CNIDARIA AND CTENOPHORA

Introduction

Plan your laboratory work to take advantage of the variety and abundance of living forms. Much more material will be provided than can possibly be studied in detail during the three days allotted to these phyla.

Keep a simple but careful record of what you do. Drawings should be adequately labeled, ordinarily including the animal's scientific name and the orientation and magnification of the parts shown, as well as pertinent structural details. Notes on experiments should be to the point yet effectively cover purpose, methods, results and conclusions. If work is included in your notebook which was done elsewhere, please so indicate. Your reports are due at the beginning of the first lab period on flatworms.

The following daily outline is to be regarded as only a suggestion. Feel free to pursue any aspect which you find interesting and informative even though this means omitting other of the suggested work.

First day:

1. Study the main features of the anatomy of *Tubularia* and *Obelia* using the directions in *Selected Invertebrate Types* (S.I.T.) and run both through the *Keys to the Invertebrates of the Woods Hole Region* in order to learn the terminology and organization of the keys.

2. During the first day a number of hydroids will be available. You may run some or all of them through the keys as you wish, but you should at least briefly examine each available species so that you can recognize it in the field.

3. Set up a small piece of any hydroid colony under a thread tied around a microscope slide. Sketch the whole piece and observe it at regular intervals for several days. Racks will be provided to hold these slides.

4. As far as time permits study structure, development, regeneration, or behavior in hydroids using S.I.T. and the comments on the next few pages.

5. In the evening observe liberation of medusae and discharge of eggs in *Pennaria*, or *Stylactis* if it is available.
Second day:

1. Study the structure and behavior of Aurelia or Cyanea, using the general directions in S.I.T.

2. Examine Conionemus or other hydromedusae available and compare their general structure with that of scyphomedusae.

3. Stauromedusae (Haliclystus or Craterolophus) may be available. If so, study their structure and behavior using pp. 508-511 in Hyman.

4. Make a comparative study of nematocysts from various species including:
   a. Aurelia or Cyanea
   b. Obelia
   c. Metridium
   d. Physalia, if available

Third day:

1. Examine the anthozoa furnished, using S.I.T. and the directions on the following pages.

2. The ctenophore Mnemiopsis is common in Woods Hole later in the summer but rather scarce at this time. If it is not now available, plan to steal a little time later in the season to work with it.

3. Extend any observations made or projects begun in the preceding days.
STUDY OF HYDROIDS

Directions for the study of four species are given in the text. The following pages point out the special features exemplified by the local forms.

Comparisons should be made in respect to:

Colony form and the growth pattern which produces it
Polymorphism
The structure of the gonosome, i.e., the parts which produce medusae, incomplete medusae, or sporosacs.
Developmental stages of hydranths and of medusae

S. I. T. contains suggestions for studies of feeding activities. For most coelenterates newly hatched nauplii of the brine shrimp, Artemia, are suitable for food. Dishes containing these will be available.

GYMNOLASTEA - ANTHOMEDUSAE

Tubularia
See S. I. T. Tubularia, like many other hydroids, shows spontaneous, periodic movements which are associated with digestion. In Tubularia this activity includes concerted tentacle movements and sometimes a tidal exchange of fluid between the stalk and the hydranth body. This activity can be best studied in small polyps using transmitted light.

Ectopleura
Similar to Tubularia except that free medusae are produced.

Pennaria
See S. I. T. for directions. A good form to study in the evening when medusae are being liberated and sperm and eggs are being shed.

Bougainvillica
This is perhaps a more generalized hydroid than any of the preceding. Its medusae are produced as buds from the hydrocaulus. Stages of their development may be studied. Illustrations are given in Borradaille and Potts.

Eudendrium
Gonosomes are complex. Sexes of the colonies are separate. The male gonophores appear to form linear series of two or more sporosacs, several of these series being attached to the hydranths just proximal to the tentacles. A spadix runs through each series as a central column. The female colonies are sporosacs clustered around the base of the hydranth or tip of the pedicel. The distally
bifurcated spadix partly encircles the eggs. In some species of Eudendrium the hydranth bearing the gonophores is reduced to a mere blastostyle.

Clava

A simple form. Tentacles with irregular distribution. The hydranths are naked and arise singly from a mesh-work of perisarc-covered hydrophizae. Gonophores are sporosacs borne below the proximal tentacles in compact clusters.

Cordylophora

One of the few hydroids occurring in fresh water. Cordylophora lacustris is extremely hardy, can be kept in a laboratory for months at a time and if the seacoast is not near at hand will serve nicely for experiments on regeneration, polarity, etc.

Corymorpha

A huge solitary hydroid much used on the West Coast for studies of regeneration. A combination of care and luck may make them available. The fleshy processes at the proximal end anchor the base in the sand or mud.

Hydractinia

Follow directions in text. Comparisons with Podocoryne and Stylactis may be made.

Podocoryne

Compare with Hydractinia. Shedding of medusae may be observed. Although Podocoryne and Hydractinia are found in the same location, Hydractinia is rarely found on rough shells such as Nassa trivitata. Podocoryne rarely on smooth shells such as Littorina littorea. Bring in a batch from the bathing beach and check this.

Stylactis

Compare with Podocoryne and Hydractinia. The hydorhizal system is a network rather than a compact mat. Like Pennaria this is a good form in which to observe liberation of medusae and shedding of gametes.

CALYPTOBLASTEA - LEPTHOMEDUSAE

Obelia

Directions in text. Most elementary textbooks use Obelia geniculate as the typical hydroid. In fact its life history is poorly known. Stages of the development of new hydranths should be observed. Hydranths are short lived, undergo regression and are replaced.
Obelia commisuralis is a species with a large branching colony.

Campanularia flexuosa

May be used instead of Obelia. Similar except that free medusae are not produced.

Campanularia calceolifera

Forms a large branching colony.

The following forms show a pattern of colony growth quite different from that of Obelia and Campanularia. The tip of each main stem or branch grows out without differentiation at its tip; behind the tip hydranths are organized.

Sertularia

Pedicels lacking. A hinged operculum closes the hydrotheca when the hydranth is retracted. The male gonosome has the blastostyle pressed to one side and carries a single gonophore with prominent manubrium and a mass of sperm. The female gonosome has the blastostyle pressed to one side and from it originate, one at a time, vestigial gonophores that in turn push toward the distal end of the gonangium and discharge their eggs into a specially constructed brood pouch, the acrocyst. By opening acrocysts with needles, stages in development up to planulae may be obtained.

Schizotriche

In addition to the typical hydranths observe the small modified hydranths called nematophores. A nematophore contains a modified hydranth with no mouth but with nemtocytes and adhesive organs used in food collection and defense.

Anthomedusae (Gymnoblastea)

Free living immature medusae are produced by Podocoryne and Bougainvilliea; medusae which are mature when liberated are produced by Pennaria and Stylactus. You may wish to examine only one as a representative, or several for comparisons. The following should be noticed: Shape of manubrium, presence of lips or tentacles on manubrium, the canal system, ocelli (light sensitive organs), arrangement and number of tentacles. Gonads may be identifiable in the walls of the manubrium. For Pennaria see S. I. T.

You may wish to keep one or two for a few days to observe the sequence in which new tentacles are added, and growth of gonads. Feed with Artemia. Study the feeding reaction.
Leptomedusae (Calyptoblastea)

Obelia is the only species likely to be available. See S. I. T. Later in the course when a study of plankton is made some other species may be seen.

Trachylina

Goneonemus is sometimes placed in the Order Trachylina, sometimes in the Hydroida. See text for directions.

SIPHONCOPHORA

Physalia

The Portuguese Man-o-War, is abundant in the Gulf Stream. Following a few days of onshore winds it may become abundant in Buzzards Bay or Vineyard Sound. Sometimes during the course a few specimens will probably be brought to the laboratory. Their ability to capture fish of moderate size can be demonstrated. The sting is most painful and human deaths have resulted from entanglement with the tentacles.

SCYPHOZOA

Aurelia

If living specimens are available they should be studied using S. I. T. Some of the suggested experiments should be performed. If living specimens of Aurelia are not available, Cyanea may be used.

Haliclystus

This, or the related Craterolophus, may be available as an example of an "attached jellyfish." Gwilliam (Biol. Bull. 119, 1960) describes spontaneous behavior and responses to stimuli in Haliclystus.

ANTHOZOA

Metridium

See S. I. T. for directions.

Nematostella

Crowell (J. Wash. Acad. Sci. 36, 1946) has indicated the desirability of using this species for studies of anemones, pointing out that it has the advantages of being hardy, small, transparent, simple in structure, etc. The following suggestions may be supplemented by reference to his paper.
The animal lives in soft mud or sand with mouth and tentacles expanded at the surface. It has been taken at only two localities: The Mill Pond at Woods Hole and on the Vineyard. A similar species is known from the Isle of Wight.

External parts: Mouth. Oral disc bearing the tentacles. Scapus (the main portion of the column). Physa (the bulbous expanded basal portion of the column).

Internal structure: Septa (How many?); How arranged with reference to the tentacles. Are all tentacles primary, i.e., joined to the pharynx or stomodeum? Pharynx. Look for small spheroid bodies called nematosomes lying in the angles where the septa are inserted in the body well. These are clusters of cells containing nematocysts. They may be dislodged and will swim about in the coelenteron. Nothing is known as to their origin and function. Observe the extent of the cnidoglandular ridges (septal filaments) on the edges of the septa.

Other observations or experiments may be performed:
- Feeding activities
- Method of burrowing
- Digestion
- Peristalsis of body wall
- Studies of the nematosomes
- Tolerance to dilution of sea water
- Locomotion

Astrangia

See S. I. T.

CTENOPHORA

Mnemiopsis is the only common ctenophore in this area. It is a large form and favorable for many kinds of study, including regeneration, polarity and dominance, nervous control of cilia and luminescence. Belonging to the Order Lobata it is considerably modified from the form of the commonly described cydippid types. Consult the account of a cydippid in the text and locate modifications in Hyman, pp. 668-9, 683.

Visualize the form of Mnemiopsis as that of a solid sphere modified in the following ways: (1) elongate in the oral-aboral axis.  (2) Compressed in one side to side axis (called the tentacular axis).  (3) Expanded at each end of the other side-to-side axis (called the stomodeal axis), into great oral lobes, so that an oral view or cross section might suggest the side view of a spool or dumb-bell. At the end of the animal where all the lobes hang free is the slit-like mouth, elongate in the stomodeal axis (or stomodeal or "sagittal" plane). At each side of the mouth, defining an axis at right angles to the stomodeal axis is a single tentacle in a small pocket. (These tentacles are much smaller than in most ctenophores and have lost the usual tentacle sheath.) Hanging free just to each side of each tentacle is a tongue-like auricle, four in all. These are fringed with well developed cilia.
The most conspicuous and diagnostic feature of ctenophores is the system of ctenophoral or comb-plate rows of ciliated swimming plates. Eight such rows radiate from the aboral pole and extend meridionally towards the oral end. One pair extends down each oral lobe, these are the adesophageal, subsagittal or terminal plate rows. A shorter row runs into the base of each auricle. These four rows are the adtentacular, subtentacular or lateral rows. Notice that the comb plate rows may be considered adradial. At the aboral pole is a sense organ, including a statocyst, presumably an organ of equilibrium, and possibly other types of receptors. The comb plate rows converge and their synchronism is effected here also.

The internal anatomy is simple. The mouth leads into a long, flattened ectodermal stomodeum. Near the aboral pole this opens into a small endodermal stomach or infundibulum. Extending aborally from the stomach is a funnel tube which opens to the outside by a tiny eccentric pore in one of its four interradial corners. The stomach gives off eight canals: one in each interradius and four in the perradii of the tentacular plane, two paragastric or pharyngeal canals which hug the sides of the stomodeum and two in the bases of the tentacles. The interradial canals dichotomize very soon into adradial canals which lie under the eight comb plate rows, extending orally beyond their ends and anastomosing in great loops, the two adesophagals of each oral lobe with each other and the adtentaculars with each other and with the pharyngeal canals. The hermaphroditic gonads are in the walls of the meridional canals. These canals are also the source of the brilliant green luminescence produced on stimulation after dark adaptation.

Ctenophores are micro-predators feeding on mollusc larvae and other plankton forms. Ciliary currents on the surface of the body carry the food into the auricular grooves where it is caught by adhesive cells or colloblasts of the numerous small tentacles of the labial ridge and transferred to the mouth. There are no nematocysts. Study the action of the comb plates, determine the direction of effective beat and of the matachrenal wave passing along the row. This relation is peculiar in ctenophores.

In order to study details of the apical and oral regions, the canals and the action of the comb plates, cut a specimen into suitable fragments and manipulate these to obtain favorable views. Make general and detailed sketches, semi-diagrammatic when necessary, Emphasize and be sure you understand the biradial type of symmetry.

Behavior of a ctenophore: Besides possessing a number of interesting features peculiar to itself, the ctenophore nervous system has been given extra significance by phylogenetic speculation such as the orthogenal theory of Hanstrum. Two forms of response are here especially suitable for study. These are ciliary activity and luminescence.
Observe carefully the activity of the comb plates in an intact specimen of Mnemiopsis. As you watch it write down a list of the characteristics of this activity (times, directions, coordination, variability, etc.) and of the questions these raise in your mind. Devise your own experiments to answer as many of these questions as you can. The following suggestions are only offered to supplement your own ingenuity.

Do the successive waves that pass down the plate rows from the apical body depend on a spontaneous rhythmic center there for their initiation? Cut out the apical body or isolate an oral lobe with its comb plate rows to see whether spontaneous waves will be initiated in them. Isolate as small a piece of plate row as you can and look for spontaneous activity. What do you think is the normal pacemaker and how are the waves coordinated? Cut across a row, leaving it in place. What happens to the activity on the two sides of the cut, in the first few seconds, after some minutes, after several and after many hours? Try this on several specimens, at different levels and in isolated oral lobes or smaller pieces.

Do you think the wave may be propagated by the mechanical stimulus each plate contributes to the next when it beats? In an isolated row or oral lobe arranged for close observation, stimulate locally by touching a plate row and notice the sinking in of the row beneath the surface as though for protection. Notice whether the plates thus covered over still beat and whether the wave is propagated beyond this region. By very careful manipulation it is possible to hold one or more still with fine needles and then to look for propagation of the metachronal wave across the immobilized region; if this is not easily accomplished try stopping a few plates by applying a few strands of cotton, holding them across a row. Is the continuity of the jelly or of any nerves that may be in it necessary for the propagation of the metachronal wave? Cut through as much of the jelly as possible in an isolated oral lobe, leaving as little continuity as you can between the two ends of the row; this must be done carefully so as not to strain the row. How fast does the metachronal wave travel? Compare this with the speeds of other kinds of conducted waves of excitation.

Look for general reflex responses of the whole plate row system. In other species ciliary arrest and reversal of beat are common and conspicuous features of this system, but in Mnemiopsis reversal is very difficult to elicit. Squirt a small quantity of clam juice or the like into the auricles of an intact specimen while watching the plates closely; look for a prompt but very transient response of the whole plate system. Notice what the animal does when it bumps into an obstacle. Does it ever change its direction of progression when swimming free in a large aquarium?

Attempt to demonstrate a difference between intact animals and preparations with the apical body removed as respects orientation to gravity.
At night or after some hours in a dark room look for luminescence in Mnemiopsis. Does it occur spontaneously? What is its character, location and pattern of response to stimuli? To what kinds of stimuli in which parts of the body will this response occur? How local or general is the response in each case? What kinds of cuts through the ectoderm alone and through the jelly will circumscribe the response, i.e., what is the simplest operation that will confine the response? What kinds of cuts can the response circumvent or have no effect on it? What happens to the ability to luminesce when the animal is exposed to light? Try various intensities or times of exposure and test for luminescence after varying periods in the dark. Try light of different colors obtained by means of filters of known transmission, compensating for lowered intensity. Speculate on the function of this elaborate mechanism in the general economy of the organism.

RESEARCH PROJECTS

Because of the unique opportunities for original research afforded by the available coelenterate material and facilities at the MBL, it is strongly suggested that those members of the class who are interested in conducting original comparative physiological investigations on animals available during this part of the course make plans for their project during the Cnidaria lab section. These plans should be discussed with the instructors before research is begun.

Some suggested projects:

A. Culture of luminous hydroids and a study of the taxonomic distribution of luminescence.

B. Biochemical investigation of luminescent forms (Mnemiopsis, Obelia); including luciferin-luciferase reaction, effects of chemical reagents, fractionation of activity, etc.

C. Studies on the electrophysiology of colonial responses (polyp contraction or luminescence) in hydroids, including speed of conduction, effects of stimulus parameters on distance of spread, refractory periods, effects of temperature and inhibitors, etc.

D. Factors affecting the growth and regeneration cycle in colonial hydroids, including temperature, food, chemical inhibitors, etc.

E. Comparison of ability to regenerate tentacles or other parts in various hydroids (Tubularia has long been a favorite animal for studies on regeneration).

F. Organization of a hydranth from coenosarc contents.

G. Comparative embryology of coelenterates.
H. Stolonic fusion or the lack of it between growing pieces of *Hydractinia*, both pieces from the same initial colony and pieces from different colonies.

I. Any other feasible experiment of your own design.
Class: Turbellaria

Of the representative forms available from the several orders of Turbellaria it is suggested that a complete and thorough study of the trielad Bdelloura be done first. After that, polyclads, rhabdocoels and acocels should be examined to whatever extent time and materials permit. The following are generally available for study:

1. Order: Trieladida

Bdelloura candida and Syncoelidium pellucidum occur in the same habitat as commensals on the book gills and elsewhere on the surface of the horseshoe crab Limulus polyphemus. They are not found on any other host. There should be available both, some naturally infested Limulus and a dish containing worms of various sizes and stages of sexual development. Bdelloura will probably be the more abundant, but either form can be studied by the directions beginning on p. 145, S.I.T.

a. **Juveniles and Adults:** The worms in the dish have been maintained in sea water for several days to clear the intestine of food. Study these first, since their internal anatomy will be much more readily observed, compared to that of the worms removed directly from the crab.

Add a small piece of fresh toadfish liver to a vial or dish containing a "starved" Bdelloura and observe and describe the feeding response. If the worm is an adult, keep it for several days in a vial of clean sea water and check for cocoon deposition. How do these cocoons compare morphologically to the ones deposited on the gills of Limulus? Cocoons deposited *in vitro* may be successfully embryonated and hatched in a Syracuse dish containing clean sea water.

b. **Cocoons:** Examine the gills of Limulus for cocoons of Bdelloura and Syncoelidium. Are cocoons more prevalent on one gill surface than the other? Is there a difference between the 2 species in mode of attachment and distribution of cocoons on the gill surface? Isolate several cocoons and study the different stages of embryonation and development which you will encounter.

2. Order: Polycladida

**Hoploplana anguillina, Stylochus sebra, Prostheceraeus maculosus**

*Euryplepa maculosa.*

The first of these is best for general study of a polyclad as it is a transparent animal with little pigmentation. It dwells ectocommensally in the mantle chamber of the whelk *Bucephalus.* Living specimens in a dish and stained permanent mounts are available for study. Laboratory directions begin on p. 156, S.I.T.

**Stylochus sebra** is a large robust polyclad living commensally in shells occupied by the hermit crab *Pacurua pollux.* A few of these may be available for general observation.
Prostheceraeus maculosus is a rather large but very delicate free-living polyclad found among growths on pilings and submerged rocks. A bucket of scrapings from the bridge pilings at the mouth of Lagoon Pond on Martha's Vineyard will be available. Examine some of these scrapings for worms.

3. Orders: Acoela and Rhabdocoela

These organisms although small, warrant very careful study. At their lower size range they are somewhat comparable to large ciliates and indeed may be mistaken for them. These small worms are often found on or just below the surface of marine bottom muck, under small stones along the tide line, or among growths on pilings.

The acoel Anaperus gardineri is sometimes available for study. These small brown worms (about 1 mm long) may be collected by taking bottom samples off the Fisheries wharf. Study a mature specimen, using directions for the study of a genus no longer prevalent in the area, Polychaera, p. 141, S.I.T. Additional information on Anaperus may be found in Hyman, The Invertebrates, vol. II, or in von Graff's monograph.

A miscellany of rhabdocoels may also be found with the acoels. Refer to S.I.T. p. 143, and to Hyman, vol. II.

Suggested Optional Problems:

1. Obtaining Müller's larvae from Prostheceraeus.
2. Regeneration in such turbellarians as Bdelloura, Stylochus, Dugesia, etc.
3. Salinity tolerance of Bdelloura.
4. Survival of Bdelloura away from host, or on isolated host gill, or on immature Limulus.

Class: Trematoda

A. Order Monogenea:

Examine the gills of the various species of fish available in the laboratory, particularly butterfish, sea robins, toadfish. Place portions of the gill in a Syracuse dish containing sea water and locate the trematodes using a dissecting microscope. Note orientation of worms to the lamellae. Mount attached and unattached worms on slide under coverslip and study. Use S.I.T., p. 158-161, Hyman vol. II, and Dawes, "The Trematoda".

B. Order Digenea:

Adults of digenetic trematodes.

1. Cryptocotyle lingua (Creplin): This worm occurs in the small intestine of fish-eating birds and mammals. Obtain several specimens from the instructor, or locate them for yourself from pieces of gull's small intestine, and mount on a slide in normal avian saline or fresh water. Vaseline the edges of the cover glass and apply slight pressure until the worms are fairly well flattened. Watch for a few minutes for egg discharge. As the worm flattens, body details may be observed more clearly. (Note: Specimens of other species of trematodes may be found in this material. If you locate other trematodes, identify them
and save for observation under section 3.) Median sagittal sections of single
specimens and copulating pairs will be available for assistance in determining
relationships of parts described in S.I.T., p. 167, and in Fig. 109.

2. Parorchis acanthus (Linton); This adult trematode from the cloacas of
piscivorous birds including the herring gull, Larus argentatus, the common gull
of Europe, Larus canus, the common tern, Sterna hirundo and the roseate tern,
Sterna dougallii, is in many ways better for class room study than Cryptocotyle
lingua. If P. acanthus is available it may be used in place of, or in addition
to C. lingua.

Obtain a specimen of this worm and mount as directed above. At the
anterior end is the prohaptor, or oral sucker, surrounded by a flared collar.
Estimate or count the number of spines in the collar. This spiny collar
characterizes the family Echinostomatidae, to which this fluke belongs, and the
nature of the spination characterizes the various species. The mouth, which is
in the center of the oral sucker, leads back into a short prepharynx, which in
turn joins the muscular pharynx. The narrow esophagus runs posteriad and shortly
branches into the two, thin-walled, narrow intestinal caeca. The large, cup-
shaped opisthaptor or acetabulum (or ventral sucker) is between the anterior
heavily cuticularized spine area and the posterior more weakly cuticularized spine
region. The genital pore opens to the ventral surface immediately anterior to
the sucker. From the pore the uterus and the vas deferens pass posteriad and finally
connect to the median ovary and testes. The testes lie side by side (Gr. par-
beside; orchis, testis) and are irregular in outline. Contiguous to the ovary posteriorly is a shell gland which is also connected by vitelline
ducts to the laterally located vitellaria. The excretory system consists of a
postero-dorsally emptying, irregularly shaped, conspicuous excretory bladder
which receives two branched excretory trunks. The ultimate unit of the
excretory system is the flame cell (solenocyte, or terminal flame bulb) visible
only under high power. Note the eggs and free miracidia in the terminal part
of the uterus. Each miracidium contains a simple, fully formed redia, and the
life cycle is therefore, without a sporocyst generation.

3. Other adult digenetic trematodes: Study and identify, if possible, any
living trematodes that you may find in the mouth, stomach, intestine, cloaca, or
excretory system, etc., of the other vertebrate hosts available for parasitological
examination. Consult the known life-cycle table below for suggestions
and identifications aids.

Larval forms of Digenetic Trematodes:

1. Redia. In a finger bowl at your desk are specimens of Littorina littorea
infected with the larval stages of C. lingua. The largest mass of these larvae
is usually to be found in the "liver" of the snail. Notice the texture and
color changes between the uninfected snail liver and the infected one. Locate
an undamaged redia and mount on a slide in a drop of molluscan Ringer’s solution,
and study as directed in S.I.T., p. 170.

2. Cercaria. Obtain a drop of sea water containing mature cercariae of C.
lingua, add a drop of 1:10,000 solution of neutral red, and cover with a
coverglass. As the water evaporates the pressure of the coverglass flattens and
quiets the cercariae, and the body details become more distinguishable. Observe
the general resemblance of the cercaria to that of the adult fluke, and determine
the usefulness to the cercaria of any adaptations you find. Study as directed
in S.I.T., p. 171.
Make a comparative study of the various cercariae in the laboratory after examining those of C. lingua. Determine the probable life-cycle of each from their cercarial specializations. Note the tail size, motility, eye spots, reaction to light and shadows, swimming motions, etc.

3. Metacercaria. The metacercariae of C. lingua are found in the skin of fishes, especially the cunner. Add C. lingua cercariae to a finger bowl containing a small cunner or Fundulus. Locate metacercariae in the fins a few hours later. Add a small piece of cunner fin to a watch glass containing cercariae; observe the cercariae during penetration and encystment. (cf. pp. 172-173, S.I.T.)

Study the metacercaria of C. lingua as it appears encysted in a cunner fin. With well sharpened needles carefully dissect a metacercaria from its cyst. Note that except for size and maturity it resembles the adult worm; can you find any point of difference? What changes occur after the metacercaria becomes established in the definitive (final) host?

The following experiments may be substituted for C. lingua metacercariae. Remove and study the metacercariae of Maritrema arenaria from the barnacle Balanus balanoides. Metacercarial cysts occur in large numbers throughout the tissues of the barnacle and may be teased out easily with needles and the aid of a dissecting microscope. Place the cysts on a slide in a drop of water. Slight pressure on the cover glass might successfully excyst the worms.

4. Life Histories of Trematodes from the Woods Hole Region.

The following table lists the trematodes from the Woods Hole area whose life cycles are known. Expose the proper metacercarial hosts to the cercaria available and determine the methods of infection. If time and hosts are available, feed the infected metacercarial hosts to the proper definitive host and reclaim the young excysted worms.

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<td>Cryptocotyle lingua</td>
<td>Littorina littorea</td>
<td>skin of fishes</td>
<td>Gulls, etc. Stunkard, 1930 J. Morph. and Physiol. 50: 143</td>
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<td>Podocotyle atomon</td>
<td>L. rudis</td>
<td>Crustaceans Fishes</td>
<td>Hunninen and Cable, 1943. Trans Amer. Micr. Soc. 62:57</td>
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<td>Parorchis acanthus</td>
<td>Urosaipinx cinereus and Thais lapillus</td>
<td>Open Gulls Cable and Hunninen 1940 Biol. 78:136</td>
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<td>Siphodera vinaledwardsii</td>
<td>Bittium alternatum</td>
<td>Flounders Toadfish Cable and Hunninen 1942 J. Parasitol. 28:407</td>
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<td>Deropristis inflata</td>
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<td>Nereis Eels Cable and Hunninen 1942 Biol. Bull. 82:292</td>
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<td>Lecithaster confusus</td>
<td>Odostomia</td>
<td>Copepods Sticklebacks and other fishes Hunninen and Cable, 1943 J. Parasitol. 29:71</td>
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<td>Monorchides cumingiae</td>
<td>Cumingia tellinoide and Tellina tenera</td>
<td>Siphons of cercarial hosts Eels and Flounders Martin, 1940 Biol. Bull. 78:338</td>
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<td>Maritrema arenaria</td>
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<td>Balanites balanoides Ruddy Turnstone Hadley and Castle 1940 Biol. Bull. 78:338</td>
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*Species name used here is that given in the paper cited*
Class Cestoda

1. Adult worm

Tapeworms available will belong either to the order Tetraphyllidea or Tetrarhynchidea. The latter have scolices equipped with four, spiny, protrusible proboscides, while the former have scolices variously equipped with suckers, bothridia, or hooks of various shapes. Tetraphyllideans which may be available include Calliobothrium from the spiral valve of the dogfish, and Phyllobothrium (=Crossobothrium) from the sand shark. Tetrarhynchs which may be available include Lacistorhynchus from the dogfish, Grillotia from the skate, and possibly other genera which may be identified by referring to Wardle and McLeod, *The Zoology of Tapeworms*. Consult S.I.T., p. 191 and 194 for morphological details.

Using the dissecting microscope, study Calliobothrium and Lacistorhynchus in a Petri dish of elasmobranch saline containing urea. What purpose does the urea serve? Carefully describe the movements of the bothridia of Calliobothrium, and the proboscides of Lacistorhynchus. Place a white paper sheet under the dish, and using reflected light determine whether either of the tapeworms contains areas of localized pigment? Color?

Mount the scolex of each species individually on a slide in elasmobranch saline, and cover with glass slip; ring with vaseline. Make detailed drawings and describe differences in the adaptive features of these holdfasts.

2. Mature proglottids and eggs

Obtain mature proglottids of Calliobothrium and Lacistorhynchus free in the spiral valve, and place in a dish of sea water. Make observations as rapidly as possible. Note any color changes that may occur, movement, and method of shedding eggs. Mount eggs on slide and study under high power. What fundamental differences exist between the eggs of Calliobothrium and Lacistorhynchus?

Prepared slides of proglottids of Phyllobothrium are available for study. If a spiral valve of a sandshark is available living proglottids of this type may be obtained, and studied by compressing them between cover-glass and slide arranged as described for trematodes. Consult S.I.T., p. 192 for details.

3. Coracidium of Lacistorhynchus

Place on a slide a drop of sea water containing embryonated eggs and hatched coracidia of Lacistorhynchus. Study stages in development of the coracidium. Find hatched eggs and describe. Observe movement of hatched coracidia and then study thin mount under high dry and oil immersion for morphological detail. How many pairs of hooks? Are flame cells present?

4. If available, study demonstration of living procercoids of Lacistorhynchus in experimentally infected copepods.

5. Plerocercoid larvae

The tetrarhynch Ochobothrium crenacolla adult lives in the spiral valve of the hammerhead shark, who sustains such infections by feeding on butterfishes.
Plerocercoids, the infective larvae, may be located as small white dots in the dorsal body musculature of the butterfish. Carefully dissect out several of these encysted forms, and tease them apart on a slide in a drop of saline solution. Note the larva essentially is a scolex without a strobila of proglottids, and exhibits considerable motility. Details of morphology of this species may be found in S.I.T., p. 194.

PHYLUM RHYNCHOCHOAELA

1. Morphology. The subclasses Anopla and Enopla of the phylum Rhynchocoela are not easy to distinguish from observations on the living specimens, except in so far as the presence of a proboscis armed with stylets suggesting the latter subclass. However, details of the position of nerve tracts in relation to integument and musculature depends upon the preparation of histological sections, and will be disregarded in this exercise. Specimens of the Enoplales Amphiporus, Tetrastemma and Zygonomertes will be available for detailed study. These worms will range in length from perhaps 5 to 25 mm, are slim-bodied, and make good study material when mounted on a slide in a drop of sea water, and covered with a coverslip. A little practice will show the amount of pressure necessary to hold the worm still enough to be studied without rupture from excessive pressure. These genera can be distinguished on the basis of number and position of eyespots, and by the size, number and shape of stylets on the proboscis. Study the morphology of a nemertean, using directions in S.I.T., beginning on p. 209.

2. In addition to the genera mentioned above there should be a number of other genera available including the large Cerebratulus, usually white or cream-colored, and Micrura leidyi, a somewhat smaller form usually a handsome rosy pink in color. Make a comparative study of the available forms, tabulating the following characteristics: color, size, body shape, position and shape of mouth, number and distribution of eyespots, character of cephalic pits or grooves, type and number of stylets, swimming ability, and any other feature which you can observe. Using the accompanying taxonomic key, which is an adaptation from Coe (1943), determine to species as many of the forms as material and time allow.

3. Suggested additional exercises:

a. Regeneration. Nemerteans vary considerably in their capacity to regenerate. Sever the body of a nemertean into several parts, keep the parts in cool clean sea water for a number of days, observing the viability of the parts.

b. Autotomy. Many nemerteans spontaneously break into pieces when handled or otherwise disturbed. Devise some tests to determine the nature and strength of stimuli necessary to induce autotomy.

c. Osmoregulation. Nemerteans vary in their tolerance to variation in salinity. Devise some tests to determine the tolerance of several species.

d. Eggs and Larvae. Suggestions in Chapter 1, Coe (1943), Biology of the Nemerteans of the Atlantic Coast of North America on obtaining eggs, sperms, fertilization, and obtaining larvae for various nemerteans may be followed. The genus Cerebratulus makes good material for such exercises.
PHYLUM ASCHELMINthes

Class: Nematoda

1. Great numbers of free-living nematode species abound in marine mud. In former years, *Metoncholaimus pristuris* was obtained in mud from shallow water of Great Harbor, and served as an excellent form for study as an introduction to the Class. It belongs to a large marine subfamily, the Oncholaiminae. Unfortunately, this nematode has become very scarce in recent years and may not be available. However, an excellent substitute has been found to occur as an ectocommensal on *Limulus*. This oncholaimid is very similar morphologically to *Metoncholaimus*, with the additional advantage that all stages in the growth cycle from egg to adult are usually readily obtained at this time of year on *Limulus*.

Obtain a young *Limulus* approximately 2 inches in width and place ventral side up on the stage of the dissecting microscope. Placing fingers on lateral edges of gill hooks, push upward and in medial direction to expose the area of confluence of gill book bases and body wall where many of the oncholaimids will be found. Masses of wriggling nematodes of all stages as well as clusters of eggs will be seen if the light source is correctly positioned: use 10X to 20X magnification. These organisms are hairlike and several mm in length. With mounted minuten nadeln or watchmaker's forceps scoop the nemas into a dish of sea water. The reason for difficulty in getting the nematodes to release from the instruments will become apparent when the tail is examined. Mount the organisms in a small drop of sea water, cover with a glass slip and remove excess fluid with lens paper. Ring slip with vaseline and examine. Consult Cobb's excellent paper (1932) on *Metoncholaimus* for the anatomical details of male and female adult worms. How do these organisms found on *Limulus* differ? Study the juvenile (larval) stages as well as the eggs.

2. *Cephalobus species*: This nematode which is a soil form (i.e. "fresh water") is grown on moist potato slices. Obtain juvenile (larval) and adult stages from dish for study. Use directions in S.I.T., p. 227, for *Rhabditis maupasi*. Note: Mount these organisms in tap water, not sea water.

3. *Syringolaimus smaragdus* Cobb, 1928 (Jour. Wash. Acad. Sci. 18:249-253) is a marine nematoda occurring among the algae on *Nassa obsoleta* shells. Scrape some of the algal coating from a heavily encrusted mud snail into a drop of clean sea water on a slide, tease the clumps apart so as to produce a uniform layer, cover and study.

4. *Dichelyne lintoni* is a transparent nematode found in the gut of *Fundulus*, and may be profitably studied as a type.
This reference list purports only to include a number of books useful for orientation in the several phyla, and such papers to which some aspect of the course makes specific reference. It is not intended to be comprehensive or exhaustive.

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PHYLA ENTOPROCTA AND ECTOPROCTA

No great difficulty should be encountered in the identification of the local Entoprocta, but the Ectoprocta are so numerous and the classification to the level of genera and species so subtle, in many instances, that considerable experience is required for proper identification. Part of the difficulty in identification is inherent in the key. A key is designed for rapid approximation and any identification made with a key should be viewed skeptically. It is necessary to check the original full description or submit the material to an expert. Another difficulty lies in the fact that the key includes only the more commonly encountered Ectoprocts. If a species not in the key is collected and identification is attempted, either considerable frustration or a completely erroneous identification will result.

Accurate identification of species is the role of the specialist, and the student should recognize this. Certain of the local forms are very easy to associate with a name currently in use at the MBL, and these forms are used for study. Naming the animal, at this stage, is the least important aspect of the exercise. It is more important to understand how the animal is built and how it works. The laboratory notes are designed to aid you in this.

1. Suggested studies on Entoprocta.

Phylum ENTOPROCTA Nitsch 1869
Family Pedicellinidae Hincks 1880
Barentsia Hincks 1880 or Pedicellina Sars 1885

General Directions:

Obtain some slides which have been immersed in the Eel Pond for two weeks or more and study them for colonies of various entoprocts and ectoprocts. Some of these slides will probably contain colonies of Barentsia, Pedicellina, Cryptosula, Bugula, and variety of other forms, including some solitary and compound ascidians.

The slides should be placed in Petri dishes containing a little sea-water, and the free behavior of the organisms studied - first with a stereoscopic microscope. Identify the forms on a slide; be sure you can distinguish between entoprocts and ectoprocts. Get an idea of their general appearance and activities, and then transfer the slide to the compound microscope for study under both low and high powers. CAUTION: Avoid dipping the 4-mm objective into the sea-water in the Petri dish.

After you have completed your studies on the slides, return them to their racks in the aquaria; you may need them again later.

In case the slides are unsatisfactory or do not contain the desired forms, return these and obtain other material (scrapings from the limb-bases of horseshoe crabs, etc.) as directed by the instructor.

Specific Directions:

Select a colony of Barentsia or Pedicellina for study. The colonies of both are stolonate; that is, the individuals (zooids) are stalked, and the stalks spring from a delicate horizontal tube or stolon lying on the substratum. In
Barentsia, the stalk is enlarged at the point where it joins the stolon. This enlargement, the muscium, is not present in Pedicellina.

Note the stolons and stalks. Do they appear to be divided by transverse walls, or septa? Are the stalks branched? i.e., do two or more zooids join the stolon by a common stalk?

The organs of the individual are contained in a distal head, or calyx. Note the location of the tentacles. These are borne on a circular ridge of the body wall known as the lophophore. Is the number of tentacles constant in different specimens?

Observe the manner of withdrawal of the tentacles. The entoprocts are characterized by the fact that the tentacles are rolled inward toward the center of the vestibule, and not retracted as a group as in the ectoprocts. The sensitivity of the tentacles may be investigated by touching a single tentacle with a fine hair or a glass needle. If one tentacle is touched, do others respond? Note that the tentacles are ciliated, and try to determine the distribution of cilia on a tentacle. The action of these cilia produces the feeding currents by which food particles are swept into the vestibule and drawn into the oral opening: this lies toward one side of the vestibule, within the circle of tentacles. Investigate the feeding currents and the action of tentacular cilia in gathering food by adding small amounts of a suspension of killed yeast dyed with Congo Red. Note the paths along which food particles are conducted around the vestibule and into the mouth.

The anterior side of the calyx is somewhat flattened, while the posterior side shows a slight outward bulge. The lophophore marks the ventral surface, and the stalk is attached to the dorsal side of the calyx. With these reference points in mind you should be able to orient your specimens properly.

The digestive tract is usually conspicuous, particularly when it contains masses of ingested food. The mouth, opening from the vestibule, lies anteriorly; from it, the esophagus passes downward along the anterior side of the calyx. The large central cavity is the stomach, which bears on its ventral (i.e., uppermost) side a sizable mass of cells, brown in color, sometimes termed the "liver". From the posterior end of the stomach the intestine curves and passes upward along the posterior side of the calyx to the rectum and anus. The anus is located posteriorly in the vestibule, in the sagittal plane, and is usually borne at the tip of a so-called anal cone.

Some of the details of the internal structure of the calyx will be seen to better advantage after scraping off a few individuals and mounting them in sea-water on a slide, for closer observation under a cover glass. If some individuals have been fed with dyed yeast cells, select these for study. Under these conditions the action of the cilia lining the digestive tract may be observed. This ciliary action, rather than muscular peristalsis, appears to be responsible for the movement of food masses through the digestive tract. Congo Red is an indicator dye which turns from red to blue under conditions of considerable acidity; do you note any color change in the yeast-cells undergoing digestion? Look for valves in the digestive tract; and note any characteristics of the process of egestion which may be related to the fact that entoprocts feed on particulate matter using ciliary currents.
At this season, many individuals will be found in stages of sexual reproduction. Females may contain developing eggs, and in many cases the vestibule will be found to contain ciliated larvae just prior to release. These are attached by a viscid secretion to the floor of the vestibule, between the mouth and the opening of the reproductive ducts, and normally remain here until well developed. Observe any larvae which may be released under the pressure of the cover glass.

Many additional details of the anatomy of a representative entoproct are described in Selected Invertebrate Types. See also Hyman, L. H. The Invertebrates: Acanthocephala, Aschelminthes, and Entoprocta (1951). Another useful reference is a paper by M. D. Rogick, 1948, Studies on Marine Bryozoa, II. *Barentsia laxa* Kirkpatrick. Biol. Bull. 94:123-142. Copies of this article are available in the laboratory.

It is suggested that as many drawings or other records be made as time permits, recording significant features of anatomy and physiology of *Barentsia* or *Pedicellina* or both. These should be retained and submitted with the completed work on Ectoprocta.

2. Suggestions for Studies on Ectoprocta.

Order Cheilostomata Bush 1852
   Family Bugulidae Gray 1843
   Genus *Bugula* Oken 1815

This member of the Cheilostomata is presented because it is very common here, and because it represents a type of growth which is in contrast to the flat, platelike, encrusting colonies of *Cryptosula*.

Two species of *Bugula* are available: *Bugula flabellata* (J. W. Thomson) 1847 or 1848, and *B. turrita* (Desor) 1848. They can be distinguished by differences in growth habit. Place a sprig of each in a finger bowl of sea-water and compare. *B. turrita* has a delicate, spiral growth pattern, while *B. flabellata* springs are stubby and fanlike.

Either or both of these species may be represented by small, young colonies of only two or three zooids on the slides already examined for Endoprocts. These are very favorable objects for study of general characteristics and activities. Examine, as before, in a Petri dish containing enough sea-water to cover the slide and allow room for the animals to expand.

1. Survey of external features of zooids and colony.
   Obtain small sprigs of *Bugula flabellata* and *B. turrita* from the water table and place in separate Syracuse dishes in sea water. Examine either species at 20 to 50X magnification, preferably with the binocular and preferably with strong light from above - and make out the gross anatomy of an expanded single zooid (polypide). Learn to recognize: the whorl of tentacles, arising from a conical support, the lophophore, in the center of which is the mouth. Note, further, that the living body of the zooid projects out of a cup-like horny case or zooecium, into which it withdraws momentarily at intervals. Note how the many zooecia making up the colony are connected together to form the flat tough branches or *zoarium*. How were the colonies attached to the substrate?
II. Comparative colony arrangement ("habit") of Bugula flabellata and B. turrita.

Under low proper study sprigs of the above two species, with an eye to seeing why they are considered separate and different. First of all, examine each colony separately and ascertain how the individual zooids (zooecia) are oriented with respect to each other and to the colony as a whole, and how the branches of the colony are related to one another. How do the two species differ in gross arrangement? Sketch briefly your observations on the features asked about above and show also, in low power "habit" sketches (2 or 3 inches high) any differences in colony form which you have observed in the two species. If you observe any other differences, such as, for example, number of tentacles per zooid, record your observations along with the sketch.

III. Zooid arrangement in a colony

In a small sprig of either species of Bugula, study the appearance of zooecia in all regions of the colony. Are there any ovicells, and if so, are they localized in the colony? Do you see any "brown bodies", and if so, where? Do you see any evidence of the origin of new zooecia? What do you conclude as to the mode of growth of the colony and the succession of phases in the life history of an individual zooid? Note also the location and arrangement of the avicularia among the profiles of the branches. At this magnification they will probably appear as refractile knobs or bumps. Watch them for a while. Do they undergo any spontaneous activity?

IV. Physiology.

Before beginning this section, fling out any little crustacea which may be lurking among the branches of the colony - otherwise they will be sure to tramp on the zooid you are observing. (Incidentally, what do you infer from the presence of these crustacea as to the feeding habits of Bugula in comparison with those of the Hydroidea?).

Under somewhat higher power (e.g., 100X) study some living zooids (either species). Numerous vorticellids and other organisms may be attached to the zooecia, and should be ignored for the time being. Observe carefully the ciliary beat on the tentacles, and record anything unusual. Observe how the zooid contracts into the zooecium. What is the motive force and how is it applied? Is there an operculum? Is the contraction or the expansion the more rapid? Are the cilia beating when the tentacles first reappear? Can you see or think of any reason for the periodic twitching of the tentacle tips? Of the contractions of the whole zooids? (If so, try to test experimentally). Time a few successive contractions in one zooid. Is the interval between contractions regular? Do the tentacles actually shorten during contraction? Return now to lower power, and taking a half-inch length of human hair in your forceps, prepare to stimulate an expanded zooid.

Remember, in the following experiments, that no valid conclusion can be drawn unless the experiment is repeated several times. Unless otherwise instructed, allow sufficient time for recovery between stimulations. Make allowances for spontaneous contractions.
If you touch one zooid gently with the hair, so that it contracts, do neighboring zooids also contract? (Be sure you touch only one zooid, preferably on the tentacles). Do the zooids ordinarily make each other contract as their tentacles touch when one contracts spontaneously? What do you conclude from this? Try a more vigorous stimulus. Result? Rub the under (or "outside") surface of the branch, or otherwise see if you can cause a general response. Describe. What do you conclude as to colony organization? Try touching separate tentacles. Will the tentacles respond individually? Is the response different if the tentacle is touched on the outside surface than if touched on the inner? How does this response differ from that given by the tentacles of a hydrozoan polyp? Now, using a gentle touch, stimulate a single zooid repeatedly (allowing it sufficient time between to expand again). Does it give an invariable response? Repeat with a violent stimulus. Result? Conclusion? We shall study the avicularia more carefully later on, but try stimulating them with the hair and record the results. Remember that their function is somewhat obscure, and that you may learn something new.

While observing the _B. turrita_ colony under 50 to 100X, add a little powdered carmine suspension (or dyed yeast or whatever else is available) from a pipette, and record anything relevant to the ciliary currents of the tentacles, or to the method of feeding.

Place a small branch of the _B. turrita_ colony on a slide and cover it with a slip supported by vaseline or fine sand grains. Under 400X (4 mm lens) sketch one of the contracted zooids; and make what observations you can on the structure and reactions of the avicularia.

V. Anatomy of a zooid.

Put the colony of _B. flabellatum_ into a watch glass of 0/2% chloretone in sea water. After five minutes test the reactivity of a few zooids with a hair. If the zooids do not retract, although individual tentacles still bend, the colony is properly anaesthetized. Since the _B. flabellatum_ branches are flat, and since the V-shaped gut of the zooids is oriented in a plane perpendicular to the branch, the trick now is to orient the zooids so that a "side view" may be obtained. If this can be done the complete digestive tract and other internal organs can be seen and drawn at once. You have already noticed that the zooarium of _B. flabellata_ consists of three or more rows of zooecia, stacked in straight tiers; with two very fine needles, separate out several single tiers of a half-dozen or so zooids each, by shredding a branch longitudinally. Be careful not to pulverize the zooids. Now mount several of these strips or tiers on the sides (profile view) together with a piece of flat branch (face view for comparison), under a cover supported with vaseline or sand. Search for a zooid clear of its neighbors and displaying both mouth and anus simultaneously. Make a careful drawing of this zooid, showing zooecium, tentacles, lophophore, mouth, pharynx, oesophagus, stomach, caecum, intestine and anus. In this, or another specimen, see if you can find the funiculus, and retractor muscles which connect the lophophore to the posterior end of the zooecium. Clear refractile bodies on the funiculus are the _ovary_, close to the caecum, and the _testis_, more proximal. If you had not done so previously, sketch an avicularium in side view, showing hinges, muscles, jaws, etc. Make outline sketches of other zooecia showing ooecium (ovicell), and brown body.
VI. Comparative study of Bryozoan types.

Several of the local species of ectoprocts will be available in the lab. Using the available keys and notes you have, identify as many of these as possible and treat in some detail the following:

a) Arborescent Cheilostome
b) Encrusting Cheilostome (e.g., Cryptosula, Electra, etc.)
c) Cyclostome (e.g., Crisia)
d) Erect Ctenostome (e.g., Bowerbankia)
e) Encrusting Ctenostome (e.g., Flustrella, Alcyonidium)
General

Although a major part of the practical work involves dissection of typical forms from each group of the Mollusca, a great deal about functional aspects of molluscan organization can only be learned from observations on living animals. It is strongly suggested that you devote at least as much time to such observations, as to the conventional deep dissections of the circulatory and nervous systems which are outlined in Selected Invertebrate Types. Anatomical studies of the latter sort can be carried out elsewhere on preserved (and injected) material, but conditions at the NDL offer an unusually favorable opportunity to study a series of representative mollusks alive and healthy.

Particular attention should be paid to the mantle cavity and its associated organs. Along with the shell, the mantle cavity is one of the most distinctive features of the Mollusca. It is primarily a respiratory chamber housing the ctenidia (gills), but alimentary, excretory and genital systems all discharge into it, and it has undergone remarkable modifications of structure and of function in different groups of mollusks. In addition to its respiratory function, it provides the feeding chamber of bivalves and of some gastropods, a marsupial brood pouch in some forms, and an organ of locomotion in a few bivalves and in the most highly organized mollusks — the Cephalopoda.

In each example, the circulation of water through the mantle cavity and gills should be studied. In addition to the cilia causing these general water currents, other ciliated surfaces are responsible for the cleansing of sediment from the cavity and its general "sanitation", besides providing the intricate ciliary feeding mechanisms of some mollusks. Water currents and ciliary tracts can be investigated using particles of various weights and sizes (e.g., carborundum, carmine and
various pigments, particulate carbon, "Aquadag", etc.).

Four days should be available for practical work on molluscs:

First day: Dissection of Busyccon (see S.I.T.); study of functioning of the ctenidium and mantle cavity in Crepidula, Acmaca, and Busyccon.

Second day: Study of the feeding mechanisms in either Venus or Mya or Modiolus; comparison with conditions in Yoldia; dissection (see S.I.T.) of one of these and of Pecten.

Third day: Dissection of Loligo (see S.I.T.); observations on living squid.

Fourth day: Investigation of functional anatomy of Chaetopleura and one other project. For example, this could involve a study of radular action in a range of gastropod species, OR of the structure and ciliation of the ctenidia and palps in a range of bivalves, OR of the functioning of the proboscis and odontophore in Busyccon, OR of the structure of the pallial siphons in different bivalves and of functional aspects of their extension and withdrawal, OR of the orientation and responses of certain gastropods, OR of the foot and burrowing movements in various bivalves, OR of the modifications of structure and function in the monomyarian bivalves such as oysters and Pecten, OR of the functioning of the ligament in various bivalves, OR of the detailed structure of the sense organs in Loligo, OR of the functioning of the chromatophores in Loligo, OR of heart physiology in a large bivalve, OR of shell growth in one or more species, OR of gonad condition in a population sample of Crepidula, OR other similar aspects of molluscan structure, physiology or behaviour.

Any day: Observations on living embryos and larvae of mollusks, as
available. Every student should spend some time examining living veligers of *Crepidula*, at least. A preparation can be made in a cavity slide or with a supported coverslip, all the internal structures can readily be distinguished, and movements of the heart, gut and ciliated velum can be studied. Egg capsules of the snails *Nassarius, Thais, Busyccon* and *Buccinum*, and of the squid *Loligo* may also be available.

Notes on practical work: Suitable methods of shell removal will be demonstrated where these are required. In the cases of *Busyccon* and *Loligo*, preserved (injected) specimens will be available for dissection as well as living or recently killed ones. As noted above, much more can be gained from study of molluscs in the latter state. Wherever possible, mark on your drawings of whole or dissected mollusks the direction of water currents and of ciliary tracts on the mantle, ctenidial palps and other organs. Make full use of the books and copies of published papers which are provided. Most of the projects listed for the fourth day will involve special reading.

**Outline classification of the mollusca**

(Typical genera in brackets, marked thus* if species in Cape Cod area, *n* if non-marine).

**PHYLUM MOLLUSCA**

A. Class **NOPLACOPHORA** *(Neopilina)*

B. Class **AMPHINEURA**

   I. Subclass **APLACOPHORA** *(Neomenia, *Chaetoderma*
                      *(Chaetopleura)*

   II. Subclass **POLYPLACOPHORA** *("chitons")

C. Class **SCAPHOPODA** *("Dentallum")

D. Class **GASTROPODA**

   I. Subclass **PROSBRANCHIA**
      a. Order Archaeogastropoda *("Diotocardia or Aspidobranchia") *(Acmaea)*
b. Order Nesogastropoda  
  (Monotocardia I or Taenioglossa) (*Littorina, *Lacuna, *Crepidula)

c. Order Neogastropoda  
  (Monotocardia II or Stenoglossa) (*Busycon, *Nassarius, *Urosalpinx)

II. Subclass OPISTHOBRANCHIA
   a. Order Cephalaspidea  
      (*Acteon, *Philine)
   b. Order Anaspidea  
      (*Haminlea, Aplysia)
   c. Order Thecosomata (Thecosomatous Pteropods)  
      (*Cavolina)
   d. Order Gymnosomata (Gymnosomatous Pteropods)  
      (*Clione)
   e. Order Sacoglossa  
      (*Elysia)
   f. Order Acochlidiacea  
      (*Hedylopsis)
   g. Order Notaspidea  
      (*Pleurobranchus)
   h. Order Acoela (Nudibranchia)  
      (*Acanthodoris, *Aeolidia)

III. Subclass PULMONATA
   a. Order Basommatophora  
      (*Helamys, *Lymnaea)
   b. Order Stylommatophora  
      (*Cepaea)

E. Class BIVALVIA (Lamellibranchia or Pelecypoda)

I. Subclass PROTOBRANCHIA  
   (*Jucula, *Yoldia, *Solemya)

II. Subclass LAMELLIBRANCHIA  
   (no longer used to designate whole class)
   a. Order Taxodonta  
      (*Anadara)
   b. Order Anisomyaria  
      (*Hytilus, *Chlamys, *Cossostrea)
   c. Order Heterodonta  
      (*Tellina, *Mercenaria)
   d. Order Schizodonta  
      (*Mya, *Unio)
   e. Order Adapedonta  
      (*Lyonia, *Pandora)
   f. Order Anomalodesmata  
      (*Cuspidaria)

F. Class CEPHALOPODA

I. Subclass NAUTILIOIDEA (TETRABRANCHIA)  
   (Nautilus)

II. Subclass AHIMONOIDEA  
   (entirely extinct)

III. Subclass COLEOIDEA (DIBRANCHIA)
   a. Order Decapoda  
      (*Loligo)
   b. Order Octopoda  
      (*Octopus)
   c. Order Vampyromorpha  
      (*Vampyroteuthis)
List of further reading on Mollusca

The most important references are:


See also the specialist journals such as: Proceedings of the Malacological Society of London; Malacologia (International Journal of Malacology); Nautilus; Veliger; Johnsonia; Journal of Conchology; etc.

General List


Morton, J. E., 1958. (see main reference above).


Yonge, C.M., 1947 (see main reference above)


INVERTEBRATE ZOOLOGY COURSE, 1965

ANNELIDA

General plan:

First day. External and internal polychaet anatomy (Arenicola and Nereis).

Second day. Comparative study and classification of representative Annelids.

Third day. Special problems and experiments with living worms.

First day.

Time spent on Nereis should be limited to half an hour, as the dissection of Arenicola must be completed in one day. The fresh specimens do not keep well over night. The primary thing to get out of the study of Nereis is a knowledge of the anatomy and terminology of the generalized polychaete head and parapodium. After the dissection of Arenicola is completed, return to the optional behavior studies on Nereis.

I. External anatomy of a typical polychaete.

1. Put a live Nereis in a finger bowl of sea water and make out the following: (a) head, with 4 pairs of whisker-like cirri (singular cirrus) projecting from it; (b) the long series of parapodia or swimming feet on each side of the body.

2. Sketch the head and next two segments and label (a) the conical prostomium, bearing two pairs of eyes, two small tentacles and two blunt, fleshy palps; (b) the peristomium (around mouth), which is considered to be the first true somite, and which bears 4 pairs of peristomial cirri (very similar, except in length, to tentacles.)

3. Put the Nereis into fresh water for a few minutes, and when it everts the proboscis (part of the pharynx), sketch its shape and position and the two strong jaws. Tiny black denticles are scattered over the proboscis. Indicate by dotted lines on your first sketch the probably position of the jaws when the proboscis is withdrawn. Return the Nereis at once to salt water (otherwise it will die) as you will need it later to make the behavior observations outlined below.

4. With a razor slice a thin transverse section bearing one or two parapodia from the preserved Nereis on the supply table. Mount it in water under a coverslip and sketch it quickly, labelling the parts underlined below. Note that the blade or fin-like parapodium is divided roughly into a dorsal lobe, the notopodium and a ventral lobe, the neuropodium, each of which is in turn partly subdivided and each of which bears a fleshy process, known respectively as the dorsal and central cirri. Finally, both the notopodium and neuropodium bear fans of setae, one of which (in each lobe), the aciculum is enlarged and extends back into the body where it is anchored to muscles. What would you guess is its function? To see the acicula you may have to remove the parapodium entirely from the body and press it under the coverslip while you observe it.

The importance of being conversant with the general head and parapodial anatomy is that most of the polychaetas we study in the
laboratory and see in the field can be considered simply as modification: reductions or elaborations on the scheme seen in Nereis. Some have fewer or more tentacles or peristomial cirri; some have gills attached to their parapodia; some have reduced neuropodia, and so on.

II. Anatomy of Arenicola.

Begin the study of Arenicola, as prescribed in S.I.T., pp. 278-289. Each student will have (if we are lucky) one anesthetized specimen for the internal anatomy, and there will be one active worm to each table for the study of external anatomy and behavior. Work carefully, taking particular pains with the vascular system. Drawings should include at least the following: (1) external view of anterior 2 or 3 segments to show the everted proboscis, prostomium peristomium, nuchal groove, etc. (2) external view of region included in somites 8 to 10 or 18 to 20 to show segments with and without gills, and to show annuli and setae. Enlarged sketches of setae and gills may be included; (3) internal anatomy, as in S.I.T. The principal blood vessels of the anterior region will require a separate drawing—preferably lateral—as will the nephridium and cells of the coelomic fluid. When you finish the study you should be equipped to draw a reconstruction of a cross-section made at any level of the body.

III. Behavior and other supplementary work on Nereis.

1. Study the swimming of Nereis. Do opposite parapodia move synchronously in the same direction? Do all the parapodia on one side move simultaneously? If not, describe how they move. How are the movements of the parapodia related to the torsions on the body? In which direction do the waves appear to travel? Study the locomotion of Nereis on wet paper on the table top. How does the locomotion of Nereis on wet paper differ from that of the earthworm? What happens when the worm crawls off the paper on to the dry wood? Explain. Diagram the motion.

2. Study the reactions of Nereis to a length of glass tube, as directed for Diopatra in Problem 1, paragraph 4, of the work for the third day.

Second Day.

Scattered about in the laboratory are named specimens of about 40 common local annelids. There should be enough for one specimen of each species to every four students, so share them about. Be sure to replace in proper dish. The purpose of today's work is to construct a key which could be used by one unfamiliar with the Woods Hole fauna to identify some of the species in the laboratory. The purpose of a key is to permit the identification to be made without the necessity of wading through the separate descriptions of all possible annelids. Its main characteristics, then must be brevity and clarity.

You have been exposed to several types of keys so far in the course (Hydroidea, Bryozoa, Platyhelminthes) and another type is used in Pratt. One of the most convenient types is the "dichotomous" (two-branched) key, in which each statement used is paired with an opposite statement. In other words, at each level in the key, one has to choose between only two possibilities.

You may use whatever characters you please, but before you begin be sure that you are thoroughly familiar with the parts of the generalize polychaete head and parapodium, as studied in Nereis. Note also that
size is very variable, as is color (particularly when one has to consider also pickled specimens); that behavior is a poor criterion since not everyone has the opportunity of seeing the specimen alive; and that the properties of a case or tube would be of no help if the worm were collected without its tube. Also, you will soon find that the structure of the parapodium sometimes varies extensively in different regions, so that if you use a parapodial characteristic it may be necessary to specify whether anterior, medial and posterior is meant. The following are some hints or suggestions:

1. Characters which have been used in the past are length, number and position of head appendages (prostomial tentacles and palp, peristomial cirri, eyes) parapodial characters (dorsal and ventral cirri, setae, gills, notopodium and neuropodium), gills etc.

2. In general it is most satisfactory to pick characters which leave related forms together. Start with an inclusive character and pass on to those less comprehensive. As a starter you might, for example, first separate the scale worms from the others; then those with gills on the anterior end only from those with gills elsewhere, etc. Or you might equally well lead off with some head or parapodial character.

3. You will find it most satisfactory to base your key on observations on the living worms, since structures can be seen so much more clearly. Use light from above, and the low power of your binocular. If the animal continues to be so active as to preclude study, it may be quieted with chloretone, but do not leave it in too long, as the same specimens must serve throughout the day, and also for the research problem on the Third Day. For the same reason, handle the specimens gently.

4. One or two alcoholic specimens of each species will be left in watchglasses on the supply table and may be borrowed temporarily to check up on structure, e.g., head appendages. Also, thin transverse slices may be cut from them with a razor to show parapodial structures. Permanent slides of the parapodia of ten species are on the supply table and will save you some work. Use only low power on these.

5. In going over the various worms if you notice any structure or activity which interests you, make a note of it, and (with the instructor's approval) you may devote part or all of the Third Day to working on it.

6. In order to prevent your having to refer back constantly to the specimen, it would be well to sketch the structures examined (e.g., head appendages, parapodia, etc.) roughly, and this will make it easier to compare different animals. The drawing may be included with the key, but cannot, of course, be an integral part of it.

7. Helpful descriptions and hints about several of the species are contained in Drew's Manual and in Liner.

8. Before you turn in the final key, it might be a good idea to have some unbiased classmate (preferably one who used rather different key characters) try to 'run' your key.

No definite number of species is required to be included in your key. The more species, the more useful the key. Fifteen species in addition to the forms studied in the laboratory (Nereis, Arenicola, Amphitrite, Dionatrala) would seem to be the minimum that would give a key of any practical value, or require any ingenuity, and at least 25 species can be included without undue heur-splitting. The careful examination of the 25 species of polychaetes should enable one to construct a fairly complete analysis of the modifications that have occurred from the basic annelid plan as seen in Nereis. Structural modifications are closely allied to the habits of the organism, and one
may study the functional morphology of this group by comparing: feeding structures and organization of the gut; coelom and organization of septa; arrangement of muscles; vascular system; respiratory pigments and associated structures; sense organs; tube-building and materials for construction, etc.

Much of this information can be found from observations without dissection, and further documentation obtained by consulting the literature available in the laboratory. A fairly complete table should result. On the following page a format of the table is illustrated.

Third Day.

These are merely suggested problems - you may pick others if the instructor approves - the main idea is to study living worms, see what they do, how they are fitted to their environments, and so on. Some of the problems suggested are roughed out in fair detail; some are real research; some are additional exercises similar to those already encountered. You may do one thoroughly or as many as seem interesting. Illustrate with drawings, tables, descriptions, measurements, graphs, etc.

Problem I. Tube building in Diopatra.

1. Obtain from the water table a Diopatra tube with the worm inside, and place it in a finger bowl full of sea water. Study the outside of the tube with regard to: (a) materials of which it is composed; (b) arrangements of materials; (c) method of consolidation; (d) physical properties (touch? elastic? flexible? be careful not to be too vigorous or you will ruin the worm); (e) geometry (larger at one end? ends closed? etc.). Use of hand lens may be helpful.

2. Very carefully slit the tube with a fine scissors, beginning at the posterior end and being sure to keep the scissor's point touching the inside wall of the tube so as not to cut the worm. Liberate the worm into the bowl and examine the inside wall of the tube, adding any new observations to those already recorded.

3. Examine the worm with hand lens and low power binocular and try to ascertain whether it has any morphological peculiarities fitting it for tube-dwelling, or at least correlated with that mode of life.

4. Supply the diopatra with a short length of glass tubing (the worm may have to be helped a little in the following). Will it enter the tube headfirst? Will it enter tailfirst? What happens when the worm enters headfirst and the head reaches the other end of the tube? What happens when the other end is blocked? What does the worm do when left undisturbed in the tube for some time?

5. Cover the bottom of another finger bowl with a thin (1/8 inch) layer of sand, mud, pebbles, shell fragments, etc., fill with sea water and introduce the diopatra. Now carefully observe the process of tube building, particularly in the early stages before the activities of the worm are concealed in his partly built case. What appendages are used to shift materials? Is more than one method of shifting used? Is there any selection of material (e.g., try broken glass)? How are large pieces moved? How large a piece can be used? Is the activity governed by sight? Is any activity other than tube-building indulged in?

6. After an inch or so of tube is completed, remove all material out of reach of the anterior end of the tube. What occurs?

Problem II. (Golfingia) (Gephyrean worm)
| GENERIC NAME | CLASSIFICATION (Errantia vs. Sedentaria) | Arrangement of SEPTA (Derived from-) | FEEDING STRUCTURES (Derived from-) | RESPIRATORY STRUCTURES (Derived from-) | RESPIRATORY PIGMENT | LOCALIZATION OF PIGMENT | TYPE OF TUBE | SENSORY STRUCTS AND LOCAT |
Golfingia is interesting as a very aberrant type of Annelid.

1. Start to anesthetize a Golfingia, as described below, and while it is going under, study the external anatomy and behavior of a fresh specimen, as directed in S.I.T., pp. 306-11. In addition, study its burrowing behavior in sand.

2. Put a Golfingia into a finger bowl and add enough sea water to just cover it. Add every minute or two a cc. of 95% alcohol, and stir. As the worm becomes flaccid, try to work out the introvert (pharynx or proboscis) with pressure from your fingers, so that it is extended. Use this worm, together with a fresh one, for dissection, as directed in S.I.T. pp. 311-317. At least half an hour in alcohol is necessary to quiet the worm, and the dissection should be carried on in the alcohol.

Problem III. Autolytus.

With live specimens, if available, or if not, with permanent slide material, study the structure of this worm and its remarkable method of asexual reproduction by budding, as directed in Drew's Manual, pp. 95-96.

Problem IV. Polychaet bloods.

Study as many species as convenient with reference to the following:

1. Presence of pigment in the blood (red = hemoglobin; green = chlorocruorin).

2. Pigment carried in corpuscles or dissolved in the plasma? For this, study a covered drop of blood on a slide.

3. Type of circulation - vessels, sinuses, lack of circulation, accessory gills, etc. (This can usually be ascertained by external examination).

4. Types of cells in the blood - red cells, amoeboid cells, etc.

5. Any correlation between either the type of circulation or the type of blood with the habit of the worm (i.e., tubicolous, errant, etc.)?

6. Indication of respiratory function of the pigment. Is color lightened (reduced) when worm is suffocated in mineral oil?

7. Results may be presented in tabular form, with sketches where necessary.

Problem V. Gill function.

Study a variety of gilled forms, e.g., Nephtys, Diopatra, Amphi-trite, Lepraea, Orbinia or Scoloplos, Cirratulus, etc. with a view to trying to answer how the aeration of the blood is accomplished. Movements may be slowed, if necessary, with chlorostone. Often the posterior gills are simpler in structure and easier to study. Strong light from above is favorable. Suggested attacks are:

1. Is the comparative complexity of branching of the gills related to their number in the different forms?

2. Is the size of the gills related to the size of the worm?

3. Is the localization of gills on the body correlated with mode of existence?

4. Why do some worms seem to require so much more gill area than others?

5. How is the blood actually circulated through the gills (there are several mechanisms). Is the circulation one-way or two-way? Are
the gills emptied periodically or is the circulation continuous? Is there any provision for changing the oxygen supply (external medium)? Dionatras has a particularly beautiful gill for observing one of the more efficient types of circulation.

6. Can you find any structure in any of the Annelids in the laboratory which, though not primarily a gill, is serving in respiration?

Problem VI. Physiology of tubicolous worms.

Study the behavior of Pectinaria, Pista, Clymenella, Dionatra, Chaetopterus, Hydrodes, Spirorhmis, etc. in their own or in artificial tubes as follows:

Test for tube circulation with carmine. What do you conclude about the mode of feeding of each worm? Is this borne out by mouth structure? Is there any correlation between the path of circulation and gill structure and arrangement?

2. In forms having a direct tube circulation, measure the time necessary for carmine to pass through a given length of tube; measure the diameter of the tube lumen; compute the volume of sea water circulated through the tube in 24 hours.

3. It may be possible to fit a right angle glass tube of the same bore to the end of one of the worm tubes and measure the rise of water in the tube. From this the pressure of the tube circulation can be calculated. Chaetopterus and Dionatra are favorable forms for this.

4. In Chaetopterus, estimate the volume enclosed in the concave side of one of the fans, time the frequency of fanning, and make a rough estimate of the volume of fluid moved through the tube in a day.

5. Study the structural modifications of the different worms (e.g., setae, paranoid, gills, posterior end, etc.) and explain how they are related to tube life. (See also Problems I and VII.)

Problem VII. Comparative structure of tubes.

Examine tubes of Pectinaria, Pista, Clymenella, Dionatra, Chaetopterus, Hydrodes, etc. with regard to the following:

1. Is there any evidence of selection of material, either as to kind, size or arrangement?

2. Can any conclusion be drawn from the tube as to the habitat of its occupant?

3. Can any conclusion be drawn from the tube as to whether its occupant was modified structurally for tube life?

4. Are the tubes impervious? Flexible? Is cement substance used sparingly or lavishly?

5. What can you conclude from the tube as to the mode of feeding of the occupant? as to his gills?

Problem VIII. Tentacular or cirral activity.

Observe in finger bowls a variety of Terebellid worms (e.g., Amphitrite, Leptaeae, Pista, Thelaus, Polycirrus, Enoplobranchus and Cirratulus) with regard to the following:

1. How are the filaments protruded and retracted?

2. Is there any system or rhythm in the sequence or direction of protrusion of a given cirrus or tentacle?

3. Is there evidence of the filaments serving more than one function?

4. How do the filaments attach to the glass?

5. Do the filaments aid in locomotion?
6. Put Pista into a finger bowl of sea water which has a thin layer of sand sprinkled on the bottom. How are the particles grasped? How released? Is the collection anything more than random? (Observe with binoculars).

7. Try Amphitrite out in a somewhat similar way to Pista, by letting it extend its filaments on glass and then dropping on them particles of mud from a pipette.

8. Cut off a tentacle. Is it capable of autonomous movement? Is the activity coordinated (directed)?

Problem IX. Feeding

Offer bits of fresh clam and clam juice to various worms, and if they feed, observe the method of feeding (i.e., grasping, tearing, swallowing, etc.) If they do not feed, do they nevertheless respond to the food particles or their juices? Is there evidence of perception from a distance? Do worms which are filter feeders react differently from those which are predators?

Problem X. Osmotic regulation.

1. Measure the length of Nereis and then pop it into 25% or 50% sea water. Observe any immediate changes in reactions, body length, metamere size, and so on. Study again after fifteen minutes, and after 1/2 hour. At the end of 1/2 hour (not longer) put the worm back in sea water and observe whether there is any recovery (reverse) of effects.

2. While the Nereis is soaking, try the same experiment on some other worms (not more than one specimen of each) e.g. Golfingia, Glycera, Polycirrus, Cirratulus, etc., and compare the results with that observed in Nereis.

Problem XI. Spawning in Nereis

If ripe male and female N. limbata are available, study their behavior and spawning when they are brought together. If the eggs are fertilized, extrusion of polar bodies, cleavage, etc. can be observed in the living eggs. Heteronereis stages can often be seen swarming at the surface at night around the dock lights on the town landing in Great Harbor.

Problem XII. Modification of behavior in Nereis virrens.

Try conditioning worms to tactile or other stimuli as done by Copeland (1930) and Copeland and Brown (1934).

Problem XIII. Early stages in development of Hydroides

The larva of Hydroides is selected for study because it is the most typical trochophore in Woods Hole waters and can be readily raised in large numbers for class study. The procedure is as follows:

Prepare about ten finger bowls of clean sea water. Remove the worms (with as little damage as possible) by breaking the calcareous tubes in which they live. As the worms are removed place each one in a separate finger bowl. The mature worms will shed their genital products at once, the gametes streaming out from either side of the more posterior segments. The sperm forms a white cloud; the eggs appear
as definite dots rather yellow or orange in a mass. After a number of
good females have shed their eggs, remove all the eggs to a single
finger bowl of clean sea water, pick out any organic debris which may
have collected and add five or six drops of water in which the sperms
are thickly suspended. A generous amount of sperm suspension seems
in this particular case to be desireable. Mix eggs and sperm with a
pipette and allow the eggs to settle. Then decant and add fresh sea
water.

The schedule of development runs somewhat as follows:

<table>
<thead>
<tr>
<th>Event</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germinal vesicle breaks down in about</td>
<td>10 to 15 minutes</td>
</tr>
<tr>
<td>1st polar body</td>
<td>20 to 25 minutes</td>
</tr>
<tr>
<td>1st cleavage</td>
<td>50 to 70 minutes</td>
</tr>
<tr>
<td>2nd cleavage</td>
<td>70 to 85 minutes</td>
</tr>
<tr>
<td>The 25-30 minutes between cleavages</td>
<td></td>
</tr>
<tr>
<td>Swimming larvae</td>
<td>6 hours</td>
</tr>
<tr>
<td>Good gastrulae</td>
<td>9 hours</td>
</tr>
<tr>
<td>Trochophores</td>
<td>20 hours</td>
</tr>
</tbody>
</table>

The larvae will not proceed further in their development unless
fed. But they will continue to live without attention for a week or
more and gradually their protoplasm clears up, until the whole trocho-
phore becomes very transparent. In this condition (4 to 6 days after
fertilization) they are best suited for study.

Hydroides trochophores tend to collect on the side of the culture
dish toward the light. In order to maintain as great a concentration
as possible of the trochophores, be careful not to disturb the culture
dishes. Remove a drop of the culture with a pipette, taking care not
to stir up the remaining contents of the culture dish. A large number
of trochophores will be found swimming about in the drop, and can just
be seen with the naked eye. Examine under lower power, noting method
of locomotion. Under high power study larvae carefully and try to
make out the following structures:

- apical organ, eye spot, prototroch, secondary ciliated circlet,
  mouth, digestive tract, anus, nephridium.

Add a drop of carmine suspension in sea water and note the
results. DRAW as seen from the right or left side.
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Neuromuscular System  

Behavior  
ARTHROPODA LABORATORY WORK

In this laboratory work on Arthropoda, special emphasis is to be placed on the diversity of form found in the crustacean groups. While a number of standard dissections will be done, they should be accompanied by careful examination, recorded in notes and drawings, of limb morphology and limb function in all groups. The major adaptive radiation during crustacean evolution has been in the use of the limbs. This radiation is stressed in the lectures and is illustrated in this laboratory work.


Fourth day: Larval stages. See key to crustacean larvae and other attached sheets. Pay particular attention to nauplius larva of Artemia, mysis stage of Homarus and zoea larva of Polyonyx macrocheles or some other crab.


CLASSIFICATION OF THE CRUSTACEA

(From Waterman and Chace, 1960. Number of recent species in each group in brackets)

Subphylum Crustacea
Class Crustacea (26,000+)
  Subclass 1. Cephalocarida (4+)
  2. Branchiopoda (800+)
    Order 1. Anostraca (175+)
      2. Notostraca (15)
      3. Diplostraca (605+)
    Suborder 1. Conchostraca (180+)
      2. Cladocera (425+)
    4. Hystacocarida (3-)
    5. Copepoda (4500+)
  Order 1. Calanoida (1200+)
    2. Harpacticoida (1200+)
    3. Cyclopoida (1000+)
    4. Notodelphyoida (300+)
    5. Nonstrilloidea (35)
    6. Caligoida (400+)
    7. Lernaeopoida (300+)
  Subclass 6. Branchiura (75)
Subclass 7. Cirripedia (800+)
   Order 1. Thoracica (550+)
      2. Acrothoracica (12+)
      3. Ascothoracica (25+)
      (4. Apoda) (1)
      5. Rhizocephala (200+)
Subclass 8. Malacostraca (18,000+)
   Series 1. Leptostraca (7)
      Superorder Phyllocarida
      Order Nebaliacea
   Series 2. Eumalacostraca (18,000+)
      Superorder 1. Hoplocarida (180+)
      Order Stomatopoda
      Superorder 2. Syncarida (6)
      Superorder 3. Peracarida (9000+)
      Order 1. Thermosbaenacea (4)
         2. Spelaeogriphacea (1+)
         3. Mysidacea (450+)
         4. Cumacea (425+)
         5. Tanaidacea (250+)
         6. Isopoda (4000+)
         7. Amphipoda (3600+)
      Superorder 4. Eucarida (8600+)
      Order 1. Euphasiacea (90+)
         2. Docapoda (8300+)
         Suborder 1. Natantia (1930)
            Section 1. Penaeidea (318)
               2. Caridea (1590)
               3. Stenopodidea (22)
         Suborder 2. Reptantia (6391+)
            Section 1. Hermit (693)
               2. Anomura (1270)
               3. Brachyura (4428)
      Superfamily 1. Brachyryynchana
         2. Oxyrhyncha
      Subsection 1. Dromiacea
         2. Oxystoma
         3. Brachygnatha

REFERENCES ON ARTHROPODA

1. Important basic references

Dahl, E. 1963. Main evolutionary lines among recent crustacea. In Phylogeny
and evolution of crustacea (ed. H.B. Whittingdon and W.D.I. Rolfe), Museum
194 figs.
Biological Reviews 33:255-337.
2. References on Crustacea (asterisked references are most useful in this course)

General Morphology and Evolution


Physiology and Cytology


Larval Forms

Cephalocarida


Branchiopoda


Mystacocarida


Ctenophoda


Ostracoda


Cirripedia


Leptostraca

Syncarida


Thermosbaenacea


Mysidacea


Cumacea


Tanaidacea


Isopoda


Amphipoda


KEY TO CRUSTACEAN LARVAE

I. Body without trace of the tagmata of the adult.
   A. 3 pairs of limbs only Nauplius
   B. More than 3 pairs of limbs Metanauplius

II. Body with tagmata of the adult partially or fully established
   A. No carapace or paired eyes
      Copepodid larva ("Cyclops" stages)
   B. A carapace and paired eyes
      1. Thorax cirripede-like, abdomen 4-segmented, a bivalve shell. "Cypris" larva
      2. Thorax and abdomen malacostracan, carapace not bivalve (Malacostracan larva)
         a. Some thoracic limbs used in swimming, none are well developed legs.
            i. Abdomen unsegmented, or with some of the anterior segments only. Thorax always segmented. Protozoa
            ii. Abdomen has all segments distinct. tho' telson may not be separated by articulation from last segment. Thorax sometimes unsegmented in forms which start at this stage
               1. Limbs behind 3rd maxilliped absent or present as biramous rudiments. Abdominal limbs 1-5 rarely present Zoa
               2. Limbs behind 3rd maxilliped present and if biramous well developed. Abdominal limbs all present.
                  i. Limbs behind 34th maxilliped uniramous. Metazoa
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ii. Well developed biramous limbs on most or all thoracic segments. Body not Zoea-like.

Schizopod larva ("Mysis" larva)

b. Thoracic limbs not used in swimming. Some are well developed legs.
   i. Legs and uropods resemble Paguridea
      Glaucothoe
   ii. Legs resemble crabs, uropods prawn-like.
      Megalopa

THE NAUPLIUS LARVA OF THE BRINE SHRIMP, ARTEMIA

I. Place a Nauplius larva in a drop of water on a cover-slip and study its characteristic swimming (and feeding) movements.

II. Add a few lens paper fibers to the drop and cover with a second cover-slip. Both the ventral and the dorsal surfaces of the larva can now be studied under high magnification.

Your attention is called to the following features:

1. The oval, unsegmented body
2. A single, median eye
3. A large, rectangular upper lip (labrum)
4. Three pairs of appendages
   a) The anterior pair are uniramous. Each is a relatively short, unjointed, appendage bearing 3 setae on the free extremity. They are probably sensory and are of little importance in swimming. In the adult they will form the antennules.
   b) The second pair are biramous. Each is composed of a thumb-like endopodite, a large subconical exopodite and a gnathobase in the form of a recurved, conical structure at the base of the protopodite. These are powerful organs for swimming and food gathering in the larva. They will form the antennae in the adult.
   c) The third pair are biramous in most nauplii. In Artemia there is a short protopodite bearing a terminal, finger-shaped endopodite. Medially directed setae serve to push food particles towards the mouth. Later these appendages metamorphose into the mandibles of the adult.
5. A digestive tract consisting of mouth, oesophagus, stomach, intestine and anus.
6. Muscles which move the appendages. They originate in the mid-dorsal region.
The term zoea is given to larvae of decapod Crustacea which swim by means of the exopodites on specially modified maxillipeds, the remaining thoracic appendages being either absent or rudimentary. More strictly the term is confined to larvae of this type in which the abdomen is segmented but lacks well developed pleopods and in which the thoracic appendages behind the maxillipeds are absent or very rudimentary. An earlier stage, in which the abdomen is unsegmented or has only one or two of the anterior segments, is called a protozoea, and a later stage which has abdominal pleopods and well developed rudiments of the posterior thoracic appendages is called a metazoea.

The zoeal stage occurs in the development of Natantia, Anomura, and Brachyura. Those belonging to the true crabs, or Brachyura, are the ones usually figured as examples of typical zoeae in text books. But the variety of types of zoeae is considerable, and no one type can be claimed as more typical than another. Those of Natantia usually have swimming setae on the two pairs of antennae as well as on the maxillipeds, whereas those of Anomura and Brachyura usually swim by the maxillipeds alone.

Frequently common in surface plankton at Woods Hole in August is the large zoea of *Polyonyx macrocheles*. The adult of this species lives in the tubes of *Chaetopterus*, and at first sight appears to be a true crab. However, it possesses uropods and certain other features which class it as an anomuran, family Porcellanidae. Its zoea is notable for the great length of the rostrum, and of the lateral spines which extend back from the carapace. Only two pairs of biramous maxillipeds, with natatory exopodites, are present in the zoea, sensu strictu. But in the metazoea the third maxillipeds, though smaller, are also natatory, a distinction from the Brachyura, in which the third maxillipeds are never natatory.

In the zoeae of Brachyura, the carapace usually has a prominent dorsal spine, in addition to the apir of lateral spines. Most species go through four or five zoeal plus metazooal instars. In the first instar the exopodite of each maxilliped carried four swimming setae, and at each subsequent molt the number of these setae increases by about two.

On the zoeae which are provided try to locate as many as possible of the following:
1. Large compound eyes
2. Carapace, with perhaps dorsal and lateral spines
3. Rostrum
4. Segmented abdomen, with pleopods in a metazoea
5. Telson, the shape of which is usually important in the identification of species.
6. Antennules
7. Antennae
8. Mandibles
9. Maxillules
10. Maxillae
11. Maxillipeds (2-3 pairs)
12. Rudiments of more posterior thoracic limbs in the metazoea

If the zoeae are alive, try to observe how the maxillipeds are used in swimming and what part in swimming, if any, is played by the rostrum and the spines of the carapace, how the telson is used, and any reactions the animals may show to the direction of incident light. Interesting observations on these matters are reported by Foxon (1934). Notice, also, the beating of the heart, beating of the scaphognathites, peristalsis of the intestine, and possibly the expansion or contraction of the chromatophores.
THE HYSSIS OR SCHIZOPOD LARVA OF THE LOBSTER

Probably only preserved specimens will be available. Study under hand lens and under binocular dissecting microscope. Notice the following features:

1. The cephalothorax, in which all somites are fused
2. The abdomen, with six free somites
3. The carapace, fused dorsally with all somites of the thorax, and extending down at the sides to cover the gills
4. The long, pointed rostrum
5. The large telson
6. The antennules and antennae, lacking the long flagella of the adult
7. The mouth-parts, not very different from those of the adult, except for the third maxillipeds, which have a leg-like endopodite and a natatory exopodite.
8. The five pairs of walking legs, with natatory exopodites on all, a small chela on the first, and minute chelae on the second and third.

Lobster larvae go through three instars in the schizopod stage. You can determine which instar you have by reference to the following:

First instar: No pleopods on abdomen. Antennules unsegmented, with the inner ramus minute.

Second instar: Biramous pleopods, without setae, on abdominal somites 2-5. Antennules with three-segmented stalk, and inner ramus almost as long as outer.

Third instar: Short setae on endopodites and exopodites of pleopods. Uropods now present.

The fourth instar is no longer a schizopod, and is much more lobster-like in appearance than are the earlier instars. The natatory exopodites on the walking legs are reduced to minute stumps. Long setae are present on the pleopods, and it is with these that the animal now swims. The posterodorsal spines of the abdominal terga have gone. The chelipeds are much larger and more lobster-like. And both pairs of antennae are provided with long flagella.

Not only the Nephropsidea, to which the lobster belongs, but also Penaeidea, Caridea, Stenopidea, Scyllaridea, and Thalassidacea, have "mysis" or "schizopod" larvae. These larvae are so named because of their resemblances to members of the Mysidacea (of which hyssis is a widely known genus), and because of their possession of biramous thoracic limbs ("schizopod" = split foot). The two orders Mysidacea and Euphausiacea formerly were united together as Schizopoda for the same reason. Even among adult Decapoda, there are certain shrimps belonging to some families of the division Caridea, in which the thoracic limbs are biramous. In all of these types there is a marked common resemblance, because all conform rather closely to the caridoid facies.

Nevertheless, distinctions can be made on the basis of certain clear-cut taxonomic characters. Mysidacea, being members of the Peracarida, have the carapace fused dorsally with not more than the first three thoracic segments, and have an elongate heart (best seen in living specimens); most of them have natatory exopodites on the last seven pairs or thoracic appendages. Euphausiacea, Caridea, and schizopod larvae, being members of the Eucarida, have the carapace fused dorsally with all thoracic segments, and have a much shortened heart located in the thorax. Euphausiacea have none of the thoracic appendages modified as maxillipeds, have natatory exopodites on all or at least the first 6-7 pairs of these appendages, have gills on the last seven pairs, projecting uncovered by the carapace and have small maxillae which are not modified as scaphognathites.
Characteristics of the phylum

Coelomates with pentameral symmetry and asymmetry superimposed on a fundamental bilateral symmetry. This appears superficially as five ambulacra with intervening interambulacra. The body wall contains calcareous spicules or ossicles which may emerge on the surface as spines, and there are highly characteristic organs such as tube-feet (podia) and pedicellariae. The coelom is well developed and differentiated into characteristic divisions such as the water vascular and peri-haemal systems which communicate with the exterior by a hydropore often in the form of a madreporite. Echinoderms lack a definite head. Much of the nervous system is diffuse, superficial in position and separable into 3 divisions: (1) superficial oral (ectoneural), (2) deep oral (hyponeural), and (3) aboral (entoneural). The central nervous system is not well defined. All are marine.

Sub-phylum: Eleutherozoa

Free living stemless forms, the tube-feet being involved in locomotion.

Class: Asteroidea

Pentagonal, with disc and arms which are not sharply differentiated, oral surface is directed downwards, the ambulacra forming prominent open grooves limited to the oral surface. They are supported by a flexible endoskeleton. The tube-feet are highly developed and serve several functions. Example Genera Asterias or Henricia

1. Note and sketch rapidly: disc, arms (rays), ambulacra, ambulacral grooves, spines, ambulacral and adambulacral ossicles (produced into spines), madreporite, mouth, anus, peristome, podia, suckers of podia, terminal (azygous) tentacle, optic cushion ("eye spot"), pedicellariae. Scrape away a few tube-feet. Note pores between ambulacral ossicles through which the tube-feet emerge.

2. Make an incision with a scalpel between the disc and one arm, at a level about half way between oral and aboral surfaces. With strong scissors cut along the side of the ray to the tip, keeping the same level. Cut round the tip and along the other side of the arm to the base. Repeat for each arm. Cut carefully round the madreporite, avoiding injury to the subjacent, axial sinus, and stone canal. Lift the one of the flaps of body wall at the tip of an arm and cut carefully through the mesenteries which join it to the underlying organs, working toward the base of the arm. Repeat for each arm and finally the disc, taking great care not to injure the underlying organs.

Note: perivisceral division of coelom, mesenteries, cardiac and pyloric portions of stomach, oesophagus, retractor muscles of stomach, hepatic caeca, intestine, rectal sac, ampullae, ambulacral ossicles, stone canal, axial sinus, axial organ, gonads.

The water vascular system may be demonstrated by injecting methylene blue into the radial canal on the cut end of one arm.
Note radial water vascular canals, stone canal, circum-oesophageal water vascular ring, Tiedemann's bodies.

3. Briefly examine other available preserved asteroids, especially those showing regeneration.

MAKE A FULLY LABELLED DRAWING OF YOUR DISPLAYED DISSECTION

EXPERIMENTAL

1. Locomotion and Righting: Study locomotion by observing the movement and operation of podia. Do isolated podia contract? Extend? Is the motor activity of the podia co-ordinated within a region, an arm, or the whole animal? Are the arms co-ordinated in the overall locomotion and righting of the organism? Study righting in detail and diagram your observations (consult the various papers of A.E. Moore on the subject). Is righting a response to geotrophic, thigmotrophic, phototrophic, or other stimulation? Design a few experiments to study this problem. To what extent are locomotion and righting mediated by the 'Central Nervous System'? Design a few simple experiments to study this problem. See various papers of J. E. Smith.

2. Sensory physiology: 1. Touch. Analyse the response of the appendages and the whole animal to graded tactile stimulations. How is propagation of the stimulus mediated? Can you directly demonstrate if the superficial nervous system is involved? (For such studies it is best to anchor animal to a piece of wire screen by means of rubber bands.)

2. Light. Study the photoresponses of several animals before and after dark adaptation. (Use healthy animals and keep them in good physiological condition.) After you have studied the normal response under various light and dark conditions, remove the ocelli from each arm and repeat some of your experiments. Are ocelli necessary for general light reception? For specific light reception, e.g. direction of light? (Consult instructor before proceeding.)

3. Chemicals. Study the response of parts and the whole animal to various shelf reagents and natural substances (consult instructor for available material). Use semiquantitative techniques. Tabulate results.

3. Ciliary currents: Chart the ciliary currents on the oral and aboral surfaces and about specific appendages, including inside and outside of papulae; also within the perivisceral coelom. Deposit heavy particles of carmine by means of a pipette on various parts of the organism and carefully observe the direction in which these particles are swept. Diagram and indicate by arrows. (First make a few observations on an anchored animal.) After you have ascertained ciliary currents under these conditions, relax the animal in 7.2% NaCl for ten minutes, then return it to sea water and proceed with a thorough study on the still animal. Is there an observable difference in the ciliary currents after relaxation of the animal? Examine a small piece of epidermal tissue under compound microscope and observe the cilia in action.

4. Coelomocytes and Clotting: Cut the tip of one or more arms of Asterias and allow the perivisceral fluid to drain into a clean test tube. (Save both the animal and arm tips.) Record colour and turbidity of this fluid. Immediately remove a drop of this fluid and examine
under high, dry and oil immersion. Examine several fields and diagram the various coelomocytes. Now examine a given field several times during a half hour period and note what happens. Examine the test tube containing the fluid at the same intervals and note what happens.

What is the significance of your observations? Now examine the cut arm. How does this observation bear upon your conclusion? (See Boccolotian, 1958, 1959; Boccolotian and Clesse, 1959). (At this time place the arm tip in a Syracuse dish and cover it with chlorox for the study of ossicles mentioned below.) Observe the circulation of coelomocytes in the papulae (40 X). Inject 1 ml. of carmine suspension into the perivisceral coelom (use a Pasteur pipette and inject through a papula). How fast does the carmine spread to other papulae? After 10 minutes remove a drop of perivisceral fluid by the Pasteur pipette and examine under high, dry and oil immersion. Where do you find the carmine particles? What is the significance of your observations? Keep the animal with injected carmine for several hours and examine the papulae later. What is the fate of the carmine particles? What does this indicate?

5. Carefully examine the reaction of a few available molluscs to the stomach juices and tissues of Asterias (see paper by Feder & Lasker, Life Sciences, Vol. 3, p. 1042, 1954).

ECHINODERMA II

Class: Echinoidea; globular, oval or discoid, without differentiation into disc and arms. The skeleton forms a characteristic “test” of closely fitting calcareous plates, the ambulacral grooves are closed and the tube feet are arranged in meridional rows. The spines are extensively developed and are freely movable on ball and socket joints, forming important locomotory organs.

Sub-class: Regularia (= Endocyclica); body nearly spherical, mouth and anus at opposite poles, anus with apical system of plates.

Genera Arbacia, Strongylocentrotus. Examples of regular echinoids.

1. Examine and sketch rapidly the whole animal, showing mouth, 5 teeth, peristome, ambulacra (radii), interambulacra (inter-radii), tube feet, buccal tube feet, external gills, buccal plates, anus, periproct, genital pores, madreporite, terminal tube feet (= “terminal tentacles”), pedicellariae, periproct plates, spines.

2. Examine a “prepared” test. Note 20 meridionally arranged rows of calcareous plates, ambulacral plates, pore pairs (passing through the ambulacral plates - a unique feature), terminal (ocular) plates, inter-ambulacral plates, genital plates, tubercles (primary and lower orders), auriculae, ambitus.

3. Remove some of the pedicellariae by scraping test gently. Examine in water under a dissecting microscope and make rapid sketches. Note: stalk (with calcareous and flexible portions), blades, adductor muscles. Distinguish 4 kinds as follows:

(a) tridactyle: largest, with long slender blades
(b) onchocephalous (“snake heads”): most abundant, with blunt scoolar-like blades produced into a basal process
I (c) trifoliate; with broad leaf-like blades
(d) glandular (gemmaform or globiferous); with wholly stiff stalk, blades bearing terminal teeth and a pouch-like gland.

4. Cut horizontally round the ambitus, keeping the two halves together. Carefully turn aside the upper half, freeing the intestine from both halves as necessary in order to place the upper half alongside, with the contents exposed.

Note: jaw apparatus ("Aristotle's lantern"); oesophagus, inferior spiral of intestine ("stomach"); superior spiral of intestine, rectum, siphon, mesentery, marginal and collateral sinuses (haemal vessels), stone canal, axial organ, circum-oesophageal vascular ring, spongy bodies or so-called polian vesicles (= Tiedemann's bodies of asteroids), madreporic ampulla, radial water vascular canals, ampullae, paired canals between each ampulla and tube foot, radial nerve cords and branches, gonads.

5. Examine Aristotle's lantern (c.f. good figure in MacBride), and note: jaws, teeth, protractor, retractor, comminator (= rotary), and rotular (rocking) muscles, circum-oesophageal nerve ring, compasses, compass elevators and compass depressor muscles, epiphyses.

MAKE A FULLY LABELLED DRAWING OF YOUR DISPLAYED DISSECTION


Examine and draw the stages you obtain and, if possible, supplement them by specimens obtained from living plankton (see Hyman, The Echinodermata 1955, and Fell, 1948).

The following corrections should be applied to S.I.T. 1957 edition

1. p. 516 The madreporite plate is as often orange as red
2. p. 516 6th line from the bottom insert "the base of" before "these spines"
3. p. 518 "Dissection". Anesthetize the animal first by placing in sea water containing the HgCl₂ or by merely adding some crystals of HgCl₂ to a finger bowl containing the starfish. It takes about 45 minutes for full anesthetization.
4. p. 519 -top- recent paper (See 1954 paper by Anderson)
5. p. 520 Line 8 change "axial organ" to "axial sinus" after "within the"
6. p. 522 Last paragraph "by cutting along the" "midline of "aboral margin" of a single arm
7. p. 522 footnote- be sure to dilute sperm; one drop undiluted to about 500 ml. in sea water
8. p. 523 Anesthetize first in HgCl₂ before beginning study of Ophiura
9. p. 525 Fig. 215 "aboral plate" should read "aboral shield"
10. p. 526 Second paragraph - line 23, granules need not be scraped since the radial shields appear to be lighter in color
11. p. 527 Line 5 - insert after "disk", "one each at the lateral edge of the point of origin of each ray" "next to arms"
12. p. 528 Line 4 - figure referred to on line 3 should probably be fig. 214. Also insert the work "intervertebral" between "four" and "muscle"
I especially like the following features:

1. The mouth and anus are not at opposite poles, the periproct being removed from the centre of the aboral surface.

2. As a result, an axis of bilateral symmetry arises; anterior and posterior ends can be distinguished. The posterior interambulacrum houses the periproct. A line drawn through the anus and passing through the centre of the test divides the animal into two equal halves. The radii can be separated into 2 groups: (a) an anterior trivium (of 3 radii), and (b) a posterior bivium (of 2 radii).

This morphological differentiation is correlated with a physiological one, the urchins move with the anterior radius (= middle ray or trivium) directed forwards and sand dollars cannot move backwards.

(c) The ambitus is typically oval or heart-shaped.

(d) The body is flattened orally, or both orally and aborally.

(e) The aboral regions of the ambulacra are modified to form petaloids, due to the varying width of the ambulacral plates which are narrow near the centre of the disc, progressively widen and then become narrower toward the ambitus.

The living irregular urchins are sub-divided into 2 orders: Clypeasteroida (sand dollars and cake urchins) and Cratangoida (heart urchins).

Order: Clypeasteroida

The following features are characteristic of the order:

(a) The test is oval or circular at the ambitus and greatly flattened in an oral-aboral direction.

(b) The spines are short, numerous and form a covering of fine "fur".

(c) The podia are differentiated into two types and show a division of labour.
1) large respiratory tube feet which are suckerless and confined to
the ambulacra, emerging by pore pairs, the two members of which
are connected by a groove ("yoked pores").

ii) small suckered podia scattered over the test and not confined to
ambulacra. They assist in feeding and play a minor role in
locomotion.

(d) The peristome and apical system are central, but the periproct is
outside the apical system (exocyclic) and situated in the posterior
interambulacrum, along which it may have moved as far as the oral
side.

(e) The oral side bears ambulacral furrows which are characteristically
branched towards the ambitus.

The spines are used in locomotion, assisted by the podia. Typically
the animals burrow in sand or soft mud.

Echinarchinus burrows by piling sand into a mound in front of itself by
means of the podia, and then rowing itself into the mound by the spines,
the podia continuing to cover the body with sand.

Mellita ("key hole urchin") elaborates this process by rotating its body
from side to side and thus slides into the sand. If turned over it is
unable to right itself, but is righted again by wave action, being
unstable when inverted.

The slits in the test are termed "lunules" and their function is not
fully understood. In one species they are essential to righting and
burrowing, by virtue of the large spines bordering them, which drive
sand through the opening. When inverted, the accumulated sand lifts the
animal to the vertical position from which it topples over.

Order: Serratoida

The following features are characteristic; compare and contrast them
with those of Clypeasteroids.

(a) the ambitus is oval or heart shaped (the broad end is anterior).
(b) the aboral surface is arched and the test is very thin.
(c) the peristome is displaced anteriorly and is bordered by a lip or
labrum.
(d) the oral ends of the ambulacra are expanded to a leaf-like shape
forming the phyllodes which bear large pores for modified podia.
(e) the anterior ambulacrum does not form a petaloid.
(f) the podia are not used in locomotion, and are of five types:

   1) respiratory, borne on the petaloids.
   ii) funnel building, borne on anterior ambulacrum.
   iii) sensory, reduced, situated in the ambital region of each
       ambulacrum.
   iv) sanitary, arising from sub-anal region.
   v) feeding, located in phyllodes.
Types ii), iv) and v) are penicillate.

(g) the spines are slender, curved, and held parallel to surface (producing a characteristic "combed back" appearance. They are used in digging. Certain club-like spines (clavules) are confined to special areas forming the fascioles. Those spines are richly ciliated and the current they produce is used for:

i) creating a respiratory stream over the tube feet
ii) removing sand from test
iii) removing faecal matter from vicinity of anus.

(h) Aristotle's lantern has disappeared.

The animals burrow in sand or mud; the walls of the burrow are supported by the broad tips of the spines and plastered with mucous from glands on the spines. A narrow opening communicates with the overlying sea, and through it are thrust the highly extensible ambulacral brushes during feeding.

See papers by Nichols.

EXPERIMENTAL

The materials available are Arbacia punctulata, Strongylocentrotus droebachiensis and the sand dollar, Echinarchniius parma. Follow the general topics covered in the case of the asteroids, modifying your techniques to suit the echinoids. Study one species in detail but examine others as time permits. (A healthy Strongylocentrotus is less sluggish than Arbacia and therefore better for physiological work.) Consider the following:

1. External organs and their behaviour.
2. Locomotion and righting as associated with neuromuscular action.
3. Sensory physiology.
4. Ciliary currents (inside as well as outside).
5. The perivisceral fluid, coelomocytes and clotting.

Note the following useful references:

Mortensen, Th. A Monography of the Echinoidea
Cowles (1911) Johns Hopkins Univ. Circular: 3.
Sub-phylum: Eleutherozoa
Class: Holothuroidea

The following are characteristic features of the class:

(a) More or less cylindrical form without arms, elongated in the oral
aboral axis, the mouth and anus being terminal.

(b) The body wall is soft and muscular with embedded microscopic
ossicles; it lacks spines and pedicellariae. The ventral surface
forms a flattened creeping sole.

(c) The radii are differentiated into 2 groups, a trivium (of three)
which normally rests on the ground, and a bivium (of two). The
radii of the trivium are closer and bear more tube feet than those
of the bivium.

(d) The mouth is surmounted by enlarged tube feet ("tentacles").

(e) The tube feet are not confined to the radii, those of the trivium
bear suckers and are used in locomotion, those of the bivium are
suckerless and sensory.

(f) The madreporite is internal.

PRELIMINARY INSTRUCTIONS FOR THE DISSECTION OF LEPTOSYNAPTA INHAEBRENS
PRELIMINARY INSTRUCTIONS FOR THE DISSECTION OF LEPTOSYNAPTA INHAERENS

1. General: The common white synaptid, Leptosynapta inhaerens, of the New England coast, provides a rather simple and yet illuminating dissection. There are many points of its physiology, reproduction, and behavior which remain to be worked out, hence this outline is intended to provide an introduction to an animal well worth knowing. These directions apply to what has been commonly known as L. inhaerens, although they should also apply in a general way to its relative, L. (Epitomapta) roseola. Both forms burrow in sand or mud, large quantities of which are ingested in feeding. Whether there is much selection in the course of the feeding is a problem awaiting observation. The burrowing activities should be observed in the laboratory before removing the animals from the storage aquaria. Leptosynapta keeps well in the laboratory, but only if provided with a layer of sand in which to burrow. Without this protection, the animals will invariably constrict off a series of posterior fragments until little remains. Although an anterior fragment will generate a new rear portion, headless segments soon die.

2. Anaesthesia: This is easily accomplished by use of isotonic MgCl₂ 10.36 Molar; 73.2 grams MgCl₂ = 6 H₂O per liter, or 34.2 grams of the anhydrous MgCl₂ per liter). If this solution is used full strength, relaxation is complete in a few minutes; to allow time for observation, it may be preferable to mix the MgCl₂ solution with an equal volume of sea water. Following immobilization, it may be well to dilute further with sea water to avoid complete flaccidity or death of the preparation.

3. External Anatomy: Carry out these observations while your animal is relaxing. Note the wormlike form and the thin body wall, roughened by small papillae and underlain by five conspicuous longitudinal "radial" muscle strands. Twelve short pinnate tentacles mark the oral or "anterior" end, and surrounded an oral disk which opens the mouth. The oral disk is set obliquely, with the dorsal edge slightly in advance. The dorsal side is further marked by a small genital papilla between the bases of the two dorsal-most tentacles, as well as by the fact that as in other holothurians, the dorsum consists of an interradial area lying between a pair of radii (here marked by the muscle bands, since tube feet are absent.) The ventral surface on the other hand, has a midventral and two ventro lateral radii, the midventral radius extending from the more posterior edge of the oral disk. Note the arrangement of pinnules and the characteristic motions of the tentacles. On the inner face of each tentacle, near its base, lie two clusters of small cup-like structures, probably sensory in function. The cavity of each tentacle may be seen to extend downward as a canal passing within the calcareous ring to join the ring canal of the water-vascular system (not visible externally). In handling the animal, note the "sticky" feel of the surface due to numerous small hooks, which may be seen under the dissecting microscope at this time, but which will be observed in more detail later.

b. Dissection: When your animal is relaxed, start a longitudinal incision in the right dorsal interradius in the mid-body region (to cut in any other interradius will either damage important structures or will lead to difficulty in seeing certain organs near the mouth). When the cut has been extended for an inch or so, pin out the body wall flat, preferably on a black surface. Now extend the cut to the anus, pinning out the body wall, and slanting the pins so as not to obstruct work. Then, very carefully make cuts at right angles, extending these to the dorsal and ventral midlines, and pin out the flaps thus freed. The last cuts should be made as far forward as possible, so as to expose the calcareous ring. If the exposure is properly made, the rest of this study can be carried out with little more than slight lifting and teasing.
5. Body Wall: On the inner surface of the body wall, note the five radial muscles, and the thin, circular layer outside of them. If anaesthetization has not been excessive, pricking with a needle will cause local muscular contractions. Note also the thickened papillae outside the circular muscular layer. At this point, examine a slide of an area of body wall to see the ossicles found therein: (a) large "anchors" and "plates" of the general surface; note position and orientation; (b) occasional nail-shaped "rods"; (c) irregular dumb-bell or C-shaped small ossicles in the papillae and especially in the longitudinal muscles. Fresh preparations may be treated with clorox to show the ossicles more distinctly if desired. The ossicles in *L. roseola* differ somewhat from those found in *L. inhaerens*. Observe in your preparation the three lines of tiny funnels on the inner side of the body wall; these will be discussed in (7) below.

6. Alimentary Tract: Starting at the simple mouth, trace back a flattened, whitish oesophagus to an elongated stomach, whose walls are thin, transparent, brownish and pleated accordion-fashion into lateral folds. Oesophagus and stomach are suspended by a dorsal mesentery from the dorsal interradius. Make a cross-sectional diagram in the region of the stomach, showing the positions of the parts thus far identified (to be added to later). Passing rearwards, the stomach merges into the narrower and less pleated intestine. At a point about midway down the length of the animal, the dorsal mesentery ceases to support the gut, although it continues as a slight fold for some distance posteriorly. The intestine from this point on, is attached to a new mesentery, which arises just to the right of the mid-ventral radial muscle. These dorsal and ventral mesenteries do not appear to be interconnected. Make a second cross-sectional diagram, showing relationships of parts in the rear half of the body. The intestine ends in a simple anal aperture, which, because of the frequent occurrence of posterior autotomy, is often in a state of disrepair.

7. Ciliated Funnel: These enigmatical structures lie in three well-defined rows, usually spaced rather closely along each row, but sparse in some parts of the body. One row lies in the dorsal interradius, just to the right of the dorsal mesentery or its posterior prolongation; a second is in the left dorso-lateral interradius just above the left ventro-lateral muscle; a third is just to the right of the mid-ventral muscle strand, and is close to the origin of the ventral mesentery in the posterior part of the body. (In *L. roseola*, the arrangement of the funnels differs somewhat; this should be checked.) The funnels are of two sizes, relatively few large funnels occurring among the more numerous small ones. Remove several funnels and examine with the compound microscope. In addition a little powdered carmine in sea water in your dissected specimen will reveal ciliary activity in the funnels. While the carmine tends to become aggregated in the strands of mucus along each line of funnels, and even to be taken into them. These organs have been considered to function as phagocytic organs, but very little is actually known about them. Add the positions of the funnels to your cross-sectional diagrams.

3. Lacunar or Haemal System: The lacunar system can be very easily traced in synaptids, perhaps more easily than in any other echinoderms. It consists of a system of thin-walled vessels, filled with a plasma-like fluid, apparently with a high protein content. Its distribution is such as to suggest a nutritive function. Whether or not it should be compared to a vascular system is questionable. Locate, on the dorsal side of the stomach in the mesentery, a vessel sending numerous branches into the stomach itself. This mesenterial vessel may show peristaltic contractions; how regular these may be in direction, and to what extent they may circulate the lacunar fluid, are questions of which further observations are needed.
As the mesenterial vessel passes rearward, to the point where the dorsal mesentery terminates, it passes around the left side of the intestine and continues aft in the ventral mesentery. A similar, but apparently non-contractile (check this) vessel lies opposite the mesenterial vessel and may be termed the ab-mesenterial vessel. It lies dorsally on the rear part of the intestine, but at the region where the anterior dorsal mesentery takes charge, the ab-mesenterial vessel passes around to the right to assume a ventral position. The ab-mesenterial vessel, like its counterpart, is connected to the gut by numerous branches, the whole system somewhat resembling the intestinal lymphatics of a vertebrate. The anterior ramifications of the system are not easy to discern; sectioning would be necessary for a complete picture. Along the oesophagus, the vessels become smaller. The mesenterial vessel runs to the point in the mesentery above the anterior oesophagus where the gonads arise, while a small strand seems to lie along the stone canal (see 10 below). Ventral to the oesophagus, the ab-mesenterial vessel seems to peter out in the vicinity of the water-vascular ring. Probably there is some sort of circumoral connection, but it cannot well be traced without special methods. Puncture one of the larger vessels and note the viscosity and high refractive index of the lacunar fluid; probably both are due to plasma proteins. The homologies of this lacunar system with the vascular systems of other invertebrates are not clear, and the common use of the term "haemal system" is perhaps unfortunate in its connotations.

9. Gonads: *Leptosynapta* is hermaphroditic (protandric?) but not self-fertilizing. The gonads are slender filamentous outgrowths from the dorsal mesentery, at a point close behind the calcareous ring, and in close association with the lacunar system. From this point of origin, an indistinct genital duct extends forward to a small external genital papilla between the bases of the two dorsal tentacles.

10. Water Vascular System: In the apodous holothurians such as *Leptosynapta*, the water vascular system consists chiefly of the conspicuous ring canal about the oesophagus, sending forward twelve large canals to supply the tentacles. A single Polian vesicle arises ventrally on the ring canal, while the short stone canal arises dorsally and extends forward on the right hand side of the dorsal mesentery. Some teasing away of tissue may be required to expose it. The stone canal ends in a flared madreporite, whose calcareous support assumes various forms. At its simplest, it has the form of a ring, folded in half to form a double horseshoe which supports two semicircular lips. In other cases, the structure is quite irregular in shape. Add a little powdered carmine in sea water to show the ciliary activity at the madreporite. With a little care, the entire canal and madreporite may be dissected out on a bit of the dorsal mesentery and mounted in a drop of sea water on a slide for microscopic study. The stone canal is lined with cilia, rapidly beating inwards, and is paralleled by a clear, brownish vessel, perhaps part of the lacunar system. Irregular ossicles occur in the walls of the stone canal, ring canal, tentacles, and the polian vesicle.

11. The calcareous Ring: The earlier dissection should have exposed the right side of the circum-oral ring, composed of small white ossicles. Five of these are radial in position and are perforated for the passage of the radial nerves. Between each two radial ossicles are two (sometimes one?) interradial ossicles. Do not attempt to remove the calcareous ring just yet. (Note: in *L. roseola*, the radial plates are not perforated but are merely notched for the passage of the radial nerves.)

12. Nervous system: This is not easy to make out in the fresh specimen, but certain elements can be seen. The radial nerves are soft, whitish strands emerging from the perforations of the radial ossicles. The main nerve ring lies internal to the calcareous ring, and probably cannot be seen. The statocysts are
associated with each of the radial nerves. With care, and employing good illumination, locate the statocysts by picking away the loose tissue anterior to the point at which each radial nerve emerges from the radial ossicle. The statocysts are clear, colorless, transparent, small, spherical vesicles, each containing a statolith. After locating one or more with the dissecting scope, transfer one to a slide and study under the compound microscope. The function of the statocysts is probably that of indicating the orientation of the animal while burrowing. Some of the experiments of Clark (1899) on the gravitational sense of Leptosynapti may be tried on intact animals. The sensory cups on the tentacles, usually assumed to be chemo-receptive, have already been mentioned in (3). Examine their structure in more detail. Leptosynapti lacks eyespots, although certain members of the family have them.

13. Experimental

Observe the following:
1. Autotomy.
2. Evisceration.
3. Regeneration of eviscerated specimens.
4. Locomotion in sand and water.
5. Feeding and respiratory movements.
6. Sensory physiology.
7. External and internal ciliary currents.
8. Coelomocytes and properties of coelomic fluid.

14. References


ECHINODERNATA IV

Sub-phylum: Eleutherozoa

Class: Ophiuroidea ("brittle stars")

Characteristics of the Class

Stellate forms with slender, usually simple rarely branched, arms, sharply marked off from the disc. The arms are relatively solid due to
the enlarged ambulacral ossicles which fuse in pairs from side to side to form "vertebral" ossicles with articulating faces. The arms bear prominent roughened spines. Ambulacral grooves are absent and the podia are reduced to suckerless "tentacles" or "pavillae." A pair of pouches (genital bursae) is found at the base of each arm. There is no anus, the madreporite is on the oral surface and difficult to distinguish. There are no pedicellariae.

Genus: Ophioderma

1. Examine and draw preserved specimens using lens. Note: slender arms, leathery skin with embedded ossicles, conspicuous roughened spines, joints, covering ossicles of arm (aboral, lateral and oral plates or arm shields), podia (tentacles), tentacle pores, tentacle scales, mouth (really pre-oral cavity, leading to mouth), mouth frame, jaws, vertical rows of teeth, bursal slits bounded by ossicles (genital plates), buccal plates, buccal tube feet.

2. Cut through one arm near base. Note large vertebral ossicle.

EXPERIMENTAL

1. Examine living specimens, noting especially activities and attitudes of arms in locomotion and activities of podia and movements of respiration.

2. Proceed as in asteroids, Experimental 1-3.

ECHINODERMATA V

Sub-Phylum: Pelmatozoa

Characteristics: Forms which are attached either throughout life or during the early part of it. They are scaly or jointed in appearance, the viscera being enclosed in a calcareous shell or theca. Most sessile forms have a stalk formed of numerous ossicles. The oral surface is directed upwards and bears both mouth and anus. The podia and ambulacral grooves serve in food gathering and not in locomotion. Most members are extinct.

Class: Crinoidea

Characteristics:

1. The theca is differentiated into an aboral cup (calyx) and an oral cover (tegmen).

2. Highly mobile, branched arms are present, sometimes bearing numerous small branches on either side (pinnules).

3. The ambulacra extend along arms and pinnules.

4. When free-moving, the region of attachment to the former stalk is visible as a centro-dorsal ossicle.
Genus: Antedon (a comatulid)

1. Examine and sketch rapidly a whole specimen, noting disc, calyx, tegment, radii, inter-radii, arms, pinnules, cirri, ambulacral grooves, mouth, anus, oral tentacles, podia ("tentacles"), centro-dorsal ossicle, brachial ossicles, saccules.

2. Examine tegmen with lens. Note: leathery skin, embedded ossicles, ciliated funnels.


Millot, N. 1954. Sensitivity to light and the reactions to change in light intensity of the echinoid, Diadema antilirum. Philippi. Philos Trans (B) 238, 137-220.


Mortensen, Th., 1931-1938. Contributions to the study of the developmental and larval forms of echinoderms. K. Dansk. vidensk., Striffer (9) 4-7: I-IV.


ADDITIONAL REFERENCES

LABORATORY INSTRUCTIONS FOR PROTOCHORDATES

A. Forms to be studied in the laboratory.

It is suggested that you spend the morning of the first day studying *Perophora*. Material has been prepared by attaching colonies to Syracuse dishes. Search for a suitable individual and use it for study of the basic anatomy of a simple ascidian.

The information obtained from a study of *Perophora* may be used as a basis for comparative study of solitary ascidians. S.I.T. provides a dissection guide for *Nolgula*. In addition, study *Styela* and *Ciona*.

The compound ascidians available for study are *Botryllus*, *Amaroucium*, *Perophora*, and *Didemnum*. Stages in the development of *Botryllus* colonies will be available on slides which have been submerged in the Eel Pond and in the sea table.

Tadpole larvae will be available. If mature *Ciona* can be obtained, eggs may be fertilized and developed to the tadpole stage observed. See Costello for directions and description of early development.

Any good-sized *Botryllus* colony will liberate larvae when it is cut up. Examine the larvae, let some attach (this happens rapidly), and follow the early development of the adult.

*Saccoglossus* will also be available for observation and dissection the morning of the second day. Dissection of the proboscis to observe the stomo-cord is feasible. You may wish to look for the ciliary organ on the proboscis (see Burden-Jones).

B. *Botryllus* life cycle.

*Botryllus* passes through one budding cycle in four days to two weeks, depending upon temperature and the availability of food. A number of changes take place in parallel during this time, and these are worth investigating. When the new generation of zooids takes over, the old zooids shrink and are resorbed and the ampullae are loaded with cells, particularly pigment cells. At the same time, the new zooids are expanding rapidly. They soon open their siphons; eggs, if any, leave special chambers and move into the atrial cavity on little pedicels. The testes continue to mature and contain motile sperm for the first time after about two days.

Follow a colony as long as you can, making frequent drawings (various slow movements do take place in addition to the obvious reorganization of the systems when the new zooids take over). Note any changes in pigmentation, circulatory activity, ampullae, testes (remove and examine under compound scope), regenerative ability (including rate), and development of larvae, if any.

C. Other projects and observations.

A number of possibilities for brief observations are available. Observation of early development has already been mentioned. In addition, the following may be suggested:
1. Feeding currents and collection and manipulation of suspended material.

2. Pattern of blood flow, descriptive aspects of heart reversal, circulatory relations in colonial forms such as Botryllus and Perophora.

3. Regeneration in Botryllus. Using a system on a glass slide, remove everything but the peripheral vascular system. Look for the regeneration of new zooids.

4. Pigment patterns in Botryllus. Examine a number of different colonies and note the various components which make up the overall pigment pattern. How stable are these characteristics? If you can collect Botryllus in various places, you may be able to make some comparisons with respect to pigmentation.

5. Colony fusion in Botryllus. The closer related two colonies are, the more likely they are to fuse vascular systems when made contiguous. If you can demonstrate fusion, consult your instructor for further experiments which now become possible.

6. Development of Botryllus eggs. Ordinarily, fertilization takes place shortly after new zooids have taken over. Eggs may be isolated and followed up to the gastrula stage without difficulty. Further survival depends upon the eggs not resting on a hard surface continuously. External fertilization may be tried by placing eggs in the presence of sperm from crushed testes or in the presence of mature colonies. Exact time to collect eggs will be described in lecture.

7. Chromosome number of Botryllus. Since this tunicate has promise for genetic experiments and since most tunicates seem to have a small number of chromosomes, it would be interesting to find the chromosome number for Botryllus. Eggs in early cleavage stages or eggs in meiotic metaphase might be squashed in hematoxylin.

8. Regeneration in Perophora. When a Perophora stolon is broken, large numbers of blood cells come out and aggregate at the cut end. A number of such raspberry-shaped aggregates might be put together to see if any differentiation takes place. A great effort should be made to get a large mass of such cells.

9. Cell culture in Botryllus or Perophora. Anyone familiar with cell culture techniques might well try them on tunicates, particularly in view of the remarkable regenerative ability of such forms as Botryllus. Consult instructor. This should not be tried unless you know the techniques thoroughly.

10. Blood cell types (see George).

11. Experiments on the ascidian heart (see Krijgsman, Waterman).

12. Records of pumping activity (see Hoyle).
Bibliography of "PROTOCHORDATES"

A. General and related books. Good compiled bibliographies are contained in the works cited in this and the next section:

Grasse, P. Traite de Zoologie, vol. XI.
Horst, C.J., 1927-33. Hemichordaten, in Bronn's Tierreich, Bd. 4, Abt. 4

B. The most recent taxonomic monographs, which also contain good summaries of the general biology of tunicates:


C. Recent studies in the biology of protochordates:

Reverberi, G. and La Snina, R., 1959. Normal larvae obtained from dark fragments of centrifuged Ciona eggs. EXPERientia 15: 122.
J. Exp. Biol. 16: 499.

Bancroft, F.V., 1903. Variation and fusion of colonies in compound 

D. Recent additions:
Comp. Biochem. & Physiol. 8: 327-330.

E. Some major workers on ascidians:
Bancroft
Berrill
Oka
Sabbadin
F.H. Scott

With the exception of Bancroft, these men are currently publishing. It would therefore be a good idea to look for recent publications of interest by these workers. In addition, certain rather obscure journals carry a good deal of work on the ascidians. One of these is the Bulletin of the Marine Biological Laboratory at Asamushi, Japan. Assorted copies of this journal are in the stacks.

F. Special topics--Biol. Abstract. volume, abstract number:
Systematic review and new spp. 42: 19660.
Distribution and Nat. Hist. 42: 8717; 44: 12984, 12985; 45: 26615, 26616
Vanadium and sulfate. 42: 5229; 44: 22349; 45: 15951.
Budding. 42: 3538.
Iodine. 44: 10157.
Parasites. 43: 16518; 44: 25522; 45: 12640.
Tunic formation. 43: 15052.
Laboratory Work on Plankton

Examine both living and preserved plankton samples. Observe the methods used to obtain these samples in the field and examine the special apparatus for quantitative work displayed in the laboratory. Many of the groups or organisms which are common in the plankton are listed below. Try to find at least one representative of each group. Record your findings with adequately labelled drawings and concise notes. Include any observations that you make on structure, adaptations and life history.

Diatoms  Barnacle larvae
Dinoflagellates  Copepoda
Foraminifera  Mysid shrimps
Radiolaria  Amphipods
Tintinnids  Isopods
Sponge larvae  Euphausioid shrimps
Nudusae and Planulae  Decapod larvae
Ctenophores  Pteropods and Heteropods
Nematodes  Veliger larvae
Bryozoan larvae  Cephalopod larvae
Chaetognaths  Echinoderm larvae
Trotchophores  Ascidian larvae
Ctenocysts  Pelagic Tunicates
Eggshells  Fish Eggs

PLANKTON REFERENCES

A vast number of publications are concerned with the biology of marine plankton. Thus, unlike most bibliographies issued for earlier parts of this course, this list does not include a representative sample of original papers, but only suggests a few books in which reasonably comprehensive bibliographies are to be found.

An excellent general text on the biology of plankton is:

On fundamental oceanography and marine biology, a dated but still valuable elementary work is:

Two good introductory accounts at a more sophisticated level are:
Sverdrup, H.V., Johnson, I.V. and Fleming, R.H., 1942 "The Oceans: their physics, chemistry and general biology," Prentice Hall, N.Y. and

For a clear statement of many of the environmental problems concerned with marine plankton productivity, read:
Harvey, W.W., 1945 "Recent advances in the chemistry and biology of water." Cambridge University Press.

A detailed account of the biology of Calanus - perhaps the most important organism in the zooplankton of much of the world's oceans is given in:


There are no good elementary texts for the identification of marine plankton, but two incomplete series provide very detailed surveys of some groups:

Conseil perm. internat. pour L'exploration de la mer (1949 to date)
Zooplankton identification cards

and

Brandt, K., Apstein, C., et al. (1901-1928) "Nordisches Plankton"

An older book which may prove valuable when examining marine plankton for the first time is:


Of course, many of the monographs quoted in earlier bibliographies for this class are on particular groups of animals with representatives, or more often larval stages, occurring in the plankton.
GASTROPODA - Radula types

Examples: groups and biology:

- Rhipidoglossan
- Docoglossan
- Trenioglossan
- Pulmonate-type
- Rachiglossan
- Toxiglossan
Work on the Porifera will include a comparative study of various kinds of sponges with respect to habit, gross structure and microstructure; an attempt to synthesize an understanding of general sponge structure by the examination of sections of living material; a look at some sponge larvae; the reconstitution of Microciona cells, separated by forcing through fine bolting cloth, into new sponges; and any additional simple experiments that seem desirable.

This work can be done most meaningfully in concert with a familiarity with what is known about sponges in general. The complex vocabulary, unfortunately, is in large part basic to effective observation; and illustrations of cell types will be helpful, too.

The study of sponge microstructure is made difficult by the poor survival of the material in the laboratory, by the generally small cell size, by the spicules (except when they are being studied), and by the poor contrast offered. On the other hand, good microscopy and effort will bring results.

I. Reassociation of Microciona cells. This exercise must be started first because it calls for periodic observation of a day-long process.

Each student needs a finger bowl, a Syracuse dish, and slides. Each table will need an additional common finger bowl. Fill the bowls 2/3 full of cool sea water. Put the Syracuse dish in the bottom of your finger bowl and put two slides on the Syracuse dish. The assistant will force Microciona through fine bolting cloth into the common finger bowl. With a pipette, take a small amount of the resulting cell suspension and deposit it on your slides. With two or three setups on each slide, you can insure against accidents; also, you can vary the amount of cell suspension used. Large masses, incidentally, don't aggregate well. When the cells have attached, gently transfer the slides to the microscope for examination. Observe any tendency for cells to aggregate; watch for filopodia extruded from groups of cells; can you relate filopodium size to cell group size? A series of sketches made during the day and evening will tell the story. Keep the preparations in the cool sea water bowls in between. A few preparations (one per table) can be kept for long-term observation.

The structure of the reunion mass and the cell types involved are discussed by Wilson and by Galtsoff in papers available in the lab. (Although it is worthwhile to examine the cell types briefly here, Jepps point out that the best way to see the various kinds of cells is to tease apart a sponge. Use microforceps or tiny needles). Since the bolting cloth holds back the syncytial surface layer, the skeletal elements, and other cell types; and since the choanocytes almost invariably lose their collars and flagella, the cells observed in this preparation are not representative of the whole variety found in an intact sponge.

Wilson classifies the cells of the reunion mass as 1) choanocytes, which form new flagella and collars and make the new choanoderm;
Porifera

2) cells with nucleoli and variable cytoplasmic granulation, which form skeletal and reproductive cells; and 3) greyish, non-nucleolate cells with uniformly fine cytoplasmic granulation, which produce the surface syncytium and the non-flagellated portions of the inner mass.

2. The asconoid grade of sponge structure (Leucosolenia). Leucosolenia comes in clusters of small, delicate, branched tubes. Examine a cluster in a small dish of cool sea water, noting the currents at the oscula as revealed by the movements of particulate matter. The absence of current indicates unhealthy, and probably degenerating, material. Make a habit sketch (about 2x) to show characteristic form and colony growth. Habit sketches are always useful. They should not show the details for which more specialized drawings are used, nor should they consume much time.

Now take a living tube with a minimum of surface debris and place on a piece of cardboard in sea water. Cut lengthwise with a sharp razor blade, and transfer the halves to a slide in a drop of water. Under the dissecting scope, gently arrange one half inside up and the other half outside up, making sure that the edges are unlikely to fold over. Now gently add a coverslip. The coverslip should not move freely, but the area under it should be filled with water. Now examine at 100x-500x for choanocytes and porocytes on the inside and for pinacocytes and porocytes on the outside. Leave the spicules till last.

The choanocytes are small, closely packed cells, whose collars won't be visible. But as you rack down on the inner surface, you will see flagellar activity before the cells come into focus. Pinacocytes form a pavement on the exterior, not always easy to see. Porocytes are visible on either side as clear cells with a sometimes crooked canal. Sometimes a membrane can be seen, leaving only a tiny central hole. In all cases, avoid the temptation to focus on a deep plane. The very surface has what you are looking for.

Now examine the spicules in terms of form, relations, and orientation with respect to the osculum. You will see tri-radiates, quadri-radiates, and large and small monaxons. What looks like a dark spot in an apparent tri-radiate is actually the fourth spine of a quadri-radiate coming out at you. Are the spicules cemented to one another?

To see the choanocytes in action, cut a fresh longitudinal half and make a C-shaped section. Work hard to get a thin one. Note the general organization and the gastric rays of quadri-radiate spicules. If you can't see collars, try teasing as suggested above. Do remember, however, that each type of preparation has its own best form of lighting, and it is silly to deny yourself the best possible view by neglecting your microscopic technique. Similarly, a healthy preparation is vital, so use the material freely.
3. The syconoid grade (Scypha). Scypha is a vase-shaped sponge, distinguished by long spicules surrounding the osculum. After general observations and a habit sketch, slice off the osculum region and observe the thin, iris-like oscular membrane that controls the size of the oscular opening. Now make further cross sections of various thickness: thin ones for cellular detail, thicker ones for higher levels of organization. Wedge-shaped ones work well, too. Note the radial canals and their openings (apopyles) into the central cavity. Note that the central cavity or spongocoel of Scypha is not homologous with the structure of the same names in Leucosolenia. Compare these two forms with respect to general organization and spicule arrangement. In Scypha, observe the cortical spicule layer and the incurrent canals.

Now make a parasagittal slice—one longitudinal cut in the middle of the body wall, the other including part of the central cavity. The cuts need not be absolutely parallel. This section will help tie things together and, specifically, should show the choanocytes and any embryos present. Note the relation of the choanocytes to the radial canals and incurrent passages.

4. The leuconoid grade (Halichondria, Haliclona, Microciona, and Cliona). Most sponges are of this grade. You won't be able to see much on the cellular level, but the spicules are interesting, and other structural aspects may be observed. These are all members of the Demospongiae; Leucosolenia and Scypha are members of the Calcarea, which have much larger cells than the other two classes. Observe all the leuconoid forms superficially. Study one or two further with the aid of Hartman (1958) or other literature.

The leuconoid plan is well described in Hyman. Try some hand sections on these species. Spicule differences will be most clear. (The best way to remember which spicule is where is to learn their names.) In Halichondria the prominent monaxons, oxae, are loosely arranged and bound together with spongin. In Haliclona, the spicules are more elaborately arranged in tracts and bound once more by spongin. Microciona has two classes of spicules: megascleres (3 types) in definite arrangements, and tiny microscleres. Cliona has distinct megascleres and may or may not have microscleres. This is the shell-boring sponge.

Dried sponges (e.g., Hippospongia, the bath sponge, with a skeleton entirely of spongin) and glass sponge skeletons (Hexactinellida, e.g., Euplectella, Venus' flower basket) may also be seen.

5. Spicules. Clorox digests provide spicule samples from each of the living forms described above. Of course the arrangement is lost. To distinguish calcareous spicules from silicaceous ones, drop into dilute acid and watch the former fizz.

6. Embryos and larvae. Leucosolenia will probably be loaded with larvae. Snip apart ten or twenty tubes in a finger bowl. The larvae will swim up to the surface layer, where they can be transferred easily to a Syracuse dish by drawing the surface layer up into a fine pipette. Some may be put under a coverslip for examination. Note the
flagellated hemispheres of smaller cells. The non-flagellated, larger
cells will overgrow the others, putting them on the inside. The larvae
may settle and grow into little sponges in a few days. Larvae left to
settle previously may have grown in something worthwhile by the time
of this exercise. If so, they will be available.

Scypha will probably also have embryos or larvae in abundance.
The same procedure may be tried with them. Perhaps some of the other
species will have some, too.

Selected references on Porifera

These are in addition to those listed in S.I.T. pp. 569-70. The
following papers represent varied approaches and this list is not
intended to be complete.


Ankel, W. E., 1949. Uber Fragen der Formbildung und der Zelldetermination

Bergmann, W., 1949. Comparative biochemical studies on the lipids of
marine invertebrates, with special reference to the sterols.

Burton, M., 1953. Suberites domunculus (Olivi): its synonymy, distribution,

de Laubenfels, M. W., 1936. A discussion of the sponge fauna of the
Dry Tortugas in particular and the West Indies in general, with
material for a revision of the families and orders of the Porifera.
Publ. Carnegie Inst. no. 467 (Papers Tortugas Lab. vol. 30), 1-225.

Hartman, W. D., 1957. Ecological niche differentiation in the boring

Hentschel, E., 1925. Parazoa. In: Kukenthal-Krumbach, Handbuch der
Zoologie, Bd. 1, 1. Halfte.

Hartman, W. D., 1958. Natural history of the marine sponges of
155 pp, 12 pl.
Porifera- 5


This is followed by a series of papers on Leucosolenia, all in Q. J. M. S.

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Old, M. C., 1941. The taxonomy and distribution of the boring sponges (Clionidae) along the Atlantic Coast of North America. Publ. Chesapeake Biol. Lab. 44: 1-30.


Other papers on the controversial matter of neurons in sponges include:


_____, 1953. Ibid., 236: 130-133.

Pavans de Ceccatty, 1953. Ibid. 236: 2342-44.


Weel, P. B. van, 1949. On the physiology of the tropical fresh-water sponge, Spongilla proliferens Annand. 1. Ingestion, digestion and excretion. Physiol. comp. et Oecol. 1: 110-126. (This journal now known as Monographiae Biologicae).
Special topics
(Biol. Abstract volume, abstract number)

Chemistry of skeleton. 65: 23065.


Additional References

Ultrastructure


Regeneration, Gemmulation, Culture, and the like


Resmont, R., 1962. The physiology of gemmulation in freshwater sponges. 20th Growth Symposium, 1-25. Ext. no. 577.014 S.S.D.G.


Sara, Bolletino Zool. 22: 43-50; 323-27. 23: 113-119; 149-61

Grafting


Gametogenesis and fertilization


Tuzet and Pavans de Ceccatty. Spermatogenesis, fertilization, etc. in Hippospongia communis (tr.). Bull. biol. 92: 331-348

General


Special


GENERAL DIRECTIONS FOR PROTOZOA

You will be presented with a representative collection of marine and brackish water flagellates, sarcodines, and ciliates; some from culture, others collected from their natural habitats. The life of a protozoan is spent in feeding and reproducing, and associated activities. Observe and describe these activities, and the organelles used in their performance, in as great detail as the material permits. Think about mechanisms of organelle function, coordination, and formation, using whatever knowledge of physiology you brought to this course and your imagination. Next, immobilize the organisms and make detailed, labeled drawings of each one from several perspectives. Caption each drawing with as complete a taxonomic description as you can.
Phytoflagellates

General


Cultivation and physiology:


Local interest:


Rhizopods


Foraminifera

General:


Some Life Cycles:


Laboratory propagation:


Local Interest:


Ciliates

General


Maupas, E. (1889) La rajeunissement karyogamique chez les cilies. Arch. zool. exp. et gén. (ser. 2), 7:149-517.


Marine


Phylum Protozoa

Subphylum Sarcomastigophora

Superclass Mastigophora

Class Phytomastigophorea

Order Dinoflagellida

Gymnodinium

An unarmoured flagellate with a transverse furrow that runs approximately across the middle of the cell and is only slightly spiral.

Gyrodinium

The transverse furrow of this unarmoured marine dinoflagellate is a steep spiral. The sections of it visible on the ventral surface are far apart, though linked by a part of the longitudinal furrow known as the intercircular area. The origins of the transverse and longitudinal furrows are, therefore, separated from one another.

Haplophidinium

The transverse furrow of this unarmoured form is shifted close to the anterior end so that the epicone is very small.

Peridinium

The transverse furrow is broad and slightly spiral, with projecting rims and is a bit below the middle of the body so that the epicone is larger than the hypocone of this armoured form.

Gonyaulax

An armoured dinoflagellate with a markedly spiral transverse furrow. Origins of flagella displaced as in Gyrodinium.

Geratium

Noctiluca*

An aberrant dinoflagellate that attains a diameter of 1.5 mm and must be examined undistorted in a depression slide. The body is shaped like a peach, covered with a thick pellicle, and indented by a groove, held uppermost in floating and marking the morphological ventral side. Towards this, normally upper, pole most of the cytoplasm is concentrated and the surface is strengthened by a rod-like pellicular thickening. Close by are grouped the nucleus, flagellar apparatus, mouth (cytostome), and various other organelles, and the whole complex is called the polar mass.

Branching and anastomosing threads of cytoplasm stretch from the polar mass across the interior of the sphere, and they exhibit streaming movements.

From the upper end of the groove, which leads to the mouth, springs a thick tentacle, cross-striated, and about as long as the body axis; its slow beat, about 4 to 8 times per minute, rotates the whole organism. Between the base of the tentacle and the mouth are crowded 3 structures of uncertain function. These are a tiny flagellum, here usually called the "cilium" which flickers with an intermittent undulating movement, a soft, cleft flap, inappropriately called the tooth, and, just behind the cilium, the lip. Noctiluca can engulf almost any planktonic animal up to the size of a copepod larva, and the tentacle is the active organ in feeding. As it swings it comes into contact with food, which adheres to its sticky surface, and eventually the accumulation of organisms is sucked in by the mouth, to which may be directed by the lip and the tooth. Defecation also takes place through the mouth. The function of the "cilium" is unknown; it alternates long periods of inactivity with phases of rapid undulation; possibly it clears the area about the mouth of faecal debris.

Asexual reproduction by binary fission is preceded by a de-differentiation of the organelles, which are regenerated by the daughter cells; the whole process takes from 12 to 24 hours.

At times Noctiluca produces swarmers from its polar mass, and there they lie like a cap until they develop flagella and break away, when the parent body dies. Each swarmer measures 12-23μ x 12-14μ, and is bilaterally symmetrical; the anterior region is separated by a constriction from the posterior, which it overhangs, and from the constriction or girdle, arises a single, narrow, ribbon-like flagellum, nearly 4 times as long as the body. Swarmers are isogametes. Noctiluca is, as far as we know, the only dinoflagellate to reproduce sexually.

A guide to some of the literature on Protozoa (excludes most parasitic groups). A selection from a list compiled by J. O. Corliss in 1958, with additions by G. G. Holz, Jr. to update the original.


Claparedo, E. and J. Lachmann (1858-1861) Études sur les infusoires et les rhizopodes. Vols. 1 & 2, Genève. (Gen. Inst. nat. geniv., 5-7.)


Dogiel, V. A. (1951) Protozoologie. Moscow. (Entirely in Russian.)


Sonneborn, T. M. (1947) Recent advances in the genetics of Paramecium and Euplotes. Advance in Genetics, 1:263-358.


Stein, F. (1859-1883) Der Organismus der Infusionsthiere... Leipzig.


