METAMORPHOSIS AND SETTLEMENT IN THE SABELLARIIDAE

Kevin J. Eckelbarger*
Harbor Branch Foundation, Inc., RR l, Box 196, Fort Pierce, Florida 33450

Settlement and metamorphosis are reviewed in the polychaete family Sabellariidae with emphasis on some ultrastructural features of the premetamorphosed larva of *Phragmatopoma lapidosa*, an intertidal reef-building species. A possible sensory structure is described from *P. lapidosa* larvae which might play a role in substrate selection. Among the factors influencing settlement and metamorphosis in sabellariid larvae are type of substrate, degree of tidal exposure, tolerance of wave energy, phototaxis, presence or absence of gregariousness in settling, and ability of larvae to detect the tube cement of their own species. The larvae of most intertidal reef-building species are induced to settle and metamorphose upon contact with the adult or larval tube cement of their own species.

INTRODUCTION

The family Sabellariidae is a relatively small group of tube-building, marine polychaetes with a cosmopolitan distribution from the intertidal to abyssal depths. Their pelagic larvae are seasonally prominent members of the nearshore plankton in many parts of the world[1-4] and have representatives in the teleplanic larval community in the open ocean.[5] Sabellariid polychaetes have played a key role in demonstrating substrate selectivity by marine invertebrate larvae through the more recent studies of Wilson [6-9] on *Sabellaria alveolata* and *S. spinulosa.*

The purpose of this paper is to review settlement and metamorphosis in the family Sabellariidae and to present new findings regarding larval metamorphosis in the reef-building species, *Phragmatopoma lapidosa.*

MATERIALS AND METHODS

Larval stages of *Phragmatopoma lapidosa* were obtained from artificially fertilized eggs using the procedures of Eckelbarger[10] and from plankton samples kindly provided in part by Dr. M. J. Youngbluth and Ms. P. I. Blades of the Harbor Branch Foundation, Inc. Specimens for scanning (SEM) and transmission electron microscopy (TEM) were prepared according to the procedures of Eckelbarger and Chia.[11,12]

SETTLING BEHAVIOR

The larval development of eight species of sabellariids has been described in the literature—three species from Europe: *Sabellaria alveolata* (Linne),[1,6,8,13] *Sabellaria spinulosa* Leukart[1,9] and *Lygdamis muratus* (Allen)[1,14-16]; three species from the east coast of North America: *Sabellaria vulgaris* Verrill,[10,17,18] *Sabellaria floridensis* Hartman,[19] *Phragmatopoma lapidosa* Kinberg[4,20]; a single species from California: *Phragmatopoma californica* (Fewkes)[19,21,22] and a single species from the Indian Ocean: *Lygdamis indicus* Kinberg.[23] Many of these reports provide some data on larval settlement behavior.

Figure 1 shows a portion of an intertidal reef on the east coast of Florida (St. Lucie County) built by *Phragmatopoma lapidosa* to illustrate the massive nature of sabellariid reefs.

Sabellariid larvae generally respond positively to a weak light source. *Sabellaria floridensis* larvae, however, seem to be neutral to photo stimuli and swim randomly in larval culture during the entire developmental period.[19] Eckelbarger[10] reported that most *Sabellaria vulgaris* larvae

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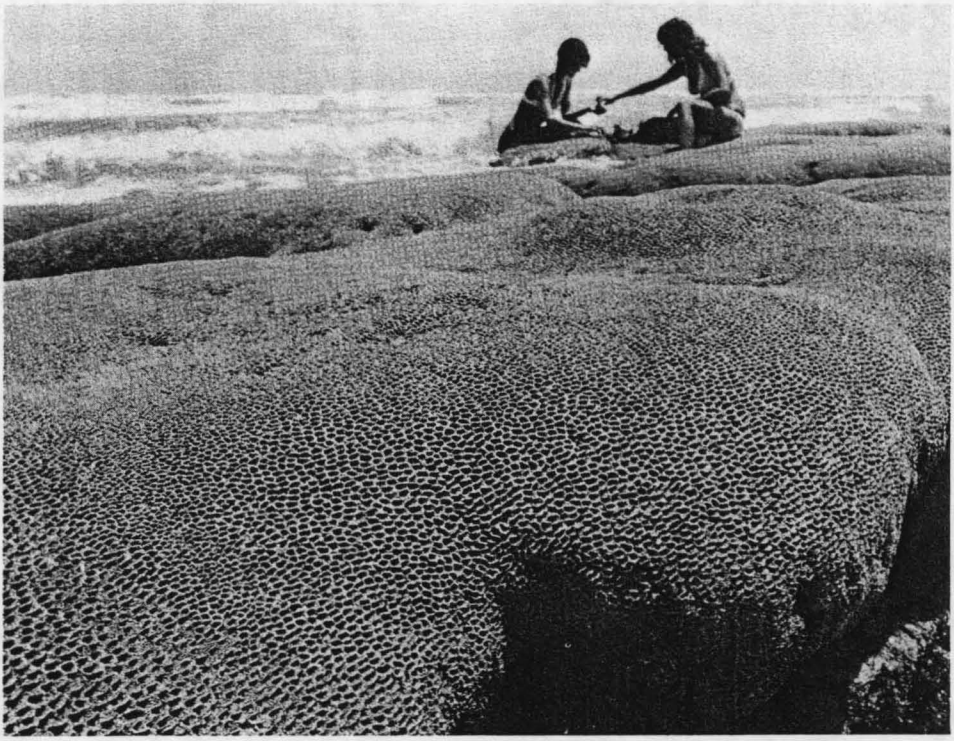


Fig. 1. Portion of *Phragmatopoma lapidosa* reef in St. Lucie County, Florida.

in laboratory cultures tended to aggregate at the water line nearest the window illumination whereas a smaller number of larvae characteristically aggregated furthest from the light source. As development proceeded and larvae approached settlement, the photopositive larvae migrated towards the bottom of the culture. The remaining larvae, presumably not as far along in development, behaved similarly by aggregating at the water line and repeating the same migratory behavior as they approached settlement. Wilson[14] reported a similar massing of *Lygdamis muratus* larvae on the light and dark sides of his laboratory cultures.

As sabellariid larvae approach the stage shown in Figures 2A, 14, and 20, they tend to swim near the bottom of the culture and periodically cease swimming and crawl over the substratum. The larva crawls on its ventral side with its mouth applied to the substratum by pushing back the building organ to allow the ciliated surface of the lips of the mouth to be applied to the bottom. The larval tentacles are turned anteriorly so that their ventral ciliated food grooves contact the substratum. The tentacles alternately touch the substrate and are lifted up in a manner suggesting feeling or testing. During crawling behavior, larvae tenaciously cling to the substrate and considerable effort is required to dislodge them with squirts of water from a fine pipette. After a few seconds or minutes of crawling, the larvae abruptly resume swimming.

Phragmatopoma lapidosa larvae approaching settlement frequently have been observed swimming nearly upside down, stopping to apply their anterior region (episphere or "hood," as defined by Wilson[1]) gently to the substratum and then swimming on (Eckelbarger, unpublished observations). Occasionally a number of larvae display this behavior when they encounter a metamorphosed larva, appearing to take special interest in its mucoid tube. Although this behavior has been observed on numerous occasions in *P. lapidosa* larvae, it has not been reported for other sabellariid species.

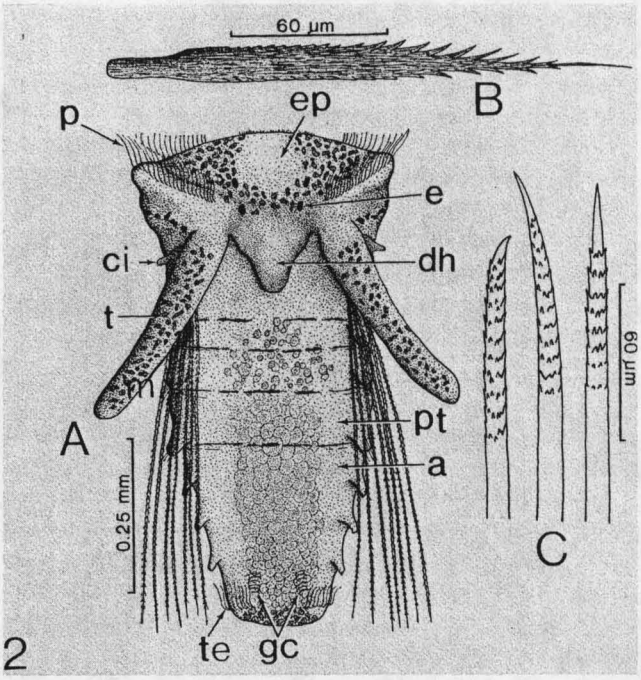


Fig 2. A. Dorsal view of late larval stage of *Sabellaria floridensis* just prior to metamorphosis. B. Primary opercular palea from setal bundle. C. Larval nuchal spines from different individuals (modified from Eckelbarger[19]).

The larvae of *Sabellaria floridensis*[19] and *Lygdamis muratus*,[14] two non-rock-adherent species from Florida and the waters of the English Channel, respectively, behave differently from other sabellariids. In each species, the larvae crawl over solid surfaces less actively than other sabellariids and tend to burrow through the substratum, frequently burying themselves under sand grains and pebbles. Generally, they do not turn their tentacles forward to test the substrate, but use them frequently when on their sides or back.

LARVAL MORPHOLOGY

Before a discussion of sabellariid metamorphosis can be presented, it is necessary to describe the general external features of the larva just prior to this event. With the exception of species within the genus *Lygdamis*,[14,16,23] the larvae of *Sabellaria floridensis* and *Phragmatopoma lapidosa* are typical of most sabellariid larvae at settlement and will be used to illustrate general morphological features.

Figure 2A shows a late stage of a *Sabellaria floridensis* larvae with four crescent-shaped red eyespots, a pair of posteriorly projecting tentacles about one-half the body length, a well-developed prototroch and telotroch, two bundles of barbed, provisional setae, a dorsal hump posterior to the eyespots, three parathoracic segments with dorsal parapodial lobes each bearing four capillary setae and three abdominal segments with dorsal uncinigerous lobes. Yellow-green chromatophores and black pigment bands are present dorsally on the posterior borders of the parathoracic segments. Hidden among the provisional setae are broad, spiny primary paleae (Fig. 2B) and a pair of small nuchal spines (Fig. 2C). The latter figure shows variation in spine morphology from different individuals. In a study of the anterior region of *Phragmatopoma californica*, Dales[22] concluded that the larval tentacles were probably prostomial in origin because they are innervated from the brain mass in the adult. He added that the pigment patches which appear on the dorsal surface of the tentacles are additional evidence for a prostomial origin because such pigment is characteristically found on the asegmental regions of the larval body (i.e., pygidium and episphere).

Grasping Cilia

One peculiar feature common to sabellariid larvae is the presence of groups of tightly clustered cilia forming two dorsal longitudinal rows over the pygidium (Fig. 2A). The cilia are continuous with the telotrochal cilia, but when viewed by light microscopy, they appear to be

fused and undergo flicking or vibratory movements as a unit rather than beating motions typical of the other telotrochal cilia. Wilson[1] referred to these cilia as "grasping cilia" and attributed their function to curling around and grasping the provisional setae when the larvae are swimming. Dales[22] referred to these cilia in the larvae of *Phragmatopoma californica* and attributed the same function to them. Cazaux[13] disputed this interpretation, but Wilson[6] reconfirmed his observations and the present author concurs with Wilson after extensive observations of the larvae of *Phragmatopoma lapidosa.* Figure 3 shows an SEM view of the grasping cilia which illustrates the close association of the cilia. The tips of the cilia in this figure and a TEM view of a longitudinal section through the cilia (Fig. 4) indicate that the cilia are not fused. Figure 4 reveals, however, that the basal bodies of the cilia appear to be fused, perhaps in order to lend greater support during the forceful flicking motions or to provide coordination of action.

Prototroch and Telotroch Cells

Figure 5 is a cross section through the prototroch cells illustrating the long, primary rootlets that penetrate deeply into the cell and appear to anchor to the nuclear membrane. Prototroch cells are large, cuboidal cells with spherical basal nuclei and numerous spherical or oblong mitochondria. Cell microvilli differ from those of other epidermal cells by their beaded appearance (Fig. 6) and appear to be less branching than those of adjacent cells. Telotroch cells are similar with long primary rootlets (Fig. 7) and branching secondary rootlets (Fig. 8). The cells are attached at their apicolateral margins by a junctional complex consisting of maculae adherens and septate desmosomes (Fig. 9).

Epidermal Gland Cells

At least two distinct types of gland cells, designated Types I and II, are present in the epidermis of premetamorphosed *Phragmatopoma lapidosa* larvae. Either of these cells may play a role in the secretion of the larval mucoid tube during or after metamorphosis. Each type is based solely upon the ultrastructure of its respective secretion droplets; no cytochemical tests were performed to characterizie or differentiate them.

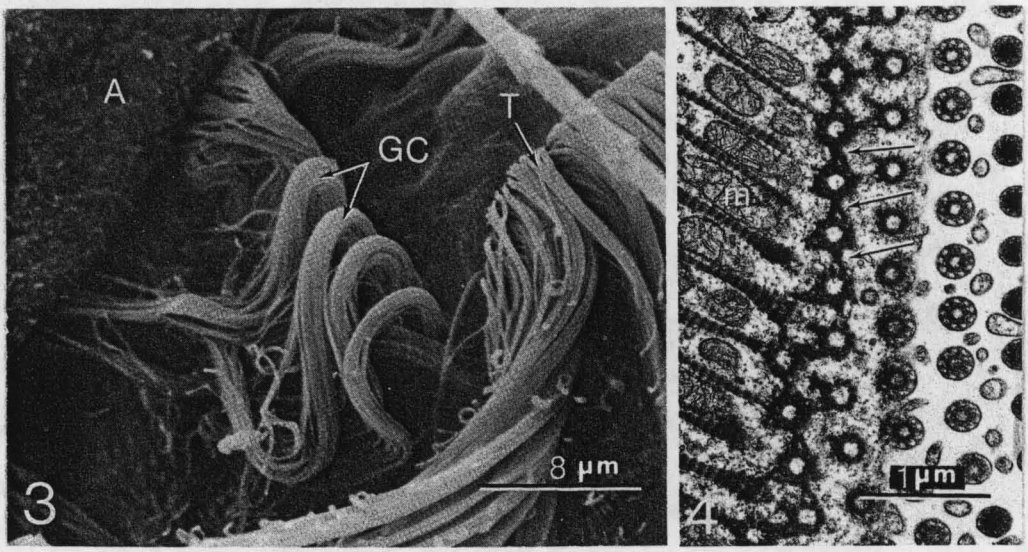
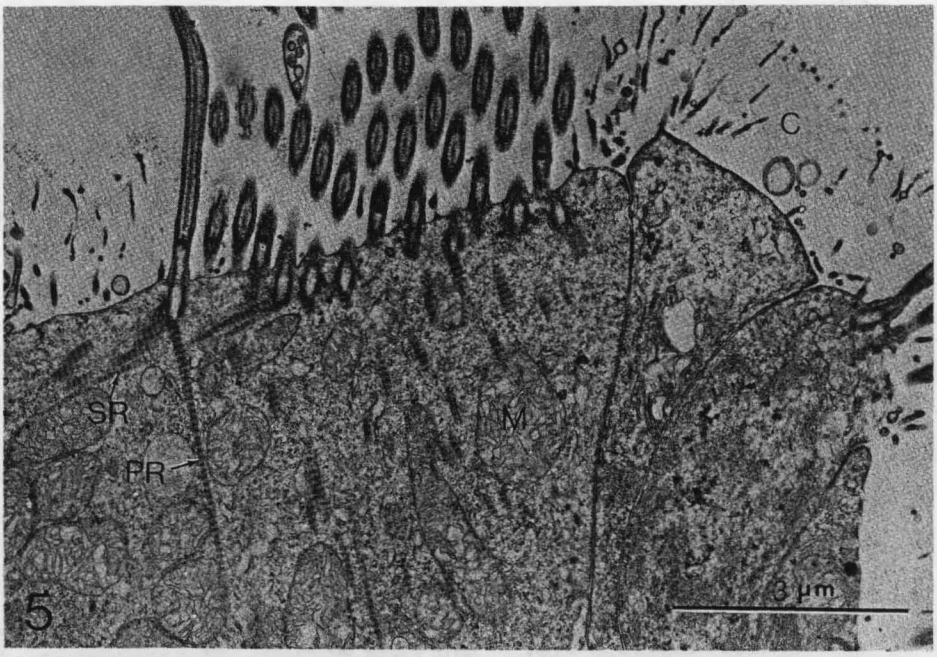


Fig. 3. SEM view of "grasping cilia" from pygidium of late stage larvae.

Fig. 4. TEM section through the base of the "grasping cilia" showing apparent fusion of basal bodies (arrows).



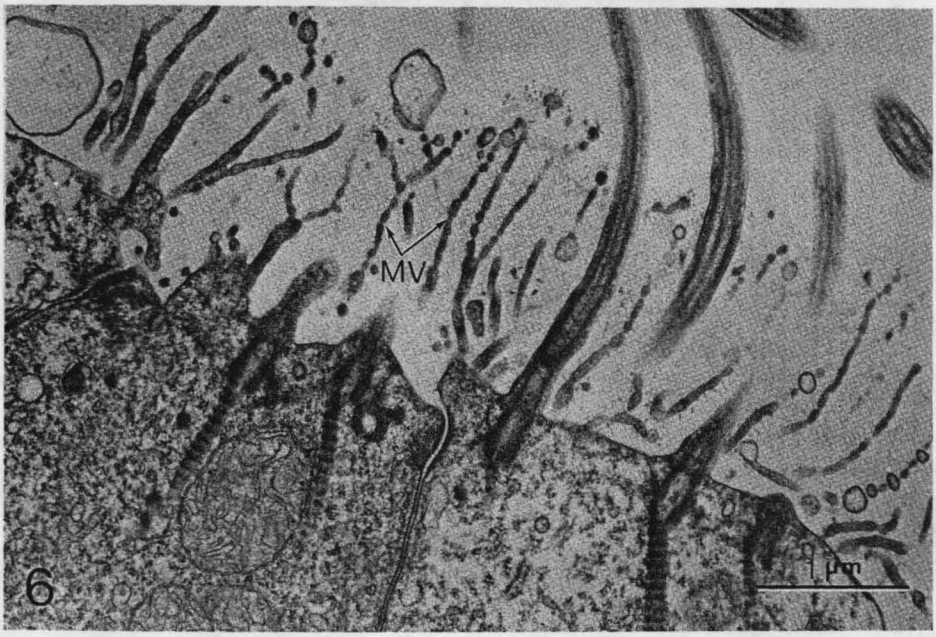


Fig. 5. Section through prototroch cell of *P. lapidosa* showing ciliary rootlets.

Fig. 6. Beaded microvilli of prototroch cell cuticle. Note unbeaded microvilli of adjacent epidermal cell to left.

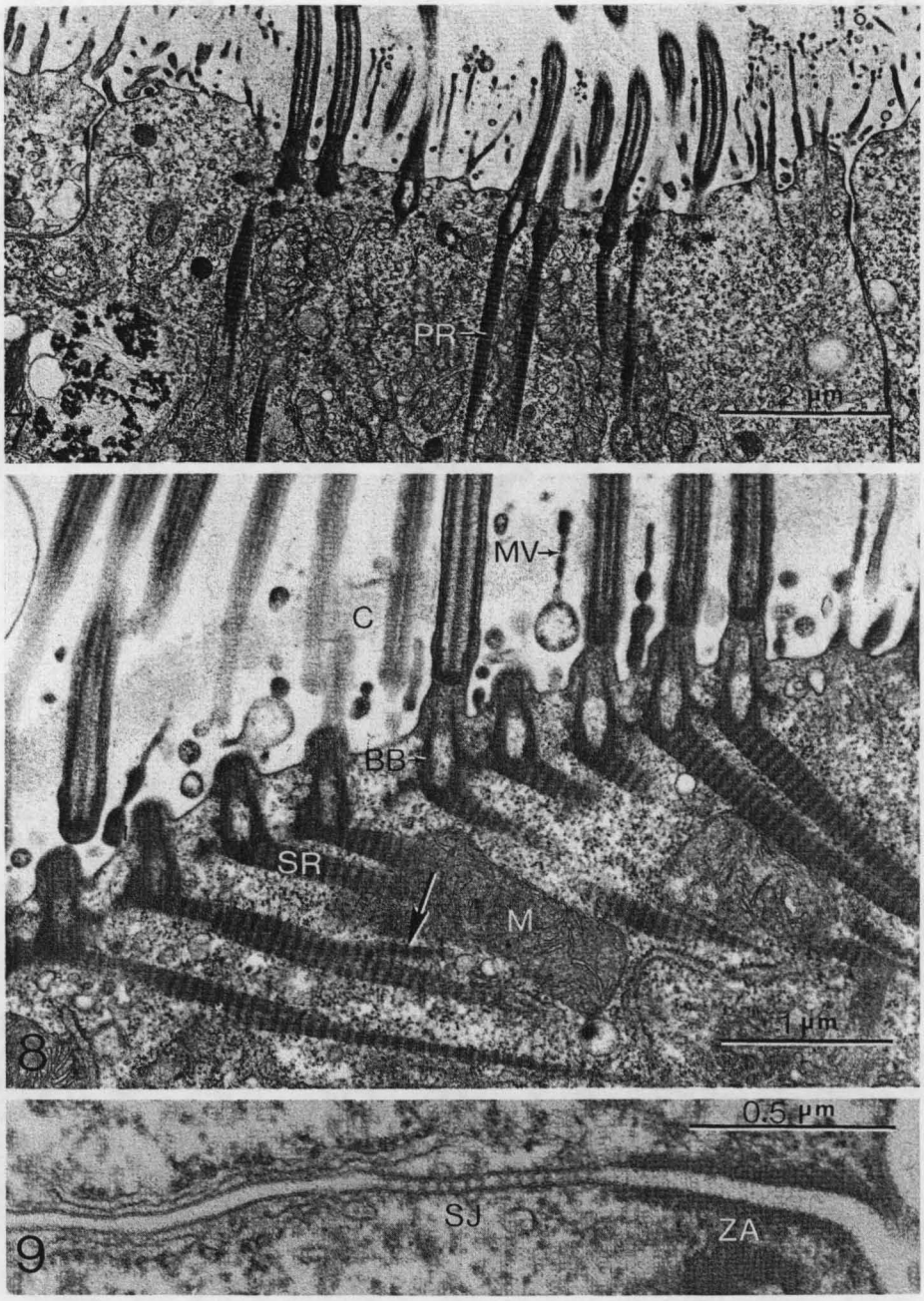


Fig. 7. TEM section through primary rootlets of telotrochal cilia.

Fig. 8. Section through secondary rootlets of telotrochal cilia. Note branching of rootlet at arrow.

Fig. 9. Junctional complex at apicolateral margins of the telotrochal cells.

Type I gland cells are limited to the ventral portion of the first parathoracic segment, and they are smaller and less numerous than Type II cells, and contain secretion products composed of whorls of moderately electron-dense fibrillar material (Fig. 10). The gland pores are recessed beneath the cuticle and are surrounded by short, nonbranching microvilli. An SEM view of the ventral portion of the parathoracic segments reveals a number of pores which are believed to represent the pores of Type I gland cells (Fig. 11).

Type II gland cells are limited to the pygidial region surrounding the anus and the episphere or hood. The cells are large and contain somewhat spherical secretions consisting of fibrillar strands aligned in randomly arranged parallel arrays (Fig. 12). The apex of the cells is elevated above the level of neighboring epidermal cells and the gland pore is wide and usually observed to be releasing the secretory product. SEM views of the surface of the episphere of the larva reveal these raised gland pores (Fig. 13).

Sensory Structures

In his detailed light microscopic investigation of larval development in *Sabellaria alveolata,* Wilson[1] referred to the presence of "sensory cilia" on a number of locations, including the cirri and tentacles, on the surface of the premetamorphosed larvae. More recently, scanning electron microscopy was utilized to examine the surface of *Phragmatopoma lapidosa* larvae nearing settlement in order to determine whether sensory-like structures existed which were not easily discernible with the light microscope.[11] Figure 14 shows an SEM photograph of a larva just prior to settlement and Figure 15 illustrates the dorsal surface of one larval tentacle having a number of sensory-like tufts composed of a variable number of circularly arranged, stiff, radiating cilia. A transverse section through a tuft reveals a single, small supportive cell from which the stiff cilia project through the larval cuticle (Fig. 16). The cells contain a basally situated nucleus, large oval or irregularly shaped mitochondria, slightly electron-dense vesicles and thick, blunt, branching and nonbranching microvilli with bundles of microfilaments (stereocilia) which project well into the cytoplasm of the cell. These microvilli differ significantly from the thin, highly branching microvilli of regular epidermal cells. The modified cilia are characterized by their stiff posture and blunt tips (Fig. 17), which contrast with the curved, pointed cilia observed in other epidermal cells. Occasionally microtubules are observed in the cells. Synaptic contacts with sensory nerve fibers have been observed at their base. Figure 18, based on a compilation of scanning electron micrographs taken from approximately 70 larvae, represents the distribution of presumed "sensory tufts" over the surface of a *Phragmatopoma lapidosa* larva in the swimming-crawling phase just prior to settlement. The greatest concentration of "sensory tufts" per unit area occurs on the dorsal surface of the tentacles where they usually form two or three rows. The dorsal hump, a raised area between the tentacle bases, is the only other region on the dorsal surface where tufts were observed. These structures are conspicuously absent from the dorsal regions of the thoracic and abdominal segments. On the episphere or anterior surface of the hood, a small number of tufts are distributed in a horseshoe-shaped pattern along with a number of gland pores. Ventrally, "sensory tufts" are scattered from the neurotroch, laterally to the neuropodia on the thoracic segments, over the surface of the building organ and the lips of the mouth. Tufts are restricted to the ventral lateral regions of the last one or two abdominal segments. The pygidium possesses a large concentration of tufts scattered around the anus in association with gland pores and in the dorsal gap of the telotroch.

It is noteworthy that in the juvenile worm, ciliary tufts are not observed on the dorsal surface of the numerous feeding tentacles formed after metamorphosis, but they are still present on the original pair of larval tentacles which are retained after metamorphosis. Dales[22] pointed out that the latter tentacles probably have a sensory function in most sabellariids except in the genus *Phalacrostemma* where they function in feeding.

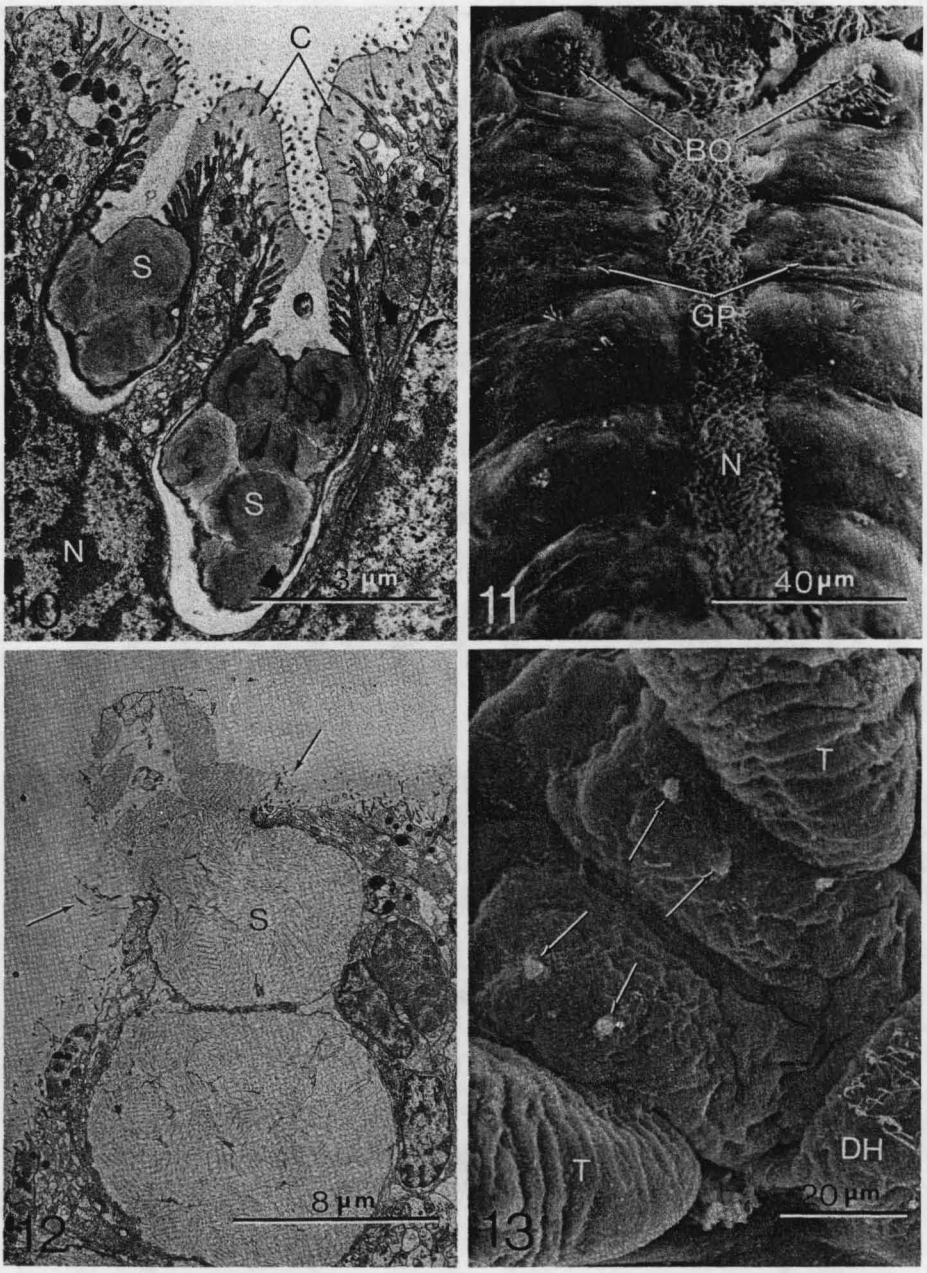


Fig. 10. TEM section through Type I ventral epidermal mucous glands of the first parathoracic segment of settling *P. lapidosa* larva. Note recessed gland pores.

Fig. 11. SEM view of ventral parathoracic segments of larva showing gland pores.

Fig. 12. TEM view of Type II epidermal gland cell from episphere of settling larva. Arrows indicate the elevation of the cuticle around the gland pore neck.

Fig. 13. SEM view of episphere of larva showing secretion droplets emerging from Type II gland pores (arrows).

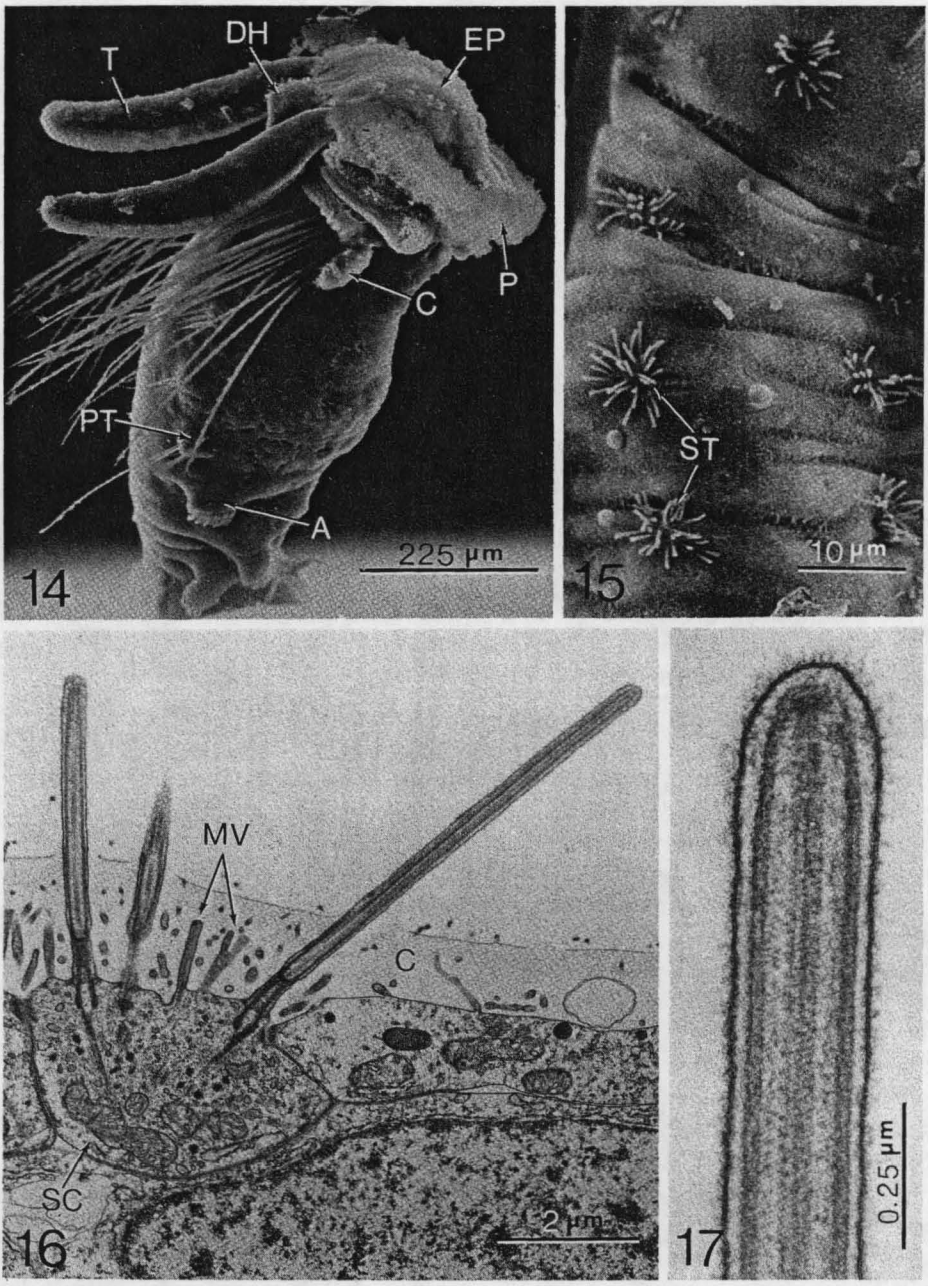


Fig. 14. SEM view of right lateral side of *P. lapidosa* larva just prior to settlement.

Fig. 15. SEM view of sensory-like tufts over the dorsal surface of tentacles of larva in Fig. 14.

Fig. 16. TEM section through "sensory tuft" of *P. lapidosa* larva.

Fig. 17. Longitudinal section through cilium of "sensory tuft" showing blunt tip.

(Figs. 14 and 15 from Eckelbarger and Chia.[11] Reproduced by permission of the National Research Council of Canada from the *Canadian Journal of Zoology*, Volume 54, pp. 2082-2088, 1976.)

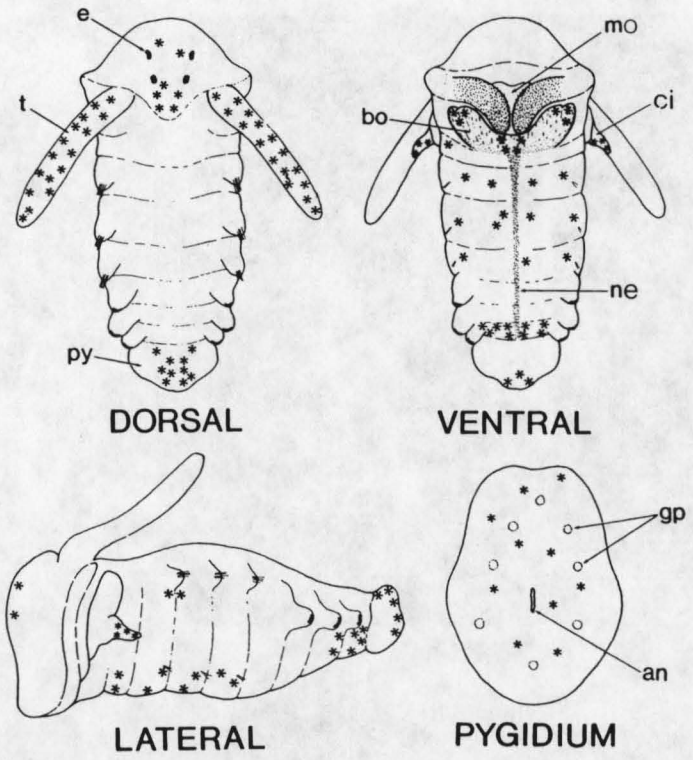


Fig. 18. Diagrammatic views of the pygidium and dorsal, ventral and lateral surfaces of a late stage *P. lapidosa* larva showing distribution of "sensory tufts " (*).

It is unclear whether the tufts described from the larvae of *Phragmatopoma lapidosa* correspond to secondary sensory cell mechanoreceptors or chemoreceptors as defined by Welsch and Storch.[24] The cilia are closely grouped together and are modified from those of other ciliated cells. The microvilli are likewise modified, but do not encircle the cilia as in some mechanoreceptors.

Wilson[6] clearly established that *Sabellaria alveolata* larvae in the premetamorphosed searching phase can detect the adult tube cement or larval mucoid tubes of other sabellariids and that the attracting factor is not detected from a distance. During the searching phase, the larvae contact the substrate with their ventral surface, mouth region and tentacles. *Phragmatopoma lapidosa* larvae appear to be unique by "testing" the substratum with their head region as well. The distribution of tufts over these surfaces in *P. lapidosa* larvae suggests that they serve a sensory function during substratum selection although fine structural evidence alone is not conclusive. The function of the pygidial tufts is unclear as larvae do not appear to contact the substratum with this surface during the searching phase.

The tufts described here from *Phragmatopoma larvae* superficially resemble epidermal "diffuse sense organs" of adult *Nereis virens* described by Langdon[25] to consist of one or more distal processes penetrating the cuticle from basally located bipolar nerve cells. These structures were particularly numerous around the mouth and over the distal surfaces of cephalic appendages of *Nereis* such as the polyps, cirri and tentacles.

Wilson[6] compared the substrate selection behavior of *Sabellaria alveolata* to that of the barnacle cypris larva. Crisp and Meadows[26,27] reported that physical contact of the cyprid with the substrate containing the metamorphosis-inducing substance, to which they respond, was

essential. Knight-Jones[28] had earlier concluded from studies of cyprid behavior that contact of the cyprid with settled barnacles or their bases was essential and that the perceived inducing substance was not water soluble. He concluded that the gregarious settling response in barnacles was probably due to contact with quinone-tanned proteins forming the epicuticle of settled animals. Crisp and Meadows[26] suggested that the cyprid is able to recognize a specific chemical structure or molecular configuration of substances attached to solid surfaces without the need for aqueous diffusion. This ability was referred to as a "tactile chemical sense."[27] Wilson[7] suggested that a similar mechanism is utilized by *S. alveolata* larvae during settlement, although the specific substance detected by sabellariid and cyprid larvae differs in that it is destroyed by cold concentrated HCl in the former case but not in the latter.

Crisp[29] questioned whether the surface would not be occluded by bacterial or other films which grow on it if larvae depend on close contact with the substratum and the settlement-inducing substance on its surface. Wilson[8] reported, however, that laboratory experiments on *Sabellaria alveolata* settlement behavior indicated that aged tube cement was less effective than fresh cement in inducing settlement, suggesting that indeed microbial growths or other surface films might prevent larvae from detecting these substances.

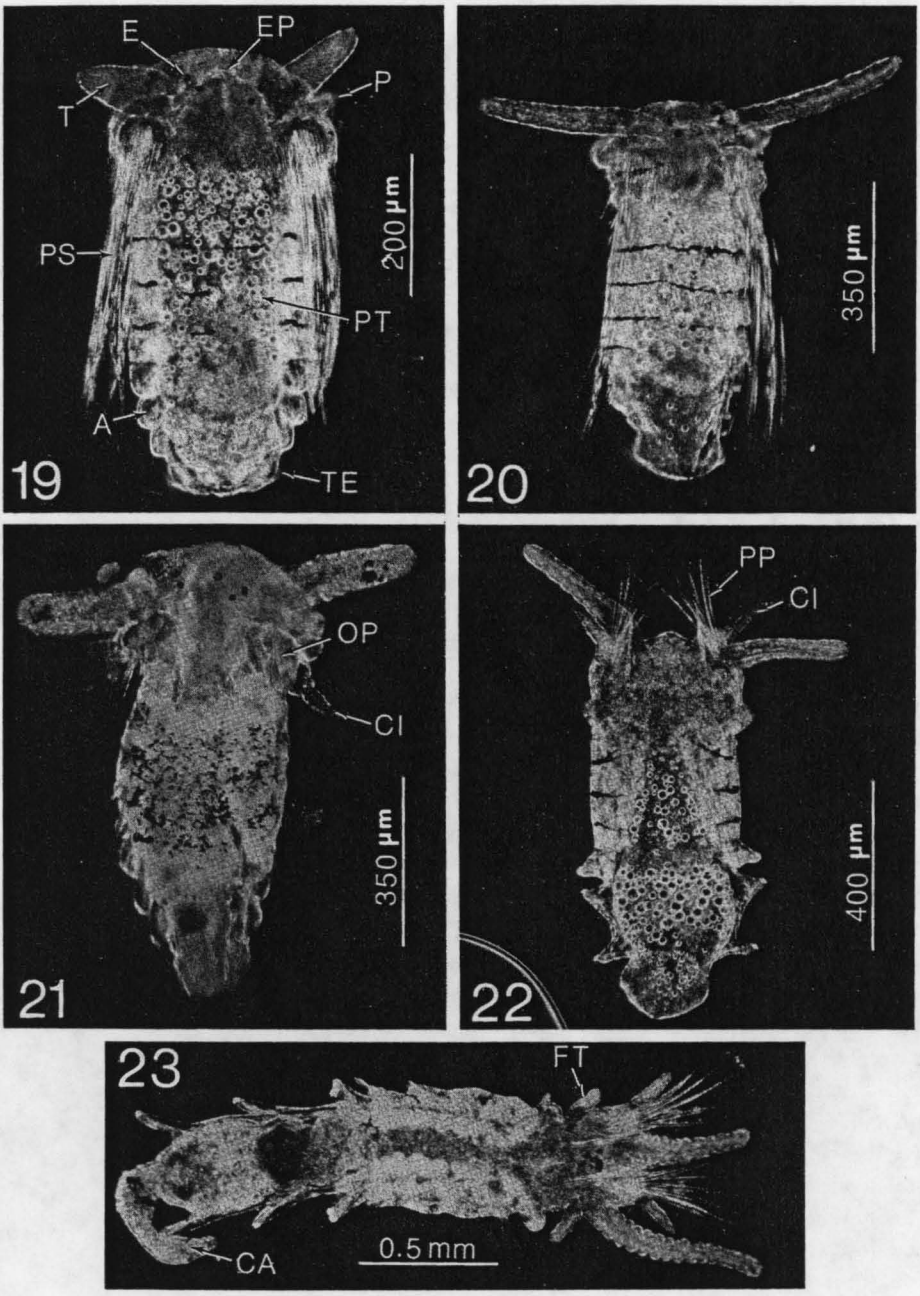
METAMORPHOSIS

The duration of larval development in sabellariids varies widely according to the literature (see Table 1): from 14-30 days for *Phragmatopoma lapidosa*[4] to over 32 weeks for *Sabellaria alveolata.*[8] It is always somewhat difficult to use laboratory development times to predict developmental rates in nature owing to the artificial conditions imposed on the organism in the laboratory setting. A thorough investigation of laboratory development times combined with field studies of spawning and settling rates is perhaps the most reasonable way to determine the true development time for a given species. After extensive laboratory and field observations over many years, Wilson[30] suggested that the normal development time for *S. alveolata* in nature (Duckpool, North Cornwall) probably ranged from 6 to 24 weeks, with the peak probably falling between 8 and 12 weeks.

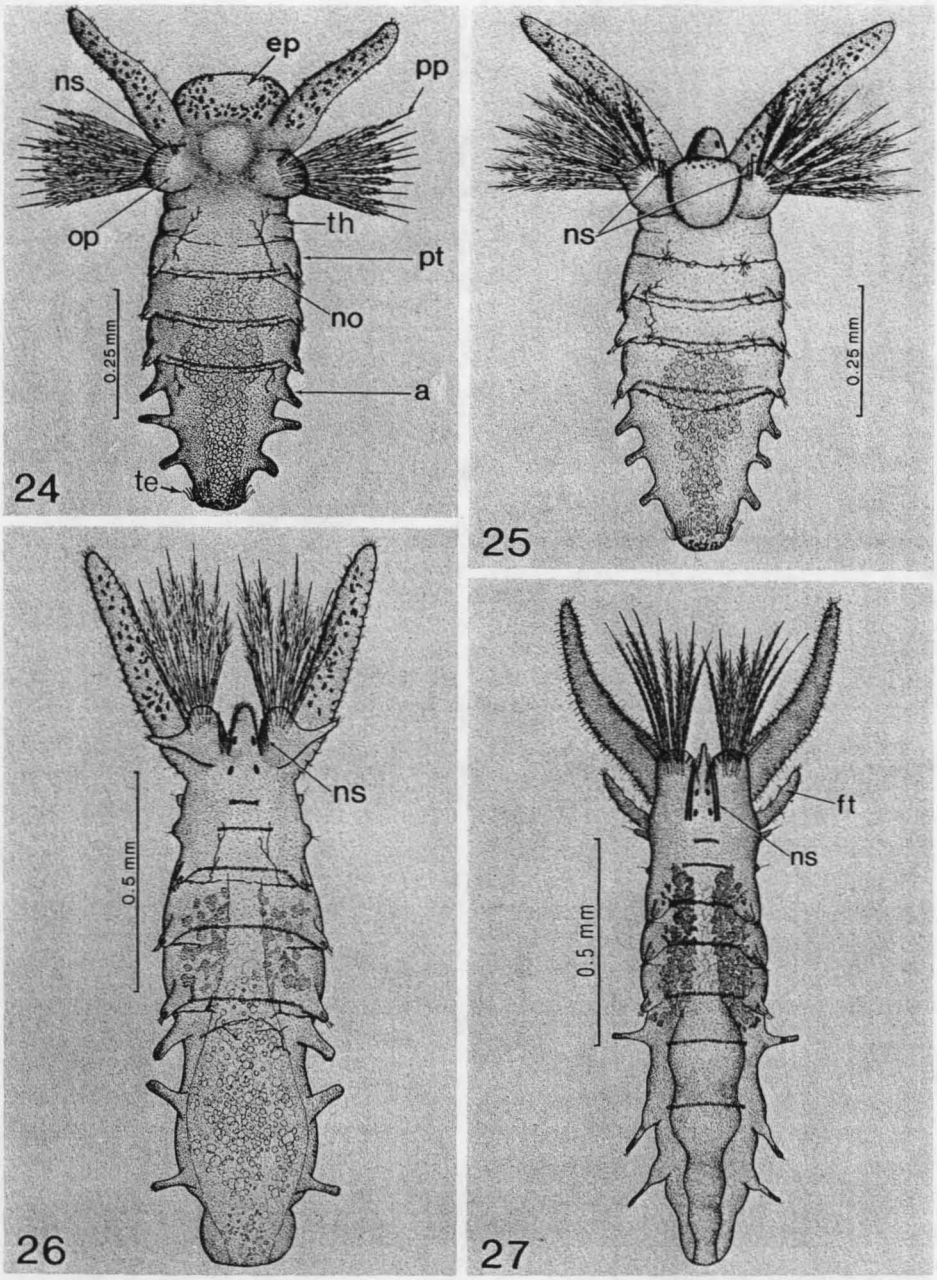
The change in larval behavior from swimming to crawling is accompanied by a series of morphological changes which constitute metamorphosis. Metamorphosis in sabellariids follows a typical, distinctive pattern with only minor variations among species. Figures 19 through 22 photographically document metamorphosis in living *Phragmatopoma lapidosa,* giving a more life-like impression of the process. For comparative purposes, Figure 23 shows a juvenile worm two weeks after metamorphosis. Figures 24 through 26 represent similar stages in the larval metamorphosis of *Sabellaria floridensis* in order to more graphically demonstrate some of the detailed morphological changes which occur. Figure 27 represents a juvenile worm approximately the same age as the *P. lapidosa* juvenile shown in Figure 23.

Metamorphosis begins with a gradual shrinkage of the episphere, loss of the prototroch and enlargement of the building organ. The tentacles rotate anteriorly and become contractile (Fig. 20). The provisional barbed setae are lost (Fig. 21) and reveal a number of pairs of primary paleae projecting from the setal sacs (more easily observed in Fig. 24). The two setal sacs with their accompanying paleae and cirri, now collectively referred to as the opercular peduncles, rotate until the paleae project anteriorly (Fig. 25). The entire head region has now shrunk and 2 of the 4 red eyespots have migrated closer together. The entire larval body lengthens and the telotrochal swelling shrinks with the loss of the telotroch (Figs. 22 and 26).

At this stage, the larva initially constructs a mucoid tube which is attached to a stable substrate. Sand grains are seized by the tentacles, conveyed to the mouth by the ventral ciliated food grooves, and cemented to the outside of the mucoid tube by secretions from the building organ. The metamorphosed larvae of *Sabellaria floridensis*[19] and *Lygdamis muratus*[14] are unusual among sabellariids in failing to attach themselves to the substrate. Rather, both encase



Figs. 19-23. Stages in the metamorphosis of living *Phragmatopoma lapidosa* larvae. 19. Larva about a week before metamorphosis; tentacles turned anteriorly during narcotization and do not represent the normal condition at this stage. 20. Larva in the crawling stage with tentacles rotated anteriorly. 21. Larva has lost provisional setae and telotroch. 22. Larva has rotated opercular peduncles with primary paleae and opercular papillae; episphere has shrunk, eyespots have migrated together, prototroch has been lost and body has elongated; larva has secreted a mucoid tube. 23. Juvenile worm removed from tube two weeks after metamorphosis; note appearance of new feeding tentacles and cauda.



Figs. 24-27. Stages in the larval metamorphosis of *Sabellaria floridensis*. 24. Larva is crawling and has rotated tentacles anteriorly and lost provisional setae. 25. Opercular peduncles bearing primary paleae have rotated anteriorly and episphere has shrunk. 26. Completely metamorphosed larva in tube. 27. Juvenile worm two weeks after metamorphosis (modified from Eckelbarger[19]).

TABLE 1

Sabellariid Ecology and Larval Behavior

Species	Development[a] Time	Development[a] Temp. (°C)	Adult Habitat	Tube-Building Habits	Type of Settler	Metamorphosis Induced by Tube Cement	References
Sabellaria alveolata[b]	6-32½ weeks	±15	Intertidal[d]	Reef-building	Gregarious	Yes	1,6,8,13
Phragmatopoma lapidosa[c]	14-30 days	21-23	Intertidal[d]	Reef-building	Gregarious	Unknown (suspected)	4,20
Phragmatopoma californica[b]	18-25 days	21-23	Intertidal[d]	Reef-building	Gregarious	Unknown (suspected)	12,21
Sabellaria spinulosa[b]	5½-12 weeks	±15	Intertidal & subtidal[d]	Reef-building/small aggregations	Gregarious	Yes	1,9
Sabellaria vulgaris[b]	19-30 days	21-23	Intertidal & subtidal	Colonial/small aggregations	Gregarious	Unknown	10,17,18,31
Sabellaria floridensis[b]	18-27 days	21-23	Intertidal & subtidal	Small aggregations/solitary	Nongregarious	No	19
Lygdamis muratus[b]	about 6 weeks	15-20	Subtidal	Solitary	Nongregarious	No	14-16

[a]Period from fertilization to settlement and metamorphosis.

[b]Warm-temperate species.

[c]Tropical species.

[d]Prefer high wave energy for tube-building.

themselves in a covering of agglutinated sand grains to form a tube which frequently is free on the bottom. Young *L. muratus* worms, unlike most sabellariids, can leave their tubes and build others. Newly metamorphosed individuals of *S. floridensis,* can crawl around in culture dishes on their tentacles dragging the tubes with them.

Among the last events in metamorphosis is the growth of the pygidium into a long, achaetous appendage which reflexes ventrally to form the cauda characteristic of the adult worm (Fig. 23). Whereas metamorphosis up to the formation of the cauda has been estimated to occur within 24 hours (*Lygdamis muratus*[14]) to two days or longer (*Sabellaria alveolata*[1]), formation of the cauda can take a week or more (*Sabellaria alveolata*[1]; *S. vulgaris*[10]).

During early larval development in sabellariids, the parathoracic segments (Figs. 2 and 24) are the first to develop, with the abdominal segments behind this region becoming recognizable with increasing age. The thoracic segments, which develop anterior to the parathoracic segments, are peculiar in that they are not clearly defined until metamorphosis.

An additional number of minor external morphological changes are part of metamorphosis in each species of sabellariid, but will not be detailed here. The reader should refer to Eckelbarger[19] for a list of references.

With the exception of the larvae of the genus *Lygdamis,*[14,16,23] late stage larvae within the genera *Sabellaria* and *Phragmatopoma* appear markedly alike to even the trained eye. As

a result, prominent chitinous structures of systematic importance such as the larval primary opercular paleae and opercular spines are of considerable importance to zooplanktologists wishing to identify sabellariid larvae to species level. Larval opercular paleae are formed in the late stages of development and are carried in bundles in each opercular peduncle, but are hidden from view by numerous barbed, provisional setae. They are homologous to the adult opercular paleae and following metamorphosis are periodically lost and replaced by new, more adult-like paleae during the succeeding juvenile stages. Figure 28 shows the variation in those larval opercular paleae that have been described from developmental studies of sabellariids.

Larval opercular spines are smaller, less numerous structures which are also found hidden within the provisional bundles in all sabellariids described except *Sabellaria floridensis.*[19] Opercular spines are formed in the late larval stages and lost at metamorphosis or soon after. It is not clear what, if any, adult structures are homologous to the spines. Figure 29 shows the variation in larval opercular spine morphology.

Although not strictly a part of metamorphosis, the development of the larval cuticle is a dynamic process spanning the entire period of larval development and continuing after metamorphosis. Eckelbarger and Chia[12] studied the morphogenesis of the larval cuticle in *Phragmatopoma lapidosa* from egg envelope formation to larval metamorphosis and determined that the egg envelope is retained as the larval cuticle through the trochophore stage and that it is then gradually lost and replaced by a new cuticle. Figure 30 diagrammatically summarizes the ultrastructural events occurring during the ontogenetic development of the egg envelope and larval cuticle in *Phragmatopoma.* Figures 31 through 34 show the variation in the morphology of the cuticle on the ventral surface of the tentacles, the ventral parathoracic segments, the dorsal parathoracic segments and the episphere of a late stage *P. lapidosa* larva (Fig. 20). Figure 35 shows the cuticle of a juvenile worm approximately 2 weeks after settlement (similar to Fig. 23).

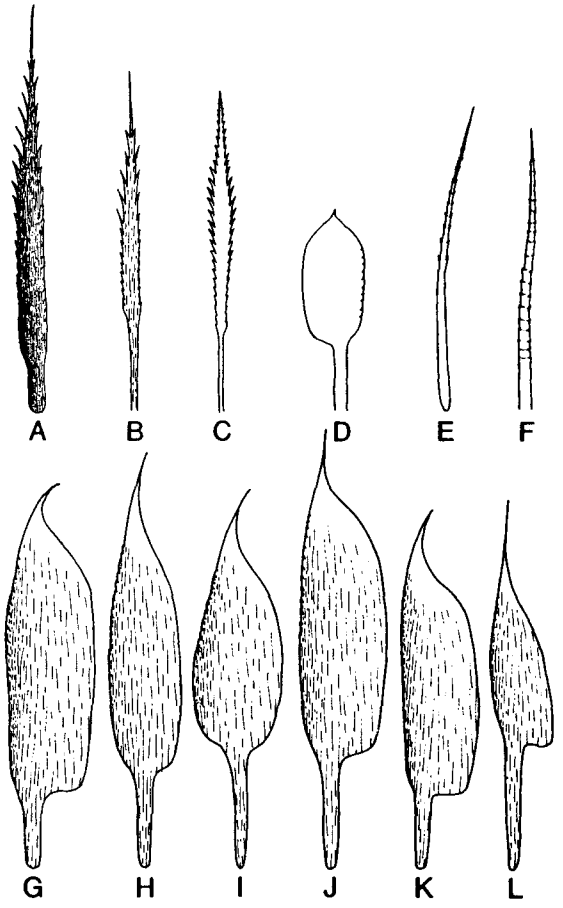


Fig. 28. Primary opercular paleae from late stage sabellariid larvae: A, *Sabellaria floridensis*[28]; B, *Sabellaria vulgaris*[19]; C, *Sabellaria spinulosa*[14]; D, *Sabellaria alveolata*[14]; E, *Lygdamis indicus*[32]; F, *Lygdamis muratus*[25]; G-I, *Phragmatopoma lapidosa*[17]; J-L, *Phragmatopoma californica.*[28]

FACTORS INFLUENCING SETTLEMENT AND METAMORPHOSIS

D. P. Wilson's pioneering studies on larval settlement behavior in the sabellarids *Sabellaria alveolata,*[6,8] *S. spinulosa,*[9] and *Lygdamis muratus,*[14] have provided most of our information on settlement behavior in this group. Arduous observations on the larvae of the reef-building species, *Sabellaria alveolata,*[6] demonstrated that purely physical factors have only

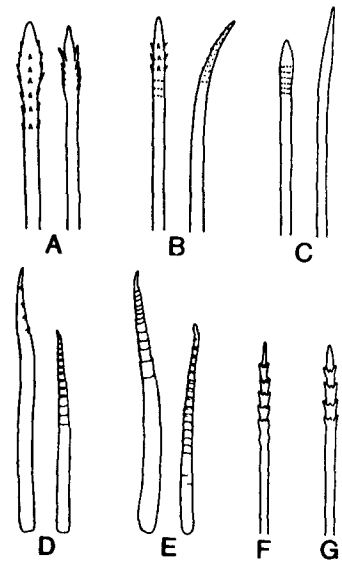


Fig. 29. Larval opercular spines from late stage sabellariid larvae: A, *Sabellaria vulgaris*[19]; B, *Sabellaria spinulosa*[14]; C, *Sabellaria alveolata*[14]; D, *Lygdamis indicus* [32]; E, *Lygdamis muratus*[25]; F, *Phragmatopoma lapidosa*[17]; G, *Phragmatopoma californica.*[28]

a minor influence on settlement. Such physical factors as clean, stable substrates washed over by sea water carrying suspended sand grains and a slight preference for shallow cracks or corners had some effect on settlement behavior. Wilson could find little preference for rough or smooth surfaces, little influence of surface bacterial slime films as a settlement attractant. In the laboratory, *Sabellaria alveolata* larvae are scarcely influenced by the mineralogical nature of adult tube walls, settling readily on tubes of their own species built of mineral grains, whereas in nature the adults build primarily with shell fragments if abundant.

Biochemical factors were found to have the most powerful influence on larval settlement patterns in *Sabellaria alveolata* larvae.[6] Some of the strongest stimuli to settlement and metamorphosis resulted from accidental contact with conspecific adult tubes, tube remnants, or with the tubes of recently settled young, whether simple mucoid tubes or newly constructed sandy tubes. Wilson found the specific factor triggering the settlement response to be the tube cement secreted by young or adult worms. Contact with the cement by larvae was essential because no evidence was found to suggest that it can be detected from a distance. Furthermore, the presence of newly metamorphosed worms on or in an attractive material makes the material even more attractive.

Wilson determined that the metamorphosis-inducing component of the cement is insoluble in water and unaffected by drying. The inducing material was destroyed by cold concentrated HCl without destroying the entire tube wall.

The settling larvae of *Sabellaria alveolata* are able to distinguish between the natural tubes of their own species and those of the sympatric species, *S. spinulosa.*[8] However, in laboratory tanks when adult worms of both species built tubes from the same shore sand, most *S. alveolata* chose *S. spinulosa* tubes in preference to their own, a phenomenon that could not be explained.

Wilson's[8] experiments on *Sabellaria spinulosa,* a non-colonial subtidal species in the Plymouth area, demonstrated that the larvae were strongly stimulated to metamorphose by the tube cement secretions of their own species and rarely failed to distinguish such secretions from those of *S. alveolata. S. spinulosa* larvae were not misled into showing preference for tube material from *S. alveolata,* and *S. alveolata* tubes were only slightly more effective in stimulating *S. spinulosa* larvae to settle and metamorphose than was sand from the seashore. Scallop shells, in particular, were shown to have some slight settlement-inducing properties especially when covered with silt and sand grains.

In larval development studies of the intertidal and subtidal non-reef building sabellariids *Sabellaria vulgaris,*[10] *S. floridensis*[19] and the intertidal reef-building species *Phragmatopoma lapidosa*[4] and *P. californica,*[19] no specific laboratory substrate experiments were undertaken. However, all these species will settle and metamorphose in the presence of sand from tubes of any of the other species.

Most sabellariid larvae are markedly gregarious in their settlement patterns and generally settle on top of or alongside the tubes of previously settled worms. However, the subtidal, non-reef building species, *Sabellaria floridensis*[19] and *Lygdamis muratus*[14] are exceptions. The

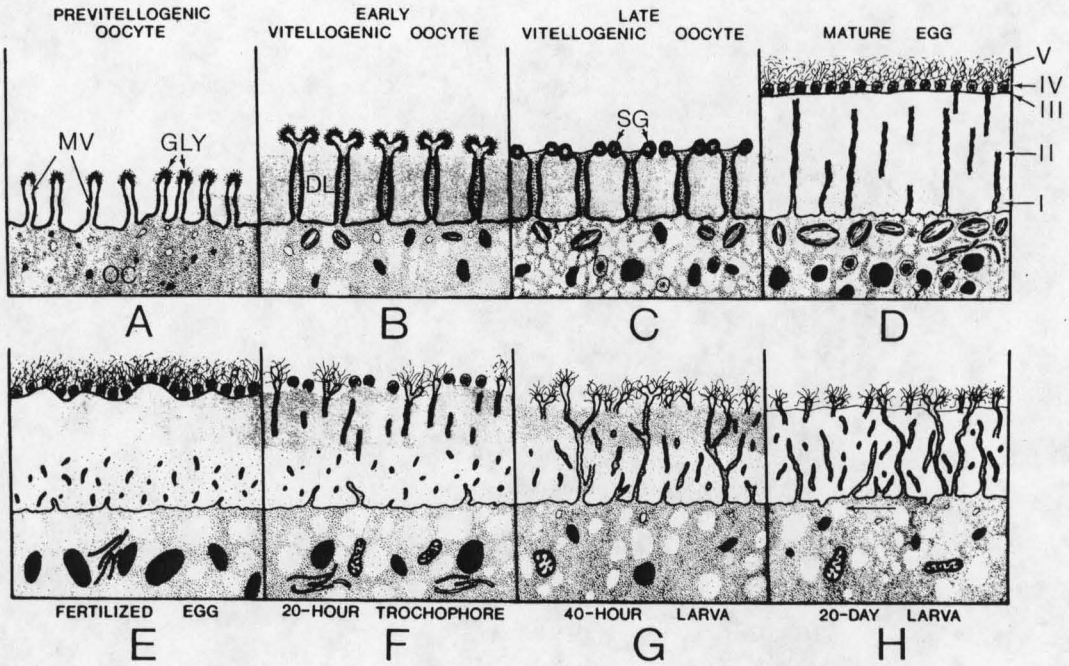
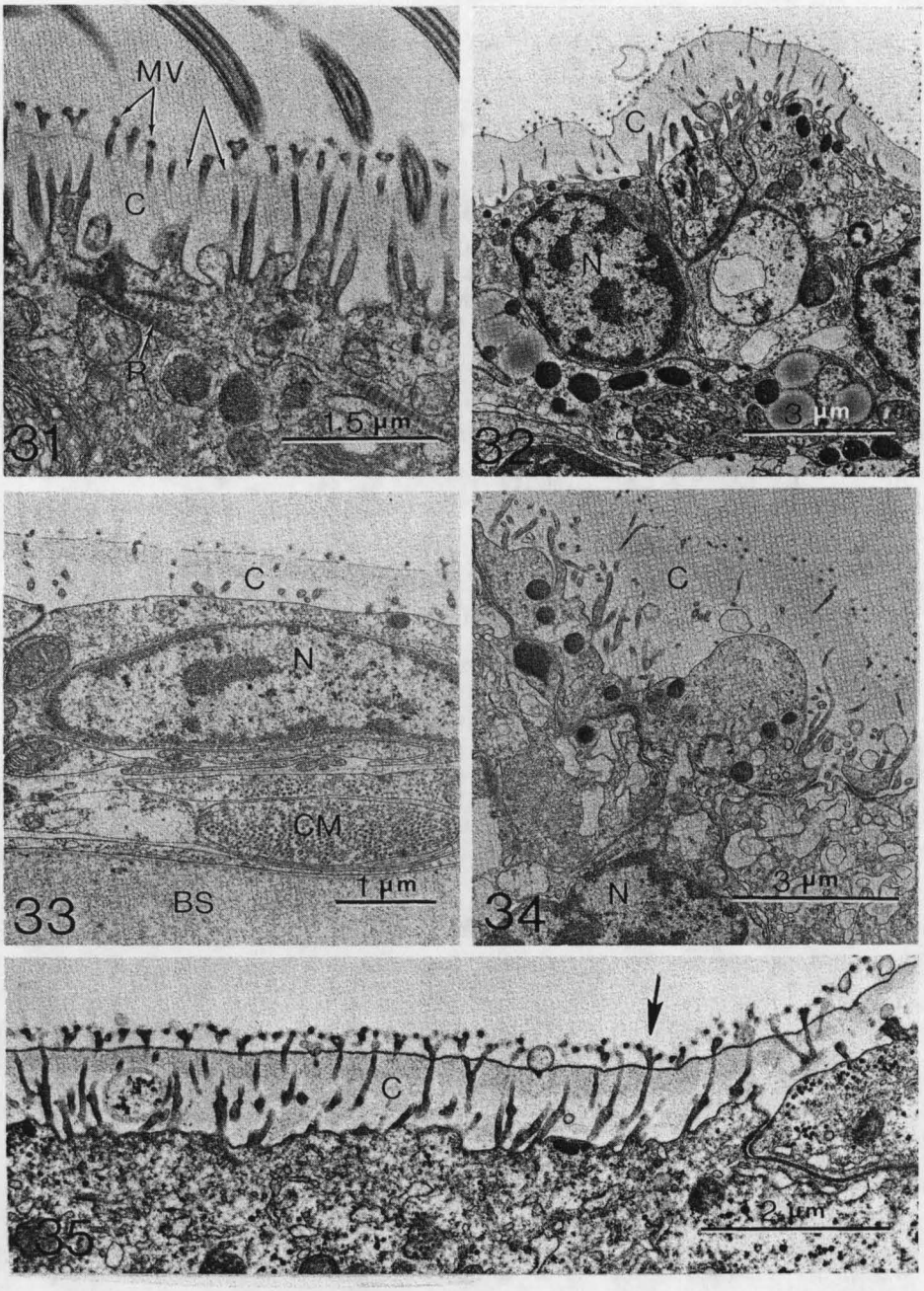


Fig. 30. A-H. Diagrammatic summary of the development of the egg envelope and its gradual replacement by the larval cuticle in *Phragmatopoma lapidosa*. A-C. Formation of surface granules by oocyte microvilli. D. mature egg showing 5 zones of the egg envelope including an outer jelly coat (Zone V), a granular layer (Zone IV), and underlying, thin, electron-dense layer (Zone III) and two inner layers (Zone I and II) of varying thickness and electron density. E. Withdrawal of microvilli into Zone I at fertilization and isolation of outer granular layer. F. Loss of jelly coat (Zone V) and Zone III and partial loss of surface granules (Zone IV); note penetration of new microvilli to surface of 20-hour trochophore cuticle. G. Complete loss of surface granules, formation of highly branching microvilli and partial loss of Zone II in 40-hour larva. H. Ventral cuticle of larva just prior to metamorphosis showing complete absence of original layers of egg envelope. Reproduced by permission of Springer-Verlag from *Cell and Tissue Research*, Volume 186, pp. 187-201, 1978.

larvae of both species crawl over and through the substratum for some period and finally build solitary tubes. It is interesting to note that although the larvae of *Sabellaria spinulosa*[9] and *S. vulgaris*[10] are gregarious settlers under laboratory conditions, they are not colonial reef-builders in nature except at some localities in the former species.[9] Substrate experiments with *L. muratus* larvae[14] demonstrated that they are not, unlike *S. alveolata* larvae, influenced by the cement of newly metamorphosed or adult worms. The larvae are capable of choosing between different kinds of deposits, favoring sandy sediments containing pebbles and mud and preferring non-sterile to sterile deposits.

Delay of metamorphosis for periods of weeks in the absence of a suitable substrate has been demonstrated in the laboratory for *Sabellaria alveolata*[6] and *Lygdamis muratus*.[14] Larvae remain in the swimming-crawling phase during this period and although some may eventually metamorphose in the absence of normal environmental stimuli, others die or metamorphose abnormally. The larvae of *Sabellaria alveolata*[8] and *S. vulgaris*[10] also survive periods of starvation by ceasing growth and resuming development when food is again available.

Agitation or circulation of larval cultures was noted by Wilson[6] to shorten larval settlement times. Eckelbarger[4,10] demonstrated that settlement times for the larvae of *Sabellaria vulgaris* and *Phragmatopoma lapidosa* were doubled in unstirred cultures, compared to stirred cultures. The stimulating effect of agitation on larval settlement probably is reflected by the tendency of



Figs. 31-35. Variation in cuticle morphology from various regions of late stage *Phragmatopoma lapidosa* larva. 31. Ventral ciliated cuticle of larval tentacle showing closely spaced, straight microvilli with bifurcated tips with glycocalyx and two-layered, faintly electron-dense outer region (arrows). 32. Ventral cuticle of parathoracic segment with branching microvilli and thin, single-layered electron-dense outer region. 33. Thin dorsal cuticle from parathoracic segment with sparse, branching microvilli and faint, electron-dense outer layer. 34. Cuticle of episphere showing irregular thickness, highly branching microvilli, noticeable absence of outer electron-dense boundary and highly vacuolated underlying epidermal cells. 35. Dorsal cuticle of juvenile worm 2 weeks after metamorphosis showing closely spaced microvilli with branching tips (arrow) and distinct, electron-dense outer boundary.

these species to select habitats in nature where some degree of wave action is present to provide tube-building materials. Agitation might serve as an additional cue to larvae in selecting an optimum habitat, particularly in intertidal reef-building species.

Additional factors apparently play a role in larval settlement in nature. Wilson[6] includes availability of clean, firmly anchored substrates continually washed with suspended sand grains. Depth and substrate preference and gregarious settlement behavior could also potentially determine the distribution of populations. Curtis[31] studied the intertidal, vertical distribution of *Sabellaria vulgaris* in Delaware Bay and found optimum colony growth was influenced by length of exposure time at low tides and availability of tube-building materials as reflected by wave action. He was not certain whether exposure affected the larval or adult stage but concluded it likely affected both. Colony formation is also limited by the strength of the wave action with such reef-building species as *Sabellaria alveolata* and *Phragmatopoma lapidosa* building strong colonies on the open coast where wave action is intense while *S. vulgaris* builds more fragile colonies that cannot withstand high wave energy.[19,31] All of the factors described above including larval substrate and tidal preferences, phototaxis, presence or absence of the ability of larvae to detect the cement of their own species, tolerance of wave action and presence or absence of gregariousness, undoubtedly contribute to ecological and reproductive isolation of natural populations.

ABBREVIATIONS

A, abdomen; AN, anus; BB, basal body; BO, building organ; C, cuticle; CA,cauda; CI,opercular cirrus; CM, circular muscle; DH, dorsal hump; E, eyespot; EP, episphere; FT, feeding tentacle; GC, grasping cilia; GP, gland pores; GLY, glycocalyx; M, mitochondrion; MO, mouth; MV, microvilli; N, nucleus; NE, neurotroch; NO, nototroch; NS, nuchal spines; OC,oocyte; OPP, opercular peduncle; P, prototroch; PP, primary paleae; PR, primary rootlet; PS, provisional setae; PT, parathoracic segment; PY, pygidium; R, rootlet; S, secretion droplet; SC, sensory cell; SG, surface granule; SR, secondary rootlet; ST, "sensory tuft"; SJ, septate junction; T, tentacle; TE, telotroch; TH, thoracic segment; ZA, zonula adherens.

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