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Some Observations on Gametogenesis, Larval Development and Substratum Selection of the Sea Pen *Ptilosarcus guerneyi*

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Abstract

Ptilosarcus (Leioptilus) guerneyi (Gray) maintained in the laboratory, were observed to spawn in late March, 1972. Gametes, developed in the leaf proper, discharged through the mouths of feeding polyps and were fertilized externally in the sea water. The sea pen's eggs are 500 to 600 μ in diameter; a large female is capable of producing over 200,000 eggs in one season. A pear-shaped and free-swimming planula larva developed 4 days after fertilization, at a temperature of 12 °C. The larvae were ready to settle and metamorphose when 7 days old if favorable substratum was available, but would remain as planulae for at least 30 days if kept in glass dishes only. The 30-day-old larvae would metamorphose if a suitable substratum (coarse sand, for example) was presented. The larvae do not feed and, hence, development is lecithotrophic. Studies of histogenesis showed that metamorphosis greatly enhanced the rate of cellular differentiation. The high fecundity, lecithotrophic development, and the ability of substratum selection by the larvae explain the success of this species in maintaining a high-density population in many areas of sandy substratum in the shallow waters of Puget Sound (USA), despite the fact that it is preyed upon by 7 species of predators.

Introduction

Ptilosarcus (Leioptilus) guerneyi (Gray), a pennatulacean anthozoan known commonly as the sea pen (Fig. 1), is abundant in many areas of sandy substratum in the shallow waters of Puget Sound, Washington (USA). The ecology of this species has been studied intensively by Birkeland (1969), who recorded that the population density may be as high as 129 individuals/m^2, and that large specimens may live for as long as 15 years and grow to a height of 50 cm. Birkeland also reported that *P. guerneyi* is able to maintain a steady-state population, in defiance of heavy predation by 3 nudibranchs and 4 sea stars.

Despite the ecological significance of this species, nothing is known about its reproduction, which is important, in our opinion, in order to understand its success. In this paper, observations on gametogenesis, fecundity, spawning, larval development, and metamorphosis are reported and the role of reproduction in coping with the problems of heavy predation is discussed.

Materials and Methods

Over 15 gravid *Ptilosarcus guerneyi*, collected off Alki Point, Seattle (USA), on March 17, 1972, by SCUBA diving, were kept in sea-water tanks at Friday Harbor Laboratories, University of Washington. These individuals spawned on March 28, 29, and 30th. Fertilized eggs were collected from the sea-water tanks, and were placed in glass dishes at a temperature of 12 °C for observations of development. The water in the culture dishes was changed periodically during the course of the development. Some metamorphosed polyps were fed *Artemia* nauplii, and others were left in slowly circulating sea-water aquaria without food.

For histological preparations, individuals at different stages of development were fixed in either 4% formalin in sea water, or in phosphate-buffered glutaraldehyde made isotonic with sea water by the addition of sodium chloride (Dunlap, 1966; Cloney and Florey, 1968). Glutaraldehyde-fixed material was post-fixed in OsO_4 buffered with bicarbonate (Wood and Luft, 1965). Formalin-fixed materials were embedded in paraffin, and glutaraldehyde-fixed materials were embedded in both paraffin and Araldite. Paraffin-embedded material was sectioned serially at 7 μ, and stained with Masson's trichrome stain. One μ sections from Araldite were stained with Richardson's stain (Richardson *et al.*, 1960).

Gametogenesis was studied from paraffin sections of leaves of gravid individuals fixed in Bouin's fluid and stained by Masson's trichrome.

Results

Gametogenesis

The sexes of *Ptilosarcus guerneyi* are separate. In both sexes, gametes develop in the leaf proper; although found throughout the leaf, more are located immediately below the polyps. The leaf is divided internally by a series of parallel septa, which originate at the base of the polyp and extend part-way across the leaf. In this way, the gastric cavity in the leaf is

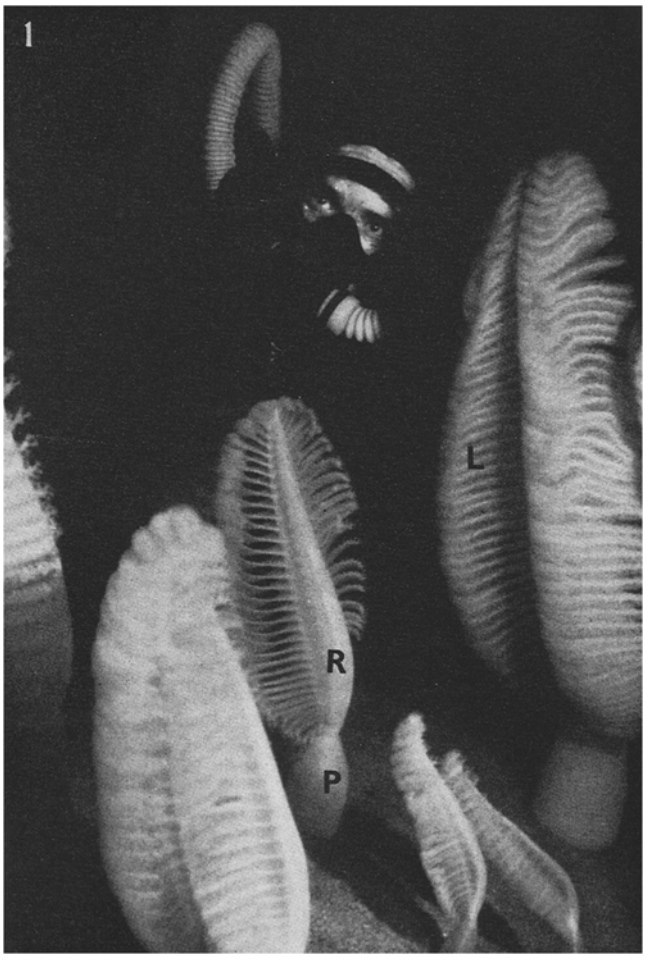

Fig. 1. *Ptilosarcus guerneyi*. Underwater photograph, showing general morphology of adults. Diver illustrates relative size of the sea pens. P: peduncle; R: rachis; L: leaf with feeding polyps. (Photograph courtesy of Dr. C. E. Birkeland, Smithsonian Tropical Research Institute, Canal Zone)

compartmented into a number of incomplete chambers, which house a series of septal filaments of different sizes. The filaments originate either from the body wall or from the septum itself. The septum and the filament consist of two layers of endodermal epithelium, sandwiching a thin layer of mesoglea. Both sex cells, as far as we can determine, are derived from endodermal cells of the filament.

Oogenesis

The oogonial cell occurs most frequently at the tip of the filaments; it is a small (4 μ) spherical cell, characterized by clear cytoplasm and a prominent nucleus with a single nucleolus. Oogonia may occur in clusters (Fig. 2: *3*). At a diameter of 20 μ, young primary oocytes begin to be surrounded by a layer of follicular covering (Fig. 2: *2*). The follicle cells at this stage are either squamous or cuboidal, and all are derived from endodermal epithelium of the filaments. The oocyte is embedded in the mesoglea, in direct contact with the basement membrane of the endodermal epithelium. In these young oocytes, the germinal vesicle (nucleus) is 8 μ in diameter and the cytoplasm is strongly basophilic.

Vitellogenesis begins around the germinal vesicle when the oocyte attains 150 μ diameter. Most of the yolk platelets are probably of the lipid type, since they form small vesicles after being fixed in Bouin's fluid and prepared by the routine paraffin methods. However, large numbers of proteinaceous yolk platelets are also found throughout the cytoplasm. At this stage, the follicle cells have become columnar in shape and bear cilia.

In oocytes 450 μ in diameter, the germinal vesicles occur in the periphery of the cytoplasm. The germinal vesicle is now 100 μ in diameter, the nucleolus 20 μ in diameter. The entire cytoplasm, except for a thin

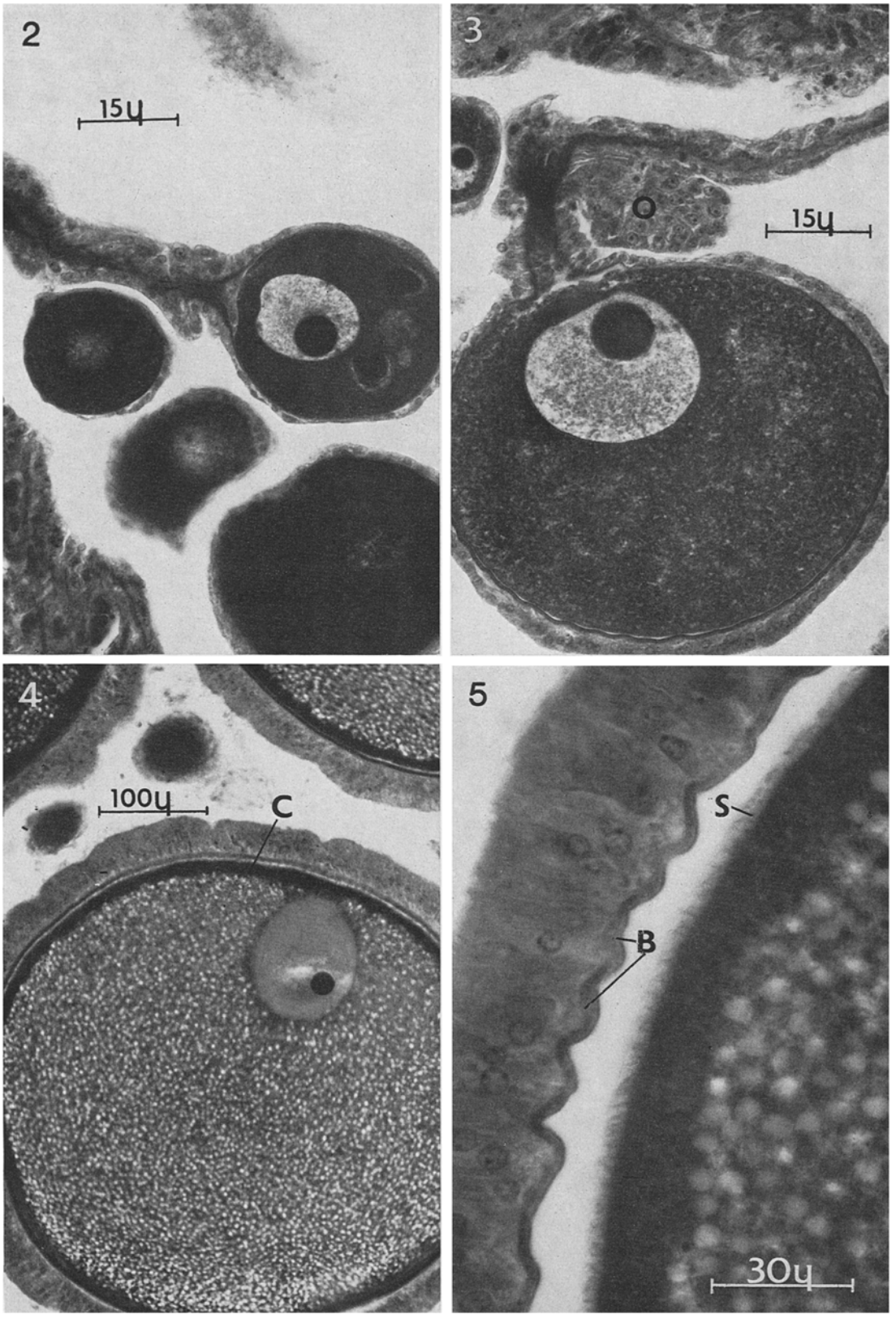

Fig. 2. *Ptilosarcus guerneyi*. *2*: Young primary oocytes at end of a septal filament; note that oocyte is covered by follicular layer of squamous cells. *3*: Cluster of oogonia (O), and a young primary oocyte covered by follicular layer of cuboidal or low columnar cells. *4*: Large primary oocyte, with numerous yolk platelets in cytoplasm; note basophilic egg cortex (C) and location of germinal vesicle. *5*: Portion of a large primary oocyte showing follicular layer of columnar cells; note basement membrane (B) of follicular epithelium and fine spines (S) on egg surface. (The publishers apologise for the fact that μ instead of μ appears on all figures. This mistake was not noticed until after the figures had been etched)

cortex 16 μ thick, is filled with yolk platelets (Fig. 2: *4*). The columnar follicular layer has grown to a thickness of 30 to 40 μ, and rests on a basement membrane which is separated from the jelly coat of the oocyte by a narrow space (Fig. 2: *5*). Radial striations seen in the jelly coat are, in fact, very fine spines of the egg membrane (Fig. 2: *5*), a common feature seen in many anthozoan eggs (Chia and Spaulding, 1972). Primary oocytes grow to a terminal diameter of 500 or 600 μ just before spawning, without further morphological changes.

In the later stages of oogenesis, most of the oocytes have separated from the septal filaments and are suspended in the leaf chambers, although the egg surface is still covered by the follicular layer. The cilia on the follicle cells are probably responsible for the movement of the larger oocytes in the gastric cavity of the leaf. At spawning, most of the eggs must have been freed from the follicle cells; they were then discharged through the mouth of the feeding polyp.

Direct count of the eggs in two large gravid females (with 92 leaves) showed that they contained 200,252 and 210,190 eggs, respectively. Since *Ptilosarcus guerneyi* reproduces annually, and since each specimen may survive and reproduce for 10 years (Birkeland, 1969), the fecundity of this species is very high.

Spermatogenesis

Like oogonia, spermatogonia are first seen in the septal filaments, where they multiply and change into primary spermatocytes and then spermatids (Fig. 3: *6—8*). In the earlier stages, the endodermal epithelium of the filaments surrounding the testis tubules consists of a layer of cuboidal cells (Fig. 3: *8*). In later stages, the epithelial covering becomes squamous. The developing sex cells in the tubules are presumably nourished by direct diffusion of nutrient from the gastric fluid through the thin epithelium.

As in other anthozoans (Chia and Rostron, 1970), mature spermatozoa in *Ptilosarcus guerneyi* are arranged into radial columns in the tubules (Fig. 3: *9*). The head of the sperm is oval in shape, and is approximately 1.5 μ long and 1 μ in diameter.

At spawning, the spermatozoa must be discharged from testis tubules into the gastric cavity of the leaf; they then escape from the mouth of the feeding polyp.

Spawning

As mentioned earlier, *Ptilosarcus guerneyi* spawned in the laboratory on 3 consecutive days in late March. In two out of three cases, several individuals of each sex spawned, and almost all the eggs in the tanks were fertilized. In each instance, the sea pens spawned between 15.00 and 18.00 hrs, after exposure to bright sunlight. Before spawning, all specimens were fully expanded. Spawning began at the upper end of the rachis, and was accompanied by a slow wave of contraction of the leaves. Gametes were expelled through the mouth of the feeding polyps. When first spawned, the eggs were fusiform; after 30 min they had rounded. Freshly spawned eggs were orange in color, and floated at the surface of the water, while sperm became dispersed soon after being discharged from the polyps.

Cleavage

Observations up to 4 h after spawning indicated that the eggs were abnormal, since the embryos resulting from the first two cleavages appeared as irregular lobed structures. At 5 to 6 h, the eggs were in the 8-cell stage (Fig. 4: *10, 11*), but cleavage was superficial as sectioned material revealed that the blastomeres were joined to one another at the center. Later cleavages were complete and, after 12 to 14 h, the embryo had formed a spherical blastula (Fig. 4: *13*) with larger cells at the vegetal pole and a small blastocoel in the center. The cytoplasm of the superficial cells of the blastula could be recognized as two parts, the outer zone (original egg cortex) of fine basophilic granules and the inner zone of yolk platelets. All the inner cells of the blastula were filled with yolk platelets. The surfaces of the superficial cells were still covered with clumped fine spines.

In general, the initial cleavage pattern was radial, although the blastomeres tended to rearrange themselves after each cleavage, often presenting a spiral appearance (Fig. 4: *11, 12*).

Endoderm Formation

At approximately 18 h after fertilization, the surface of the embryos became wrinkled or folded. The convolutions increased in complexity over the next 48 h (Fig. 4: *14*). In early gastrulae (18 to 20 h), there was no distinct separation between the superficial cells and the inner cells, and the yolk granules were more or less evenly distributed throughout the embryo. In later stages, however, the inner cells, containing most of the yolk, were separated from the layer of superficial cells, a columnar epithelium, by a basement membrane. At this stage, the outer layer can be considered as the ectoderm and the inner layer the endoderm. The endoderm probably originated from the superficial cells by a process of delamination, similar to that in *Renilla*, described by Wilson (1883). The endoderm was, however, poorly defined — cell boundaries were not clear and only a few nuclei were visible. The small blastocoel, filled with cellular debris, later expanded to become the gastrovascular cavity. Between 65 and 72 h of development, the embryos became smooth-surfaced and ciliated and began to swim sluggishly.

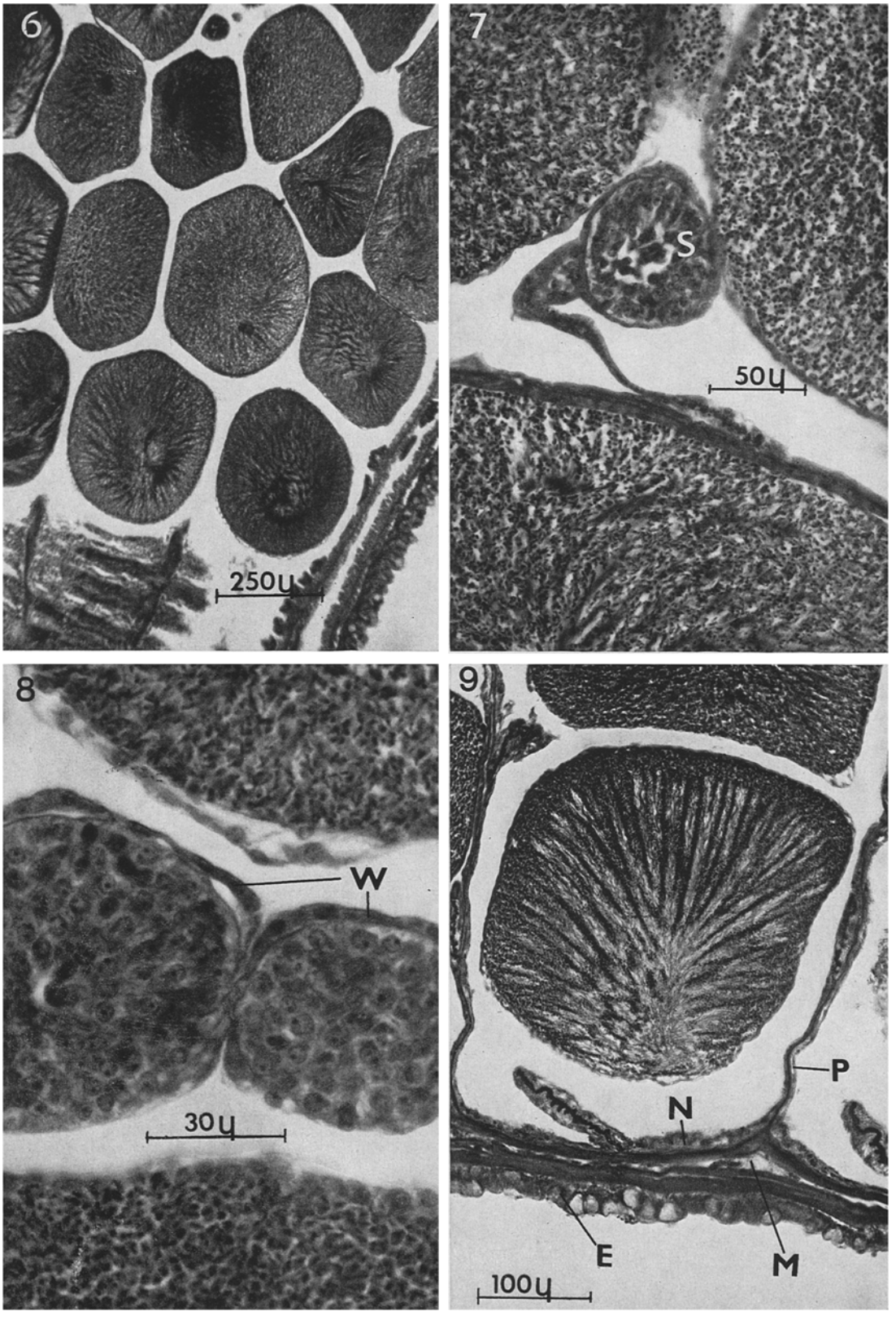

Fig. 3. *Ptilosarcus guerneyi*. *6*: Cross section of testicular tubules from gastrovascular cavity of a leaf in a gravid male specimen. *7*: Cross section of testicular tubules, showing primary spermatocytes (S) in a small tubule and spermatids in other tubules. *8*: Cross section of testicular tubules showing cuboidal epithelium of tubule wall (W). *9*: Cross section of mature testicular tubule showing radial arrangement of spermatozoa in the tubule; note ectoderm (E), mesoglea (M) and endoderm (N) of the body wall; note also the septum (P) from the body wall

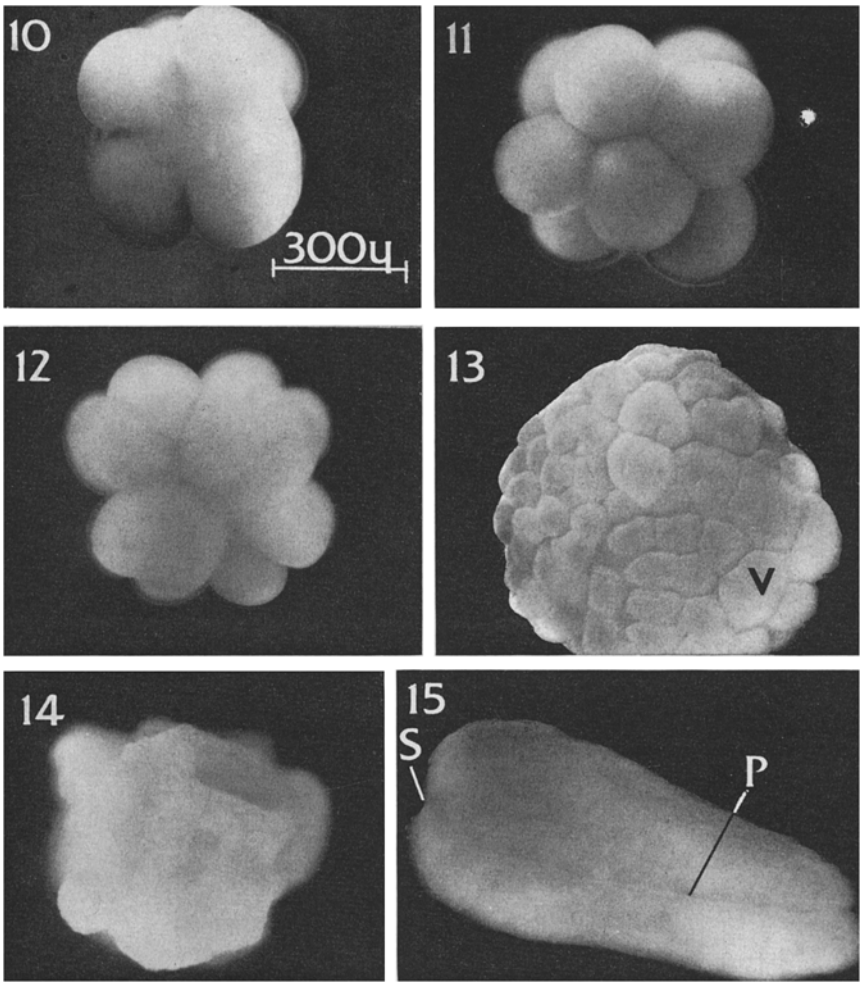

Fig. 4. *Ptilosarcus guerneyi.* Larval development, all from living specimens, same magnification. *10*: Four-to 8-cell stage, showing incomplete cleavage. *11*: Eight-cell stage, showing apparent spiral rearrangement of blastomeres. *12*:. Sixteen-cell stage. *13*: Blastula, showing larger blastomeres at vegetal pole (V). *14*: Gastrula, showing wrinkled surface. *15*: Free-swimming planula, showing areas of septum (P) and stomodeum (S)

Planula

On the 4th day of development, the embryo had changed from spherical to egg-shaped and then to pear-shaped, forming a planula. The planula swam with the pointed end forward; it then elongated further and also flattened. The stomodeum arose by invagination at the blunt end of the larva (Fig. 4: *15*).

By the 7th day, the gastrovascular cavity had expanded and the cellular nature of the endoderm was more apparent. Eight septa were formed, joining the pharynx to the body wall. All septa were formed from inward foldings of the basement membrane and the overlying endodermal epithelium. The single peduncle septum formed at the anterior end of the planula grew toward the pharynx, where it became continuous with 2 of the 8 pharyngeal septa. The pharyngeal septa appeared at the time of stomodeal invagination, and grew anteriorly.

At this time, planulae were capable of metamorphosis into a polyp if presented with a suitable substratum (see below). If metamorphosis did not occur, they would remain basically as planulae. However, later, 8 small tentacular buds would appear around the mouth.

In general, planulae of all ages tend to rest vertically on the bottom of the culture dishes with the mouth down, but we have no evidence that they feed at this time. Larval nutrition depends entirely on the yolk which was stored in the egg. The development is, thus, lecithotrophic.

Effect of Substratum on Larval Settlement

In order to test the effect of substratum on larval settlement, we performed two experiments. In the first experiment, ten 6-day-old planulae were placed in each of 4 culture dishes containing, respectively: (1) fine sand (median particle diameter 150 μ) from False Bay, San Juan Island, where there were no sea pens; (2) the same as above but mixed with mucous from adult sea pens; (3) medium sand (median particle, diameter 300 μ) from a sea pen bed at Friday Harbor, San Juan Island; (4) tubes of the polychaete *Phylochaetopterus* sp., a substratum which has been effective in inducing metamorphosis in the anemone *Tealia crassicornis* (Chia and Spaulding, 1972). One more culture dish without substrate served as a control.

Planula larvae placed on sand (Dishes 1—3) began to swim actively within 1 h, but all had returned to the substrate after 12 h. The larvae placed on medium sand were sticky to the touch, and appeared to be covered with mucous. At first, they were attached to the substrate by their sides but, 48 h later, the pointed end of the planula (peduncle) had burrowed into the sand and the mouth was pointed upwards. Planulae placed on fine sand mixed with mucous (Dish 2) required 4 to 5 days to settle, while those on fine sand only (Dish 1) required 1 to 2 weeks. *Phylochaetopterus* tubes caused a weak swimming response in some larvae, but did not induce metamorphosis. Planulae kept in control glass dishes remained unchanged for at least 1 month; they appeared to be normal, and would settle and metamorphose when presented with a suitable substratum.

The results of this experiment indicate that there may be two factors responsible for larval settlement; size of the sand particles, or the organic components on them. The second experiment was designed to test which of these factors was more important. In this experiment, sand was collected again from the sea pen bed at Friday Harbour, and divided into 5 size classes on the basis of particle diameter: 75—100, 100—210, 210—500, 500—1000 μ, and greater than 1000 μ. Each size sample was divided into two parts: one-half was placed in a culture dish without treatment; the other half was boiled in concentrated sulfuric acid for 10 min, washed in running tap water for 2h and in running sea water for 1 h, and then placed in a second dish. Ten 6-day-old planulae were then introduced into each of a total of 10 culture dishes.

The larvae in each of the 5 dishes containing non-treated sand behaved in the same manner as those in medium sand (Dish 3) in Experiment 1; all had settled within 2 days. The planulae in all 5 dishes containing acid-treated sand began to settle only after 17 days. By this time, the treated sand had probably already aquired a coating of organic matter. Thus, the results of the second experiment suggest that the organic content of the sand is probably the most important factor in inducing larval settlement in *Ptilosarcus guerneyi*; particle size appears to have no effect. Soft sandy substratum is, however, important, since the planulae do not settle in glass dishes without sand.

Development of Young Polyps

Three days after settlement, 8 short tentacles had developed in the young polyp. At this stage, the polyp would contract into the sand when disturbed. Pinnae developed on the tentacles by the seventh day after settlement, and 2 secondary polyp buds appeared below the primary polyp 5 days later. Ciliary movement was observed in the gastric cavity at this stage.

Initially, the larvae settled about equally on the sand and the sides of the glass dish but, soon after, the polyps on the glass relocated in the sand.

Table 1. *Ptilosarcus guerneyi. Chronology of development in the laboratory at a temperature of 12 °C*

Time	Developmental events
1 h	Spawning and fertilization
3— 5 h	Early cleavage
5— 6 h	8-cell stage
6— 7 h	16-cell stage
12—14 h	Blastula
18—20 h	Early gastrula
1— 2 days	Late gastrula, free swimming
3— 4 days	Planula
6— 7 days	Larval settling when suitable substratum available
9 days	Young polyp with peduncle in sand and mouth upwards
10 days	Development of 8 short tentacles surrounding mouth
14 days	Development of tentacular pinnae
19 days	Development of 2 secondary polyps

Some young pens in one culture dish lived for at least 3 months without being fed. During this time, no growth was observed, in fact, they appeared to shrink slightly. Other pens, fed occasionally with *Artemia* nauplii from the third month, developed a fourth polyp in the fifth month but little growth was noted. These individuals were obviously undernourished, as those in the field of approximately the same age were more advanced in development. All the cultures were terminated on the eight month after fertilization, for technical reasons. The general morphological events of development from fertilization to a young sea pen with 3 polyps is summarized in Table 1.

Histogenesis

There was little cellular differentiation at the stages of late gastrula or young planula. The ectoderm, including the lining of the stomodeum, consists of a layer of ciliated simple or pseudostratified columnar

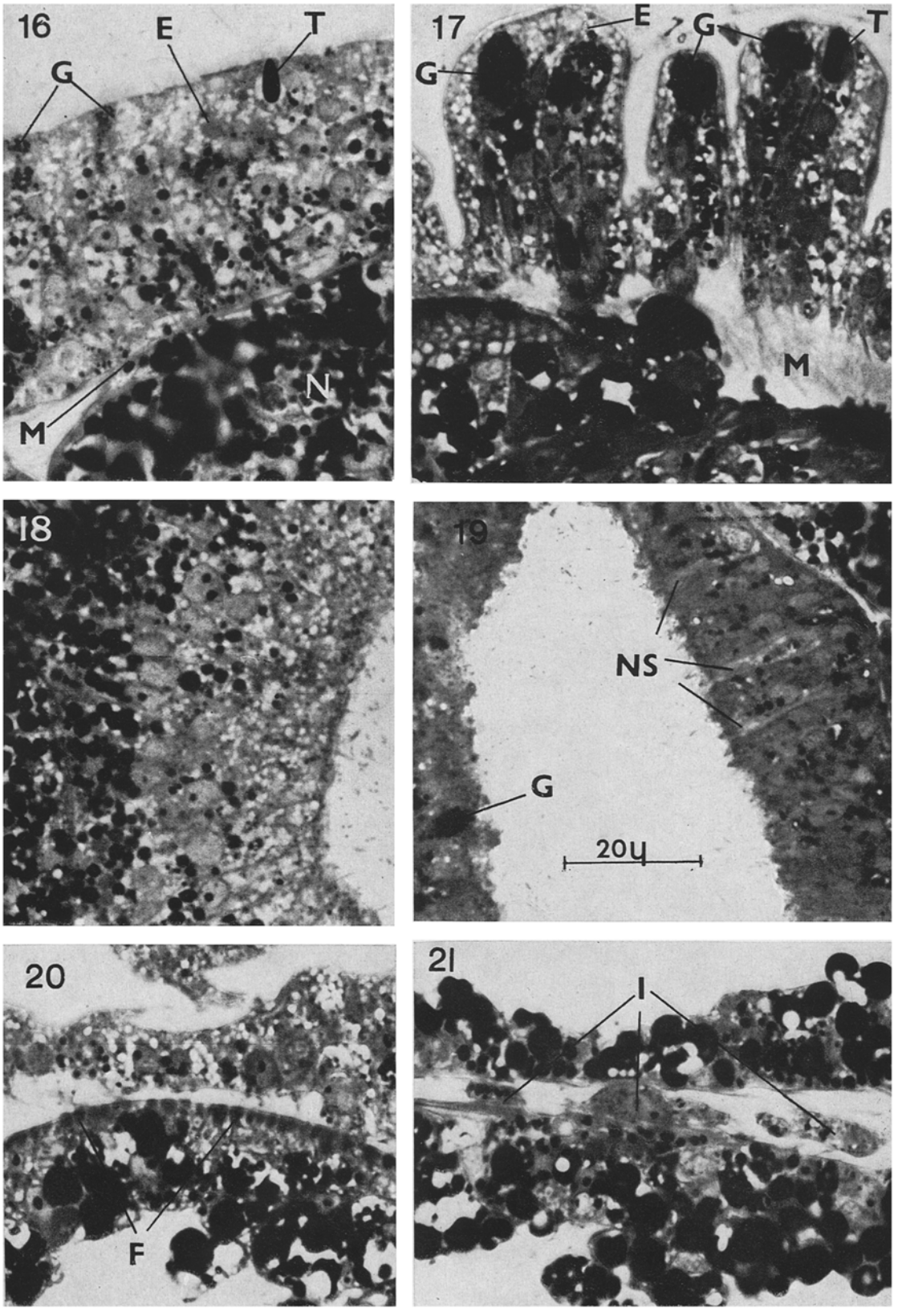

Fig. 5. *Ptilosarcus guerneyi*. Comparative histology of planula and polyp of same age, same magnification. *16*: Body wall of 18-day-old planula, showing ectoderm (E), mesoglea (M), and endoderm (N); note glandular cells (G) and nematocyst (T) in the ectoderm. *17*: Body wall of 18-day-old polyp, showing folded nature of ectoderm (E), and the much thicker mesoglea (M); note morphology and location of glandular cells (G) and nematocysts (T). *18*: Pharyngeal ectoderm of 18-day-old planula. *19*: Pharyngeal ectoderm of 18-day-old polyp; note glandular cell (G) and flask-shaped cells (NS). *20*: Body wall of 18-day-old polyp, showing smooth muscle fibers (F) in basal ends of the endoderm cells. *21*: Peduncle septum, showing interstitial cells (I) in the mesoglea of the septum

epithelium, still rich with yolk. The mesoglea is a thin layer of amorphous material without any cellular components, and the endoderm consists of large irregular ciliated cells with numerous yolk platelets.

In an older planula, e.g. 18 days of age, a few glandular cells and nematocysts occur in the ectoderm of the general body surface (Fig. 5: *16*) but not in the pharynx (Fig. 5: *18*). The ectoderm is still ciliated, and the cells have a microvillus border. The majority of the ectoderm cells contain clear vacuoles at the apical portion of the cytoplasm, and yolk granules at the basal portion (Fig. 5: *16, 18*). No change was noted in the endoderm or the mesoglea of the body wall. Traces of smooth muscle fibers are, however, apparent in the endoderm of the septa.

In contrast to the planula, there is a considerable degree of cellular differentiation in the young polyps. For example, in an 18-day-old polyp, identical in age to the planula described in the preceding paragraph, the ectoderm consists clearly of 4 cell types: supporting cells, interstitial cells, glandular cells, and nematocysts. Both glandular cells and nematocysts are larger and more numerous than those in the planula. The ectoderm, except the lining of the pharynx, has now lost its cilia; it is a cuboidal epithelium in expanded specimens, but remains as a columnar epithelium in contracted ones. When contracted, the ectoderm is thrown into numerous folds, with the nematocysts and glandular cells at the apex of the folds (Fig. 5: *17*). A few glandular cells as well as pale flask-shaped cells are found in the pharyngeal ectoderm (Fig. 5: *19*) The flask-shaped cells are possibly neurosensory in nature.

No apical organ was found at any stage of development. The sensory receptors for substratum selection are probably located throughout the ectoderm, as electron microscopy has revealed a number of cells here which appear to be neurosensory.

The endoderm of the 18-day polyp consists of a layer of irregular ciliated cells, and is still rich with yolk granules; however, bundles of smooth muscle fibers have developed in the basal ends of the cells (Fig. 5: *20*). These muscle fibers must be responsible for the contractile ability of the polyp. No glandular cells are found in the endoderm.

The mesoglea is much thicker in the polyp than in the planula. There are still no cellular components in the mesoglea of the body wall, but large interstitial cells are disernible in the mesoglea of the peduncle septum (Fig. 5: *21*). These cells are probably responsible for the secretion of the calcareous style in the sea pen, as similar cells were reported to be responsible for the secretion of spicules in *Renilla* (Wilson, 1883).

Discussion

As mentioned previously, *Ptilosarcus guerneyi* is preyed upon by 7 species of predators. Birkeland (1969) has asked: "How can the prey species prevent being overexploited by its complex system of predators?" After 5 years' study, he concluded that the major defense mechanism for *P. guerneyi* is to settle unpredictably in both time and space, and so make itself generally unavailable to predators. Our data on the substratum selection of the planula larvae explain, at least in part, the mechanism of unpredictable settlement. As we have shown, the larvae are very sensitive to the nature of the substratum; they are ready to settle when 7 days old if there is suitable substratum, but will delay metamorphosis at least as long as 1 month when suitable substratum is not available. The delaying of metamorphosis is, of course, common in many other marine invertebrates (Birkeland *et al.*, 1971). Considering the settling behavior of the larvae on one hand and the continuous changes on the surface of the sandy substratum on the other, one can begin to appreciate why one area may provoke settlement in one year but not in the next. In other words, it is the delicate and changing nature of the substratum at the crucial time of larval settlement that dictates the success of the recruitment in any given area, and hence the patchiness of distribution.

Our findings on fecundity and larval biology in general also explain how *Ptilosarcus guerneyi* is able to cope with the problem of overexploitation by predators. Firstly, a large adult is capable of producing over 200,000 eggs in one season, so the total output of eggs by a large population with a maximum density of 129 pens/m^2 is indeed very impressive. Secondly, the eggs are large (600 μ in diameter), and development is lecithotrophic. This means that the larvae are nutritionally independent, and larval mortality by starvation is thus greatly reduced; it means also that planktonic larval life is shortened, and hence mortality by predation is reduced. Thirdly, the larvae are capable of delaying metamorphosis until favorable conditions for settlement are encountered. Combine these factors, and it becomes clear that *P. guerneyi* has indeed adapted a high ability to replenish its stock and to maintain its population in a steady state despite heavy predation.

The morphological events of larval development in *Ptilosarcus guerneyi*, including histogenesis, resemble closely those of *Renilla*, which were described by Wilson (1883). One interesting facit of our study should be stressed, that is, the enhancement of the rate of cellular differentiation by settlement. It was shown that the number of glandular cells and nematocysts are greater in the ectoderm of a settled polyp than in a non-settled planula, although both of them are identical in age. Furthermore, the cilia are lost from ectoderm cells once they have settled. This observation affirms the concept that cellular differentiation or the rate of cellular differentiation is dependent on some exogenous factors and, in this case, settlement, which is controlled by the substratum.

Summary

1. The gametes of the sea pen *Ptilosarcus (Leioptilus) guerneyi* (Gray) originate from endodermal cells of the septal filaments in the leaves.

2. Pens freshly collected from the field spawned in the laboratory in late March. The eggs are 500 to 600 μ in diameter, and bear surface spines. A large female is capable of producing over 200,000 eggs per season.

3. The first 3 cleavages were superficial, but later cleavages were complete, forming a blastula with a small blastocoel.

4. Endoderm appeared to be formed by delamination, and the surface of the gastrula is highly wrinkled. A pear-shaped free-swimming planula was formed 4 days after fertilization.

5. Seven-day-old planulae were able to settle and metamorphose if presented with a suitable substratum (for example, sand), but they remained as planulae for at least 30 days if kept in culture dishes without sandy substratum. These larvae, although 30 days old, would metamorphose if a suitable substrate was presented.

6. Larval settlement and metamorphosis greatly enhanced the rate of cellular differentiation.

7. The high fecundity, lecithotrophic development, and the ability of substratum selection by the larvae explain their success in maintaining a steady-state population despite heavy predation by at least 7 species of predators.

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