

## SETTLEMENT SITE SELECTION BY OYSTER LARVAE, *CRASSOSTREA VIRGINICA*: EVIDENCE FOR GEOTAXIS

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**ABSTRACT** Settlement of larval oysters, *Crassostrea virginica*, with respect to upper and lower surfaces of natural substrates, was studied in the field and in the laboratory. Enclosures were used to retain pediveligers of *Crassostrea* under controlled field conditions, until they settled. About 62% of these larvae settled onto rough (outer) surfaces of natural oyster shell substrate; this closely matched the proportion of substrate oriented with the rough surface downward. In the laboratory, about 83% of larvae settled onto the lower surfaces of similar shell substrates, in the absence of light, regardless of how the shell substrate was positioned. Both field and laboratory results suggest that geotaxis is a stronger settlement orientation cue than either phototaxis or rugotaxis, in *Crassostrea*.

**KEY WORDS:** *Crassostrea virginica*, larvae, settlement, geotaxis

### INTRODUCTION

Oyster larvae (Ostreidae) have long been studied as a model of bivalve settlement processes but are in fact nearly unique among the more than 90 bivalve molluscan families. Oysters cement permanently to the substrate on selection of a settlement site (Cranfield 1973, Kennedy 1996). In most other bivalve taxa, in contrast, there is at least an epifaunal plantigrade postlarval phase (Carriker 1961, Loosanoff 1961, LaBarbera and Chanley 1971), and for many, there is a planktonic postlarval phase (Sigurdsson et al. 1976, Yankson 1986, Beukema and de Vlas 1989, Martel and Chia 1991). Only for oysters, apparently, is settlement an irrevocable process. What selective advantage this provides is unclear, although some successful nonmolluscan taxa, such as barnacles (Crisp 1961, Le Tourneaux and Bourget 1988) and ascidians (van Duyl et al. 1981), also settle irreversibly. The immediate implications are clear, however—an oyster larva that chooses poorly is doomed.

It is not surprising, therefore, that oyster larvae have exhibited clear responses to environmental cues during settlement. Settlement may be facilitated by water-soluble chemical cues from conspecifics (Crisp 1967, Vietch and Hidu 1971, Coon et al. 1985, Shpigel et al. 1989, Bonar et al. 1990) or bacterial films (Fitt et al. 1990).

Physical cues may also be important. Ritchie and Menzel (1969) reported negative phototaxis in settling *Crassostrea virginica*, in the laboratory. In the natural environment, this behavior would result in highest settlement onto the lower, shaded surfaces of shells of adult oysters, which contain chemical settlement inducers (above). In this orientation, the juvenile oyster would experience a lower risk of burial by sedimentation events, in estuarine environments. Although it has not been tested for oysters, negative geotaxis while settling (often not clearly separable from movement in response to pressure, or barokinesis) could produce similar settlement orientation patterns (Crisp and Ghobashy 1971, Mann and Wolf 1983, Pires and Woollacott 1983).

Substrate texture has been suggested to be a settlement cue for some nonoyster taxa, including shipworms (Dons 1944), brachiopods (Wisely 1969), and barnacles (Wethey 1986). There are two scales of surface texture on an oyster shell, a common substrate for

settling *Crassostrea*. One is the very large-scale texture (compared with a 300- $\mu$ m larva) of shell concavity, with inner surfaces generally being more concave than outer surfaces, and the other is the small-scale rugosity of the outer shell surface, compared with the smooth inner surface (Carriker 1996). Unlike most bivalves, both the inner and the outer surfaces of *Crassostrea* shells are foliated calcite, although some prismatic calcite remains on the outer surfaces of younger oyster shells (Carriker 1996). Rugosity, therefore, is the primary difference between inner and outer shell layers.

Field observations of oyster settlement have often been contradictory. Bonnot (1937) reported that *Ostrea lurida* recruited more on upper surfaces of artificial substrates, but Hopkins (1935) reported the opposite, for the same species. Cole and Knight-Jones (1949) reviewed early literature, which generally reported higher oyster recruitment on lower surfaces. This is in contrast to their own studies (Cole and Knight-Jones 1949), in which they reported higher settlement for *Ostrea edulis* on upper surfaces, when shaded from above (although their results are not significant at  $\alpha = 0.05$ ; this author, reanalysis). Shaw (1967) examined settlement by *C. virginica* onto facing surfaces of paired plates, suspended horizontally. When the plates were 10 cm apart, settlement was higher on the surface facing downward, but placing the plates 2.5 cm apart reversed this trend. However, this trend was not statistically analyzed, nor was variance reported, so the validity of these results cannot be assessed.

Most prior studies of oyster settlement orientation fail to address actual settlement patterns in the field onto natural substrates—typically, the shells of adult conspecifics. This study examined settlement data of a cohort of *C. virginica* onto shells of conspecifics, in a larval enclosure deployed in the field. The field data were compared with settlement patterns onto similar substrate, observed in the laboratory.

### MATERIALS AND METHODS

Oyster settlement was observed within larval enclosures placed in the field in the York River estuary, Chesapeake Bay, VA. Larval enclosures were larger than, but similar in principle to, the enclosures used by Young and Chia (1982). Each enclosure was 1 m on a side and 15 cm deep and was covered with snug but removable lids. All construction was clear, 6.5-mm Plexiglas acrylic, and the lid was perforated with four 40-mm circular holes, covered with 150- $\mu$ m mesh size to permit water exchange. An input port in the

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center of the lid, to permit the injection of larvae, was fitted with a screw-on cap (Fig. 1).

Dead oyster (*C. virginica*) shells from a York River reef were defaunated by air exposure for several months. Oyster shells have a rough, usually convex outer surface and a smooth, concave inner surface. A 5- to 10-cm layer of defaunated shells was placed into each of four enclosures, at 1.5-m mean tidal depth, and allowed to shift via wave sorting for 24 h. Lids were left off of the enclosures for this period.

*C. virginica* pediveliger larvae were in the Virginia Institute of Marine Science oyster hatchery. Several hours before larvae were used, they were stained by neutral red stain, which was introduced to the larval water to a concentration of about 10 ppm. Neutral red, a vital stain, has been shown to have little or no harmful effects on oyster larvae but stains larvae for at least several days postsettlement, facilitating sampling (Baker 1991). Enclosure lids were put in place, and approximately equal aliquots (about 110,000 larvae each) were introduced to each enclosure by a diver via lengths of stoppered tubing, through the central input port.

After 24 h, the enclosure lids were removed, and a 10 × 10 grid was placed over each enclosure. Three 10 × 10 cm random samples of shell substrate were removed from each enclosure; edges of the enclosures were not sampled, to avoid possible edge effects. Shell substrates that overlapped grids were assigned to the grid that contained the largest proportion of that shell.

Data on shell substrate orientation in the field samples were not available because of handling techniques; therefore, a separate estimate of substrate orientation was made subsequent to the field sampling (below). The orientations of the rough (outer) surfaces of relatively intact substrate shells were recorded from four separate samples (one random sample from each enclosure), of 15–23 shells each. Grid points previously sampled were avoided. Each orientation was scored as rough surface upward or downward. A goodness-of-fit  $\chi^2$  test (Zar 1996) was used to test the null hypothesis that the orientation pattern did not differ from random (50% with rough surfaces facing primarily up, excluding shells lying on edge).

Shell substrates from the field were examined under a dissecting microscope, and juvenile *Crassostrea* were recorded. The numbers of juveniles on rough (outer) and smooth (inner) surfaces

of large, relatively intact shell surfaces were recorded and expressed as proportions; juveniles not clearly on one surface or the other were not included in the analysis. A one-sample *t*-test (Zar 1996) was used to examine the null hypotheses that the proportion of juveniles on rough surfaces did not differ from the observed proportion of shells in the field enclosures with downward-oriented rough surfaces. Proportion data were normalized with an arcsine-square root transformed before analysis (Zar 1996).

A laboratory settlement assay was used to examine settlement onto rough and smooth surfaces of defaunated adult *Crassostrea* shells, with shells in differing orientations. Intact lower valves (the deeply cupped valve that attaches to the substrate), from the same source as shells for the enclosures, were conditioned for 1 day in flume seawater, at the same time as the field enclosures were being prepared. Conditioning permits bacterial growth, which enhances *Crassostrea* larval settlement (Fitt et al. 1990). One shell substrate was placed individually into each of 12 chambers, with 1 L of 150- $\mu$ m filtered York River water, in a laboratory at ambient temperature (mean = 29°C). The shell substrate positions were alternated, so that half had the rough surface facing up, and half were facing down. Approximately 500 stained *Crassostrea* larvae from the same cohort used in field enclosures (above) were placed into each chamber. Chambers were covered with black fabric, in a darkened laboratory, for the duration of the trial. After 24 h, *Crassostrea* juveniles settled onto upper and lower surfaces were recorded by use of a dissecting microscope. Two-factor analysis of variance (Zar 1996) was used to test the null hypothesis of no larval orientation (choice of upper or lower surface of shell) or ‘‘rugosity’’ preference (choice of settlement on rough outer surfaces vs. smooth inner surface of shell).

## RESULTS

Defaunated oyster shells in the field enclosures tended to lie with the rough surface oriented downward (mean percent oriented downward = 63.9%, SD = 5.0%,  $\chi^2 = 68.6$ ), which differed significantly from random at  $\alpha = 0.05$ . Approximately 1% of shells in the enclosures were on edge, with no clear upward or downward orientation.

Variability of total settlement between samples was high (mean juveniles per sample = 1,791, SD = 2,284). The proportions of larvae that settled on a particular surface, however, had a much lower variance; the mean proportion on rough surfaces of shell substrates was 61.8% (SD = 15.5%), which did not significantly differ from 63.9%, the observed proportion of shells in the field in which the rough surface was oriented downward ( $p = 0.71$ ). However, the proportion settled on rough versus smooth surfaces did significantly differ from random, or 50% on either surface ( $p = 0.0017$ ).

Settling *Crassostrea* larvae in the laboratory significantly favored the lower surfaces of shells, regardless of whether the rough surface was up or down ( $p < 0.0005$ ), but did not favor smooth surfaces to rough surfaces ( $p = 0.66$ ). The mean proportion of the original cohort that settled on any surface was 64.6% (SD = 16.7%) in 24 h. A mean of 250 juveniles per chamber settled on upper and lower shell substrate surfaces combined; only 3.2 per chamber settled on the edges of the shell substrates, while 70 per chamber settled on the sides of the dishes themselves. Of those larvae that settled on the shell substrates (except for edge specimens), 83.3% (SD = 8.42) settled on lower surfaces, regardless of whether the rough surface of the shell substrate was oriented up-

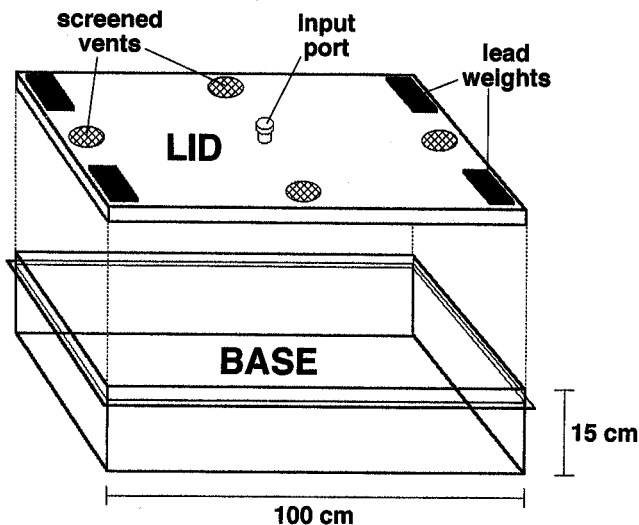


Figure 1. Field settlement enclosure design.

TABLE 1.

Summary of two-factor analysis of variance of the effects of shell substrate orientation (upper or lower shell substrate surface) and rugosity (smooth vs. rough shell substrate surface) on proportional settlement of *Crassostrea* larvae.

Source of Error	df	SS	MS	F	p
Orientation	1	1.69279	1.69279	113.65	<0.0005
Rugosity	1	0.00310	0.00310	0.21	0.660
Interaction	1	0.01636	0.01636	1.10	0.325
Error	8	0.11916	0.01490		
Total	11	1.83141			

DF, degrees of freedom; SS, sums of squares; MS, means squared.

ward or downward. Results of the two-factor analysis of variance on larval settlement site choice are summarized in Table 1.

### DISCUSSION

It can be strongly inferred from both field and laboratory data that *Crassostrea* prefers to settle on lower substrate surfaces. This suggests that if pediveliger larvae locate the upper surface of a substrate, they either swim or crawl considerable distances to try to find a lower surface (consider the 70- to 100-mm width of an adult *Crassostrea* shell compared with the 300 +  $\mu\text{m}$  body length of a pediveliger). In the field, this settlement behavior could have been accounted for by differing light levels between upper and lower shell substrate surfaces, as reported by Ritchie and Menzel (1969), but the absence of light in the laboratory trial precluded light as the dominant cue in this study. Barokinesis, or swimming in response to barometric pressure, has been demonstrated for nonestuarine bivalve larvae (Mann and Wolf 1983), but even modest waves in shallow water would make barometric pressure unreliable. Gravity, in contrast, is constant, and geotaxis can account for the settlement patterns observed in this study.

There is prior evidence that *Crassostrea* larvae exhibit geotaxis while settling (Crisp and Ghobashy 1971, Pires and Woollacott 1983). Both *Crassostrea* and *Ostrea* pediveligers have been shown to possess pedal statocysts, which are believed to be geosensory (Cranfield 1973, Eble and Scro 1996). The function of bivalve

statocysts is reviewed by Cragg and Nott (1977), who also describe the ultrastructure of scallop (*Pecten*) pediveliger statocysts, which are similar to those in oyster larvae. Pediveliger larvae are strongly negatively buoyant when not swimming (Cragg and Gruffydd 1975, Mann and Wolf 1983), making gravity an important cue even before settling. If geotaxis is the primary cue used during settlement, exploring pediveliger larvae must process the information that (1) the foot is in contact with appropriate settlement substrate, and (2) the body of the larva is either above or below the substrate.

In contrast to the shell orientation results, laboratory data failed to detect a preference by *Crassostrea* larvae for rough, convex outer surfaces of adult oysters versus smooth, concave inner surfaces. In the absence of currents, larvae are unlikely to be able to detect the concavity of an entire oyster shell, which is several orders of magnitude larger than themselves, and it may be that they are unable to detect the finer-scale rugosity found on the rough outer surfaces of all adult *Crassostrea* shells. Alternately, substrate rugosity on that scale may not be an important settlement cue for *Crassostrea* larvae, although it has been shown to be a cue for other taxa under some conditions (Dons 1944, Wisely 1969, Wethey 1986). A healthy oyster reef, for example, may present primarily living oysters as settlement substrate, with no opportunity for a larval settlement choice between smooth internal and rough external portions of adult shells.

The ecological value, for oysters, of settling on the lower surfaces of substrates is clear. Most *Crassostrea* populations occur in estuaries, which have a high sediment load. In addition, oysters settle gregariously (Crisp 1967, Vietch and Hidu 1971, Coon et al. 1985, Shpigel et al. 1989, Bonar et al. 1990), and adult oysters produce copious feces and pseudofeces, which enhance local sedimentation. Death by siltation is thus a very real possibility to the early juvenile oyster. If a lower surface of a substrate is free, however, it suspended slightly above the sediment and is free from at least normal sedimentation. Chemical cues could help a pediveliger larva locate a reef of conspecifics but would be of little use for selecting lower versus upper surfaces. Other cues could conceivably permit a pediveliger larva to select a site, but on the basis of this study, it appears that geotaxis by itself can account for observed patterns of settlement and that gravity is the primary cue when choosing the final settlement site.

### LITERATURE CITED

- Baker, P. 1991. Effect of neutral red stain on settlement ability of oyster pediveligers, *Crassostrea virginica*. *J. Shellfish Res.* 10:455-456.
- Beukema, J. J. & J. de Vlas. 1989. Tidal-current transport of thread-drifting postlarval juveniles of the bivalve *Macoma balthica* from the Wadden Sea to the North Sea. *Mar. Ecol. Prog. Ser.* 52:193-200.
- Bonar, D. B., S. L. Coon, M. Walch, R. M. Weiner & W. K. Fitt. 1990. Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. *Bull. Mar. Sci.* 46:484-498.
- Bonnot, P. 1937. Settling and survival of spat of the Olympia oyster, *Ostrea lurida*, on upper and lower horizontal surfaces. *Calif. Fish Game* 23:224-228.
- Carriker, M. R. 1961. Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve *Mercenaria*. *J. Elisha Mitchell Scientific Soc.* 77:168-241.
- Carriker, M. R. 1996. The shell and ligament. pp. 75-168. In: V. S. Kennedy, R. I. E. Newell, and A. F. Eble (eds.) *The Eastern Oyster, Crassostrea virginica*. Maryland Sea Grant, College Park, MD.
- Cole, H. A. & E. W. Knight-Jones. 1949. The setting behavior of larvae of the European flat oyster *Ostrea edulis* L. *U. K. Ministry Agriculture & Fisheries Fish. Invest. Ser.* 2, Vol. 17, No 3. 39 pp.
- Coon, S. L., D. B. Bonar & R. M. Weiner. 1985. Induction of settlement and metamorphosis of the Pacific oyster *Crassostrea gigas* (Thunberg) by L-DOPA and catecholamines. *J. Exp. Mar. Biol. Ecol.* 94:211-221.
- Cragg, S. M. & L. D. Gruffydd. 1975. The swimming behaviour and the pressure responses of the veliconcha larvae of *Ostrea edulis* L. *Proc. 9th Eur. Mar. Biol. Symp.* 43-57.
- Cragg, S. M. & J. A. Nott. 1977. The ultrastructure of the statocysts in the pediveliger larvae of *Pecten maximus* (L.) (Bivalvia). *J. Exp. Mar. Biol. Ecol.* 27:23-36.
- Cranfield, H. J. 1973. Observations on the behaviour of the pediveliger of *Ostrea edulis* during attachment and cementing. *Mar. Biol.* 22:203-209.
- Crisp, D. J. 1961. Territorial behavior in barnacle settlement. *J. Exp. Biol.* 38:429-446.

- Crisp, D. J. 1967. Chemical factors inducing settlement in *Crassostrea virginica* (Gmelin). *J. Anim. Ecol.* 36:329-335.
- Crisp, D. J. & A. F. A. A. Ghobashy. 1971. Responses of the larvae of *Diplosoma listerianum* to light and gravity. *4th Eur. Mar. Biol. Symp.* 433-465.
- Dons, C. 1944. Marine boreorganismer. VIII. Teredo-angrepensenes avhengighet av treverkets struktur og stilling. *Norske Vidensk. Selsk. Forh.* 17:40-42 (Engl. abstract).
- Eble, A. F. & R. Scro. 1996. General anatomy. pp. 19-73. In: V. S. Kennedy, R. I. E. Newell, and A. F. Eble (eds.) *The Eastern Oyster, Crassostrea virginica*. Maryland Sea Grant, College Park, MD.
- Fitt, W. K., S. L. Coon., M. Walch, R. M. Weiner, R. R. Colwell & D. B. Bonar. 1990. Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. *Mar. Biol.* 106:389-394.
- Hopkins, A. E. 1935. Attachment of larvae of the Olympia oyster, *Ostrea lurida*, to plane surface. *Ecology* 16:82-87.
- Kennedy, V. S. 1996. Biology of larvae and spat. pp. 371-421. In: V. S. Kennedy, R. I. E. Newell, and A. F. Eble (eds.) *The Eastern Oyster, Crassostrea virginica*. Maryland Sea Grant, College Park, MD.
- LaBarbera, M. & P. Chanley. 1971. Larval and postlarval development of the corrugated jewel box clam, *Chama congregata* Conrad (Bivalvia: Chamidae). *Bull. Mar. Sci.* 21:733-744.
- Le Tourmeaux, F. & E. Bourget. 1988. Importance of physical and biological settlement cues used at different spatial scales by the larvae of *Semibalanus balanoides*. *Mar. Biol.* 97:57-66.
- Loosanoff, V. L. 1961. Partial metamorphosis in *Anomia simplex*. *Science*. 133:2070-2071.
- Mann, R. & C. C. Wolf. 1983. Swimming behavior of larvae of the ocean quahog *Arctica islandica* in response to pressure and temperature. *Mar. Ecol. Prog. Ser.* 13:211-218.
- Martel, A. & F.-S. Chia. 1991. Drifting and dispersal of small bivalves and gastropods with direct development. *J. Exp. Mar. Biol. Ecol.* 150:131-147.
- Pires, A. & R. M. Woollacott. 1983. A direct and active influence of gravity on the behavior of a marine invertebrate larva. *Science* 220: 731-732.
- Ritchie, T. P. & R. W. Menzel. 1969. Influence of light on larval settlement of American oysters. *Proc. Natl. Shellfish Assoc.* 59:116-120.
- Shaw, W. N. 1967. Seasonal fouling and oyster setting on asbestos plates in Broad Creek, Talbot County, Maryland, 1963-65. *Chesapeake Sci.* 8:228-236.
- Shpigel, M., S. L. Coon & P. Kleinot. 1989. Growth and survival of cultch-less spat of *Ostrea edulis* Linnaeus, 1750 produced using epinephrine and shell chip. *J. Shellfish Res.* 8:355-357.
- Sigurdsson, J. B., C. W. Titman & P. A. Davies. 1976. The dispersal of young post-larval bivalve mollusks by byssal threads. *Nature* 262:386-387.
- van Duyl, F. C., R. P. M. Bak & J. Sybesma. 1981. The ecology of the tropical compound ascidian *Trididemnum solidum*. I. Reproductive strategy and larval behavior. *Mar. Ecol. Prog. Ser.* 6:35-42.
- Vietch, F. P. & H. Hidu, 1971. Gregarious setting in the American oyster *Crassostrea virginica* Gmelin: I Properties of a partially purified "setting factor." *Chesapeake Sci.* 12:173-178.
- Wethey, D. S. 1986. Ranking of settlement cues by barnacle larvae: influence of surface contour. *Bull. Mar. Sci.* 39:393-400.
- Wisely, B. 1969. Preferential settlement in concavities (rugophilic behavior) by larvae of the brachiopod *Waltonia inconspicua* (Sowerby 1846). *N. Z. J. Mar. Freshwater Res.* 3:273-80.
- Yankson, K. 1986. Observations on byssus systems in the spat of *Cerastoderma glaucum* and *C. edule*. *J. Mar. Biol. Ecol.* 66:277-292.
- Young, C. M. & F.-S. Chia. 1982. Factors controlling spatial distribution of the sea cucumber *Psolus chitonoides*: settling and post-settling behavior. *Mar. Biol.* 69:195-205.
- Zar, J. H. 1996. *Biostatistical Analysis*. 3rd ed. Prentice-Hall, Upper Saddle River, NJ. 918 pp.