling are significantly longer for *Arenicola cristata* and longer at the $p < 0.061$ level for *Mercenaria mercenaria* juveniles on contaminated sediments (Table 2). Also, a significant percentage of both the *M. mercenaria* and *Mulinia lateralis* juveniles rejected the contaminated sediments, while all individuals established themselves in the control sediments (Table 1). We demonstrated previously that such increases in time to initiation of burrowing and failure to burrow both result in significantly higher erosion rates of juveniles (Woodin et al. 1995).

These organisms, *Arenicola cristata*, *Mercenaria mercenaria*, and *Mulinia lateralis*, all live within the same estuary as the capitellid polychaete, *Notomastus lobatus*, that produces the bromophenols contaminating sediments near its burrow. Similarly, *Thelepus crispus*, the haloaromatic producing species, lives within the same embayment as *Nereis vexillosa*. The spatial distributions of all of these species display a similar pattern. In the habitats near the Friday Harbor Laboratories in Washington State, USA, if a shovel of sediment contains *T. crispus*, it will not contain *N. vexillosa*, and in the habitats near the Baruch Field Laboratory in South Carolina if the shovel of sediment contains *N. lobatus*, it will not contain *A. cristata*, *M. mercenaria*, or *M. lateralis*. These casual observations on adult distributions are congruent with our data from both systems on juvenile responses to haloaromatic contaminated sediments and suggest the existence of

Table 2. *Arenicola cristata, Mercenaria mercenaria.* Times in seconds at which individuals initiated burrowing into control (Control sed.) or bromophenol contaminated sediments (Exper sed.). Means with standard errors in parentheses are given. N: number of individuals which burrowed in that treatment. Individuals which did not burrow within the observation period are omitted. Significance values are from Kruskal-Wallis tests. P: polychaete; B: bivalve

| Species | Control sed. | N | Exper. sed. | N | Significance |
|---|---|---|---|---|---|
| *A. cristata* (P) | 65.3 (7.9) | 15 | 120.0 (19.2) | 13 | $p < 0.025$ |
| *M. mercenaria* (B) | 19.9 (5.1) | 10 | 70.1 (23.9) | 5 | $p < 0.061$ |

spatially segregated communities whose composition is determined by the presence or absence of infauna producing and releasing haloaromatic compounds into the sediments. We are currently exploring this possibility as well as its corollary, the coexistence or exclusion of species from an assemblage based on their ability to detoxify haloaromatic compounds.

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