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

ARTHROPODA

(CRUSTACEA)

Balanus eburneus *

LIVING MATERIAL:

This form is quite commonly found in the Woods Hole, Mass., region; the shell is low and broad in form, with a smooth, yellow-white exterior. It usually occurs at or below the low-water mark, on stones, shells, timbers, etc. Burbanck *et al.* (1956) collected *B. eburneus* in abundance at Rand's Harbor, in areas of fresh water inflow.

BREEDING SEASON:

Fish (1925) states that the breeding season of *B. eburneus* at Woods Hole extends from August to mid-November. At Beaufort, North Carolina, this barnacle apparently breeds from July to September (Costlow and Bookhout, personal communication).

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The animals should be kept in aquaria supplied with running sea water.

B. Procuring Embryos: Carefully chip away the calcareous portion of the basis. If the eggs are ripe and in the process of development, the egg lamellae are firm and, with care, may be removed intact. They should be placed in a finger-bowl of sea water; the eggs may be teased out with a needle.

C. Preparation of Cultures: The embryos and larvae are sensitive to temperature and oxygen changes. Therefore, the sea water in which they are kept should be changed frequently, and the cultures kept cool on a sea water table. Despite these precautions, the mortality rate is high. The addition of 200,000 to 400,000 units of penicillin per liter appears to reduce bacterial growth in the cultures (Costlow and Bookhout, 1957).

Aqueous extracts of mantle wall or body tissues, but not of egg-masses themselves, have been found to promote hatching and liberation of the nauplii (Crisp, 1956).

NORMAL DEVELOPMENT:

A. Fertilization and Cleavage: The available evidence indicates that barnacles normally are not self-fertile; however, Barnes and Crisp (1956) have collected some data which indicate that *B. perforatus* eggs occasionally develop either parthenogenetically or as a consequence of self-fertilization.

Fertilization in *B. eburneus* is internal; in the youngest stages of development, it is possible to observe the formation of the polar bodies, and the approach of the germ nuclei. This is best seen in preparations which have dried somewhat, so

* Dr. J. D. Costlow, Jr. and Dr. C. G. Bookhout supplied much of the information on which this section is based; we should like to express our appreciation to them.

that the eggs are slightly flattened. Cleavage apparently is similar to that in the egg of Lepas, and gastrulation is by epiboly.

B. Time Table of Development: The over-all time for development of *B. eburneus* is quite short, as compared with that for many barnacles, and requires from 7 to 13 days (Costlow and Bookhout, 1957). At 26° C., the following durations for the naupliar and cyprid stages were recorded by Costlow and Bookhout (1957):

Stage	Duration
First naupliar	15 minutes to 4 hours
Second naupliar	1 to 2 days (average: 1 day)
Third naupliar	1 to 4 days (average: 1.5 days)
Fourth naupliar	1 to 4 days (average: 2 days)
Fifth naupliar	1 to 5 days (average: 2.6 days)
Sixth naupliar	2 to 4 days (average: 2.5 days)
Cyprid	1 to 14 days

C. Later Stages of Development: A three- and a five-segment stage are undergone by *B. eburneus* (Costlow and Bookhout, personal communication). The organogeny of the developing embryo is complex; Groom (1894) gives diagrams of the early development and later phases of *B. perforatus.*

Free-swimming stages: Costlow and Bookhout (1957) describe the six naupliar and one cyprid stage of *B. eburneus,* giving setation formulae, specific morphological characteristics, frequency of molting, duration of intermolt periods, and time of complete development in the laboratory. The use of a motile source of food, such as Arbacia plutei and Chlamydomonas, is necessary in order to maintain the animals throughout the larval period.

Setting and metamorphosis: Costlow and Bookhout (1953, 1956) describe methods for collecting the cyprid stage of *B. improvisus* and *B. amphitrite niveus.* They use six-inch plastic squares, which are suspended in water known to be inhabited by the adults. Small squares of plastic, containing individual cyprids, may be cut from the larger collecting-square and observed, from either surface, under the microscope. Metamorphosis of the cyprid into the "pin-head" stage is described for *B. amphitrite niveus* by Doochin (1951). The subsequent development of the characteristic six mural plates of the adult barnacle is described for *B. improvisus* by Costlow (1956).

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ARTHROPODA

(CRUSTACEA)

Emerita (formerly *Hippa*) *talpoida*

LIVING MATERIAL:

The sand crab, Emerita, is common on sandy beaches; the animals migrate, with the tide, up or down the beach. The females are about twice the size of the males; according to MacGinitie (1938), the males outnumber the females by a ratio of three to one during the early part of the breeding season.

BREEDING SEASON:

On the California coast, in May and June the season is at its height. However, females with eggs in young stages have been found as late as October. Females carry their eggs for a period of four to five months. It appears that in general, mating of the California forms takes place in the late spring or early summer, although females with eggs are found throughout the year.

The breeding season for this form in the Woods Hole, Mass., region has not been accurately determined, although females with eggs have been found in July and August (Bumpus, 1898).

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: Animals may be kept in large fingerbowls which are three-fourths filled with sand. An abundant supply of running sea water, led in under the sand, should be provided. Molted exuviae occasionally appear on top of the sand layer; these should be removed promptly.

B. Procuring Gametes and Embryos: For details concerning the mating habits of Emerita, see the paper by MacGinitie (1938). The time of mating can be detected by observing the behavior of the animals; several males will gather around a female for as many as five days in advance of egg-laying. Sperm will be deposited in ribbons of mucus on the ventral side of the female; shortly afterward, the female begins the egg-laying process. Eggs are deposited under the telson, on the ventral surface of the abdomen; the process may occur over a period of three days. Diagrams of the male reproductive system of *Hippa pacifica* are presented by Matthews (1956).

C. Methods of Observation: Embryos may be removed from the egg-mass at any time, by holding the female gently, prying up the telson, and removing a few embryos with forceps.

Cleavage stages are more clearly observed if the embryos are first treated with 1% chromic acid, washed in water and mounted under mica coverslips. Older stages are more difficult to study satisfactorily; they should be killed, to whiten the embryonic area, using mercuric chloride. The embryonic regions begin to whiten in a few minutes; strong aceto-carmine (with a little sea water) should then be used. After a few minutes, put the eggs in 50% glycerine, which causes the stain to fade.

NORMAL DEVELOPMENT:

A. The Unfertilized Ovum: The eggs are orange to scarlet in color, and measure approximately 380 microns in diameter. They are attached to the egg-mass by thin stalks, and remain on the ventral surface of the female until the zoeae hatch; presumably, the eggs do not develop further if they are detached from the egg-mass.

B. Cleavage and Gastrulation: Divisions appear to be total, but there is some question as to whether the cleavage furrows actually penetrate the yolk mass. Cleavage stages are more frequently found in Emerita eggs than in Libinia eggs.

Gastrulation is similar to that in Libinia, and there is a corresponding development of the embryonic rudiments in the embryonic area. Fixation of the cells with mercuric chloride (as noted above) and study by reflected light will facilitate observations.

C. Later Stages of Development: The zoea larvae hatch from the egg membranes and may be kept in fingerbowls supplied with pieces of Ulva; the larvae should be fed diatoms.

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ARTHROPODA

(CRUSTACEA)

Lepas anatifera

LIVING MATERIAL:

The goose barnacle is not indigenous to the Woods Hole, Mass., region; however, during the summer, timbers and wooden boxes with barnacles attached may sometimes drift into the harbor from the Gulf Stream.

BREEDING SEASON:

The limits of the season are not known. Bigelow (1902) states that he found maturation stages early in June, whereas by July and August, young nauplii were prevalent.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: Sections of timbers with adherent barnacles may be placed in aquaria supplied with running sea water, in the laboratory, or larger timbers may be anchored in the Eel Pond. Detached animals will also live in an aquarium in the laboratory.

B. Procuring Gametes and Embryos: Lepas is hermaphroditic and can fertilize its own gametes; these embryos are then deposited in the mantle cavity in sheet-like ovigerous lamellae, where they continue to develop until they hatch as nauplii. If one wishes to examine the gametes, they can be procured by slitting a barnacle along the plate hinges and exposing the visceral mass. The testes lie at the stalk side of the body, and when the animal is in a breeding condition, they are white and swollen. The ovary is found by cutting the stalk lengthwise; young egg-masses are bright blue. In this condition, unfertilized eggs can be found; it has not been ascertained whether such eggs can be artificially inseminated. For cleavage stages, it is better to study the eggs which are obtained from the ovigerous lamellae.

If enough animals are available, a complete series, from egg to hatching larva, may be obtained at the same time. During development, there is a striking color change in the eggs of the lamellae: early cleavage stages are medium blue, later cleavage stages are light blue to blue-lavender. In pink-lavender lamellae, larvae can be seen inside the egg cases. Hatching stages are pink in color, while young to mature swimming nauplii accumulate in peach-colored masses. The chemistry of this color change has been studied by Ball (1944).

The lamellae lie as two sheets which are at first closely applied to the lower portion of the visceral mass and later are extended to cover the entire inside of the mantle cavity. When the lamellae are old enough to contain hatching stages, they are extruded from the body when the tentacles are molted. The bottom of the aquarium should therefore be inspected for these peach-colored sheets, which are about thumb-nail size.

The various stages may be isolated in separate fingerbowls. Bigelow (1902)

states that the early embryos will not contir ıe development outside the brood-chamber for a period of longer than five to ten hours. Late nauplii, however, will live for some time in fingerbowls of sea water.

C. Methods of Observation: To aid in the study of cleavage stages, it is helpful to stain with strong methyl green; the micromeres stain deeply, the macromeres, faintly. The stain, however, is transient.

NORMAL DEVELOPMENT:

A. The Unfertilized Ovum: The eggs are distorted while they are still in the ovary, but become spherical in shape after they are placed in sea water. According to Bigelow (1902) the first polar body is given off when the eggs leave the oviducts; whether or not fertilization occurs at this time, the vitelline membrane is elevated and lies, therefore, between the egg surface and the first polar body.

B. Fertilization and Cleavage: After fertilization, waves of slow contraction can be seen in the egg; Groom (1894) states that this process separates the protoplasmic portions of the egg from the yolk. The egg has a rounded point at the vegetal pole and is blunt at the animal pole. Cleavage is total, unequal, and regular. For a complete account of cleavage and of the cell lineage of this form, see the paper by Bigelow (1902).

Costello (1948) has pointed out that the cleavage of Lepas can be homologized with that of spirally cleaving eggs by considering it to be cleavage by "monets" rather than by quartets.

C. Later Stages of Development and Metamorphosis: Groom (1894) gives an account of later development (as well as maturation and early cleavage stages). The papers of Bigelow (1902) and Groom (1894) contain illustrations of stages from the unfertilized ovum to the early unhatched larva. Groom continues the series to the mature nauplius larva.

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ARTHROPODA

(CRUSTACEA)

Libinia emarginata and *L. dubia*

LIVING MATERIAL:

Libinia emarginata may be distinguished from the less commonly found *L. dubia* by the presence on its back of nine median spines, as contrasted with the six median spines characteristic of *L. dubia.* The animals occur on mud flats, and are abundant. The eggs are carried by the females on the legs; those egg-masses which are bright vermilion in color are best for study, since they contain the early stages. Later stages of development are chocolate-brown in color. Zoea and megalops larvae (of this and other crabs) are sometimes obtained by towing in the Hole at Woods Hole, Mass. Frequently, an electric light shining near the surface of the Eel Pond water (as, for example, the Nereis-collecting light commonly used) will attract vast numbers of larvae, which may then be dipped up.

BREEDING SEASON:

Bumpus (1898a) reported that females with eggs were collected at Woods Hole during the month of May and (1898b) that oviposition was observed as late as August 7. Thompson (1899) states that the larvae had disappeared from his collections by September 4.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The animals should be kept in large aquaria supplied with running sea water.

B. Methods of Observation: Forceps may be used to pick the eggs and larvae from the legs of the female. Cleavage and subsequent stages of development can be studied in the living egg only with considerable difficulty, and it is therefore advisable to fix the embryos. The following methods are useful:

1. Place the eggs in strong aceto-carmine (in a very little sea water). After about ten minutes, transfer them to 50% glycerine, which causes the stain to fade.
2. Early embryos are readily studied after the addition of 1% chromic acid to a drop of sea water in which the embryos are contained. After about five minutes, wash the embryos in water and mount them on glass slides under mica coverslips.
3. Drop the older stages into *strong* mercuric chloride solution; the embryonic area will whiten in a few minutes.

Use *reflected* light and a low power of the microscope for observation of all stages which have been fixed.

Study of the zoea stage in the living condition requires that the larvae be anaesthetized (with magnesium sulfate or other agents). With the use of the higher powers of the microscope, such details as the muscles, compound eyes, contractile heart and intestine can be observed.

NORMAL DEVELOPMENT:

A. Early Stages of Development: The early development of the centrolecithal egg of Libinia is essentially the same as that of the crayfish, Astacus. Since the paper by Reichenbach (1886) is in a journal which is not readily accessible, this account is based upon the summary given by MacBride (1914), and on the description by Brooks and Herrick (1892) of the cleavage of Alpheus and Stenopus.

The zygote nucleus occupies a central position in the fertilized egg and there divides. Protoplasmic division is said not to begin until after the fourth nuclear division, by which time the nuclei have migrated to the periphery. The daughter nuclei are at first internal but gradually migrate outward until they reach the surface. At this time the egg is imperfectly divided (by radiating planes of cytoplasm between masses of yolk granules) into a series of pillars, each of which contains one of the daughter nuclei. These are referred to as "columnar blastomeres." The yolk pyramids persist for only a short time; then the dividing planes disappear, and a flattened "skin" of cells remains, surrounding a large mass of yolk. This "skin" of cells is termed a "blastoderm." This stage corresponds to a blastula, the blastocoele being filled with an unsegmented mass of yolk.

Preceding the formation of the gastrula, there is an increase in the number of the blastoderm cells on one side of the egg; they are also thicker here and this becomes the ventral surface of the embryo. They press on one another laterally as they increase in number and become columnar in character, to form the ventral plate. This is on the future neural side of the embryo. Five circular areas develop in this ventral plate, in each of which the cells are arranged in concentric curves, and in lines radiating from a central point. These areas may be clearly distinguished in an embryo of this age after fixation with mercuric chloride, if it is examined by reflected light. The two anterior areas are the "cephalic lobes," or the rudiments of the paired eyes and cerebral ganglia. The thoracico-abdominal thickenings posterior to these constitute the next pair of rudiments. Just posterior to these, on the mid-line, is the central disc, or endodermic rudiment.

At the anterior margin of the endodermic rudiment, a groove develops. This is the beginning of the blastopore. The appearance of this groove (the endodermic groove) marks the beginning of the process of gastrulation. The endodermic groove later becomes a complete circle, as the periphery of the endodermal disc is invaginated, giving rise to the "endodermal button." As the button is carried in, a circular blastopore forms, later changing into an elliptical blastopore. The front border of the endodermic rudiment is the point of origin of the mesoderm. The endodermal tube becomes pinched off as a blind sac; much later, the proctodeum and stomodeum grow through to it. The proctodeum appears in between, and just posterior to the thoracico-abdominal rudiments, where the blastopore formerly was located. Other embryonic areas develop a short time after the first five. Three of these are the rudiments of the anterior paired appendages: first antenna (antennule), second antenna, and mandibles, and are characteristic of all crustacean larvae. When these rudiments appear, the stage is called a nauplius.

B. Rate of Development: About one month passes between spawning and the zoea stage.

C. Later Stages of Development: The nauplius stage has the first three pairs of appendages; later stages have five or more pairs of appendages, and the

stomodeum, ventral fold, dorsal shield, telson and ganglia may be found. It is necessary to supply the older larvae with Ulva and diatoms.

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