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Boring Mechanism of *Polydora websteri* Inhabiting *Crassostrea virginica*

**Sarah A. Haigler**

*Virginia Institute of Marine Science, Gloucester Point, Virginia 23062*

**Synopsis.** The boring mechanisms of species of polydorid polychaetes are little understood due to lack of experimental evidence and direct observations. In the present studies the boring mechanism of adults and metamorphosing larvae of *Polydora websteri* was investigated by (1) inducing adults and larvae to settle against test substrates, (2) observing behavior in natural burrows and in "artificial blisters" composed of transparent "Pliobond" films surrounding Iceland spar substrates, (3) removing the giant setae of worms prior to tests of boring, (4) applying the giant setae to substrates, and by (5) testing for production of acid.

All the layers of oyster shell, including conchiolin, were bored. Calcareous substrates and Iceland spar were penetrated rapidly by adults without the assistance of the giant setae. Nor were these organs essential to the boring of a larva. A characteristic type of behavior involving close contact with the substrate during backwards and forwards movements and periods of immobility always preceded boring. The worms produced acid, probably some common product of metabolism, which can account for these results.

The "mud worm*, Polydora websteri*, is a small polychaete borer which lives in the shells of oysters and other molluscs. Larval worms settle on the outer surfaces of oysters, but adult worms are responsible for the two types of unsightly "mud blisters" which lower the quality of raw bar oysters and interfere with shucking. Most "mud blisters" are formed by oysters in response to adult worms that bore into the shell cavity. Blisters of another type, common in heavily infested oysters in the late summer, result when adults crawl between the mantle and inner shell surfaces. The oysters secrete conchiolin followed by calcite layers to wall out the intruders. Concurrently, the worms fill the pockets with loose mud, and then compact it, leaving U-shaped channels leading to the environment. Continued boring can be detected soon after the blisters are formed.

The boring mechanisms of *Polydora* species in calcareous substrates are unknown or disputed despite a century of study. Varying emphasis has been placed on mechanical abrasion by modified chitinous setae, in most species by the characteristic giant setae located on the fifth setiger (Fig. 1), and chemical action by a secretion. The evidence supporting the numerous hypotheses is almost entirely circumstantial and subject to conflicting interpretation. Most studies merely confirm chosen viewpoints and fail to account for inconvenient evidence. The experimental evidence and direct observations needed to resolve the long controversy are difficult to obtain, for boring activity in nature is intermittent and visibility is obscured by the opaque shells.

**Identity of Species**

*Polydora* species have often been misidentified. The Virginia specimens of adult *P. websteri* used in this study corresponded to the description of Hartman (1943) except that the ventral hooded hooks commence on the seventh instead of the eighth setiger. For rapid sorting *P. websteri* were distinguished from the ever-present *P. ligni* by the fine longitudinal lines of black pigment on the palps as opposed to the diffuse, brown lines on the palps of *P. ligni*.

Characteristics for distinguishing larval *P. websteri* from other polydorids in the...
region were established after extensive comparison and rearing of larvae. The only species with which a confusion is conceivable is *P. ligni* which bears a similar series of black dorsal bars and stellate chromatophores beginning on the third setiger. *P. websteri* were readily distinguished by weak ventral pigmentation not confined in chromatophores, a single dorso-lateral bar between the first and second setiger, the absence of anal papillae, heavier black pigmentation dorsally on the pygidium, and a pair of transverse black bars on the most distal region of the lips. In addition, late larvae were distinguished by setal characters and 5 pairs of dorsal bars although the fifth pair often has some stellate characteristics.

**MATERIALS AND METHODS**

New and improved techniques dependent upon the limitation of tube-building materials were required to make boring predictable and observable. Adult *P. websteri* removed from their burrows and placed on test substrates constructed opaque tubes from any loose materials available. Because the tubes were only loosely attached to the substrates, the worms did not bore. When instead, tube-building materials were withheld, occasional worms formed short mucous tubes directly against the substrates and bored. The posterior segments of these worms were usually amputated to prevent the curling movements which broke the mucus connections to the substrates. Since worms vacated tubes formed against conchiolin substrates, they were allowed to settle on adjacent or overlying calcareous regions. This technique was primarily used to demonstrate which substrates were bored and how rapidly, but was not applicable to worms whose giant setae were removed; hence artificial blisters (Fig. 2) were constructed to hold them. These consisted of pieces of Iceland spar (pure calcite) completely surrounded by films prepared from the household adhesive trademarked "Pliobond" (Goodyear Tire and Rubber Co.). "Pliobond" was dropped on fresh water to form thin, transparent layers which were lifted out on and sealed around the test.
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substrates. Rinsing in fresh water removed the adhesive solvent, methyl ethyl ketone, and sea water caused the coatings to swell. Openings were then cut in the "Pliobond" layers without marring the substrates, and the worms were inserted within. Septa of compacted mud from natural blisters were provided for traction, but loose tube-building materials were withheld to promote direct contact with the substrates. The preparations were kept at approximately 28°C in standing membrane-filtered sea water without the addition of food.

Correct spacing was essential for demonstrating boring within artificial blisters, for worms rejected blisters with insufficient space and did not bore the substrates of loose preparations. Since "Pliobond" is elastic and the septa are compressible, some adjustment of this factor was possible. Because tube-building materials were withheld and both the films and substrates were transparent, direct observations of behavior were possible.

In experiments to determine whether the giant setae are essential for boring, the setae were extirpated with small-bore syringe needles. After anesthetization with 5% magnesium chloride on glass slides, most of the liquid was pipetted off, and the worms were bent to each side to expose the setal fascicles of the fifth setiger. Incisions were made on each side without damaging the medial organs. Returned to sea water, the worms recovered rapidly and the wounds healed quickly.

Fascicles of giant setae and single setae were brushed against Iceland spar and the various natural substrates to determine their capacity to remove substrate particles. Whole fascicles were held in fine forceps. Individual giant setae were teased from fascicles, cleaned of muscle fibers, dried and mounted with "Duco Cement" (E. I. duPont de Nemours & Co.) on fine steel pins mounted in turn within glass capillary tubes.

Considerable preparation was necessary before the boring of metamorphosing larvae could be studied in the laboratory. Larvae were obtained from capsules laid in nature during the summer spawning season, or laid in the laboratory out of season. The capsules of P. websteri which are laid in the dwellings of parent worms contain two types of spawn requiring different rearing techniques, but yielding larvae identical in appearance. In one type the eggs develop at similar rates and the larvae are released at the 3-setiger stage (Hopkins, 1958) without feeding upon undeveloped eggs which are occasionally present. Capsules of this type were generally produced in the laboratory out of season when shells containing P. websteri were lightly cleaned of fouling organisms and kept in membrane-filtered sea water at 25°C. At this temperature the P. lini, which can never be completely removed by cleaning the shells, were prevented from spawning. During the few months prior to a new spawning season, when the eggs degenerate within the bodies of female worms in nature, shells over-wintered at about 20°C were used for production of larvae. Whether obtained from nature or produced in the laboratory, these larvae were reared to swimming-crawling stage on algae: Dunaliella, Phaeodactylum, and Monochrysis. Faster growth was obtained when larvae were kept with shells during the younger stages.

The second, unreported, type of spawn was easier to rear. The eggs develop at variable rates and, initially, undeveloped eggs are always present. The larvae are usually brooded until some reach a very advanced stage, when the undeveloped or nurse eggs are disintegrated into a brei and consumed as food. Since no pelagic existence was required for the oldest larvae within such capsules, they were maintained on the internal food source.

P. websteri larvae selected calcareous substrates in the presence of weak water currents produced by organisms or an apparatus. Occasional worms which settled on non-calcareous substrates repeatedly vacated their tubes to form new ones. The larvae selected any crevices available and often settled between the test substrates.
and the glass culture dishes. Therefore, to observe settlement behavior, larvae in the swimming-crawling stage were provided with carefully selected and prepared substrates. Cleaned dried substrates without crevices were soaked to remove trapped air and mounted, still wet, in “Pliobond” on pieces of glass. The weak water currents required for settlement were provided by an apparatus based on the design of Hannerz (1952-1953). The cultures were maintained at about 28°C and both food and tube-building materials were withheld to increase visibility.

Despite the small size and fragility of the larvae, it was also possible to extirpate their giant setae, but the operations had to be performed very rapidly to avoid desiccation and death of the larvae.

The acid production of *P. websteri* and of non-boring species of worms was determined by a 1% agar-sea water medium containing phenol red as an indicator and antibiotics to prevent bacterial growth. Adults were forced into the soft medium, while larvae were maintained in a few ml of sea water above the medium. When the color of the medium changed from red to yellow, the worms were removed and rhombohedrons of Iceland spar were introduced to test the etching capacity of the secretions.

**RESULTS AND CONCLUSIONS**

*P. websteri* penetrate all layers of the oyster shell, including prismatic, calcite-ostracum, hypostracum, periostracum, and internal conchiolin layers of visible thickness, as determined by examinations of natural burrows and duplicated in direct tests against fresh substrates. Worms induced to settle directly on test substrates at room temperature bored chalky deposits within 24 hr, calcite-ostracum and hypostracum within 1 week, and conchiolin layers within a month. This evidence, in addition to that readily obtained by observing the extension of natural burrows, refutes the common supposition that boring is a slow process.

Boring was also demonstrated within artificial blisters on Iceland spar substrates. The first signs of etching were detected after 4 days, but several weeks were required for channels to form (Fig. 3). In one experiment to determine whether the giant setae are essential for boring, these setae were removed from two sets of worms. One set was allowed to regenerate new unworn setae; the regenerated setal sacs of the other set were removed before new setae could be secreted. A third set of worms served as controls. Since the worms without giant setae produced borings identical to those produced by the two sets of worms with giant setae, these organs are not essential for boring in this hard substrate. Further, no particulate products were found; these would have resulted if the giant setae had assisted in substrate removal. No signs of wear were detected on the new giant setae, although the fragile, regenerated, companion setae were frayed. Apparently, all the calcite was removed by dissolution, even when the giant setae were retained. Preliminary results from other experiments with artificial blisters show that the giant setae are not essential for the penetration of either chalky deposits or calcite-ostracum.
In the many direct observations of adults within artificial blisters, the giant setae seldom contacted the substrates. For long periods, however, the worms lay motionless with their ventrums appressed to the substrates, and sometimes slowly moved backwards and forwards, still maintaining close contact with the substrates. At such times the worms were especially unresponsive to tactile stimuli. Since this characteristic type of behavior always preceded the detection of boring, whether observed in natural or artificial burrows, or after direct settlement by adults or larvae, it is believed to be the true boring behavior.

Attempts to abrade substrates with isolated giant setae and setal fascicles were mostly ineffective. Brushed against unetched substrates with light pressure, the tips of the setae wore down or broke off. Only those substrates which offered slight resistance, such as the prismatic layers and chalky deposits, were affected. Certainly setal action alone cannot account for the rapid boring of such hard substrates as calcite-ostracum, which comprises the major portion of oyster shell. On the other hand, the setae were very effective against etched substrates, including Iceland spar, although they were not used to remove substrate in the experiments with artificial blisters. The only situation in which the giant setae of adult worms removed substrate was during settlement on chalky deposits and prismatic calcite. Most of the particles were lifted and trapped by mucous strands, but some were accidentally caught on the giant setae and other setae during locomotory movements. All the particulate calcite was incorporated into the mucous tubes, including that which passed through the gut and was expelled as feces. After tubes had been constructed, the same behavior occurring in artificial blisters was observed.

The giant setae were not essential to the boring of a metamorphosing larva of 16 setigers which was induced to settle satisfactorily and bore into a cube derived from a chalk deposit. Its behavior differed in one respect from that of normal larvae in which the giant setae were often seen vigorously removing substrate particles during settlement and in the presence of mucous secretions. This behavior is more likely related to attaining firm attachment of the primary tubes, than to boring. As soon as the larvae had constructed primary tubes, however, and long before metamorphosis was completed, they began to move backwards and forwards very rapidly, with the ventrums of their bodies closely appressed to the substrates. These conspicuous motions alternated with periods of quiescence. No particles were removed and the giant setae were not applied to the substrates. Unlike the adults, metamorphosing larvae responded to tactile stimulation by vacating the primary tubes.

The fragility of the giant setae and the ability of adults and larvae to bore without them proves that a chemical agent is involved in boring. This chemical is not concholinase, for Iceland spar is composed of pure calcite and lacks the concholin matrix which binds calcium carbonate crystals together in most oyster shell layers. The adults and larvae of *P. websteri* did produce acid in sea water-agar medium with phenol red indicator, and pieces of Iceland spar introduced into the medium after the worms were removed were etched in a manner indistinguishable from etched areas in artificial blisters. Non-boring species of worms, including adult and larval *P. ligni*, also produced acid capable of similarly etching Iceland spar. This suggests that an acid product of metabolism was responsible for boring. If the chemical agent in boring is not specific, the manifestation of boring potential in *P. websteri* probably depends upon behavioral adaptations, such as the observed capacity to maintain intimate contact with substrates for extended periods.

**DISCUSSION**

By means of new techniques and careful observations, it has been shown that *P. websteri* bores rapidly into calcareous substrates. As recently as 1967, Landers stated...
that *P. websteri* apparently does not bore. Actually, the boring species he used was probably *P. websteri*, since Pettibone (pers. comm.) has preferred including *P. websteri* under the name *P. ciliata*.

Artificial blisters, composed of cover glasses cemented to oyster shell substrates, were used by Mortensen (1945) who attempted to determine the boring mechanism of a species she called "*P. ligni.*" When the worms did not bore after 7 weeks in these substitute dwellings, she concluded that boring was an extremely slow process probably effected by carbonic acid resulting from respiration. Although these particular experiments were probably conducted with *P. websteri* rather than *P. ligni* (which does not penetrate calcareous substrates or stimulate formation of blisters), any number of other factors could have prevented boring. Tube-building materials were added instead of limited, the spacing may have been unfavorable, and an illustration shows a tube formed against the glass cover instead of the test substrate.

Assumptions that *Polydora* species penetrate calcareous substrates mechanically have been based on weak evidence which does not detract from my own conclusion that the boring mechanism of *P. websteri* is largely chemical. Although Hempel (1957) assumed that calcite would be more easily penetrated by mechanical than by chemical means, *P. websteri* rapidly bored hard Iceland spar without using the giant setae. Theoretically, giant setae are capable of assisting in the removal of soft and softened calcareous substrates, but evidence is lacking to prove this function. Impressive as the wear on the giant setae may be, this fact in itself speaks equally well to the ineffectiveness of the setae against hard substrates. The giant setae of *P. ligni* dwelling in mud tubes constructed against oyster shell are worn, yet they do not penetrate the substrate. The same species, collected in intertidal sand has severely frayed and worn setae, but no one would conclude that therefore the worms have the capacity to penetrate sand grains. Unless the setae are seen to remove substrate, the only valid conclusion is that worn setae have been in contact with hard materials.

Several direct observations of so-called mechanical boring (Wilson, 1928; Hannerez, 1956) involve metamorphosing larvae whose behavior during settlement can be interpreted quite differently. Wilson has emphasized the brief duration of the behavior. Söderström (1920) observed the boring of an adult *P. ciliata* in a thin-walled burrow. His observations of the same sliding motions described in this study have been practically ignored. He also saw strokes of the giant setae in what seemed to be mechanical action against the substrate, though no definite scratch or removed substrate was detected. In a later paper (1923) Söderström placed far less emphasis on the mechanical component of boring, stating that the giant setae function primarily as organs for support and adhesion, as during ventilation or feeding movements. Although vigorous action of the giant setae has been observed by Dorsett (1961), and especially Hempel (1957) during the penetration of predominantly clay substrates, such evidence does not require mechanical and preclude chemical action with respect to predominantly calcareous substrates. Without direct observations, Hempel assumed that *P. ciliata* used their giant setae in the same way against clay and calcite, but did not explain why *P. ligni* did not do likewise. The "argillaceous difficulty" has probably been overemphasized, and in any case, presents no great problem to the understanding of how *P. websteri* bores, for this species has not been reported from clays, shales, sandstones, or from soft wood. Even in the occasional penetration of conchiolin layers, the chemical may be of considerable importance. One often finds such layers detached from the floors of the channels and the underlying calcium carbonate etched. Whether such layers are removed by setal action or by prolonged chemical action is not known.

Particulate calcareous products clearly
the result of mechanical boring have not been observed. The few granules found in the septa of *P. websteri* burrows could have been detached by mucus or gathered from outside the shell. The argument that such particles would necessarily be dissolved (Prell, 1926; Hempel, 1957) if chemical means were employed, is opposed by the argument (Mortensen, 1945; Dorsett, 1961) which requires direct application of the chemical to avoid dilution and neutralization by currents of sea water passing through the burrows.

The over-all contours of the burrows are smooth. Scratches in calcareous substrates which might be attributed to the giant setae (Lamy and André, 1937; Hempel, 1957) have seldom been detected. The few seen on the walls of *P. websteri* channels were associated with soft etched areas of calcite-ostracum. The roughness of the walls (which may appear smooth in reflected light) can be duplicated without setal action.

The main weakness of the chemical hypothesis is that no acid other than mucus has been demonstrated before. Lankester (1868a, b), an early proponent of a chemical-mechanical hypothesis believed carbonic or sulfuric acids might be involved in boring. Although he obtained an acid reaction in a crude test of *P. ciliata* against litmus paper, this result could not be duplicated by others (McIntosh, 1868; Elliott and Lindsay, 1912; Söderström, 1920), and he withdrew his hypothesis (McIntosh, 1868).

Many glands have been shown to have acidic contents. Although the podial glands most often cited have been thought by some to produce sulfuric acid (Lankester, 1868b; Söderström, 1920), according to Hannerz (1956) and Dorsett (1961) they produce mucus, which the latter author says is secreted to line the tubes. A single observation casts doubt upon the idea that the chemical is confined to the podial glands. In one artificial blister, a *P. websteri* constructed a tube with a short dead-end offshoot. Only the posterior end of the worm was observed within this section, which seemed too short and narrow to hold the anterior end. The calcite-ostracum floor beneath was bored, although the podial glands in the posterior setigers are rudimentary. Dorsett (1961) has suggested that a chelating agent linked to the biochemistry of mucus produced by other glands is the chemical agent. In *P. websteri* very little mucus is present at the boring sites except during periods of settlement and tube-building. Although several deep channels in Iceland spar were eventually lined with mucus tubes similar to those observed in *P. ciliata* borings, even with *P. ciliata* these linings are absent in areas where boring is probably occurring (Dorsett, 1961). Hannerz (1956) suggested that the acidic contents of a set of glands associated with the giant setae of metamorphosing larva aid boring at that stage, yet a larva of *P. websteri* bored after those regions were removed.

In light of the results and conclusions reached in this study, the boring mechanisms of other *Polydora* species should be carefully re-examined.

**REFERENCES**


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