Costello, Donald P. and Catherine Henley (1971) *Methods for Obtaining and Handling Marine Eggs and Embryos.* Woods Hole, MA: Marine Biological Laboratory, 247 pp.

BRYOZOA *

(ENTOPROCTA)

Barentsia laxa

LIVING MATERIAL:

The colonies of this form are tan or grey in color, and are composed of numerous pin-like individuals crowded together; the colonies are approximately one-half inch in height, and from one-half to one inch in extent. They are often found in association with Venus shells which are encrusted with the sponge, Cliona (Rogick, 1948), and are obtained by dredging in the Hole at Woods Hole, Mass. Barentsia is very similar to Pedicellina, but is larger, hardier, and easier to find.

BREEDING SEASON:

Rogick (1948) reports that free-swimming larvae and embryos in various stages of development were obtained between July and September, but that very probably they are also obtainable earlier and later during the summer. It is not certain whether Barentsia is sometimes hermaphroditic (Rogick, 1948), but in many instances, at least, the sexes are separate.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The animals are hardy and easy to keep in the laboratory if they are supplied with running sea water. They do not require feeding.

B. Procuring Embryos: Embryos and larvae are found within the calyx of the adult; larvae are released through a channel opening into the atrial cavity in front of the anal opening (Rogick, 1948).

NORMAL DEVELOPMENT:

The development of this form is apparently very much like that of Pedicellina (Rogick, personal communication), except that the embryos, larvae and adults are easier to study. Calyx regeneration is a conspicuous phenomenon in Barentsia and one which, as Rogick points out, is probably worthy of detailed study.

REFERENCES:

OSBURN, R. C., 1910. The Bryozoa of the Woods Hole region. *Bull. U. S. Bur. Fisheries,* **30**: 205–266.

ROGICK, M. D., 1948. Studies on marine bryozoa. II. *Barentsia laxa* Kirkpatrick 1890. *Biol. Bull.,* **94**: 128–142.

(See, also, references for Pedicellina.)

* We are grateful to Dr. Mary D. Rogick for much of the information on which this and succeeding sections concerning bryozoan development are based.

BRYOZOA

(ENTOPROCTA)

Pedicellina cernua (P. echinata)

LIVING MATERIAL:

These colonial bryozoans are moderately common on shells and algae in shallow water. The zoids arise independently from a branched, creeping stolon, and have cup-shaped calyces and yellow-red stalks. Hyman (1951) reports that Pedicellina is sometimes hermaphroditic, sometimes dioecious.

BREEDING SEASON:

Although this has not been determined for the Woods Hole, Mass., region, it probably occurs during the summer months.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The animals are moderately hardy in the laboratory, if they are supplied with running sea water.

B. Methods of Observation: Cleaving eggs and young larvae can be dissected from the egg capsules; older larvae will be released if fertile colonies are allowed to stand in fingerbowls of fresh sea water. Harmer (1887) states that the larvae will not attach in small amounts of water. He suggests placing the ripe colonies, together with the algae to which the colonies are attached, in glass jars covered with fine bolting silk. These jars are then suspended in live-boxes, and examined in about a day. At that time, a large number of attached and metamorphosing larvae should be present.

NORMAL DEVELOPMENT:

A. The Ovum: The ripe eggs measure 40 by 50 by 60 microns, the shortest axis being apico-vestibular.

B. Fertilization and Cleavage: There is some disagreement as to whether the colonies are bisexual or unisexual (see "Living Material" above). Marcus (1939) suggests that they are unisexual, although hermaphroditism occasionally occurs. Fertilization is internal, and at least the first maturation division occurs within the ovary. After its release from the ovary, the egg passes through a short vagina where it is enclosed in a soft shell with a long stalk. The shelled eggs are attached by means of the stalks to the floor of the brood-chamber, which consists of the walls of the vestibule between the tentacles and the vestibular groove. Cleavage is equal and spiral, closely resembling that of the annelids. Five quartets of micromeres and one quartet of macromeres are produced (Hyman, 1951). A hollow blastula is formed by the 67-cell stage. Gastrulation is by invagination and starts at about the 90-cell stage.

C. Later Stages of Development: Following gastrulation, there is an apical-oral elongation of the embryo, accompanied by closure of the slit-like blastopore. An apical plate, bearing cilia, develops at the aboral pole but invaginates by the time

the larva is set free. Shortly after this organ is formed, the stomodeum and proctodeum invaginate on the oral surface, and the pre-oral or dorsal organ appears as an invagination on the anterior side of the larva above the mouth. At this time, the egg shell vanishes and the larva is freed. When released, the larva swims with the aid of an oral ring of strong cilia, the corona. The digestive tract is complete and feeding occurs. Shortly after release, a well-marked invagination appears between the mouth and anus. This is the vestibule or atrium; it is bordered by the corona. Both the mouth and the anus are borne on long projections called the epistome and anal cone, respectively.

After about a day, the free-swimming larvae attach by means of the atrial surface, and undergo a complicated metamorphosis which involves a degeneration of the apical plate and dorsal organ, and a complete upward rotation of the entire larval body. For further details concerning the larvae and metamorphosis, see the papers by Harmer (1887), Cori (1933) and Hyman (1951).

REFERENCES:

CORI, C. J., 1933. Dritter Cladus der Vermes Amera. Kamptozoa. Allgemeines über den Cladus Kamptozoa. Erste und einzige Klasse des Cladus Kamptozoa; Kamptozoa = Bryozoa Entoprocta = Calyssozoa. *Kükenthal Handbuch d. Zool.*, **2**: Hft. 1 (5), 1–64.

HARMER, S. F., 1887. On the life-history of Pedicellina. *Quart. J. Micr. Sci.*, **27**: 239–263.

HATSCHEK, B., 1877. Embryonalentwicklung und Knospung der *Pedicellina echinata*. *Zeitschr. f. wiss. Zool.*, **29**: 502–549.

HYMAN, L. H., 1951. The Invertebrates: Acanthocephala, Aschelminthes, and Entoprocta. The Pseudocoelomate Bilateria. McGraw-Hill Book Co., Inc., New York.

MARCUS, E., 1939. Briozóarios Marinhos Brasileiros. III. *Bol. d. Fil. Ciên., e Let., Univ. de São Paulo, Zool.*, **3**: 111–291.

BRYOZOA

(ECTOPROCTA)

Bugula flabellata and *B. turrita*

LIVING MATERIAL:

Bugula flabellata is found on the lower surfaces and edges of horizontal submerged timbers in Eel Pond, at Woods Hole, Mass.; *B. turrita* is found in similar habitats in Vineyard Sound or on the rocks at Stony Beach. Colonies of *B. turrita* are about 30 cm. high, yellow in color, and composed of flat branches growing in spirals. The flesh-colored colonies of *B. flabellata* are slightly shorter and are composed of broad, flat branches, each of which contains three to seven rows of zooecia. Both species have stalked avicularia shaped like birds' heads. Ripe colonies can be recognized by the prominent ovicells.

BREEDING SEASON:

At Woods Hole, these animals have been found to release larvae from the first or second week of June until November 1 (Grave, 1930).

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The animals should be provided with running sea water.

B. Methods of Observation: Early stages of development can be obtained only by dissection of the ovicells, but larvae are easily obtained. Breeding colonies should be collected on the afternoon of the day previous to that on which they will be used. If they are placed in fingerbowls of sea water and left overnight in front of a window, the larvae will be released some time between 5 and 10 A.M. the next morning. If the fingerbowl is left in a dark-room during the night, the larvae will not be released at dawn, but will be retained until the colony is exposed to light. However, this release of larvae in response to light decreases after noon. The released larvae, which gather at the lighted side of the dish, can be pipetted to fingerbowls or other receptacles where they will attach. After attachment, the larvae should be transferred to vessels provided with running sea water, if further development is desired.

NORMAL DEVELOPMENT:

A. Early Stages of Development: Taxonomic confusion makes it uncertain whether the species *B. flabellata* found at Woods Hole is identical with *B. calathus,* studied by Vigelius (1886). If the two species are identical, the animals are hermaphroditic, and the internally fertilized eggs are transferred to ooecia where development occurs. The cleavages are regular and equal as far as the 32-cell stage, producing a flat, two-layered plate of cells. Gastrulation is probably by epiboly, and further development leads to the formation of a rather degenerate free-swimming larva.

B. Later Stages of Development: The ciliated larvae of the two species of Bugula found around Woods Hole are almost spherical and measure approximately

180 microns. Larvae of both species have a stiff circle of cilia surrounding a convex apical organ on the pole which is carried foremost in swimming. The opposite pole is depressed and bears a central invagination called the internal sac. This depression extends up one side of the larva to the equator in the form of a glandular groove. At the apex of this groove is a tuft of long flagella (the vibratile plume). The lateral groove and the plume make up the so-called "pyriform organ." The larva of *B. turrita* has, in addition, four or six eyespots: two or four located on the anterior borders of the lateral groove, and two on the opposite side. When they are first released, the larvae are strongly attracted by light, but after two or three hours, they gradually become negatively phototropic. If they are left undisturbed, the larvae also show a moderate negative geotropism.

After a swimming period of four to six hours, the larva attaches. A temporary attachment is first made by the lateral groove; then the internal sac is suddenly everted and fastened to the substrate. About an hour after fixation, the larva begins to elongate, and in four to eight hours the body cavity is visible and the first polyp begins to form. The primary zoid is completely formed in 24 hours. From this time on, secondary individuals are produced rapidly by asexual budding, until a large colony is formed. Sexual maturity is attained about one month after attachment.

REFERENCES:

CORRÊA, D., 1948. A embriologia de *Bugula flabellata*. *Universidade de São Paulo Bol. d. Fac. Fil., Ciên., e Let., Zool.*, **13**: 7–73.

GRAVE, B. H., 1930. The natural history of *Bugula flabellata* at Woods Hole, Massachusetts, including the behavior and attachment of the larva. *J. Morph.*, **49**: 355–383.

GRAVE, B. H., 1937. *Bugula flabellata* and *B. turrita*. *In:* Culture Methods for Invertebrate Animals, edit. by Galtsoff *et al.*, Comstock, Ithaca, pp. 178–179.

HASPER, M., 1912. On a method of rearing larvae of Polyzoa. *J. Mar. Biol. Assoc.*, **9**: 435–436.

LYNCH, W. F., 1947. The behavior and metamorphosis of the larva of *Bugula neritina* (Linnaeus): Experimental modification of the length of the free-swimming period and the responses of the larvae to light and gravity. *Biol. Bull.*, **92**: 115–150.

LYNCH, W. F., 1952. Factors influencing metamorphosis of Bugula larvae. *Biol. Bull.*, **103**: 369–383.

LYNCH, W. F., 1955. Synergism and antagonism in the induction of metamorphosis of Bugula larvae by neutral red dye. *Biol. Bull.*, **109**: 82–98.

MCDOUGALL, K. D., 1943. Sessile marine invertebrates of Beaufort, North Carolina. A study of settlement, growth, and seasonal fluctuations among pile-dwelling organisms. *Ecol. Monog.*, **13**: 321–374.

MAWATARI, S., 1951. The natural history of a common fouling bryozoan *Bugula neritina* (L.). Misc. Reps. Res. Inst. Natural Resources, Nos. 19–21, Keita Shibata Memorial Numbers, Feb., 1951, pp. 47–54.

MILLER, M. A., 1946. Toxic effects of copper on attachment and growth of *Bugula neritina*. *Biol. Bull.*, **90**: 122–140.

VIGELIUS, W. J., 1886. Zur Ontogenie der marinen Bryozoen. *Mitt. Zool. Stat., Neapel*, **6**: 499–541.

VIGELIUS, W. J., 1888. Zur Ontogenie der marinen Bryozoen. *Mitt. Zool. Stat., Neapel*, **8**: 374–376.

BRYOZOA

(ECTOPROCTA)

Crisia eburnea (*Crisiella* sp.)

LIVING MATERIAL:

This species grows in the form of upright, white, bushy tufts, 8 to 25 mm. high. The tube-like zooecia are arranged in two alternate rows. There is some question as to whether the species is dioecious or monoecious and protandrous. The conspicuous ovicells make the fertile "female" colonies easy to recognize. The form is common at Woods Hole, Mass.

BREEDING SEASON:

In San Francisco Bay, the animals breed from late February to May. The limits of the breeding season have not been investigated in the Woods Hole region, although Rogick and Croasdale (1949) report that they found embryos in the ovicells as late as August 8.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The colonies may be kept in aquaria supplied with an adequate amount of running sea water.

B. Methods of Observation: Although the animals must be sectioned if early developmental stages are desired, both the primary embryo and the secondary embryos can be dissected from the ovicells with fine needles.

NORMAL DEVELOPMENT:

A. Early Stages of Development: The early development of the eggs occurs in ovicells, which are highly modified zooecia. These structures are usually the modified second or third zooecium of an internode, although they are not present in each internode. The ovicell is a vase-like structure, consisting of a narrow stalk, a bulging mid-region and a short neck. The neck contains an opening through which the free-swimming larvae are released. The opacity of the ovicells, and the minute size of the eggs and developing embryos, make it impracticable to study early development by methods other than histological.

The eggs, measuring 18 microns or less in diameter, become associated with young zoid buds which are transformed into gonozoids. Only one egg develops in each gonozoid. Fertilization is internal, but the details of maturation and fertilization are not known. The early cleavages are unusual, in that the small follicle cells, which apparently serve as food for the developing embryo, actually penetrate between the blastomeres and separate them completely. The follicle cells start to disappear in the 24-cell stage and by the 60- or 70-cell stage, the embryo consists of a solid ball of cells, measuring about 43 microns in diameter. The increase in cell number continues until the embryo contains about 200 cells.

B. Later Stages of Development: When the primary embryo reaches the 200-cell stage, it sends out processes which bud off as secondary embryos. When they

are first formed, these secondary embryos are solid balls of cells, measuring between 25 and 35 microns in diameter. They develop into very degenerate free-swimming larvae, which leave the gonozoids through the open mouth. The larvae of *Crisia eburnea* have apparently not been described, but if they resemble those of *C. ramosa,* they are simple, ciliated, sac-like structures, consisting of an inner and an outer layer of cells. One surface bears a large invagination or sucker, by which attachment is effected; the opposite pole, which develops into the mouth cavity of the primary zoid, is flattened and non-ciliated. For a further description and figures of the larvae, see the papers of Barrois (1877) and Harmer (1893).

REFERENCES:

BARROIS, J., 1877. Recherches sur l'embryologie des Bryozoaires. Travaux de l'Inst. Zool. de Lille et de la Station Maritime de Wimereux. Monograph.

BORG, F., 1926. Studies on recent cyclostomatous Bryozoa. *Zool. Bidrag Från Uppsala,* **10**: 181–504.

HARMER, S. F., 1893. On the occurrence of embryonic fission in cyclostomatous Polyzoa. *Quart. J. Micr. Sci.,* **34**: 199–241.

ROBERTSON, A., 1903. Embryology and embryonic fission in the genus Crisia. *Univ. Calif. Pub. Zool.,* **1**: 115–156.

ROGICK, M. D., AND H. CROASDALE, 1949. Studies on marine bryozoa, III. Woods Hole region bryozoa associated with algae. *Biol. Bull.,* **96**: 32–69.

SILÉN, L., 1944. The anatomy of *Labiostomella Gisleni* . . . with special regard to the embryo chambers of the different groups of Bryozoa and to the origin and development of the bryozoan zoarium. *Kungl. Svenska Vetensk. Handlingar, Tredje ser.,* **21**: no. 6, pp. 3–111.

BRYOZOA

(ECTOPROCTA)

Electra (formerly *Membranipora*) *pilosa*

LIVING MATERIAL:

These animals form flat, encrusting colonies on sea-weeds and stones. They can be recognized by the ovate zooecia which are punctured by minute oval pores. The mouth of each zooecium is rimmed by a circle of from four to twelve short spines; a single, longer spine lies directly below the opening. The zoids are hermaphroditic and possibly protandrous. Breeding colonies can be recognized by the presence of eggs within the body cavity of the zoids.

BREEDING SEASON:

This has not been determined for the Woods Hole, Mass., region. In European waters, the season lasts from June until December, with the peak in November.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: Colonies can be maintained easily in the laboratory, if an adequate supply of running sea water is provided.

B. Methods of Observation: Breeding colonies should be placed in fingerbowls of fresh sea water and examined at periodic intervals under the dissecting microscope, to ascertain whether shedding of eggs has begun. Since these eggs are small, they may not be visible to the unaided eye. Just (1934) warns that they are few in number and rather sensitive to environmental changes.

NORMAL DEVELOPMENT:

A. The Unfertilized Ovum: Although the eggs are irregular in outline while they are in the body cavity, they soon round up after they are shed. When freshly shed, the eggs are greyish in color, and measure 79 microns in diameter.

B. Fertilization and Cleavage: Fertilization presumably occurs within the body cavity, before the eggs are shed. Although it is absent in body-cavity eggs, a membrane rises from the surface of shed eggs; when this membrane is fully formed, it is connected to the egg surface by fine radiating lines. The polar bodies are given off within a few minutes of shedding. The cleavages are regular and equal as far as the 16-cell stage. At this time, a bilateral symmetry becomes apparent, and by the 32-cell stage, the future ectoderm and endoderm cells are distinguishable and the cells are arranged in a flat plate. Gastrulation is by epiboly.

C. Later Stages of Development: The larva is a typical "cyphonautes," with a complete digestive tract. It is conical in shape, the broad end being termed the oral surface. At the aboral pole, a tuft of long cilia springs from the apical organ. The margin of the oral surface is rimmed with a circle of powerful cilia, forming the corona. A bivalve shell and a peculiar pyriform organ are formed soon after the free-swimming stage is reached; the internal sac seems to be associated solely with metamorphosis and appears late in development. Details and figures of the

development of the eggs and larvae are given by Prouho (1892), Kupelweiser (1905) and MacBride (1914).

REFERENCES:

BONNEVIE, K., 1907. Untersuchungen über Keimzellen. II. Physiologische Polyspermie bei Bryozoen. *Jenaische Zeitschr. f. Naturw.,* **35**: 567–598.

JUST, E. E., 1934. Fertilization in *Membranipora pilosa. Carnegie Inst. Year Book,* **33**: 268–270.

KUPELWEISER, H., 1905. Untersuchungen über den feineren Bau und die Metamorphose des Cyphonautes. *Zoologica,* **19**: Hf. 47, pp. 1–50.

MACBRIDE, E. W., 1914. Text-Book of Embryology. Vol. I. Invertebrata. Macmillan and Co., Ltd., London.

PROUHO, H., 1892. Contribution à l'histoire des Bryozoaires. *Arch. de Zool. Exp.,* **20**: 557–656.

SILÉN, L., 1944. The main features of the development of the ovum, embryo and ooecium in the ooeciferous Bryozoa Gymnolaemata. *Arkiv f. Zool., K. Svenska Vetenskap.,* **35A**: no. 17, pp. 1–34.