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

COELENTERATA *

(Hydrozoa)

Introduction

Living Material:

There is much fluctuation from year to year in the availability of material, certain species, only, being available some years. The species listed in this manual include forms showing the range from types such as Obelia, with perfect medusae, to types such as Hydractinia, in which the medusae are degraded to mere sporosacs. Unfortunately, species such as Gonionemus and Eutima, in which the hydroid generation is very inconspicuous and the medusa generation is the important phase of the life history, are not common at Woods Hole.

The "typical" hydrozoan exhibits an alternation of generations—the succession of two morphologically different adult types, each characterized by a different manner of reproduction. In the hydroid generation, the animal is usually attached and colonial; individual members of the colony may be alike, or the colony may exhibit polymorphism with some members being specialized for securing food and others for reproduction. Asexual reproduction, by budding, results in formation of the medusa; in general, a colony produces either male or female medusae. These individuals leave the colony and carry on a free-living existence; gonads develop on the radial canals or on the sides of the manubrium, and liberation of the gametes from these gonads results in sexual reproduction. The fertilized egg typically develops into a planula larva, which eventually becomes attached and develops a hydranth; lateral budding occurs, and in this manner a new colony is formed.

In general, the Hydrozoa are very sensitive to environmental conditions and do not survive well in the laboratory, even in aquaria containing running sea water. When they are removed to fingerbowls or other smaller vessels, the animals are likely to die very soon unless they are kept at a temperature below that of the room. In working with hydroids, it is advisable to use only a few stems in a large volume of sea water, and to change the sea water frequently. *Do not crowd* either the adults or the eggs and embryos.

Breeding Season:

The height of the hydroid breeding season for some species is during the spring, and warm weather seems to be detrimental to these colonies. A good many forms are available through the summer months for embryological work, however, and the individual breeding season for each species (as far as it has been ascertained) is indicated in the following accounts. Colonies which are excellent for work on regeneration problems are available in the fall.

* Much of the information describing the development of coelenterates was furnished by Dr. W. W. Ballard, to whom we are most grateful.

COELENTERATA

(Hydrozoa)

Bougainvillia superciliaris and *B. carolinensis*

(Perfect but relatively inconspicuous medusae)

Living Material:

The trophosome of *B. superciliaris* is about two inches tall, irregularly branched and light green in color. The pale rose hydranths have 15–20 tentacles in a single whorl about an inconspicuous hypostome. *B. carolinensis* is usually three to six inches tall; it is by far the commoner species at Woods Hole, Mass. The colony is light brown in color, with a greenish tinge; the red hydranths bear about 12 stiff, filiform tentacles on a long, flexible eversible proboscis. Both species are found attached to Fucus and floating timbers, and *B. carolinensis* has also been collected from the dock of the U. S. Fish Commission. See the paper by Nutting (1901) for details and diagrams.

Breeding Season:

June, July and August. The medusae are reported to be present in August; the hydroids are available all summer, although some years they are difficult to find in June.

Procuring and Handling Material:

A. Care of Adults: The hydroid colonies should be kept in large dishes, supplied with running sea water. They should not be crowded.

B. Methods of Observation: Medusa development can be studied by clipping off the gonophores and mounting them on slides under a coverslip. Withdrawing a little of the water from under the coverslip (by absorbing it with a piece of lens paper) will exert a slight pressure on the gonophores and will bring out the structural details of the buds. The mature medusa buds can be dissected from the gonophores for study.

Normal Development:

A. Asexual Reproduction: The gonophores are borne singly or in clusters on the main stem and branches; they have the appearance of stalked sacs, and a single medusa is produced within each. The buds are scattered irregularly throughout the colony and there is no orderly arrangement as to age. The medusae develop within the gonophores until all the organs except the gonads are fully formed; then they break away and take up a free-swimming existence. Details concerning formation of the medusae are to be found in the papers by Goette (1907) and Hyman (1940).

The medusae of both species are similar, being sub-globular in shape and possessing four radial canals and a velum. The tentacles are in four groups, each group having a conspicuous eyespot at its base. The brick-red manubrium of *B. carolinensis* is shorter and more slender than that of *B. superciliaris,* but both

species have four branched mouth tentacles. The free-swimming life lasts one or two months, during which time the gonads develop along the manubrium.

B. Sexual Reproduction: In *B. superciliaris* the fertilized eggs are retained in folds on the manubrium until the planula stage. A series of cleavages leads to the formation of a single-layered coeloblastula, which is transformed to a solid mass by a multipolar migration of cells. For details of this process, see the paper by Gerd (1892).

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COELENTERATA

(Hydrozoa)

Campanularia flexuosa

(Degenerate medusae)

Living Material:

This form is so similar to Obelia that one cannot differentiate between the two, using the anatomy of the feeding individuals as the sole criterion. Even the gonosomes are similar in the two genera, each consisting of a transparent gonotheca with the blastostyle extending from base to tip, and the gonophores budding from it. The gonophores of Campanularia are very degenerate, however, and their medusa-like structure can be distinguished only in sections. Because the gonophores are so inconspicuous and the embryos so obvious, the colonies which produce female gonophores and which later contain embryos are loosely spoken of as "female" colonies, although actually they are asexual.

The colonies are very abundant on floating sea-weed and timbers in shallow water.

Breeding Season: June and July.

Procuring and Handling Material:

A. Care of Adults: The colonies can be kept in large dishes or beakers supplied with sea water.

B. Methods of Observation: The eggs and planulae may be studied either by mounting a gonosome in sea water on a slide, or by dissecting the embryos from the gonophores with fine needles.

The mature planulae will metamorphose readily if two or three are placed in a covered Syracuse dish of sea water. Once the planulae attach, the water in the dish should be changed at least twice a day.

Normal Development:

A. Asexual Reproduction: The gonosomes of a female colony are larger than those of the male, and contain a series of embryos which are budded off from the blastostyle in a regular order, so that the older buds are closer to the mouth of the gonotheca. A single egg or embryo develops in each gonophore.

The smaller, oval, male gonosomes resemble those of the female, and bear rounded gonophores filled with milky-grey sperm which become active when they are discharged into sea water.

Details of gonophore development may be found in the paper of Goette (1907).

B. Sexual Reproduction: The maturation and development of the eggs have been studied by Hargitt (1913). There are no nurse cells present; a conspicuous release of chromidia from the nucleus occurs during the maturation process. The large, irregularly-shaped eggs (approximately 160 microns in diameter) are fertilized *in situ,* cleave, form a morula, gastrulate by delamination, and reach a free-

swimming planula stage within the gonophores. Mature planulae are two or three times longer than they are broad, and show maggot-like movements while they are still within the gonotheca.

C. Later Stages of Development and Metamorphosis: Four to ten hours after leaving the gonotheca, the mature planulae will attach. Each then opens a mouth, puts forth tentacles, secretes a hydrotheca and perisarc, and becomes a fully-formed individual polyp in two or three days.

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COELENTERATA

(HYDROZOA)

Clava leptostyla

(Degenerate medusae)

LIVING MATERIAL:

The colony consists of solitary brick-red hydroids branching from a filiform hydrorhiza. The polyps are about two cm. high, and have approximately 20 filiform tentacles arranged irregularly about a long hypostome. See the diagram by Hyman (1940).

The colonies are found in shallow water, attached to fronds of rock-weed (*Fucus nodosus*).

BREEDING SEASON: June and July.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The colonies should be kept in large dishes, supplied with sea water.

B. Methods of Observation: All stages of development up to the mature planulae can be teased from the female gonophores with fine needles. Larvae, released from ripe colonies, should be pipetted to fingerbowls of fresh sea water. They will then attach and metamorphose.

NORMAL DEVELOPMENT:

A. Asexual Reproduction: The gonophores develop in dense, bud-like clusters just below the tentacles. Those of the male colonies are pink, those of the female colonies purple. In both sexes they are very degenerate, retaining few traces of the medusa structure. Although there are only a few eggs present in each of the female gonophores, there is no evidence of a phagocytosis of nurse cells. Details of gonophore development are available in the papers by Harm (1902) and Kühn (1910).

B. Sexual Reproduction: The large eggs (131 microns in diameter, according to Hargitt, 1919) possess a delicate blue pigment, which appears during maturation and increases and spreads during the cleavage process. Fertilization is internal, and regular or irregular cleavage leads to the formation of a solid morula. The endoderm is formed by a secondary delamination within this mass (Harm, 1902). The oval embryo then elongates, becomes ciliated, and acquires the beginning of the coelenteron. At this stage, the planulae burst from the gonophores.

C. Later Stages of Development and Metamorphosis: The free-swimming planula creeps about for some time, exhibiting marked muscular contractions. Gland cells and nematocysts develop in the ectoderm, and the coelenteron increases in size. Eventually the larva becomes fixed at its broad anterior end, loses its cilia, and flattens out somewhat. It then elongates in the direction of the main

body axis, and the first tentacles appear at the free end; this produces a functional hydroid. See the paper by Harm (1902), for further details.

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COELENTERATA

(HYDROZOA)

Eudendrium ramosum

(Degenerate medusae)

LIVING MATERIAL:

The colonies are profusely branched, and between 10 and 15 cm. in height. The hydranths have a trumpet-shaped hypostome surrounded by a single row of about 20 filiform tentacles. They are very common on piles or docks in shallow water.

BREEDING SEASON: July and August.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: Colonies should be provided with adequate supplies of fresh sea water.

B. Methods of Observation: The release of planulae from ripe colonies occurs about mid-day, if the colonies are collected in the morning. Few larvae can be obtained from colonies collected late in the afternoon.

Eggs dissected from the gonophores will not cleave normally; if a study of normal development is desired, it is necessary to clip off not only the intact gonophore but also a considerable portion of the hydroid colony.

NORMAL DEVELOPMENT:

A. Asexual Reproduction: The gonosomes are rudimentary, sessile medusaforms or gonophores, borne at the bases of special hydranths which lose their tentacles and degenerate during the period in which the gonophores are ripening. The gonophores are strikingly different in the two sexes. The "female" colonies bear loose irregular tufts of sporosacs attached to the stems, each ripe sporosac being bright orange in color. "Male" colonies bear strings of light pink sporosacs (two to four or more per string), radiating like the spokes of a wheel from a common point on the base of the degenerate hydranth. Hyman (1940) shows illustrations of the gonophores; the paper of Goette (1907) may be consulted for the details of gonophore development.

B. Sexual Reproduction: The eggs are fertilized within the gonophores; they are large (230 microns in diameter) and very opaque, due to the presence of a large supply of orange-colored yolk. The details of cleavage can be ascertained only by histological study, but it has been shown that cleavage is very similar to that of insects and crustaceans, involving the formation of yolk-pyramids and an early period during which nuclear, but not cytoplasmic, divisions occur. Following a migration of nuclei to the surface of the syncytial mass, an outer layer of ectoderm is cut off. The inner mass remains syncytial for a considerable period of time and the endoderm is not fully differentiated until the time of metamorphosis.

C. Later Stages of Development and Metamorphosis: When first liberated, the ciliated planulae are elongated and pyriform; however, they become slender after a few hours of free-swimming existence. They show a marked positive phototropism when they are first released, but this later declines. Fixation normally occurs after two or three days; the broader aboral pole is attached to the surface and fixed there by a slimy secretion. Metamorphosis is completed in 12 to 24 hours.

For details of cleavage and planula formation, see the paper by Hargitt (1904); metamorphosis is described by Hargitt (1904), and by Allman (1871).

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COELENTERATA

(Hydrozoa)

Eutima mira

(Conspicuous medusa generation)

Living Material:

The medusae were formerly abundant at Woods Hole, Mass., in August, but at the present time they are relatively rare. Adult medusae are low and bell-shaped, with four long, tapering marginal tentacles. Three rudimentary tentacles are also present in each quadrant. The four-lobed mouth is located at the apex of a long manubrium which is borne on a gelatinous peduncle. The manubrium is two or three times the height of the bell. The gonads are located along the mid-section of the four radial canals. (See the paper of Hargitt, 1904, for a diagram.)

Breeding Season:

This species breeds during the summer months at Beaufort, N. C., but the breeding season at Woods Hole has not been determined.

Procuring and Handling Material:

A. Care of Adults: An adequate supply of fresh sea water is important.

B. Methods of Observation: Fertilized eggs can be obtained by placing several ripe animals in a small aquarium or battery jar of sea water. Shedding usually occurs between 7:30 and 8:30 P.M. on the night when the animals are collected.

Normal Development:

A. The Unfertilized Ovum: The eggs are said to be very clear and transparent, so that it is possible to observe details of development quite completely.

B. Cleavage and Gastrulation: Fertilization is apparently external. Segmentation is total but not entirely regular, leading to the formation of a hollow, ciliated, one-layered blastula. The endoderm layer first appears at the posterior pole, but it is not clear as to whether this is a process of delamination or of cellular migration.

C. Time Table of Development: Details of the developmental time sequence are not available, although development is rapid. Eggs shed at 8 P.M. develop into rapidly swimming planulae by the next morning.

D. Later Stages of Development and Metamorphosis: About 12 hours after fertilization, a ciliated, elongate planula is formed. The cleavage cavity is reduced in the larva, but is still visible between the endoderm cells. The anterior end of the planula is the broader, and on the opposite end an invagination soon appears. This invagination is gradually pushed toward one side of the larva (termed the "ventral" side) by the enlargement of one of the lips of the invagination. At the time of attachment, the invaginated "ventral" sac is everted and the glandular cells which cover it secrete an adhesive cement. The free end of the attached

"root" then increases in length and the first hydranth develops at its apex. Subsequent hydranths are produced in a regular order behind this first individual.

The hydroid colonies are inconspicuous. The hydroids have a prominent round manubrium surrounded by a single circle of ten tentacles. The elongate, cylindrical hydranth body is not covered by the perisarc, and the stem lacks annulations.

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COELENTERATA

(Hydrozoa)

Gonionemus murbachii

(Conspicuous medusa generation)

Living Material:

Gonionemus was formerly very abundant in the Eel Pond at Woods Hole, Mass., but has now practically disappeared from the vicinity; this seems to be associated with the disappearance of eel grass.

The adults measure about 20 mm. in diameter and are about 10 mm. high; they possess four radial canals on which are located the yellow, ribbon-like gonads. The marginal tentacles vary in number from 16 to 80 and have characteristic adhesive pads near their tips. The sex can be ascertained by examining the animals with a dissecting microscope; the ovaries have a granular appearance, while the testes are homogeneous and translucent.

Breeding Season:

July to the last week of September; the height of the season is from mid-July to mid-August.

Procuring and Handling Material:

A. Care of Adults: The adults are hardy and will survive in aquaria for several months. Neither larvae nor adults need running sea water, but they seem to survive best in balanced aquaria where they have a constant supply of diatoms for food.

B. Methods of Observation: Adults gathered in the morning will normally shed their eggs between 6 and 8 P.M. on the day of collection. They can be induced to shed in the morning by keeping them in artificial light during the night to prevent shedding, and then placing them in the dark for an hour. If eggs are desired in the afternoon, the adults should be gathered in the morning and then placed in the dark for an hour, beginning at about 3 P.M. A single animal kept in the laboratory will produce eggs nightly for as long as a week, although the number of eggs obtained decreases after the third day.

Since the fertilized eggs adhere to the surfaces with which they come into contact, it is suggested that the dishes in which shedding animals are placed contain microscope slides. These slides, with the attached eggs and larvae, are then easily manipulated for study.

Normal Development:

A. The Unfertilized Ovum: The eggs are yellowish in color and measure about 70 microns in diameter. When shed, they are covered with a sticky jelly membrane; after fertilization they rapidly sink to the bottom and adhere to the container. Maturation apparently takes place before shedding, since polar bodies are not found on shed eggs.

B. Cleavage and Gastrulation: Cleavage is total and equal. At the 8-cell stage, there is a rotation of some of the blastomeres to form a flat plate of cells. Continued cleavage gives rise to a hollow blastula consisting of a single layer of cells. These cells develop cilia and, while the blastula is still rotating within its membrane, the endoderm is formed by multipolar delamination. With the formation of this inner layer the blastocoele is eliminated.

C. Time Table of Development: Perkins (1902) gives the following schedule. The times are given in minutes after fertilization; no temperature is specified.

Stage	Time
First cleavage	60 minutes
Second cleavage	110 minutes
Third cleavage	160 minutes
Planula	12 hours
Attachment	2 weeks
First tentacles on hydroid	3 weeks

D. Later Stages of Development and Metamorphosis: After leaving the membranes, the larvae elongate and the anterior end (aboral pole) broadens. At this time the planulae are bottom-swimmers. The coelenteron begins to form in the posterior portion of the larva by a rearrangement of the endoderm cells, shortly before metamorphosis. At the same time the cell boundaries of the posterior (oral) pole disappear and this region becomes syncytial. At metamorphosis the larva stops swimming, loses its cilia, and attaches by the broad anterior end. A mouth forms at the free end, and is surrounded at first by two tentacles and later by four. The polyps remain solitary but may give off non-ciliated planula-like buds (frustules) which creep along the bottom and develop into hydroids.

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COELENTERATA

(HYDROZOA)

Gonothyrea loveni

(Imperfect medusae)

LIVING MATERIAL:

The colony consists of an erect, irregularly branching stem about one-half to three-fourths of an inch high. The hydrothecae are bell-shaped and have a toothed margin. The hydroids possess a single whorl of filiform tentacles. Gonothyrea is not common at Woods Hole, Mass.

BREEDING SEASON: July and August.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: The colonies should be provided with an adequate supply of fresh sea water.

B. Methods of Observation: The eggs and larvae are difficult to dissect from the gonophores, and can best be studied by mounting whole gonophores on slides. Free-swimming planulae will readily attach and metamorphose in the laboratory if they are placed in covered Syracuse dishes of sea water.

NORMAL DEVELOPMENT:

A. Asexual Reproduction: The gonangia, which are borne in the axils of the branches, consist of a central blastostyle surrounded by a long, oval gonotheca. The gonophores, which are actually degenerate medusae, do not break away from the blastostyle but remain attached to it by means of short stalks, projecting outside the gonotheca in groups of three or four. In the development of the gonophores of both sexes, there are traces of radial and ring canals, and stubby tentacles are present on the distal ends of the sporosacs.

B. Sexual Reproduction: The eggs, of which there may be one or more present in each gonophore, are comparatively small (102 microns, according to Hargitt, 1919). They are fertilized *in situ* before maturation by sperm which are released by the male gonophores. Cleavage may be either regular or irregular. If it is regular, a coeloblastula is formed and the endoderm is formed by multipolar migration; if it is irregular, a solid morula is formed and both ectoderm and endoderm differentiate directly from this. In either type of development the resulting planula is a solid, oval mass of cells. This soon elongates, becomes ciliated, and develops traces of a coelenteron. At this time it escapes from the gonophore.

C. Later Stages of Development and Metamorphosis: The planula, which shows a well-developed coelenteron, swims about for 6–12 hours and then attaches by its broader, anterior pole. At the time of attachment, it loses its cilia and flattens out. The widened base soon begins to secrete a perisarc. Following these events, there is an elongation perpendicular to the attachment, and the development of a mouth and tentacles at the free end. Details of gonophore formation are available

in the paper by Goette (1907) and the entire development, through metamorphosis, is described by Wulfert (1902).

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COELENTERATA

(Hydrozoa)

Hydractinia echinata

(Degenerate medusae)

Living Material:

Colonies of this form are fairly common on the Littorina shells inhabited by the small hermit crab, Pagurus. There are three types of individuals in the fully-developed colony: (1) ordinary polyps (feeders), with a single whorl of tentacles; (2) thread-like coiling forms with no mouth and an apical knob of nematocysts (stingers, commonest around the lip of the shell); and (3) gonosomes. All three types arise singly from a hydrorhiza network covered by a rust-red spine-studded crust (Nutting, 1901).

The snails on which the colonies grow are common in the littoral near Woods Hole, Mass., and can be gathered in considerable numbers at Sheep Pen Harbor and Tarpaulin Cove.

Breeding Season: June, July and August.

Procuring and Handling Material:

A. Care of Adults: Colonies may be kept in large beakers or other deep vessels, supplied with running sea water.

B. Methods of Observation: If a number of snail-shells bearing "male" and "female" colonies are placed in a large uncovered dish of sea water and left overnight, eggs will usually be shed and fertilized between 7 and 9 A.M. on the following morning. Colonies kept in running sea water have been known to shed daily for a week before becoming exhausted. The shedding can be controlled by light, however, if eggs are desired at some other time of day. Colonies should be kept in running sea water, under a glowing 100-watt bulb, from the time of collection until gametes are needed. They should then be placed in the dark for one or more hours and subsequently re-exposed to light. By the use of a hand lens or dissecting microscope, the sexes can be segregated to separate fingerbowls of fresh sea water. The males will shed 50 minutes after re-illumination, the females five minutes later. The eggs should be transferred to fingerbowls of fresh sea water and inseminated with one or two drops of water taken from a dish of shedding males. Ballard (1942) gives further details of this method for controlling shedding.

Normal Development:

A. Asexual Reproduction: The gonosomes, or reproductive individuals, are usually without tentacles and have a large knob of nematocysts on the proboscis; each bears a number of gonophores, which are medusa-buds reduced to the status of sporosacs. Ripe "male" and "female" colonies can be distinguished from one another with the unaided eye, since the eggs within the sporosacs are dull green

against the red hydrorhiza, and the sperm, when mature, are a white mass. For details of gonophore development, see the papers by Goette (1907, 1916).

B. Sexual Reproduction: The maturation of the eggs within the gonophores occurs as a direct response to light and can be seen in eggs dissected from colonies placed in the light after several hours of darkness. In such eggs, the large germinal vesicle begins to break down soon after the exposure to light. The first polar body is given off 45 minutes after exposure to light, the second polar body ten minutes later. Occasionally the first polar body may divide. The eggs are shed immediately after the second maturation division (Ballard, 1942).

The eggs are yolky and usually green; occasionally grey, orange or pink ova are shed. Teissier and Teissier (1927) give the average egg-diameter as between 160 and 170 microns. When shed, the eggs are covered by a highly transparent, radially striated jelly, which swells on exposure to sea water. The swelling of this layer causes the polar bodies to be lifted from the egg surface and they are soon lost. Cleavage may be irregular, but usually the somewhat amoeboid egg undergoes three equal, total cleavages, each of which is at right angles to the preceding one. The separating pairs of blastomeres tend to retain broad protoplasmic connections with one another on the side opposite the cleavage furrow, until just before the succeeding cleavages begin. There is much variation in the time and degree of shifting of positions of the blastomeres, but the bizarre cleavage patterns often seen in the laboratory are commonly the result of evaporation of the sea water, or other unfavorable factors.

Mitotic synchronism quickly disappears. Gastrulation is said to start as early as the 16-cell stage, by mixed delamination and multipolar proliferation. The gastrula loses its spherical form and remains for a few hours an irregular mass; then it returns to the spherical form and gradually lengthens into the planula form. For illustrations of the cleavage pattern, see the papers by Beckwith (1914) and Bunting (1894).

C. Later Stages of Development and Metamorphosis: At the end of 24 hours the embryo is a "preplanula" (Teissier and Teissier, 1927) with an elongated oval form, recognizable polarity and ciliation. During the course of a few days, it lengthens, one end becoming progressively slimmer, while it rolls and crawls along the bottom like a planarian. The large end (which goes first in this movement) is the end which later produces the adhesive disc by which the larva attaches for metamorphosis; it becomes the aboral end of the polyp.

Following attachment of the attenuated planula, there is a delay of a few hours to several days, and then the tapering free end shrinks down almost to the substrate, where it produces a mouth and a succession of tentacles. The new polyp elongates, its attached end meanwhile actively sending out a number of anastomosing and encrusting hydrorhiza processes from which branch new polyps. For further details of planula development and metamorphosis, see the paper by Teissier and Teissier (1927).

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COELENTERATA

(Hydrozoa)

Obelia commissuralis and *O. geniculata*

(Perfect but relatively inconspicuous medusa generation)

Living Material:

The colonies of *Obelia commissuralis* are tree-like in form, and reach a height of 6 to 8 inches. The annulated, rather sparse side branches are given off at right angles from a long central trunk. Colonies of *O. geniculata* are not more than 30 mm. high and usually consist of a single stem bearing alternate hydranths on broad processes. Hydroids of both species have cup-like hydrothecae and a single row of filiform tentacles surrounding the hypostome.

The hydroid colonies are commonly found attached to docks, sea-weed or floating timbers at Woods Hole, Mass. The medusae are often caught in tow nets during the summer.

Breeding Season: June, July and August.

Procuring and Handling Material:

A. Care of Adults: Colonies are easily maintained in the laboratory, if they are provided with an adequate supply of fresh sea water.

B. Methods of Observation: To obtain medusae, ripe hydroid colonies should be placed, without crowding, in large fingerbowls of sea water. Within an hour or two, swimming medusae are usually released; these can be removed to depression slides for study. Young stages of medusa formation must be teased from the gonosomes with needles; older gonophores will usually be released if a well-matured gonosome is mounted on a slide under a coverslip and pressure applied to the coverslip with needles.

To obtain fertilized eggs, several mature medusae should be placed together in a jar of sea water. Although it has not been demonstrated for the Woods Hole form, Merejkowsky (1883) states that the Mediterranean species of Obelia always sheds in the early morning hours.

Normal Development:

A. Asexual Reproduction: The gonosomes of both species are borne in the axils of the branches, and are several times as large as the hydranths. Each consists of a vase-like gonotheca surrounding a central blastostyle whose expanded tip forms a loose plug for the mature gonotheca. The gonophores which mature as medusae develop as buds along the blastostyle, the most mature buds being found toward the neck of the gonotheca. When fully mature, they break loose and escape past the blastostyle plug to the outside. Details and diagrams of the development of the medusae can be found in the paper of Goette (1907).

B. Sexual Reproduction: When first shed, the medusae of *O. geniculata* have 24 tentacles, while those of *O. commissuralis* have only 16. Both species have

four radial canals and a rather short manubrium. The velum is reduced to a narrow and somewhat lobed membrane near the bases of the tentacles. This reduction of the velum makes possible an eversion of the bell when the medusa comes to rest, so that the manubrium protrudes from the center of the convex surface. The gonads are not visible when the medusae are shed, but slowly mature during the two months of free-swimming existence. When mature, they appear as oval structures hanging from the mid-part of the radial canals. Diagrams of the medusae of the two species are presented by Nutting (1901).

Merejkowsky (1883) gives the details of sexual reproduction in an undesignated species of the genus Obelia. The eggs (about 130 microns in diameter, according to Hargitt, 1919) are fertilized externally after the gametes have been shed. No fertilization membrane is produced. A single polar body is given off after the eggs are shed. Regular and total cleavage leads to the formation of a one-layered coeloblastula, which soon becomes ciliated and motile. A few hours after the blastula is formed, the endoderm develops by an inward migration of cells from the posterior pole of the larva, which is now elongated. Continued multiplication of the endoderm cells leads to the formation of a typical solid, ciliated planula, with a broad anterior end and a narrow, pointed posterior end. This planula remains as a surface-swimmer for about a day, gradually developing nematocysts and epithelio-muscular cells. It then drops to the bottom, loses its cilia, and becomes affixed by the broader anterior end. Tentacles and mouth develop at the free end of the attached larva, and eventually asexual budding leads to the formation of a new hydroid colony.

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COELENTERATA

(Hydrozoa)

Pennaria tiarella

(Imperfect medusae)

Living Material:

The colonies vary from two to six inches in height, and grow in a branched, fan-like pattern. The terminal hydranths are large, with vermilion bodies and white tentacles. The hypostome is long and is covered with stubby, knobbed tentacles. In addition, there is a basal ring of about 12 long, slender tentacles. Diagrams are given by Hyman (1940) and Nutting (1901). The adult colonies are very common at Woods Hole, Mass., and can be collected from Fucus or other sea-weeds, or from pilings three feet below sea level. They are abundant on the vegetation of the Spindle.

Breeding Season: Mid-July to September.

Procuring and Handling Material:

A. Care of Adults: The animals are extremely sensitive and should never be crowded. Place a few colonies bearing the best embryological material (large eggs and full spermaries) in large fingerbowls on a water table, allowing a gentle stream of sea water to flow through the dishes.

B. Methods of Observation: About 3 P.M. on the second afternoon after collection, cut a few small stems from ripe "male" and "female" colonies, carefully rinse them in sea water, and place them together in a fingerbowl containing filtered sea water. Cover the dish and return it to the water table. Examine the dish during the evening for evidence of shedding, and pipette the fertilized eggs to a fingerbowl of fresh sea water as soon as possible after they are shed. If unfertilized eggs are desired, the male and female colonies can be isolated in separate fingerbowls. Eggs procured in this manner can be artificially inseminated with a few drops of sperm solution taken from a dish containing a shedding male colony.

It is possible to modify the time of shedding and to procure gametes at any time of day which is convenient (Baker, 1936; Ballard, 1942). If colonies are placed in the dark for 24 hours, as soon as they are brought into the laboratory, and then exposed to continuous light (either artificial or natural), shedding can be expected 10–14 hours after the return to light. Since the animals do not live long in the laboratory, this procedure can be used only once.

The free-swimming larvae should be transferred to Syracuse dishes of sea water and kept in a moist-chamber on a sea water table until attachment. After attachment has occurred, the dishes can be stacked in an inverted position, in wooden racks placed in aquaria of running sea water.

Normal Development:

A. Asexual Reproduction: The gonophores bud off singly from the hydranth body just above the proximal tentacles; there may be one to three per gonosome.

A single colony bears gonophores of one sex only, but in living individuals sex is difficult to ascertain until the gonophores are mature; the eggs will be pink and the sperm white (Smallwood, 1899). "Male" and "female" colonies are actually asexual, bearing male and female gonophores, respectively. For illustrations and details of gonophore development, see the paper of Goette (1907).

The mature medusae are similar in the two sexes; they have an elongate bell, a velum, four radial canals and four rudimentary tentacles. The vermilion manubrium (spadix), to which the gametes are attached, is short and there is no mouth. In southern waters, Pennaria medusae generally break away from the colony and swim about during the discharge of the sex products; in Woods Hole, however, they usually remain attached, and the eggs may not be ejected until long after fertilization.

At the onset of spawning, the ripe medusae gradually begin a rhythmic twitching. The males emit puffs of white sperm, the females eject three to six eggs. The spent medusae finally drop off, swim feebly if at all, and shrivel and die in a few hours.

B. Sexual Reproduction: The newly shed eggs are opaque and usually pale pink in color, although this can vary from a cream-white to orange. The irregular shape makes accurate measurements difficult; C. W. Hargitt (1900) gives 400–500 microns as the diameter, while G. T. Hargitt (1919) states that the average diameter is only 237 microns. The large size of at least some of the ova is probably associated with the fact that certain of the maturing oöcytes absorb other oöcytes during development. The polar bodies are given off and lost before the eggs are shed. Fertilization is external and is accompanied by amoeboid movements of the egg; no fertilization membrane is formed.

The cleavages, which begin about 30 minutes after insemination, are rapid. Although at times they may be quite regular until the 8-cell stage, they become chaotic and without pattern after this stage. Nuclear division with delayed cytoplasmic division is quite common. The embryo has a flat, disc-like appearance during the later cleavages, but rounds up to form a solid spherical morula. This differentiates an ectodermal layer, which becomes ciliated. In 12–24 hours, the pyriform embryo becomes a young free-swimming planula. See the papers of C. W. Hargitt (1900) and G. T. Hargitt (1909) for details of early development.

C. Later Stages of Development and Metamorphosis: The early, free-living planula is a solid, ciliated organism, but eventually the endoderm differentiates from the central mass and the beginnings of a coelenteron become visible. In about five days the larva attaches and begins to secrete a delicate perisarc. Two days later, the rudiments of the proximal tentacles of the first hydranth are visible about the mouth, which develops at the free end of the metamorphosing larvae. Hargitt (1900) presents diagrams and describes metamorphosis in detail.

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COELENTERATA

(Hydrozoa)

Podocoryne carnea

(Perfect but relatively inconspicuous medusa generation)

Living Material:

This genus is very similar to Hydractinia. The colony is composed of an encrusting mat of stolons covered with a perisarc which is beset with jagged spines. From these hydrorhizae arise the feeding polyps, each having a single whorl of tentacles around a conical proboscis; the slender protective hydranths lack tentacles but are armed with batteries of nematocysts. The reproductive hydranths bear clusters of gonophores just below the tentacles. For a further description, see the paper of Hargitt (1901).

The species is rare at Woods Hole, Mass.; it is occasionally collected with colonies of Hydractinia from Sheep Pen Harbor. Like the latter species, it is found as an encrusting mat on snail shells, but the ratio of Hydractinia to Podocoryne colonies is about 100 to 1.

Breeding Season: June and July.

Procuring and Handling Material:

A. Care of Adults: The colonies should be maintained in adequate supplies of sea water.

B. Methods of Observation: If ripe colonies are isolated in fingerbowls of sea water, the medusae are often discharged. Stages of medusa development can be obtained by dissection from the gonophores.

Normal Development:

A. Asexual Reproduction: A single medusa develops within each gonophore, and only male or female medusae are produced by any one colony. The details of medusa development are described by Goette (1907). When they are fully formed, the nearly perfect medusae escape into the water. At the time of release, they have a marked bell-shape, a definite velum, and a short, reduced manubrium. At the end of the four radial canals can be seen four marginal tentacles, and between these there are four interradial tentacles. Although some strains of this species have mature gonads at the time when they are set free, the strain available at Woods Hole has only very immature gonads visible along the radial canals. Goette (1916) states that the medusae may give rise to several additional generations of medusae by budding before they produce gametes. Diagrams of the medusae are available in the paper by Hargitt (1901).

B. Sexual Reproduction: This phase of the life history has been studied by de Varenne (1882), in one of the strains having a free-swimming life of only a few hours. Fertilization is external and occurs before the breakdown of the germinal vesicle. The egg is said to exhibit marked amoeboid movements. Total

cleavages lead to the formation of a solid, oval morula; an internal cavity soon appears and the elongating, ciliated larva quickly develops into a free-swimming planula. After a few hours the planula loses its cilia, fixes by its anterior end, and secretes a perisarc. The free end develops the mouth and tentacles of a typical hydroid.

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COELENTERATA

(HYDROZOA)

Tubularia crocea

(Imperfect medusae)

LIVING MATERIAL:

The colonies form dense tufts of long, tangled, sparsely branched stems, three to four inches high. The hydranths have two rows of short tentacles: a row about the mouth and a proximal row of 16–25 shorter tentacles. See the diagram by Hyman (1940). The colonies grow profusely on piles and docks in the Woods Hole, Mass., area.

BREEDING SEASON: June and July.

PROCURING AND HANDLING MATERIAL:

A. Care of Adults: Select a good-sized colony, examine it with a hand lens or dissecting microscope, and clip off those branches containing the best embryological material. The hydroids should not be crowded, and the sea water should be changed frequently. They are very sensitive to increases in temperature.

B. Methods of Observation: Motile sperm can be obtained by crushing the gonophores on a slide; eggs and larvae can be teased from the female gonophores with fine needles. Older larvae, close to the time of hatching, are visible *in situ.* Actinulae escaping from the parent colony will not develop without special feeding. If mature colonies are allowed to stand for several hours in a large dish, the actinulae will be shed first, followed by earlier and earlier developmental stages, until finally cleavage stages are obtained.

NORMAL DEVELOPMENT:

A. Asexual Reproduction: The gonosomes of a mature specimen are long, branched stalks which grow in a dense cluster between the two circles of tentacles. The gonophores are budded off along the length of the gonosomes; they are quite markedly reduced medusae which never become free-swimming, and usually show no signs of radial or circular canals. The male and female gonophores are produced by separate colonies. The male gonophores are balloon-like structures lacking tentacles; within them, a cloudy mass of sperm can be seen surrounding the dark red spadix, which is actually the manubrium of the medusoid. The female gonophores, containing the eggs and developing larvae, usually have four blunt, knob-like tentacles at the distal end, although occasionally one or more of these is slightly elongated. Details of gonophore development can be found in the paper by Goette (1907).

B. Sexual Reproduction: Although many oöcytes are present in the young gonophores, only a few reach maturity. These favored oöcytes engulf and absorb the "nurse" eggs, which are arrested in the primary oöcyte stage. The sperm probably enters the egg before the polar bodies are given off, although there is

some controversy on this point. When ripe, the egg is very large (approximately 400 microns) and somewhat irregular in shape (Allen, 1900; Hargitt, 1909, 1919). Fertilization and early development take place within the modified medusa. Cleavage is often irregular and apparently either a coeloblastula or a solid morula can be formed (Lowe, 1926; Hargitt, 1909). Gastrulation of the coeloblastula is described as a mixture of delamination and multipolar proliferation (Benoit, 1925; Hargitt, 1909). The embryo is thus converted into an oval, solid mass of cells which eventually flattens to a disc. Irregular coalescing spaces appear in the endoderm, marking the beginning of the adult coelenteron. Blunt protuberances at the edge of the disc are rudiments of the aboral tentacles of the adult; these rapidly elongate and bend toward the future aboral end of the body.

C. Later Stages of Development and Metamorphosis: The entire embryo elongates in the direction of the oral-aboral axis and becomes cylindrical. The oral end is perforated by the mouth and a series of small protuberances, the oral tentacles, develop about this opening. When this stage is reached, the "actinula," which may be considered a precociously metamorphosing form, part planula and part polyp, leaves the gonophore. It then creeps about on the substrate by means of the aboral tentacles; although the mouth is carried downward at this time, the attachment is made by the aboral pole. The attached polyp increases rapidly in height, and buds off daughter hydroids along its sides. The creeping stolons, which bud off secondary upright shoots, are developed from the base of the primary polyp. Details of actinula formation are described by MacBride (1914).

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COELENTERATA

(Hydrozoa)

Turritopsis nutricula

(Conspicuous medusa generation)

Living Material:

This is not a common Woods Hole form. The adult medusae are square in shape and have a large manubrium which nearly fills the upper part of the subumbrella cavity. Although the young medusae have only 8 tentacles, the adults have over 100. The large, oval, red-orange reproductive organs surround the upper portion of the manubrium and are found along the four radial canals. The branching hydroid colonies are 8 to 12 mm. high and bear yellowish-red hydranths. The medusa buds are found on the stem at the bases of the hydranths.

Breeding Season:

This has not been ascertained for the Woods Hole region. At Beaufort, North Carolina, the animals breed during the summer months.

Procuring and Handling Material:

A. Care of Adults: The adults are relatively hardy and live well in aquaria, although they are very voracious.

B. Obtaining Gametes: The eggs are shed by dehiscence from the gonads about 5 or 6 A.M.

Normal Development:

A. The Unfertilized Ovum: Approximately 20–35 eggs are shed at one time; they are spherical and measure 116 microns in diameter. The inner, dense, yellowish yolk-mass is surrounded by an outer clear ectoplasmic layer, but there is no visible fertilization membrane. Two polar bodies are produced shortly after shedding, but these soon disintegrate and are lost.

B. Cleavage and Gastrulation: Cleavage is total and approximately equal until the 8-cell stage, after which time it becomes very irregular. As in the case of Gonionemus, there is a rotation of cells at the 8-cell stage, so that a flat plate is formed. A solid, syncytial morula, with no trace of a cleavage cavity, develops in six to eight hours. Both the outer ectoderm and the inner endoderm develop from this syncytium.

C. Time Table of Development: The following time table is taken from the paper of Brooks and Rittenhouse (1907). No indication as to the temperature is given.

Stage	Time
Polar bodies	A few minutes after shedding
First cleavage	25–30 minutes after polar bodies
Second cleavage	50–60 minutes after the first cleavage

Stage	Time
Third cleavage	75–85 minutes after the second cleavage
Oval, morula-like embryo	6–8 hours
Free-swimming planula	11 hours
Top-swimming, contractile planula	24 hours
Attachment	48–60 hours
First hydroid well formed	72 hours

D. Later Stages of Development and Metamorphosis: The young, oval, ciliated embryo changes by the eleventh hour into a solid, bottom-swimming planula, which is elongated and has a broad anterior end. The planula continues to elongate, and after a day has the ability to contract. At this time, it becomes a top-swimmer. Forty to sixty hours after shedding, a cavity becomes visible in the endoderm of the planula, starting at the anterior end and extending posteriorly. Soon after this time, the larva again sinks to the bottom, and after a short interval, during which it glides along the bottom, the cilia are lost and the larva attaches by its side to the substrate. The root-like planula increases in size and a bud develops from the mid-region of its upper surface. Twenty-four hours after attachment, this bud becomes a young hydroid with three whorls of tentacles. The primary bud gives rise to a branching colony (much like that of Tubularia in general appearance) by asexual budding.

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COELENTERATA

(Scyphozoa)

Aurelia aurita (*A. flavidula*)

Living Material:

The number of animals available at Woods Hole, Mass., varies from year to year; at times they are abundant, at other times they are very scarce. Mature females are recognizable by the presence of pale-pink or purplish gonads; the gonads of the males are milky-white. The animals are usually abundant in the spring, but disappear in July.

Breeding Season:

This is reported to extend from mid- to late summer.

Procuring and Handling Material:

A. Care of Adults: Aurelia is very difficult to maintain alive in the laboratory for any period of time; large aquaria, supplied with adequate amounts of running sea water, are probably desirable.

B. Methods of Observation: Eggs and developing planulae can be dissected from the brood-pouches located on the inner surfaces of the oral arms; they should be mounted in a drop of sea water for microscopic observation.

Active planulae, obtained in the above manner, will readily attach and metamorphose as far as the scyphula stage, if they are placed in clean watch glasses of sea water. Gilchrist (1937) gives directions for culturing the scyphistoma stage.

Normal Development:

A. Early Stages of Development: The pinkish, transparent eggs measure about 120 microns in diameter. Fertilization occurs in the gastric pouches, and the eggs are transferred to small brood-pouches on the inner surfaces of the oral arms. Cleavage is total and quite regular, forming a hollow, single-layered blastula. Gastrulation is by invagination, occasionally accompanied by an in-wandering of cells. At the time of completion of the gastrulation process, the blastopore, which becomes the mouth of the scyphistoma, is almost completely closed. The spherical embryo develops cilia and elongates, taking on an egg-shape; the blastopore is located at the more pointed end. At this stage, the larva leaves the brood-pouch and takes up a free-swimming existence. For details of the early stages of development, see the papers by Hargitt and Hargitt (1910) and Smith (1891).

B. Later Stages of Development: In about two days the planula attaches by the aboral end and gradually flattens to a cup-shape, losing its ciliation; it is now a young scyphistoma. The mouth and a short hypostome soon become visible. Four primary tentacles arise in a circle about the gaping mouth, and, alternating with these, four endodermal ridges (the taeniolae) project into the coelenteron. The tentacle number is increased to 24 in about a month. Lateral buds can be produced, which either become free or form stolons from which new scyphistomae

arise. In the older scyphistomae, the gastric pouches can be seen to communicate by means of holes in the taeniolae, forming the ring sinus just below the oral disc. Sense organs are visible at the bases of each of the eight primary tentacles, appearing first as wart-like buds. Eight lobes from the ring sinus grow out as lappets towards the sense organs, each lappet eventually enclosing a sense organ in its forked tip. When this stage is reached, strobilization occurs. For details of scyphistoma formation, see the paper by Hein (1900).

C. Strobilization and the Ephyrula: The scyphistoma stage of Aurelia is long and strobilization does not usually occur until about April. At this time, the primary tentacles are lost and the tubular body is divided into a series of plate-like discs. Each of these discs becomes free as a small ephyrula, or young jelly fish. The ephyrula bears eight forked lappets, each of which is tipped with a sensory tentacle. The four gastric pouches, lined with gastric filaments which develop from the free edges of the original taeniolae, are clearly visible. Further growth over a period of four months leads to the formation of an adult jelly fish. Details of ephyrula formation and strobilization can be found in the papers by Friedemann (1902) and Percival (1923); the entire development of Aurelia is reviewed by MacBride (1914).

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COELENTERATA

(Scyphozoa)

Cyanea capillata (*C. arctica*)

Living Material:

The abundance of these animals varies from year to year; adults have been captured at all times of the year, but are not found near the surface during stormy weather.

Breeding Season:

Usually in March and early May, but some animals in the breeding condition can be taken as late as July.

Procuring and Handling Material:

A. Care of Adults: No information is available.

B. Obtaining Embryos: Mature animals can be recognized by the white or cream-colored gonads lining the gastric pockets. Eggs and developing larvae are found in the brood-pouches along the oral lobes; they appear as greyish specks to the unaided eye, and can be dissected out into a drop of sea water on a slide, for examination. The early cleavage stages and blastulae are found in the region of the mouth.

C. Methods of Observation: If active planulae are placed in clean Syracuse dishes of sea water, they will attach and metamorphose. If the watch glasses with the attached scyphistomae are removed to aquaria and the larvae fed echinoderm larvae, copepods, etc., they will live for several months.

Normal Development:

A. Early Stages of Development: Maturation probably occurs in the gonad. The mature eggs, each with the second polar body clinging to the delicate egg membrane, then dehisce into the gastric pouches where fertilization occurs; the eggs lodge in folds of the oral lips, where they continue to develop until the planula stage. Cleavage is total, and may or may not be equal; often it is slightly irregular. A cleavage cavity appears early and a hollow, single-layered blastula is formed. Gastrulation is by invagination, although at times this may be accompanied by delamination. The spherical gastrula soon becomes oval and elongates into an active planula which leaves the oral lobe. See the paper by Hargitt and Hargitt (1910) for details of early development; Okada (1927) describes the details of gastrulation.

B. Later Development: The free-swimming planulae are orange-red in color, well ciliated and opaque. The anterior end is distinctly broader than the posterior end, and the old blastopore, which develops into the mouth, may be visible. After a free-swimming life of from 20 to 40 days, the larva settles down and attaches by the narrow end to the substrate. There is an elongation of the body, followed by the acquisition of tentacles about the gaping mouth. The larva is now in the

scyphula or scyphistoma stage. The number of tentacles increases from two to twenty-four. Agassiz (1862) gives diagrams of the planulae and young scyphulae.

Apparently, stolonization and colony formation occasionally occur; strobilization is inconspicuous, and often only one strobilus is given off at a time, although occasionally three to five are released. Ephyrae can be produced 20 days after attachment, but normally this process takes 30 to 40 days.

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COELENTERATA

(Anthozoa)

Metridium dianthus (*M. marginatum*)

Living Material:

Metridium is a large yellow-brown anemone, which is common along the Atlantic coast. It has a broad pedal disc and a lobed oral disc bearing many short tentacles.

Breeding Season: Probably June and July.

Procuring and Handling Material:

A. Care of Adults: These anemones are easily kept in the laboratory for considerable periods of time, if they are maintained in aquaria with adequate supplies of running sea water.

B. Methods of Observation: The sex of the mature animals cannot be ascertained macroscopically. If a number of ripe individuals are placed together in large dishes containing sea water, natural spawning and fertilization will occur. The same individuals have been shown to spawn several times, at intervals of two to ten days, for a month. Since the eggs are heavy, they will sink to the bottoms of the containers; they can be picked up and transferred to fingerbowls with a pipette. The jelly, which may surround the eggs when they leave the stomodeum, soon dissolves. The larvae can be maintained in the laboratory if they are changed to dishes of fresh, aerated sea water at intervals of a few days.

Normal Development:

A. The Unfertilized Ovum: The eggs are mature and surrounded by a delicate membrane at the time of shedding; they are spherical, opaque, and usually pink in color. McMurrich (1891) reports that they measure between 100 and 160 microns in diameter.

B. Fertilization and Cleavage: Fertilization occurs soon after the eggs are shed, as the eggs are sinking to the bottom. Cleavage is total and slightly irregular, being either equal or sub-equal. The hollow, single-layered blastula is converted into a gastrula by invagination.

C. Time Table of Development: No details of the exact developmental rate are available, although the first cleavage is said to occur 45 minutes after insemination. Fixation occurs in about a month.

D. Later Stages of Development: The young gastrula is top-shaped, the oral surface being somewhat flattened. In the older larva the body is lengthened, and a tuft of long, stiff cilia appears on the anterior (aboral) pole. The stomodeum is well formed, and the two lateral mesenteries appear as conspicuous folds in the gastric cavity. Nematocysts are present at both the oral and the aboral poles of the developing larva. Although temporary attachments (probably for feeding

purposes) are made by the oral surface, the permanent attachment occurs at the aboral pole.

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