Invertebrate Zoology Course Materials 1949

STAFF

Instructors

F. A. Brown, Jr., Professor of Biology, Northwestern University, In charge of course. Annelida

W. D. Burbanck, Professor of Biology, Drury College, Porifera, Entoprocta, Ectoprocta

C. G. Goodchild, Professor of Biology, South West Missouri State College, Helminths

L. H. Hyman, Curator in Invertebrate Zoology, American Museum of Natural History, Turbellaria

L. H. Kleinholz, Associate Professor of Biology, Reed College, Protochordata

J. H. Lochhead, Assistant Professor of Zoology, University of Vermont, Arthropoda

Madelene E. Pierce, Associate Professor of Zoology, Vassar College, Mollusca

W. M. Reid, Professor of Zoology, Monmouth College, Echinodermata

T. H. Waterman, Assistant Professor of Zoology, Yale University, Coelenterata, Ctenophora

Assistants

Robert S. Howard, Assistant in Zoology, University of Miami

Marie Wilson, Assisnat in Zoology, Northwestern University

INVERTEBRATE ZOOLOGY COURSE - 1949

Name	Institution	Seat	Team
Amberson, Barbara Dorsett	Pennsylvania State Univ.	1	I
Amos, Patsy Dawn	Oberlin College	2	I
Anderson, Betsy Jayne	Drury College	3	I
Anderson, Victor Elving	University of Minnesota	4	I
Auerbach, Earl	Northwestern University	5	I
Barber, Saul Benjamin	Yale University	6	I
Bellisario, Joseph	Washington & Jefferson Col.	7	I
Bouwman, Frederick L	Michigan State College	8	I
Bullock, Jane Ann	University of Oklahoma	9	I
Burnett, Frances Lowell	Cornell University	10	II
Birch, Robert L.	West Virginia University	11	II
Chaet, Alfred B	Univ. of Massachusetts	31	IV
* D'Ambrosio, Gloria Irene	Northwestern University	13	II
Davis, Earle Andrew Jr.	University of Illinois	14	II
Dawson, William A.	Swarthmore College	15	II
Donnelly, Orville Wright	Williams College	16	II
Fienemann, Barbara Joane	Smith College	17	II
Fingerman, Milton	Northwestern University	18	II
Foster, Richard	Harvard University	19	III
Frost, Robert Carlton	The Rice Institute	20	III
Gerold, Nicholas John Jr.	Cornell University	21	III
Getman, Herbert Clapsaddle	Brown University	22	III
Gilmartin, Rosemary Therese	Yale University	23	III
Godfrey, Robert Riggs	New York University	24	III
Goldstein, Lester	Univ. of Pennsylvania	25	III
Grant, William C. Jr.	Dartmouth College	26	III
Grun, John	Brooklyn College	27	III
Hankins, Robert M.	University of Kansas	29	IV
Hansen, Bruce Winston	Monmouth College	30	IV
Holle, Paul August	University of Notre Dame	32	IV
James, Carolyn Louise	Vassar College	33	IV
Jones, Elizabeth Jane	Oberlin College	34	IV
Kallen, Eugene Joseph	Columbia University	35	IV
Leonard, Barbara Hopkins	Smith College	36	IV
Mabel, Judith Balsam	Goucher College	37	V
Most, Sylvia Nussbaum	Univ. of Pennsylvania	38	V
Nanney, David L.	Indiana University	39	V
Patten, John A.	University College, N.Y.U.	40	V
Peck, Harry Dowd Jr	Wesleyan University	41	V
Pulley, Thomas Edward	University of Houston	42	V
Rieck, Alvin Frank	Marquette University	43	V
Said, Rushdi	Cairo University, Egypt	44	V
Schneiderman, Howard Allen	Harvard University	45	V
Sedar, Albert William	State University of Iowa	46	V
Sengbusch, Howard George	New York University	47	VI
Sindermann, Carl James	Univ. of Massachusetts	48	VI
Sippell, Theodore Otto	Yale University	49	VI
Spiegel, Melvin	University of Rochester	50	VI
Stafford, Nancy Jeanette	Tufts College	51	VI
Telfer, William Harrison	Harvard University	52	VI
Tinker, Milton Elliott	University of Minnesota	53	VI
Weyl, Hilda	Hunter College	54	VI
Williams, Austin B.	University of Kansas	55	VI
Wilson, Katherine	Washington University	56	VI
* Currier, Joanne Elizabeth	Vassar College	12	II

Front row sitting (left to right): L. Goldstein, R. R. Godfrey, W. C. Frost, B. B. Amberson, E. J. Kallen, S. B. Barbor, B. W. Hanson, M. E. Tinker, E. J. Jones, F. D. Amos, H. Weyl, M. Fingerman, G. I. D'Ambrosio, E. Auerbach, and A. B. Chaet. Second row sitting (Left to right): R. T. Gilmartin, H. G. Bongbusch, J. A. Patten, M. J. Stafford, C. L. James, B. J. Anderson, B. H. Leonard, E. A. Davis, Jr., S. N. Most, V. E. Anderson, F. L. Burnett, C. J. Sinderman, H. D. Peck, Jr., H. A. Schneiderman, W. A. Dawson, J. A. Bullock. Third row sitting (left to right): J. E. Currier, J. B. Mabel, H. C. Cetman, P. A. Holle, M. Wilson, A. F. Rieck, J. Grun, Dr. J. H. Lochhead, W. H. Telfer, K. Wilson. Standing (left to right): W. C. Grant, Jr., R. Said, T. O. Sippell, R. M. Hankins, Dr. W. M. Reid, F. L. Bouwman, T. E. Pulley, R. Foster, N. J. Gerold, Jr., A. W. Sedar, Dr. C. G. Goodchild, Dr. M. E. Pierce, Dr. L. H. Kleinholz, R. S. Howard, A. B. Williams, D. L. Nanney, M. Spiegel, Dr. T. H. Waterman, J. Bellisario, R. L. Birch, C. W. Donnelly, Dr. F. A. Brown, Jr.



ABBREVIATIONS REGARDING THE NATURE OF THE SUBSTRATUM
TO USE ON LARGE CHART

S --- sand
M --- mud
G --- gravel
Sh -- shells
St -- stones larger than gravel
P --- peat with well-preserved stems in it
dP -- decayed peat
O --- non-peaty organic matter present } . . . not specially odor-
Ø --- non-peaty organic matter abundant } iferous
F --- foul-smelling organic matter present
B --- black in color
D --- dark in color, but not black
f --- firm
s --- soft
W --- attached seaweed present
U --- unattached (drifting) seaweed present
a --- above

Combinations should be patterned as follows:

MS --- sandy mud
SM --- muddy sand
MG --- mud with some gravel
GS --- gravel with some sand
} i.e., the most abundant constituent always placed first, the least abundant last

GSSh - gravel with sand and less shell

fSBM - firm sand with black mud
DdPS - dark decayed peat with sand
} i.e., adjectives preceding words to which they apply

fSMFUaP - firm muddy sand with foul-smelling organic matter and drifting seaweed, above peat with well-preserved stems in it. (I.e., "above" is used between two layers which are one above the other; seaweed comes at the end (of the upper layer), even though it may be more abundant than some other constituents.

If you are forced to invent other abbreviations, write these below, but be sparing in this.

SUGGESTIONS FOR LACKEY'S BAY TRIP

It is suggested that this trip be planned as an introduction to quantitative ecology. Experience in previous years has shown that the type of quantitative study for which Lackey's Bay seems best suited consists in taking samples at a series of stations extending across the mud flats at right angles to the shore. Theoretically, this ought to show zonation of the various species, in accordance with the distance from low tide mark, somewhat modified by local variations of depth and stratum. It has been found, however, that in the short time available, a single team cannot take a sufficient number of sufficiently large samples to provide an adequate picture of the distribution of even the commoner species. Thus, it would seem desirable that the six teams should run parallel lines of stations within a relatively small area, the spacing of the stations being kept the same in each line. In this way, the results of the teams will be additive, and it is hoped that some reasonably significant results may be obtained. On the map posted in the lab, the proposed location and arrangement of the six lines of stations has been marked. The location has been chosen to give a fairly uniform increase in depth, over as uniform a substratum as seems to be available at Lackey's Bay. Satisfactory digging and seiving of samples can be continued out to a depth of about 90 cm., and it is thought that in the proposed location this depth will be reached at about 60 m. out from the low tide mark, if we can judge from incomplete figures in the reports of previous years. This would make possible a line of 21 stations at 3 m. intervals, as indicated on the map. Fewer stations or more may be worked, according as time and water depth permit. It is suggested that Station 1 be located just below low-water mark, or if this is too stony, at the first point out from shore where digging becomes feasible. If any team gets out to a depth beyond which digging and seiving becomes impracticable, and still has time left for further work, they may return to Station 1 and run a line of stations at 1 m. intervals back up the beach toward high water mark.

Perhaps a quick survey will be taken before starting work, to see whether another 60 m. stretch can be found, more uniform as to slope and substratum, and ending at a depth not exceeding 90 cm. Once the location has been chosen, a few members from each team should set up the three lines of stakes, as indicated on the map, the other members of the teams at first staying on shore, and either getting themselves organized for work, or making a preliminary study of the beach.

The stakes have been assembled in three bundles, each containing 21 stakes, tied to a strong cord at 3-meter intervals. A few loose stakes are also available for use, should it be desired to extend the line of stations out farther than the proposed 60 m. If possible, the stakes should be set up from boats, so as to cause a minimum of disturbance to the bottom.

Three boats would be required, each with a crew of three, and an additional fourth man should remain on shore opposite the center line of stakes. Procedure should be as follows: First, select the center bundle of stakes (these are slotted to receive number-cards at the top). Plant stake number 1 of the center row in the desired place. Then plant a separate stake higher up on the beach, placing it carefully so that by sighting stake number 1 against it, the correct line to follow for the center row will be obtained. Record the compass bearing of the line selected. Now plant the number 1 stake of each of the lateral rows, getting the correct distance from the center row by using the 6 m. cords which are provided, and taking care that the three number 1 stakes form a straight line exactly at 90° to the line chosen for the center row (another compass bearing may help here). Two men in the stern of each boat should now start unrolling their bundle of stakes while the oarsman takes the boat slowly out from shore. When the second stakes are reached, the one of the center row should be planted first, sighting carefully against the stakes already set up. This sighting can be done both by one of those on the boat, and by the fourth man staying on shore. The second stakes in the two lateral rows can now be set up at correct distances to either side by use of the 6 m. cords. Probably it will be found convenient to loop the ends of these cords over nails or pegs in the stern of each boat. It would also be well for one of the men in the stern of each boat to have a pole with which to push the boat this way or that. Each stake in the center row should receive its appropriate number card, inserted in the slot at the top, and facing outward, toward the lateral rows. Stakes in all rows should be pushed deep enough into the mud to withstand the necessary pulling they are subjected to when the cords are pulled tight, but not so deep as to be hard to pull out. For further clarification regarding the above directions, consult the diagram posted in the lab.

As soon as stakes for the first four stations have been set up, the teams on shore can start digging at station 1. Each team should be organized on a basis rather different from the customary arrangements on other field trips. Duties of individual team members should be as follows:

No. 1. Shovel. Must be able to deliver standard-sized shovelfuls on all types of bottom and in all depths of water up to three feet. Should also aid in identification of specimens.

No. 2. Seive. Should be careful not to lose any portion of the samples. Seiving should be gentle, especially where there are stones, to avoid washing too many of them clear through the seive. Where peat is met with, this will have to be broken up and the specimens picked out by hand, since very little of it will pass through the seive. No. 2 should also aid in identification of specimens and should be equipped with forceps secured by a string long enough to permit easy use to aid in

identification of specimens and should be equipped with forceps secured by a string long enough to permit easy use to aid in transferring specimens to the finger bowl or ark.

No. 3. Second shovel. Duties as for No. 1. May also be asked to help set up the stakes.

No. 4. Second seive. Duties as for No. 2. May also be asked to help set up the stakes.

No. 5. Species angel. Should concentrate on getting an accurate record of the number of each species taken at each station, not forgetting always to record the station number, and the bottle numbers in which the specimens were put.

No. 6. Physical environment angel. Should record the station number and time, water depth, and nature of the substratum at each station. Will require a watch, meter stick, and pad and pencil. Substratums likely to be met with include sand, gravel, mud (of various consistencies), peat, and various mixtures of these, with varying proportions of organic matter, large stones, etc. The records taken should be sufficiently detailed to show any differences between stations that might affect the populations of animals living there.

No. 7. Ark. Should carry a finger bowl, watch glass, forceps, pipette, and a sufficient supply of bottles. Should aid in transferring specimens from the seives to the finger bowl or watch glass, aid in their identification, and transfer to bottles all specimens to be saved. The forceps and pipette should be secured, each with a separate string. The watch glass should be carried in a safe pocket and probably will be used very little. It should be remembered that the total supply of bottles is sufficient to permit the use of an average of only two bottles or tubes per station.

No. 8. See bucket. Should go in advance of the rest of the team, observing the nature of the undisturbed substrate surface at each station where digging will be done, and making counts or estimates of the number of worm tubes, worm castings and surface forms of life met with in each three-meter length of a strip one meter wide. The dividing lines between successive three-meter strips should be chosen halfway between successive digging stations (estimating this by eye), and each strip can then be given the same number as the station which it includes. A meter length of wire, equipped with a handle for holding it close to the bottom, will aid in maintaining an even width.

No. 9. Substrate surface angel. Should record the observations made by the "see bucket."

At the first few stations, each team will probably have only one shovel and seive in action, but a second shovel and seive will become available as soon as all the stakes have been set up. Two adjacent stations can then be sampledat one time, individual team members taking up positions as indicated on the diagram posted in the laboratory. One

full-sized shovelful should be taken at each station but two or more shovelfuls (all seived together) may be taken at stations where the material sifts easily and where specimens seem not to be too abundant, if the team feels it has time to do this. In that event, the angel must be sure to record the number of shovelfuls taken, and the final results should be expressed as multiples of the average number for a single shovelful. Thus, if 9 specimens of a certain species were taken in two shovelfuls, this should be reported as $2x4\frac{1}{2}$.

At the first few stations, team members 8 and 9 should stay reasonably close to the rest of the team, so that all can see how the see-bucket is being used. Thereafter, these two should try to go ahead as fast as they can and when they have reached the end of the line of stations, should return to the shore, load the ark and its remaining bottles into a skiff, and proceed out to where their team is working. No. 7 thus will not need to wade ashore to exchange his full bottles for empty ones, and some of the team members may find that in the deeper water, they can work better sitting in the skiff than when standing in the water. It is hoped that four skiffs will be available, which should be assigned as follows: No. 1, team I; No. 2, teams II and III; No. 3, teams IV and V; and No. 4, team VI.

So far as possible, all identifications should be done in the field, but wherever there is any doubt, the specimens should be brought back to the lab for a further check. This applies not only to single specimens, but also to any number of specimens that may be taken, even when all appear in the field to belong to a single species; a more careful check in the lab may sometimes show that two or more species are involved.

While in the field, the trip captain will record air and water temperatures, and also any rise or fall in the tide at selected time intervals. This will make it possible to convert water depths reported by physical environment angel (who also records the time at which each depth is measured) to a common "low tide" basis.

After completing a laboratory check-up, each team should prepare a chart showing for each station the water depth, nature of substratum, and number of each species taken. They then should transpose these results to a large master chart that will be posted. So far as possible, the work involved should be shared among different team members. When all teams have recorded their results on the large master chart, computations will be made for each station in regard to the average depth, average nature of substratum, and total number of each species taken. After all this has been recorded, those interested may inspect the chart to see to what extent individual species show zonation correlated with depth, distance from shore, or nature of substratum. If desired, the quantitative distribution of a few selected species may be shown by drawing a graph, or by constructing diagrams of the type often used to show the vertical distribution of plankton.

INSTRUCTIONS FOR FILLING IN MASTER CHART

Each team should fill in for each station:

a. The water depth (in cm.).
b. The nature of the substratum (following the scheme of abbreviations posted here).
c. The number of each species taken. If any particular species is not already listed on the chart, write its name in an appropriate column.

In writing in the above data, be careful to use the space intended for your team. The numbers 1 to 6 in the diagram below show where teams 1 to 6 are supposed to record their respective data:

STATION NUMBER	Distance (in meters) from low tide line	Depth (in cm.) of water at low tide (or height above low water).		Nature of substratum.			ANNELIDA	
		1 3 5 2 4 6		1 2	3 4	5 6	Neanthes virens 1 3 5 2 4 6	etc.

EQUIPMENT NEEDED FOR LACKEY'S BAY TRIP

A. General (not assigned to individual teams):

3 bundles of 21 5-ft. stakes, tied at 3-meter intervals to strong cord
4 extra stakes, at least
1 compass
2 6-meter cords, each ending in aloop at each end
3 poles, for poling skiffs
21 large cards, bearing numbers 1 to 21
4 skiffs (three of them with a strong nail or peg in the center of the stern

B. To be assigned to teams:

6 x 2 shovels
6 x 2 seives
6 x 1 meter rulers.
6 x 1 arks
6 x 1 knapsacks
6 x 3 pairs forceps, on strings
6 x 1 pipettes, on separate strings
6 x 1 small finger bowls
6 x 1 watch glasses
6 x 1 angel pads and pencils
6 x 2 special angel pads and pencils
6 x 1 wrist watches
6 x 1 see-buckets
6 x 1 1-meter wires, with handles attached to right angles
6 x 1 hand lenses

C. Needed by trip captain:

1 watch
1 30-cm. ruler
1 small notebook and pencil
1 3-4 ft. stake
5 18-in. stakes

D. Needed in lab (to be distributed or posted before the trip:
*6 copies of "Suggestions for Lackey's Bay Field Trip"
*6 copies of "Diagram Showing Method for Setting up Stakes"
*6 copies of "Diagram Showing Locations to Be Taken up by by Individual Team Members"
1 large chart for recording all results
*7 copies of "Instructions for Filling in Master Chart (one copy to be posted beside this chart)

EQUIPMENT NEEDED FOR LACKEY'S BAY TRIP (cont.)

*7 copies of "Abbreviations Regarding the Nature of the Substratum, to Use on Large Chart" (one copy to be posted)
*7 copies of "Equipment Needed for Lackey's Bay Trip" (one copy to be posted)
*6 copies of map of Lackey's Bay, with proposed locations for the lines of stations marked

DIAGRAM SHOWING METHOD FOR SETTING UP THE STAKES REQUIRED IN QUANTITATIVE ECOLOGICAL SURVEY AT LACKEY'S BAY

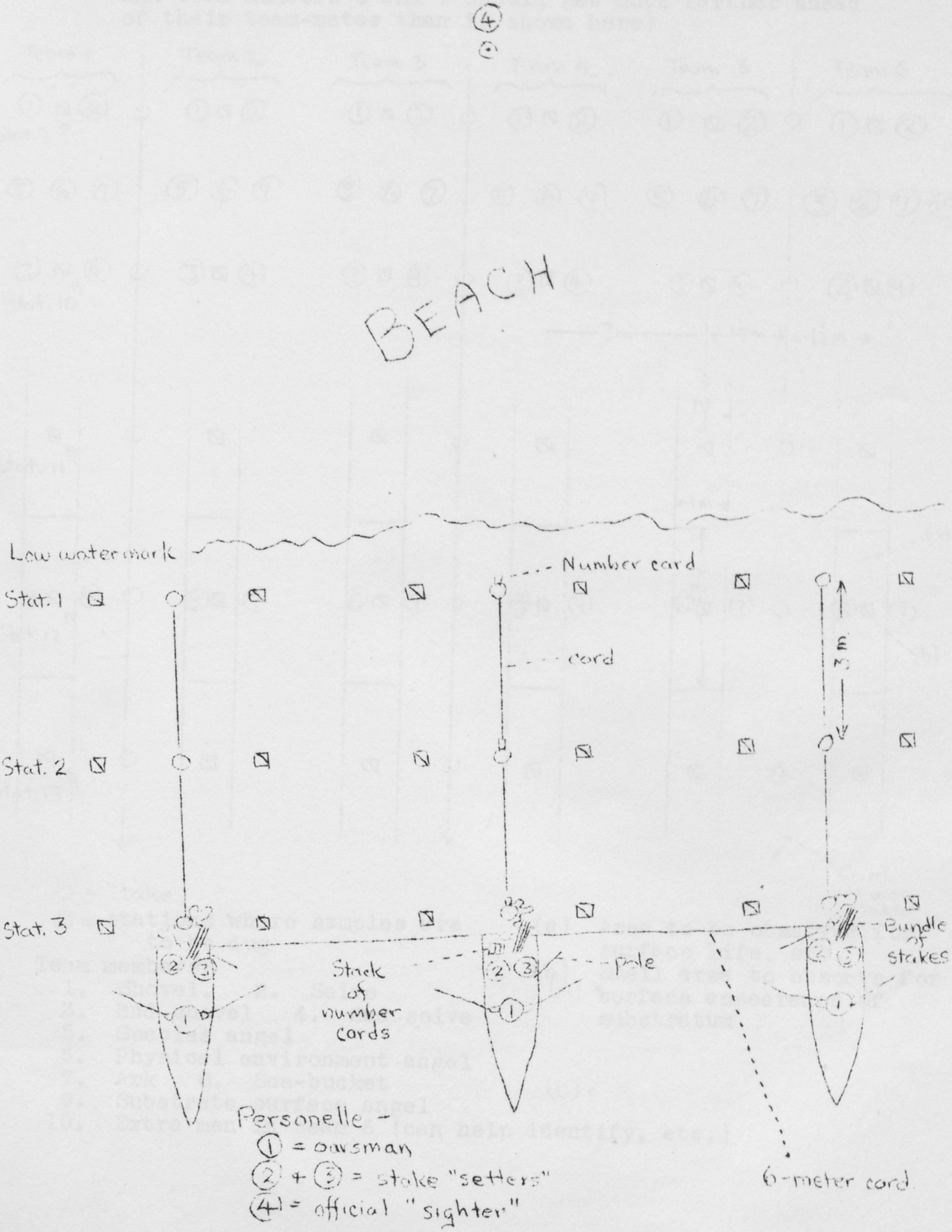
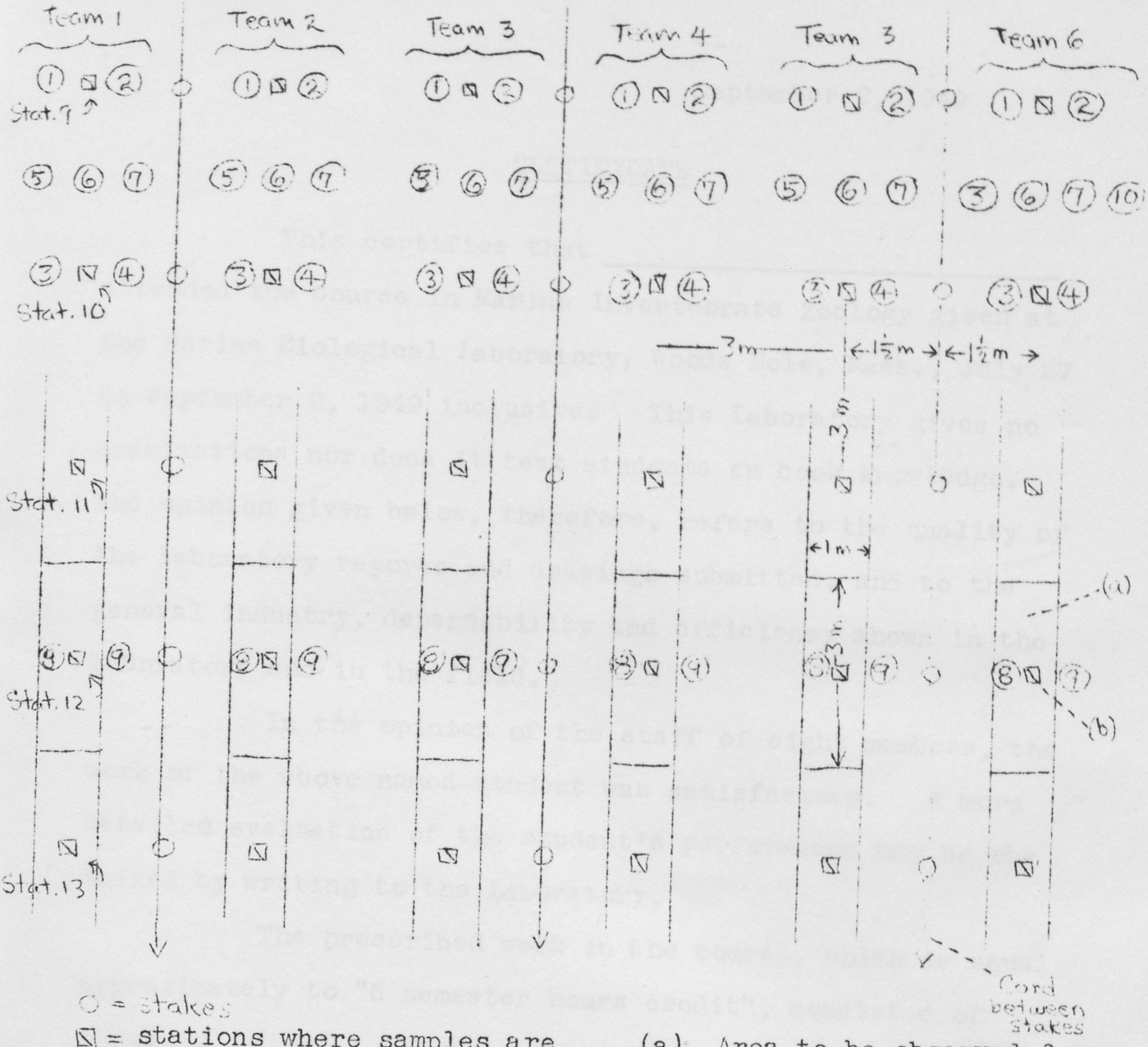


DIAGRAM SHOWING LOCATIONS TO BE TAKEN UP BY INDIVIDUAL TEAM MEMBERS WHILE CARRYING OUT QUANTITATIVE ECOLOGICAL SURVEY AT LACKEY'S BAY

(In actual practice, some teams will get ahead of others, and team members 8 and 9 should get much farther ahead of their team-mates than is shown here)



○ = Stakes

▨ = stations where samples are to be dug

Team members:

1. Shovel. 2. Seive
3. 2nd shovel 4. 2nd seive
5. Species angel
6. Physical environment angel
7. Ark 8. See-bucket
9. Substrate surface angel
10. Extra man in team 6 (can help identify, etc.)

(a) Area to be observed for surface life, etc.

(b) Small area to observe for surface appearance of substratum

MARINE BIOLOGICAL LABORATORY
WOODS HOLE, MASS.

September 2, 1949

CERTIFICATE

This certifies that _____
attended the Course in Marine Invertebrate Zoology given at the Marine Biological Laboratory, Woods Hole, Mass., July 27 to September 2, 1949 inclusive. This Laboratory gives no examinations nor does it test students on book knowledge. The opinion given below, therefore, refers to the quality of the laboratory reports and drawings submitted, and to the general industry, dependability and efficiency shown in the laboratory and in the field.

In the opinion of the staff of eight members, the work of the above named student was satisfactory. A more detailed evaluation of the student's performance may be obtained by writing to the Laboratory.

The prescribed work in the course, which is equal approximately to "6 semester hours credit", consisted of about

33 lectures

142 hours of supervised laboratory work (4½ hours daily and 2 hours evenings after field trips)

9 field trips (4 full days, 5 half-days)

_____
Instructor in Charge

INVERTEBRATE ZOOLOGY CLASS PROGRAM

1949

(Subject to change according to weather)

Tues.	July	26	7:30 P.M. Introductory remarks and lecture on the Sea.
Wed		27	Porifera (Burbanck, Kleinholz, Howard)
Thur		28	Coelenterata (Waterman, Pierce, Wilson)
Fri		29	Coelenterata (Waterman, Pierce, Wilson)
Sat		30	Coelenterata and Ctenophora (Waterman, Pierce, Wilson)
Sun		31	**********
Mon	August	1	Nobska (8:00-8:55-10:30); Lab review 11:00-12:30 Platyhelminthes (Hyman, Goodchild, Howard) 1:45-4:15
Tues		2	Platyhelminthes (Hyman, Goodchild, Howard)
Wed		3	Stony Beach (8:30-9:39-10:30); Lab review 11:00-12:30 Platyhelminthes (Goodchild, Reid, Howard) 1:45-4:15
Thur		4	Platyhelminthes (Goodchild, Reid, Howard)
Fri		5	Nemathelminthes (Goodchild, Reid, Howard) 9:00-12:00 Entoprocta and Ectoprocta (Burbanck, Brown, Wilson) 1:45-4:15
Sat.		6	Entoprocta and Ectoprocta (Burbanck, Brown, Wilson)
Sun.		7	**********
Mon		8	Annelida (Brown, Kleinholz, Wilson)
Tues		9	Annelida (Brown, Kleinholz, Wilson) 9:00-12:00 Hadley Harbor (1:30-3:09-4:00) Lab review 7:00-8:00
Wed		10	Annelida (Brown, Kleinholz, Wilson)
Thur		11	Annelida (Brown, Kleinholz, Wilson) Half day. Dredging (Teams I-III 9:00-11:30) (Teams IV-VI 1:45-4:15)
Fri		12	Mollusca (Pierce, Burbanck, Howard)
Sat		13	Mollusca (Pierce, Burbanck, Howard)
Sun		14	**********
Mon		15	Mollusca (Pierce, Burbanck, Howard)
Tues		16	Mollusca (Pierce, Burbanck, Howard)
Wed		17	Arthropoda (Lochhead, Goodchild, Howard)
Thur		18	Lagoon Pond (8:30-12:01-2:00) Lab review 4:00-5:00
Fri		19	Arthropoda (Lochhead, Goodchild, Howard)
Sat		20	Arthropoda (Lochhead, Goodchild, Howard)
Sun		21	**********
Mon		22	No. Falmouth (9:00-12:45-3:00) Lab Review 7:00-8:00
Tues		23	Arthropoda (Lochhead, Goodchild, Howard)
Wed		24	Cuttyhunk (9:00-2:16-3:00) Lab review 7:00-8:00
Thur		25	Echinodermata (Reid, Brown, Wilson)
Fri		26	Echinodermata (Reid, Brown, Wilson)
Sat		27	Echinodermata (Reid, Brown, Wilson)
Sun		28	**********
Mon		29	Tarpaulin Cove - Class Picnic
Tues		30	Plankton (Waterman, Burbanck, Lochhead, Kleinholz, Wilson)
Wed		31	Plankton (Waterman, Burbanck, Lochhead, Kleinholz, Wilson) 9:00-12:00 Protochordates (Kleinholz, Waterman, Wilson) 1:45-4:15
Thur	Sept	1	Lackeys Bay (8:00-9:26-11:00) Lab review 7:00-8:00 Protochordates (Kleinholz, Waterman, Wilson) 1:45-4:15
Fri		2	Protochordates (Kleinholz, Waterman, Wilson)

INVERTEBRATE ZOOLOGY COURSE - 1949

Teams and Instructors for Field Trips

Trip	FAB	THW	WDB	CGG	JHL	MEP	MWR	LHK
Robska Mon. Aug. 1 8:00-8:55-10:30	3	1	2	X	4	5	Capt.	6
Stony Wed. Aug. 3 8:30-9:39-10:30	Capt.	6	4	5	2	1	X	3
Hadley Harbor Tues. Aug. 9 1:30-3:09-4:00	2	3	X	6	1	Capt.	5	4
Dredging Thurs. Aug. 11 9:00-11:30 1:45-4:15	X X	X X	X 5	3 X	X 6	1 X	2 4	Capt.
Lagoon Pond Thurs. Aug. 18 8:30-12:01-2:00	1	Capt.	3	4	5	6	2	X
North Falmouth Mon. Aug. 22 9:00-12:45-3:00	3	5	6	Capt.	2	X	4	1
Cuttyhunk Wed. Aug. 24 9:00-2:16-3:00	X	4	Capt.	1	3	2	6	5
Lackey's Bay Thurs. Sept. 1 8:00-9:26-11:00	X	6	1	2	Capt.	4	3	5

Before the start of each field trip all members of each team are to provide themselves with the proper collecting apparti (according to the "Team Organization" list) and report to the proper instructor (according to the above chart) in the laboratory. Students are not to proceed to the boat until given specific permission by the Team's instructor. N.B. that each team member is entirely responsible for the care and return in clean condition of his or her assigned piece of equipment.

Immediately upon return from a trip, the team members must help the Ark Angel remove all specimens to cool, fresh, salt-water in approximately sized finger bowls, watch glasses, etc. with numbered labels (pencil on paper) corresponding to the Ark bottle numbers. This must be done at once, before leaving for meals or to change clothes.

Teams will meet in the laboratory with their respective instructors, usually at 7:30, to review the names and characteristics of the forms collected, to preserve specimens where desirable, to identify small and doubtful forms, and to prepare field trip reports when and as directed. Angel must check and hand in team collecting record.

SUGGESTIONS FOR FIELD TRIP ETIQUETTE

1. Care of the equipment: The equipment which is used on field trips is expensive and hard to replace. Each team member should feel a personal responsibility for the preservation and use of the particular materials assigned to his position on the team. The care of equipment begins with seeing that it is carried from lab to field, and ends with cleaning it and stacking it neatly by the desk at least by 10 pm of the night of the trip. Constant check on equipment should be made in the field, especially in moving from area to area.

2. The ark: Ark bottles often have a tendency to float away to sea. In dealing with deep mud bottoms, or with water of uncertain depth, the ark should be left on safe ground and bottles taken collecting site in the knapsack. Mixing bottle numbers with cork numbers can give rise to great confusion in the angel record and in checking arks after the trip. The ark members should be sure to match bottles and corks at all times. By 10 pm of the night of the trip the ark bottles should be washed and arranged in order in the arks.

3. The angel: The recording angel should take particular care in writing up the trip, being careful (1)to keep the pad dry, (2)to report accurately the name of specimens collected, and (3)to record any ecological data given by the team captain. By 10 pm of the night of the trip the angel pads and team checklists should be neatly in order and placed on the angels' desks to be collected by the assistant.

4. The specimens: Specimens in ark bottles should be protected from heat and suffocation by shading the ark and changing water when necessary. Upon returning to lab, specimens should be placed in fingerbowls with fresh sea-water and accurately labeled with number and field identification. After working over the collection in lab with the team captain, the specimens should be disposed of as desired and the glassware cleaned and returned to the back table -- this should be accomplished by 10 pm of the night of the trip.

5. Forceps and pipette: These instruments are especially subject to loss and should be kept on string around the neck of the carrier. Forceps should be washed with fresh water upon return to the lab, if not sooner.

6. Knapsack: In the field the knapsack is of course used to carry ark bottles. In assembling equipment after a trip it is especially useful as a holder for white pan, fingerbowl, watch glass, forceps, and pipette.

7. See-bucket: The staff suffers damaged reputation every time the glass in the bottom of the see-bucket has to be replaced. This piece of equipment was not designed as a seat or rock container.

8. Miscellaneous: All equipment has a tendency to pick up sand, dirt, etc. Team members who rinse their equipment with fresh water after trips are likely to gain the favor of the assistants.

CHECK LIST OF INVERTEBRATE ANIMALS

Commonly or occasionally found by the

Invertebrate Zoology Classes

at the

Marine Biological Laboratory
Woods Hole, Mass.

Forms marked (*) are most commonly found or are conspicuous for other reasons. Where a name used in Pratt's "Manual of the Common Invertebrate Animals" (revised edition) differs from the name used in the check list, the name used in Pratt follows the check list name, and is enclosed in parentheses.

Woods Hole, Mass.

****

PHYLUM PROTOZOA
(not listed)

PHYLUM PORIFERA
Class Calcarea
*Leucosolenia botryoides
*Sycon ciliatum
Class Demospongia
*Chalina arbuscula
Cliona celata
*Halicondria panicea
*Microciona prolifera
Suberites compacta
Tethya gravida

PHYLUM COELENTERATA
Class Hydrozoa
Abietinaria abietina
*Bougainvillia
B. caroliensis
B. superciliaris
*Campanularia
C. calceolifera
C. flexuosa
Clava leptostyla
Clytia
C. edwardsi
C. johnstoni
(C. bicophora)
Cordylophora lacustris
Corynitis agassizi
(Gemmaria gemmosa)
Ectopleura ochracea
*Eudendrium
E. album
E. carneum
E. ramosum
E. tunue
Eutima mira
Gonionemus murbachi
Halecium halecinum
*Hydractinia echinata
Nemopsis bachei
*Obelia
O. commissuralis
O. geniculata
O. bicuspidata
*Pennaria tiarella
Physalia pelagica
Podocoryne
P. carnea
P. fulgurans
*Schizotricha tenella
*Sertularia pumila
Stylactis hooperi

Thuiaria argentea
*Tubularia
T. crocea
T. larynx
Turritopsis nutricula
Class Scyphozoa
Aurelia aurita
Cyanea capillata
Dactylometra quinquecirrha
Class Anthozoa
Alcyonium carneum
*Astrangia danae
*Edwardsia
E. elegans
E. leidyi
Eloactis producta
Halcampa farinacea
*Metridium dianthus
*Sagartia
S. leucolena
S. luciae
S. modesta

PHYLUM CTENOPHORA
*Mnemiopsis leidyi

PHYLUM PLATYHELMINTHES
Class Turbellaria
Sub-class Acoela
*Polychoerus caudatus
Order Tricladida
*Bdelloura candida
Procerodes wheatlandi
Syncoelidium pellucidum
Order Polycladida
Eustylochus ellipticus
Planocera
P. inquilina
P. nebulosa
Stylochus zebra

PHYLUM NEMERTEA
Order Paleonemertea
Cephalothrix spiralis
Order Heteronemertea
*Cerebratulus lacteus
*Lineus
L. bicolor
L. ruber
L. socialis
*Micrura leidyi
Order Haplonemertea
Amphiporus ochraceus
*Tetrastemma
T. candidum
T. vermiculum

PHYLUM NEMATHELMINTHES
(not listed)
PHYLUM TROCHELMINTHES
(not listed)

PHYLUM MOLLUSCOIDEA
Class Entoprocta
Barentsia
*Pedicellina
Class Ectoprocta
*Acanthodesia tenuis
Aetea anguina
Aeverrillia setigera
Alcyonidium
*Bowerbankia gracilis var.
*Bugula
*B. flabellata
*B. turrita
Callopora aurita
*Crisia eburnea
*Cryptosula pallasiana
Electra
E. hastingsae
*E. pilosa
Flustrella hispida
Hippodiplosia
H. americana
*H. pertusa
Hippoporina contracta
Hippothoa hyalina
Membranipora
M. crustulenta ? (M. lacroixii)
M. tuberculata
Microporella ciliata
Schizmopora avicularis
Schizomavella auriculata
Schizoporella unicornis
*Smittina trispinosa var. nitida
*Stephanosella biaperta
Tegella unicornis
PHYLUM ANNELIDA
Class Chaetopoda
Sub-class Polychaeta
Ampharete setosa
*Amphitrite
A. brunnea
A. ornata
Anaitides catenula
*Arabella iricolor
*Arenicola
A. cristata
A. marina
*Autolytus sp.
Chaetopterus variopedatus
*Cirratulus grandis
*Cistenides gouldi

*Clymenella torquata
*Diopatra cuprea
Dodecaceria coralii
*Enoplobranchus sanguineus
Eteone sp.
*Eulalia annulata
Eumidia sanguinea
Flabelligera affinis
*Glycera
G. americana
G. dibranchiata
Harmothoe imbricata
*Hydroides dianthus
Laonice viridis
Lepidometria commensalis
*Lepidonotus squamatus
Lumbrineris
L. hebes
L. tenuis
Maldane urceolata
Marphysa sanguinea
*Neanthes
N. succinea
N. virens
Nephtys
N. bucera
N. incisa
Nereis pelagica
Nicolea zostericola
Ninoe nigripes
Notomastus
N. filiformis
N. luridus
Orbinia ornata
Parasabella microphthalmia
Pholoe minuta
Pista palmata
Platynereis dumerillii
Podarke obscura
*Polycirrus eximius
Polydora sp.
Pseudopotamilla oculifera
Sabellaria vulgaris
Haploscoloplos
H. fragilis
H, robustus
Spio setosa
*Spirorbis spirorbis
Stauronereis sp.
*Sthenelais leidyi
Syllis sp.
Terebella rubra
Terebellides stroemi
Thelepus cincinnatus
Travisia forbesi
Sub-class Oligochaeta
Enchytraeus albidus
Lumbricillus agilis

PHYLUM ANNELIDA (Cont'd)
Class Gephyrea
*Phascolosoma gouldi

PHYLUM MOLLUSCA
Class Amphineura
*Chaetopleura apiculata
Class Pelecypoda
*Anomia simplex
*Arca
A. campechiensis pexata
A. transversa
Astrarte castanea
Barnea truncata
Brachidontes demissus plicatilis
Cardium pinnatulum
Corbula contracta
*Cumingla tellinoides
Ensis directus
Gemma gemma
Gouldia mactracea
Laevicardium mortoni
Lyonsia hyalina
Macoma
M. balthica
M. tenta
Mactra solidissima
*Modiolus modiolus
Mulinia lateralis
*Mya arenaria
*Mytilus edulis
Noetia ponderosa
Nucula sp.
Ostrea virginica
Pandora gouldiana
*Pecten irradians
Periploma leanum
Petricola pholadiformis
*Solemya velum
Tagelus
T. divisus
T. gibbus
*Tellina tenera
*Teredo navalis
*Venus mercenaria
Yoldia limatula
Class Gastropoda
Acanthodoris sp.
*Acmaea testudinalis
Aeolidia sp.
Alexia myosotis
Anachis avara
*Bittium alternatum
Busycon
B. canaliculatum
B. carica

Class Gastropoda (Continued)
Caecum pulchellum
Carithiopsis
*C. adamsii
C. greeni
C. subulata
*Coryphella sp.
Crepidula
*C. fornicata
C. glauca
C. plana
Elysia sp.
Epitonium sp.
Eupleura caudatum
Facelina bostoniensis
Haminosa solitaria
*Lacuna vincta
Littorina
*L. littorea
L. obtusata
L. saxitilis
*Melampus lineatus
Melanella intermedia
*Mitrella lunata
*Nassa obsoleta
N. tribittata
N. vibex
Natica clausa
*Odostomia sp.
Polinices
*P. duplicata
P. heros
Rissoa ap.
Thais lapillus
Turbonilla sp.
Turritella sp.
*Urosalpinx cinereus
Vermicularia spirata
Class Cephalopoda
Loligo pealeii

PHYLUM ARTHROPODA
Sub-phylum Crustacea
(Sub-order Cladocera and Classes Ostracoda, Copepoda, and Branchiura not listed.)
Class Cirripedia
Order Thoracica
*Balanus
B. balanoides
B. eburneus
*Chthamalus fragilis
Lepas
L. anatifera
L. fascicularis
Order Mysidacea
*Heteromysis formosa
Michtheimysis stenolepis

Order Cumacea
Diastylis sp.
Order Tanaidacea
Leptochelia savignyi
Tanais cavolinii
Order Isopoda
Chiridotea
C. caeca
C. tuftsii
Cirolana concharum
Cyathura carinata
Edotea triloba
Erichsonella filiformis
*Idothea
I. baltica
I. metallica
I. phosphorea
*Ja era marina
Limnoria lignorum
Philoscia muscorum
Probopyrus pandalicola
Sphaeroma quadridentatum
Order Amphipoda
Aeginella longicornis
Allorchestes littoralis
Ampelisca macrocephala
Amphithoe
A. longimana
A. rubricata
*Caprella acutifrons
Carinogammarus muoronatus
Chelura terebrans
*Corophium cylindricum
Elasmopus levis
*Gammarus
G. Annulatus
G. locusta
G. marinus
Sub-phylum Crustacea
Class Malacostraca
Order Amphipoda
Grubia compta
Haustorius arenarius
Jassa marmorata
Lembos smithi
Leptocheirus pinguis
Lysianopsis alba
Melite nitida
Microdeutopus gryllotalpa
*Orchestia platensia
Stenotho
S. cypris
S. minuta
Talorchestia longicornis
Unciola irrorata
Order Decapoda
Callianassa stimpsoni

PHYLUM ARTHROPODA (Cont'd)

Order Decapoda

*Callinectes sapidus

*Cancer

C. borealis

C. irroratus

*Carcinides maenas

*Crago septemspinosus

Emerita talpoida

Eurypanopeus depressus

Heterocrypta granulata

Homarus americanus

*Libinia

L. dubia

L. emarginata

*Neopanope texana

Ovalipes ocellatus

*Pagurus

P. acadianus

P. longicarpus

P. pollicaris

P. pubescens

Palaemonetes vulgaris

Panopeus herbsti

Pelia mutica

Pinnixa

P. chaetopterana

P. cylindrica

P. sayana

*Pinnotheres maculatus

*Uca

U. minax

U. pugilator

U. pugnax

Upogebia affinis

Virbius zostericola

Order Stomatopoda

Chloridella empusa

Sub-phylum Insecta

Class Apterygota

Order Collembola

*Anurida maritima

Sub-phylum Arachnida

Class Xiphosura

*Limulus polyphemus

Class Acarina

Family Halacaridae

Various species

Class Pseudoscorpionidea

One species (Chelonethida)

Class Pycnogonida

Anoplodactylus lentus

Pallene empusa

Tanystylum orbiculare

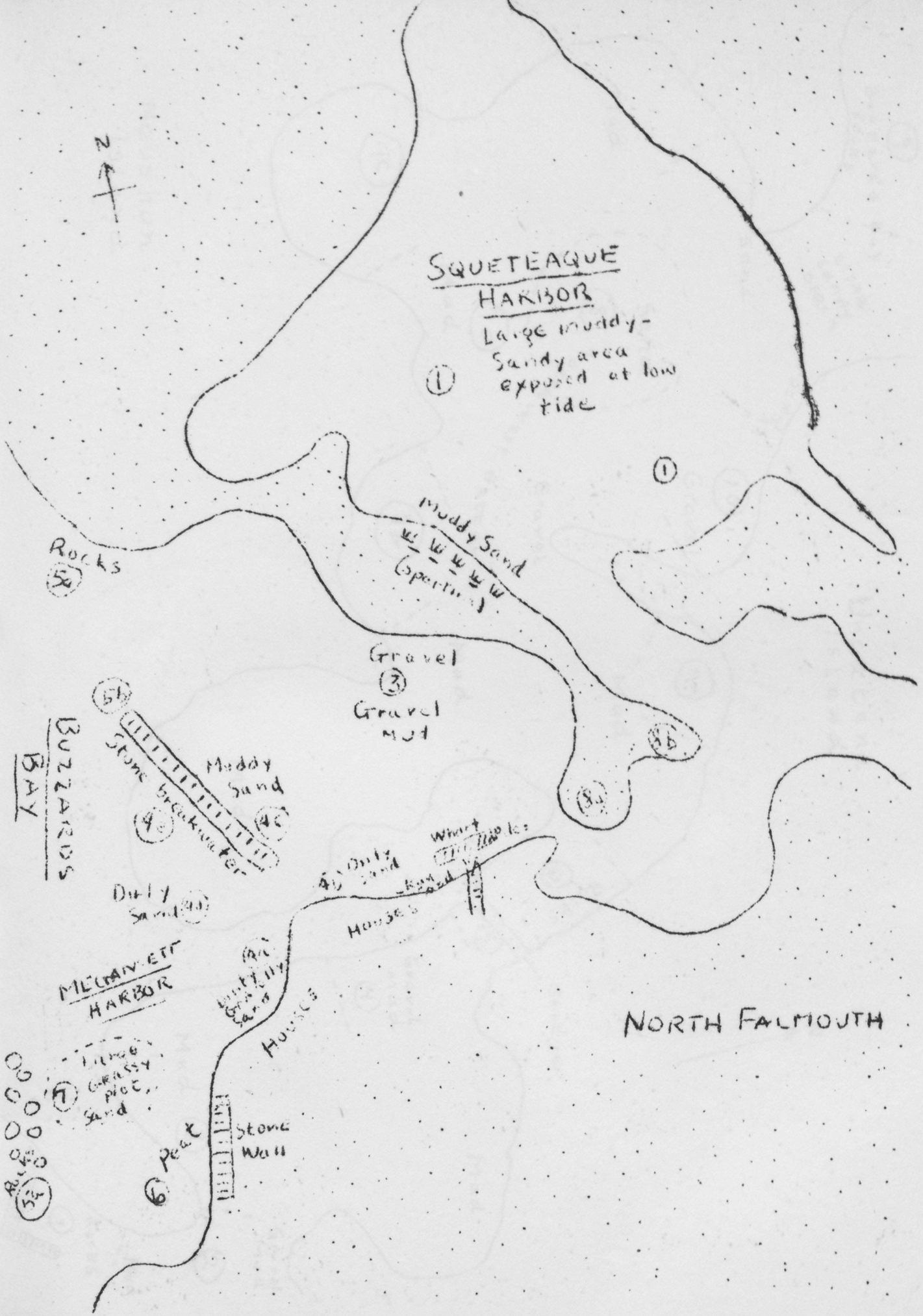
Incertae sedis

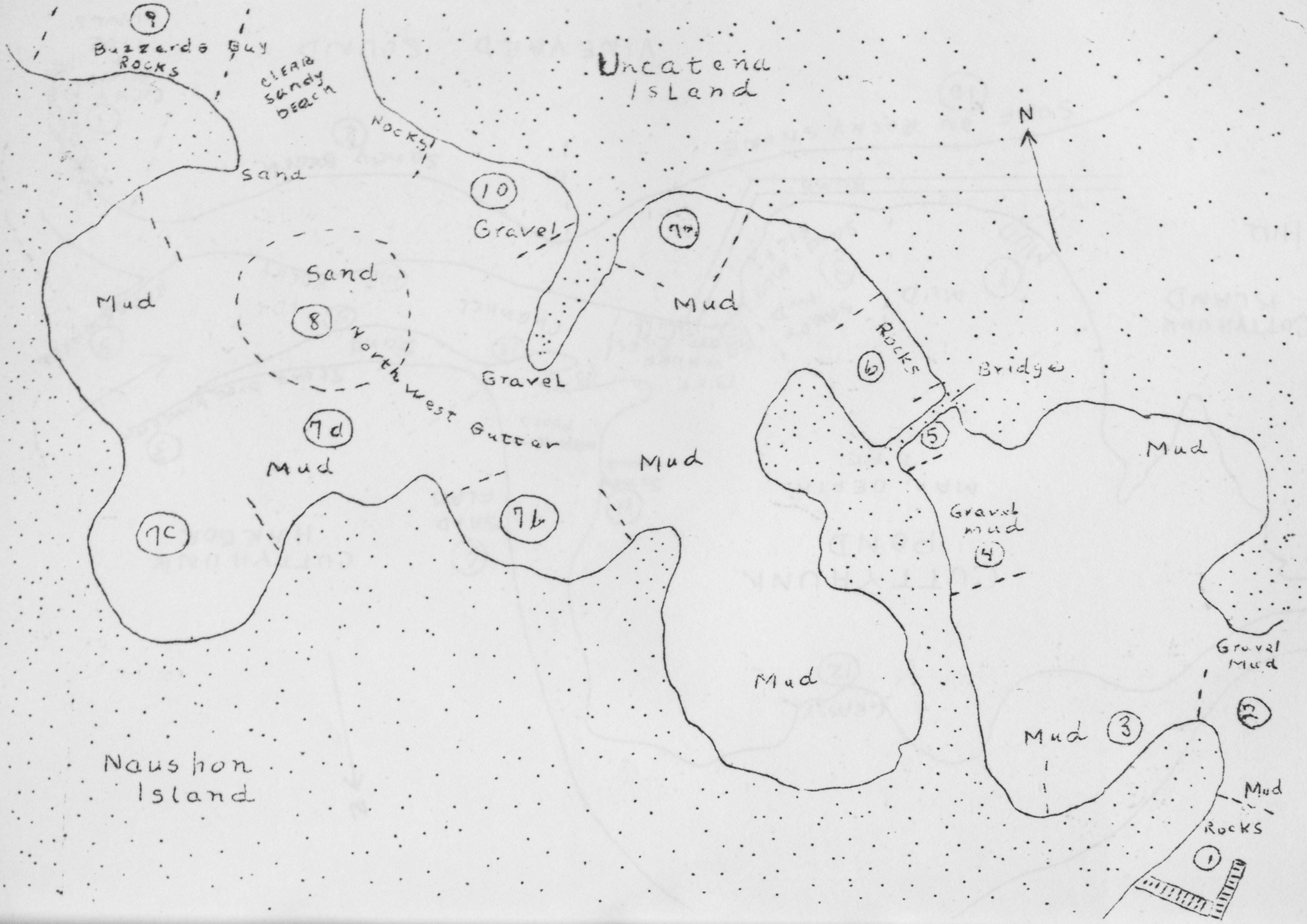
Class Tardigrada

Various species

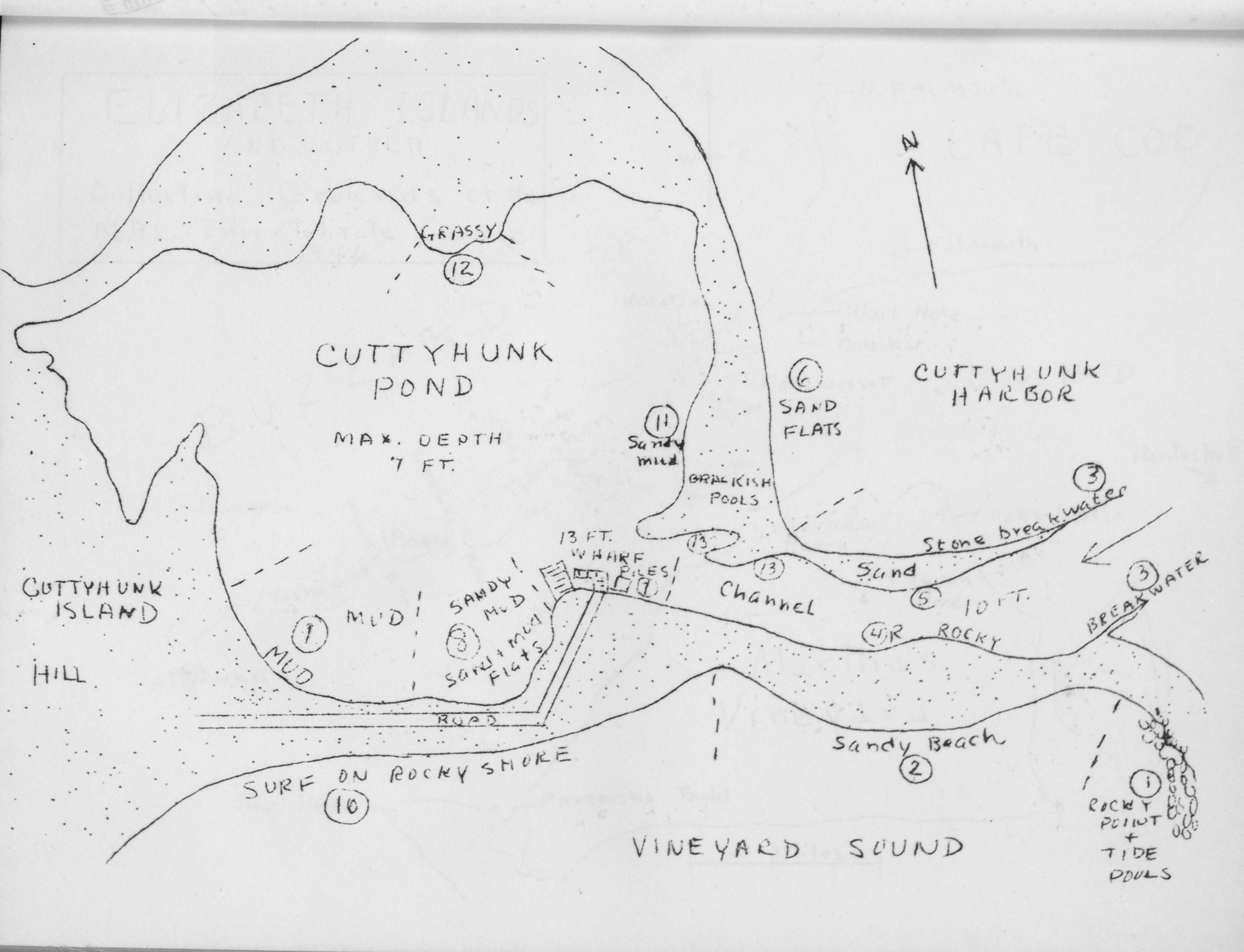
PHYLUM ECHINODERMATA
Class Asteroidea
*Asterias
A. forbesi
A. vulgaris
*Henricia sanguinolenta
Class Ophiuroidea
*Amphipholis squamata
*Ophioderma brevispinum
Ophiopholis aculeata
Ophiura sp.
Class Echinoidea
*Arbacia punctulata
*Echinarachnius parma
Strongylocentrotus drobachiensis
Class Holothuroidea
*Leptosynapta
L. inhaerons
L. roseola
*Thyone briareus

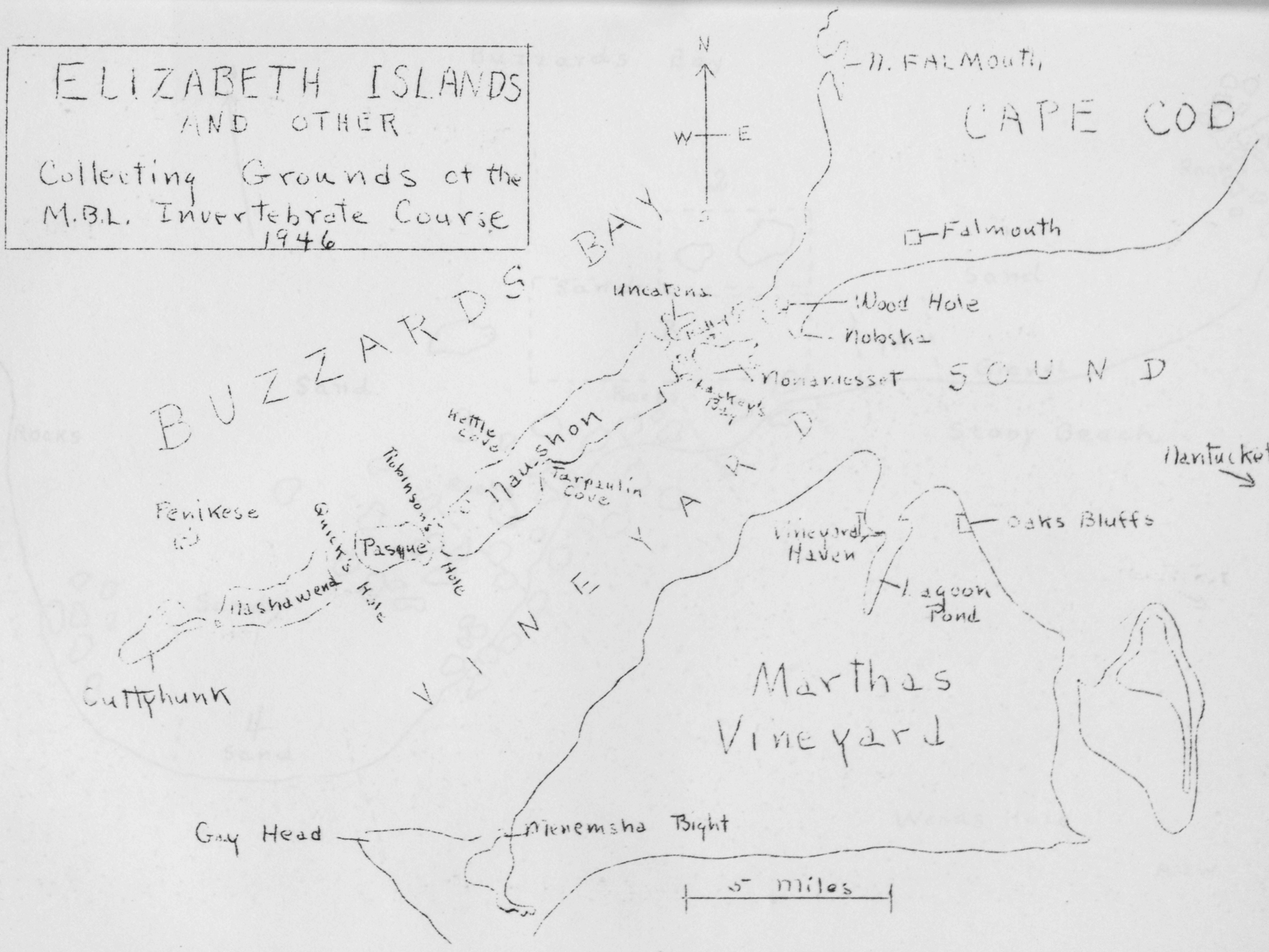
PHYLUM CHORDATA
Subphylum Enteropneusta (Hemichordata)
*Saccoglossus kowalevskyi
Subphylum Tunicata (Urochordata)
Amaroucium
*A. constellatum
A. pellucidum
A. stellatum
Appendicularia
*Botryllus schlosseri
Ciona intestinalis
Didemnum
*D. candidum lutarium
D. albidum
Molgula
M. arenata
*M. manhattensis
*Perophora viridis
Salpa
*Stvela partita

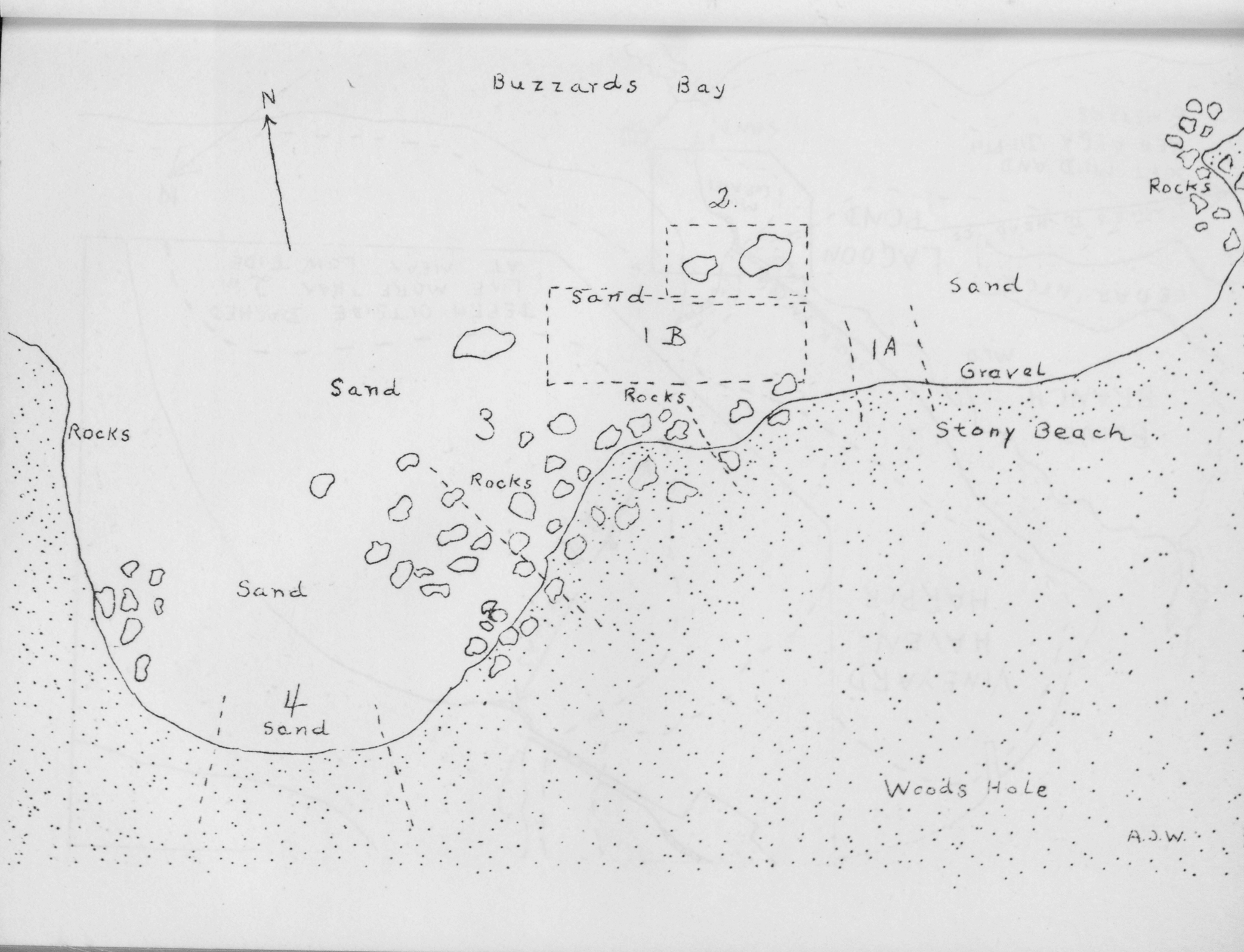

N
SQUETEAQUE HARBOR
Large muddy-Sandy area exposed at low tide
1
1
Muddy Sand
(Spartina)
Rocks
5a
Gravel
3
Gravel Mud
5b
Stone breakwater
Muddy Sand
4c
4c
BUZZARDS BAY
Dirty Sand
4d
Dirty Sand
Wharf
Houses
MEGANSETT HARBOR
Dirty Gravelly Sand
Houses
NORTH FALMOUTH
Large Grassy plot, Sand
7
Peat
Stone Wall
6
5a

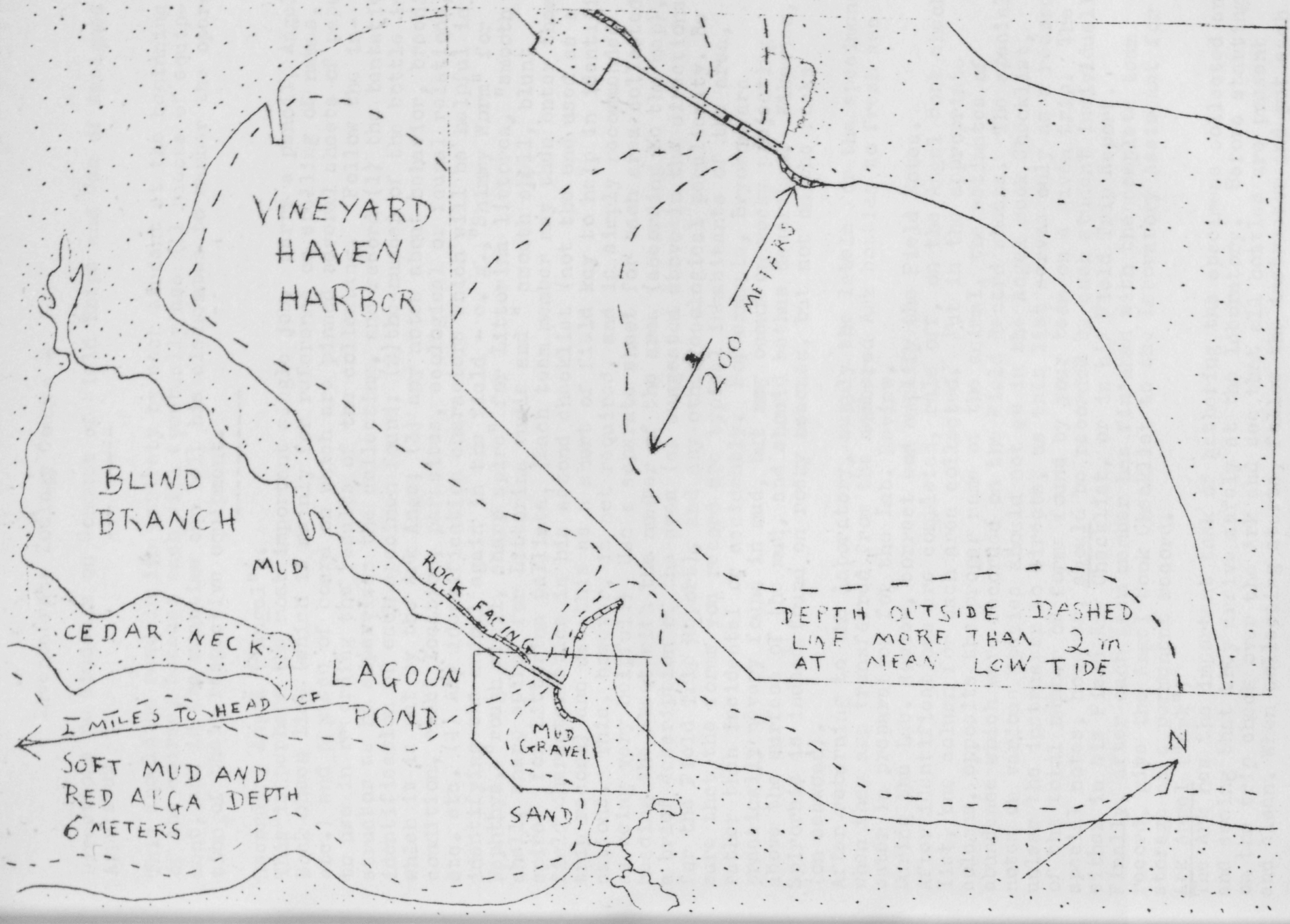


9
Buzzards Bay
Rocks
Clear Sandy Beach
Uncatena Island
N
Rocks
Sand
10
Gravel
7a
Sand
Mud
8
North West Gutter
Mud
Gravel
Rocks
6
Bridge
5
7d
Mud
Mud
Mud
7c
7b
Gravel mud
4
Mud
Gravel Mud
2
Mud
3
Mud
Rocks
1
Naushon Island


N
GRASSY
12
CUTTYHUNK POND
MAX. DEPTH 7 FT.
11
Sandy mud
6
SAND FLATS
CUTTYHUNK HARBOR
BRACKISH POOLS
3
Stone breakwater
13 FT. WHARF
PILES
1
13
Sand
Channel
5
10 FT.
3
BREAKWATER
CUTTYHUNK ISLAND
1
MUD
MUD
SANDY MUD
8
SAND + MUD FLATS
4R
ROCKY
HILL
ROAD
Sandy Beach
2
SURF ON ROCKY SHORE
10
1
ROCKY POINT + TIDE POOLS
VINEYARD SOUND


ELIZABETH ISLANDS
AND OTHER
Collecting Grounds of the
M.B.L. Invertebrate Course
1946
N
W
E
S
N. FALMOUTH
CAPE COD
BUZZARDS BAY
Falmouth
Uncatena
Wood Hole
Nobska
Nonamesset
SOUND
Kettle Cove
Naushon
Tarpaulin Cove
Robinsons Hole
Penikese
Quick's Hole
Pasque
Nashawena
VINEYARD
Nantucket
Vineyard Haven
Oaks Bluffs
Lagoon Pond
Cuttyhunk
Marthas Vineyard
Gay Head
Nenemsha Bight
5 miles


Buzzards Bay
N
2.
Sand
1B
1A
Sand
Gravel
Rocks
Rocks
Sand
Rocks
3
Rocks
Stony Beach
Sand
4
Sand
Woods Hole
A.J.W.


VINEYARD
HAVEN
HARBOR
200 METERS
BLIND
BRANCH
MUD
ROCK FACING
CEDAR NECK
LAGOON
POND
1 MILES TO HEAD OF
SOFT MUD AND
RED ALGA DEPTH
6 METERS
MUD
GRAVEL
SAND
DEPTH OUTSIDE DASHED
LINE MORE THAN 2 m
AT MEAN LOW TIDE
N

Invertebrate Zoology Course -

Directions to Students on Conduct of Field Trips and Use of Assigned Apparatus

---

This should be read in its entirety by each student at the beginning of the course, because each will eventually use all pieces of equipment, and in the meantime can help his classmates to remember the operation of their respective equipment.

Recording Angel ("Angel").
This is perhaps the most important single job. Carry a pencil, Angel Book Check list (which is mainly for reference on spelling of names, etc.) and a piece of board on which are pinned several sheets of paper to use in recording the results of the collecting. Follow the instructor as he supervises the collecting, and record (1) the tentative identification of each specimen found; (2)the number of the bottle into which it is put by the Ark Ange; (3) any notes about behavior, breeding condition, exact location, parasites, ecological or faunal relations, etc. etc. (4) Any identification characters which will be helpful in identifying the animal again in the field - e. g.,"Shimmy Worm" for Nephthys, "rough shell, sharp spire" for Littorina littorea, "smooth shell, sharp spire" for Littorina rudis and " smooth shell, blunt spire" for Littorina palliata. (Each team member may then enter these field characteristics in his second checklist (not the one used as a trip record) and use this as a short of field key to help in identifications. This, however, is not required, and is simply recommended as being very helpful.) Use a separate sheet for each area collected, heading the sheet with the number of the area (according to the map), a brief description of the area (as suggested above in the directions for the Field Trip Report), and any other ecological peculiarity. Be sure that the forms you record are typical inhabitants of the area, rather than incidental or accidently. For example, Bryozoa are practically nevery found in mud, but may occur on rocks projecting above the surface of the mud, and should bethus described. Likewise, Spirorbis is indeed found on rocky beaches, but not on the rocks (on seaweed).
After returning to the laboratory, supply the labels for the specimens when they are transferred from the numbered Ark bottles to fresh sea water in preparation for the Lab. Review.
During the Lab. Review, correct and amplify the Field Notes.
After identifications are completed, rule off, on the Angel Book checklist, one column for each area collected. Put in the appropriate columns, opposite the proper name of the animal, the estimates of abundance which were recorded on the Field Record sheets. The special notes on various species should not go in the Angel Book Checklist, unless the instructor so directs, as this list serves only as a record of the total number of forms found by your team on a given trip. The special notes, however, should be recorded by each student individually, either in his Field Key Checklist, or in his Field Trip Report.
Finally, after each team member has finished with the complete team record, give the Angel Book Checklist to the Laboratory Assistant for storage as a permanent record.
Ark Angel ("Ark")
The Ark has the important task of gathering the specimens collected and and seeing that they arrive safely at the laboratory. Before starting on the trip check over the Ark and see that all bottles are present and clean. When collecting starts, follow the instructor and put each

specimen, as collected, into a bottle of appropriate size. Make certain that the Recording Angel puts down the number of the bottle in which the specimen is put. Do not crowd the bottles - worms and crustacea, in particular, need plenty of space. Leave some air space in the bottles. If "doubling up " becomes necessary, be very careful not to mix up the records by putting two animals of the same species from different areas together, and be careful not to mix animals which will prey on one another. Be sure the bottles are securely corked while the Ark is in motion, but whenever a lengthy pause occurs (particularly at lunch time or on the trip home) take the opportunity to uncork the bottles to permit aeration, replace turbid water, and splash the bottles in cool water. Don't dip the whole Ark, as the bottles will float away. It is your responsibility to see that the animals reach home in good shape. You may either carry the whole Ark with you (watch out for slippery rocks) continuously, or set it on shore and just carry out a knapsack full of bottles, returning for a new load when necessary. In any case, be sure the Ark, when not being carried, is on a flat solid support, out of reach of the tide, and in the shade, if possible; and be sure to dash water over it frequently to keep the bottles cool.

Immediately upon return from a trip, and before changing clothese or leaving for supper, transfer all specimens from the bottles to fresh sea water in watch glasses or finger bowls. Be sure each is accompanied by a label (in pencile) bearing its proper bottle number, and be certain that the bottles are really empty - small forms often stick around the shoulder of the bottle or on the cork, Gastropods and some crustaces will often climb out, so should be loosely covered, Don't mix forms which will prey on one another. Rinse out Ark bottles return in proper order, and replace the Ark in the Storeroom.

Sea Bucket

Do not use the See Bucket for carrying anything, and do not let others put anything in the bucket, as the window breaks easily and is difficult to replace. Be careful not to sink it or let it float away from you. Never try to fill any buck when the boat is in motion. In using the See Bucket be sure the window is clean. Visibility is improved by wetting the upper surface of the glass and by putting the face close to the glass.

Scrape Net

Be careful not to catch the netting between the steel edge and the piling.

Sieve

Hold by bottom, rather than by hand holes, as shovel may bruise your fingers. Shake gently, particularly when gravel is present, as otherwise delicate animals will be macerated. With thick mud, water should be splashed in from above.

White Pan, Finger Bowl, Watch Glass, Forceps, etc. These are for examining more closely the animals caught in net or sieve. Stick close to the instructor and be ready to take charge of the animal and transfer it to the Ark Angel when the identification is made.

INVERTEBRATE ZOOLOGY COURSE - Student Field Trip Report

Name _____ Team ___ Trip _____ Date _____

Time of Low Tide _____ Collected from ____m. to ____m. Air Temp.___

Water temp._____(indicate local variations from this below under habitat description) Weather: Wind_____ Sky_____

Precipitation _____

I. Describe each of the habitats studied. Refer to appended map. Include such factors as: nature of substratum (be more specific than "mud" or "rocks"); degree of exposure to or protection from wave action (estimate importance of scouring and impact), strong currents; temperature fluctuations, direct sunlight, salinity fluctuations, silt, pollution; tidal levels involved; important plants.

II. List the most abundant species, in the order of abundance, in each habitat. If more than one natural grouping or definable association of abundant species occurs in a habitat, distinguish them. Distinguish the few (3 or 4) most important species which characterize or define each association.

	Species	Ecologic notes
Area No.,Habitat or Association 1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
Area No.,Habitat or Association 1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

Trip No..... Locality..... Map area.....

Date..... Weather.....
Temp. (a) Air....°C Water....°C: Low tide .....m: Collected .....m.
Exposed to (a)Waves?.....(b)Stg.Currents?.....(c)Silt?.....
Any fresh water?..... Pollution..... Team.....
General description of area.....
.....
.....

Field identification	Bottle	Exact Habitat (Use key)	Abun-dance	Ecological notes etc.

(Continued over)

Field identification	B.	E.Hab.	Abund.	Ecological notes etc.

Area No., Habitat or Association	Species	Ecologic Notes
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

III. Probable or possible ecological correlations or questions: Think about the area and the animals studies, their physical and biotic environment, their needs, adaptations and problems. Using your imagination and common sense, but without "loose" thinking, suggest pertinent questions about this area and attempt explanations of the presence and absence of particular species. Some examples of general questions: Are predators, herbivores, scavengers, or plankton feeders markedly more important here, one than the other? Why? What general groups of animals are absent or poorly represented? Do they have a common denominator which might explain this? What are the most important problems of existence of each habitat which all its fauna must overcome? What differences are discernible between the exact situation and habit of life of species belonging to genera or families of which we have more than one member in this vicinity? Point out correlations between structure or habit of life and physical conditions imposed by the environment (e.g. byssus threads in mussels which live exposed to currents, permanent tube-dwellers more common, wandering burrowers less common in clay-mud where gas exchange below surface is probably poor and cohesion of particles may impede burrowing). Use back of sheet or extra pages if necessary.

IV. Notes about rare, striking or otherwise interesting forms not covered elsewhere.

Habitat Symbols to be used in angel sheets and check lists.

1. In sand or mud:

CS = Clear sand
MS = Muddy sand
M = Pure Mud
PS = Peaty sand
G = among grass roots.

Use figures to indicate depth in inches, e. g. MS_6 = 6" down in muddy sand; CS_0 = at surface of clean sand

2. Attached to or crawling on rocks in still water:

Ex = On exposed surface
U = On underside

3. Attached to or crawling on rocks exposed to waves or to strong currents

Ex = Exposed surface
U = On underside

4. On or among algae in still water.

U - Ulva
A = Ascophyllum
F = Fucus
E = Enteromorpha
R = Red Alga

NR = Not recognized

5. On or among algae exposed to waves or strong currents

Use same key letters as in category 4

6. Free Swimming or floating:

TP = in tide pools
S = Over sand or mud
O = In open water (Tow net collections)
SP = Spray pools

7. On pilings

T = In tidal zone
B = Below tidal zone
U = Unattached

8. Dredged in deep water

S = On sand bottom
G = On gravel bottom
A = On algae

PORIFERA

The Laboratory outline is prepared for a full day of work on the Porifera, therefore, organize your work so that at the end of the day you will have accomplished those parts of the laboratory study which are most interesting and beneficial to you.

The regeneration experiments on Microciona will be started first in order that the material may be observed during the day.

Regeneration of Microciona. Each student will need a finger bowl, a Syracuse watch-glass and a slide. The group at each table will need an additional finger bowl to be used in common. Fill the finger bowls about two-thirds full of sea-water. Place the watch-glass on the bottom and the slide on top of the watch glass. Into the common bowl, cells and broken fragments from Microciona will be pressed through fine meshed bolting cloth. Pipette a small quantity of this material into the finger bowls containing the slides. As soon as the cells have had time to settle on the slide, carefully lift it out and examine it. Return it carefully to the finger bowl. Study several times during the day. Have the cells shown a tendency to aggregate?

The structure of the reunition mass and the cells involved are discussed in papers by Wilson and Galtsoff. Many of the cell types, the syncytium which forms the surface layer and all of the skeleton are retained by the bolting cloth. Wilson considers three types as represented in the free cells, one, the choanocytes which remain specific and produce a new gastral epithelium, two, cells with nucleolate nuclei and possessing granules to a variable degree in their cytosomes and, three, non-nucleolate cells possessing in their cytosomes fine granules of uniform size which gives to the cells a grayish color. He regards the nucleolate type as a primordial cell which produces in the reunition mass, as in the adult, skeletal and reproductive cells. The non-nucleolate cell produces the syncytium which covers the body and lines those canals not occupied by choanocytes. Some indication of cell types can be learned from fresh preparations but fixed and stained material has been found more satisfactory. Do not spend too much time trying to identify the different types of cells.

Ascon type. (Leucosolenia). Note relation of individuals to each other and prevalence of buds. Make a habit sketch.

Select an individual which is relatively free from debris. With a razor or sharp scalpel cut it lengthwise and place the halves on a slide so that both inner and outer surfaces may be viewed under the same cover glass. Note the different types of spicules and especially their general arrangement. Note, also the lack of cementing material (spongin) to hold them together such as will be found in some monaxonid sponges, e. g. Halichondria and Chalina. Focus on the gastral surface and observe the beating of the chonocyte flagella (The details of the collar cells may be studied better in Sycon). Note the numerous porocytes with their intracellular openings. Draw a porocyte and some adjacent choanocytes.

A spicules in this form and also in Sycon, Chalina, Halichondria and Microciona can be studied best by examining the residue which remains

after sponges have been boiled in potassium hydroxide. Material has been treated in this way for you and is available on the preparation table. Draw acetic acid under the cover glasses of your several preparations. Note the results. Illustrations and names of some typical spicule shapes are found in Pratt's Manual and Hyman's Invertebrates, p. 298. Compare the descriptions given in the text for species represented in Woods Hole with your observations. Draw each type of spicule found in each of the sponges available. For the non-calcaria refer to Vosmaer's The sponge of the Bay of Naples. Write the s cientific term under each spicule. See pp. 95-105 in Delage and Herouard. Emphasis has been placed on the form of the spicules because the spicule is frequently the most constant morphological structure available in the classification of some sponges.

Sycon type. (Sycon called Grantia in older texts and Scypha by de Laubenfels). Suggestions for study of this sponge are given in Drew (pp. 37-40, 5th edit.) The remarks here are supplementary to the text. The relative sizes of incurrent and radial canals and their relation to each other may be seen clearly in a tangential section of a dried skeleton made as follows: Two cuts are made parallel to the long axis; the plane of the first passes tangentially to the middle of the body wall, the plane of the second, parrallel to the first, is made sufficiently near the long axis so as to include part of the inner surface.

Study of living material. Observation on the direction of current flow can be carried out either in Sycon according to directions in Drew or in Halichondria which shows a more vigorous flow of current if good material is available.

It is suggested that, instead of tangential sections mentioned in Drew for living material that you try to make cross-sections sufficiently thin to allow study with a 4 mm. objective and yet, in making the sections, to retain typical relations of the animal's structures. Sections a quarter to a fifth of a millimeter are about right. The choanocytes lining the radial canal will be visible and with proper adjustment of condenser and illumination one may observe flagella, collars, a nd the prosopyles. Draw a choanocyte if you did not observe one in the fragments of Microciona.

Observe also in this preparation germ cells and developing embryos which may be present. Compare those amphiblastulae with the larvae of Microciona. Those latter may be obtained by dissecting pieces of the adult sponge with needles. When freed from the parent the larvae swim out into the surrounding water. Draw whatever embryonic stages, either in sycon or microciona that may be available in your material.

Leucon type. 1. (Chalina). Observe the general organization of the colony. Indentify the oscula. Only a rough idea can be gained of the anatomy of the sponge from thin cross-sections but such a p reparation will reveal the manner in which short monaxonid spicules are effectively held together by spongin. Use magnification of about 400 x. Make a drawing to illustrate this.

2. (Halichondria). Place a piece of a colony in a Syracuse watch glass and look for an osculum. Note the vigorous flow of water from it. A thin membrane which forms the osculum is a syncytium. It covers the surface of the sponge forming its dermal

layer. The spicules beneath support it like so many tent poles. Numerous ostia are present in the membrane which lead into the extensive subdermal space below. From this lead the incurrent canals directing the water toward the flagellated chambers.

Other sponges. Examine such other sponges as may be available in the laboratory, probably Tethya and Cliona. Cliona is the sulphur sponge- smell it. It is able to bore into shells, but apparently not by the use of an acid.

All records of your work on the Porifera are due not later than to- morrow morning at 9:00 a. m.

REFERENCES

Clarke, F. W. and W. C. Wheeler. 1922. The inorganic constituents of marine invertebrates. Dept. of Interior, Professional Paper No. 124. 2nd edit.

Dellage, Y. and Ed. Herouard. 1899. Spongiaires in Traite de Zoologie Concrete, Paris.

Fincher, J. A. 1940. The origin of the germ cells in Stylotella heliophila Wilson (Tetraxonida) Jour. Morph. 67:175-197.

Galtsoff, P. S. 1923. The Amoeboid movement of dissociated sponge cells. Biol. Bull. 45:153-161.

" " 1925. Regeneration after dissociation (an experimental study on sponges). 1. Behavior of dissociated cells of Microciona prolifera under normal and altered conditions. Jour. Exp. Zool. 42:183-221.

" " 1925. Regeneration after dissociation (an experimental study on sponges) 11. Histogenesis of Microciona pro- lifera, Verr. Jour. Exp. Zool. 42:223-255.

" " 1929. Heteroagglutination of dissociated sponge cells. Biol. Bull. 57:250-260

Hyman, L. H. 1940. The Invertebrates: Protozoa through Ctenophora. McGraw-Hill Book Co. N. Y.

Parker, G. F. 1910 Reactions of sponges with a consideration of the origin of the nervous system. Jour. Exp. Zool. 8

" " 1914. On the strength of water currents produced by sponges. Jour. Exp. Zool. 16.

Rentschel, E. 1923-1925. Parazoa in Kukenthal and Krumbach's Handbuch der zoologie, I. 307-418

Vosmaer, G. C. J. 1932-35. The sponges of the Bay of Naples, pori- fera incalcaria. Vol. 1-3. Capita Zoologica.

Wilson, Henry Van Peters, and James T. Penny. 1930. The regeneration of sponges (Microciona) from dissociated cells. Jour. Exp. Zool., v. 56: 73-147.

Wilson, H. V. 1932 Sponges and biology. Amer. Nat. 66:159-170.

Wilson, H. V. 1935. Some critical points in the metamorphosis of the halichondrine sponge larva. Jour. Morph. 58:285-353.

Wilson, H. V. 1938. Behavior of the epidermis in sponges (Microciona) when treated with narcotics or attacked by aquarium degeneration. Jour. Exp. Zool. 79.

Classification of the Phylum Porifera

Class 1. Calcarea of Calcispongia, the calcareous sponges. Skeleton composed of separate calcareous spicules, one-, three-, four-rayed, not divisible into megascleres and microscleres.
Order 1. Homocoela, the ascon sponges. Structure asconoid.
Order 2. Heterocoela. Structure syconoid or leuconoid.

Class 11. Hexactinellida or Triaxonida or Hyalospongiae, the glass sponges. Skeleton composed of triaxon (six-rayed) siliceous spicules or some modification of the triaxon form, separate or united into networks; choanocytes limited to finger-shaped chambers arranged in a simple or folded layer; without surface epithelium.
Order 1. Hexasterophora. With hexasters, without amphidisks.
Order 2. Amphidiscophora. With amphidisks, no hexasters.

Class 111. Demospongiae. Skeleton of siliceous spicules or horny fibers or both; siliceous spicules not triaxon, generally differentiated into megascleres and microscleres; flagellated chambers mostly small, round, of the leuconoid type.
Subclass 1. Tetractinellida. With tetraxon spicules; no sponging; spicules sometimes wanting.
Order 1. Myxospongida. Without spicules; structure simple.
Order 2. Carnosa or Homosclerophora or microsclerophora. Megascleres and microscleres not sharply differentiated; mostly without trianes; asters may be present.
Order 3. Choristida. With long-shafted triaenes; megascleres and microscleres distinct.
Suborder 1. Astrophora. Microscleres include asters.
Suborder 2. Sigmatophora. Microsclores when present are sigmas.
Subclass 11. Monaxonida. Megascleres monaxonial; with or without spongin.
Order 4. Hadromerina or Astromonaxonellida. Megascleres mostly tylostyles; microscleres when present some form of aster; without spongin. (Cliona, Suberites, Tethys).
Order 5. Halichondrina. Megascleres mostly of two or more kinds; microscleres wanting or are rhaphias, with little spongin. (Halichondria)
Order 6. Poecilosclerina. Megascleres often of two or more sorts, localized in distribution; reticulate, united by more or less spongin; often with echinating spicules; microscleres include sigmas, chelas, and toxas. (Microciona)
Order 7. Haplosclerina. Megascleres of one kind, diactinal, without special localization; with or without microscleres; spongin generally present. (Chalina)
Subclass 111. Keratosa, the horny sponges, Skeleton composed of spongin fibres, without siliceous spicules.

Taken from "The Invertebrates" by L. H. Hyman.

CLIONA, HALICHONDRIA, CHALINA

Since the three leuconoid sponges, Cliona*. Halichondria, and Chaline are all in the subclass Monaxonida, it is not surprising that they resemble each other in various ways. However, their spicules, gross anatomy, and ecology differ in so many respects that a comparison of the three genera is valuable.

When pieces of each sponge are boiled in 5% KOH, the spongin is dissociated allowing the siliceous spicules to remain in the residue. A microscopic study of this sediment in alcohol reveals that Cliona has megascleres made up of tylostyles and spirasters while Halichondria possesses megascleres of both diactinal and monactinal oxea. Chalina also has oxea. The last two may be further differentiated by examining small macerated pieces. These reveal that the oxea of Halichondria are strewn through the flesh paralleling the surface while those of Chalina form a meshwork of single or parallel oxea united by spongin.

In shallow water, both Cliona and Halichondria grow on rocks forming low encrustations while in deep water their growth is massive. Often clam shells, particularly of Venus, are completely riddled by Cliona. The broken surface of fragments show extensive tunnels eroded by the sponge. Each tunnel has an osculum slightly raised above the mass of sponge encrusting the surface of the shell. The color of Cliona is light yellow and the substance of the sponge is very compact with a sulfurous odor. Halichondria on the other hand even when taken from deep water is soft and brownish-yellow in appearance with high delicate oscula through which water pours with more vigor than is true of either of the other two sponges. Lastly, the branching yellowish-brown spongin ladened flesh of Chalina perforated with many osucla has resulted in its picturesque common name of Dead Men's Fingers. Except when broken from its stalk and washed ashore, this sponge is not found in shallow water.

MICROCIONA

This large spectacular sponge is found in two forms. In shallow water it forms a soft thin encrustation on rocks and shells while in deeper more quiet water, the colonies grow on the same substratum, but become massive measuring up to 15 cm. in height. Although the siliceous spicules of Microciona prepared by boiling pieces of the animal in 5% KOH are interesting because of the several types present (isochelas, toxas, acanthostyles), the most unusual characteristic of this animal is its tremendous capacity to regenerate.

Even in 24-48 hours, interesting studies may be made on Microciona by microscopically examining cells thoroughly dissociated by forcing a piece of the living animal through bolting cloth (heavy silk used in processing wheat flour) onto slides supported by syracuse dishes in fingerbowls of clean seawater. Apparently, only choanocytes, nucleated archaeocytes, and granular amoebocytes are able to survive such strenuous maceration. It is still an open question whether archaeocytes or amoebocytes may form chonaocytes if the latter are completely removed by careful and laborious methods.

2--*Microciona*.

During reorganization, small filopodia may be seen to extend to scattered cells and upon contact with them withdraw pulling a cell or cells into the small clump of already aggregated cells. After several days, flickering choanocyte flagella may be seen under oil immersion within even tiny cell aggregations.

In early August, swimming larvae may be observed upon macerating adult specimens in sea water and studying the resulting preparations with the microscope.

LEUCOSOLENIA

The small, delicate sponge, Leucosolenia, grows in shallow water below low tide mark, on large boulders and jetties. In spite of its apparent frailty, this sponge is found where wave action is intense and does not live in calm water.

The colony of Leucosolenia consists of clusters of slender whitish tubular individuals joined together irregularly. It is difficult to define an individual because of the budding type of growth typical of representatives of this genus. Individual branches may be up to 25 mm. in height. Examination with the unaided eye reveals that there is a simple circular opening, an osculum, at the distal end of each mature tube. Carmine suspended in sea water containing a living individual is carried away from the osculum instead of into it. The opening serves, therefore, as a vent or exhalent siphon rather than a mouth.

Favorable sections made with a razor, or better, stained permanent slides of a cross-section of Leucosolenia, help explain the structure of and water circulation through the animal. It is now seen that the water issuing from the osculum must have entered the internal cavity, or spongocoel, of the animal through the many fine pores in the body wall. The pores in Leucosolenia are different from those in more complex types of sponges by being intracellular. These modified epidermal cells are aptly named porocytes. Although the chief function of these specialized cells is to admit water, they can ingest such organisms as diatoms. Usually, the cells are described as resembling a doughnut in shape; actually, they more closely resemble a short piece of hollow macaroni.

Pieces of the macerated living sponge may exhibit independent and erratic movements. Under high magnification, one of the pieces which has come to rest may be seen to be made up in part of uniflagellated cells, the choanocytes.

Around the bases of the flagella of some of these cells, hyaline collars project from the cell bodies, but in other choanocytes, their collars may be retracted. The beat of the flagella of the choanocytes pumps water through the sponge. Another equally important function of the choanocytes depends on the ability of the cell body to engulf small particles of food around the outer edge of the base of its collar. The food is then digested and its derivatives are passed on to wandering amoebocytes. Finally, the choanocytes take an active part in the nourishment of the ova and sperm and facilitate their union.

The general physiological condition of the animal determines the activity of the flagella, and the flow of water is controlled also by the porocytes and by a perforated membrane which is stretched across the osculum. There is no coordinating nervous system, the individual cells responding to stimuli as independent effectors. Sponges with this porocyte-spongocoel-osculum arrangement are typical of the simplest Porifera, and are called ascon sponges.

Many sponges are maintained in a turgid semi-rigid condition by the pressure of the current of water propelled through the system of pores and canals. To some extent this may be true of Leucosolenia, although the chief means of support appears to be small interlaced calcareous rods or spicules. Those which are elongate and pointed at each end are the monaxon type while those with three rays are the triaxon type. Found less frequently are tetraxon spicules with rays radiating in tetrahedral arrangement.

Spicules dissected from the living specimen may show amoebocytes which secrete spicules and are called calcoblasts. This is an appropriate name, for when an acid is added to the spicules, an effervescence follows which indicates that the spicules are composed of calcium carbonate. The monaxon spicules may be seen to be formed by two calcoblasts working in cooperation while the triaxon type is formed by three.

Leucosolenia, as a typical ascon sponge, represents a stage through which the more complex sycon and leucon sponges pass during their development. Because of this and other resemblances, reference will be made to this simple sponge during the discussion of the more complex ones which follow.

SCYPHA (= Sycon)

Scypha[1] is a small marine sponge belonging to the Class Calcarea. It resembles Leucosolenia and occupies the same shallow water habitat where wave action provides the animals with an adequate supply of food and with well oxygenated water.

In both size and anatomical complexity, Scypha surpasses Leucosolenia. Individuals of Scypha are 20-25 mm. in height and 5-6 in diameter. Asexually reproducing animals may have one or two sizable buds attached to the base of the parent. Branching, however, is by no means as extensive as in the ascon sponges.

In Leucosolenia, the single tubular spongocoel is lined with collar cells. In Scypha, and other sycon-type sponges, there is still a tubular spongocoel, but the choanocytes are located in many regularly spaced radial canals which radiate from the spongocoel. Perhaps, therefore, the increased mass of the syconoid sponges has resulted from this adaptation, which, in proportion to the mass of the body, presents a far larger number of choanocytes to the food-laden water, than occurs in ascon sponges. Longitudinal sections through both the living and dried specimens reveal the openings of the radial canals into the spongocoel. These are the apopyles or internal ostia. In cross-sections, it can be seen that the outer ends of the radial canals end blindly. Careful scrutiny may also reveal tiny openings between individual collar cells in the layer of choanocytes, the prosopyles. Externally, the animal possesses over

---

[1] Formerly misnamed Grantia.

its entire surface innumerable pores or ostia. In Leucosolenia, the pores are intracellular; in Scypha, they are intercellular. Water entering the ostia goes directly into blind pouches, incurrent canals, which have no choanocytes but are lined with epidermis. The incurrent canals are of approximately the same size and shape as the radial canals, and interdigitate among the latter in the body wall. The prosopyles mentioned earlier connect the incurrent and radial canals. The water propelled by the choanocytes flows from the former into the latter. Finally, it flows through the apopyles into the spongocoel and out through a relatively large osculum at the distal end of each individual.

Around the osculum of Scypha there are giant monaxon spicules arranged to form a funnel-shaped collar. With this exception, the spicules are morphologically and developmentally very similar to those in Leucosolenia. The spicules of Scypha are arranged in a definite manner in respect to its canal system. At their distal, blind ends, the radial canals fuse with the epidermis to form a cortex. This cortex has monaxon spicules partly projecting beyond its surface giving the animal a bristly appearance. Along the radial canals, triaxon spicules are arranged in a pattern with one point directed toward the distal end of the canal. Finally, around the apopyles, next to the spongocoel, tetraxon spicules occur along with the triaxon spicules.

Scypha is a hermaphroditic organism, in which spermatozoa and ova arise from specialized amoebocytes called archaeocytes. In macerated living Scypha and in stained sections, sperm spheres and segmenting eggs are frequently found. Swimming amphiblastula larvae are common during the summer months on the north eastern Atlantic coast of United States. Stained whole mounts of the larvae show plainly that the flagellated cells are smaller and more numerous than the nonflagellated ones. When the larva finally comes to rest, an invagination takes place and an osculum breaks through at an end opposite a point of attach-

ment. The flagellated larval cells develop collars and become functional choanocytes. At this time, S cypha closely resembles the simple ascon sponge.

Laboratory Program for Cnidaria and Ctenophora

The material available in the laboratory and described in the outline provides more than ample subject matter for a full schedule. Thus systematic, anatomical, histological, and various physiological observations could each of them afford a more than full three days. It is suggested that the student adapt his work to the background and interests that he brings to the course. Except for suggesting that he acq uaint himself with enough systematic and morphological jargon and analysis to identify the most common species, the student is left with pretty much of a free hand to browse and study, provided, of course, that concrete evidence in the form of drawings and notes shows that something worthwhile was accomplished.

The main levels on which the work may be carried out are suggested by the following topics (outline references where pertinent experiments or techniques are given are indicated by the Roman numerals):

1. Identification of hydroids from the key.
2. Comparative morphology of the cnidarian body plan.
3. Growth, habitat, coloration, etc.
4. General histology.
   a. Teased fresh material. 1, Vl
   b. Macerated tissue lll, Vl
5. Nematocysts: comparative structure and physiology. Vll
6. Flagellar and ciliary currents. 1, Vl, Vll
7. Feeding reactions 1, ll, V, Vl, Vll.
8. Digestion 1, Vl, Vll
9. Nervous system and sense organs. 1, V, Vl, Vll
10. Neuromuscular system. 1, Vl, Vll
11. Reproduction and development. 1, V, Vll
12. Regeneration 1, ll

Additional material for study (not specifically included in the outline) will include: 1. Physalia (feeding and polymorphic colonies). 2. Cyanea (Swimming and feeding). 3. Astrangia (structure and feeding).

Ctenophore study will be maily of the local Mnemiopsis in which locomotion, morphology, and grafting experiments deserve attention.

Dichotomous Key to the Hydroids Most Commonly Found in Shore Collecting in the Vicinity of Woods Hole.

(Talbot Waterman, 1948) (Data from Fraser, 1944)

1. A. Hydranths and gonophores not protected by cup-shaped or vase-shaped parts of the periderm. Sub-order Gymnoblastea. 2
   B. Hydranths and gonophores protected by hydrothecae and gonothecae. Sub-Order Calyptoblastea. 19

2. (1)A. Hydranths with filiform tentacles only. 3
   B. Hydranths with capitate tentacles. 18

3. (2) A. Hydranths with scattered filiform tentacles. Clavidae. 4
   B. Hydranths with distinct whorls of filiform tentacles. 6

4. (3) A. Gonophores clusters of subspherical sporosacs just below proximal tentacles or ovoid sporosacs on the stem. 5.
B. Gonophores single pedicelate medusae. Turritopsis nutricula.

5. (4) A. Hydranths arising singly from a stolon. Clava leptostgla.
B. Hydranths in branching colonies. Cordylophora lacustris.

6. (3) A. Hydranths with a single whorl of filiform tentacles. 7
B. Hydranths with more than one whorl of tentacles. 15

7. (6) A. Colonies without periderm around pedical of zooids. Mydracti-
B. Colonies with periderm cut to base of hydranth nidae. 8. sup.10

8. (7) A. Stolons covered by crust of free coenosarc. 9
B. Stolons not so covered. Stylactis hooperi.

9. (8) A. Gonophores (dioecious) borne on pedicel of reproductive zooids with reduced tentacles; spines rough. Hydractinia echinata.
B. Gonophores borne on pedicel of nutritive zooid (hydranth); spines smooth. P odocoryne carnea.

10. (7) A. Hydranths with conical proboscis. Atractylidae Bougainvillea. 11
B. Hydranths with trumpet shaped proboscis. Eudendridae Eudendrium. 12

11. (10) A. Stem simple Bougainvillea superciliaris.
B. Stem fascicled (in bundles). Bougainvillea carolinensis.

12. (10) A. Stem Simple. 13
B. Stem but not main branches fascicled. 14

13. (12) AStem branches and pedicels annulated at the base. Eudendrium tenue.
B. No distinct annulations. Eudenidrium album.

14. (13) A. Reproductive hydranths with gonophores aborted. Eudendrium carneum.
B. Reproductive hydranths with gonophores not, or but little, aborted. Eudendrium ramosum.

15. (6) A. Hyrandths with proximal whorl of tentacles longer than distal. Tubularidae. 16
B. Hydranths with two or more whorls of tentacles similar in size. Margelopsidae Margelopsis gibbesi.

16. (15) A. Oral tentacles number about 24, proximal about 30. Ectopleura dumortieri
B. Oral and distal tentacles about the same in number. Tubularia. 17

17. (15) A. Apical processes of gonophores laterally compressed, stems not annulated. Tubularia crocea.
B. Apical process of gonophores conical, stom usually annulated. Tubularia larynx.

18. (2) A. Hydranths with both filiform and capitate tentacles. Pennaridae Pennaria tiarella.
B. Hydranths with capitate tentacles only. Corynidae Corynitis agassizi.

19. (1) A. Hydrothecae free from the stem or branches. 20
B. Hydrothecae sessile and more or less adnate to the stem or branches. 27

20.(19) A. Hydrothecae bell-shaped, diaphragm at base of hydranth but no operculum on hydrotheca. Campanularidae. 21
B. Hydrothecae reduced to saucer-shaped hydrophores, no diaphragm, no operculum. Halecidae Helecium halecinum.

21.(20) A. Gonothecae corrugated: hydrothecal teeth simple; free swimmin medusae released with 4 tentacles. Clytia. 22
B. Gonothecae not corrugated; hydrothecal teeth absent or bicuspid; no free medusae or with 16 or more tentacles on release. 23

22.(21) A. Stems branched; hydrothecal teeth long, slender, rounded blunt tip, hydrotheca deeper than wide. Clytia edwardsi.
B. Stems usually unbranched, hydrothecal teeth sharp or only slightly rounded, hydrotheca about as wide as deep. Clytia, johnstoni.

23.(21) A. Hydrothecal teeth bicuspid. Obelia bicuspidata.
B. Hydrothecal margin without teeth. 24.

24.(23) A. Gonothecae reduced distally by collar. 25
B. Gonothecae without distal collar. 26

25.(24) A. Stems branched. Obelia commissuralis.
B. Stems unbranched. Obelia geniculata.

26.(24) A. Terminal part of gonothecae bent over. Campanularia calceolifera.
B. Terminal part of gonothecae not bent over. Campanularia flexuosa.

27.(19) A. Stems and branches without nematophores (tiny dactylozooids with adhesive cells and nematocysts). Sertularidae. 28

28.(27) A. Hydrothecae in two rows with 1 opposite pair in each. Sertularia pumila.
B. Hydrothecae althernate in 2 longitudinal rows. 29

29.(28) A. Ovoid gonotheca. Abietinaria abietina.
B. Conical gonotheca widening distally. Thuiaria argentea.

PHYLUM CNIDARIA (COELENTERATA)

This phylum comprises the hydroids, sea anemones, jellyfish, corals, sea pens, and their congeners. All are specialized aquatic carnivores feeding mainly on zooplankton. As ubiquitous inhabitants of most marine and some fresh-water biotopes, and particularly as outstandingly successful forms in much of the eulittoral zone in the tropics, their ecology and biogeography have attracted wide-spread attention. Similarly, the Cnidaria are of particular interest from a comparative and phylogenetic point of view since their organization is of the simplest type found in true metazoan animals.

In habit cnidarians are free-swimming or attached, solitary or colonial animals. There are about 10,000 recent species. Within the wide range of differences in detail they all fall into one of two morphological and ecological types: the polyp and the medusa. The polyp usually is a sessile columnar form bearing tentacles distally around the mouth; it reproduces by various asexual methods such as budding, stolonization, fission and the like, or by sexual means. The medusa, on the other hand, is a saucer - or bell-shaped free-swimming creature with tentacles and a mouth on the lower or exumbrellar margin of the disc. It usually reproduces sexually. Despite the definite distinctions between polypoid and medusoid cnidarians their basic structural organizations, as will be seen later, is closely similar, and in many forms both occur in the same life cycle. This polymorphism both within the phylum and within species in it is a complex and interesting phenomenon which will be discussed below.

Systematically the animals in this group may be classified as follows: (simplified from Hyman, 1940)

Phylum Cnidaria: tentacle-bearing radiate animals with intrinsic nematocysts.

Class 1. Hydrozoa: hydroids and hydromedusae, usually with a velum and epidermal gonads, chiefly marine, 2700 species.

Order 1. Hydroida: hydroids and hydromedusae.
Order 2. Milleporina: colonial polypoid with massive calcareous skeleton, ephemeral incomplete medusoid stage, tropical eulittoral. Only one genus, Millipora.
Order 3. Stylasterina: somewhat similar to Millepora but zooids distinctive, no free medusa.
Order 4. Trachylina. Medusa with velum and gonads in gastrodermis. Hydroid stage reduced or absent.
Order 5. Siphonophora. Polymorphic colonial free-floating or swimming. Medusoids and polypoids occur in same colony attached to a stem-like primary zooid.

Class 2. Scyphozoa: True jellyfish without velum, with endodermal gonads, marginal sense-organs (rhopalia) present. Polypoid reduced or absent in most. All marine, 200 species.

Order 1. Stauromedusae. Polypoid adult develops directly from sessile scyphistoma larva, attached by aboral stalk; gastrovascular cavity divided by four septa.

Order 2. Cubomedusae. Free-swimming medusae with velumlike velarium; septa present; 4 tentacles on pedalia.
Order 3. Coronatae. Scalloped margin separated from rest of bell by furrow; septa and pedalia present.
Order 4. Semaeostomeae. 4 circumoral lobes; pedalia and septa absent.
Order 5. Rhizostomeae. Oral lobes contain many small mouths replacing true mouth overgrown by their fused bases; no septa, pedalia, or tentacles.

Class 3. Anthozoa: All polypoid with no trace of medusoid stage. Septa divide gastrovascular cavity into more or less complete compartments. Actinopharynx present. Exclusively marine, 6100 species.

Subclass 1. Alcyonaria. Octamerous symmetry, pinnate tentacles; colonial with endoskeleton consisting at least in part of calcareous spicules.

Order 1. Stolonifera. Polyps not fused except through basal mat of stolons.
Order 2. Testacea. Long axial polyps with lateral polyps.
Order 3. Alcyonaceae. Soft Corals.
Order 4. Coenothecalia. Blue coral.
Order 5. Gorgonacea. Horny corals, sea fans, and sea feathers.
Order 6. Pennatulacea. Sea pens and sea pansies. Many lateral polyps borne on a long axial polyp.

Subclass 2. Zoantharia. Sometimes hexamerous symmetry, tentacles usually unbranched. Septal pattern never simple octamerous type of Alcyonaria.
Order 1. Actiniaria. Sea anemones, solitary, no skeleton.
Order 2. Madreporaria. Stony corals, solitary or colonial, compact calcareous exoskeleton.
Order 3. Zoanthidea. Anemone-like forms with distinctive septal pattern.
Order 4. Antipatharia. Black corals. Deep-water forms superficially similar to gorgonians.
Order 5. Coriantharia, burrowing solitary anemone-like forms without pedal disc.

Having reviewed the various kinds of animals which make up the [illegible] phylum, the basic characteristics of the whole group will be considered in more detail before a series of type animals is discussed.

Specifically characteristic of all members of the phylum is the presence of nematocysts. These minute and complex organelles are the so-called nettle cells or stinging cells with which cnidarians are able to hold and paralyze prey. All members of the phylum possess nematocysts. No other metazoan animals are known which produce these offensive weapons although some flatworms and nudibranchs which feed on cnidarians contain nematocysts within their tissues.

In basic body plan Cnidaria are radially symmetrical sac-like organisms. The only body cavity is that of the digestive tract and hence is an enterocoele. Because of this body plan the phylum has frequently been called the coelenterata. However, as different zoologists have variously used this name to include Porifera, which are

not Eumetazoa, and Ctenophora, which are quite distinct from the Cnidaria, it seems most appropriate to use the latter term as the formal name of the phylum. The term Cnidaria is derived from a synonym for nematocysts: cnidae.

The digestive cavity is provided with one main external opening, the "mouth", which develops ontogenetically from the embryonic blastopore. In most forms the mouth is surrounded by one or more rows of tentacles which are active in feeding. Small secondary openings not involved in ingestion or egestion may be present, as in the familiar brown hydra, Palmatshydra, or the cinclides of anemones.

The axis of symmetry which may become modified to bi-radial or even bi-lateral in certain forms is the oral-aboral axis. No head is present and little of the concentration of nervous and sensory tissue associated with cephalization occurs. Correlated with this lack of well organized sensory and coordinating mechanisms is the diffuse elementary character of coelenterate behavior patterns. Spontaneous behavior is limited to locomotions and feeding reactions.

Locomotion even in the swimming forms is of a simple type mainly adapted to maintaining or changing the animal's vertical position in the surrounding water mass. Feeding also is of a sort requiring little coordinated activity. All coelenterates are carnivores and collect food by a sort of passive filtering action of the tentacles, by mucous-ciliary currents or by a combination of the two. It should be noted that two of the important elements in coelenterate feeding mechanisms, namely the nematocysts and cilia are independent effectors which apparently operate without benefit of nervous or sensory cells. In keeping with the lack of central coordination of complex behavior is the fact that isolated parts, such as tentacles, may carry out many of the characteristic responses of the intact animal as if they were part of it.

The body wall is three-layered, consisting of two epithelia, the gastrodermis which lines the enterocoel and develops from the embryonic endoderm, and the epidermis which covers the outer surface of the body and develops from the embryonic ectoderm; between them lies the mesoglea. The latter is a mesenchyme, mainly of ectogermal origin. In hydrozoans it consists of an acellular mesolamella but in the other coelenterates it is a cellular layer of fibrous or gelatinous connective tissue. Although the mesoglea is a distinctly organized cellular tissue in the majority of cnidarians, it does not give rise to skeleton. muscle, and transport systems as does the mesoderm of higher groups. The latter, unlike typical mesoglea, is of endodermal origin.

Histologically, the tissues of cnidarians are well differentiated. General epithelial, absorptive, excretory, skeletogenous, neuronal, ameboid, muscular, fibrous and gelatinous connective tissue cells are wide-spread in occurrence. However, the structural differentiation and organization of Cnidaria has only reached the tissue rather than the organ stage of development. The sense organs of medusae, are the only real organs in the phylum. It should be noted, nevertheless, that the concept of an individual organism is not precise which applied to highly polymorphic, simple, colonial animals of this sort. Thus, a siphonophore colony like the Portuguese man-of-war, Physalia, in its individuation and its well developed division of labor among constituent parts, strikingly approximates an organism in the higher animal

phyla. Mainly in its plant-like indeterminate growth habits and the close resemblence of the plan of its various specialized parts to that of complete individuals in other cnidarians, does such a living system seem a colony rather than an organism. In any case certain of the cell types found in coelenterate tissues are of particular interest because they are peculiar to or characteristic of this phylum.

The cnidoblast, the cell which produces the organoid nematocyst, is foremost of these. Cnidoblasts develop from undifferentiated interstital cells; these lie in the general supporting epithelia and also give rise to the germ cells and epitheliomuscular elements. The nematocyst and associated intracellular structures comprise a remarkably complex effector mechanism. When mature they are so oriented in the distal part of the cnidoblast that any prey contacting the surface in which they lie causes them to discharge sticky or poisonous threads of minute size. These entangle or pierce the food-animal. In many species poisons having severe and fatal effects, even in some cases on so large an animal as man, are hypodermically administered by batteries of such nematocysts.

Another interesting cnidarian cell type is the epithelial cell which becomes partly differentiated into a second kind of cell. Epithelio-muscular cells in the epidermis, nutritive-muscular elements in the gastrodermis, and even (in the pedal disc of hydras) glandular-muscular cells. All of these types have their basal portions broadened and flattened so that they can contain myofilbrils; the distal parts remain epithelial or may be digestive or glandular depending on their location. These partly contractile elements have frequently been considered as primitive forerunners of completely differentiated muscular cells. They do not, however, occur in the sponges and may perhaps be considered in cnidarians more a consequence of the absence of an endomesoderm from which to develop muscular tissue. However, partly contractile, partly conductive cells are prominent in another pro-coelomate phylum, the Nematada, which do have an endomesoderm in their development. In many scyphomedusae and anemones completely differentiated muscle cells are of frequent occurrence; they are of epithelial origin but may sink down into the mesoglea so they do not lie directly in the epidermis or gastrodermis. Histologically cnidarian muscle may be either smooth or in some of the more definitely organized aggregates of muscular tissue like the swimming muscles of medusae, they may be cross-striated.

Cell types which seem already in the Cnidaria to have reached a degree of development comparable to that found on through all the higher groups of animals are neurons and gametes. The former are bipolar or multipolar. They lie in loosely organized plexuses mainly. Although they may be denser in certain regions or oriented in strands following a particular direction there are no true nerves as in higher animals. Cell bodies of the neurons are scattered throughout the network rather than being aggregated in ganglia or nuclei distinct from nerves or tracts. Structurally the nervous system is made up of discrete cells with definite (although morphologically undifferentiated) synaptic junctions between them. The concept that they form a continuous cyncytial nerve net is erroneous.

The gametes, as typical large non-motile egg and small flagellated swimmin sperm, are similar to the small cell types found throughout a large part of the animal kingdom. Full gamete evolution obviously

occurred at a very early stage in phylogeny. In coelenterates gametogensis occurs in typical fashion. Gonads are not organized as true organs but are simply an aggregation of interstitial cells which develop into gametes. Cleavage of the zygote is adequal and development indeterminate.

The life history of Cnidaria varies markedly from group to group and even among some closely related species. Many of the phylogenetic generalizations dealing with the lower invertebrates were based on inadequate knowledge of the facts of these life-cycles. Thus the concept of a primitive diploblastic hollow organism formed by simple invagination of a hollow blastula is made less convincing by the fact that the majority of coelenterates develop a solid gastrula, the stereogastrula, which at first is mouthless and archenteron-less. Endoderm arises not by invagination in many of these but by wandering or delamination of the cells comprising the blastular wall. In addition, a majority have a well-developed cellular mesenchyme in the adult stage.

Similarly the application of the concept of alternation of generations or more specifically metagenesis (discovered in and clearly exemplified by the salps) to the cnidarians raises many difficulties. Metagenesis, in the sense of a strict alternation of morphologically distinct sexual and asexual generations occurs in only a few cases like they hydroid, Corymorpha or the medusa, Aurelia. The methods of growth and budding in colonial forms may greatly modify such a simple plan. In many forms either polypoid or medusoid stages are suppressed. Furthermore, if the actinula, a polypoid larva which occurs in a number of cnidarians, is considered to be the forerunner of all the polyp-like coelenterates, then the so-called alternation of generations becomes merely a type of metamorphosis. From this point of view corals, for example, which have no trace of a medusoid form would be considered peristent larval types which have developed a sort of pedogenesis. But since a majority of cnidarians either have no medusoid stage (Stauromedusae, Anthozoa) or have it markedly reduced (many hydroids), a slightly different analysis would seem to be more acceptable. Namely, that the polypoid actinula originally became an adult animal capable of sexual and asexual reproduction. Then in its adaptive radiation this form evolved in two main directions.

On the one hand it exploited the possibilities of a benthonic existence by developing into a fixed polyp type. In the corals, sea anemones, and their relatives, this has been done completely; after the free-living larval stage, there are no pelagic stages or forms. On the other hand the creeping polypoid ancestral actinula also began to undertake a pelagic existence. In some forms like most trachyline medusae, direct metamorphosis of actinula into medusa exemplifies this trend clearly. However, less direct production of free-swimming forms is more nearly the rule in recent species of cnidarians. Thus the formation of scyphomedusae is nearly always preceded in the life history by a fixed stage, or, perhaps generation, which in polypoid form shows marked similarities to anthozoan zooids. In the Stauromedusae the life history stops at this stage. But in the rest of the group the polypoid either metamorphoses into one or divides asexually into many medusoid individuals. In the hydrozoans the tendency toward pelagic existence is preceded and quite frequently completely overshadowed by the fixed polypoid form. Fundamentally the hydromedusae develop as

specially modified hydranths which by a sort of division of labor carry out the sexual reproduction of the species, often as free-swimming medusae. But many species illustrate a secondary trend in this cnidarian class towards the regression of the free-living medusoid sexual individuals. This reaches its extreme expression in forms like the hydras where even the vestiges of the sexually reproducing individual have apparently been lost and the polyp itself bears eggs and sperm directly.

It will be readily seen from the foregoing that the wide variety of life-cycles shown by cnidarians renders the notion of alternation of generations as unsatisfactory in many ways.

Nor do the known facts of paleontology help as yet in untangling the relations of primitive characteristics and phylogeny in this phylum. One Precambrian form and many Cambrian species are available but have not been as thoroughly studied as they deserve. Progress has been made, however, in the demonstration of the protochordate nature of the graptolites, thus removing them from a position as dubious members of the hydrozoan coelenterates. It is to be hoped that further work on the fossil primitive invertebrates will shed some light on the interrelationships of recent forms and the origins of metazoan animals.

Tubularia crocea (Agassiz)

1. Classification. Class Hydrozoa, Order Hydroids, Suborder Gymnoblastes, Family, Tubularidae.

2. Habit and occurrence. Colonial hydroid of stolonial habit. Forms dense tufts of irregularly and sparsely branched long stems bearing hydranths, arising from tangled mass of prostrate stolons. Common in shallow water, particularly on wharf pilings, from Arctic regions to Cape Hatteras; British Columbia to San Diego.

3. Anatomy and physiology.

A. External structure. Large hydranths borne on long slender stems which may branch sparsely giving rise to other hydranths. Periderm (= perisarc), the external secreted non-living protective layer is thick and extends distally nearly to the base of the hydranth, which arises from a slightly enlarged section of the distal pedicel. There are two whorls of filiform tentacles of which the oral ones are considerably shorter. The reproductive individuals (gonophores or collectively gonosome)arise on the hypostome of the hydranth between the two whorls of tentacles. They are borne in elongate clusters with a central stalk supporting them. No free medusae are present in the life cycle. The gonophores develop into medusoid structures but never break loose from the rest of the colony.

In studying these features of Tubularia examine a small part of a colony under low power with a dissecting microscope. Note the relation between thelation between the prostrate hydrorhiza or stolon and the upright stem or hydrocaulus. The primary zooid of the colony forms the stolon and secondary hydranths bud off from it. Make a diagram of this growth habit labelling the primary zooid 1 for first generation, its asexual offspring 2, their progeny 3, etc. Compare the cycle of generations so established by this growth pattern with that of the other cnidarians studied.

Under higher power ascertain the detailed relation between tentacles, mouth and gonophores in the mature hydranth. A diagram of this arrangement and a sketch indicating color pattern would be a useful addition to the laboratory notes.

B. General body organization. In the colonial hydroids the gastrovascular cavity of the hydranths is continuous through the stem as is the body wall of the zooids. This shared part of the body wall is the coenosarc and consists as does the body of the hydranth of epidermis and gastrodermis with an intermediate non-cellular layer, the mesolamella. Outside the living wall is the periderm, a chitinous transparent layer secreted by the epidermis. The tentacles are solid consisting of an epidermal epithelium with many nematocyst-bearing cells and a solid core of gastrodermis.

Prepared histological sections are desirable for examining these details most effectively. However, a number of interesting features may be observed by teasing out fragments of fresh tissue of Tubularia and examining them microscopically under moderately high power. Stain various pieces by drawing a drop or two of different vital dyes under

the cover-slip. 1% sea-water solutions of methylene blue, bismarck brown, methyl green, and neutral red used separately, are suitable for this purpose. Just enough dye should be added to color the water lightly. Observe the tissue and remove the stain when enough has been absorbed. Such facts as the distribution pattern of the nematocysts, the structure of the mesoglea, and the obvious cell types of the epidermis and gastrodermis can be determined in thisway.

C. Respiratory system. No special respiratory mechanisms are present in Tubularia. This is in keeping with the unusually large surface to (living)volume ratio found in most snidarians because of their essentially epithelial organization. Flagellar currents are present internally, however, and act among other things to provide respiratory transport within the zooids and the coenosarc as well. These currents are driven by the activity of flagellated gastrodermal cells.

Their effectiveness may be readily demonstrated by introducing a small amount of a suspension of fine carmine or india ink particles. By this means the rate of transport of gastrovascular fluid as well as any patterns of flagellar currents may be determined. A diagram of an appropriate section of a colony indicating such currents would summarize this data for Tubularia. In this species as in other large hydroids, like Corymorpha, the surface area of the stem in increased by special structural devices. A slice of Tubularia stem cut crosswise will indicate the arrangement in this hydroid.

No respiratory pigments aiding the transport or storage of respiratory gases are known in coelenterates. But some heme pigments like those which function in the enzyme systems of cellular respiration in higher animals are present. Carbonic anhydrase which aids in CO_2 transfer in higher metazoans has also been found in Cnidaria; its function has not been demonstrated however.

Metabolic rate of cnidarians is low but not surprisingly so, considering the little work they do and the relatively minute amounts of active protoplasm present in many. Respiration is aerobic and in general proportional to the amount of oxygen present over a considerable range although in corals it is maintained at a constant level until the concentration of oxygen falls below half its normal value when it is reduced proportionately until all the oxygen is used up.

D. Circulatory system. The only extracellular body fluid in Tubularia, as in the rest of the phylum, is that of the enterocoele. It is moved about by the flagellar currents mentioned in the previous section. Also muscular movement of the hydranths may move this medium about. Certain types of transportation which in higher animals might be effected by the circulatory system are carried out in hydroids and other Cnidaria by wandering cells which either actively migrate through the body or are carried passively by body fluid to another region where they settle down. Such amebocytes may have been already observed in the preparations of hydranth fragments suggested in section B. above. They are commonly to be found in the gelatinous or fibrous mesoglea of the other two classes of coelenterates.

E. Excretory system. No specially organized excretory system is present. The amoboid wandering cells mentioned above have been seen in some hydroids to carry waste matter to the exterior. As in amy of

the other aquatic invertebrates, ammonia is apparently the major nitrogenous waste product. Being entirely carnivorous, these animals have to excrete not only large amounts of nitrogen but also appreciable amounts of phosphorus and sulfur. Biochemical details of coelenterate metabolism, including those concerned with such metabolites as these, are poorly known.

F. Feeding mechanism and digestive system. In tubularians, as in most other cnidarians, zooplankton which comes in contact with the tentacles is caught and paralyzed by the nematocysts. In polyps muscles of the tentacles bend the tips in toward the mouth and the hypostome contracts to bring the mouth over to meet the food being brought to it. The prey is then engulfed by the mouth and passes into the enterocoele.

These typical feeding reactions can be elicited in hydranths with small bits of Mytilus meat or juice. Experiments should be tried to determine whether actual contact with the food is essential to evoke these responses and whether the zooid distinguishes between food, filter paper soaked in clam juice and plain filter paper. This can be done with fine forceps by offering the food to large hydranths under the binocular microscope. The results obtained will depend among other things on the nutritional state of the hydranth as repeated trials of the same procedure will demonstrate mechanical stimulation with food is an excellent way to cause nematocyst discharge. The structure of these organelles may be studied after these experiments by then staining with methyl green or methylene blue. Note that there is a cushion of gastrodermal tissue at the base of the hydranth which constricts the body cavity at this point thus preventing large food particles from passing into the stem.

Digestion is partly extracellular. Ingested food masses, which may be surprisingly large, are rapidly disintegrated by a protease secreted into the coelenteron by cells of the gastrodermis. This brei is then engulfed by phagocytic action of the walls of the digestive cavity and digestion completed over a longer time intracellularly. Proteases, peptidases, weak lipases, and at least in some forms, weak glycogenases are the digestive enzymes involved. In other words, the battery of digestive enzymes is consonant with the purely carnivorous diet which provides proteins, fats, and glycogen to be digested.

The progress of extracellular and intracellular digestion can be readily followed by staining small copepods or similar zooplankton with neutral red or brom-thymol blue. Their fate is clearly demonstrated by the stain. Tubularia is near the practical limit of size for such study and these experiments as well as digestive enzyme analysis can more profitably be carried out on larger coelenterates like Aurelia or anemones.

G. Nervous system and sense organs. The nervous system consists of a diffuse network of neurons arranged in an epidermal and a gastrodermal plexus interconnected by fibers coursing through the mesoglea. Neurosensory and neuroeffector junctions are made by the constituent neurons which are discret cells with bipolar or multipolar neuritis. In preparations suitably stained with methylene blue, as suggested in Section B. above, these cells may be seen. Their arrangement may also be determined by testing the reactions of the hydranths to a fine needle or brush touched to various parts of the surface of zooids and

stems. Such study can indicate presence or absence of discrete pathways of conduction, directionality of impulses over these, facilitation, spread of excitation, areas of greatest sensitivity and so on.

In addition neurosensory cells occur with receptor terminations in either epithelial layer and neurites synapsing with the subepithelial neuronal plexus. Epithelial sensory cells and perhaps free nerve endings are also present but no specific sense organs occur in Tubularia or any other of the polypoid cbelenterates. Structurally organized special receptors appear for the first time in Metazoa in medusae.

H. Skeleto-muscular systems. Except for the periderm which forms a transparent chitinous exoskeleton there are no skeletal structures in Tubularia. The muscular system is epitheliomuscular in nature. The general orientation of its contractile fibrils is longitudinal in the epidermal elements and circular in those of the gastrodermis. It thus roughly consists of two cylinders of muscular tissue one inside the other. Some of the contractile elements are believed to be independent effectors, at least in the tentacles; thus they would be like the muscle cells of sponges, which have no neurons. If so, they and some of the gland cells of cnidarians may be added to the cilia and nematocysts as effectors which are not under nervous control.

The details of the arrangement of the muscles of Tubularia have to be studied in histological sections although the observations on structure (Section B) and feeding reactions (Section F) above will have yielded some useful information on this point.

The movements carried out by Tubularia with its muscular system are limited to expanding and contracting reactions and feeding behavior since the adults have no locomotor ability. These reaction patterns have already been studied in connection with Section F.

4. Reproductive system and reproduction. Since no free-living medusae are released by Tubularia crocea its life cycle is one that illustrates the trent towards reduction and final elimination of the medusoid stage. As has already been observed the gonophores are borne in elongate clusters springing from the hypostome just above the proximial whorl of tentacles. In these gonopho clusters the distal buds are the oldest. Thus moving from the base to the tip of the cluster, a summary of gonophore development may be observed. It starts with an outpushing of epidermis, mesolamella, and gastrodermis, then proceeds to the formation of an incomplete medusa. The latter, attached by an aboral stalk has the typical tetramerous symmetry of hydromedusae indicated by knoblike tentacle-buds. The sex cells develop from interstitial cells in the epidermis of the manubrium and are shed into the exumbrellar cavity which remains closed by the velar plate until considerable larval development has occurred. The planula, the typical ciliated mouthless stereogastrula of many cnidarians, never becomes free-living but metamorphoses into a polypoid tentaculate actinula larva, which is then set free. The latter larva is of unusual interest since it appears to be closely similar to the stem-form of coelenterate from which all present-day members of the phylum evolved. With a pair of fine needles tease out some of the gonophores of Tubularia and observe as much as possible

of this life history. It is also worth-while to allow some actinulae to settle down and attach in a finger bowl of sea-water (renewed from time to time). Each becomes a primary zooid and if fed, will begin to form the typical stolonial colony, thus completing the cycle.

The factors involved in growth and differentiation of tubularia have been widely studied by means of regeneration experiments. The interstitial cells which form cnidoblasts and sex cells also are active in regenerating hydranths. Some simple experiments of this sort may easily be carried out. If clean, vigorous-looking polyps are used regeneration takes place rapidly. Determine extent and type of regeneration in lengths of stem which have neither hydranth nor proximal parts present. See if the proximal and distal ends behave similarly and test whether a fine thread tightly tied around the middle of the piece affects this. Also test the influence of a terminal hydranth on proximinal regeneration with and without such a thread. Further interesting results are obtained by varying the length of stem left attached to an isolated hydranth or by removing the tentacles.

5. Methods of collection, study, etc. As with many other sessile organisms interesting early stages of development and growth may be obtained (in proper season) for hydroids like Tubularia by exposing a rack of clean glass slides in shallow water near rocks or piles where the adults are growing. Examination of these surfaces from time to time will reveal their gradual colonization by large numbers of forms among which may be found the specific ones in question.

In preparing Tubularia, and in fact most other cnidarians, for preservation it is necessary to anesthetize the animals before introducing the fixing fluid. This may be done most effectively by gradually introducing $MgSO_4$ into the dish in which the animal, maximally expanded, has been placed. One method of doing this is to suspend a small cloth bag of crystals of this salt in the water over the specimen so that as the magnesium goes into solution it will gradually diffuse out and into the water. Care must be taken that the effect of the salt is sufficient to prevent contraction before fixation is attempted. Animals so fixed in the expanded state may then be stained and whole-mounted or prepared in any of the standard ways for histological study.

II. Hydractinia echinata (Fleming)

1. Classification. Class Hydrozoa, Order Hydroida, Sub-order Gymnoblastea, Family Hydractinidae.
2. Habit and occurence. Colonial hydroid of stolonial habit. Forms encrusting mat of stolons from which the polymorphic zooids arise on unbranched pedicels. Common in shallow water on rocks, wharf pilings, and gastropod shells from Arctic regions to Dry Tortugas on Atlantic coast.
3. Anatomy and physiology.
   A. External structure. The stolons of this species fuse to form a network of gastrodermal tubes covered above and below by sheets of epidermal epithelium resulting from coalescensce of the epidermis of neighboring individual stolons. A secreted layer of periderm attaches the colony to the substratum and also projects from the colony's surface in the form of jagged spines which are a diagnostic structural feature. There is no periderm around the zooids, which emerge from this stolonial base. Five types of persons occur in the colonies although not all in one, since the species is dioecious. The feeding hydranths, the trophozooids, bear one whorl of filiform tentacles at the base of a conical hypostome. They are larger than gonozooids. The latter have a short rounded hypostome and no tentacles although knobs of large nematocysts simulate vestigial tentacles around the base of the hypostome.

   The gonophores are sporosacs, i.e. the medusoid stage is completely supressed and represented only by simple evaginations of the blastostyles which bear eggs and sperm in different colonies. These structures are borne on the stem of the gonozooid below the reduced hydranth.

   Similiar in general structure to the reproductive persons of the colony are the spiral zooids. They lack gonophores, however, are more elongate, and frequently recurve on themselves, whence the name. They are presumed to be specialized defensive zooids.

   In Hydractinia colonies growing on snail shells inhabited by hermit crabs, there may be found near the margins of the shell still another type of polyp: the tentaculozooid. These are even more elongate than spiral zooids, also lack tentacles and in addition have no nematocyst knobs. These polyps may be sensory in function.

   To study the polymorphic colonies fragments of snail shell encrusted with Hydractinia provide a convenient source of material. Note that both sexual and asexual reproduction within the colony is restricted to certain zooid types. A diagram showing their relation to the growth habit and other polyp types should be made, labelling, as before, the successive generations.

   B. General body organization. Except for the structure of the stolonial mat and its spines, the basic plan of Hydractinia's organization is closely similar to that of Tubularia. Techniques described for that hydroid can profitably by employed in this case to study the differences in tissue organization and distribution of cell types in the various forms of the Hydractinia zooid.

C, D, and E. General comments and some suggestions for observations on respiration, circulation, and excretion will be found under the appropriate sections for Tubularia.

F. Feeding mechanisms and digestive system. Repeat on Hydractinia the experiments on feeding behavior suggested for Tubularia. In the present form it is of interest to note any differences in reactions to food and/or mechanical stimulation elicited in the different types of zooids present. Information should be obtained by this means for or against the suggested special functions of spiral zooids and tentaculozooids.

The frequent occurrence of Hydractinia echinata on gastropod shells inhabited by hermit crabs has led many zoologists to believe that this relationship is of some commensal significance. Feeding by both animals, however, is apparently quite independent and either can flourish without the other. The relationship is thus merely a facultative one.

G. Nervous system and sense organs. The possible function of tentaculozooids as sensory polyps is a point that should be studied further. Evidence for greater concentration of sensory and nerve elements in these zooids could be obtained by appropriate staining techniques (e.g. intra-vitam methylene blue) and by observations on reactions like those suggested in the previous section.

H. Skeleto-muscular system. The specialized peridermal developments of the encrusting part of Hydractinia colonies have already been mentioned. The movement and behavior patterns of the zooids habe been observed in section F. Note that movement and contractile ability of the zooids is extensive in this species, due in part to the absence of periderm on the zooids and their stalks.

4. Reproductive system and reproduction. The free-living medusoid, as in Tubularia, is absent from the life cycle of Hydractinia. However, in the two other genera of the Hydractinidae, Podocoryne and Stylactis, free medusae are produced. Closely similar relations may be found in other hydroid groups. Species with nearly identical hydroid forms produce gonophores of quite different degrees of differentiation. In addition, even if complete free medusae are achieved, they may have quite distinctive morphology dispite similarity of the polyps. These facts demonstrate that the trend towards reduction of the free-living sexual generation is expressed independently in a number of parallel cases. Also they indicate independent evolution of the medusoid once it has become free of the sessile colony.

As a practical matter these relations make the systematics of the hydroids complex and difficult. The only sure way of identifying a new medusa with a particular polyp is to see it develop from it or reproduce it. As this has by no means always been possible hydroids and hydroid jellyfish of the same species may become assigned to quite different genera. Thus even the suborders, Gymnoblastea and Calyptoblastea, established for the polypoids, have parallel medusan suborders, Anthomedusae and Leptomedusae respectively.

About all that can be observed in the gonophores of Hydractinia is a simple structure, homologous with the manubrium of a complete hydromedusa. On this are borne the sex cells which are actively shed when ripe, through muscular contraction of the walls of the gonophore. Several interesting experiments may be done on the spawning of this hydroid.

Since the species is dioecious it would obviously be an advantage if both male and female colonies spawned at the same time in any given neighborhood. This does indeed occur in nature; the spawning is synchronized by some photosensitive mechanisms. Only when it is exposed to light for a certain period after a preceding conditioning period in the dark does a colony shed its gametes. Using ripe colonies of both sexes determine by exposure to experimentally controlled periods of light and dark what the necessary sequence is for spawning. Check these results with spawning time in control colonies just exposed to normal night-day light sequence. Note whether time of day influences ability of a given light sequence to induce spawning. Involvement of gonophore musculature in these reactions may be demonstrated by repeating some of these experiments in calcium-free sea-water, since muscle cannot contract in the absence of this cation.

After spawning, early cleavage stages, planula larvae and initial colony formation may be seen if fertilization has been effected. Evidence for the indeterminate nature of the early cleavage in this phylum can be adduced from the subsequent development of isolated blastomeres, teased apart with fine needles.

Regeneration in this species, while basically like that of Tubularia, has an additional feature of importance: polypoids are readily regenerated but fragments of each type will grow back only their own kind of zooid. Experiments on this hydroid are very difficult to carry out however, because of their considerably smaller size.

5. Methods of collection, study, etc. The discussion in Section 5 for Tubularia is equally pertinant here. Note that while the polymorphic zooid of Hydractinia are relatively easy to identify in living colonies unless fixation has preserved the polyps in fully expanded condition, killed material is almost useless for this purpose.

For collecting this species the shell-houses of hermit crabs are a convenient and common substrate. It is necessary to identify the species, however, since Podocoryne and Stylactis, which are quite closely related, are also common on shells from similar habitats.

About all that can be observed in the gonophores of Hydractinia is a simple structure, homologous with the manubrium of a complete hydromedusa. On this are borne the sex cells which are actively shed when ripe, through muscular contraction of the walls of the gonophore. Several interesting experiments may be done on the spawning of this hydroid.

Since the species is dioecious it would obviously be an advantage if both male and female colonies spawned at the same time in any given neighborhood. This does indeed occur in nature; the spawning is synchronized by some photosensitive mechanisms. Only when it is exposed to light for a certain period after a preceding conditioning period in the dark does a colony shed its gametes. Using ripe colonies of both sexes determine by exposure to experimentally controlled periods of light and dark what the necessary sequence is for spawning. Check these results with spawning time in control colonies just exposed to normal night-day light sequence. Note whether time of day influences ability of a given light sequence to induce spawning. Involvement of gonophore musculature in these reactions may be demonstrated by repeating some of these experiments in calcium-free sea-water, since muscle cannot contract in the absence of this dation.

After spawning, early cleavage stages, planula larvae and initial colony formation may be seen if fertilization has been effected. Evidence for the indeterminate nature of the early cleavage in this phylum can be adduced from the subsequent development of isolated blastomeres, teased apart with fine needles.

Regeneration in this species, while basically like that of Tubularia, has an additional feature of importance: polypoids are readily regenerated but fragments of each type will grow back only their own kind of zooid. Experiments on this hydroid are very difficult to carry out however, because of their considerably smaller size.

5. Methods of collection, study, etc. The discussion in Section 5 for Tubularia is equally pertinant here. Note that while the polymorphic zooid of Hydractinia are relatively easy to identify in living colonies unless fixation has preserved the polyps in fully expanded condition, killed material is almost useless for this purpose.

For collecting this species the shell-houses of hermit crabs are a convenient and common substrate. It is necessary to identify the species, however, since Podocoryne and Stylactis, which are quite closely related, are also common on shells from similar habitats.

III. Pennaria tiarella (Ayres)

1. Classification. Class Hydrozoa, Order Hydroida, Suborder Gymnoblastea, Family Pennaridae.
2. Habit and occurrence. Colonial hydroid forming loose bushy growth by twice pinnate branching; colonies up to 15 cm. in height. Free medusa present in life cycle. Common in shallow water; on piles, rocks, and seaweed; from Bay of Fundy to Gulf of Mexico.
3. Anatomy and physiology.
   A. External structure. Hydranths have large conical hypostome bearing scattered tentacles with terminal knobs (capitate) and a basal whorl of filiform tentacles. Just distal to the latter the gonophores are borne. They develop into relatively large medusae with four knob-like tentacles. Spawning may occur while the medusae are still attached but they may bread free first. The species is dioecious.

      Growth in Pennaria colonies is monopodial, i.e. the growth zone is just proximal to the terminal hydranths of the main stem and its branches. After an internode of stem growth has occurred in this region a hydranth buds off (alternately on one side then the other of the stem). If it is to form a branch, this subterminal hydranth develops a growth zone just proximal to itself and then proceeds to form a branch just as the main stem has been formed by the primary zooid. The latter is thus the oldest polyp, and the terminal zooid on the branch nearest the base of the colony is the next oldest. The youngest obviously are the penultundate hydranths of the stem and branches. Diagram a portion of a colony of Pennaria and compare the succession of generations with that found in other Cnidaria.

   B. General body organization. Many of the basic structural features of Pennaria are not strikingly different from those already observed in other gymnoblastean hydroids. The capitate tentacles of this species are of interest, however, for the marked aggregation of nematocysts in their tips. These may be more readily observed if the tentacles are stained with methyl green of methylene blue.

      Details of the cell types making up the tissues of this hydroid may be demonstrated by the following maceration technique (which may be applied aswell to other coelenterates).

      1. Place the tissue to be studied in i sea-water solution of equal parts of 0.05% osmic acid and 0.2% acetic acid for 3 minutes.
      2. Wash in several changes of 0.1% acetic and leave in latter 12 hours.
      3. Wash in water.
      4. Stain with acid carmine and mount in glycerine.
      5. If cells are not sufficiently isolated, tapping the cover-glass gently will separate them further.

      The above procedure provides only a temporary mount; for permanent preparations a different stain and dehydration must be used. This technique should clearly demonstrate cell shapes and such details as the muscle fibers of the hydromedusa may be observed for the first time in the present series of hydroid types. It is bell or thimble-shaped and as mentioned above, has four reduced tentacles present on its margin. The outer

surface, from whose apex an aboral stalk attaches it to the hypostome of the parent hydranth, is called the exumbrellar surface; the inner surface, the subumbrellar, bears the mouth centrally at the end of a tubular pendant structure like a bell clapper. This is called the manubrium. Extending from the margin of the bell towards the oral-aboral axis is a shelf-like double layer of epidermis, the velum, which partly closes off the subumbrellar cavity from the exterior. It functions in these small medusae to produce a sort of jet propulsion on the contraction of the bell. Both ex- and subumbrellar surfaces consist of epidermis.

The gastrovascular system comprises a central gastric cavity and four radial canals which join a ring canal running around the margin. The mesohlea is much thicker than it is in the polypoid colony although in hydromedusae it is still an acellar layer.

C.-H. The analyses and suggestions made for the preceding hydroids apply generally to the Pennaria polyp as well. Further comment and observations on the hydromedusae will be given for a form, Gonionemus, more suitable to detailed study, described below.

4. Reproductive system and reproduction. In Pennaria the medusoid stage usually becomes free-living but has only a brief pelagic life during which it feeds little if at all. Growth of the medusa, maturation of the gametes, and even sometimes their release occur while the gonophore is still attached to and dependent on the polyp colony. Thus although somewhat less suppressed, the medusoid stage is only a step less so that in Tubularia, for example.

5. Methods of collection, study, etc. Some of the techniques and data given for other hydroids will also be found pertinent to the present species and useful for its further study.

IV. Obelia geniculata (Linnaeus)

1. Classification. Class Hydrozoa, Order Hydroida, Suborder Calyptoblastea, Family Campanulariidae.
2. Habit and occurrence. Colonial hydroid with simple geniculate stem. Hydranths on pedicels arising alternately from shoulders of internodes. Free living medusae produced in gonangium with axial collar. Common in shallow water, on piles, rocks, and seaweed; Arctic regions to Gulf of Mexico.
3. Anatomy and physiology.
   A. External structure. Hydranth with single whorl of filiform tentacles at base of hypostome. Latter swells distally into subspherical structure. A hydrotheca, part of periderm, forms a bell-shaped cup around base of hydranth. At the base of this hydrotheca the polyp rests on a shelf-like projection which constricts the cavity within the periderm at this point. Gonophores which develop into free discoidal medusae are borne on modified polyps called blastostyles. Each of the latter is enclosed in a peridermal protective cup, in this case a vase-shaped gonotheca which is constricted by a collar. Gonotheca, blastostyle, and gonophores together form a gonangium. Gonangia arise in an axillary position between the simple stem and the pedicels of hydranths.

   The growth of the Obelia colony is sympodial, i.e., each new hydranth arises as a bud from the stem just proximal to the next youngest polyp. The gonangia arise as secondary buds in the axils so formed. Thus the youngest hydranths are the most distal ones and the stem of the colony, which in monopodial forms is all produced by the primary zooid, is the composite effort of all the hydranths it bears. Each stem internode is contributed by one hydranth.

   Diagram a portion of an Obelia colony indicating the succession of generations, as with the other forms studied. Compare with other cnidarian life-cycles.

   In the higher calyptoblastean hydroids like Plumularia, which is not included here in the type series, another pattern of colonial growth occurs. This is a second kind of monopodial development in which a permanent growing tip is maintained at the distal end of the stem and branches. This itself never differentiates into a hydranth but continually buds off polyps in characteristic pattern as it grows. This growth plan, along with the stolonial, monopodial, and sympodial growth patterns studied above, completes the basic methods of hydroid colony development.

   B. General body organization. Most of the important characteristics of the Obelia trophosome have been mentioned above. One further prominent feature of the periderm should be observed, namely that stems, pedicels and gonangial stalks are all annulated by ring-like constrictions. These presumably add flexibility to the colony but their significance is not clear.

   Like the anthomedusan of Pennaria the medusa of Obelia has a marked tetramerous symmetry. It has 24 marginal tentacles when liberated from the gonangium; four radial canals which bear the sex cells (unlike those of Anthomedusae which occur in the epidermis of the manubrium); 8 closed statocysts around the bell margin (these do not occur in Anthomedusae). When the medusa is not actively swimming the bell is usually

everted so that the manubrium projects from the umbrella, at least in young specimens. The more usual relationships are assumed, however, when the animal swims by the pulsations of the disc.

The gonophores are released from the gonangium as complete active small medusae but do not have mature gametes. Hence, an appreciable period of pelagic existence is necessary before spawning can occur. Of the hydroids studied so far in this series, Obelia has the most complete and relatively most independent sexual stage of the group. In the the next form to be considered, however, the medusoid is definitely the predominant generation while the polypoid is a small solitary form.

C. and following sections are omitted here because the main techniques and procedures pertinent to the hydroid Obelia have already been given for the prededing forms. More detailed discussion of the hydromedusae will be found under Gonionemus.

V. Gonionemus murbachii Mayer

1. Classification. Class Hydrozoa, Order Hydroida, Suborder Leptomedusae (Limnomedusae), Family Olindiidae.
2. Habit and occurrence. Hydroid minute (1 mm.), solitary with 4 tentacles. Medusa 2 cm. in diameter with 16 or more marginal tentacles and gonads of typical ruffled appearance running length of the 4 radial canals. In shallow water attached to seaweed (particularly eel grass, Zostera) by adhesive discs. Before the wasting disease of Zostera nearly wiped out the species, Gonionemus was very common in certain localities.
3. Anatomy and physiology.
   A. External structure. As far as known, the polypoid never develops beyond a minute hydra-like creature with four tentacles, each with a solid core of gastroderm. Gonophores are borne in gymnoblastean fashion as buds on the column of the hydroid. The medusa of Gonionemus is a typical tetramerous hydromedusa with a short manubrium bearing 4 short frilled oral lobes. Special pads of adhesive tissue occur distally on a sort of knee on each tentacle. This may be studied by placing an appropriate piece of tentacle under a cover slip on a slide and examining in a drop of glycerine under high power. At the same time note the nearby batteries of nematocysts and the fact that the medusan tentacles are hollow in this species.

   B. General body organization. As in other hydromedusae the peripheral parts of the gastrovascular system, the radial and ring canals, are tubes of gastrodermis imbedded in the mesogloea. The latter forms only a thin layer like the hydroid mesolamella between the gastroderm and subumbreal surface but is a thick gelatinous stratum between the exumbrellar epidermis and the gastrodermis. Between the radial canals the gastrodermis forms a thin sheet of flat epitheliał cells (one layer thick) like a part of the gastrovascular system whose cavity had been obliterated and walls collapsed to a single sheet of tissue.

   At the bases of the marginal tentacles are swellings called tentacular bulbs; they contain part of the gastrovascular cavity and apparently function in digestion and also serve as sites of nematocyst formation. These bulbs also serve some sensory purposes but they are not ocelli with photosensory function as frequently stated. The relation between these bulbs and the rest of the gastrovascular canals should be determined. Sense organs in the form of statocysts do occur in the bell margin between the tentacle bases.

   C.-H. Since Gonionemus is difficult to obtain in the living state, observations on the behavior and physiology of medusae had best be made on more readily available animals. The behavior of the Gonionemus medusais, however, interesting on several counts. First, the chemical stimulation of food apparently excites the animals to greater activity. Random swimming movements, poorly, if at all, oriented to the stimulus, are increased. Second, this medusa has been described as actively fishing. To do so it swims upwards to the

surface, turns over and sinks downward with its tentacles trailing. Both of these feeding activities are worthy of notice since many medusae display feeding reactions only when they actually come in chance contact with food.

4. Reproductive system and reproduction. Note that as in the Scyphomedusae and trachyline medusae, the hydroid stage of the life cycle is much reduced. The polyp can reproduce asexually by budding off frustrles, which in turn develop into more polyps, but each individual remains solitary and bears gonophores on its pedicel. The medusae, which are so produced, in turn produce eggs and sperm in gastrodermal gonads on the radial canals. The zygote gives rise to a ciliated planula which swims about, settles down on its aboral end and gives rise to the polypoid stage. When ripe living material is available all these early stages may readily be observed in the finger bowls in which the medusae are kept.

5. Methods of collection, study, etc. No special comment.

VI. Aurelia aurita Lamarck

1. Classification. Class Scyphozoa, Order Semaeostomeae, Family Ulmaridae.
2. Habit and occurrence. Discoidal, large (30 cm.) medusae with 4 frilly oral arms, short marginal tentacles, and alternate branched and unbranched radial canals connected peripherally by a ring canal. Polypoid form reduced to a solitary trumpet-shaped scyphistoma, 5 mm. high. Common from Greenland to the West Indies in continental waters.
3. Anatomy and physiology.
   A. External structure. The symmetry of the Aurelia medusa is strongly tetramerous. The mouth is square in shape and from its four corners the oral arms arise. The four radii so delineated are called the perradii; at 45° to these are 4 interradii, and halfway between per- and interradii are 8 adradii. The gastric pouch is four lobed with each of its outpocketings lying along an interradius. From it arise a definite pattern of branched and unbranched radial canals which distally join a common circular ring canal. The perradial canals, arising between the gastric pouches, and the interradial canals, arising from the distal end of each pouch, show a characteristic branched pattern. At the ends of their center branch are niches in the margin of the medusa which scallop the bell into 8 lobes. In the 8 indentations thus formed complex sense organs called rhopalia occur. These will be discussed below. In the adradii run eight unbranched canals, 2 of which arise from each of the four gastric pouches. The gonads appear as 4 horseshoe-shaped aggregates in the floor of these pouches. Beneath them are shallow cavities, the subgenital pits, on the subumbrellar surface. They do not, however, appear to be functionally connected with the gonads of gamete discharge.

   The oral arms are large and gelatinous, being about as long as a radius of the bell. Along their subumbrellar surface runs a groove, the two edges of which are convoluted and bear many short tentacles. The remaining tentacles are in a marginal position on the medusa - simple, short and numerous.

   B. General body organization. The basic plan and the main types of cells and tissues of the Scyphomedusae are similar to those of the other cnidarians studied. The mesoglea, however, is cellular as well as being much larger in volume. Fibers present in the gelatinous matrix may give it a consistency as stiff as cartilage.

   Staining and maceration techniques described for some of the previous coelenterates are a useful adjunct to examination of the whole animal if cell and tissue details are to be observed under moderate magnification.

   The body organization of the polypoid form is rather different, however. Here there are four septa, called tenioles, which divide the gastric cavity into four perradial pouches. Ectodermal invaginations of the subumbrellar surface, called subumbrellar funnels, push into these septa. It will be seen that the interradial position of these invaginations of the polypoid stage is the same as that of the subgenital pits of the medusa. Note, however, that the gastric pouches of the latter are not perradial as are those formed by the septa, but interradial in position. Aurelia does not have septa or

funnels present in the medusa; they disappear during the metamorphosis of the strobila into ephyrae (mentioned below). Both septa and funnels are, however, characteristic of adults in the Stauromedusae, Cubomedusae and Coronatae.

C. Respiratory system. The sub-genital pits and the possibly homologous subumbrellar funnels of Scyphozoa increase the surface area of the body especially in the region of the gonads. It has been suggested that this is of respiratory importance. Experimental support for this notion is lacking. The highly branched gastrovascular system of this medusa is obviously of importance for respiration since it permits the circulation of coelenteric fluid in direct contact with the exterior through the mouth to all parts of the gastrodermis.

Even in a fairly massive jellyfish, note that the active tissues are still epithelial in their organization and that the mesoglea which is thick, contains relatively minute amounts of active protoplasm. Even entire jellyfish probably contain only about 1% organic matter, largely protein. In some species that have been analyzed about 96% has been found to be water. The statements in the literature that marine jellyfish may contain 99.8% water are not correct since even sea water itself is somewhat less than 97% water usually! However, it has been shown for Aurelia that in a brackish environment of 0.6% salinity in which this medusa sometimes lives, it may consist of 98% water. Thus overall oxygen requirements would be expected to be small especially in a gelatinous tissue containing only a few wandering cells.

D. Circulatory system. Coelenteric fluid is moved about in the gastrovascular cavity by the swimming contractions of the disc, movements of the oral arms, etc.. In addition flagellar currents flow distally through the straight radial canals and centrad in the branched ones. A suspension of fine carmine or india ink particles injected into the coelenteron and ring canal will demonstrate this. Diagram the pattern of circulation as determined, indicating the speed of particle movement in the various channels.

As mentioned for Tubularia wandering cells also play a transport role, perhaps nourishing and scavenging the regions of the mesoglea which are too remote from epidermis or gastrodermis for diffusion to be effective.

E. Excretory system. As in hydroids there are no structural specializations for excretion and the physiology and biochemistry of the problem are imperfectly known.

F. Feeding mechanisms and digestive system. The oral lobes of Aurelia with their tentacles play a major part in its feeding. Characteristic of the scyphozoans is the presence of gastrodermal tentacles (gastric filaments) on the wall of the coelenteron. In Aurelia these occur on the floor of the gastric pouches just central to the gonads. These bear nematocysts and hence function in quieting prey which is brought into the gastric cavity. They also contain gland cells which secrete most of the digestive enzymes for extracellular digestion. Furthermore, at least in some species, intracellular digestion also occurs in the gastric filaments. These various phenomena may be observed to advantage if some copepods or other zooplankton stained with neutral red or

brom-thymol blue are introduced into the coelenteron or even just the oral lobes of the medusa.

Also the specific activity of the enzymes can be tested either by making extracts of various tissues like the gastric filaments and then measuring their activity or more crudely by introducing drops of olive oil, pieces of denatured egg white, grains of starch, glycogen, etc. onto the surface of the gastrodermis and observing whether or not they are affected by enzymes.

G. Nervous system and sense organs. As in the hydrozoans the nervous system consists of subepithelial diffuse nerve plexuses. In forms like Aurelia there is a concentration of neurons near each rhopalium, forming a sort of ganglion, and radial strands of the network running towards the oral aboral axison the subumbrellar nerve net extends onto the oral arms and tentacles. Conduction of impulses via this nerve net has been measured at 23 cm./sec. for Aurelia (cf. 12,000 cm./sec. in some mammalian myelinated fibers). If a doughnut preparation is made by removing the margin (including rhopalia) and the center of the bell, an impulse once started around the ring in a given direction will circulate around for a long time without fatiguing or changing its amplitude.

The specific sense organs of Aurelia are the eight marginal tentaculocysts, the rhopalia. These structures which are presumably specialized tentacles consist of a club shaped lithostyle which has equilibratory function, upper and lower sensory pits, and an ocellus. To observe the gross structure of this organ place an appropriate piece of the umbrellar margin in a watch glass under a dissecting scope. The rhopalium isenclosed in a hood and two flanking pairs of tab-like lappets.

The rate of contraction of the disc is increased under certain conditions when the illumination is increased. A demonstration of the photosensory function of the ocelli may be obtained by a controlled experiment of this sort on medusae with and without rhopalia.

Experiments on the equilibritory function of the lithostyles have shown that they normally act as pacemakers for the rhythmic contractions of the medusa. This can be observed when all but two rhopalia are removed from an Aurelia. Swimming movements can be seen to originate near the rhopalia. In such a medusa too, compensatory responses to tipping of the bell are mediated by the rhopalia; these disappear if these latter organs are removed.

H. Skeleto-muscular system. Subumbrellar muscle of ectodermal origin provides the main contractile tissue of Scyphomedusae. Radial as well as circular fibers are present. Histological examination of fresh or macerated tissue will demonstrate the type of cell structures involved.

The behavior of the Aurelia neuromuscular system may be readily studied with a strip preparation prepared from the ring described above by a radial cut. Stimulation of such a strip produces a contraction which lasts about 1 sec., The refractory period is nearly as long as this so that summation or fusion of twitches does not take place. Facilitation does occur, however, and subsequent responses grow for the first few. Demonstrate these facts by conducting some simple experiments of this type.

4. Reproductive system and reproduction. The gametes of Aurelia are shed into the gastrovascular cavity and passed out through the mouth. In female medusae the developing larvae may be retained for a considerable period in the grooves of the oral arms. Examine Aurelia specimens for evidence of such brooding. The gastrula develops by a "typical" invagination; the planula attaches to a substratum by its anterior, aboral end and metamorphoses into a scyphistoma. The polypoid structure of this stage has been outlined above. It winters over the first year and may give rise asexually to other scyphistomae. Then it begins distally to differentiate a serial array of medusae, forming as many as a dozen or more, (strobilization). As these develop, the most distal one becomes free first and swims away as an ephyra.

This is a young medusoid with the characteristic tetramerous symmetry well-developed. It gradually matures into the adult medusa. Study a series of these stages in Aurelia's life cycle so that both their individual structure and their interrelations are understood.

5. Methods of collection, study, etc.. No special comment.

Metridium dianthus (Ellis)

1. Classification. Class Anthozoa, Subclass Zoantharia. Order Actiniaria, Family Metridiidae.

2. Habit and occurrence. Large sessile solitary polyp. No trace of medusoid stage. Numerous short tentacles. From tide pools to 90 fathoms. Labrador to New Jersey, on rocks.

3. Anatomy and physiology.

A. External structure. Typical anemone organization with attached base, upright column, disc with central mouth and marginal tentacles. The column is differentiated into two portions, a distal, thin-walled, short capitulum and a proximal thick-walled scapus. These two regions are separated by a groove and collar. The wall of the scapus is perforated by small openings called cinclides, and the whole animal is firmly, but not immovably fastened to the substratum by the pedal disc. Normally the cinclides are closed but when the anemone contracts on being disturbed water and acontia may be discharged from these openings. The mouth is an elongate slit, which causes the symmetry to deviate from radial. It opens into a coelenteron through a throat-like actinopharynx. The latter has one or more flagelated grooves, siphonoglyphs, which run longitudinally from mouth to lower end of pharynx. Not that if only one siphonoglyph is present the symmetry of the oral disc is reduced to bilateral.

B. General body organization. As in the basic body plan of scyphozoans, the coelenteron of anthozoans is divided by a series of longitudinal partitions. These septa originate as folds of mesoglea covered with gastrodermis from the wall of the column and project inward toward the oral-aboral axis. Septa which reach and join the actinopharynx are called primary mesenteries. There are usually six pairs ofthese in Metridium. Those which insert near the siphonoglyph are called the directive mesenteries; if there are two siphonoglyphs or more, each pair of primary mesenteries associated with a siphonoglyph is called a directive pair. On the side of the mesenteries are longitudinal retractor muscles. On the directives these are on the surfaces of the pair facing out, on the other primaries they are on the sides facing in.

At their upper end the septa are each pierced by two ostia so that the chambers between them are in communication. Below the actinopharynx they are in communication also. There the free edges of the mesenteries are thickened to form the mesenterial filaments which basally becomes free as threadlike acontia. The latter bear nematocysts and can be partly extruded through the cinclides.

Between the primary mesenteries are successivcly shorter cycles of secondary, tertiary, and quartenary pairs. Each bears filaments and basally acontia. Distally they each have only one (the marginal) ostium. Gonads are borne on the mesenteries parallel to the filaments but back from the edge of the septum.

Study these features of Metridium's organization by cutting cross-sections of an expanded specimen through the pharynx and below the pharynx. This may be done effectively with a large pair of

scissors. If the resulting section is not a smooth one at right angles to the longitudinal axis trim it with the scissors to make it so. Diagram the various structures revealed by this means. Unfortunately, Metridium's habit of reproducing asexually by pedal lacerations produces some specimens which have more than two siphonoglyphs and a resultant irregular number of mesenteries. Longitudinal section of a specimen will demonstrate the ostia, relation of pharynx, septal filaments, and acontia.

In anthozoans the mesoglea reaches its highest degree of differentiation. In addition to its non-cellular elements it contains stellate amebocytes and connective tissue cells. Maceration, teasing and staining procedures described for other coelenterates may be used to advantage in studying this tissue.

C. Respiratory system. Definite respiratory currents are maintained by inward beating of the flagella borne in the siphonoglyphs. This direction of beat is the reverse of that found over most of the surface of the disc and actinopharynx under normal conditions. It seems likely that the elongation of the mouth, and hence the deviation from radial symmetry, arose from the need to isolate a special inward flowing respiratory current. The presence of such a circulation is apparently a result of the restricted access to the coelenteron and the heavier tissue construction of anemonis.

D. Circulatory system. The main structure of interest has been mentioned in Section C. Note that the ostia of the mesenteries and the well developed musculature of the column and septa both aid in circulation of coelenteric fluid.

The activity of the siphonoglyphs and various other mechanisms in circulation may be readily observed by the usual techniques with fine carmine or idia ink suspensions.

E. Excretory system. There is good evidence, particularly in corals, that certain areas of the mesenteric filaments are the main site of excretion, at least of solid material. It will be shown below that they also function prominently in digestion and absorption of food. As in other cnidarians, the biochemistry of metabolites and their final fate is not well known in anemones.

F. Feeding mechanisms and digestive system. The basic type of feeding and digestion described for the previous coelenterates is found in Metridium. Because of its large size and abundance these phenomena have been widely studied in this species.

Using Mytilus juice and meat, sand grains, filter paper, and filter paper soaked in Mytilus juice determine the reactions of the tentacles, oral disc, and lips to stimulation by these objects. The reversal of ciliary beat to be observed on the edge of the mouth isan example of application of ciliary currents both to cleansing the disc (centrifugal currents) and transporting food particles to the mouth (centripetal currents). Such ciliary reversal has also been found in some corals but is not known to occur in metazoans other than Cnidaria.

The progress of extracellular and intracellular digestion may be studied as previously suggested by various techniques. In a young active Metridium the following may be tried: 1) measure time necessary for extracellular digestion of animals or meat fed to the anemone 2) if these have been stained observe their intracellular fate from the coloration of gastrodermal cells 3) test the digestive ability of the anemone by noting digestion, or its absence, for specific proteins, fats, or carbohydrates 4) measure the digestive activity of aqueous extracts made from various specific tissues such as tentacles, mesenterial filaments, acontia, et. In the latter, controls must be run with boiled extracts in which the enzymes would be destroyed.

Metridium provides a good coelenterate in which to study the stimuli which cause the discharge of the prey-catching nematocysts. Isolated tentacles observed under the microscope in depression slides are suitable. If care is taken the following facts may be demonstrated: 1) a human hair touched to the tentacle surface will cause many nematocysts to discharge 2) a clean (flamed before used) glass rod of comparable size does not cause discharge unless stimulus is violent 3)food juices introduced with a minimum disturbance, do not evoke discharge 4) in the presence of food juices or other substances such as saliva (1:10 in sea water) mechanical stimulation by a clean glass rod will evoke discharge. This mechanical stimulation aided by a threshold lowering effect of chemical substances seems to be the effective stimulus for the discharge of nematocysts. The nature of the sensitising substance is unknown but may be lipoidal.

To observe more details of nematocysts draw a metridium tentacle across a slide on which some saliva has been dried. Stain with methylene blue or acid fuchsin. The cnidoblast is probably the most complicated metazoan cell known from a morphological point of view. A comparative study of nematocyst structure is of considerable interest. Certain types are of systematic importance and phylogenetic significance. However, the details of their structure are near the limit of resolution of the light microscope even under oil immersion. The various categores of nematocysts should be identified from a classification such as that given by Hyman 1940, p. 383. Compare these organelles from Metridium with those which can be found in any of the other available cnidaria.

As a matter of interest the tips of the cerata of nudibranchs should be examined for these coelenterate structures. Certain of these molluscs with a cnidarian diet have evolved the habit of retaining some of these nematocysts within their own tissues where they presumably function as defensive organs or as distasteful deterrents to the nudibranch's enemies.

G. Nervous system and sense organs. There are no specific sense organs in the Anthozoa. Their nerve net is of the basic type found in coelenterates. Conduction has been found to occur at the rate of 13 cm/sec in this system. The organization of this neuronal network into distinct aggregation or pathways can be determined histologically. However, it is perhaps of greater interest to demonstrate such facts physiologically. This may readily be done as follows: 1) test various parts of the body for sensitivity to mechanical and electrical stimulation 2) note the effects of strength of stimulus on its spread to remote parts of the anemone 3) make a series of cuts, in the

tentacles on the margin, on the column, etc. to see how they restrict the transmission of excitation. Tongue-like strips cut out of similar places will also help identify any directional properties of the system.

H. Skeleto-muscular system. There is no skeleton present in anemones. The muscular system is well developed. Epidermal musculature which is not prominent in any case is restricted in most forms to tentacles and oral disc; gastrodermal musculature is well developed. It consists of circular muscles in the tentacles, oral and pedal discs, and column as well as strong longitudinal retractor and some radial fibers in the mesenteries. The mesenteric retractors are the main muscles which contract the anemone.

Note that although anemones are in general sessile, they move about. Burrowing forms use the physa (which replaces the pedal disc) for digging themselves in while forms like Metridium can creep by means of contractile waves which pass over the pedal disc.

Nuromuscular responses of anemones differ in two important respects: 1) no response is obtained by a single stimulus to the column but if it is followed soon enough by a second, a contraction occurs. Facilitation will continue to build up at a rate dependent on the numbers and rate ofstimulation until a steady level is reached. This decays when stimulation stops 2) The refractory period is shorter than the slow twitch so that mechanical summation is a prominent feature of the response. These facts may be readily demonstrated by stimulating the column of a Metridium with shocks of various frequencies and intensities, meanwhile recording the resulting tension developed. Note that the anemone's neuromuscular system differs from that of the medusa discussed above by having a twitch that is tonic rather than clonic in character and by having synapses in the system which require more than one impulse or series of impulses to effect transmission.

4. Reproductive system and reproduction. The life cycle of Metridium is simple. As mentioned above, gonads are borne on the mesenteries, but in Metridium not on the directives and usually not on the primaries. Gametes which originate from gastrodermal intertitial cells mature in the mesoglea. The zygote develops into a free-swimming planula which begin to metamorphose into anemones, settle down and attach at their aboral end.

Asexual reproduction occurs commonly in Metridium. As previously stated pedal laceration is the predominant method. The anemone moves away from its point of attachment leaving behind it parts of the pedal disc and septa. These regerate a new anemone in the old site and the parent regrows a pedal disc in a new location. Laceration of this sort may be quite irregular when the frequency of modifications in the basic septal and siphoglyph plan in this species.

5. Methods of collection, study, etc. No special comment.

PLATYHELMINTHES - LABORATORY DIRECTIONS

Class: Trematoda.

A. An adult digenetic trematode.

1. Cryptocotyle lingus (Creplin): This worm occurs in the small intestine of fish-eating birds and mammals. Obtain several specimens from the instructors 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 coverglass and apply slight pressure until the worms are fairly well flattened. Watch 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.)

By using the supplementary laboratory directions identify all the structures of the adult Cryptocotyle lingua.

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 dougalli, 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 it on a slide in a small quantity of avian salt solution. Place a cover glass on the preparation and while looking through the microscope carefully withdraw the excess solution from the edge of the cover glass with a piece of absorbent paper; continue the withdrawal until the worm is flattened, quiet and relatively transparent. Now carefully vaseline the edges of the cover glass. The following parts should be identified and studied. At the anterior end is the oral sucker surrounded by a

flared collar. Estimate, or count, the number of spines in the collar. 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 (sing. caecum). The large, cup-shaped acetabulum (ventral sucker) is between the anterior heavily cuticular spined region and the posterior more weakly cuticular spined area. The genital pore opens to the ventral surface immediately anterior to this sucker. From the pore the uterus and 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 lying vitellaria. The excretory system consists of a posterio-dorsally emptying, irregularly shaped, conspicuous excretory bladder which receives, antero-laterally, two much-branched excretory trunks. The ultimate unit of the excretory system is the flame cell (solenocyte, 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 single, fully-formed redia and the life cycle is, therefore, without a sporocyst generation. Study the miracidium and understand its full importance.

3. Other 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 identification aids.

B. 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 Cryptocotyle 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. Note the pharynx, short intestine, antero-lateral birth-pore, and the developing cercariae, ranging from undifferentiated germ-balls at the posterior end to mature cercariae near the birth pore. Draw a mature redia containing cercariae in various stages of development.

2. Cercaria.

a. 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 cover glass. As the water evaporates the pressure of the cover glass flattens and quiets the cercariae; the body details then become more distinguishable. Observe the general resemblance of the body of the cercaria to that of the adult fluke. Find oral sucker, pharynx, intestinal crura, penetration glands (how many, and of what use?) with ducts leading forward to empty near the anterior tip, cystogenous glands (of what function?) scattered over the whole body surface, germinal mass, excretory bladder, eye spots, and tail. Note the distribution of the fin on the tail, and spines on the body. See part b. for physiological experimentation suggestions for use with this cercaria. Determine the usefulness to the cercaria of any adaptations you find.

b. Make a comparative study of the various cercariae in the laboratory after examining the cercaria of C. lingua. See part 3b. Determine the probable type of life-cycle of each form from their cercarial specializations. Note: tail size and motility, eye spots, reaction to light and shadows, swimming motions, etc.

3. Encystment of the cercaria to form the metacercaria:

a. 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 and make a series of sketches showing the activities of the cercariae during penetration and encystment.

b. Other cercariae and hosts will be available for additional experimentation. The following table lists the trematodes from the Woods Hole area whose life-cycles are known. Expose the proper metacercarial hosts to the cercariae available and determine the methods of infestation. If time and hosts are available feed the infected metacercarial hosts to the proper adult host and reclaim the young excysted adult worms.

Adult Worm	Cercaria in*	Metacercaria in	Adult in	See
Zoogonus rubellus	Nassa obsoleta	Nereis	Fishes	Stunkard, H. W. 1938 Biol. Bull. 75: 398-334
Himasthla quissetensis	Nassa obsoleta	Nereis and bivalve molluscs	Gulls& Herons	Stunkard, H. W. 1938. Biol. Bull. 75: 145-164.
Lepocreadium setifercides	Nassa obsoleta	Turbellaria and annelids	Flat Fishes	Martin, W. E. 1938. Biol. Bull. 75: 463-474
Stephanostomum tenue	Nassa obsoleta	Menidia (liver)	Puffer & other fish	Martin, W. E. 1939. Biol. Bull. 76: 131-144
Gynaecotyla nassicola	Nassa obsoleta	Talorchestia	Sandpiper Plovers Gulls	Rankin, J. S. Jr. 1940, Biol. Bull. 79: 439-451
Cryptocotyle lingua	Littorina littorea	Skin of fishes	Piscivorous birds Mammals	Stunkard, H. W. 1930, J. Morph. and Physiol. 50: 143-191.
Podocotyle atomon	Littorina rudis	Crustaceans	Fishes	Hunninen, A. V. & R. M. Cable 1943. Trans. Amer. Micr. Soc. 62: 57-68
Opecoeloides manteri	Mitrella lunata	Amphipods	Fishes	Hunninen, A. V. & R. M. Cable 1941. Biol. Bull. 80: 415-428
Zoogonoides laevis	Columbella (= Mitrella) lunata	Nereis	Tautog	Stunkard, H. W. 1943. Biol. Bull. 85: 277-237.
Parorchis acanthus	Urosalpinx cinereus & Thais lapillus	Open	Gulls	Cable, R. M. & A. V. Hunninen 1940. Biol. Bull. 78: 136-157.
Siphodera vinal-edwardsii	Bittium alternatum	Flounders	Toadfish	Cable, R. M. & A. V. Hunninen 1942. Jour. Parasitol. 28: 407-420.
Deropristis inflata	Bittium alternatum	Nereis	Eels	Cable, R. M. & A. V. hunninen 1942. Biol Bull. 82: 292-312

Lecithaster confusus	Odostomia	Copepods	Stickle-backs and other fish	Hunninen, A. V. & R. M. Cable 1943. Jour. Parasitol. 29: 71-79.
Monorchoides	Cumingia tellinoides & Tellina tenera	Siphons of cercarial hosts	Flounders and eels	Martin, W. E. 1940. Biol. Bull. 79: 131-144
Maritrema arenaria	Undiscovered	Barnacles	Ruddy Turnstone (shore bird)	Hadley, C. E. & M. R. Castle, 1940 Biol. Bull. 78: 338-348

* The species name used here is that given in the paper cited.

4. Metacercaria.

a. Study the metacercaria of C. lingua as it appears encysted in a Cunner fin. With well-sharpened needles, carefully remova a metacercaria from its cyst and study it. 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 final host?

b. The following experiments may be substituted for C. lingua metacercarial experiments with the permission of the instructor.

1) Remove and study metacercariae obtained as a result of your experimentation under part 3B.

2) Remove and study the metacercaria 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 mechanically excyst the worms.

Class: Cestoda.

A. Scolex: Examine living scolices of Rhynchobothrium and Calliobothrium (both from the spiral valve of the smooth dogfish),

and if available, Crossobothrium from the sand shark. Look for bothria, hooks, suckers, proboscides with their sheaths and contractile bulbs, and unsegmented neck region, excretory tubes with adjacent flame-cells, and the nerve trunks. Consult the supplementary laboratory manual for additional morphological details.

B. Mature proglottid: Study prepared slides or, if available, examine a mature living proglottid by compressing carefully between slide and cover glass. Follow the supplementary laboratory manual as far as possible.

C. Plerocercous (Cysticercoid) larva: The hexacanth embryos of the tapeworm, Otobothrium, from the Hammerhead shark, enter the body of the butterfish, their intermediate host to form the cysticercoid larvae which are small white dots in the dorsal body muscles. Remove a cysticercoid from its sheath of host connective tissue and carefully tease it apart with needles. Cover and study in the flattened position. As the larva unfolds, notice the fine proboscides similar to those in Rhynchobothrium. Also note the ear-shaped bothria provided with accessory suckers. The striking locomotion of the larva aids it in moving through the thick intestinal chyme of the shark host.

Class: Nemertea.

A. Amphiporus or Tetrastemma: Follow the directions in the supplementary laboratory manual for determining the morphology and physiology of these nemertines. The chief difference in gross morphology between the two genera is the larger number of eye-spots in Amphiporus.

B. Make a comparative study of the available nemertines by completing a table containing, at least, the following headings: color; size; shape of body; shape of head; shape and position of mouth opening; eye spots; type of proboscis; type and number of stylets; swimming ability; character of gut; character of gonads.

Class: Namatoda. Metoncholaimus pristiurus is a free living nematode formerly found in abundance in mud from shallow water of Great Harbor. It belongs to the large marine sub-family, the Oncholaiminae. If specimens are available then study several in a syracuse dish with a binocular dissection microscope to observe the continual coiling and uncoiling characteristic of many nematodes and to distinguish the blunt anterior from the more pointed posterior end. Note that some large specimens contain several large bead-like structures at about the middle of the body. These are eggs and indicate the animal is an adult female. Place such a specimen in a drop of fresh water for one to two minutes until quiet and then mount at once in clear sea water. Flatten the animal slightly by removing water from under the cover-glass. Under these conditions the worm should be quiet except for slow movements of the digestive tract which will help observations.

A. The Digestive System:

Note that the posterior end tapers very rapidly and is slightly curved. The anterior end tapers gradually. Along the sides of both ends are numerous sensory setae. At the truncated extremity of the anterior end is seen the mouth opening. Behind it is the short pharynx in which there are three sharply-pointed teeth, the onchia. The thick-walled tube running backward from the pharynx is the oesophagus. At its posterior end is a sphincter valve (the cardia) marking the beginning of the intestine, which is a yellowish-brown tube running throughout nearly the entire length of the body. Careful focusing on the anterior part of the intestine will show that its wall is composed of typical columnal epithelium. The inner ends of most of the epithelial cells are filled with granules which give the color to the intestinal wall. About halfway along the tapering tail (ventrally)

is seen the anus and running forward from it at an angle is the rectum.

B. The Tail and Spinneret:

The tail is first conoid and then cylindroid in the posterior fourth where it ends in the somewhat blunt, almost imperceptibly swollen ampullae of the three caudal glands. The cement-like secretion of these glands is poured out of the minute pore at the extreme tip of the tail to be used in temporarily cementing the worm by the tail to the substratum. A spinneret valve (hemispherical posteriorly and tapering anteriorly) is fastened by a contractile fiber to the ampullae and the contraction of this fiber withdraws the valve to allow outflow of the secretion. The caudal glands are ellipsoidal, arranged in tandem fashion from a point about five body diameters anterior to the anus to a point about 10 body diameters anterior to the anus. Each caudal gland is connected with an ampulla by a long, slender caudal gland duct.

C. The Excretory System

This system consists of a single "renette" cell which is a fusiform, ventral cell located ventrally about four body-widths behind the neck. This cell is connected by the renette duct to a single, ventral excretory pore located about one-fourth the distance from the anterior end of the body to the nerve ring. The renette duct enlarges near the excretory pore to form a minute excretory visicle.

D. The Nervous System.

The chief concentration of the nervous system is the thick, semi-translucent nerve-ring which encircles the oesophagus about midway of its length. Before and behind the nerve-ring are numerous distinctly nucleated ganglion cells. Other ganglion cells may be seen along the ventral nerve and in connection with sensory setae

of the tail may be seen in demonstration specimens stained with methylene blue. The longitudinal nerve cords are not well developed in Metoncholamimus.

E. The Reproductive Systems:

1. The female reproductive system:

a. The genital organs. A short distance proximal to the large thick shelled eggs, which are in the uterus, may be seen a row of cuboidal cells nearly as large as the diameter of the body. The most proximal of these cells, the oöcytes, marks the posterior end of the ovary. From this point, anteriorly, the ovary continues forward showing progressively more advanced stages in egg development. The broad reflexed ovary is continuous with the much narrower oviduct which turns posteriorly to connect with the uterus near the posterior end of the ovary. Posteriorly, the uterus connects by a short, transverse vagina to the slightly elevated vulva, the ventrally located external opening of the female system.

b. The Demanian system. This system is found only in the female where it elaborates a copious, elastic, sticky, non-water-soluble secretion possibly utilized during copulation and also presumably to protect and preserve the eggs after oviposition. The system consists of the following structures: A short distance anterior to the rectum are two large, clear, cross-striated tubes, the moniliform glands, which open posteriorly by separate pores. Anteriorly these tubes unite near the conspicuous, rosette-like uvette. From the uvette a tube runs to the intestine, and another tube, starting as a wide ampulla, soon narrows rapidly to form a thin tube, the efferent uterus which joins the uterus in the vicinity of the vulva.

Make a large drawing of the female Metoncholaimus to show as many of the above features as you have been able to identify.

In the manner already described mount a male specimen and examine. The demanian system is absent although possibly represented by obscure homologous structures. The tail of the male diminishes suddenly in size at the arms and is armed with about ten small "supplementary organs" ventrally located, which give the tail a serrated appearance, giving rise to the specific name, pristiurus (saw-tailed). There are also about thirty short ventral sensory setae. Supplementary organs and setae are alike sensory in function. The opening of the male genital system is just anterior to the anus. Extending forward from it are two slender, rod-like spicula, about sent times as long as the anal body diameter. There are two testes, the anterior testes and the posterior, extending in opposite directions along the middle third of the body. The two testes join the long vas deferens which connects with the ejaculatory duct which in turn opens posteriorly thru the genital pore. In each testis there is a progression of stages in sperm development from the blind end of the testis toward the junction with the vas deferens.

Draw to show the male genital organs and the extreme posterior end of the male worm.

KEY TO THE MORE COMMON NEMERTEA OF THE WOODS HOLE AREA

NEMERTEA. Unsegmented, tapelike or filiform, externally ciliated, soft-bodied, contractile worms, often brightly colored, and with creeping, swimming or burrowing habits; intestine opens at a posterior anus; formidable proboscis often everted from anterior end upon stimulation. Key adapted from Coe (1943).

(1) (28) Mouth posterior to brain; central nervous system imbedded in body wall, either between the muscular layers or external thereto; proboscis not armed with stylets (Class Anopla). . . . . .(2)

(2) (9) Body musculature composed either of 2 layers (outer circular and inner longitudinal) or of 3 layers (outer circular, longitudinal and inner circular); cutis absent (Order Paleonemertea). . .(3)

(3) (6) Mouth situated immediately behind brain; nephridia with single pair of large collecting tubules and efferent ducts. . . . . .(4)

(4) (5) Lateral nerves situated at base of body epithelium or external to circular muscles of body wall, at least in anterior portion of body; internal circular musculature relatively thin; lateral sense organs present; rhynchocoel vessels absent. . . . . .
Tubulanus pellucidus (Coe, 1895)

(5) (4) Lateral nerves situated outside muscular layers in anterior portion of esophagral region but imbedded in longitudinal muscles in nephridial region and posteriorly; internal circular musculature enormously developed in nephridial region; lateral sense organs absent; rhynchocoel vessels present. . . . . .
Carinoma tremaphoros Thompson, 1900

(6) (3) Mouth situated far behind brain; nephridia with very numerous minute efferent ducts; body filiform; head sharply pointed. . . . . (7)

(7) (8) With inner circular musculature surrounding blood vessels and gonads in foregut region of body; worms coil body in a spiral when disturbed. . . . . .
Procephalothrix spiralis (Coe, 1930)

(8) (7) Inner circular muscles absent; worms do not coil body in a spiral when disturbed. . . . . .
Cephalothrix linearis (Rathke, 1799)

(9) (2) Body musculature of 3 layers (outer longitudinal, circular and inner longitudinal) to which thin inner circular and outer oblique layers are sometimes added; cutis well developed. . . . . (Order Heteronemertea). . (10)

(10) (13) Without longitudinal cephalic grooves. . . . . .(11)

(11) (12) Caudal cirrus present; head narrow; without oblique cephalic grooves. . Zygeupolia rubens (Coe, 1895)

(12) (11) Caudal cirrus absent; head broad; with oblique cephalic grooves..Parapolia aurantiaca Coe, 1895

(13) (10) With longitudinal cephalic grooves. . . . . (14)

(14) (19) Caudal cirrus absent; body long and slender, filiform in some species, rounded or flattened in others, very contractile; ocelli present in most species..(15)

(15) (16) With conspicious median dorsal stripe, but without transverse markings; dark brown or olive, with median dorsal stripe of white or yellow extending whole length of body and head. . . . . Lineus bicolor Verrill, 1892

(16) (15) Without conspicious median dorsal stripe. . . . . (17)

(17) (18) Head rather broad, cephalic grooves short; body contracts by shortening and thickening--not by coiling in spiral; oblique musculature thin. . . Lineus ruber (O. F. Müller, 1771)

(18) (17) Head narrow, cephalic grooves long; body contracts by coiling in spiral; oblique musculature relatively thick. . . . . .Lineus socialis (Leidy, 1855)

(19) (14) Caudal cirrus present; body not very slender, ocelli present or absent. . . . . (20)

(20) (27) Body rather soft, usually flattened; lateral margins not thin; not adapted for swimming; mouth small and round; proboscis sheath often shorter than body, but proboscis very slender and much longer than body, ocelli usually present. . . . . (21)

(21) (22) Head with a row of 4 to 6 ocelli on each side. . . Micrura affinis (Girard, 1853)

(22) (21) Head without ocelli. . . . . .(23)

(23) (26) Color of body red or reddish. . . . . (24)

(24) (25) Pale red, yellowish red or brownish red. . . . . Micrura caeca Verrill, 1895

(25) (24) Deep red or purplish red. . . . . Micrura leidyi (Verrill, 1892)

(26) (23) Color of body whitish or pale yellowish, often with a tinge of red or orange anteriorly. . . . . Micrura albida Verrill, 1879

(27) (20) Body firm, long and ribbon-like; much flattened in intestinal region, with very thin lateral margins and well adapted for swimming; body less contractile than in other genera; mouth large and elongated, ocelli absent..Cerebratulus lacteus (Leidy, 1851)

(28) (1) Mouth anterior to brain; central nervous system situated internal to body musculature; proboscis usually armed with one or more sharply pointed stylets (Class Enopla). . . . . .(29)

(29) (58) Intestine straight and with paired lateral diverticula; stylet apparatus present; mouth and proboscis opening separate; posterior sucking disk absent (Order Hoplonemertea). . . . . (30)

(30) (31) Proboscis rudimentary, without accessory stylets; parasitic on gills and egg-masses of the lady crab, Ovalipes ocellatus, and other crabs. . . . . Carcinonemertes carcinophila (Kölliker, 1845)

(31) (30) Proboscis eversible, provided with accessory stylets; not parasitic on crabs. . . . . (32)

(32) (33) Body long and slender; cerebral sense organs small, situated in front of brain; proboscis and proboscis sheath nearly as long as body. . . . . Oerstedia dorsalis (Abildgaard, 1806)

(33) (32) Body usually relatively short and broad; cerebral sense organs large, situated beside, behind or in front of brain. . . . . (34)

(34) (47) Intestinal diverticula branched; intestinal caecum usually with long anterior branches; gonads irregularly grouped; ocelli usually numerous. . . . . (36)

(35) (36) Ocelli extend posteriorly along lateral nerve cords beyond brain; basis of central stylet cylindrical and sharply truncated or concave at posterior end. . Zygonemertes virescens (Verrill, 1879)

(36) (35) Ocelli do not extend posteriorly beyond brain; basis of central stylet truncate conical or pear-shaped and usually rounded at posterior end. . . . (37)

(37) (44) Ocelli few; in mature individuals not more than 12 on each side of head. . . . . (38)

(38) (39) Only 1 pair of ocelli, situated near tip of head; color of body orange-red. . . . . Amphiporus bioculatus McIntosh, 1873

(39) (38) Ocelli 6 to 12 on each side of head. . . . . (40)

(40) (41) Ocelli in a single row along each side of head; blood vessels bright red and conspicious in life; color of body usually pale yellow or rosy. . . . Amphiporus cruentatus Verrill, 1879

(41) (40) Ocelli in an irregular double row on each side or scattered. . . . . .(42)

(42) (43) Epidermis very thick and soft, secreting much viscid mucus when stimulated; movements of body often leech-like. . Amphiporus griseus (Stimpson, 1857)

(43) (42) Epidermis thin and firm, secreting but little mucus when stimulated; moves by creeping. . . . . Amphiporus ochraceus (Verrill, 1873)

(44) (37) Ocelli numerous; usually more or less separated into 2 groups on each side of head. . . . . .(45)

(45) (46) Anteromarginal ocelli in elongated cluster; cerebral group in more compact cluster; color of body reddish brown dorsally; posterior border of head with conspicuous white spot on each side. . . . . Amphiporus angulatus (Fabr., 1774)

(46) (45) Anteromarginal ocelli in single irregular row; cerebral group in more compact cluster; color of body pale yellow or orange; head broad and flat and without white spots. . . . . Amphiporus lactifloreus (Johnson, 1828)

(47) (34) Intestinal diverticula unbranched; intestinal caecum with short anterior branches or none; gonads alternate regularly with intestinal diverticula; ocelli usually 4. . . . . .(48)

(48) (49) With 4 or 6 ocelli; fresh-water species, often common in ponds in the Woods Hole area. . . . . Prostoma rubrum (Leidy, 1850)

(49) (48) With 4 ocelli; marine species. . . . . (50)

(50) (53) Body usually with more or less conspicuous longitudinal stripes. . . . . (51)

(51) (52) Body rather slender, yellow, with 2 broad longitudinal brown stripes. . Tetrastemma elegans (Girard, 1852)

(52) (51) Body short and broad; usually green, with 1 or 2 longitudinal yellow stripes and 6 green stripes near tip of head. . Tetrastemma vittatum Verrill, 1874

(53) (50) Body without well-defined longitudinal stripes. . .(54)

(54) (55) White or translucent with superficial flecks of white. . . . . .Tetrastemma wilsoni Coe, 1943

(55) (54) Yellow, rosy, red or green. . . . . .(56)

(56) (57) Pale green or yellowish, head white or cream colored. . Tetrastemma candidum (Müller, 1774)

(57) (56) Yellow or rosy, often spotted with brown; with band of dark pigment connecting the two ocelli on the same side of head. Tetrastemma vermiculus (Quatr., 1846)

(58) (29) Intestine convoluted and without diverticula; stylet apparatus absent; mouth and proboscis opening united; posterior sucking disk present (Order Bdellonemertea). One rather rare local species parasitic in mantle cavity of Venus, Mya and occasionally Ostrea. . . . . Malacobdella grossa (O. F. Muller, 1776)

PLATYHELMINTHES AND NEMATHELMINTHES
SELECTED BIBLIOGRAPHY

(Good reference lists, original research and investigations based on the Woods Hole fauna have been the criteria used in compiling this bibliography. It is not an exhaustive review of the literature of these groups, but is one designed for quick orientation in the several groups.)

GENERAL

Dawes, Ben. 1946. The Trematoda, with special reference to British and other European forms, Cambridge Univ. Press. London.

Kükenthal, W. and T. Krumbach, 1928. Handbuch der Zoologie, Vol. 2, first half. An excellent general discussion of the morphology, physiology, and taxonomy of all groups included under the Platyhelminthese and Nemathelminthes. Following each group discussion is a good selected bibliography up to 1930.

Class: Trematoda

Order Monogenea

Alvey, C. H. 1936. The morphology and development of monogenetic trematode (Sphyranura oligorchis (Alvey, 1933) and the description of Sphyranura polyorchis n. sp. Parasitol., 28: 229-253.

Jahn, T. L. and L. R. Kuhn. 1932. The life history of Epibdella melloni MacCallum, 1927, a monogenetic trematode parasitic on marine fishes. Biol. Bull. 62: 89-111.

Odhner, T. 1912. Die Homologien der weiblichen Genitalwege bei den Trematoden und Cestoden. Zool. Anz. 39: 327-251.

Paul, A. A. 1938. Life history studies of North American Freshwater polystomes. Jour. Parasitol., 25: 489-510.

Order Aspidogastrea

Stafford, J. 1896. Anatomical structure of Aspidogaster conchicola. Sool. Johr. Abt. f. Anat. und ontogenic der thiere. 9: 477-542.

Stunkard, H. W. 1917. Studies on North American Polystomidae, Aspidogastridae, and Paramphistomidae. Ill. Biol. Monographs 3: 1-114.

Van Cleave, H. J. and C. O. Williams. 1943. Maintenance of a trematode, Aspidogaster conchicola, outside the body of its natural host. Jour. Parasitol. 29: 127-130.

Williams, C. O. 1942. Observations on the life history and taxonomic relationships of the trematode Aspidogaster conchicola. Jour. Parasitol. 28: 467-475.

Order Digenea

Bosma, N. J. 1934. - The life history of the trematode Alaria mustelae n. sp. Trans. Amer. Micr. Soc., 53: 116-153.

Cable, R. M. 1934. - Studies on the germ cell cycle of Cryptocotyle lingua. II. Germinal development in the larval stages. Quart. Jour. Micr. Sci., 76: 573-614.

Cable, R. M. and A. V. Hunninen, 1939. Studies on the life history of Spelotrema nicolli. Biol. Bull. 77 (2): 309.

Cable, R. M. and A. V. Hunninen. 1940. Studies on the life history of Spelotrema nicolli (Trematoda: Microphallidae) with the description of a new microphallid cercaria. Biol. Bull. 78 (1): 136-157.

Cable, R. M. and A. V. Hunninen, 1942. Studies on Deropristis inflata (Molin), its life history and affinities to trematodes of the family Acanthocolpidae. Biol. Bull. 82 (2): 292-312.

Cable, R. M. and A. V. Hunninen. 1942. Studies on the life history of Siphodera vinaledwardsii (Linton) (Trematoda: Cryptogonimidae). Jour. Parasitol. 28 (5): 407-420.

Chen, Pin-dji. 1937. The germ-cell cycle of the trematode, Paragonimus kellicotti Ward. Trans. Amer. Micr. Soc. 56: 208-236.

Dobrovolny, C. G. 1939. Life history of Plagioporus sinitsini Mueller and embryology of new cotylocercous cercariae (Trematoda) Trans. Amer. Micr. Soc. 58: 121-155.

Ferguson, M. S. 1943. - Development of eye flukes of fishes in the lenses of frogs, turtles, birds and mammals. Jour. Parasitol. 29 (2): 136-142.

Goodchild, Chauncy G. 1943. The life-history of Phyllodistomum solidum Rankin, 1937, with observations on the morphology, development and taxonomy of Gorgoderinae (Trematoda), Biol. Bull. 84 (1): 59-86.

Hadley, C. E. and M. R. Castle, 1940. Description of a new species of Maritrema Nicolli. 1907, Maritrema arenaria, with studies of the life history. Biol. Bull. 78 (2): 338-348.

Hsu, D. Y. 1937. Life History and morphology of Macravestibulum eversum sp. nov. (Pronocephalidae, Trematoda). Trans. Amer. Micr. Soc. 56: 478-504.

Hunninen, A. V. and R. M. Cable. 1941. Studies on the life history of Anisoporus manteri Hunninen and Cable, 1940 (Trematoda: Allocreadiidae). Biol. Bull. 80: 415-428.(Note: Anisoporus = Opecoelides)

Hunninen, A. V. and Cable, R. M. 1943. The life history of Podocotyle atomon (Duodolphi) (Trematoda: Opecoelidae). Trans. Amer. Micr. Soc. 62 (1): 57-68.

Hunninen, A. V. and R. M. Cable. 1943. The life history of Leithaster confusus Odhner (Trematoda: Hemiuridae). Journ. Parasitol 29 (1) 71-79.

Hussey, Kathleen L. 1943. Further studies on the comparative embryological development of the excretory system in digenetic trematodes. Trans. Amer. Micr. Soc. 62 (3): 271-279

LeRue, G. R. 1927. A trematode with two ani. Jour. Parasitol. 12: 207-209.

LeRue, G. R., 1938. Life history studies and their relation to problems in taxonomy of digenetic trematodes. Jour. Parasitol. 24: 1-11.

McMullen, D. B. 1939. Observations on precocious metacercarial development in the trematode superfamily Plagiorchioidea. Jour. Parasitol. 24: 273-280.

Martin, W. E. 1938. Studies on trematodes of Woods Hole: The life cycle of Lepocreadium setiferoides (Miller and Northrup), Allocreadiidae, and the description of Cercaria cumingiae n. sp. Biol. Bull. 75: 463-474.

-1939 - Studies on the trematodes of Woods Hole. 11. The life cycle of Stephanostomum tenue (Linton). ibid. 76: 65-73

-1940- Studies on the trematodes of Woods Hole. 111. The life cycle of Monorcheides cumingiae (Martin) with special reference to its effect on the invertebrate host. ibid. 79 (1): 131-144.

-1944- Studies on trematodes of Woods Hole. 1V. Additional observations upon Cercaria loossi Stunkard developing in an annelid. Trans. Amer. Micr. Soc. 63 (3): 237-243.

-1945- Two new species of marine cercariae. Trans. Amer. Micr. Soc. 64 (3): 203-212.

Nicoll, W. 1909. - Studies on the structure and classification of the digenetic trematodes. Quart. Jour. Micr. Soc. 53:391-487.

Odhner, T. 1928. - Weitere Trematoden mit anus. Arkiv. f. Zoolog. (Stockholm) 20: 1-6.

Olivier, Louis, 1940. - Life history studies on two strigeid trematodes of the Douglas Lake region, Michigan. Jour. Parasitol 26: 447-477.

Pratt, H. S. 1909 - The cuticula and subcuticula of the trematodes and cestodes. Amer. Nat. 43: 705-729.

Rankin, J. S., Jr. 1939. - The life cycle of the frog bladder fluke, Gorgoderina attenuata Stafford, 1902 (Trematoda: Gorgoderidae) Amer. Midl. Nat., 21: 476-488.

Rankin, J. S. Jr. 1940. - Studies on the Trematode family Microphallidae Travassos, 1921 1V. The life cycle and Ecology of Gynaecotyla Nassicola (Cable and Hunninen, 1938) Yamaguti, 1939. Biol. Bull. 79 (3): 439-451.

Rankin, J. S., Jr., 1946. Examination of tube-dwelling polychaete annelids for larvae trematode infections. Jour. Parasitol. 32 (1): 92.

Ssinitzin, D. T. 1911. - Parthenogenetic generation of trematodes and its progeny in molluscs of the Black Sea. Records of the Imperial Academy of Sciences, 8th series, Physico-Mathematical Department, 30 (5). (Original in Russian. Translations may be obtained from the secretary, Department of Zoology, University of Michigan.)

Stunkard, H. W. and R. M. Cable. 1932. - The life history of Parorchis avitus (Linton) a trematode from the cloaca of the gull. Biol. Bull., 62: 328-338.

Stunkard, Horwace W. 1938. The morphology and life cycle of the trematode Himasthla quissentensis (Miller and Northup, 1926) Biol. Bull. 75 (1): 145-164.

Stunkard, H. W. 1938. Distomum lasium Leidy, 1891 (syn. Cercariaeum lintoni) Miller and Northrup 1926, the larval stage of Zoogonus rubellus (Olsson, 1868) (syn. Z. mirus Looss, 1901) Biol. Bull. 75 (2): 398-334.

Stunkard, H. W. 1943. - The morphology and life history of the digentic trematode, Zoogonoides laevis (Linton) 1940. Biol. Bull. 85 (3): 227-237.

Stunkard, H. W. 1946. - Possible snail hosts of Human Schistosomes in the United States. Jour. Parasitol. 32 (6): 539-552.

Stunkard, H. W. 1930. - The life history of Cryptocotyle lingua (Creplin), with notes on the physiology of the metacercariae. Jour. Morph. and Physiol. 50: 143-191.

VonWicklen, J. H. 1946. - The trematode Genus Opecolleides and related Genera, with a description of Opecoeloides polynemi N. sp. Jour. Parasitol. 32 (2): 156-163.

Willey, C. H. 1930. - Studies on the lymph system of digenetic trematodes. Jour. Morph. and Physiol. 50: 1-37.

Williams, Charles O. 1942.-Observations on the life history and taxonomic relationships of the trematode Aspidogaster conchicola. Jour. Parasitol. 28 (6): 467-474.

Class: Cestoda

Benham, B. 1901. - Platyhelmia. A Treatise on zoology edited by E. Ray Lankester.

Linton, E. 1938. - Cestode holdfasts. Livro Jubilar Prof. Travassos, 3: 275-278.

Lühe, M. 1909. Parasitische Plattwurmer. Trematodes. Die Susswasserfauna Deutschlands, 17: 100.

Stunkard, H. W., 1937. - The life cycle of Moniezia expansa. Science 86:312.

Stunkard, H. W. 1940. The morphology and life history of the cestode, Bartiella studeri. Amer. Jour. Trop. Med. 20: 305-333.

Stunkard, H. W. 1941. - Studies on the life history of the anoplocephaline cestodes of hares and rabbits. Jour. Parasitol. 27:299-325.

Thomas, Lyell, J. 1947. - The life cycle of Diphyllobothrium oblongatum Thomas, A tapeworm of gulls. Jour. Parasitol. 33 (2): 107-117.

Venard, C. E. 1938. - Morphology, Bionomics, and taxonomy of the cestod Dipylidium caninum. Annals N. Y. Acad. Aci., 37: 273-328.

Young, T. 1935. - Some unsolved problems of cestode structure and development. Trans. Amer. Micr. Soc. 54: 229-239.

Class: Nemertea

Coe, W. R. 1930. - The peculiar nephridia of the nemerteans of the genus Cephalothrix. Zool. Anz. 89: 103-108.

-1930a- Assexual reproduction in nemerteans. Physiol. Zoool. 3: 297-308.

Coe, W. R., 1934. - Analysis of the regenerative processes in nemertean Biol. Bull. 66: 304-315.

Coe, W. R. 1943. - Biology of the Nemerteans of the Atlantic Coast of North America. Trans. Conn. Acad. Arts and Sci., 35:129-328.

Dakin, W. J. and M. G. C. Fordham. 1936. - The anatomy and systematic position of Gorgonorhychus repens, gen. n., sp. n., a new genus of nemerteans characterized by a multi-branched proboscis Prof. Zool. Soc. London, 1936 (2): 461-483.

Friedrich, H. 1935. - Beobachtungen uber eigenartige Bewegungsweisen bei Nemertinen. Biol. Zentralbl. 55: 84-86.

-1935a.- Studien zur Morphologie, Systematik, und Okologie der Nemertinen der Kieler Bucht. Arch. Naturgesch, (Arch. wiss. Zool. Abt. B) 4: 293-375.

Hopkins, Sewell H. 1947. The nemertean Carcinonemertes as an indicator of the spawning history of the host, Callinectes sapidus. Jour. Parasitol. 33 (2): 146-150

Stiasny-Wijnhoff, G. 1934. - Some remarks on North Atlatic nonpelagic Petystylifera. Quart. Jour. Micr. Sci. 77: 167-190.

Phylum Nemathelminthes
Class; Nematoda and Acanthocephala

Chitwood, B. G. and M. B. Chitwood, 1937-1938. - An introduction to Nematology, Baltimore.

Cobb, N. A. 1914. - Nematodes and their relationships. Yearbook, U. S. Department of Agriculture, 1914: 457-490.

Taylor, A. L. 1935. - A review of the fossil nematodes. Prof. Helminth Soc. Wash., 2: 47-49.

VanCleave, Harley J. 1947. - A critical review of terminology for immature stages in Acanthocephalan life histories. Jour. Parasitol 33 (2): 118-125.

Yorke, W. and P. A. Maplestone, 1926. - The nematode parasites of vertebrates. Philadelphia.

Ecology and Physiology of Helminth Parasites

Addis, C. J., Jr. and Asa C. Chandler. 1946.-Further studies on the vitamin requirement of tapeworms. Jour. Parasitol. 32 (6): 581-584.

Brand, T. V. and G. F. Otto, 1938. - Some aspects of the carbohydrate metabolism of the hookworm, Ancylostoma caninum, and its host, Amer. Jour. Hyg. 27: 683-689.

Brand, T. V. 1937. - The anaerobic glycogen consumption in Ascaris females and males. Jour. Parasitol. 23:68-72.
-1937a. - The anaerobic glycogen consumption in Ascaris females and males. Jour. Parasitol. 23: 316-317.

Brand, T. V. 1942. - Physiological observations upon a larval Eustrongylides. Biol. Bull., 82: 1-13.

Cameron, T. W. M. 1945. - Fish carried parasites in Canada. I. Parasites carried by fresh-water fish. II. Parasites carried by salt-water fish. Canadian Jour. Comp. Med. 9:245-254; 10: 283-286; 11: 302-311.

Campbell, D. H. 1936. - Active immunization of albino rats with protein fractions from Taenia taeniaeformis and its larval form Cysticercus fasciolaris. Amer. Jour. Hyg. 23: 104-113.

Campbell, D. H. 1936. - An antigenic polysaccharide fraction of Ascaris lumbricoides (from hog)Jour. Inf. Dis. 59: 266-280.

Campbell, D. H. 1937. - The immunological specificity of a polysaccharide fraction from some common parasitic helminths. Jour. Parasitol. 23: 348-353.

Culbertson, James T., Harry M. Rose, and Constance R. Demarest, 1944. Filariasis bancrofti: Its diagnosis by immunological tests with antigen derived from Litomosoides carinii. Amer. Jour. Hyg. 39 (2): 156-162.

Culbertson, J. T. 1938. - Recent contributions to the immunology of helminthic infections. Archives Pathology, 25: 85-117,256-280.

Davey, D. G. 1938. - Studies on the physiology of the nematodes of the alimentary canal of sheep. Parasitol 30: 278-295.
-1938a. - The respiration of nematodes of the alimentary tract. Jour. Exper. Biol. 15: 217-224.

Davis, D. J. 1936.-Pathological studies on the penetration of the cercaris of the strigeid trematode, Diplostomum flexicaudium. Jour. Parasitol. 22: 329-337.

Eisenbrandt, L. L. 1937. - On the serological relationship of some helminths. Amer. Jour. Hyg., 27:117-141.

Ferguson, M. S. 1940. Excystment and sterilization of metacercariae of the avian strigeid trematode, Posthodiplostomum minimum, and their development into adult worms in sterile cultures. Jour. Parasitol. 26: 359-372.

Giovannola, A. 1936.-Energy and food reserves in the development of nematodes. Jour. Parasitol. 22: 207-218.

Glaser, R. W., E. E. McCoy, and H. B. Girth. 1942. The biology and culture of Neoaplectana chresima, a new nematode parasitic in insects. Jour. Parasitol. 28: 123-126.

Hoeppli, R., L. C. Feng, and H. J. Chu. 1938. - Attempts to culture helminths of vertebrates in artificial media. Chines Me. Jour. Supplement 11: 343-374

Kuntz, Robert E. and M. A. Stirewalt. 1946. - Effects of DDT on Cecariae of Schistosoma mansoni. Jour. Parasitol. 32 (6): 529-538.

McCoy, O. R. 1935. - The physiology of the helminth parasites. Physiol. Review. 15: 221-241.

Metcalf, M. M. 1929.-Parasites and the aid they give in problems of taxonomy, geographical distribution, and paleogeography. Smithson, Misc. Coll., 81 (8): 1-36.

Rankin, J. S. Jr. 1937.- An ecological study of parasites of some North Carolina salamanders. Ecol. Monogr. 7: 169-269.

Rankin, J. S. Jr. 1939.- Ecological studies on larval trematodes from Western Massachusetts. Jour. Parasitol 25: 309-328.

Rothschild, M. 1936.-Gigantism and variation in Peringia ulvae Pennant 1777, caused by ingection with larval trematodes. Jour. Mar. Biol. Assoc. United King. 20: 538-546.

-1938- Further observations on the effect of trematode parasites on Periangia ulvae (Pennant)1777. Novit. Zool. 41: 84-102.

Sandground, J. H. 1936. - On the potential longevity of various helminth with a record for a species of Trichostrongylus in man. Jour. Parasitol. 22: 464-470.

Smyth, J. D. 1947. Studies on Tapeworm physiology. 11. The cultivation and development of Ligula intestinalis in vitro. Parasitology 38: 173-181.

Stephenson, W. Survival of Fasciola hepatica L. in vitro. Nature (London)155 (3930): 240-241. 1945.

Stephenson, Wm. 1947a. Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. I. Survival in vitro. Parasitology 38: 116-122.

Stephenson, Wm. 1947b. Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. 11. Feeding. Parasitology 38: 123-127.

Stephenson, Wm. 1947c. Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. 111. Egg-shell formation. Parasitol. 38: 128-139.

Stephenson, Wm. 1947d. Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. 1V. The Excretory system. Parasitol. 38: 140-144.

Stunkard, H. W., Lowell T. Coggeshall, Thomas T. Mackie, Robert Matheson, and Norman R. Stoll. 1943.-Parasitic diseases and American participation in the war. Ann. New York Acad. Sci. 44 (3) 189-262.

Stunkard, H. W. 1937. - The physiology, life cycles and phylogeny of the parasitic flatworms. Amer. Mus. Nov., No. 908: 1-27.

Taliaferro, W. H. 1940.-The mechanism of acquired immunity in infections with parasitic worms. Physiol. Reviews, 20:469-492.

Wardle, R. A. 1937. - The physiology of tapeworms. Manitoba Essays, 60th Anniversary Commemmoration Volume, Univ. Manitoba, Winnepeg, Canada; MacMillan Co., Toronto: 338-364.

Wilhelmi, R. W. 1940.-Serological reactions and species specificity of some helminths. Biol. Bull. 79: 64-90.

Wright, Willard H., K. B. Kerr, and Leon Jacobs, 1943. Studies on trichinosis. XV. Summary of the findings of Trichinella spiralis in a random sampling and other samplings of the population of the United States. Publ. Health Repts. 58 (35): 1293-1313.

Cryptocotyle lingua (Creplin, 1825) Fischoeder, 1903

The digenetic trematode, Cryptocotyle lingua, is a widely distributed intestinal parasite of fish-eating birds and mammals. It occurs commonly in European and North American loons, gulls, and terns, and often may be found in these birds taken far inland. Young nestling or fledgling birds are usually heavily infected and a single bird may yield hundreds of worms. Rats, cats and dogs are suitable mammalian hosts.

Study sectioned specimens and preserved whole mount or living worms obtained from recently killed infected hosts by gently scraping with a scalpel the mucosa of the small intestine. The elliptic worms are small (1 mm. long by 0.4 mm. wide) and light brown in color. When you have located a fluke, mount it on a slide in normal avian saline solution and after carefully compressing the worm by withdrawing excess fluid from the edge of the cover glass with absorbent paper, seal the edges of the cover glass with vaseline to prevent evaporation. Other temporary, vitally-stained preparations should be made in a similar way by adding a small drop of dilute neutral red (1:5000 in distilled water) to the avian saline.

Detailed labeled drawings of the adult worm, the redia, the cercaria and metacercaria, as well as a drawing of a median sagital section of the adult worm showing the ventro-genital pit are required.

Morphology

1. Integument. The cuticula which is secreted by the underlying hypodermis is thin and transparent. The anterior half of the worm is covered with small scale-like spines readily seen in profile at the margins; the spines gradually decrease in size posteriorly and finally disappear at the level of the acetabulum.

2. Muscular system. This system in most trematodes consists of several definite peripheral layers under the hypodermis and scattered bundles of muscles running from the dorsal to the ventral body surfaces. Usually, the outermost layer is the circular muscles beneath which is a thin group of diagonal muscles and finally there is an innermost layer of longitudinal muscles. These layers, as well as the dorso-ventral muscles, are not conspicuous in Cryptocotyle.

Near the middle of the body is an invaginated muscular ventro-genital pit used during the sperm interchange act of copulation. The acetabulum or ventral sucker in this worm is small and forms an anterior, crescent shaped, internal portion of the pit. The muscular fibers of the ventro-genital pit are complexly arranged. Radial muscular fibers are present in the wall of the acetabulum while muscle units resembling sphincters occur near the outer lips of the pit and allow this structure to function during copulation as a genital sucker.

The oral sucker and pharynx are muscular feeding organs and show chiefly a radial muscular fiber system.

Cryptocotyle lingua (Creplin, 1825) Fischoeder, 1903

The digenetic trematode, Cryptocotyle lingua, is a widely distributed intestinal parasite of fish-eating birds and mammals. It occurs commonly in European and North American loons, gulls, and terns, and often may be found in these birds taken far inland. Young nestling or fledgling birds are usually heavily infected and a single bird may yield hundreds of worms. Rats, cats and dogs are suitable mammalian hosts.

Study sectioned specimens and preserved whole mount or living worms obtained from recently killed infected hosts by gently scraping with a scalpel the mucosa of the small intestine. The elliptic worms are small (1 mm. long by 0.4 mm. wide) and light brown in color. When you have located a fluke, mount it on a slide in normal avian saline solution and after carefully compressing the worm by withdrawing excess fluid from the edge of the cover glass with absorbent paper, seal the edges of the cover glass with vaseline to prevent evaporation. Other temporary, vitally-stained preparations should be made in a similar way by adding a small drop of dilute neutral red (1:5000 in distilled water) to the avian saline.

Detailed labeled drawings of the adult worm, the redia, the cercaria and metacercaria, as well as a drawing of a median sagital section of the adult worm showing the ventro-genital pit are required.

Morphology

1. Integument. The cuticula which is secreted by the underlying hypodermis is thin and transparent. The anterior half of the worm is covered with small scale-like spines readily seen in profile at the margins; the spines gradually decrease in size posteriorly and finally disappear at the level of the acetabulum.

2. Muscular system. This system in most trematodes consists of several definite peripheral layers under the hypodermis and scattered bundles of muscles running from the dorsal to the ventral body surfaces. Usually, the outermost layer is the circular muscles beneath which is a thin group of diagonal muscles and finally there is an innermost layer of longitudinal muscles. These layers, as well as the dorso-ventral muscles, are not conspicuous in Cryptocotyle.

Near the middle of the body is an invaginated muscular ventro-genital pit used during the sperm interchange act of copulation. The acetabulum or ventral sucker in this worm is small and forms an anterior, crescent shaped, internal portion of the pit. The muscular fibers of the ventro-genital pit are complexly arranged. Radial muscular fibers are present in the wall of the acetabulum while muscle units resembling sphincters occur near the outer lips of the pit and allow this structure to function during copulation as a genital sucker.

The oral sucker and pharynx are muscular feeding organs and show chiefly a radial muscular fiber system.

cross fertilization is probably more common. In C. lingua, copulating adult worms are often observed. If possible, locate such pairs of worms and determine the uses of the terminal genitalia about to be described.

As previously noted, the ventral sucker and the genital sinus are located within the ventro-genital pit, a median invagination of the body wall. The genital sinus receives the vas deferens and the terminal uterus which pass through the dorsal part of a papilla-like accessory structure, the gonotyl. This gonotyl is somewhat acorn-shaped with a broad cup-shaped base and a conical tapering tip and being protruded during copulation serves to hold the worms together as well as to align the genital pores to facilitate sperm exchanges.

A. Male system: The two testes are lobed, somewhat diagonally placed, and are located in the posterior part of the worm. In uncompressed specimens, the lobation may be indistinct and the testes somewhat separated. Fine efferent sperm ducts, the vasa efferentia, which are impractical to search for, run forward from the testes and unite, behind the genital pit, to form the vas deferens. This larger sperm duct widens behind the genital pore to form a sperm-packed seminal vesicle and finally terminates in the ejaculatory duct which then opens into the genital sinus.

B. Female system: The female reproductive system is more complex and accordingly more difficult to interpret. The ova are produced in the ovary, a lobed organ lying usually on the right side and on the same side as the posterior testis. The oviduct leads away from the ovary and quickly receives a short duct from the large ovoid or spherical seminal receptacle usually packed with sperm and located immediately behind the ovary. From this point of junction, at which point fertilization occurs and which marks the end of the oviduct and the beginning of the uterus, a short Laurer's canal runs dorsally to open at the surface near the midline at the level of the ovary. It has been postulated that excess sperm are eliminated through this canal. Between the ovary and seminal receptacle will be found a vitelline, or yolk duct, which meets a similar duct from the opposite side in the expanded vitelline reservoir located just ahead of the anterior testis. Notice the granular vitelline material in the vitelline reservoir. The fertilized egg now passes in the uterus alongside the vitelline reservoir and receives yolk material through the short common yolk duct from the reservoir. An ootype, an egg-shaping organ found in many trematodes, is not present in C. lingua. The uterus occupies most of the space between the posterior sex organs and the ventro-genital pit.

As the eggs proceed through the convoluted uterus toward the genital pore, they increase in size, form brownish egg shells probably from some of the vitelline materials, and undergo the first few cleavages. The eggs finally pass into the genital sinus and out into the small intestine of the host to be carried with the faeces to the outside.

Larval Stages

1. Miracidium and Sporocyst. Cleavage and development of the larva continue for about ten days after the egg reaches the outside. A free-swimming miracidium does not occur in this life cycle; rather, the fully-embryonated egg must be injested by a snail, usually the edible periwinkle, Littorina littorea which is exceedingly common along the North Atlantic coasts of Europe and North America.

The miracidium, as seen in fully embryonated eggs, is ovate and about as long as the egg. It is covered with long cilia spirally arranged but missing on the anterior tip. Several large glands with apically opening ducts occur in the central portion of the larva.

After being eaten, the infective larva hatches and invades the tissues of the snail host. From other completely-worked life cycles, we can infer that the miracidium here transforms into a sporocyst, a simple sac-like larva which lacks a digestive tract and which in turn produces the rediae.

2. Redia. Rediae occur in great numbers in the snail's liver which is altered in color and texture as a result of the mass infection. Crush an infected snail in molluscan Ringer's solution and mount several of the small, colorless, sausage-shaped rediae on a slide and examine under high power. Locate a small anterior oral sucker and a short sac-like intestine restricted, in a mature redia, to the anterior end. On the ventral side immediately behind the oral sucker is the birth pore provided with small enclosing lips and through which cercariae can emerge. A nerve commissure is located on the dorsal side of the intestine immediately behind the oral sucker. A double excretory system with lateral excretory pores situated about one-third the body length from the posterior end and internal ducts and flame-cells may be apparent in favorable specimens.

The interior of the redia is filled with developing cercariae. In the posterior end, germinative cells give rise to small, undifferentiated germ balls which slowly develop into tailed cercariae as they move anteriorly. Study the posterior end of carefully compressed specimens under high power and determine the character of the germinative cell wall layer. Also examine the germ balls and note their resemblance to early cleavage stages and morulae in other forms. Now trace the development of the cercaria by examining selected stages anteriorly. Notice the formation of the tadpole-shaped body, the thinner caudal region, and the heavily pigmented eyespots.

3. Cercaria. The cercariae are not fully mature when they emerge through the birth pore. As they migrate through the host tissues they complete their maturation by acquiring cuticular spines, a tail fin and glandular secretory granules.

Locate a favorable specimen which has not become detached from its tail and examine it under high power. Note the morphological resemblance between the cercarial body and the adult.

The cercaria has several larval specializations which enable it to find and penetrate its next host. The two conspicuous eye-spots composed of black pigment granules are located on the dorsal side of the developing esophageal commissure and function as light-sensitive organs. The larvae are positively phototropic and will collect on the light side of the container. They also react immediately to shadows by swimming actively in the direction of the shadow casting agent; this reaction would serve in nature to locate and attach to fish, their next host. The tail, which is the organ of locomotion, is long, muscular and provided with dorsal and ventral fins each having a thin delicate keel apparently stiffened by "bristles." Examine a bristle critically and determine that it is merely an optical illusion formed by light refraction at a crest in the homogeneous keel. The center of the larva contains about eighteen gland cells with excurrent ducts opening beside the oral sucker. The gland cells are filled with a granular secretion, readily visible in neutral red stained larvae, which is undoubtedly histolytic and dissolves the flesh of the fish beneath the spot of cercarial penetration. Located peripherally are many small gland cells whose secretion probably aids in the formation of the metacercarial cyst.

The larval morphological specializations are transitory and cease to function at the time of metacercarial formation; however, some cercarial structures are permanent and persist through the adult stage. The cercarial sucker and excretory system, consisting of posterior bladder, ducts and flame cells, are visible in most larvae. The pharynx, a group of cells near the eye spots, and gut caeca are not well-formed. A group of cells immediately anterior to the excretory vesicle is believed to give rise to all the genital organs.

4. Metacercaria. Free-swimming cercariae readily penetrate the skin and especially the fins of fishes. Most fish of the intertidal zone, particularly sluggish swimmers such as the Cunner, are naturally infected with these metacercariae. Examine a Cunner and note the peppering of black dots in the fins and flesh; each spot indicates the presence of a metacercarial cyst which is made more evident by encircling black pigment cells formed by the host.

Cut a 3 mm. square of healthy fin from a stunned Cunner and place it in a watch glass together with several cercariae concentrated in a small quantity of sea water. Observe the random swimming motions of the cercariae under the binocular microscope and locate cercariae crawling on the surface of the fin. Determine if there is any positive chemotaxis or if the finding of the fin by the cercariae is purely chance. When the cercarial tail is shed and active penetration of the fin is begun, transfer the vessel to the stage of the compound microscope and record in a series of sketches your observations on the method of penetration and encystment.

Place an encysted metacercaria in a drop of normal saline on a glass slide and with well-sharpened needles carefully remove the young worm from the cyst. Place a cover glass on the animal and study it under high power. Note the absence of cercarial organs and the continued development of adult organs. As you draw the metacercaria, mentally compare its anatomy with that of the sexually mature adult and the cercaria both previously studied.

Tetrastemma candidum (Müller, 1774)

The proboscis worms, of which Tetrastemma is an example, are flattened, elongate, free-living or parasitic, carnivorous worms living in the ocean, in fresh water or on land. Many nemertine species are large and opaque; they are not desirable for student use because the internal organs are difficult to determine. The smaller Tetrastemma is selected for study because, in whole mounts, the internal structures are easy to determine, because it is commercially available and because in gross morphology it resembles species of Amphiporus, another commercially available genus, and it resembles closely the fresh-water nemertine species, Prostoma rubrum (Leidy, 1850) which can be rather easily cultured in inland colleges. These directions with slight, readily-apparent modifications can be used to study Tetrastemma, Amphiporus or Prostoma.

Living worms and preserved whole mounts as well as cross sections through the middle of the body should be available for study. If time permits and living specimens are available, regeneration experiments should be attempted. Try cutting animals in two through the middle, and also cut off segments anterior to the brain. Isolate the fragments in clean water which should be periodically changed. Record the regenerative changes. Your labeled drawings will include the following: a drawing at least six inches long of a dorsal view of the whole worm, and enlarged drawings of the head end and stylet region.

Adult

The yellow, white or light green adult Tetrastemma is dorso-ventrally flattened and is about 2 cm. long and 0.5 cm. wide. The front end is rounded and provided with four conspicuous reddish-brown ocelli which form the corners of a square; the posterior end tapers to a rounded point.

Body Wall and Parenchyma. The body wall consists of an outer layer of ciliated columnar epithelium and gland cells resting on a basement membrane of hyaline connective tissue. Underlying the basement membrane are two layers of somatic muscles consisting of an outer circular and an inner longitudinal layer. Dorso-ventral muscles may be apparent in the section. A mesenchymatous parenchyma, like that in flatworms, occupying the spaces surrounding the body organs should be identified and studied. The musculature of the proboscis and proboscis sheath will be discussed in the next section.

Proboscis and Proboscis Sheath. The proboscis is a long eversible organ of a formidable offensive and defensive nature contained, in its retracted state, in a proboscis sheath located dorsal to the gut. The proboscis is formed by an invagination of the anterior body wall, and the muscular layers, therefore, are continuous with those of the body wall muscles. A retractor muscle originates on the lateral proboscis sheath wall and is

inserted on the tip of the retracted proboscis. This retractor muscle aids the return of the everted proboscis back into the sheath. The center of the proboscis is hollow and is lined with a glandular epithelium. In Tetrastemma the proboscis is large in comparison to the small size of the body, is provided with sharp-pointed stylets and has an anterior duct, the rhynchodeum, which opens with the mouth. Three distinct anteroposterior chambers occur in the proboscis. The anterior chamber is the eversible region and terminates posteriorly at a cross partition which bears a median sharp, conical stylet with a pear-shaped basis imbedded in the tissue and which bears also two lateral pouches containing as many as four accessory stylets. The cross partition, or diaphragm, is perforated by a slender canal which connects the anterior and middle chambers and transports immobilizing or other types of secretions from the large posterior chamber to the base of the everted stylet.

The proboscis sheath is a closed tube with a wall of several layers and a central cavity, the rhynchocoel, which houses the proboscis, and is filled with a corpusculated fluid. The sheath is nearly as long as the body and by muscular wall pressure on the fluid within causes a rapid eversion of the anterior portion of the proboscis. Only the region back to the diaphragm turns inside out as the proboscis is extended.

Digestive System. An anus makes its first permanent appearance in the nemertines, and food materials such as minute organisms or body juices of larger prey pass from front to back ends in an orderly fashion; accordingly, the enteron, which is located ventral to the proboscis sheath, is differentiated anteroposteriad into storage, digestive, absorptive and eliminative regions and is lined with ciliated columnar epithelium throughout.

The mouth is situated in front of the brain. A narrow esophagus connects to an expanded stomach and the latter gives off posteriorly a tapered pylorus which parallels the intestine some distance dorsally before joining it. The blind anterior end of the intestine, the intestinal caecum, is provided with lateral branched diverticula as is the intestine proper. Posterior to the intestine is a tapered rectum which opens at the terminal anus.

Excretory and Vascular System. Tetrastemma has a protonephridial excretory system with a pair of lateral collecting tubules and many terminal tributary ducts and flame cells. Parts of this system will be apparent in living organisms, may be seen in sections but will be generally invisible in stained whole mounts.

The development of a blood vascular system is another important permanent evolutionary advance made by nemertines. In Tetrastemma this system consists of a single dorsal vessel and paired lateral vessels which join immediately behind the brain before sending a narrow cephalic lacunar loop forward into the interocular region. In the intestinal region there are regularly arranged transverse

vessels, alternating with the gut diverticula, which connect the dorsal and lateral vessels. A colorless vascular fluid containing floating nucleated corpuscles fills the blood vessels. This fluid ebbs and flows with body movements; there is no definite vascular circuit and a heart is missing although muscle fibers in the blood vessel walls occur generally over the whole system.

Nervous System and Sense Organs. In correlation with the active, predaceous life of Tetrastemma the nervous system and sense organs are highly developed. The nervous system consists of a four-lobed brain in the cephalic region with fused dorsal and ventral paired ganglia and with commissures above and below the rhynchodeum uniting the ganglionic pairs. Five main longitudinal nerves proceed posteriorly from the brain. Large paired lateral trunks, from the ventral ganglia, will be visible subjacent to the longitudinal body muscles even in whole mounts; they are connected, in the rectal region, by a large dorsal commissure. The smaller dorsomedian nerve, from the dorsal commissure, and the paired dorsolateral nerves, from the dorsal ganglia will be visible in cross section, if not in whole mounts. A nerve fiber network connects these various trunks and, in addition, peripheral motor and sensory nerves emerge from the central nervous system.

These peripheral nerves include general somatic nerves and, in addition: 1) cephalic nerves, from the dorsal cerebral ganglia, to the ocelli and other sense organs of the head; 2) esophageal nerves, from the ventral brain lobes, to the mouth and esophagus; 3) ten proboscidial nerves, from the ventral cerebral region, to the proboscis.

The outer surface of the body is provided with diffuse, generalized sensory cells while the head possesses concentrated, specialized ocelli, cerebral sense organs and cephalic grooves. Each of the four ocelli is composed of a cuplike group of sensory cells surrounded by reddish-brown pigment cells and connected to the cephalic nerves. The paired cerebral sense organs consist of surface pores in the deep lateral cephalic grooves and small canals leading medioposteriad to the expanded, highly-specialized sense organs lying in contact with the dorsal lobes of the brain. The oblique cephalic grooves at the level of the posterior eyes are sometimes marked by brown pigment granules.

Reproductive Organs. The nemertines are usually dioecious and, in contrast to the flatworms, have simple reproductive organs. Gonads and germ cells develop from lateral mesenchyme between the gut diverticula. As the animal reaches maturity the gonads become distended with spermatozoa or ova, according to the sex of the worm. A narrow efferent genital duct runs from each gonad to the dorsolateral body surface. Eggs and sperms are discharged into the water where fertilization occurs. Varieties of Prostoma are hermaphroditic and possess well-developed ovotestes.

Crossobothrium laciniatum Linton, 1889

This adult tapeworm inhabits the small intestine (spiral valve) of the sand shark, Carcharias littoralis. Examine a living or a preserved specimen and note the flared scolex and segmented body. The scolex possesses four elongate, concave attaching structures with somewhat thickened rims, called bothria; the bothria are borne on short thick pedicels and each bothrium bears a single accessory terminal sucker. The muscular action of bothria and accessory suckers should be observed and described in the living scolex, if available. Make a labeled drawing of the complete scolex.

The profoundly emarginate body is composed of proglottids each narrower in front and characterized by four overhanging marginal flaps on the posterior border. In general, the proglottids increase in size from the scolex posteriorly but there is not a graduated increase as in the taenioid cestodes. The proglottids are produced discontinuously from the neck region, and in some worms several zones of maturing proglottids will occur along the length of the body. The posterior proglottids are roughly triangular in surface view with a drooping, wavy posterior margin consisting of the four lobes; moreover, they are tenuously joined and become easily separated from each other. Detached and independent proglottids can be recovered from the small intestinal chyme which is often rendered milky-white by their abundance.

Certain details of the anatomy, such as the canals and flame cells of the protonephridial excretory system, are most easily seen in the living worm; other parts, such as the hermaphroditic reproductive systems, are easier to determine in well-flattened and stained specimens.

Mature Proglottid. Study an isolated stained proglottid. Locate the heavy secreted outer cuticle, the middle parenchyma, and muscle fibers which are responsible for the trematode-like locomotion of the detached living proglottid. Clear sinuous excretory canals may be visible, in favorable specimens, on the lateral borders, especially anteriorly. If they are not immediately apparent, do not waste time searching for them or for their smaller tributaries and flame cells which are usually invisible. The proglottid, of course, lacks any trace of a digestive system. As is usual in flatworms, the reproductive systems are large and most complex; they will be described separately.

Male System. Many small spherical testes encircle a clear central proglottid space. Examine a follicle under high power and note the internal tubules containing sperm cells in all stages of development. Leading away from the follicles are fine efferent sperm ducts which unite slightly anterior to the center of the proglottid to form the wider, sperm-filled vas deferens. This extremely-convoluted duct runs to the base of the long slender marginal penis. The penis, when lying in the clear bulbous penis pouch, is very much coiled, but is often seen protruded from the

common marginal genital pore. See a demonstration of this protrusion; notice the minute spines which cover the penis and find the internal duct which opens apically and is an extension of the vas deferens.

Female System. Immediately ahead of the marginal male opening is the beginning of the female copulatory tube, the vagina, which initially parallels the vas deferens but soon describes a smoothly arched medioposteriad course to near the posterior end where it dilates as the pre-ovarian cylindrical seminal receptacle. The ellipsoidal ovary is a deeply-stained organ composed of polydendritic lobes, located in the median plane slightly ahead of the posterior marginal notch, and surrounding the small compact oötype and posterior portion of the seminal receptacle. The oötype receives short oviducts from the ovary, a narrow seminal receptacle duct, and a common vitelline duct formed by left and right vitelline ducts running mediad from many lateral vitellaria which are grouped in the posterolateral margins and extend in a thin lateral band forward to near the anterior end. Eggs are fertilized and receive vitelline material in the oötype chamber; they are also here provided with shells by secretions from small encircling shell glands. When fashioned, the eggs pass into the short anteriorly directed uterus which is greatly expanded distally to form the central sacculate uterus. In the gravid proglottid, this space is filled with eggs. Make labeled drawings of mature and gravid proglottids showing all the parts mentioned.

Gravid proglottids pass out of the shark into the sea water. The life-cycle is not completely known.

ENTOPROCTA AND ECTOPROCTA

REVISED CHECK LIST OF BRYOZOA most commonly encountered around Woods Hole --- their present-day names and the names previously used.

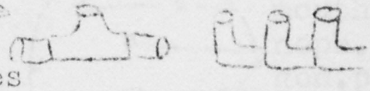
MODERN TERMINOLOGY	PREVIOUS TERMINOLOGY
Phylum Entoprocta	
Barentsia	
Pedicellina	
Phylum Ectoprocta	
Acanthodesia tenuis	Membranipora tenuis
Aetea anguina	
Aeverrillia setigera	Buskia or Hippuraria
Alcycnidium	
Bowerbankia gracilis	
Bugula flabellata	
Bugula turrita	
Callopora aurita	Membranipora aurita
Crisia eburnea	
Cryptosula pallasiana	Lepralia pallasiana
Electra hastingsae	Membranipora monostachys
Electra pilosa	Membranipora pilosa
Flustrella hispida	
Hippodiplosia americana	Lepralia americana
Hippodiplosia pertusa	Lepralia pertusa
Hippoporina contracta	Lepralia serrata
Hippothoa hyalina	
Membranipora crustulenta?	Membranipora lacroixii
Membranipora tuberculata	Membranipora tehuelcha
Microporella ciliata	
Schizmopora avicularis	Cellepora americana
Schizomavella auriculata	Schizoporella auriculata
Schizoporella unicornis	
Smittina trispinosa var. nitida	
Stephanosella biaperta	Schizoporella biaperta
Tegella unicornis	Membranipora unicornis

Roughly about 60 more species occur around the Woods Hole region but the above are the more common forms encountered.

Pedicellina Sars 1835 or Barentsia Hincks 1880

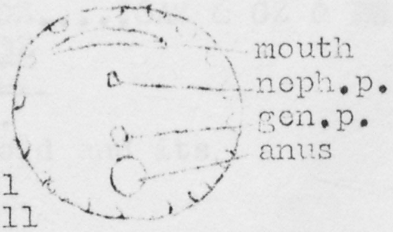
A. General directions:
1. Obtain some slides which have been immersed in sea water for 2 weeks or so and studythem for Bryozoa colonies. Some of these slides will undoubtedly contain colonies of Barentsia, Pedicellina, Cryptosula, Bugula, and a variety of other forms and phyla.
2. These slides should be placed in Petri dishes containing sea water so that the free behavior of the specimens can be studied.
3. Study the Petri dishes first under a binocular dissecting microscope to get a general view of the slide. Identify the forms found on it, then transfer the Petri dish and its contents to the stage of the compound microscope, first using the 50x then later the 100x magnification.
4. After you have completed your study of the slides return them to the water table because later in the period you may need them again.
5. In case the slides are not satisfactory or do not contain the desired form return them and obtain some material from the central tank table.

B. Specific directions:
1. Answer the questions asked below through observation. (They need not be turned in.)
2. Select a Pedicellina or Barentsia colony for study.
3. The colony of Pedicellina is stolonate, that is, the individuals or zoids are stalked and the stalks are connected by a delicate tube or stolon. The same condition obtains in Barentsia.
4. Is the stolon perfectly straight or may it bend and twist about?
5. Is the stolon entirely adherent, everywhere touching the substratum?
6. Are there any septa or partitions along the stolon? Are there any septa in the immediate vicinity of the stalk or peduncle?



7. Are there any spines or hairy processes along the stolon, peduncle, or calx?
8. Does the stolon continue as a single line or does it branch, sending side lines outward?
9. Are the peduncles of uniform diameter from stolon to calyx? If you have a Barentsia specimen there will be a marked enlargement at the base of the peduncle. Also there might be "rings" or a ringed appearance around the enlargement.
10. Are the peduncles of the same diameter as the stolons?
11. Are the peduncles hollow?
12. Is the stolon hollow?
13. Do any peduncles branch?
14. Is the head or calyx slightly flatter on one side than on the other?
15. Is the head like a ball (contracted) with tentacles folded inward, or is it like a flower, with tentacles unrolled outward, like petals?

16. Are there any peduncles without heads?
17. Where are younger peduncles placed on the stolon? Are they at one end or do they seem to occur haphazardly?
18. How can you tell a young peduncle from an old one?
19. Take a fine dissecting needle or a hair and touch a tentacle of an expanded zooid. Repeat several times, after a period of rest, to see if you get the same response.
20. If one tentacle is touched, do the others on the same head retract or fold in?
21. What is the nature of the reaction -- i.e. does the tentacle shorten or does it merely fold in?
22. If one tentalce is touched does the response involve other heads on the same stolon?
23. Count the nomber of tentacles on several individuals. Is there a constant number for the entire stolon, or is there variation?
24. Compare the tentacle number with that obtained by your neighbor.
25. Does size of calyx seem to have anything to do with tentacle number?
26. Are tentacles ciliated all around or just on one side?
27. Is there a definite pattern of ciliary action?
28. Do the tentacles show any ability for independent action or do all do the same thing upon stimulation -- in the same manner and at the same time?
29. The tentacles are borne on a ridge of body wall known as the lophophore. Is the lophophore circular or horseshoe-shaped in these forms?
30. Observe the plane or the slant of the lophophore. Is it at right angles to the main axis of the stalk and calyx? If set obliquely it is not Pedicellina but another genus.
31. If the specimen is so oriented that the flat and gibbous sides of the calyx are both evident then either the right or the left side of the specimen will be facing you. The esophagus runs along the flat side, the intestine along the gibbous side. Figure outwhether you are looking at the right or the left side of the animal.
32. The tentacles fold or curl into the atrium or vestibule, a space within the circlet of tentacles at the top of the calyx. There are several openings into this atrium from the inside. See diagram at right. The openings are the mouth, nephridial pore, genital pore, and anus.



33. A brood room is present around the anal opening. In some calyces it may be small in others large.
34. The tentacle ring is interrupted in the region of the anal opening. This is evident only in the top view of the lophophore.
35. Is the mouth round or crescent shaped?
36. A scarcely evident fold of skin, the epistome, partly guards the mouth from the tentacular side.
37. If the animal can not be conveniently studied in the Petri dish you might gently scrape off an area under the dissecting scope, place the material on a slide with cover slip and sea water.

38. The digestive tract is simple, consisting of mouth, esophagus, stomach, intestine, rectum, and anus. The mouth as well as much of the rest of the tract is ciliated.

Suggested work on Podicellina or Barentsia:

a. Answer such questions as you can from observation.

b. Suggested drawings: (make as many as time will permit.)

Fig.1. Habit sketch showing stolon, peduncles, calyces of several individuals of a colony either of Podicellina or Barentsia or both.

Fig.2. Draw a patch of stolon on both sides of a peduncle and also a peduncle, enlarged.

Fig.3. Sketch a top view of a calyx showing tentacles extended, the vestibule and the various openings.

Fig.4. Side view of calyx, enlarged, showing the digestive tract, musculature and such other organs as you can find.

PHYLUM ECTOPROCTA Nitsche 1869
Order STENOSTOMATA, formerly CYCLOSTOMATA
Family CRISIIDAE Johnston 1847
Genus Crisia Lamouroux 1812

It is better to study young Crisia colonies from submerged slides, but if these are unsatisfactory then colonies from sea weed will be suitable. The colonies are delicate, white, lacy, branching masses.

Suggested figures for drawing:

Fig. 5. Make a "mathematical formula" or diagram showing the exact arrangement and mode of branching of autozooids, gonozooids, branches, etc. using the following symbols:

PA -- parent zooid, if any AZ -- autozoid
GZ -- gonozoid BR -- branch
..... joint

Rough example: PA & BR & 3AZ & GZ.....6AZ & GZ & BR
2AZ & GZ

Fig.6. Make a habit sketch of a colony.

Fig.7. Make a detailed view of a gonozoid and its ooeciostome.

PHYLUM ECTOPROCTA Nitsche 1869
Order CTENOSTOMATA Busk 1852
Family VESICULARIIDAE Johnston 1838
Bowerbankia gracilis Leidy 1855

Obtain colonies of Bowerbankia from the side table. They will form a soft grayish fuzz on vegetation, on Bugula, hydroids, rocks, etc. Study first in a watch glass with some sea water, under the dissecting scope.

The zooids are closely clustered together, giving the impression of tall transparent columns or sky-scrapers. Their bases are so close together that it is difficult to study them. The zooids are irregularly arranged along a slender stolon, occurring sometimes on both sides and other times along one side of the stolon. Sometimes the zooids are bilaterally placed, other times irregularly placed. Are there any zooids on the top of a stolon?

Suggested work for this form:
Fig.8. A habit sketch of the colony.
Fig.9. An enlarged view of one or two zooids attached to the stolon.

PHYLUM ECTOPROCTA Nitsche 1869
Order CHEILOSTOMATA Busk 1852
Family ESCHARELLIDAE Levinsen 1909
Cryptosula pallasiana (Moll 1803)

1. Obtain slides which have been immersed in Eel Pond for a week or two. Place the slide in a Petri dish containing enough sea water to cover the specimens so colonies can expand their tentacles.
2. On these slides will be some other forms in addition to Cryptosula. See Pratt, p. 301 for small sketch of this species.
3. Cryptosula colonies are flatly encrusting, calcareous. In this young stage they will be translucent in spite of their calcification.
4. Each colony is flat and becomes circular in time. It consists of a number of compartments or zooecia arranged side by side and spreading out radially from the ancestral zooid, the ancestrula.
5. How many zooids or zooecia are there in the biggest colony on the slide?
6. Can you identify the ancestrula from which the colony sprang?
7. Are all the zooecia of approximately the same size?
8. How many zooids touch the ancestrula directly?
9. Are the zooids at the very edge of the colony as complete as ancestrula so far as walls are concerned?
10. The biggest opening or hole in each zooid is the zooecial aperture. The shape of the aperture is one of the most important key characters in Bryozoan classification. Each species has a characteristic aperture. What is its shape in this species? Is the aperture longer than wide?
11. There is a delicate membrane, the operculum, closing the aperture. It has a reinforced chitinous rim. It is open or closed, depending upon the location of the tentacles. If the tentacles are protruding the operculum is open, and vice versa. How does the operculum open, sideward, downward, upward, inward, or outward? Do you see any muscle attachments to the operculum -- like the chains of a draw-bridge?
12. The lateral margin of the aperture has a small tooth, denticle, or cardelle (synonyms here) on each side, toward the posterior border. The operculum pivots on these cardelles. The cardelles occur at the angles formed by the wider outslung lower border and the flatter upper narrower half of

the aperture. The narrower upper part of the aperture, through which the tentacles are extruded, is the porta. The wider lower half of the aperture is the vanna. The vanna is the opening into the compensation sac or compensatrix. The anter is the part of the operculum closing the porta. The poster is the part of the operculum closing the vanna.

13. The compensatrix becomes filled during the extrusion of the polypide. The compensation sac is crescent shaped from the front and located around and beneath the widest part of the aperture, around the vanna. Can you identify it in some of the most transparent individuals? In the Ascophora, to which Cryptosula belongs, the polypide can emerge from the zooecium only if an equal volume of water compensates this extrusion. Cany & Bassler, 1920, p.58, state that when the polypide withdraws again into the zooecium the water is emptied again from the compensatrix.

14. Borg, 1930, gives these measurements for the aperture of Cryptosula pallasiana: Length 0.23 mm, Width 0.18 mm at widest part. Check these measurements if your microscope is calibrated.

15. The calcareous front wall of the zooecium is the tremocyst. It is perforated by a number of large pores, the tremopores. How many tremopores are there? Is the number constant, i.e. identical for all zooecia in the colony? Check with your neighbors.

16. The two patches of muscle attached to the operdular anter are the occlusor muscles. Each is fan-shaped, with the narrow end attached to the operculum.

17. How many tentacles does your specimen have? Calvet says 16-17 but Canu & Bassler think there might be more. Compare with your neighbor.

18. In the most translucent specimens can you identify the digestive tract? If there is a colony on the reverse side of the slide study that for a moment. If not, then turn the slide over carefully and study the back of the colony which has been observed up to now. Are the internal organs more visible in this view? Are there any pores visible on the basal or back wall?

19. Are pores visible on the distal zooecial wall -- the one above the aperture? On the proximal wall? We will come back to this point later when studying mature calcined colonies.

20. Find a very young colony on your slide where the calcification has not progressed far. Are the tremopores much larger and differently shaped than in older specimens?

21. Obtain a dried mature colony from the supply table. This very probably will be attached firmly to a rock or shell. If convenient, place the rock or shell under your binocular. If the specimen is too large for that you should make a calcined mount of it. Directions for calcining are given on page 8 - "Directions for permanent preparations of calcareous Bryozoa".

22. Do you find any globose bodies, ovicells, distal to the aperture? Canu & Bassler say ovicells and avicularia are present but Osborn says they are wanting. If you find one be sure to call the instructor's attention to it, as it will settle a debated point.

23. Take a small fragment of the dry or calcined colony and either crush it gently or pick off the frontal wall so that the lateral, distal, and proximal walls of the zooecium show. The calcined colony is preferable to the natural untreated colony. You may have to turn the slide about or hold it at an angle so that you can see the various walls plainly. Wall perforations called septulae are present. If they consist of a single pore or hole they are uniporous. If they consist of a series of pores in a plate they are multiporous or rosette plates. Which types occur along the distal wall? In the proximal wall? If possible break off a lateral, distal and proximal wall to study under 100x and 430x.

24. Suggested work on Cryptosula:

a. Answer all questions as you make your observations. Do not turn in answers.

b. Possible drawings:

Fig.10. A habit sketch of the colony.
Fig.11. A zooid from a very young colony showing as much of the internal anatomy as possible.
Fig.12. Enlarged view of septulae.
Fig.13. Several contigous zooecia of the calcined or mature well calcified specimen.

PHYLUM ECTOPROCTA Nitsche 1869
Order CHEILOSTOMATA Busk 1852
Family BUGULIDAE Gray 1843
Genus Bugula Oken 1815

This second member of the Cheilostomata is chosen because it is exceedingly common here.

Two species of Bugula are available: B.flabellata (J.W. Thompson)1847 or1848, and B.turrita (Desor)1848. They can be distinguished by their mode of growth. Place a sprig of each in a fingerbowl of sea water to see their growth habit better. B. turrita has a delicate spiral growth habit while B.flabellata is stubby and fan-like.

Place a very small sprig of each in a watch glass containing enough sea water to cover them. Place this on your dissecting scope, allowing sufficient time for polypides to protrude their tentacles. Then they do protrude, count them and record for each species.

Spread a small sprig of each species on a slide, arranging it in such a manner that there are some zooecia "face" up and others "face" down. Cover with a coverslip. Try to answer these questions from observation.

Suggested work on Bugula:

Drawings suggested:

Fig.14. Habit sketch showing mode of growth of colony.
Fig.15. Enlarged view of the front surface of the zooid.
Fig.16. Enlarged view of the back surface of the zooid.
Fig.17. An ovicell, enlarged.
Fig.18. An avicularium, enlarged.

1. Supplies needed:
   a. a blowpipe, either of metal or glass, the former preferable.
   b. an alcohol or gas burner (Bunsen burner) and matches.
   c. an old spoon or crucible for holding tiny specimens.
   d. Binocular dissecting scope, or compound microscope of low power.
   d. forceps, dissecting needles or small brush, glass slides, labels.
   f. dry specimens of calcareous bryozoa.

2. Purpose of exercise:
   a. To prepare permanent slides of the skeletons of calcareous bryozoa.
   b. To burn off the organic matter which obscures the diagnostic features of the colony.
   c. To lift the firmly attached colonies from the substratum without doing too much damage to the colony.

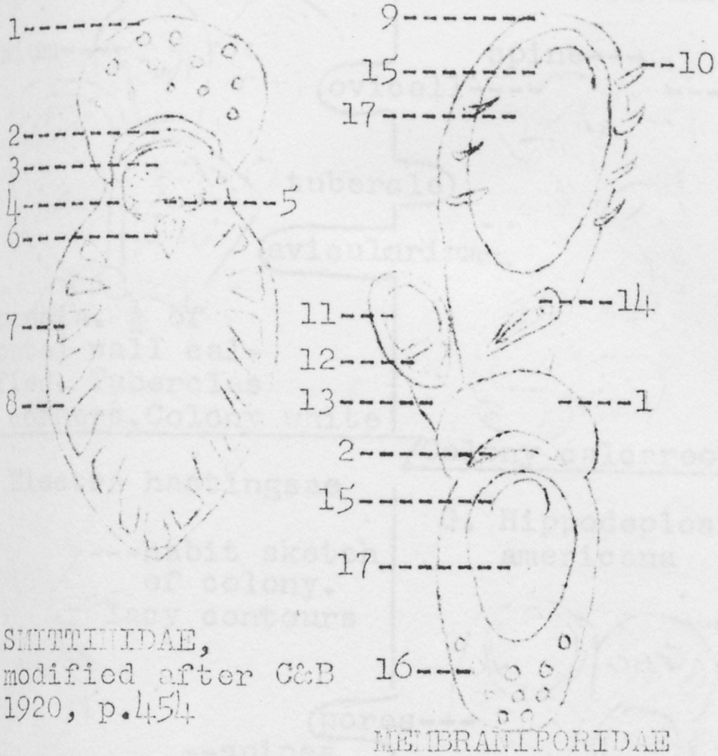
3. Method of procedure:
   a. Have all supplies on hand before starting.
   b. Select a good-sized dry rock with a vigorous colony on it.
   c. The idea is to blow through the blowpipe in such a manner that the flame of the lamp or burner will be bent toward the colony and focused directly upon it. The colony will turn black at first, then may become red hot, then turn white. By that time it should be lifting off or cracking off the rock substratum. Be careful that rock fragments do not hit you in the face.
   d. Do not burn the colony until it becomes a crumbly snowy white mass, because by that time the calcining will have gone too far and your colony will be useless. Continue the calcining only as long as necessary, when some white begins to show and as long as the colony seems to retain its fine diagnostic pattern without any crumbling flakiness. Placing the colony under the microscope will aid you in determining when to stop.
   e. If the colony comes off in chips save these. Gently transfer them to a slide on which you have placed a thick drop of balsam. Set the specimen on this balsam and allow to harden. It might be an excellent idea to mount a natural uncalcined fragment near the calcined one on the same slide, for comparison. Make slides of a number of different bryozoan species. Leave slides at your table for inspection.

GLOSSARY

1. ANCESTRULA -- The primary zooid resulting from the metamorphosis of a larva. It is the ancestor of the entire colony.
2. APERTURA -- An opening into the zooid which is entirely closed by an operculum.
3. AREOLAE -- Pores bordering the edge of a calcified frontal wall.
4. AUTOZOID -- A normal individual in a bryozoan colony.
5. AVICULARIUM -- Avicularia are incomplete, variously shaped individuals:bird's head, oval, elliptical, spatulate, etc. They may occur in various locations: on the front, at the side, and between zooecia; around the apertura in various locations; on or at the sides of ovicells, etc. They may be imbedded in the calcareous wall or may swing freely from the zooid.
6. BROOD CHAMBER -- Spaces or compartments in which bryozoan female germ cells develop. Is a more inclusive or more general term than ovicell, ooecium or gonozoid.
7. BROWN BODY -- A mass of tissue representing a degenerated or degenerating polypide.
8. FRONTAL SIDE -- The uppermost surface of an attached encrusting colony. It is the side on which the apertura is located.
9. FUNICULUS -- A large strand of tissue connecting the aboral end of the alimentary canal with the aboral wall of the zooecium.
10. GONOZOID -- A zooid or individual adopted or transformed for the production of embryos. Another name for ovicell or ooecium.
11. HETEROZOID -- Any individual which differs distinctly in some way or other from the normal zooids but which because of various reasons like mode of origin, must be considered as homologous with the normal zooids. Ex., gonozoids, kenozoids.
12. INTERNODE -- The part between joints or nodes of the Cyclostomata. It contains the zooecial tubes.
13. LOPHOPHORE -- The stage or disk surrounding the mouth and bearing the tentacles.
14. LYRULA -- A tooth or plate of various shapes or dimensions located at the posterior, inner part of the apertura, in the midline. It protects the opening of the compensatrix. Found in Smittina.
15. MUCRO -- A skeletal bump or cone-shaped growth present on the frontal furface of a zooid, near or before the apertura.
16. NODE -- The joint in a Crisiidae colony.
17. OOECIOPORE -- The actual external orifice of the gonozoid.
18. OOECIOSTOME -- The tube leading from the ooeciopore into the ooecium, if the ooecium happens to be of such a type.
19. OOECIUM -- The brood chamber or ovicell of the Cheilostomata.
20. OPERCULUM -- A delicate membranous valve closing the apertura. May be calcified in some spedies.
21. OPESIUM -- The uncalcified or more transparent frontat area of a zooid. Part of it is closed by an operculum. When its opening is completely closed or filled by the operculum it becomes the apertura.
22. OVICELL -- Brood chamber for developing larvae. Often used aynonymously with brood chamber, ooecium, gonozoid, but is a very inappropriate term for some Bryozoa.
23. PLEUROCYST -- A calcareous deposti, often granular, on the zooecia. Often forms the interareolar costules. Rarely porous. Part of calcified wall of zooecium. In Smittina and others.

GLOSSARY (Con't)

24. POLYPIDE -- A somewhat indefinite term meaning the inner organs of an autozoid. It includes the tentacles, tentacle sheath, lophophore, ganglion, digestive tract and some writers add musculature.
25. ROSETTE PLATE -- Multiporous communication plates located in the walls between zooecia.
26. SEPTULAE -- Pores, either uniporous or multiporous occurring in the walls between zooecia.
27. STOLON -- A tubular strand connecting zooecia which are not in juxtaposition or a tubular strand from which zooecia bud.
28. TREMOCYST -- One of the calcareous layers of the bryozoan zooecial wall. It is often punctured by pores.
29. TREMOPORE -- One of the pores puncturing the tremocyst as in Cryptosula pallasiana.
30. VIBRACULUM -- A bristle-like appendage or heterozooecium formed of a cavity, with chitinous or calcareous walls and of a very long flagellum, seta, or whip.
31. ZOARIUM -- The entire bryozoan colony.
32. ZOOECIUM -- This is the external skeleton of a bryozoan individual. A zoarium would consist of a number of zooecia. The zooecium is the compartment or tube housing the polypide.
33. ZOOID -- The bryozoa individual consisting of both polypide and zooecium, or the hard and soft parts. A number of zooids constitute a colony or zoarium.

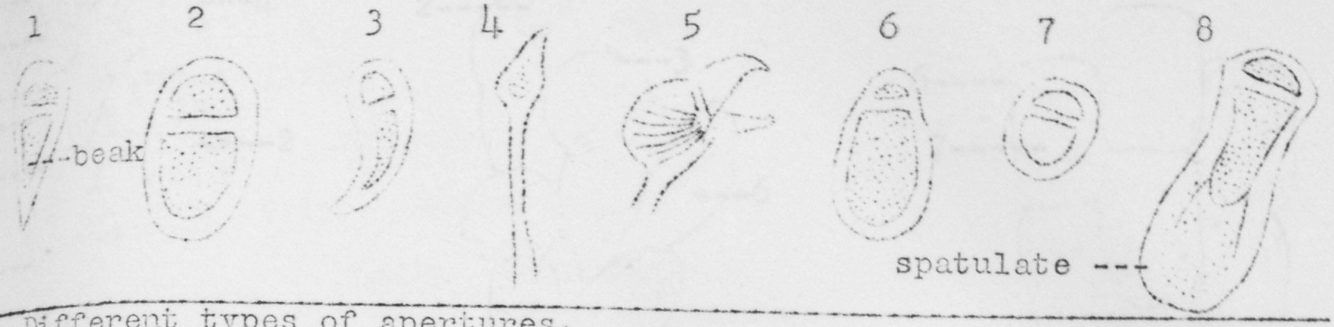


SMITTINIDAE,
modified after C&B 1920, p.454

1. ovicell
2. ovicell orifice
3. apertura
4. cardelle
5. lyrula
6. median avicularium
7. areola
8. interareolar costula
9. distal end of zooecium
10. spine
11. beak of avicularium
12. pivot of avicularium
13. interzooecial avicularium
14. frontal avicularium
15. operculum
16. tremopore
17. opesium

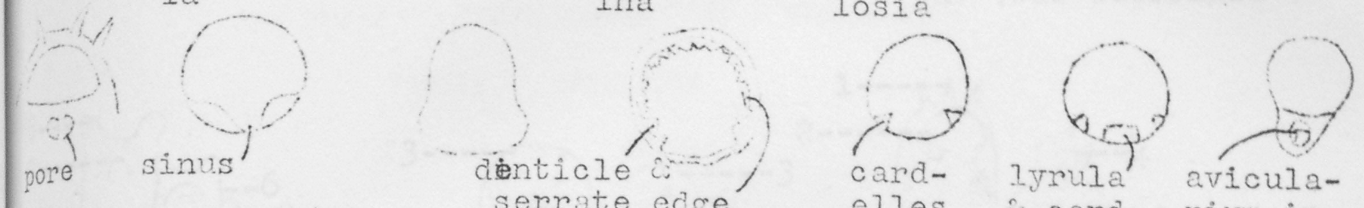
MEMBRANIPORIDAE
modified after C&B 1920, p. 3

A. Different types of avicularia.

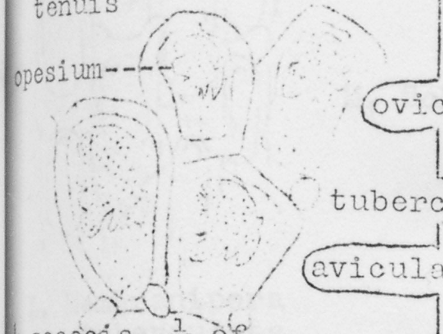


B. Different types of apertures.

1. Microporella
2. Schizoporella
3. Cryptosula
4. Hippeporina
5. Hippodiplosia
6. Smittina
7. Perella

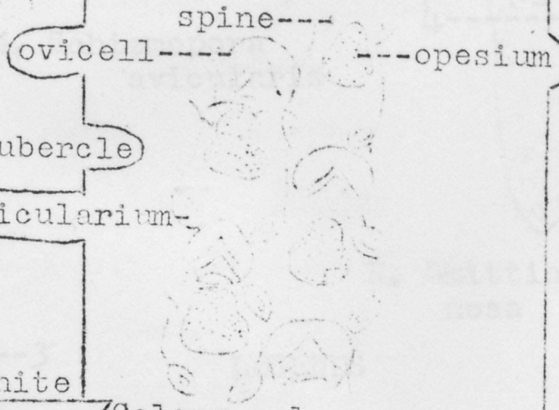


C. Acanthodesia tenuis



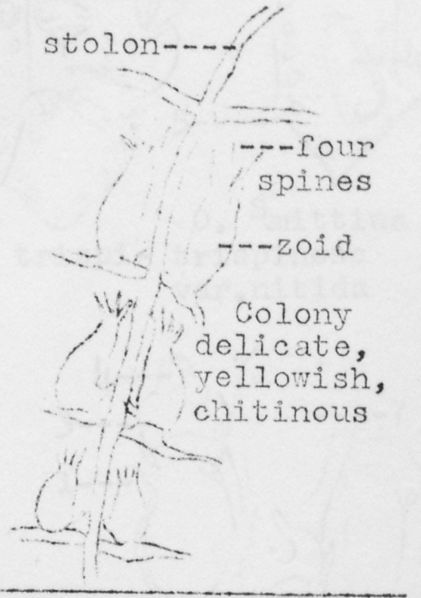
4 zooecia. ½ of frontal wall calcified. Tubercles at corners. Colony white

D. Callopora aurita



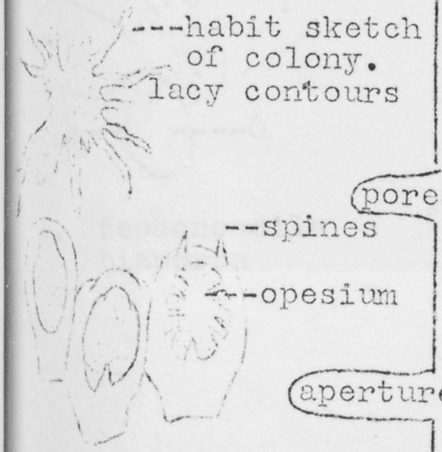
Colony calcareous

E. Aeverrillia setigera



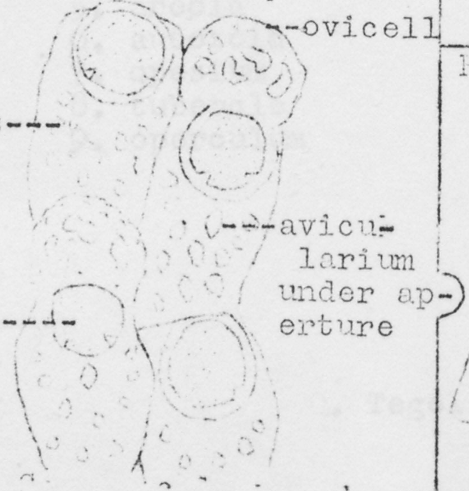
Colony delicate, yellowish, chitinous

F. Electra hastingsae

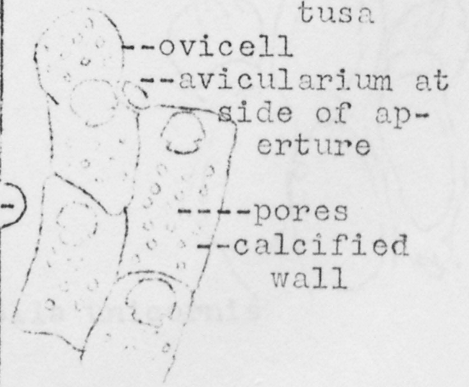


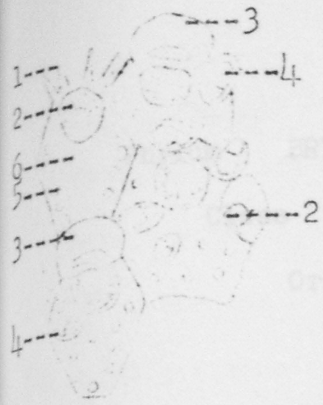
calcified zoids

G. Hippodeplosia americana

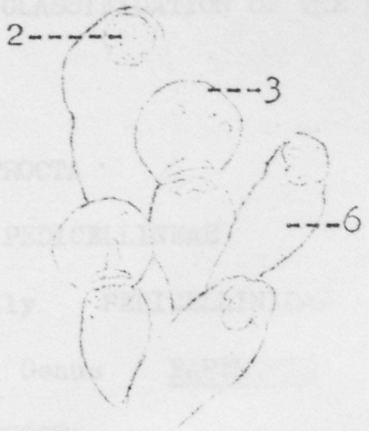


H. Hippodiplosia pertusa

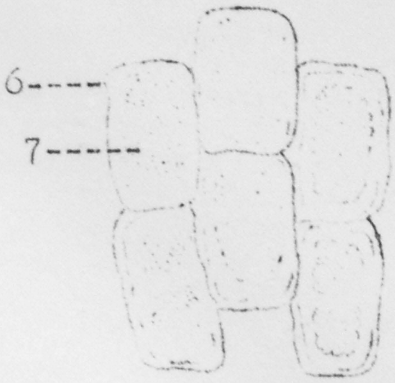




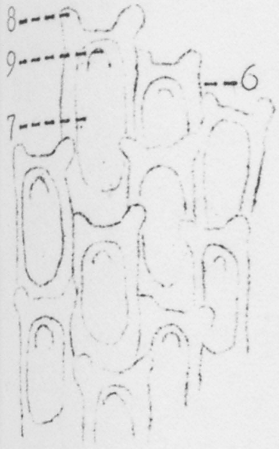
I. Hippoporina contracta



J. Hippothoa hyalina



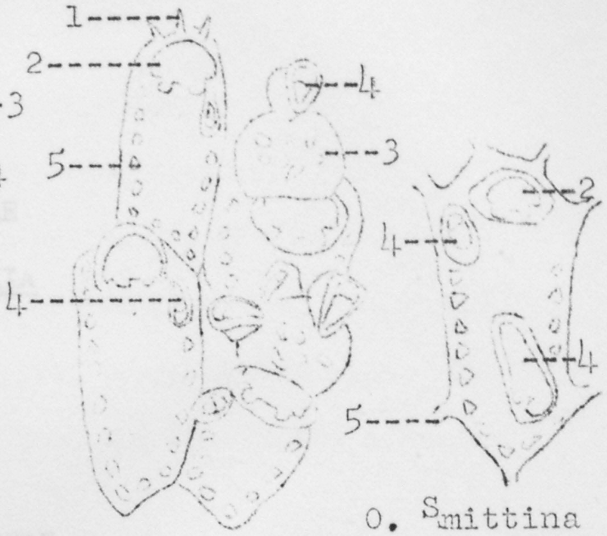
K. Membranipora crustulenta? (was lacroixii?)



L. Membranipora tuberculata

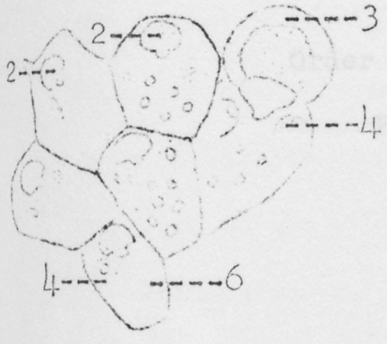


M. Schizmopora avicularis



N. Smittina trispinosa

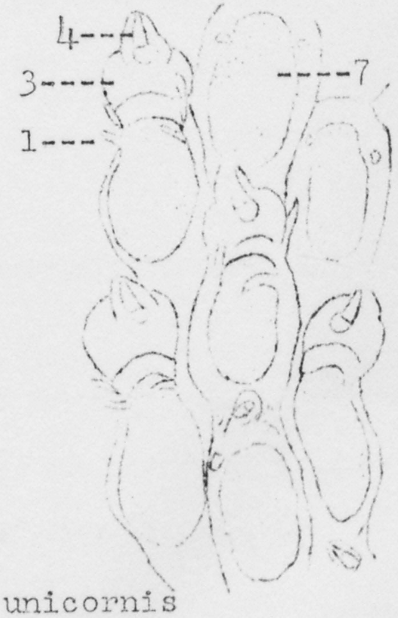
O. Smittina trispinosa var. nitida



P. Stephanosella biaperta

LEGENDS

1. spine
2. aperture
3. ovicell
4. avicularium
5. areola
6. autozoid
7. opesium
8. tubercle
9. operculum



Q. Tegella unicornis

CLASSIFICATION OF THE BRYOZOA

Phylum BRYOZOA

Class ENTOPROCTA

Order PEDICELLINEAE

Family PEDICELLINIDAE

Genus *BARENTSIA*

Class ECTOPROCTA

Order STENOSTOMATA or STENOLAEMATA

Family CRISIIDAE

Genus *CRISIA*

Order CTENOSTOMATA

Family VESICULARIIDAE

Genus *BOWERBANKIA*

Order CHEILOSTOMATA

Family ELECTRINIDAE

Genus *ELECTRA*

Family BICELLARIELLIDAE

Genus *BUGULA*

Order PHYLACTOLAEMATA

Family PLUMATELLIDAE

Genus *PLUMATELLA*

BARENTSIA

Barentsia forms soft, tufted, brownish or grey colonies ranging up to half an inch in height, on various submerged objects in sea water. Scrape some of the Bryozoan material from the substratum on to a slide. Add water and a cover slip and study under the compound microscope, using magnifications of 20x to 430x.

The individuals (zoids) consist of calyx (head), stalk and swollen base (musclium) and are connected with each other basally by stolons. Incomplete septa separate the stalks from the musclia and the musclia from the stolons.

The calyx measures 0.22 mm. to 0.78 mm. in height and 0.15 mm. to 0.52 mm. in width. It is gibbose posteriorly and flattened anteriorly and dorsally. It is very transparent, thin-walled and if lost can be regenerated. It bears the 13 to 23 slender active tentacles, the digestive tract, reproductive organs, nephridia, ganglia, nerves and embryos. It is at the tip of the long slender stalk which connects it with the enlarged base.

The slender ciliated tentacles can be durved inward into the atrium upon occasion and the velum or calyx rim can be contracted over them. The mouth and anus are within the tentacular circle as are the openings into the reproductive and nephridial ducts. The two latter sets of openings are very hard to see. The atrial floor is ciliated.

A small amount of parenchyma tissue, resembling extremely diffuse mesenchyme, is found here and there in the calyx.

The most conspicuous permanent organs in the calyx belong to the digestive system. They are the mouth, the wide pharynx, the narrowing tubular esophagus which is vertically placed along the flattened side of the calyx,

the large sac-like horizontal stomach whose upper wall is considerably yellowed and thickened into a "liver" and whose lower wall follows the ventral contours of the calyx closely, the tubular to elliptical intestine vertically placed against the gibbose side of the calyx, and the similarly shaped rectum which is bent horizontally forward over the gonads or brood chambers, below the floor of the atrium. The inner epithelium of the entire gut is ciliated, the cilia being longer and more prominent in the stomach, near the esophageal valve.

Barentsia feeds on Protozoa, Bacteria, other small organisms and debris. The food mass revolves in the stomach continuously, as does the fecal mass in the intestine and rectum, first in one direction for a number of turns, then in the other.

The reproductive organs are absent in some calyces. When present, they occur as large masses above the liver, in front of the intestine and beneath the rectum. If the calyx is male, the testes appear as two very large, elliptical, opaque masses, one on either side, above the liver. Within the testes are numerous, fine, thread-like, densely packed sperms which are in constant motion as time for release approaches. If the calyx is female, the ovaries are in relatively the same position as the testes, only slightly more forward. They consist of a number of developing eggs of oval or pear shape. Such a zoid may also be carrying embryos in various stages of development, just back of the ovary, closer to the intestine. Those most advanced in development push upward toward the atrial floor to be released. Sometimes three or more of these large opaque larvae may be lined up in the reproductive passages prior to release. Released larvae are partially ciliated, somewhat translucent and provided with a digestive tract which is already functional. Their gut resembles that of the adult calyx very closely. The larvae swim

about very actively for a time after their release before their permanent attachment. The larvae measure about 0.09 mm. in width and 0.15 mm. in length.

The ganglia are located in the spaces between the ovaries. These and the nerves are difficult to see.

The nephridia are above and in front of the ovaries, back of the pharynx and esophagus. They are slender, short, tubular processes with internal, intermittently flickering flagella which can be discerned only after the flagella are active.

Muscle fibers occur in the body wall, tentacles, digestive tract wall, stalk and enlarged muscular base.

The stalks bearing the calyces are long, slender, transparent, cylindrical and of varying degrees of flexibility or rigidity, depending upon the age of the zoid and the species. Their length may range from 0.37 mm. to 9 mm., depending upon age and other factors. The uppermost flexible part of the stalk nearest the calyx is the pedicel, the rest of the stalk is the peduncle and the swollen, barrel-shaped enlargement at the basal end of the stalk is the musclium. Muscle fibers are plainly evident in the stalk and musclium.

Occasionally, in addition to the basal musclium there may be one or two other muscles inserted along the peduncle. These may sometimes have stolons arising from their sides.

Each basal musclium is connected with the musclium of another zoid by a slender, transparent, cylindrical stolon of varying length (0.07 mm. to 1 mm. in length and 0.03 mm. to 0.06 mm. in width). From one to four stolons may arise from the base of the basal musclium.

There may be some live stalks which have lost their calyces. Sometimes new heads may form under degenerating heads before the degenerated ones drop off. Other times, the calyx may be broken off before a new one has begun to develop at the pedicel tip. In the latter case, just as in the former, a new head is regenerated if conditions are suitable.

CRISIA

Crisia forms dense bushy white dentritic masses on sea weeds, hydroids and other submerged objects. The colonies (zoaria) may attain a height of about 20 mm. Study them first under the dissecting then under the compound microscope.

The zoarium attaches to sea weed by a small rounded calcareous primary disc which in some instances is separated from the erect primary zoid by a constricted chitinous joint or basal tubulus. In addition to the basal disc there are sometimes slender calcareous rootlets (rhizoids) helping to attach the colony to the substratum.

The primary zoid gives rise by budding to autozoids which by repeated budding produce the branching tufted colony. The branches of the dendritic zoarium consist of groups (internodes) of zoids, each group or internode being separated from the ones below, above or to the side by a yellow or brown narrowed chitinous joint or node. The number of zoids in an internode varies from one to over thirty, depending upon species, location in the zoarium and other factors.

The zoarium consists of several kinds of zoids: autozoids, gonozoids and rhizoids. The autozoids are by far the most numerous and common. The other two types are few in number and hard to find but are taxonomically important.

The autozoids are long, narrow, cylindrical, calcified tubes whose aperture is terminal and whose walls are marked by numerous widely spaced but small pseudopores where the calcareous layer of the body wall is interrupted. The large terminal aperture is closed by a soft delicate terminal membrane which forms part of the body wall of the soft polypide which occurs inside the calcareous tube. If the living colony is undisturbed for a time the tentacular crown with its eight slender tentacles may be protruded through the terminal aperture.

The gonozoids (oecia) are extremely large, swollen, vase-like zoids which serve as brood chambers for developing embryos. Their walls are marked by extremely numerous pseudopores which are placed very close together. The gonozoids have the following parts: the proximal, middle and distal portions. The p roximal part is a narrow, tapering tube wedged in between neighboring autozoids and sometimes partly hidden by them. The middle part, or brood chamber proper, is very greatly dilated but tapers gracefully into the proximal part. The distal part, or coeciostome, is an extremely narrow, constricted tube, usually very short, through which the larvae leave the gonozoid.

BOWERBANKIA

Bowerbankia is a soft colonial bryozoan, forming a dense gray or brownish fuzz only one or two millimeters high on sea weeds, hydroids, rocks, and other submerged objects. Scrape a small fragment from the colony on to a slide. Add some sea water and a cover slip and study with the compound microscope. If available, slides which have been immersed in a likely salt water locality for two weeks or so may have young colonies of few individuals which would be ideal for study.

The zoarium consists of numerous tall cylindrical zoids closely packed together. These arise by budding from a basal stolon which is easily discernible in young colonies but hard to see in old ones because of zoecial crowding. The zoids range from 0.42 mm. to 1.5 mm. in height and from 0.16 mm. to 0.24 mm. in width, while the diameter of the stolon is from 0.07 mm. to 0.11 mm.

The zoids are flexible, transparent, thin-walled, cylindrical and frequently with distal tips squared. They arise singly, in pairs or sometimes even in groups, parallelling each other, usually from the sides of the stolon. Their basal end may or may not be drawn out into a narrowed caudal process.

The zoid has eight to ten ciliated retractile tentacles which are borne on a circular lophophore. When protruded they are surrounded by a short delicate collar which is almost imperceptible because of its transparency. The tentacles can retract completely down into the body cavity by the action of powerful retractor muscles. The body wall directly below the tentacles is also pulled inward, inverting into the body cavity to form a sheath for the tentacles.

Above the pulled-in tentacular sheath is a narrow channel, the vestibule, also formed from the partially inverted body wall. Toward its lower end are several groups of muscle fibers, the parieto-vaginals, leading from the outer body wall to its wall. There are several other bands of muscle fibers, the parietals, arranged hoop-like, horizontally along the body wall proper.

The digestive tract consists of a mouth, pharynx, esophagus, gizzard or proventriculus, stomach, intestine and anus. The mouth opens within the tentacular circle. The anus opens outside the circle, down along the tentacular sheath. The pharynx is directly beneath the tentacular crown and tapers into the long narrow esophagus which ends in the enlarged, bulbous gizzard which is lined with teeth. The gizzard is of brown or yellow color. Its wall is very firm and provided with a conspicuous muscular sheath. The stomach is a large, wedge-shaped, elongate soft sac extending downward to the base of the zoid. A slight constriction, the pylorus, separates the stomach from the long narrow intestine which parallels the esophagus up within the tentacular sheath. When the tentacles are retracted into the body cavity the digestive tract is thrown slightly out of position. The esophagus folds into a loop and the stomach and intestine are also slightly bent.

The stolon is slender, transparent, and occasionally septate, giving off buds which develop into zoids. These buds are in varying sizes and stages of development. Some are simple transparent sacs, others have very short young tentacles and a tiny digestive tract.

ELECTRA

Electra forms a fragile, white, flatly-encrusting calcareous patch on sea weeds, stones and other submerged objects. Young colonies are fan-shaped. Older colonies are more rounded but may sometimes be quite irregular peripherally and consist of hundreds of zoids. The zoids bud in rows, somewhat radially, from the few central ones.

Each zoid consists of two parts: the zooecium and the polypide. The zooecium is the firm outer partially calcified case housing the soft inner polypide. The polypide includes the tentacular crown, digestive tract and associated musculature.

Each zooecium has the following surfaces: (1) an attached surface by which the zoid is fastened to the substratum, (2) an upper free surface, (3) two or more lateral surfaces, (4) a proximal and (5) a distal surface. The proximal surface is nearest the parent zoid. The distal surface is the one farthest from the parent zoid. The lateral surfaces are perforated by a few pores and represent the side walls of each zoid. The upper surface consists of two parts: a calcified proximal perforate part (the gymnocyst) and a distal elliptical membranous area (the aperture). The latter large area is covered by a transparent lightly chitinized membrane, whose distal part has a hemispherical flap or operculum through which tentacles may be protruded. The crescent-shaped distal border of the operculum is more heavily chitinized. There is a circle of several short curved spines bordering the frontal area and curving over it. In some zoids these may be broken off. In addition to these spines there is another spine (basal spine or flagellum) of varying length and stoutness but always bigger than the other spines, placed proximally

below the frontal area. The gymnocyst is perforated by a number of large pores in Electra pilosa, tiny ones in Electra hastingsae.

BUGULA

Bugula forms soft yellowish dendritic colonies on sea weeds, piles and other submerged objects. Some species have a spiral growth habit, others appear flabellate and still others have an irregular bushy appearance.

The colony (zoarium) consists of fronds of numerous box-like or roughly tubular zoids which arise from the basal one by repeated budding. These zoids are arranged in one to four rows on each frond. The zoarium is attached to the substratum by a firm holdfast or irregular shape. Slides which have been submerged in a suitable salt water locality for one or two weeks are an excellent source of young Bugula colonies of few individuals and show the holdfasts beautifully.

The yellowish cuticula forming the outer wall of each zoid is so transparent that the internal organs can be readily studied. There is a large U-shaped area, the aperture, occupying the upper half or two-thirds of the frontal wall of each zooecium. It is covered over by a thin membrane which is a part of the body wall of the animal. Tentacles can be extruded through the inconspicuous operculum near the top of the aperture. A specialized individual, the avicularium, consisting of head, beak and stalk and shaped like a bird's head, is attached by a short stalk somewhere alongside the aperture. The head part contains fan-shaped muscles whose fibers coverage toward the beak or mandible. The avicularia in living specimens nod constantly and snap their jaws but their function is unknown.

Small shallow hemispherical or beret-shaped ovicells overhang the distal end of some of the zooecia. These ovicells or coecia are brood pouches for developing embryos. If embryos are still in them, the ovi-

cells look opaque or reddish. If embryos have left, the ovicells look completely empty and transparent.

There are about 14 tentacles. They are borne on a circular ridge of flesh, the lophophore, which surrounds the mouth. They can withdraw into the body cavity, pulling in after them a part of the outer body wall, the introvert, which forms a tentacular sheath for them. This retraction is accomplished by two fairly thick bands of retractor muscles which are attached at one end to the lophophore and at the other end to the body wall.

The digestive tract is V-shaped. It consists of mouth, pharynx, esophagus, stomach, caecum, intestine and anus. The caecum is that part of the stomach which forms the bottom of the V and is anchored to the body wall by a double strand, the funiculus. The pharynx is just below the circle of tentacles. The anus is near the mouth and pharynx but outside the tentacular circle.

Both male and female reproductive organs may occur in the same zoid. The testes are soft fluffy growths, consisting of germinal tissue and actively moving sperms, on the funiculus. The ovary is a lump of tissue attached to the body wall more distally than the testis and near the middle part of the body cavity. The egg which is produced in the ovary is fertilized by the sperms supposedly from the same zoid and then passed out into the ovicell where it develops further.

A small ganglion is present just slightly under the tentacular crown, between the upper part of the pharynx and intestine.

M. B. L. - INVERTEBRATE ZOOLOGY LABORATORY DIRECTIONS

ANNELIDA

General plan:

First Day.	External and internal polychaete 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 the Arenicola must be completed in one day. The fresh specimens do not keep well overnight. 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.

1. 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 rapidly the head and next two segments and label (a) the conical prostomium, bearing two pairs of eyes, 2 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 the tentacles.)

3. Pop 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 coverglass 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 subdividedand each of which bears a fleshy process, known respectively as the dorsal and and ventral 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 polychaetes we study in the laboratory and see in the field can be considered simply as modifications, 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 Drew, pp. 102-107. Each student will have (if we are lucky) one anesthetized specimen for the internal antomy, 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 Drew. If you are drawing on cards, at least 2 will be needed to show the structures at sufficient magnification, although certain segments may be omitted if they simply duplicate others (i.e. no sense in drawing the whole length of the worm). 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 of the body? In which directions do the waves appear to travel? Study of the locomotion may be facilitated by putting the worm on a sheet of 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.

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 25 common local annelids. There should be enough for one specimen of each species to every four students, so share them about. 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 thus 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.

To illustrate how such a key is constructed, we shall consider a simple key for separating 9 races of dogs. In this, the material which would actually be included in the key is in capital letters, while the part in small type is explanation or commentary. Thus, to see what the finished key would be like, read only the capital type.

KEY TO NINE DOGS

1. SHORT HAIRED 2

LONG HAIRED 6

Now, in this first "couplet" you see we have contrasted two statements. The numbers on the extreme right refer us to the couplets in which short haired (2) and long haired (6) dogs, respectively, will be considered separately in more detail. Obviously we are lucky in having only dogs which can be separated sharply into short and long haired. A large sample would doubtless contain dogs of intermediate hair length (e.g., Airdale). However, with a limited sample the best we can do is to construct the key to fit the forms on hand.

2.(1)WITH VERY SHORT MUZZLE 3

WITH LONG MUZZLE 4

We have thus begun to divide the short haired dogs into smaller subdivisions. The "(1)" following the 2 in the left column reminds us that the statements in 2 are derived from couplet 1, so that we can refer back to couplet 1 in case neither half of couplet 2 seems to fit our dog.

3.(2)WITH MARKEDLY UNDERSHOT LOWER JAW. . . . . .ENGLISH BULLDOG

WITH SLIGHTLY UNDERSHOT LOWER JAW. . . . . .BOSTON BULLDOG

Here, you see, we have disposed of all the short haired, short muzzeled dogs in our sample, and hence need no further index numbers in the right hand column. Admittedly the last dichotomy is not based on a very "safe" character, since "markedly" and "slightly" are not precisely defined. As before, if the descriptions in couplet 3 do not seem to apply to our dog, the "(2)" tells us to check back to couplet 2.

4.(2)WITH LONG FLOPPY EARS 5

WITH PRICK EARS . . . . . .GERMAN SHEPHERD

Now we are dealing with the long muzzled short haired dogs.

5.(4)WITH VERY SHORT LEGS AND VERY LONG BODY . . . . .DACHSHUND

WITH ORDINARLY PROPORTIONS. . . . . .BEAGLE

This finishes the short haired dogs in our sample. Obviously, "with ordinary proportions" is not a very complete description of a Beagle, but since he was the only short-haired, long muzzled,

long eared dog in our sample, the description is adequate. On the other hand, you might object that the description of the Dachshund in the last couplet is adequate at once to define a Dachshund, without bothering about his hair, nose and ears. This is true, but since most races require a more complicated combination of characters for identification, it is convenient to have all the dogs indexed under one system. Moreover, the key has the advantage of usually throwing closely related animals together, and thus emphasizing their kinship (if the key characters are chosen astutely.

6.(1)WITH LONG SLENDER MUZZLE. 7

NOT AS ABOVE. 8

Now we are dealing with the long haired dogs. Here "slender" is a bad term because it is too flexible. If you had never seen but the one dog you were trying to identify, you would not know whether his nose was slender or wide, compared to the average dog's. Here, also, the "not as above" is not as satisfactory a statement as would be some definite descriptive phrase such as were used in couplets 1, 2, 3, 4, etc. The "(1)", of course, refers back to couplet 1, from which 6 was derived.

7.(6)WITH LONG SLENDER LEGS AND BODY AND SOFT CURLY HAIR * RUSSIAN WOLFHOUND

WITH LEGS AND BODY OF ORDINARY PROPORTIONS AND WITH VERY THICK HAIR, PARTICULARLY ON SHOULDERS AND NECK COLLIE

8.(6)WITH CURLY HAIR COVERING FACE AND EYES..... POODLE

WITH STRAIGHT FLUFFY HAIR NOT OVER EYES..... SPITZ

This completes our key. To be sure the characters chosen at the dichotomies will probably not serve to identiy our nine dogs out of a larger sample, but that need not worry us. Note that we might have keyed out the Spitz (for example) more simply in one step by specifying that it has a black tongue, but that would have emphasized an obscure character of minor importance, whereas it is usually desirable to use as conspicuous a character as possible. Moreover, it is usually desirable to use more or less the same sorts of characters throughout the key (above, e. g., we used only external features.)

Now, turning to the Annelids, the same sort of system can be applied if proper characteristics are chosen. You may use whatever characters you please, but before you begin be sure that you are thoroughly familiar with the parts of the generalized 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 every one has the opportunity of seeing the speciment alive; and that the properties of a case or tube will be of no help if the work comes alone. 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 palps, 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" problems 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 watch glasses 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 of 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 drawings 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.

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. Ten species in addition to the forms studied in the laboratory (Nereis, Arenicola, Amphitrite, Diopatra) would seem to be the minimum that would give a key of any practical value, or require any ingenuity, and at least 20 species can be included without undue hair-splitting.

Third Day.

These are merely suggested problems - you may pick others if you wish- 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) arrangement of materials; (c) method of consolidation; (d) physical properties (tough? 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 if 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") 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. Phascolosoma. (Gophyrean worm).

Phascolosoma is interesting as a very aberrant type of Annelid.

1. Start to anesthetize a phascolosoma, as described below, and while it is going under, study the external anatomy and behavior of a fresh specimen, as directed in Drew, p. 119. In addition, study its burrowing behavior in sand.

2. Put a phascolosoma into a finger bowl and add enough sea water to just cover it. Add every minute or two a c.c. of 95% alcohol, and stir. As the worm becomes doped, 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 Drew, pp. 120-121. 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, pp. 95-96.

Problem IV. Polychaete 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 circulations - 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., Nepthys, Diopatra, Amphitrite, Lepraea, Aricia or Scoloplos, Cirratulus, etc. with a view to trying to answer how the secretion of the blood is accomplished. Movements may be slowed, if necessary, with chlorestone. Often the posterior gillsare simpler in structure and easier to handle, and 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)? Diopatra 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 Vl. Physiology of tubicolous worms.

Study the behavior of Cystenides, Pista, Clymenella, Diopatra, Chaetopterus, Hydroides, Spirorbis, etc. in their own or in artificial tubes as follows:

1. 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 type 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 Diopatra 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, parapodia, gills, posterior end, etc.) and explain how they are related to tube life. (See also Problems 1 and Vll.)

Problem Vll. Comparative structure of tubes.
Examine tubes of Cystenides, Pista, Clymenella, Diopatra, chaetopterus, Hydroides, 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. Are the tubes impervious? Flexible? is cement substance used sparingly or lavishly?
4. Can any conclusion be drawn from the tube as to whether its occupant was modified structurally for tube life?
5. What can you conclude from the tube as to the mode of feeding of the occupant? As to his gills?

Problem Vlll. Tentacular or cirral activity.
Observe in finger bowls a variety of Terebellid worms (e.g., Amphitrate, Lepraea, Pista, Thelepus, 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 binocular).
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 lX. 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. Trochophore larva.
Trochophore larvae of Hydroides can be readily obtained in about 20 hrs. These may be studied to gain a clear idea of trochophore structure. Entangle in cotton and study under a coverglass. Draw from lateral aspect.

Problem Xl. Osmotic regulation.
1. Measure the length of a nereis and then pop it into fresh water. Observe any immediate changes in reactions, body length, metamere size, and so on. Study again after fifteen minutes, and after half an hour. At the end of half an hour (not longer!) put the worm back in sea water and observe whether there is any recovery (reversal of effects).

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

Problem XII. Spawning in Nereis.

1. 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.

Problem XIII. Modification of Behavior in Neanthes Virus

Neanthes will survive well in glass dishes in the laboratory provided it is given glass tubing of appropriate bore in which to live. Try conditioning a worm to tactile or other type of stimulus by technics comparable to those of Copeland (1930) and Copeland and Brown (1934)

DISSECTION OF AMPHITRITE

From a doped or freshly killed specimen obtain some of the coelomic fluid by making a small slit in the body wall. What is the color and consistency of this fluid? Examine under the low and high powers of the microscope, and observe the varieties of cells present and their movements. Some of them are reproductive products.

Pin down the specimen, either fresh or preserved, under sea water with the dorsal or bristle-bearing surface upward. With the scissors cut along the mid-dorsal line, piercing only the body wall. Pin the body wall out flat as you cut from head to tail.

Coelom. Are there any transverse septa or diaphragms? Note the dorsal and the ventral longitudinal mesenteric strands which hold the digestive organs in place. Cut the dorsal once. How do the details of internal segmental organization correspond to the external organization?

Circulatory System. This must be observed before further dissection is done. Find the large paired subintestinal vessils. Trace them backward as far as you can, and forward into the single gastric vessel, which continues into the dorsally situated heart. From the heard the branchial vessels go forward to the three pairs of gills. Find the ventral vessel, and note its transverse branches, some of the encircling the digestive organs. You may be able to see also the dorsal neural, lateral, and esophageal vessels, all longitudinal.

Digestive System. Is the mouth ventral or dorsal? It leads into the straight muscular esophagus. How is the latter held in place? Is there a protrusible pharynx? The esophagus leads into the wide straight stomach. Does it show any segmentation? The winding portion of the alimentary tract is the intestine, which continues to the anus. Cut open the stomach and notice the character of its walls.

Muscles. Transverse-oblique, longitudinal, setal, circular. By means of a rough diagram reconstruct a transverse section of the animal through the stomach region, showing the relative positions of the muscles, stomach and blood vessels which you have observed.

Nephridia. Cut through the oblique muscles in the anterior few semites and expose the nephridia. Quickly sketch the natural shape and position of one of them. How many external nephridial openings or nephridiopores can you find? Plut a nephridium from a freshly killed worm under the microscope, and see if there is any ciliary activity. Try to find the nephrostome. How do you think the nephridium functions in the living body? The nephridia may also serve as ducts for the extrusion of genital products. The gonidial glands are small white bodies ventral to the esophagus. Ventral to them are the larger and yellowish "Stomach glands".

Nervous System. Displace the digestive organs to one side, and find the ventral nerve cord. Has it ganglia? Can you find any paired, transverse branches? What features of the nervous system are metameric How far does the ventral cord go posteriorly? Anteriorly try to find the sub-esophageal ganglion, the circum-esophageal connectives, and the supra-esophageal ganglion or brain. Where would you expect to find sense organs in Amphitrite?

Make a list of the special features of the anatomy which are particularly significant of the sedentary habit in a tube. Compare the anatomy with that of Nereis. Which of the two species do you consider to be most highly differentiated; and which the better fitted for self-maintenance and self-preservation, from what you know of the conditions of life of the two species? Make a table of the functions performed by the body of Amphitrite under two headings; those without which the animal or species cannot survive, and those which are luxuries. Are any of these functions performed in a way that you consider more efficacious by animals you have already studied in this course?

EARLY STAGES IN THE DEVELOPMENT OF THE ANNELID HYDROIDES

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 low 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 result. DRAW as seen from the right or left side.

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 the water in which the sperms are thickly suspended. A generous amount of sperm suspension seems in this particular case to be desirable. 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:

Germinal vesicle breaks down in about 10 to 15 minutes
1st polar body - 20 to 25 minutes
1st cleavage - 50 to 79 minutes
2nd cleavage - 70 to 85 minutes
The 25 to 30 minutes between cleavages
Swimming larvae - 6 hours
Good gastrulae - 9 hours
Trochophores - 20 hours

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 trochophore becomes very transparent. In this condition (4 to 6 days after fertilization) they are best suited for study.

SELECTED CURRENT AND RECENT LITERATURE CONCERNING ANNELIDS

BLOOD AND RESPIRATION:

Alsterberg, B. (1922) Die Respiratorischen Mechanismen der Tubificiden. Eine experimentell-physiologische Untersuchung auf oekologischer Grundlage. Lunds. Univ. Aaraskr. N. F. Avd. 2. 18: 1-176.

Barcroft, J. and H. Bancroft (1924) The blood pigment of Arenicola. Proc. Roy. Soc. London, B 96: 28-42

Borden, M. A. (1931) A study of the respiration and of the function of haemoglobin in Planorbis corneus and Arenicola marina. J. Mar. Biol. Assoc. Plymouth 17: 709-738.

Dolk, H. E. and F. van der Paauw (1929) Die Leistungen des Hamoglobins beim Regenwurm. Z. vergl. Physiol. 10: 324-3 43.

Florkin, M. (1933)) Recherches sur les hemerythrines. Arch. Inv. Physiol 36: 247-328.

Fox, H. M. (1926) Chlorocruorin: a pigment allied to hemoglobin. Proc. Roy. Soc. London, B 99: 199-220.

Fox, H. M. (1932) The oxygen affinity of Chlorocruorin. Proc. Roy. Soc. London, B 111: 356-363.

Hall, V. E.1931) The muscular activity and oxygen consumption of Urechis caupo. Biol. Bull 61: 400-416.

Harnish, O. (1935) Versuch einer Analyse des Sauerstoffverbrauche von Tubifex tubifex Mull. Z. Vergl. Physiol. 22: 450-465.

Lindroth, A. (1938) Gibt es bei den Polychaten intestinale Atmung per anum? Z. vergl. Physiol. 25: 283-292.

Lindroth, A. (1938) Studien uber die respiratorischen Mechanismen von Nereis virens Sars. Zool. Bidrag Uppsala 17: 367-497.

Lindroth, A. (1939) Beobachtungen an Capitelliden, besonders hinsichtlich ihrer Respiration. Zool. Anz. 127: 285-297.

Redfield, A. C. and M. Florkin (1931) The respiratory funtion of the blood of Urechis caupo. Biol. Bull. 61: 185-210.

van Dam, L. (1938) On the utilization of oxygen and regulation of breathing in some aquatic animals. Diss. Croningen.

van Dam, L. (1940) On the mechanism of ventilation in Aphrodie aculeata. J. Exp. Biol. 17: 1-7.

Zoond, A. (1931) Studies in the localization of respiratory exchange in invertebrates. 11. The branchial filaments of the sabellid, Bispira voluticornis. J. Exp. Biol. 8: 258-262.

DIGESTIVE SYSTEM:

Ambache, N., A.St. J. Dixon, and E. A. Wright (1945) Some observations on the physiology and pharmacology of the nerve endings in the crop and gizzard of the earthworm with special reference to the effects of cooling. J. Exp. Biol. 21: 46-57.

Robertson, J. D. (1936) The function of the calciferous glands of earthworms. J. Exp. Biol. 13: 279-297.

Wu, K. S. (1939) On the physiology and pharmacology of the earthworm gut. J. Exp. Biol. 16: 184-197.

NERVOUS SYSTEM AND BEHAVIOR

Bovard, J. F. (1918) The function of the giant fibers in earthworms. Univ. Calif. Publ. Zool. 18: 135-144.

Bullock, T. H. (1945) Functional organization of the giant fiber system of Lumbricus. J. Neurophysiol. 8: 55-71

Collier, H. O. J. (1938) The immobilization of locomotory movements in the earthworm, Lumbricu terrestria. J. Exp. Biol. 15: 339-357.

Collier, H. O. J. (1939) Central nervous activity in the earthworms. 11 Properties of the tension reflex. J. Exp. Biol. 16: 300-312

Collier, H. O. J. (1939) Central nervous activity in the earthworms 1. Response to tension and to tactile stimulation. J. Exp. Biol 16: 286-299.
Copeland, M. (1930) An apparent conditioned response in Nereis virens. Jour. Comp. Psych. 10: 339.
Copeland, M & F. A. Brown Jr. (1934) Modification of behavior in Nereis virens. Biol. Bull. 67: 356-364
Eccles, J. C., Granit, R. and J. Z. Young (1933) Impulses in the giant fibres of earthworms. J. Physiol. 77: 232-24P
Gray, J. (1939) Studies in Animal Locomotion: V11 The kinetics of locomotion of Nereis diversicolor. J. Exp. Biol. 16: 9-17
Gray, J., H. W. Lissmann and R. J. Pumphrey (1938) The mechanism of locomotion in the leech (Hirudo medicinalis Ray) J. Exp. Biol 15:408-430
Gray, J. and H. W. Lissman (1938) Studies in animal Locomotion. V11. Locomotory reflexes in the earthworm. J. Exp. Biol. 15: 506-517
Holst, E. v. Untersuclungen über die Funlctionesen des Zeutralnervensys-tems beim Regenwurm. Zool. Jb. 51: 547-589 (1932)
Holst, E. v. (1933) Weitere Versudie zum nervösem Medianismus der Bewegung beim Regenworm (Lumbricus terr. L.) Zool. Jb. 53: 67-100
Johnson, M. L. (1941) The respiratory function of the haemoglobin of the earthworm. J. Exp. Biol. 18: 266-277
Prosser, C. L. (1934) The nervous system of the Earthworm. Quart. Rev. Biol. 9: 18-300
Rushton, W. A. H. (1945) Action potentials from the isolated nerve cord of the earchworm. Proc. Roy. Soc. London Ser. B. 132: 423-437
Swartz, Ruth D. (1929) Modification of behavior in earthworms. Jour. Comp. Psychol. 9
Wells, G. P. and Isablel C. Ledingham (1940) Studies on the Physiology of Arenicola marina L. 11 Accommodation to Magnesium Concentration in the isolated extrovert. Jour. Exp. Biol. 17: 353-363
Wells, G. P. and I. C. Ledingham (1942) Studies on the physiology of Arenicola marine L. 111 The potassium relations of the isolated ex-trovert (with remarks on the effects of varying the lever weight). J. Exp. Biol. 19: 176-185
Wells, G. P., I. C. Ledingham and Mary Gregory (1940) Physiological effects of a hypotonic environment 11 Shock effects and accommodation in cilia (Pleurobrachia, Mytilus, Arenicola), following sudden salinity change. J. Exp. Biol. 17:378-385
Wu, K. S. (1939) The action of Drugs, especially acetylcholine, on the annelid body wall (lumbricus, Arenicola). J. Exp. Biol. 16: 251-257

HORMONES

Hanstrom, B (1939) Hormones in Invertebrates. Oxford Univ. Press
Hess, W. N. & R. L. Bacon (1940) Sex hormone of the earthworm, Eisenia foetida. Anat. Rec. 78: Suppl. 150
Koller, G. (1936) Beobachtungen au den Nephridren von Physcosoma japonicum. Naturwiss 24
Koller, G. (1938) Hormone bei Wirbellosen Tieren. Leipzig 143 pp

OSMOTIC AND IONIC REGULATION

Adolph, E. F. (1936) Differential permeability to water and osmotic exchanges in the marine worm Phascolosoma. Jour. Cell. & Comp. Physiol. 9: 117-135
Adolph, E. F. (1943) "Physiological Regulations" Jaques Cattell Press pp. 138-144
Beadle, L. C. (1931) The effect of salinity changes on the water content and respiration of marine invertebrates, J. Exp. Biol. 8: 211-227.

Beadle, L. C. (1937) Adaptation to change of salinity in the Polychaetes 1. Control of body fluid volume and of body fluid concentration in Nereis diversicolor. J. Exp. Biol. 14: 56-70
Bethe, A. and E. Berger (1931) Variationen im Mineralbestand verschiedener Blutorten. Pflug. Arch. ges. Physiol. 227: 571-584
Bethe, A. (1934) Die Salz-und Wasserdurchlassigkeit der Korperoberflachen verschiedener Seetiere in ihrem gegensertigen Uerhaltnis. Pflug. Arch. ges. Physiol. 234: 627-644
Dekhuyzen, M. C. (1921) Sur la semiperméabilite biologique des perois extorieures des Sipunculides. C. R. Acad. Sci. Paris 172: 238
Ellis, W. G. (1933) Calcium and the resistance of Nereis diversicolor to brackish water. Nature 132: 748
Ellis, W. G. (1937) The water and electrolyte exchange of Nereis diversicolor (Muller). J. Exp. Biol. 14: 340-350
Schlieper, C. (1929) Ueber die Einwirkung mederer Salzkongen-trationen auf marine Organismen. Z. vergl. Physiol. 9: 478-514
Wolf, A. V. (1940) Paths of water exchange in the earthworms. Physiol. Zool. 13: 294-308

REPRODUCTION

Clark, L. B. & W. N. Hess (1940) The reactions of the atlantic Palolo, Leodice fucata, to light Carn. Inst. Washington. Publ. 524: 71-81.
Clark, L. B. & W. N. Hess (1940) Swarming of the Atlantic Palolo worm, Leodice fucata (Ehlers). Carn. Ins. Washington. Publ. 524: 21-70
Galloway, T. W. & P. S. Welch (1911) Studies on a Phosphorescent Bermudan Annelid, Odontosyllis enopla verrill. Trans. Amer. Micr. Soc. 30: 13-39
Martin, E. A. (1933) Polymorphism and methods of asexual reproduction in the annelid, Dodecaceria, of Vineyard Sound. Biol. Bull. 65
Myers, R. J. (1935) Behavior and Morphological changes in the leech Placobdella parasitica, during hypodermic insemination. J. Morph 57
Okada, Yo. K. (1933) Two interesting syllids with remarks on their asexual reproduction. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. 8

A KEY TO THE MORE COMMON POLYCHAETES OF THE WOODS HOLE SECTION

(Nomenclature according to Dr. Olga Hartman)

Many terms are defined in the Glossary in Drew. To these may be added the following:

DORSAL CIRRUS - A soft process from the dorsal part of a parapodium

VENTRAL CIRRUS- A soft process from the ventral part of a parapodium

PALPS - Processes from the ventral side of the prostomium

PERISTOMIAL CIRRI - Paired processes arising from the first segment (peristomium)

TENTACLES - Processes usually arising from the anterior end of the prostomium and more dorsal than palps

GILLS - Thin walled, often red, usually either branched or elongated processes from the dorsal side of the head or body; sometimes difficult to distinguish from dorsal cirri

KEY

1. Scales on dorsal side of body ..... POLYNOIDAE
   a. 12 pairs of scales..... Lepidonotus
   a. 15 pairs of scales..... Harmothoe
   a. 40 - 50 pairs of scales, body black..... Lepidametria
   a. 100 or more pairs of scales..... Sthenelais
1. Anterior end concealed by feathery tentacles
   a. Several anterior segments differentiated into a "thoracic membrane"; an operculum present; forming calcareous tubes.....
   .....SERPULIDAE
      (1) Minute; in small flat-coiled tubes... Spirorbis
      (1) Larger; tubes irregularly sinous, not coiled..... Hydroides
   a. Without a thoracic membrane; no operculum; in sandy tubes.....
   .....SABELLIDAE
      (1) Collar notched dorsally; in small encrusting sand tubes..... Parasabella
      (1) Collaar without dorsal notch; in small parchment tubes among shells; dredged with Hydroides..... Pseudopotamilla
1. Anterior end concealed by chitinous spines forming an operculum
   a. Thorax with two pairs of pectinate (comb-like) gills; tail annulated and with setae; operculum formed of a horizontal row of a few strong spines on the head; in solitary, cone shaped sand tubes..... PECTINARIIDAE..... Cistenides
   a. Thorax without pectinate gills; many filiform gills on anterior end; tail smooth, slender; operculum formed of whorls of many closely spaced spines; in masses of tubes more or less firmly cemented together..... SABELLARIIDAI..... Sabellaria
1. Anterior end with numerous long, spinous setae, not forming an operculum but projecting forward beyond prostomium; eight small tentacles..... FLABELLIGERIDAE....Flabelligera

1. Anterior end with long filamentous outgrowths
   a. Tentacular filaments concentrated on head; often with branching gills on head also..... TEREBELLIDAE
      (1) Without branching gills on head; slender, usually blood-red
         (2) Setae on first 25 segments only; parapodia small; blood-red.....Polycirrus eximius
         (2) Setae on first 24 segments only; parapodia very inconspicuous; lemon yellow.....P. phosphorea
         (2) Setae on all segments; branching red parapodia in midbody region; blood-red..... Enoplobranchus
      (1) With branching, dorsal gills among or close behind filaments; not slender; body and head appendages not the same color
         (2) Three pairs of branching gills
            (3) Setae on first 40 segments only..... Amphitrite Ornata
            (3) Setae on first 23 segments only..... A. brunnea
            (3) Setae extend to posterior end..... Terebella
         (2) Two pairs of branching gills
            (3) Setae on 4th to 20th segments..... Pista
            (3) Setae on 3rd segment to posterior end..... Thelepus
         (2) Two pairs of gills on a single base, look like one gill with four parts.....Terebellides
   a. Tentacular filaments on some to many segments of the body as well as in anterior region; prostomium bare; no branching gills on head..... CIRRATULIDAE
      (1) Four to six pairs of elongate dorsal cirri, beginning on second segment; body usually blackish anteriorly changing to brownish posteriorly; constructs calcareous tube masses..... Dodecaceria
      (1) Many long dorsal cirri; color yellowish; in sand, not forming calcareous tubes..... Cirratulus
1. Otherwise; prostomium generally not concealed..... 2
2. (1) Head naked or with minute tentacles..... 3
2. (1) Head with readily visible appendages..... 6
3. (2) P rostomium naked..... 4
3. (2) Prostomium with minute tentacles
   a. Prostomium blunt, with 4 very small tentacles; body flattened; no conspicuous proboscia..... Nephtyidae
      (1) Sprinkling of gray pigment dorsally at anterior end; successive parapodia sharply set apart..... Nephtys incisa
      (1) Dorsum unpigmented; successive parapodia with apposed setae..... N. bucera
   a. Sharply pointed prostomium, apparently without appendages; body cylindrical; contractile gills on parapodia..GLYCERIDAE
      (1) Each parapodium with one dorsal and one ventral curved unbranched gill.. Glycera dibranchiata

(1) One dorsal branched gill between parapodia..... G. americana

4. (3) Mid-body segments very long (at least twice as long as broad); bamboo-like nodes; ends truncated..... MALDANIDAE

a. Anus dorsal to flattened, smooth, caudal plate; worms dark brown; live in mud tubes; length up to a 30 cm. or more..... Maldane

a. Anus enclosed in collar with 20-25 cirri; worm has 22 segments, 18 setigerous; cream color with red nodes; live in sand tubes; may reach 10 cm. ..... Clymenella

a. Similar to Clymenella, but 42 segments, and body circled by 3 or 4 chocolate-colored bands in segments 4-9..... Praxillella

a. There is a green maldanid, somewhat larger than Clymenella, and living in mud, which is otherwise practically indistinguishable from Clymenella, but probably a distinct species.

4. (3) Segments not elongate; branched gills in mid-body; posterior third without parapodia (ARENICOLIDAE)..... Arenicola

4. (3) Segments not elongate; gills unbranched or absent; parapodia rudimentary or well developed..... 5

5. (4) No gills; parapodia inconspicuous; marked iridescence; smooth elongate, cylindrical body; conical prostomium.. LUMBRINERIDAE

a. No eyes on prostomium..... Lumbrinoris

(1) Red; length up to 1 meter, diameter up to 1 mm; parapodia very small anteriorly, enlarging posteriorly..... L. tenuis

(1) Color a beautiful coppery iridescence, diameter up to 6 mm..... L. hobes

a. 4 minute eyes in transverse row on prostomium ..... Arabella

5. (4) Dorsum covered largely with fuzz of simple pointed gills; anterior region of body often flattened dorso-ventrally..... ..... ORBINIIDAE

a. Red; anterior portion flattened; gills begin on 6th to 9th segment..... Orbinia ornata

a. Yellowish; anterior portion flattened; gills begin on 26th segment; may attain length of 30 cm..... Scoloplos robustus

a. Yellowish; anterior portion cylindrical; gills begin on segment 16; up to 10 cm. in length..... Scoloplos fragilis

5. (4) Gills, if present, inconspicuous and restricted to posterior; elongate slender worms; bright red; anterior part cylindrical and thicker, posterior region squarish in cross-section; bulbous, eversible proboscis (CAPITELLIDAE)..... Notomastus

a. Very long and thread-like (diameter less than 1 mm.) ..... N. filiformis

a. Diameter up to 2-5 mm. simple short finger-like gills on all four "corners" of each segment in posterior region of body..... N. luridus

5. (4) With inconspicuous lateral gills on parapodia, not restricted to posterior region; short, stubby body (one inch in length or less); middle portion often inflated (OPHELIIDAE)..... ..... Travisia

6. (2) Body divided into several very different regions; soft; white (CHAETOPTERIDAE)..... Chaetopterus

6. (2) No gills. (Sometimes with broad leaf-like or long, dark, spine-like dorsal cirri)..... 7

6. (2) With many segmental gills, near parapodia (not necessarily on all segments)..... 8

7. (6) With flat leaf-like, sometimes overlapping, dorsal cirri partly overlying dorsale surface; parapodia uniramous; body slender and flattened; head appendages odd or even in number..... ..... PHYLLODOCIDAE

a. 4 small prostomial tentacles; 4 peristomial cirri ..... Eteone

a. 4 small prostomial tentacles; 8 peristomial cirri .....Phyllodoce or Anaitides*

a. 5 small prostomial tentacles; 8 peristomial cirri .....Eulalia or Eumida*

* There are numerous species of these, not well known, which cannot be distinguished critically except by looking at the surface of the proboscis (this in Eumida is smooth; in Eulalia papillate; in Phyllodoce with diffusely distributed papillae; in Anaitides with papillae in longitudinal rows). See checklist modifications at end of key for names of our common forms.

7. (6) Dorsal cirri short and simple; parapodia biramous; body somewhat thicker; head appendages even-numbered.....NEREIDAE

a. Dorsal division of notopodium below cirrus leaf-like; jaws black.....Noanthos virens

a. Dorsal division of notopodium below cirrus leaf-like; jaws amber colored..... Neanthes succinea

a. Dorsal division of notopodium below cirrus connical; jaws black .....Nereis pelagica

7. (6) Dorsal cirri long, dark, stiff; parapodia uniramous; body short, br ad, dark, head appendages odd-numbered (HESIONODAE) .....Podarko

8. (6) Prostomium with one pair of long tentacle-like processes, usually curling over back like ram's horns; no other conspicuour head appendages; often in tubes..... SPIONIDAE

a. 5th segment elongate with row of deeply-set, short, heavy setae; other segments with slender setae on parapodia..... Polydora

a. 5th segment not different from others; gills on all segments, commence on segments 4 or 5..... Spio

a. 5th segment not different from others; gills commence on segment 2; body usually olive green, with red gills folding over back..... Laonice

8. (6) Head with several pairs of appendages, including a median unpaired one..... 9

9. (8) Branched segmental gills in anterior region; seven prostomial tentacles the modian five of which are large and have annulated bases; large worms (more than 5 mm. wide)..ONUPHIDAE ..... Diopatra

9. (8) Small, simple segmental gills; five prostomial tentacles with no distinct bases; large (more than 5 MM wide).. EUNICIDAE .....Marphysa

9. (8) Long, simple gills (cirri); prostomium with 3 tentacles, 2 palps, 4 eyes; peristomium with 4 cirri; small worms (less than 1 mm wide), usually brightly colored.....SYLLIDAE
a. Tentacles and cirri segmented (beaded).....Syllis
a. Tentacles and cirri not segmented.....Autolytus

VARIATIONS IN POLYCHAETE HEADS

A. Prostomium - Typically conical but exceptions, for example:
Glycera - elongate and annulate
Nephtys - approximately square

1. Palps
Large - Nereids, Aphroditids (scale-worms), Eunico, Syllis.
Much branched to form long, mobile feathery filaments - Hydroides, Pseudopotamila, Parasabella
Reduced or wanting in many forms.

2. Tentacles
Absent - Scoloplos, Orbinia, Arabella, Lumbrineris, and majority of forms outside of the nereid-like groups.
one - Stenelais, Lepidometria.
Two - Nereids
Three - Lepidonotus, Harmothoe, Autolytus, Syllis
Four - Nephtys, Glycera, Phyllodoce
Five - Diopatra, Marphysa, Eulalia, Leodice (Eunice)
Eight - Flabelligera
Numerous - Amphitrite, Terebella, Polycirrus, and most other Terebellids.

3. Eyes
Absent - Orbinia, Scoloplos, Amphitrite, Lumbrineris, Pista, Nephtys, Travisia, Dodecaceria
Two - Chaetopterus, Eulalia, Phyllodece, Marphysa
Four - (2 pairs) Scale-Worms, Syllis, Autolytus, Podarke, Nereis, Spio, Polydora
Four (in a transverse row) - Arabella
Numerous - Tholepus, Terebella
Numerous, on branched palps - Parasabella, Pseudopotamilla
Two to Five - Arenicola

B. Peristomium
Usually asetigerous but setae in Nephtys, Sabellari
Peristomial cirri rare outside of Nereid-like groups but found in Spio, Polydora, Chaetopterus

C. "Head" includes several segments - Flabelligera due to forward Growth of long, slender setae.

VARIATIONS IN PARAPODIA

A. Notopodium lacking - Phyllodoce
B. Notopodium reduced and neuropodial setae enlarged and set in a transverse row in body wall - Arenicola, Clymenella, Terebellids
C. Modifications of parapodial cirri
1. Dorsal Cirrus
Absent - Nephtys
Degenerate - Glycera
As gills
Arborescent - Diopatra (anterior part), Arenicola, Terebellids

Short, Simple - Scoloplos, Orbinia, Spio, Polydora, Sabellaria

Long, Slender - Cirratuls

As elytra - some segments of Aphroditids (scale-worms)

2. Both (dorsal and ventral) foliaceous - Eulalia, Phyllodoce

VARIATIONS IN WORM TUBES

A. Calcareous tubes - Hydroides, Spirorbis
B. Membraneous tubes, sometimes with external material - Diopatra, Chaetopterous, Nereis pelagica
C. Sand, agglutinated with adhesive material - Clymella, Cistenides, Sabellaria, Parasabella, Polydora, Pista
D. Mud, agglutinated with adhesive material - Maldane, Polydora
E. Many burrowing forms are occasionally found with thin fragile sand or mud tubes or envelopes (e.g. Glycera, Amphitrite, Orbinia) and some worms will build tubes with a typical material if in "abnormal" habitat.

NOMENCLATORIAL CHANGES RECOMMENDED BY DR. OLGA HARTMAN

Check list and / or P ratt Name	Revised Name
Arabella opalina	Arabella iricolor
Aricia ornata	Orbinia Ornata
Chaetopterus pergamentaceus	Chaetopterus variopedatus
Eulalia pistacia	Eumida sanguinoa
Hydroides hexagonus	Hydroides dianthus
Lepraea rubra	Terebella rubra
Lumbrinereis	Lumbrineris
Marphysa leidyi	Marphysa sanguinea
Nephthys	Nephtys
Nereis Virens	Neanthes virens
Nereis limbata	Neanthes succinea
Nicolea simplex	Nicolea zostericola
Polycirrus eximeus	Polycirrus eximius
Trophonia affinis	Flagelligera affinis

CLASSIFICATION OF ANNELIDA

Phylum: Annelida

Class 1: Polychaeta

Order 1: Polychaeta errantia

Neanthes virens (Family: Nereidae)

Order 2: Polychaeta sedentaria

Arenicola cristata (Family: Arenicolidae)

Amphitrite ornata (Family: Terebellidae)

Class 2: Archiannelida

Class 3: Oligochaeta

Lumbricus terrestris (Family: Lumbricidae)

Class 4: Hirudinea

Order 1: Rhynchobdellida

Order 2: Gnathobdellida

Hirudo medicinalis (Family: Hirudinidae)

Class 5: Gephyrea

Order 1: Echiurida

Order 2: Sipunculoidea

Phascolosoma gouldii

Order 3: Priapulida

NEANTHES VIRENS

Neanthes virens, commonly called the clam worm, is one of the largest and most common of the marine annelids of the North Atlantic coast of North America. Large individuals range up to 15 to 18 inches in length and to nearly half an inch in width. Their coloration ranges from steel-blue, through greenish with tinges of orange and red, especially on the parapodia. In general the tendency towards development of an orange or reddish coloration is greatest in females during the breeding season.

Neanthes normally lives in tubes which it forms in the sand or mud. These excavations are made by activity of the protrusible pharynx and are lined with mucus. The worms remain within the tubes most of the time, occasionally protruding the anterior end of their bodies in search of food. The worms appear to be omnivorous.

Only during the breeding period do these worms commonly leave their burrows and become free-swimming. This phase of their life cycle is often associated with the differentiation of two distinctly different regions of the body, an anterior atoke and a posterior epitoke. The latter portion bears the gametes, and both its parapodia and chaetae are larger and more leaf-like, an adaptation to the swimming habit. This special reproductive individual is called a heteronereid.

Study, if live specimens are available, the patterns and rhythms of locomotory activity. Locomotory patterns appear to be of three types. 1) Slow ambulation. In this, only parapodial action is observed. Each parapodium shows during slow (and also rapid) ambulation a characteristic type of circular movement involving an effective and a recovery stroke. In the effective phase the parapodium is protruded, and moved posteriorly and ventrally in contact with the substrate. In the recovery phase it is

retracted, lifted upwards, and moved anteriorly. Observe the character of each phase. What is the order of sequence of parapodial activity as a quiescent worm becomes active? What direction do the rhythmical activity waves pursue over the body? Is activity on the two sides of the body in the same or alternate phase? 2) Rapid ambulation. This involves lateral undulations of the body of small amplitude in addition to the parapodial activity discussed under slow ambulation. 3) Swimming. This involves lateral undulatory waves of large amplitude in addition to the usual parapodial activity. Determine for the lateral undulations of the last two types of locomotion the direction taken by the wave of initiation of the pattern and also determine the direction of movement of the activity wave itself.

Place a glass tube of a bore slightly greater than the diameter of the worm in the container with the worm. Observe the readiness with which the worm enters the tube and its reluctance to leave it. Study the intermittent undulatory activity which pumps water effectively through the tube. By means of a little added carmine suspension determine the direction the water takes through the tube. In what ways does this rhythmical activity pattern differ from the locomotory ones?

With forceps hold a small piece of mollusc flesh or other natural food at the tube entrance. Observe the manner of feeding in Neanthes.

External anatomy. For this study a specimen freshly killed by immersion in 7% alcohol is best, though a preserved specimen is quite satisfactory.

The body is composed of an anterior prostomium followed by a variable number of more or less similar segments ranging sometimes to as many as 200 or more. Segments are increased in number during the growth of the worm

by their production just anterior to the caudal one. With the exception of the first segment or peristomium, and the caudal one, these segments possess lateral appendages, the parapodia.

The prostomium bears a pair of small tentacles at its anterior tip. Laterally it bears a pair of stout, fleshy, and somewhat contractile palps. Dorsally are located two pairs of pigmented eyes.

The peristomium bears four pairs of tentacular cirri, two dorsal and two ventral. The two longer dorsal cirri can extend backwards to the fifth to ninth segment.

The remaining segments of the body bear parapodia. These increase in size over the first few segments, are large in the mid-region of the body, and decrease in size towards the posterior end. Obtain from the instructor a segment cut from the mid-region of a preserved specimen and study it carefully with the aid of your microscope. The parapodia are biramous, possessing a dorsal division or notopodium and a ventral one or neuropodium. Each division contains a large black, tapering, supporting rod or chaeta largely imbedded within it. This chaeta is called an aciculum. At its proximal end each aciculum has attached musculature whose activity results in protrusion and retraction of the parapodium. Both rami of a parapodium typically possess, in addition, a bundle of chaetae projecting from chaetigerous sacs also possessing associated muscle fibers capable of bringing about movements of these chaetae. The chaetae are jointed structures, having a blade and a shaft. Two types of chaetae are present. What are their differences? The first and second pairs of parapodia lack the notopodial chaetae.

Each notopodium has two flattened, richly vascularized lobes, the superior and inferior ligulae. Dorsally, at the base of the superior one is the dorsal cirrus. The neuropodium has a single ligula, narrower than

the dorsal ones, and a ventral cirrus. Other lobes, at the bases of the chaetal sacs, are called the chaetigerous lobes.

Nephridiopores may be seen upon the ventral surface of each segment except the first and the last, close to the base of the ventral cirrus.

The last segment, bearing the terminal anus, has no parapodia but has a single pair of elongated ventral cirri, the anal cirri.

Coelomic cavity and its fluid. Make a short, longitudinal incision immediately to the right or left of the dorsal mid-line near the mid-region of the body. Place a small drop of the coelomic fluid on a slide and examine it with the compound microscope. What kinds of cells do you find? The fluid contains ameboid corpuscles and, especially during the breeding season, numerous reproductive cells in various stages of their development. If reproductive cells are present, what is the sex of your specimen?

Now continue the dorsal incision anteriorly to the end of the worm, pinning out the body wall as you proceed. The specimen should be submerged during the dissection and study.

The coelom in Neanthes is an extensive perivisceral cavity. It is divided incompletely into compartments by segmental septa which pass from the body wall to the visceral organs. Perforations in the septa allow passage of the fluid from one region to another. A visceral peritoneum encloses the digestive tract which is suspended within the coelom. Segmentally arranged through much of the length of the worm there are areas of ciliated peritoneum covering the dorsal longitudinal muscle tracts. These patches are known as the dorsal ciliary organs which are believed to be vestiges of the primitive coelomoducts. Search for these if your specimen has been freshly killed.

Body wall and musculature: Study the structure of the body wall. The body is covered by a thin cuticle whose irridescence is due to the possession of numerous striations. There are also numerous pores through which hypodermal gland cells discharge their products to the exterior. The hypodermis is a single-celled layer, thicker on the ventral surface where its contained glands are larger and more numerous. A portion of the dorsal hypodermis is well vascularized by a plexus of blood vessels, thereby appearing to assist the ligulae in respiratory gaseous exchange. Internal to the hypodermis is a thin layer of circular muscle. Internal to the latter are four longitudinal muscle tracts, two dorso-lateral and two ventro-lateral. Oblique muscle fibers originate in the median ventral line and pass dorso-laterally into the lateral body wall. A parietal peritoneum covers the musculature on its inner surface, lining the coelom.

Alimentary canal: The mouth is located ventral to the prostomium and is bordered by the peristomium which forms the buccal ring. The mouth opens into a buccal cavity. The pharynx lies just posterior to the latter and has a highly muscular wall. The posterior portion of the pharynx, the dental region, possesses special muscle tracts in which are imbedded laterally the hollow bases of a pair of large, powerful jaws. All of the foregoing regions of the gut, constituting the foregut, are lined with a cuticle continuous with that of the exterior. Numerous regularly arranged denticles are also on this cuticle of the dental region. The fore-gut may be completely everted as a proboscis by means of protractor muscle bands connecting the pharynx with the peristomial wall and by elevated internal hydrostatic pressure. As the dental region in everted the two large jaws open widely. As the proboscis is retracted by retractor muscle bands which extend to the body wall from the posterior dental region, the jaws are brought together and cross one another, effectively

holding anything which comes between them. Food organisms can be killed and torn apart by their action. The pharynx opens posteriorly into a narrower oesophagus. At the anterior end of the oesophagus two large oesophageal caeca open into it. After passing through a few segments the oesophagus enters the intestine which extends the remainder of the length of the body with little change. A sphincter muscle lies at the junction of the oesophagus and intestine. The intestine has both longitudinal and circular muscle fibers, the former lying nearer the peritoneum, the latter nearer the gastric mucosa. The mucosa is a columnar epithelium. The intestine is segmentally constricted by septa. The intestine in the last body segment is called the rectum and it, like the pharynx, has a cuticular lining.

Circulatory system: The circulatory system of Neanthes comprises a highly differentiated system of vessels containing blood which is red due to hemoglobin in solution in its plasma. The blood is propelled through the system by peristaltic waves passing over the vessels. The principal vessels of the system are longitudinal and in the median line. One is dorsal and the other ventral to the intestine. The blood is propelled anteriorly within the dorsal vessel and posteriorly within the ventral one. The peristaltic waves of the dorsal vessel are often clearly evident in an intact living specimen. As the blood flows anteriorly in this vessel it receives in every segment except the first four or five a pair of lateral vessels. These lateral vessels bring blood dorsally from the ventral vessel. In addition each lateral vessel receives, shortly before entering the dorsal vessel, a vessel bearing aerated blood from the notopodium and the vascularized dorsal body wall. In each segment also, the dorsal vessel has vessels connected with an anastomosing network of

vessels upon the intestinal wall. The dorsal vessel at the anterior end of the worm bifurcates to produce vessels passing latero-ventrally to a vascular reticulum upon the oesophageal wall and then continuing as lateral vessels to join the ventral vessel in the fifth segment.

The ventral vessel, like the dorsal, possesses connections in each segment with the intestinal network of vessels. From each circum-intestinal segmental vessel shortly after it leaves the ventral vessel a branch passes into the neuropodium to provide blood to the respiratory capillary system of this appendage. At the posterior extremity of the body the ventral vessel is continuous with the dorsal one through a simple circum-rectal vascular ring.

Nephridia: In every segment of the body except the first and the last is a pair of nephridia. Each nephridium consists of a somewhat convoluted, ciliated tube imbedded in a syncytial mass of protoplasm. The internal opening is in a ciliated funnel, the nephrostome, which is located in the segment just anterior to that containing the bulk of the tubule. The external opening, or nephridiopore, is located on the ventral surface of each parapodium, close to the point of origin of the ventral cirrus.

Reproductive system: . Neanthes is dioecious. The gonads are simply seasonal outgrowths of the ventral coelomic lining of many segments. They become enlarged during reproduction. At other times they are not evident. The gonads possess no ducts but simply discharge their products into the coelom which may at times become quite filled with them. While floating in the coelomic fluid the reproductive cells undergo development. The ripe gametes are discharged to the exterior by rupture of the body wall and fertilization occurs externally in the sea-water. There is often a modification to the heteronereid form during the breeding season, as described earlier.

Nervous system and sense organs: Carefully remove the gut to expose the ventral nerve cord.

The ventral nerve cord lies along the median floor of the body cavity immediately ventral to the ventral blood vessel. From the first three ventral ganglia two pairs of lateral nerves arise, an anterior pair which branch to supply the anterior septum of the segment and the next anterior segment, and a posterior pair passing within the same segment to the parapodium and to the skin. In the remaining segments three pairs of nerves arise in each segment, the anteriormost innervating the segment next anterior, and the remaining two are intrasegmental.

Anterior to the ganglia of the first segment two circumpharyngeal connectives pass upwards to the brain located dorsally in the prostomium. The brain sends nerves anteriorly to the tentacles and palps and four short optic peduncles extend dorsally to the four eyes.

The circumpharyngeal connectives near their point of junction with the brain bear small ganglia from which arise nerves supplying the ventral pairs of peristomial cirri. Also from these small ganglia arise nerves passing to a system of ganglia over the dorsal surface of the proboscis. A pair of nerves arising from the connectives nearer their ventral ends supply a similar but ventral ganglionated plexus of the proboscis. Arising from the first ventral ganglion and parallelling the connectives through much of their course is a pair of accessory connectives. Near the brain these give rise to nerves supplying the dorsal pairs of peristomial cirri.

The prostomium bears upon its dorsal surface over the brain two pairs of conspicuous eyes and ciliate nuchal pits. The eyes are pigmented vesicles containing a transparent refractive body. The wall of the vesicles comprises the light receptive cells which are continuous with the optic nerve fibers.

The nuchal pits are considered to be chemo-receptive in function. It is highly probable that the tentacles, palps, and cirri also possess important sensory functions.

ARENICOLA CRISTATA

The lug-worm, Arenicola cristata is found along the Atlantic coast of the United States from Florida to Cape Cod. It is the most common species of the genus in regions south of Cape Cod. North of the Cape a smaller species, A. marina, replaces it. The latter rarely occurs south of the Cape and typically in deeper water.

Arenicola cristata dwells in regions of muddy sand near or slightly below the low-tide mark. It occupies tubes which it forms by burrowing and then secreting a lining of mucus. The tube is usually U-shaped, one or two feet deep, with one opening to the surface possessing castings about it, the other having a funnel-like form. The worm pumps aerated water through its tube by peristaltic waves which pass rhythmically anteriorly over the body. Its delicate gills are partially protected by the notopodial chaetae which project over them.

Arenicola both burrows and feeds by forcefully everting its proboscis into the sand and then retracting it with its load of sand. The burrow is enlarged by firm muscular peristaltic waves which pass anteriorly. Organic material in the ingested sand is digested and absorbed as the material passes through the gut.

During the breeding season large numbers of eggs in elongated gelatinous masses are found at the mouth of the burrow.

Arenicola is best studied while freshly killed by immersion for an hour or two in 7% ethyl alcohol in sea-water. Preserved specimens are, however, quite satisfactory for study of most aspects of their morphology.

External anatomy: Study the general form and organization of the worm. The body is seen to comprise four general regions. 1) An anterior head region comprising the prostomium, peristomium and first achaetous segment. Posterior

to this region is a region 2) Possessing six segments bearing parapodia. 3) A third region possesses eleven segments bearing both parapodia and gills. Finally, the most posterior region 4), the tail, has a variable number of segments and bears neither parapodia nor gills.

The prostomium is small and is located anteriorly in the mid-dorsal line. It is trilobate and is bordered laterally and posteriorly by a deep groove, the nuchal groove. The prostomium bears no appendages. The peristomium possesses a single annulus which may be sub-divided in two. The mouth is a transverse slit ventral to the prostomium and largely bounded by the antero-ventral edge of the peristomium. It is bordered by papillae. The anterior region of the gut may be everted as a proboscis.

Following upon the peristomium are two annuli the anterior one of which may be sub-divided into two. These represent a first body segment which has lost its chaetae early in development.

The first three chaetigerous segments possess respectively 2, 3, and 4 annuli; the remaining segments characteristically have 5 annuli per segment with parapodia located, when present, in the next to the last. Therefore, in general, the parapodia are located quite far posteriorly in their segments.

The annulation of the tail region is quite variable. The number of annuli per segment increases from the anterior region backwards. Near the posterior end of each segment in the tail is seen a larger and more heavily pigmented annulus. This annulus appears to correspond to the chaetigerous annuli of the more anterior body regions.

The anus is terminal in the last tail segment.

The chaetigerous body segments each possess a pair of reduced biramous parapodia. A dorso-laterally located notopodium is conical in form and a

small bundle of needle-like chaetae project from a setal sac opening at its tip. The setal sac is controlled by retractor and protractor muscles to be studied later. The notopodial chaetae are very sharply pointed; their distal portions bear a serrated edge and often also are blade-like.

The neuropodia are ventro-laterally located and comprise dorso-ventrally elongated muscular ridges. Each neuropodium has a longitudinal slit-like opening into a setal sac. The sac contains large hook-like chaetae, or crochets. These chaetae are formed at the ventral end of the sac, older and more fully formed ones being found in the dorsal portion.

The parapodia of Arenicola have neither acicula nor cirri.

Nephridiopores may be readily seen in the 5th to 10th chaetigerous segments, inclusive. These comprise oval slits lying slightly dorsal and posterior to the dorsal ends of the neuropodia.

Eleven pairs of gills are intimately associated with the notopodia in chaetigerous segments 7 to 17 inclusive. These are red in color due to their contained blood. Each gill is an extensively branched outgrowth of the body wall and contains an extension of the coelomic cavity. It it thin-walled; it consists of thin epidermal and peritoneal layers between which lie fine muscle strands and fine interconnecting branches of the afferent and efferent branchial vessels. In the living worm the gills may be seen to undergo a rhythmic contraction.

In the mid-ventral line, a shallow groove parallels the ventral nerve cord; in the achaetous body segment this groove joins with others that pass dorso-anteriorly over the peristomium parallelling the circumpharyngeal connectives.

Coelomic cavity and its fluid: Make a small longitudinal incision in the dorsal line near the mid-region of the body. Collect some of the coelomic fluid, place it upon a microscope slide, and examine it with a compound micro-

scope. Determine the sex of the specimen from the character of the reproductive cells which are almost always present in this fluid. In females oocytes are observed in varying stages of growth. Their diameters range from about 20 μ to over 150 μ. The coelomic fluid of males contains clusters of spermatogonia each possessing anywhere from 8 to myriads of cells. Ripe flagellated sperms occur in very characteristic discoidal masses. The abundance of reproductive cells in the coelomic fluid varies seasonally, with huge concentrations present during the breeding season.

Coelomic corpuscles, of two types, are also present. These are large fusiform cells from 40 to 50 μ in length and smaller ameboid. The latter often contain yellowish or brownish granules. These ameboid cells are phagocytic.

Now carefully continue the mid-dorsal incision anteriorly to within 3 or 4 mm of the anterior end and posteriorly a short distance into the tail-region. In the last the gut will be entered unless extreme precaution is exercised.

The coelom is very extensive and extends throughout the length of the worm. Anteriorly it is incompletely divided into compartments by three fenestrated septa known as the diaphragms. The first of these marks the anterior boundary of the first chaetigerous segment, the second, the posterior boundary of the second, and the third, of the third chaetigerous segment. All three diaphragms contain muscle fibers. The fenestrations are of such size as to permit free passage of the coelomic fluid and its smallest cells. The first diaphragm bears two finger-like pouches projecting backwards but opening into the anteriormost coelomic space. The pouches are contractile and richly vascularized. Short dorsal and ventral mesenteries containing the dorsal and ventral blood vessels are present in the region of the first and second diaphragms.

The coelom is spacious and undivided by septa between the third diaphragm and the tail. Vestiges of septa appear to be present as strands of connective tissue following the branchial and nephridial vessels, especially the posteriormost ones.

The tail-region possesses transverse septa which are incomplete ventrally in the region dorsal and dorso-lateral to the nerve cord. Dorsal and ventral mesenteries bearing the dorsal and ventral vessels connect the gut to the dorsal and ventral body wall in the mid-line.

Body-wall and musculature: Study these in your specimen. The body is covered by a distinct cuticle secreted by the underlying hypodermis. The latter possesses numerous differentiated areas in which the cells are more deeply columnar and include abundant mucus and pigment cells. These areas are responsible for the macroscopically observed pattern of elevated pigmented patches in the skin of the worm. Underlying the hypodermis is a thin layer of connective tissue followed by a layer of circular muscle. Internal to the last is a layer of longitudinal muscle bands. These are separated from the coelomic space by the coelomic peritoneum.

Between the third diaphragm and the tail another group of muscles is very conspicuous. These muscles, the oblique, arise at the sides of the ventral nerve cord and pass dorso-laterally into the body wall at the level of the notopodia.

Associated with each notopodial sac is its musculature. This includes a number of protractor fibers and a single retractor one.

Digestive system: This tract extends from mouth to anus supported by the septa and mesenteries in the anterior and posterior regions of the body, but lying moderately freely through most of the mid-region. In the latter region this freedom is increased by the great lengths of the branchial and nephridial vessels. At the anterior end of the stomach, however, are found a pair of fine

connective tissue strands following the nephridial vessel and firmly attaching the stomach to the body wall.

The mouth opens into a buccal cavity whose surface bears conspicuous sensory papillae. The buccal cavity is followed by the pharynx. The pharynx is encased in a sheath of musculature, the buccal sheath. From this fibers extend posteriorly, penetrate the first diaphragm, and become attached to the body wall between the first and second diaphragms. These fibers serve as retractor muscles for the buccal mass and pharynx which, when everted constitute the proboscis. Protrusion of the proboscis is probably in part effected by hydrostatic pressure increase in the body fluids and in part by contraction of longitudinal muscle fibers of the body wall. The buccal cavity and pharynx possess a cuticular lining.

Posterior to the pharynx is a long tubular oesophagus apparently capable of considerable extension required by proboscis eversion. The oesophagus passes through the three diaphragms. The posterior portion of the oesophagus has a glandular and ciliated columnar epithelial lining. Opening into the posterior region of the oesophagus is a pair of oesophageal glands. These are hollow, dorso-lateral diverticula lined with a secretory epithelium which is plicated, thus providing extensive internal surface. The oesophagus possesses layers of both longitudinal and circular muscle fibers.

The stomach follows the oesophagus. It is an elongated portion of the gut whose outer surface is extensively provided with yellowish chlorogogue tissue separated into patches by a regular network of blood-sinuses. Musculature is poorly developed in the stomach. The mucosa is richly glandular particularly in its anterior region. A ciliated groove begins in the mid-region in the ventral mid-line and continues posteriorly to the anus. Lateral ciliated-groove tributaries join with the ventral groove. The cilia maintain a current in the direction of the anus.

The intestine continues from the stomach to the anus with little or no differentiation other than possessing the ventral ciliated groove. Glandular cells are not abundant in its lining.

In view of the weak musculature of the gut proper, it appears reasonable to presume that activity of the somatic musculature is largely responsible for the passage of injested material through the gut.

Circulatory system: By carefully stretching the digestive tract to right or left in your dissected specimen nearly all the major vessels of the circulatory system may be traced and their interconnections determined. The vessels are red due to the blood plasma being richly provided with hemoglobin. The blood contains a low concentration of colorless nucleated corpuscles 5 to 10 μ in diameter.

The dorsal blood vessel, carrying blood anteriorly, extends from the posterior to the anterior tip of the worm. Peristaltic waves rhythmically pass anteriorly over this vessel. In the tail it receives segmental vessels originating in the ventral vessel. Passing over the intestine anterior to the tail it receives branchial efferents from the last 7 pairs of gills and several intestinal vessels per segment. Passing over the stomach the vessel joins with numerous lateral vessels from the gastric plexus. Over the oesophagus it gives rise to lateral vessels to the oesophageal glands, to the first three nephridia and body wall to the second diaphragm and adjacent notopodial sac region and to the body wall in the region of the first setal sac. The dorsal vessel finally breaks up into a capillary network in the buccal region.

The ventral blood vessel originates in the anterior tip of the worm and conveys blood posteriorly to the posterior extremity. It collects blood from the buccal region and as it passes posteriroly it receives branches from the first diaphragm and body wall, the oesophagus, the second diaphragm and the lateral neural vessels. The ventral vessel supplies the afferent branchial and

nephridial vessels. Branches of the last also supply the body wall. At the level of the hearts the vessel receives vessels from the ventricles. In the tail the ventral vessel gives rise to the segmental vessels which connect directly with the dorsal vessel in this region.

The gastric plexus is differentiated into certain longitudinal tracts. A gastric lateral vessel is evident on the anterior portion of the stomach. This passes anteriorly opening into a thin-walled distension, the heart-auricle and then continues directly anteriorly as the lateral oesophageal vessel supplying the oesophageal glands and the oesophagus, finally terminating immediately posterior to the first diaphragm.

Sub-intestinal, longitudinal vessels are found in the plexus. These, at the level of the stomach, receive the anterior four branchial and nephridial efferents.

The ventricles of the heart are highly muscular bodies capable of considerable distension. They propel blood from the auricles into the ventral vessel.

Some longitudinal vessels are found in the body wall. A dorsal lateral vessel lies just dorsal to the row of notopodial sacs. A nephridial lateral vessel lies just ventral to the level of the nephridiopores. Lateral neural vessels are located dorsal to each edge of the ventral nerve cord. These peripheral longitudinal vessels are interconnected by commissural vessels in those segmental annuli bearing the chaetae.

Nephridia and gonads: There are 6 pairs of large nephridia located in chaetigerous segments 5 to 10 inclusive. Study these carefully in situ in your specimen both before and after removing some of the oblique musculature which partially obscures them. Observe carefully their blood supply, supporting attachments and relation to the gonads. Finally, remove one of the organs and study it with the aid of your compound microscope.

Each nephridium comprises three distinct regions: nephrostome, excretory tube, and bladder. The nephrostome is located anteriorly and has an opening directed antero-medially between dorsal and ventral lips. The nephrostome is richly vascularized over its whole surface, hence has a pinkish cast. The fimbriated dorsal lip of the nephrostome is especially well supplied. The lips and internal surface of the nephrostome possesses a ciliated epithelium which maintains a current from the coelom into the organ.

The blood vessels of the nephrostome unite to produce the gonidial vessel passing posteriorly from the posterior end of the dorsal lip. On this vessel immediately posterior to the nephrostome of each nephridium except the first is a minute gonad, usually only a fraction of a millimeter in length.

The nephrostome opens into the more posterior and laterally located excretory portion of the nephridium. The lining of this portion is a ciliated epithelium the cells of which are charged with yellowish and black granules.

The excretory portion opens into the bladder. This portion, unlike the preceeding portions, has muscle fibers in its walls. It is capable of considerable distension.

The nephridiopores are oval apertures located just dorsal and posterior to the neuropodia.

The nephridia are supported in the coelom by attachment of the nephrostome to both the ventral body wall and to the overlying oblique musculature.

The minute gonads slough off reproductive cells in a very early stage in their development. The development of the gametes occurs largely while the cells float freely in the coelomic fluid.

The oocytes at the time they break away from the ovaries are only 15 to 20 μ in diameter. At the completion of their development they are large

discoidal bodies approximately 90 to 150 μ.

The spermatogonia first freed into the coelomic cavity comprise groups of 8 or more cells all attached to a protoplasmic mass, the blastophore. The spermatogonia multipy and undergo transformation into fully differentiated sperm cells while still held together in these groups. The result is the production of discoidal masses of flagellated sperm.

The gametes at the height of the breeding season may largely fill the coelom. They escape to the exterior by way of the nephridia, whose bladders may at times be observed to be enormously distended by these products.

Nervous system: Remove the digestive system and expose the whole central nervous system. Study its organization.

The ventral nerve cord lies longitudinally in the ventral median line. It occupies a position just interior to the circular muscle layer. The cord is dorso-ventrally flattened. It is non-ganglionated and appears superficially to be single. An examination of sections shows, however, that as in other polychaetes it contains two longitudinal tracts. Ganglion cells are moderately uniformly distributed along the cord on its ventral and ventro-lateral surfaces. The cord sends off small lateral nerves at the level of each inter-annular groove. At the level of each parapodium a pair of larger nerves leave the cord.

Near the anterior end of the worm the cord bifurcates giving rise to the circumpharyngeal connectives. Along their course these connectives give off numerous nerves to the stomatogastric system of the buccal and pharyngeal complexes. A nerve supplies the conspicuous otocysts. These latter organs are easily found and lie near the connective at the place where it crosses the inter-annular ring of the peristomium. Other nerves pass to the peristomial epidermis and its underlying musculature. Remove an otocyst and examine first it and then its inclusions with the microscope.

The connectives continue dorsally and enter the anterior lobes of the brain which are situated in the prostomium. Posterior lobes of the brain extend postero-ventrally to a position ventral to the nuchal groove. The brain is a small organ, usually less than a millimeter in diameter. Nerves from the brain pass anteriorly into the eversible region of the buccal mass.

Eyes are usually present, closely associated with the dorsal surface of the anterior brain lobes. These eyes are usually not visible externally due to their small size and the pigmentation of the overlying epidermis.

AMPHITRITE ORNATA

Amphitrite ornata is common along the eastern coast of the United States between North Carolina and Cape Cod. It dwells near the low-tide level in tubes buried in sand or mud. The tubes are the burrows of the worm which have been lined with mucus. Another common species of the genus, with somewhat overlapping distribution, is A. brunnea whose distribution is largely north of Cape Cod. South of the Cape it is usually found in deeper water than is A. ornata.

Very commonly and symbiotically sharing the tube of A. ornata is one of the scale worms of the family Aphroditidae, Lepidometria commensalis.

External anatomy: The body of Amphitrite, usually about 20-30 cm long, can be seen to comprise three distinct regions, 1) a head 2) a thorax and 3) an abdomen. The head is formed by a fusion of the prostomium and peristomium. The thorax comprises that thicker portion of the body lying posterior to the head, all of whose segments except those close to the head bear notopodia containing bristles. The abdomen is a long, many-segmented region without bristles which in specimens out of their tubes is usually strongly coiled. Determine the number of segments in the thorax and abdomen.

The head bears numerous long, greatly extensible tentacles on its antero-lateral surfaces. These are outgrowths from regions of two laterial lobes of the prostomium and are believed homologous with the palps of more generalized polychaetes. The mouth is located on the anterior surface of the head among st the tentacles and possesses thick dorsal and ventral lips of peristomial origin. The tentacles are feeding organs. They are hollow containing an extension of the coelom filled with the coelomic fluid and coelomic corpuscles. They are poorly vascularized and hence yellowish-buff in color. The tentacles are slightly flattened orally-adorally and carry a

ciliated groove on their oral surface. Mucus cells are abundant at the groove. The tentacles have a well-developed musculature; a circular muscle layer underlies the epidermal layer. Internal to the circular is a layer of longitudinal muscle. Three longitudinal nerve tracts lie in the adoral wall. The tentacles are capable of very considerable extension and rapid contraction, and show writhing activity for long periods following isolation from the worm.

The first three segments of the thorax bear dorso-laterally a pair of gills. Each gill is attached to the body by a single large stalk, but branches repeatedly and profusely away from the body to produce foliaceous organs. The gills are believed homologous with dorsal cirri. The finer branches are usually much coiled. The gills are hollow outgrowths of the body containing, like the tentacles, extensions of the coelom, and are filled with coelomic corpuscles. The epithelial walls contain numerous mucus glands. Internal to the epidermis is a layer of longitudinal muscle fibers. On opposite sides of each gill filament are branches of the afferent and efferent blood vessels which unite at the gill tips. The gills normally exhibit a rhythmical contraction, with change of color from deep red to lighter red as blood is rhythmically forced out of the organs.

Note on which segments the bristled notopodia and the elongated uncini-bearing neuropodia commence and upon what segments they become discontinued. With fine forceps remove a few chaetae from organs in the mid-region of the thorax. The notopodial chaetae possess blade-like, serrated terminations. Observe carefully the form of the stout hook-like uncini of the neuropodia. These are oriented in the neuropodia in such ways as to facilitate the movement of the worm in its tube.

On the ventral surface of the first few thoracic segments are the ventral shield glands. These are supplied with mucus for the lining of the burrows by

the yellow ventral glands conspicuously seen projecting internally into the coelomic cavity in the worm. This will be conspicuously evident when the worm is opened later.

Does the abdomen possess any neuropodia? The anus is found terminally in the last abdominal segment as a dorso-ventrally elongated aperture.

Coelom and coelomic fluid: Make a longitudinal incision through the dorsal body wall immediately to the right of the mid-line. Place a small amount of the coelomic fluid upon a microscope slide and examine the preparation for the three contained types of coelomic corpuscles. The largest and most abundant type is the pinkish eleocyte. These cells are approximately spherical and charged with granules. A second type is a smaller amebocyte. The third type is a fusiform cell darkly granular except for clear ends. Are sex cells present in the coelomic fluid? If so, are they oocytes or developing sperm cells?

Now continue the dorsal incision anteriorly close to the anterior tip of the worm and posteriorly for some distance into the abdomen. Stretch out and pin down the body wall. Note in so doing that the gut is attached to the dorsal median body wall by thin suspensory muscles.

Note that transverse septa are almost completely absent from the anterior portion of the worm. Only at about the level of the fourth or fifth segment is there a thick diaphragm separating the coelom into an anterior and a posterior chamber. Find the muscular sacs projecting posteriorly from the diaphragm and opening into the anterior chamber. How many are there?

The posterior portion of the abdominal region is divided completely by numerous intersegmental septa.

Body wall and musculature: The body is covered with a thin cuticle secreted by an underlying hypodermis in which are numerous unicellular mucus glands. Immediately internal to the latter layer is the circular musculature.

Internal to the last is the longitudinal musculature which throughout most of the length of the body is clearly differentiated into four columns of fibers, two dorso-lateral and two ventro-lateral. Both sets of tracts terminate in the head at the level of the brain.

Oblique musculature, comprising fibers extending from the ventral midline to the lateral body wall just ventral to the dorsal longitudinal tracts, is found through most of the length of the worm dividing the coelom rather effectively into dorsal and ventral portions. Dorsal and ventral suspensory muscle fibers anchor the gut in the dorsal and ventral median lines, respectively.

The chaetal sacs of the notopodia are each provided with a number of protractor fibers and a single retractor.

Later, when the nervous system is studied, a median ventral longitudinal muscle will be observed lying immediately dorsal to the nerve cord and extending the full length of the body.

A somatic peritoneum covers all the foregoing musculature of the body wall.

The digestive system: The digestive tract is differentiated into six regions. The mouth opens into a dorso-ventrally compressed chamber, the pharynx. The line of division between this and the narrower tubular oesophagus is the diaphragm. Both these regions are ciliated internally and contain musculature. There are numerous mucus cells in their lining epithelium. Food coated with mucus is swept posteriorly by peristaltic and ciliary movement to the third region, the anterior stomach. This is a thinner-walled, less muscular region. Its internal layer of cells contain numerous secretory ones apparently sources of digestive enzymes. This region is covered externally with a yellowish chlorogogue tissue. The posterior stomach possesses a thick muscular layer and internally a thick secreted

membrane. This region appears to act as a powerful churn, and, with the cooperation of sand grains, as an effective gizzard. The posterior stomach opens into a coiled anterior intestine. The anterior intestine is covered with chlorogogue tissue. Both intestinal portions are relatively non-muscular, possess a ciliated ventral groove, and probably function primarily in absorption.

Circulatory system: The blood of Amphitrite is red; it contains hemoglobin.

A large amount of blood is found in an extensive gut sinus which occurs over the stomach and intestine between the gut epithelium and musculature. The blood moves slowly anteriorly in this sinus. Ventral to the gut in these regions is a pair of large sub-intestinal ventral vessels having numerous short vessels connecting it with the gut sinus. Blood flows anteriorly in this vessel as well. At the anterior end of the stomach the subintestinal vessels curve dorsally over the gut and join to form a large median dorsal vessel over the oesophagus. This short dorsal vessel has projecting into it a large mass of cells comprising the heart-body, of unknown function. Speculations have contributed a valve action and a hemoglobin destructive function to this body. Immediately anterior to the diaphragm the dorsal vessel divides into right and left basal branchial vessels each of which divides into three afferent branchial vessels supplying the three gills of that side of the body.

The three pairs of efferent branchial vessels unite to form a large median ventral vessel conducting blood posteriorly. In the stomach region the ventral vessel sends numerous pairs of segmental ventral ring vessels to a pair of longitudinal lateral vessels of this region. The latter have numerous dorsal ring vessels connecting them with the gut sinus. In the region of the intestine, to which the lateral vessels do not extend, blood is carried by segmental ring vessels directly to the gut sinus.

In the intestinal region the ventral vessel supplies, by segmental connectives, a small supra-neural vessel.

The oesophagus and pharynx is vascularized by a plexus and by vessels arising directly from the anterior end of the gut sinus.

Nephridial and reproductive systems: Carefully cut away the oblique muscles in the anterior thoracic region to expose the numerous pairs of nephridia. Do nephridia lie both anterior and posterior to the diaphragm? How many nephridia are there?

Each nephridium opens into the coelom by a nephrostome which is attached to the body-wall near the mid-lateral line. The nephrostome possesses ciliated and fimbriated dorsal and ventral lips. The nephrostome leads into an excretory sac which bends sharply back upon itself. The cells of the excretory sac include many granule-containing and many ciliated ones. Judging from the lack of vascularization of the nephridia, these organs must, in their functioning, deal exclusively with coelomic fluid. Nephridiopores open to the exterior between the notopodium and neuropodium.

The gonads are on the ventral surface of the anterior thoracic cavity just posterior to the diaphragm. These organs are numerous and interspersed among the yellow ventral glands.

The growth and development of the gametes occurs while the cells are floating freely in the coelomic fluid. The gametes are conveyed to the exterior by way of the nephridia.

Nervous system: The nervous system occupies a primitive position, in contact with the epidermis. The whole system is quite difficult to study in dissections. In fact, it is usually futile to attempt to study the supra-pharyngeal ganglia located in the prostomium and the circum-pharyngeal connectives by any method other than in histological sections. The supra-oesophageal ganglia send tentacular nerves to each tentacle and nerves to the dorsal and ventral lips.

The ventral nerve cord begins anteriorly in the mid-ventral peristomium where it receives the connectives. It then extends posteriorly as a pair of cords with segmental commissures. This portion of the nervous system can often best be studied by pinning out the specimen ventral side up and teasing away the epidermis to expose the double cord lying directly beneath it.

The ventral cord is not distinctly ganglionated. Lateral nerves are given off at brief intervals and pass between the circular muscle fibers to innervate to the body wall.

PHASCOLOSOMA GOULDII

Phascolosoma gouldii is found in Massachusetts Bay and southward into Long Island Sound. It is most abundant in muddy sand just below the low tide mark. In some highly localized patches they are so abundant that a dozen or more may be brought up in a single shovel-full of sand. It burrows vertically, obliquely, and horizontally in an irregular fashion, often going as deep as two feet or so.

Their general manner of burrowing is readily observed if they are placed in a jar containing sea water and an appropriate sand and mud mixture. The anterior end of the animal is capable of being progressively rolled outwards or inwards. In burrowing the everting anterior end of the worm is forced into the sand where it is anchored by a localized dilation of the anterior tip of the body. The body is then pulled toward the new excavation by contraction of the general longitudinal musculature. This process is repeated over and over as the worm progresses.

Preparation of specimens for study: Phascolosoma is a relatively hardy animal that may be readily procured alive from biological supply houses. It is best studied in the fresh condition, though formalin-preserved specimens are quite satisfactory. It is easily shipped by express and can be kept alive in containers of sea water in the laboratory. If the animals are to be kept for some length of time before use they can be maintained alive satisfactorily by placing the containers in a refrigerator.

The extraordinary contractility of these worms make them difficult specimens to study and dissect while fresh unless they are thoroughly anaesthetized. One good way of doing the latter is to place them in 7% ethyl alcohol in sea water, or in sea water to which is added gradually a saturated solution of chloroform in sea water. In response to this treat-

ment the animals become well extended and the general musculature relaxed. The internal organs including the cilia remain in a living state for several hours. The dissection should be performed in sea water. It is desireble to have available a source of intense illumination and, if possible, both dissecting binocular and compound microscopes.

External anatomy: Study carefully the external form of your specimen. Phascolosoma gouldii is typically whitish or light gray in color. Adult specimens, fully extended, measure about 25 to 30 cm. in length and 0.6 to 0.8 cm. in greatest diameter. The body is divisible into two general regions, 1) an anterior introvert, which is capable of being rolled into 2) the posterior portion. The body wall of the introvert is less muscular than the remainder and extends posteriorly almost as far as the pair of conspicuous latero-ventral nephridiopores of the single pair of giant nephridia, and the dorsal anus of the essentially U-shaped digestive tract. The anus is located in the mid-dorsal line. (In a fully contracted living specimen the body is characteristically curved, with the maximum convexity along the dorsal mid-line.) At the interior tip, the mouth is terminal and surrounded by an oral disk possessing tentacles (See Fig. 1).

Just posterior to the tentacular crown and on the median dorsal surface is the cephalic lobe. It comprises a ciliated elevation divided into four portions by three longitudinal grooves. This organ lies dorsal and, in part, posterior to the brain. At its anterior end the longitudinal ridges overhang a horizontal transverse ciliated pit or groove. At the lateral edges of this groove tubes of cells, terminating blindly, pass downward into the brain. In the latter region the cells of the tube wall appear to possess the form of typical ganglion cells. The function of the pits is unknown. The ciliated cells of the dorsal ridges appear richly innervated and hence there is suggestive evidence of a sensory function.

The coelomic cavity and fluid: With fine-pointed scissors make a short longitudinal incision through the body wall just posterior to the introvert and slightly to the right of the midline. With a pipette remove some of the contained body-fluid and examine it with a compound microscope for coelomic corpuscles and reproductive cells. The coelomic fluid contains several types of cells and has a pinkish cast. The color is due to hemerythrin located in abundant, disc-shaped, nucleated corpuscles (See Fig. 3). The latter vary considerably in size, ranging in diameter from 6 to 24 μ. These cells, singly, when fresh appear optically homogeneous and slightly yellowish. Other, and less abundant, corpuscles are small and large granular amebocytes showing clear pseudopodia and some giant (up to 123 μ) multinucleate disc-shaped bodies. Freshly drawn coelomic fluid shows the capacity to form a clot rather rapidly. The ameboid cells appear to participate in this process, in part through agglutination of their pseudopodial processes.

Reproductive cells are also usually abundant in the coelomic fluid. In the female these vary from small immature eggs of about 25 μ in diameter, and having large germinal vesicles, to large mature eggs (to 200 μ) possessing a thick gelatinous capsule and with nucleus obscured by yolk granules. In the male, sperm cells in various stages in development may be found. These cells may occasionally be so abundant as to give the coelomic fluid a milky appearance. They usually become motile only after being put in sea water.

Now continue the longitudinal incision to the posterior end of the worm, and also nearly to the anterior tip and determine the extent of the coelom. Pin out the body wall. The coelom is of an extensive perivisceral nature undivided by septa. It is lined by peritoneum which, especially over the gut, is covered by a thin, unicellular layer of chlorogogue cells. The latter are easily broken away from it. These chlorogogue cells contain yellowish granules. The nuclear region of peritoneal cells over most of the peritoneum possesses

tufts of long cilia which produce a circulation of the coelomic fluid.

The body wall and musculature: The animal is encased in a thick cuticle possessing pores to the exterior scattered abundantly but irregularly over the surface (See Fig. 2). The pores are associated with thickened regions of the underlying hypodermis which have differentiated into spheroid or ovoid cell masses possessing histological evidences of secretory activity. The cuticle over these organs is usually considerably thinner than over the remainder. Nearer the anterior and posterior extremities of the body are epidermal differentiations of a structural character appearing more probably sensory. Immediately internal to the hypodermis is a thin connective tissue layer separating it from a thick, continuous layer of circular muscle fibers. Internal to the latter are the longitudinal muscle fibers. These are separated into 30 or 40 longitudinal bundles, possessing many interconnecting bundles, by longitudinal grooves which penetrate partially or completely outwards to the circular muscle layer. A peritoneal membrane covers the muscle layers completely on their inner surface, bounding the extensive coelomic cavity.

The retractor muscles comprise four dorso-ventrally flattened band-like muscles with origins in the longitudinal muscles of the body wall and insertions near the anterior tip of the introvert. At this latter region the four muscles are fused to form a nearly continuous sheath about the pharynx. It is completed dorsally and ventrally by connective tissue. There are a number of connective tissue fibers also connecting the anterior end of the oesophagus with this muscular sheath. The retractor muscles are covered with a peritoneal layer essentially like that of the body wall with which they are continuous.

Vascular system: Very little of this system can be worked out by the ordinary technics of dissection. A system of blood vessels is restricted

to the anterior end of the animal. This comprises an oral-disc plexus of vessels which connects with a larger circular vessel surrounding the pharynx. There are three vessels in each tentacle. Blood actively circulates through the tentacles by action of ciliated tufts on the lining epithelium of the vessels. The circumpharyngeal vessel opens into a larger dorsal vessel immediately ventral to the brain. This dorsal vessel, clearly evident in dissected specimens, continues posteriorly along the dorsal surface of the pharynx and oesophagus, finally ending blindly over the posterior portion of the latter. This longitudinal vessel, called the blood sac, possesses contractile walls in addition to internal ciliated tufts. The fluid contained within the above system of vessels is far richer in red corpuscles than the coelomic fluid, and hence is distinctly more reddish to the eye. The fluid also contains amebocytes substantially like those of the coelomic fluid, and shows a similar clotting ability.

In view of the organization and activities of the vascular system, together with a reported observation that in oxygen-poor sand the tentaculated crown may be extended out of the sand, it would appear that the tentacles may serve as functional gills.

Digestive system: Study the alimentary tract, carefully tracing it from mouth to anus. The mouth is anterior and terminal and is located within the tentaculated oral disc. The oral disc and the median concave faces of the tentacles are ciliated as is also the lining of the pharynx. The tentaculated crown is withdrawn into the pharynx when the animal retracts. The remainder of the alimentary canal comprises the oesophagus, stomach, descending and ascending limbs of the intestine, and the rectum (See Fig. 1). The oesophagus is long. Its anterior portion is surrounded by the four retractor muscles to which it is attached by connective tissue fibers. The remainder lies free except for a single connective tissue thread of attachment. Internally its epithelial lining is thrown into a number of longitudinal folds. The stomach is short, and is characterized by the presence of four longitudinal pink bands which are due to four corresponding longitudinal internal ridges containing radial muscle fibers. The stomach lies at about the level of origin of the ventral retractor muscles. The descending intestine

extends from the stomach to the posterior tip of the animal. The longitudinal red bands are here less conspicuous. Cilia are numerous within the tract. Curious ciliated pits occur in the longitudinal ridges of this region. These become deeper in the more posterior portion, even to the extent often of forming numerous minute externally visible diverticula in this area. The ascending intestine possesses a ciliated groove. This is visible externally as a light line between two pinkish longitudinal bands. The bands are the result of a concentration of radial fibers on each side of the groove in such a manner that the depth of the groove would appear capable of alteration by their activity. Cilia in the groove maintain a current of fluid moving in the direction of the rectum. The ciliated groove terminates at the rectal end of the intestine as a small, externally evident, diverticulum - the rectal caecum. The ascending intestine has associated with it a longitudinal mesentery containing muscle fibers. This is known as the spindle muscle. Its contraction results in tighter coiling and hence shortening of the ascending intestine, and simultaneously of the descending intestine which has numerous connective tissue points of attachment with the former. Such freedom of movement is facilitated in that the greater part of the gut hangs quite freely in the coelomic cavity. The only supports other than those already mentioned are three elastic threads attaching the ascending intestine to the body wall near its rectal end, and the anal connective tissue fibers.

On the external surface of the ascending intestine are numerous ciliated crescentic funnels known as "pseudostomata." Look for one of these with your compound microscope. These are believed to have a role in removing particulate matter from the coelomic fluid inasmuch as carmine particles injected into the coelom appear to become concentrated in the chlorogogue cells found within these ciliated funnels.

The rectum possesses a lining of ciliated epithelium thrown up into numerous longitudinal ridges. There are no pseudostomata nor is there a ciliated groove in the region.

Nephridia: The single pair of giant nephridia hang freely into the coelom from their attachment at the point of the external opening (See Fig. 1). The free end is usually directed posteriorly and is closed. The internal opening, or nephrostome, is very close to and just anterior to the external opening. Thus the tubule is essentially U-shaped. The nephrostome is a slit lying between a semicircular flap of the anterior arm of the tubule and body wall. Both sides of the nephrostome are ciliated. The tubule walls contain well-developed circular and longitudinal muscle fibers lying between an internal secretory layer of cells and the covering peritoneum. After studying a nephridium in situ remove it and examine it for action of the cilia on the nephrostome.

The reproductive organs: The sexes are separate. The gonads are minute ridges of tissue lying transversely on the posterior faces of the ventral retractor muscles near their point of origin (See Fig. 1). Find them. These ridges are continuous with one another across the median line beneath the ventral nerve cord. The gonads comprise small masses of reproductive cells supported in connective tissue. The largest cells of the gonads, those about to be liberated into the coelomic space are nearest the free tips of the fimbriated organs. The sexes can be distinguished in them by the fact that the largest cells in an ovary (24 μ) are nearly twice the size of the largest cells of a testis. The breeding season lasts from June through August. Sperm cells are forcefully ejected from males by way of the nephridia. The presence of the discharged male products induces the female to eject her eggs. Egg-laying normally occurs at night between the hours of 8 P.M. and 5 A.M. A few hours

prior to liberation of eggs or sperm the nephridia become enormously distended with these sexual products.

The nervous system: The ventral nerve cord lies freely in the coelom attached to the body wall only by its lateral nerves (See Fig. 1). Examine it carefully. The cord is more or less ovoid in cross section in the posterior portion of the body but is wider and more dorso-ventrally compressed in the region of the introvert. It terminates posteriorly in one or two posteriorly projecting nerve fibers; anteriorly in the region of the pharynx it branches to form the circumpharyngeal connectives which pass dorsally and enter the suprapharyngeal ganglion or brain. The ventral cord and lateral nerves are loosely invested in a peritoneal sheath. Between the sheath and the cord proper is connective tissue. In the posterior region of the body a single median muscle tract lies within the sheath dorsal to the cord. As the cord enters the introvert the muscle tract bifurcates, the two branches going to right and left sides of the cord. These arrangements of the muscle tracts of the cord in the two regions account for the differences in cross-sectional form. Contraction of these muscle fibers results in cord-shortening.

The lateral nerves pass from the dorso-lateral aspects of the cord. They are numerous but appear to come off quite irregularly (See Fig. 4). The cord shows no evidences of segmentation. The lateral fibers pass to a position between the two muscle layers of the body wall where they produce "ring-nerves" passing around the circumference of the body. The "rings" send off numerous branches. Some branches of the "ring nerves" pass to produce a series of longitudinal nerve fibers in the connective tissue underlying the hypodermis. There is considerable fiber anastomosis here also. The sub-hypodermal, longitudinal fibers send branches to the hypodermal

sensory organs and to the circular muscle; the ring nerves innervate the longitudinal muscle fibers. These two major nerve plexuses are continuous with the plexuses of the gut and of the retractor musculature.

The brain (See Fig. 4) lies dorsal to the pharynx with its overlying "blood sac," and between the dorsal retractor muscles of the introvert. It is a relatively large structure, being about 2 mm. in diameter. The dorsal aspect of the brain is separated from the coelom by only the peritoneum and a thin layer of connective tissue. Carefully expose the brain and the circumpharyngeal connectives. The brain has numerous fibers passing to the cephalic lobes anteriorly and to the pharynx and dorsal retractors posteriorly.

A number of large nerves originating in the circumpharyngeal connectives innervate the tentacles, the pharynx and probably also the ventral retractor muscles.

MOLLUSCA

General directions for the study of the MOLLUSCA in the laboratory

The main part of the laboratory work will center about seven representative forms for which you have detailed directions for study. In addition, many other forms which may be available from time to time, will be present on the water table. Take advantage of the supply of the living additional forms, and make as many comparative studies as your time permits.

In addition to the directions under the seven representative forms a few suggestions for special study are given below.

GASTROPODA

Activity of the foot

1. Busycon, see outline
2. Nassa
   Allow Nassa to become attached to a glass plate. Observe with a lens the creeping surface of the foot. Pour sea water into the dish until the surface film is level with the ventral surface of the foot. Observe the foot with a binocular microscope, and note ciliary activity, direction of movement of carmine particles, waves of muscular contraction, etc.
3. Polynices
   Cover the specimen with sea water, and observe the gradual swelling of the foot. How much of the shell does it cover? Touch the animal to make him withdraw his foot. Repeat the experiment, and this time measure the amount of water exuded.
4. Nudibranch, see outline.

Activity and comparative study of the radula

The radula is an important characteristic of most mollusks, and therefore a comparative study of this structure is worth while. See directions under digestive system of Busycon.

PELECYPODA

1.

Comparative Study

Probably Mytilus, Modiolus, Mya, Ostrea, Solemya, Nucula, and Mactra will be available, in addition to the three forms for which you have detailed outlines. Make a comparative study of the Lamellibranchs. Pay particular attention to the following structures, gills, siphons, palps, and feet.

Gills

Protobranchia, e. g. Yoldia

Filobranchia, e. g. Modiolus, Mytilus, Pecten
Fulamellibranchia, e. g. Venus, Mya

In each case notice the gross anatomy, number of gills, form of gill sheet; filaments, shape, reflected or not reflected, ostia, distribution of cilia, inter-filamentar junctions, inter-lamellar junctions. Study the filaments from mounts of living and preserved material.

Siphons

Notice whether separate or united; whether extensible to any great length. By using carmine particles suspended in sea water, determine the directions of currents through the siphons.

Palps

Notice shape, size, whether extensible as in Yoldia; ciliation on surface, etc.

Foot

a. Byssogenous foot of Mytilus or Modiolus. This is a small foot capable of spinning the threads by which the animal attaches to the substrate. Cut off the foot during expansion and mount upon a slide. Note the ciliary activity, muscular movements, and the mucous glands containing yellow spher ules.

b. Burrowing foot
Primitive type of Yoldia. See outline
Specialized type, e. g. Venus or Ensis. See outline

c. Degenerate type, e. g. Mya or Ostrea., or Pecten.See outline.

2. Experiments with the Pelecypod heart

Directions of this experiment will be found under the Circulatory system of Venus. A limited number of kymographs etc. are available for this experiment. Anyone who is particularly interested in carrying out this study should speak to the instructor.

EMBRYOLOGY

A study of several stages of the development of molluscan eggs and larvae is advisable. Records and drawings should be made of as many details as it is possible to make out in the time available. Consult your outlines under the reproductive systems of Eolis, Busycon, and Venus, for more detailed directions.

AMPHINEURA

Chaetopleura apiculata (Say)

Chaetopleura, a member of the family Ishnochitonidae, is a small chiton about 2 centimeters in length. It lives in the shallow waters

of the Atlantic coast from Cape Cod to Florida. By means of its broad flat foot, this small Mollusk can cling fast to the rocks or empty shells upon which it lives, or creep slowly over their smooth surface in search of diatoms and algae. Chitons are easily collected either by examining the small stones and shells at the low tide level, or by dredging in water up to four or five fathoms over a substrate which is composed largely of old shells.

A living specimen can best be studied when it is attached to a glass plate. In order to prevent the animal from curling into a ball, transfer it quickly from its original stone so that it will attach to the glass while at flat position. Slight pressure on the shell from above may induce the animal to remain flat. Later study the natural behavior of the chiton when it is allowed to move its plates freely.

The dorsal surface of Chaetopleura is covered by a shell, which is composed of eight calcareous transverse plates overlapping posteriorly. These overlapping plates allow the animal to bend slightly during locomotion, or to curl up into a ball if pried loose from the substrate. They are surrounded and kept in place by a muscular integumental fold called the girdle.

On the ventral surface, the most conspicuous organ is the flattened, elliptical foot which is adapted for creeping and clinging. Locomotion is accomplished by waves of muscular contraction and ciliary action. Adherence to the substrate is maintained by suction and by the aid of mucous secreted by numerous glands. Projecting over the sides of the foot is a fold called the mantle. This fold encloses a deep furrow, the mantle cavity, in which the gills are located. They are composed of a series of twenty-four short transverse ridges. The head fold, containing the mouth, occupies the anterior portion of the ventral surface. If the mouth is enlarged by cutting, the radula, a chitinous ribbon bearing rows of teeth, can be seen. This ribbon should be removed and mounted on a slide for further study. (The radula mechanism will be explained in detail under the study of the Gastropod, Busycon.) The anus is located at the posterior end of the ventral side.

The breeding habits of Chaetopleura make it a satisfactory form for embryological study. At Woods Hole, spawning may occur from early June to late September, but the sex products are most abundant from July 10 to August 20. If the specimens are placed in finger bowls of clean sea water, they will usually shed in the early evening from 8 to 11 o'clock. Adult males and females are indistinguishable from one another. However, since no evidence for interdependent stimulation exists, isolated individuals spawn satisfactorily. Eggs can then be fertilized and studied. The entire embryological development is completed in 6-10 days.

GASTROPODA

Eolis Cuvier

Eolis, first described by Cuvier, belongs to the order Opisthobranchia, the sub-order Nudibranchia, and the family Aeolididae. It is often referred to as a nudibranch, for it is a member of that large and varied group of mollusks which have, as the name so aptly suggests, no shell to cover the gills. It is also sometimes called a sea slug. Eolis is a small nudibranch, one or two centimeters in length, which avoids the light whenever possible. It is often seen crawling about on the under side of sea weeds, or more frequently creeping about in masses of hydroids upon which it feeds. It inhabits the shallow waters of the Atlantic coast from Cape Cod northward and also the northern coasts of Europe.

In studying the specimen, allow it to crawl freely, but in a somewhat limited space like a watch glass. Small nudibranchs often crawl easily on the under side of the surface film. This is a particularly profitable position from which to study one of them. Later, if the specimen is small, you will find it advantageous to mount it on a slide and depress it slightly with a cover glass. If it is desired to keep live nudibranchs for several days, they should be kept in cool running sea water, or in the ice box.

The soft body, which is without a shell, mantle, or true gills, is covered with a transparent integument. The cerata, called adaptive gills, are the respiratory organs. These are cylindrical extensions of the integument containing hollow extensions of the liver and nematocysts derived from ingested hydroids. The cerata have a characteristic arrangement. They may occur in any transverse rows, or in a few longitudinal rows. The color also varies, shading from brown, through salmon to red. Often the ends are tipped with white. Mount one of the cerate on a slide and study it.

The head bears cylindrical tentacles, usually two pairs, one of which may be retractile. These pairs may be of different lengths. If so, the anterior or oral pair is the longer. The posterior or dorsal pair, often called rhinophores, bear simple sense organs which appear to test the surrounding water. At the base of each rhinophore is an eye spot. The mouth is prominent and can be most easily seen from the ventral view. On the right side of the head is the rather conspicuous genital opening, and just posterior and dorsal lies the smaller anus.

The foot is a muscular organ on the ventral side of the animal. Its proportions vary with the species. Locomotion is accomplished by waves of muscular contraction.

The extent to which the internal organs can be observed varies with different individuals and species. The following are usually visible in most specimens.

The dorsal systemic heart is usually beating actively, between 50-100 beats per minute. Blood is sent, somewhat indirectly, to the cerata and to the skin generally for aeration. Upon its return to the hear it is mixed with blood from the viscera, so that only partially aerated blood enters the auricle.

The most conspicuous organ of the digestive tract is the mouth. It is guarded by fleshy lips, which cover both the radula and the horny lateral jaws within the buccal cavity. The power jaws cut the soft hydroids into smaller pieces which the radula then carries back into the buccal cavity and towards the esophagus. Although the esophagus, the stomach, and the intestine are well defined, they are not easily seen through the transparent skin. The liver, already identified, has numerous delicate ducts from the cerata which form trunk channels eventually uniting to enter the digestive tract.

The nudibranch is hermaphroditic and the common gonad is protandric. In the posterior part of the abdominal cavity the numerous eggs of the large ovary are usually visible. Copulation in these Mollusks is an exchange of sex products, and spawning takes place a day or two following. Nudibranchs are most prolific. The eggs are extruded in a long, ruffled, string of sticky gelatinous substance. Because of its consistency, this jelly adheres firmly to the substrate upon which the eggs are deposited and therefore the egg masses are saved from undue buffeting. Nudibranch egg masses are commonly found upon the hydroids on which the Mollusks have been living. In the laboratory, nudibranchs will often deposit eggs upon the sides of the dishes in which they are kept.

If several groups of eggs from different individuals are examined, a representative series of embryological stages can be obtained. The veliger larvae, especially, are excellent. Due to their transparency, many structures are visible. (See more detailed directions for study under Busycon, Reproductive system.)

GASTROPODA

Busycon canaliculatum

Busycon Röding, formerly known also as Fulgur Montfort, Sycopsis Conrad, and Sycotypus Gray, is a large whelk belonging to the order Prosobranchia and the family Galeodidae. It occurs commonly in shallow waters off the east coast of the United States from the south shore of Cape Cod to the Gulf of Mexico. By means of its tremendous foot, it can plow through the sand in search of bivalves upon which it feeds.

Observe the behavior of a living specimen. Notice the large, tough muscular foot on which the animal glides along the walls of the aquarium. If the head is extended, watch the motion of the tentacles and the proboscis. Touch the specimen and study the position and function of the horny plate, the operculum, which fits into the aperture of the shell as the animal is withdrawn.

THE SHELL

In your study examine the entire shell and one which has been sawn along its axis. The shell is a structure somewhat like a spiral staircase. The apex lies at the peak of the spire which is composed of several whorls. The number is constant for the species,

but a young specimen may not possess the complete number. Notice the increase in size of each whorl from the apical region to the body whorl which is both the largest and most recently formed. The large opening of the body whorl is called the aperture. The axis around which the whorls are wound is the columella. This can be studied better from the sawn shell. The siphonal canal is the elongated end opposite the apex. On the external surface, notice the lines of growth which represent successive periods of growth. In the sawn half, identify the three layers characteristic of Molluscan shells, the outer rough periostracum largely worn off; the smooth inner nacreous layer lining the shell; and the thick, middle prismatic layer.

To determine whether your specimen is wound dextrally or sinistrally hold the shell with the apex above, the siphonal canal below, and the aperture toward you. If this aperture is on your right, the animal is considered dextral.

SURFACE ANATOMY

Compare a specimen which has been removed from its shell with the shell just studied, and try to understand their relationship. On the right side of the animal notice the columellar muscle which attached the animal to its shell and thus enabled it to withdraw. This muscle continues into the large, flat, ventral foot, which is composed almost wholly of muscle fibres. The foot is used primarily for locomotion and attachment and its flat, pliable surface is eminently modified for the purpose. By a series of rhythmic contractions of muscle, the foot is lifted off the substrate, moved forward, and set down again. These pedal waves can be observed in a living specimen. As an organ of attachment, the foot adheres firmly to the rocks by means of suction and the aid of copious mucus secreted by the cells of the pedal gland. The opening of this gland, the pedal groove, lies near the anterior end of the sole of the foot. Near the posterior surface of the foot, is the operculum, a thin, horny disc, which serves as a trap door to lock the animal securely within its shell. By means of it he is protected not only from mechanical injury and chamical irritants, but also from excessive dryness.

The head, an anterior projection above the foot, bears a pair of triangular tentacles, on the lateral surface of which are the pigmented eye spots. From beneath the tentacles the proboscis may protrude, but this will be true only in a relaxed specimen. At the tip of the extended proboscis, the triangular mouth opening can be easily identified. Frequently the teeth of the radula can be seen through this opening. If the specimen is a male, the large penis will project to the right of the right tentacle.

Above these structures lies the visceral mass which in the living animal occupies the spire of the shell. This soft area is covered by a thin, transparent, closely applied membrane, the mantle. Near the base of the visceral mass the mantel becomes thicker and forms a collar which extends around the body along a line corresponding to the aperture of the shell. It is at the free edge of the mantle that the most active secretion of the shell occurs. The space enclosed within the mantle is called the mantle cavity. The elongated portion of the mantle is the siphon and forms the lining of the siphonal canal.

It is through this canal that water is drawn into the mantle cavity for aeration of the gill.

Through the mantle many of the structures of the visceral mass can be identified. Beginning at the apex, notice the grayish brown liver, which occupies the majority of the first two whorls. Its right lobe fills the apex; its left lobe lies below the next whorl. Between the two lobes is the gonad, usually conspicuous because of its orange color. On the outer surface of the left liver lobe and partly covered by it, lies the stomach. This is neatly curved to fit the contour of the whorl. Notice the large brown kidney which lies along the dorsal surface of the mantle. It is somewhat rectangular in shape and is composed of two regions, a small tubuliferous region lying to the left of the larger acinous region. These two parts can be seen more clearly after the mantle cavity has been opened. To the left of the anterior end of the kidney lies the periocardial cavity. The heart is often visible through the thin dorsal wall. Anterior to both the pericardial cavity and the kidney lies the large obvious gill which is oblong in shape and usually brownish in color. If the specimen is injected, the left portion will be red, the right, blue.

Open the mantle cavity by cutting the mantle along the medial edge of the gill. Continue cutting until the pericardium is reached. Take care to leave the heart undisturbed at this time. Turn aside the mantle and reexamine the gill. Between the anterior end of it and the base of the siphon lies a small elliptical organ, the osphradium, over which all water passes before reaching the gill. The osphradium is composed of 90-100 leaflets covered with epithelium containing sensory, glandular, and ciliated regions. It has an abundant nerve supply. Although the function has not been satisfactorily determined, the organ appears to test the water entering the mantle cavity. Along the median side of the gill lies a modified glandular area of the mantle called the hypobranchial gland. This gland secretes mucus rapidly and copiously and thus protects the gills by removing dirt and other foreign particles. If the specimen is a female, the large yellow nidamental gland will easily be seen on the right side of the mantle cavity.

The opening of the gland, which is the genital aperture, is located on a slight elevation near the edge of the mantle. Cut open the nidamental gland by making an incision parallel to the oviduct within. From this point trace the oviduct into the ovary. Posterior and to the left of the genital opening lies a conspicuous papilla which encloses the a nus. Probe through the anus into the broad rectum.

CIRCULATORY SYSTEM

Try to obtain an injected specimen. Open the mantle cavity if this has not been done, and carefully cut the pericardial wall to expose the heart lying in its chamber. The heart is composed an anterior, triangular thin-walled auricle, and a posterior, round thick-walled ventricle. The auricle receives blood from two large vessels: the nephridio-cardio vein returns blood from the tubuliterous portion of the kidney; the efferent branchial artery returns blood from the gill. The latter artery can be seen coursing along the lateral

margin of the gill to its posterior end. Here the artery turns sharply medially along the border of the pericardial cavity where it enters first the auricle, and then the ventricle. From the ventricle a single large aorta emerges. Near its origin notice a branch, the visceral artery, which soon divides into several smaller arteries supplying the visceral hump. Trace there as far as practicable. The main aorta bends sharply anteriorly and continues parallel with the esophagus below the floor of the mantle cavity. Soon both aorta and esophagus dip deeply into the head region. Here the aorta enlarges to form the secondary heart. Trace the smaller branches into the head and foot. (Dakin, 1912) The heart, a typical Molluscan one, consists of smooth muscle fibres. The blood is carried by arteries to all parts of the body. The venous blood is returned by less well defined vessels, the large lacunae or sinuses. From them, blood passes into the kidney, through the gill for aeration, and finally reaches the auricle. Since blood also enters the auricle directly from the kidney, the blood of Busycon is mixed.

EXCRETORY SYSTEM

The two parts of the kidney have already been studied. However, observe at this point that the tubuliferous part appears to be composed of a series of parallel structures. These are the tubules. The thicker walls of the acinous part are composed of lobules. Between these two portions of the kidney, the slit-like opening of the organ lies entering into the mantle cavity.

DIGESTIVE SYSTEM

The proboscis is an extensible, retractible organ, the walls of which are composed of a superficial integument and two layers of muscle, an outer longitudinal and inner circular one. Slit the head fold between the tentacles thereby freeing the proboscis as far as possible without removing it. At its posterior end are numerous strong muscles, the retractors, which attach it to the integument. Functioning of the proboscis is as follows: Shortening is caused by a contraction of the longitudinal muscles within the wall of the proboscis; retraction of the proboscis itself within the animal by the retractor muscles already mentioned. Elongation is the result of simultaneous relaxation of the longitudinal muscles with contraction of the circular ones. Protraction is not satisfactorily understood. It is thought that when blood flows rapidly into the sinus of the head fold, the contraction of these muscles forces the enclosed proboscis out of the animal.

During this dissection the paired, yellow salivary glands, lying on either side of the esophagus, may be conspicuous. Trace their long slender ducts forward to their entrance into the anterior lateral wall of the esophagus. More posteriorly, on the right side of the esophagus lies a small glad, the pancreas. Complete the dissection of the digestive tract by tracing the esophagus to the stomach and identifying the intestine, rectum and anus.

Turn to the study of the nervous system before completing the internal dissection of the proboscis. If necessary, carefully remove the proboscis with the attached esophagus.

Cut the proboscis along the mid-ventral line, reflect the skin, and pin it flat on a dissecting tray. This will expose the buccal mass. Superficially this mass comprises several sets of muscles which conceal three important organs, the esophagus, the odontophore, and the radula. The esophagus lies flat along the inner median dorsal wall of the proboscis. The odontophore is a forked cartilage, the ends of which can be seen near the posterior end of the buccal mass. It is the support for the ribbon-like radula which bears many rows of teeth used in procuring food.

Study the various sets of muscles which control the action of the odontophore and radula. The odontophore protractors, seven or eight slender muscles on each side, run forward from the odontophore to the lateral walls of the proboscis. The odontophore retractors, two broad flat muscles which are attached to the posterior horns of the odontophore, run posteriorly and dorsally to the base of the proboscis. The Radula protractors, 3 pairs of slender muscles on the ventral surface of the buccal mass, a ttach anteriorly to the radula sac. Posteriorly the lateral protractors attach to the horns of the odontophore; the medial protractors attach to the base of the proboscis. The radula retractors, a strong set of muscles, attach the buccal mass to the posterior wall of the proboscis. These muscles can be seen most clearly from the dorsal aspect. Since the effective stroke of the radula is the back stroke, these muscles are particularly strong and bulky. Carefully remove the muscles and examine the odontophore, radula, and radula sac. The odontophore is the cartilaginous support over which the radula is pulled back and forth during the feeding process. The radula is a long flat ribbon-like structure bearing a series of short rows of teeth. On the ventral side of the odontophore the radula lies flat. As it is pulled dorsally over the tip of the odontophore, the greatest width is exposed and comes in contact with the food. As the ribbon passes dorsally and posteriorly, the lateral edges fold in upon themselves, and therefore all teeth are completely enclosed in the loose membranous radular sac. Since the teeth point posteriorly, the effective stroke of the radula is the back stroke (retraction). As the anterior teeth are worn away, new rows which are constantly being formed by odontoblasts at the dorso-posterior end, move forward to replace them. At first the young teeth are chitinous, but later become hardened by deposition of mineral salts.

As the number and arrangement of teeth on the radula are used as criteria in the classification of many Gastropoda, a comparative study of radulae is worth while. Littorina, Urosalpinx and any nudibranch offer characteristic differences. The directions for preparation of the radulae are simple. Kill the animal by immersion in hot water for a minute or two. Remove the radula sac and boil it gently in 10% KOH for several minutes, until the radula sac is dissolved. The radula should then be mounted in a drop of glycerine. As you examine the teeth on the radula, keep in mind the habitat, type of food, and feeding activity of the animal under observation.

In a living specimen, the rasping action of the radulacan be demonstrated by placing a few drops of macerated clam at the tip of the proboscis. In a living, but narcotized specimen, the same reaction can be elicited by alternate stimulation of the retractor and protractor muscles.

NERVOUS SYSTEM

The nervous system of this form shows a high degree of cephalization. With the single exception of the visceral pair, the ganglia are concentrated in close proximity to each other around the anterior part of the esophagus. They form an irregular brownish-yellow mass of tissue which completes a ring around the esophagus. With a hand lens, notice on the ventral side of the esophagus the small paired buccal ganglia, which are located most anteriorly. These connect with each other and with the cerebral ganglia. Posterior to this pair lie the much larger pedal ganglia, which are fused, yet distinctly paired in outline. The pedal ganglia possess numerous large nerves which supply the foot muscles as well as smaller connectives to the cerebral and pleural ganglia. On the dorsal surface of the esophagus is a conspicuous commissure which unites the paired cerebral ganglia. These ganglia are the most centralized for they connect directly with the pleural, pedal, and buccal ganglia, and indirectly with the parietal and visceral. On the left side, posterior and ventral to the cerebral, lies a smaller ganglion, the left pleural. It extends nearly to the ventral surface of the esophagus. On the right side, four ganglia lie close together. The right cerebral and right pleural are fused, but retain a distinct constriction. Posterior and dorsal to the right pleural lies the right parietal. Short connectives unite these two ganglia. The remaining ganglion, the left parietal, lies ventral and slightly anterior to the right parietal. It nearly touches the right pleural and right pedal. The visceral ganglion, probably the fusion of a pair, lies below the external opening of the kidney. The elongated visceral commissures connect the visceral ganglion with the parietal ganglia.

REPRODUCTIVE SYSTEM

The gonad, ducts, and external openings have already been described. The sexes are separate, and fertilization is attained internally by means of the large penis. In the Cape Cod area, eggs are laid during August, but in the Carolinas they may be deposited as early as May or June. Like many Prosobranch Mollusks, Busycon protects its eggs by surrounding them with a tough case and attaching the cases to a stable object. As the eggs pass out of the oviduct and through a temporary fold in the foot, they are covered by a secretion from the nidamental gland. This secretion forms not only the disc-shaped egg cases, but also a stem or cord, along which the discs are attached in a series. One end of the cord is fastened to a buried object. Often as many as 70 discs, each containing 20 or more eggs, are found on a single cord. The rate of formation may be as high as 3 hours per disc.

The eggs of Busycon are very large, but they contain enormous quantities of yolk, cleave slowly, and in general are not particularly satisfactory for embryological study in the class room. Development is completed within the egg case. The young Mollusks emerge as tiny replicas of the adult.

The eggs of another Gastropod, Crepidula, are classic forms for the study of Molluscan embryology, and fortunately Crepidula can be obtained throughout the summer. By pulling apart the individuals from the groups into which they are usually aggregated, the yellow egg

masses may be found deposited on the shells of the underlying animal. Various stages in development may be discovered by examining several different masses of eggs. In the veliger larva of this, or any form, try to identify the following structures:

1. Velum, with large powerful cilia around the margin
2. Transparent shell
3. Head vesicle and eyes (dorsal)
4. Foot (ventral)
5. Mouth, just above the foot
6. Heart, usually beating (dorsal)

Among the fresh water forms, the common pond snail, Physa, will live in a laboratory aquarium and often deposit its transparent gelatinous egg cases upon the walls of the aquarium. These eggs are excellent material for embryological study.

PELECYPODA

The clams, mussels, and scallops of this group are often referred to as the Lamellibranchs because of their flat, plate-like gills. The general directions for the dissection of a typical lamellibranch will be given for Venus, a common clam of this area. However these directions may be used profitably in the study of other lamellibranchs for which there will be brief directions limited to the structures particularly characteristic of each animal.

Venus mercenaria

Venus mercenaria is known by a variety of common names such as quahog, hard shelled clam, and little neck clam. This species belongs to the order Eulamellibranchia and the family Veneridae. Its entire range includes the shallow protected waters of the Atlantic coast from the Gulf of St. Lawrence to Texas, but it is most abundant from Cape Cod southward. By means of its powerful foot, this form burrows about over muddy or sandy flats near the lower portion of the intertidal zone. Although it lies buried a few inches, it keeps its siphonal end above the surface of the mud in order to maintain a current of sea water through its body. This current is essential for respiration and feeding.

SHELL

The shell of Venus is composed of two symmetrical halves called valves. Because of this arrangement, the mollusks possessing two valves are called bivalves, as distinguished from snails which are called univalves. At the dorsal margin the valves are joined by a brown, external ligament. On either side of this ligament there is a swelling in each valve called the umbo. This ends in a point, the beak, which is directed anteriorly. Growth has proceeded from the beak as an origin and has resulted in concentric lines of growth. The lunule, a heart-shaped configuration, lies anterior and ventral to the beak (absent in fresh water clam). This is a useful character for field identification. Before opening the valves, determine the correct orientation, right, left, dorsal, ventral, anterior, and posterior. Obtain, if possible, a young Venus for comparative shell study.

Pry the two valves apart and try to insert your knife between the shell and the mantle which lines it. Remove the mantle carefully, and at the anterior and posterior ends, cut the adductor muscles. Since the contraction of the adductors close valves, they will now gape and be readily separated. The gaping is due to the action of the ligament which opposes the adductor muscles.

On the inside of the shell are various markings indicating the former attachment of organs. At each end of a valve is the large, round scar of the adductor muscle. Closely a ssociated with these are the two smaller scars of the retractor muscles of the foot. The small impression of the anterior retractor is separated from the larger one of the anterior adductor, and lies dorsal and a little posterior to it. (In the fresh water clam, the scar of the anterior

retractor is ventral and slightly posterior to that of the adductor). The impression of the posterior retractor is usually merged with that of the corresponding adductor. Running from one of these large scars to the other, is a distinct line called the pallial line. This line, parallel to the margin of the shell, marks the attachment of the pallium, or mantle, to the shell. At its posterior end, the line forms a triangular sinus, the pallial sinus. (Absent in the fresh water clam) This indicates the position of the retractor muscle of the siphons.

Along the free ventral edge notice the tiny teeth. This arrangement is the result of secretion by a fringed edge of the mantle. The crenulated margin insures a firmer closing of the valves. Along the dorsal edge notice the larger teeth which a re of two kinds. Anterior to the ligament are the prominent cardinal teeth; below the ligament are the less obvious lateral teeth which extend posteriorly for some distance. Notice that the teeth fit tightly and form an efficient interlocking mechanism. In Venus, the thin periostracum, or outer layer of the shell, is partly worn away by constant friction with the sand. The innermost layer, the laminated nacreous or pearly layer, is very thick but does not appear as shining and lustrous as in many mollusks. The middle, or prismatic, layer is present but not easily visible. (In the fresh water clam, the pearly layer is often lustrous and colorful, shading from a silvery white, through salmon and pink, to lavendar. It is very thick and often composes the greater part of the shell.)

MANTLE

With one valve removed, place the specimen in a dish of sea water. The mantle, now exposed, forms a thin covering for the soft parts. The mantle consists of two lobes of tissue, one of which is applied to the inner surface of each valve. These two lobes are continuous dorsally. The free border of each lobe is thickened and contains muscles which attach along the pallial line. These muscles serve the function of a washer. The posterior edges of the mantle lobes are also thickened and fused at two points, one above the other, to form a double tube, the siphons. (In the fresh water clam the ventral siphon is not a perfect tube. It is only by the contact of the two ventral posterior edges of the mantle that the"tube" is maintained). In Venus, these siphons are very short but are easily identified by their dark pigmentation. Water enters through the ventral, incurrent siphon, and after circulating through the branchial chambers leaves by the dorsal, excurrent siphon. A few grains of powdered carmine placed near the siphons of an undissected, living specimen, will demonstrate these currents clearly.

VISCERAL MASS AND FOOT

Carefully cut the mantle and reflect the parts. The ventral portion of the median mass now visible is the foot, which is somewhat hatchet-shaped. Identify the small foot muscles, the attachments of which have already been seen on the shell. These muscles may be seen at the extreme anterior and posterior margins of the foot, where they extend dorsally toward their attachment on the valves. The dorsal portion of the median mass is called the visceral mass. As its name

suggests it contains the viscera which will be studied in logical sequence.

RESPIRATORY SYSTEM

With the mantle removed, the gills are the most conspicuous organs. They consist of two pairs of thin membranous folds, ridged in appearance, which lie on each side of the visceral mass. They are attached to the body on each side along a line extending from the wall which separates the two siphons, to a point even with the beaks on the exterior. Only the dorsal edge is attached; the ventral edge hangs freely. The outer gills are attached to the mantle lobe near its dorsal region; the inner gills are attached to the visceral mass. The entire space enclosed between the right and left lobes of the mantle is called the mantle cavity. Since the gills are attached to the mantle, to each other, and to the visceral mass, their attachments form a continuous horizontal partition separating off a small cavity, the supra-branchial or cloacal chamber, from a much larger ventral cavity, the branchial chamber. Into the latter the incurrent siphon leads, and the gills hang freely. The supra-branchial chamber is difficult to see at this point. It is a median, dorsal tube, extending antero-posteriorly, and connecting with the excurrent siphon.

Each gill is a double fold, the inner and outer surface being composed of parallel ridges or filaments. Each surface is referred to as a lamella. Each gill, therefore, consists of two lamellae, an inner and an outer one. The inner and outer lamellae of a single gill are separated by a thin space, which at regular intervals is divided vertically into a series of narrow tubes, the water tubes. These tubes connect dorsally with the cloacal chamber. The partitions forming the walls between the tubes are called inter-lamellar junctions, and they extend dorso-ventrally between the lamellae. They contain blood vessels. From one gill, cut a transverse section 1 mm. wide, and stand the section on edge. If this is examined under a dissecting microscope, many of the above mentioned structures can be seen.

Separate one lamella from the other. This can best be done by working with the free dorsal border of the inner lamella of the inner gill. Mount a small piece of a slide and study it under the low power of the microscope. Try to identify several structures already seen grossly, as well as the following. The filaments extend the width of the gill and between them are connecting bridges, the inter-filamentar junctions. The inhalant ostia are small but frequent openings bounded by filaments and their junctions. It is through these ostia that water enters the gills, the surface of which is abundantly ciliated. (Fig.) A few grains of powdered carmine placed at the ventral scalloped edge of the gill will demonstrate the current produced by the prominent cilia present. Due to the constant beating of the cilia, a continuous current of water is pumped from the incurrent siphon into the branchial chamber, through the ostia, and into the water tubes. From here the water enters the supra-branchial chamber and is carried out by the excurrent siphon.

Not only oxygen but also food particles are transported by these currents through the branchial cavity. The strength of these currents is markedly influenced by change in temperature of the surrounding water. At 22° C, Venus is actively pumping for 96% of the day, but

with a drop to 5°C ciliary activity is decreased, and below 5°C hibernation of the individual occurs. (Loosanoff, 1939)

CIRCULATORY SYSTEM

Near the dorsal midline and just anterior to the posterior adduct or muscle, there is a thin-walled triangular chamber, the pericardium, within which lies the heart. Carefully open the pericardium. If the heart is still beating, perhaps you can determine its rate. The heart is composed of a single median ventricle, and lateral paired auricles. The thick-walled ventricle is somewhat pyramidal in shape with its apex pointing anteriorly. It surrounds the intestine. Two aortae leave the ventricle; the anterior one runs dorsally to the intestine, the posterior one ventrally to the intestine. Posteriorly to the heart, but within the pericardial cavity, the posterior aorta forms a conspicuous swelling, the bulbus arteriosus. (Not present in Elleptio) The paired auricles, triangular in shape, are very thin-walled, and can be distended to a considerable extent.

The circulatory system of Venus is an open system. This means that arteries communicate with veins by way of large spaces called sinuses or lacunae. Blood is pumped by the ventricle of the heart through the two aortae to the viscera and neighboring sinuses. From here, by a network of veins, blood is returned through the kidneys to the gills, and finally brought back to the auricles of the heart.

The Pelecypod heart is an excellent subject for physiological study. The heart can be perfused with various solutions and the effect upon rate of beat observed. If heart levers and kymographs are available, a record of the experiment can be made.

Carefully remove one valve and open the pericardial cavity. Place the animal in a finger bowl containing Molluscan Ringer's solution. Observe the beat and time it. Remove the animal to a second bowl and observe the effect of dropping M/2 KCl on the heart. Add M/2 $CaCl_2$ and note the effect. Return the animal to Ringer's solution for recovery. How does the effect of these salts on the Pelecypod heart compare with their effect on the vertebrate heart? (Clark, 1927; Mobley, 1934; Walzl, 1937, 1938).

Repeat the experiment using different drugs such as nicotine, adrenalin, acetylcholine, and atropine in varying dilutions. (Prosser, 1940; Jullien and Vincent, 1938)

EXCRETORY SYSTEM

Ventral to the pericardium and between it and the posterior adductor muscle, lies a pair of dark glandular organs, the nephridia. Each communicates with the pericardium by a minute opening which is difficult to find, and also with the branchial cavity by another opening more easily seen. Reflect both gills dorsally. This second opening is located on a small papilla which will be described under the genital system. The nephridia are not homogeneous in their composition, but consist of a broad U-shaped tube. The ventral anterior

portion communicating with the heart is glandular; the dorsal posterior part is thin-walled and functions as a bladder. (These organs are much more satisfactorily seen on the fresh water clam.)

The coelom of the Mollusks consist of the sinuses of the blood system, the pericardial cavity, and the lumen of the gonad. Only the last two possess endothelia of their own. The pericardium remains connected with the mantle cavity only by way of the kidney.

DIGESTIVE SYSTEM

On either side of the antero-dorsal edge of the visceral mass lie two triangular flaps, the labial palps. (These are more nearly oval in the fresh water clam.) Both the inner and the outer flap unite with the corresponding structure of the other side, above the mouth, which lies slightly posterior to the dorsal border of the anterior adductor muscle. The palps are abundantly ciliated and cause streams of water to pass into the mouth. They therefore continue the transfer of food particles already brought in by the currents from the gills.

For dissection of the alimentary canal, pick off carefully the tissue from one side of the visceral mass and foot. As you dissect, consult the direction for the nervous system so that you will not destroy the cerebral ganglia. The mouth leads directly into the esophagus. There is no radula or buccal mass in this form. The esophagus soon widens into a thick-walled stomach, lying dorsal to a large brown gland, the liver. The liver connects with the stomach at its anterior end. The stomach continues posteriorly and ventrally, and gradually narrows into the intestine. Trace the loops of the intestine through the visceral mass, until the tube turns dorsally and joins with the portion already identified in connection with the pericardium. From the posterior wall of the pericardium, trace the intestine dorsal to the adductor muscle and finally to its end, the anus, which lies at the base of the dorsal siphon. Return to the pyloric region of the stomach where there is a diverticulum, the caecum, which contains the crystalline style. This style is a solid gelatinous rod which is very conspicuous in some forms (Mya, Mactra) but is not always seen in Venus. It is concerned with the liberation of certain enzymes, and its presence appears to be correlated with an abundant supply of oxygen.

Venus is a typical strainer. This means that food, consisting of Diatoms, Protozoa, and organic detritus, is brought in by the respiratory currents. As the food particles are passed anteriorly along the edge of the gills, the smaller ones become entangled in strings of mucus; the larger ones are dropped to the edge of the mantle and discarded. As the mucous strings approach the mouth, the cilia on the palps continue their transportation into the mouth. Digestion is chiefly intra-cellular by phagocytes lining the dig stive tract, and by wandering leucocytes. Digestive juices from the liver are known to occur, and in addition, the crystalline style liberates an enzyme aiding in the breakdown of carbohydrates.

NERVOUS SYSTEM

Although possessing no well marked head region, the bivalves

display a central nervous system similar to that of other Mollusks. It consists of three paired ganglia and their connectives. Carefully dissect the visceral mass away from the sides of the esophagus. The cerebral ganglio will be seen lying posterior to and about 3 mm. from the dorsal edge of the anterior adductor muscle. It is a round, yellowish organ, about the size of the head of a pin. It is connected with the corresponding ganglion of the other side by a commissure which passes anterior to the esophagus. Two conspicuous connectives pass from each ganglion. One of these courses posteriorly to unite with the visceral ganglion of the same side. The other passes into the foot to unite with the pedal ganglion. The cerebral ganglia also furnish the anterior pallial nerves. With a razor, make a median sagittal section through the foot and into the visceral mass. This should expose the pedal ganglia which lie embedded in the foot dorsal to the muscular portion. To find the third pair of ganglia, cut the united lamellae of the gills ventral to the posterior adductor muscle. On the anterior surface of this muscle, the visceral ganglia will be seen. They are slightly pear-shaped bodies lying so close together that they appear to be single. Trace all connectives as far as practicable.

REPRODUCTIVE SYSTEM

The genital glands consist of a pair of simple gonads, either testes or ovaries, which occupy varying amounts of space in the visceral mass. During the breeding season they are large and conspicuous and fill the spaces around the loops of the alimentary canal. It is difficult to distinguish the paired nature of the reproductive glands. Reflect the gills and follow dorsally along the posterior edge of the foot. Under the attachment of the inner gills lies a small projection, the genital papilla, which contains the opening of the genital ducts. A minute opening, the nephridiopore, is also located on this papilla, but is difficult to see. It is, of course, the external opening for the kidney.

The spawning of Venus occurs in the Cape Cod region from late June to early August, when the temperature of the water has risen to a critical level, about 75°F. Eggs and sperms, numbering into the millions, are freely shed into the sea where fertilization takes place. Spawning in this species is not necessarily completed by one discharge, but may occur several times during the summer. The early embryology of this form is typical for the bivalves. The trochophore and veliger stages are rapidly completed, and during the late summer the young quahog becomes temporarily attached to a substrate by a byssus thread. It soon regains a free existence. For a period of two years the young clam lies relatively dormant during the winter, but grows rapidly during the warm months. By the third summer, it has matured and is ready to spawn.

Mactra solidissima, close relative of Venus, possesses mature eggs and sperm in the late summer, and is therefore available for embryological studies. The sex products can be easily obtained from the ovary (usually pinkish) and from the testes (white) of mature clams. The eggs and sperms should be placed in separate finger bowls of sea water. If the eggs are then fertilized with a dilute sperm suspension, development will proceed. Within 10 minutes of

insemination, the large gern vesicle reacts; within 30 minutes the polar bodies begin to form; and after about an hour the first cleavage takes place. A swimming form is produced in 5 hours. With careful attention, these embryos can be carried over to later stages. (Shecter, 1941).

PELECYPODA

Yoldia limatula Say

Yoldia limatula, a member of the family Nuculidae, belongs to the most primitive order of the bivalves, the Taxodonta. Geographically this small clam has a wide distribution, for it is found not only on the Atlantic coast from Labrador to North Carolina, but also on the Pacific coast and in Europe. It lives in the sandy mud of shallow coves and inlets, and may be found in water up to 5 fathoms in depth. It is most commonly found in the intertidal zone near low tide level, where it can burrow rapidly by means of its large and powerful foot.

Place a lively specimen in a dish of sea water and study its behavior. Observe the foot which is thrust out repeatedly. Notice its general shape, but particularly the contour of the bottom. The temporary broadening at the distal end, together with the temporary concavity of the sole, results in rapid and efficient burrowing. The mantle is open along the greater part of its margin. If the specimen is allowed to remain quiet for a while the elongated, united siphons may be extended. If the animal can be induced to feed, the long feeding appendage may be protruded. This structure is an extension of the outer palp and should not be confused with the siphons. During the process of feeding the appendage is thrust out and inserted into the mud some distance from the shell. Cilia within the groove carry mud mixed with micro-organisms to the mouth.

Remove one valve to study the internal anatomy. The gills, the most primitive type, are small thick organs composed of a double series of parallel plates, suspended from the body wall by a thin muscular membrane. The motion of the gills of Yoldia is characteristic and can be observed through the sheel of young specimens. As blood is forced into the gill plates they are pressed ventrally so that water passes through the gills. Contraction of the suspensory membrane then pulls back the gill plates to their dorsal position. This results in a discharge of water through the dorsal excurrent siphon and inflow of water through the ventral incurrent siphon to the mantle cavity. This respiratory action is rhythmical.

Along the hinge line numerous tiny teeth are present. This condition is considered a primitive characteristic.

PELECYPODA

Pecten irradians Lamarck

Pecten irradians is the small edible bay scallop with the fluted shell. Although its range extends from Maine to the Gulf of Mexico, for commercially importance it is sufficiently common only from Cape Cod to North Carolina. It is included in the order Anisomyaria and the family Pectinidae. Pecten inhabits the shallow waters of protected bays where the bottom is usually a mixture of sand and mud. Although it is often found swimming about in the eel grass where water may be only a foot deep, it may be dredged from depths up to 60 feet. Unlike most bivalves Pecten is often an active swimming form. Usually it swims with a fairly regular motion which is the result of two jets of water sent out through temporary openings formed by the mantle edges near the hinge line. Since these jets are sent dorso-anteriorly and dorso-posteriorly, the scallop progresses ventrally (forward). Occasionally, if startled, the scallop swims by a series of quick, violent jerks, the result of a rapid closing and opening of the valves. This motion is possible because of the striated muscle fibres within the single, highly modified and enlarged adductor muscle. In this case, water within the mantle cavity is ejected ventrally and the animal therefore moves dorsally (backward). The scallop is an early example of jet propulsion.

Normally the scallop rests on its right valve. On each side of the beak are projections of the shell called wings. The posterior wing has a broad connection with the valve; the anterior wing possesses a notch or indentation, with a resulting narrow connection to the valve. If the specimen is allowed to relax, the valves will gape slightly. Along the edges of the mantle, now slightly separated, notice the velar fold, the pallial eyes, and the tentacles. The velar fold, which guards the tentacles, appears to be sensitive to certain irritating chemical stimuli. To test this sensitivity, place a few drops of extract of starfish near the volum. (Gutsell 1930). The tentacles are particularly sensitive to mechanical stimulation. Test this by touching one or two with a needle. The eyes are sensitive to a decrease in light, such as a shadow passing over the animal. However, only the eyes directly in line with the light are affected, and stimulation of at least two eyes is necessary to elicit a response. Structurally, the eye shows considerable differentiation for it is composed of a cornea, lens, retina, and optic nerve. These are not comparable to the structures of the same name in the vertebrate eye.

Pry open the valves and cut the huge adductor muscle which is composed of two sections, a large anterior striated section used in rapid swimming, and a small posterior unstriated section used for holding the valves closed over a long period of time. The gills are crescentic structures located on the antero-ventral margin of the visceral mass. Study their muscular movement which allows them to be drawn together during swimming. This contraction results in the formation of frequent vertical folds which cause temporary elevations and depressions along the surface of the lamellae. The gills are composed of a series of parallel filaments loosely attached to each other. There are two types of filaments, the larger principal filaments, and the smaller common filaments. (Fig.) The former usually

lie at the bottom of each depression. Examine a mounted portion of each gill. Between the principal filaments, the inter-lamellar junctions are present. Distinguish in addition the inter-filamentous junctions. Near the periphery of the gills they are simple clumps of modified cilia, but near the inter-lamellar junctions, they become much larger and more prominent. There are no siphons. Water flows freely through the mantle cavity. At the anterior dorsal edge of the visceral mass, lie two small organs of similar size, the degenerate foot, and the mouth, surrounded by a fringed palp. A dissection of the entire digestive tract is not always practicable. However, the large posterior end of the intestine, posterior and dorsal to the adductor muscle, is easily seen. Identify the hear, which lies in the median dorsal line.

REPRODUCTIVE SYSTEM

The hermaphroditic gonad is a conspicuous organ lying anterior and ventral to the adductor. The ovary occupies the anterior and ventral portion, and is bright pink when the eggs are ripe. The cream colored testis lies slightly dorsal to the ovary. In the scallop there is often a dense black pigmentation in the membranes which cover the gonad.

Spawning in this form occurs in the late summer and early autumn. Although it is hermaphroditic, both eggs and sperms are shed into the sea where fertilization occurs. Development is rapid with the formation of a veliger larva in a few days. Like many bivalves the young scallop passes through a short period of attachment by its byssus thread to eel grass or other vegetation., but soon becomes free. It grows rapidly, spawns in its second summer, and usually dies near the end of its second year.

CEPHALOPODA

Loligo pealeii Lesueur

Loligo pealeii, commonly known as the squid, belongs to the order Dibranchiata and the famil Loliginidae. It is a littoral form inhabiting the east coast of the United States from Maine to South Carolina. Although the eggs may be found in water as deep as 25-50 fathoms, the adult squid is usually found as an actively swimming form of shallow waters. The winter habits are little know, but in the spring and early summer the squid appear forming schools of 10-100, and breed near shore.

Study a living specimen in the aquarium or in a large finger bowl at your desk. Notice its position as it is resting, and watch the characteristic rhythmic, gentle, movements of the lateral fins. These rhythmic contractions of muscles insura a continuous supply of water for the gills which are within the mantle cavity. Touch the squid so that he swims actively. Try to observe the funnel through which water is ejected in spurts. When the funnel is ejecting water backward, the animal moves forward; when it is ejecting water forward, the animal moves backward. The squid was an early perfector of jet propulsion. Observe the movements of the arms which help in steering the animal.

Notice the rapidity of the color change in the integument. This is due to the simultaneous expansion or contraction of many chromatophores. Their activity can best be studied on a newly hatched specimen placed under the microscope. The chromatophores in the squid are more like small organs than the individual cells of the vertebrates. Each is composed of one pigment cell surrounded by a membrane to which 10-30 muscles are attached. Innervation is from the mantle nerves. Contraction of the musclescells results in the dispersion of the pigment within the chromatophore and the consequent blush of color over the animal.

If the animal becomes irritated he may eject the contents of his ink sac.

Allow your fingers to become contacted with the suckers of the arms. Place a few small fish, 1-2 inches, in the aquarium with the squid and observe his reactions.

ORIENTATION

If possible dissect a fresh specimen. It is important to orient the squid in terms not only of its morphology but also of its activity since these are not identical in this animal. Hold the animal with its pointed end uppermost, its head down. The apex of the long, cone-shaped body is the most dorsal point of the animal. The arms, Tentacles, and funnel form the ventral surface. The mouth indicates the anterior surface, the funnel the posterior. When the animal swims, he moves forward and backward, with the antero-dorsal side uppermost. Functionally, therefore, the morphological antero-dorsal side is dorsal; the postero-dorsal side is ventral; the ventral surface is anterior; and the dorsal surface is posterior. The functional meaning of the terms will be used in the following directions.

EXTERNAL ANATOMY

At the end of the head is the modified foot, which is drawn out into five pairs of arms surrounding the mouth. These arms are of two types, four pairs of true arms which are sessile, and of about equal length, and one pair of elongated retractile tentacles. Each arm bears two rows of suckers which decrease in size from the base to the tip of the arm. Each sucker is composed of a cup which is attached to the arm by a pedicle. Remove a sucker and study it under the microscope. Notice the toothed chitinous ring which supports the edge of the cup, and the piston which forms the central basal portion of it. Compare the elongated peduncle and terminal club of the tentacle with an arm. If the specimen is an adult male, the lower left arm (4th left) will show a modification of the suckers called hestocotyly. This results in a decrease in the size of the suckers with a simultaneous increase in the size of the pedicles. It is thought that the hectocotylized arm is modified for the transference of sperm to the horseshoe organ on the buccal membrane of the female.

Turn back the arms and tentacles and observe the muscular membrane extending from their bases toward the mouth. This membrane is divided into an outer part, the buccal membrane, and the inner part, the peristomial membrane. The buccal membrane consists of seven projections, each with suckers on the inner surface. The peristonial membrane surrounds the mouth opening through which the chitinous beaks are usually visible. If the specimen is a female, notice the horseshoe organ or sperm receptacle which lies on the buccal membrane, in the median line below the mouth.

On the head is a pair of large well developed eyes, in which the cornea, iris, pupil, and lens can easily be identified. Behind the eye is a fold of tissue, the free edge of which is covered by the mantle. This is the olfactory crest. Within its concavity is a special sense organ, to which has been attributed the function of olfaction, but experimental evidence in proof of this is lacking. In front of the eye is the small aquiferous pore which communicates by the aquiferous canal with the outer chamber of the eye. The activity of the pore is regulated by a sphincter muscle. On the upper side, projecting from beneath the mantle, lies the funnel. Although the funnel of the squid and the siphon of the clam both eject water from the mantle cavity, and are therefore similar in function, the two organs cannot be homologized. The funnel is a modification of the foot, while the siphon is an extension of the mantle.

The mantle completely envelopes the remaining part of the body. The free anterior edge of the mantle is called the collar. Notice the three scallops into which the collar is divided. The projections on either side of the funnel mark the position of the pallial cartilages. The single projection on the dorsal midline indicates the anterior end of the pen, or internal shell which will be described under the skeletal system. Posteriorly the mantel extends as a long cone, flanked at the apex by two lateral fins. These consist of three sets of muscles, longitudinal, transverse, and vertical, which result in strong yet flexible fins. Not only are they able to make slow, undulating movements which propel the squid

forward or backward, but they can also make swift, powerful strokes which instantly reverse the direction of progression.

INTERNAL ANATOMY

Open the mantle cavity by cutting the mantle a little to one side of the mid-ventral line from the edge of the collar below the funnel to the apex of the cone. The thick wall of the mantle is composed, almost entirely, of a sheet of circular muscles covered both inside and outside by integument. The funnel contains both circular and longitudinal fibres. The latter are continuations of the large prominent pair of retractors of the funnel. These are attached dorsally to the pen. There is also a pair of small protractors which attach the funnel to the cephalic cartilage of the head. Cut open the funnel in order to find the muscular valve on the dorsal wall near the external opening. The valve prevents water from entering the funnel from the exterior. Notice the valve-like extension of the muscular side walls of the funnel into the mantle cavity. These prevent the escape of water from the mantle cavity by way of the space between the collar and the funnel. Medial and dorsal to the retractors of the funnel are the cephalic retractors which extend from the head cartilage to the pen.

The contraction of muscles in the mantle control the passing of water into and out of the mantle cavity. When the circular muscles relax and the longitudinal muscles contract, the mantle cavity enlarges, and consequently water is drawn in between the collar and the head. When the circular muscles contract and the longitudinals relax, the cavity decreases in size, the collar is drawn tightly around the head, and water is expelled through the funnel.

The visceral mass occupies most of the space in the mantle cavity. For ease in further study identify the following obvious organs contained therein, although they may seem to have little relation to each other at this time. Extending for a short distance into the funnel is the white rectum, the opening of which lies between two small flaps of tissue, the rectal paillae. Dorsal to the rectum is the silvery ink sac. Avoid breaking it. The paired gills lie along the lateral border of the visceral mass to which their proximal ends are attached. The distal ends extend anteriorly and are attached to the lining of the mantle. At the base of each gill is a rounded organ, the branchial heart.

If the specimen is a female, a pair of large, white, elliptical nidamental glands will cover a considerable portion of the anterior viscera. Each gland has a small opening at its anterior end, in front of which lies a small accessory nidamental gland. The accessory glands, which are often bright red before egg laying, have a small opening on the ventral surface. The function of the nidamental gland is to secrete the outer capsules for the egg masses, but the function of the accessory glands is not determined. Lift the left nidamental gland to expose the large, flaring opening of the oviduct. Slightly posterior to the opening, the wall is swollen to form the oviducal gland, the function of which is to produce the spherical capsules of the individual eggs. The conical portion of the viscera contains a large mass of eggs, which are considered to

be lying within the ovary and early parts of the oviduct.

If the specimen is a male, the slender penis will lie at the left of the rectum and slightly posterior to it. Between the bases of the gills are located the paired, somewhat triangular kidneys.

RESPIRATORY AND CIRCULATORY SYSTEMS

Obtain an injected specimen. The paired branchial hearts have already been identified. They receive venous blood from the large veins which drain the body. From the head, a single large vein, the anterior vena cava, proceeds in the median line to the kidneys. As it meets the kidneys it divides into a right and left precava, which enter and pass through the nephridial sacs. As the precavae emerge from the kidney, they diverge and enter the corresponding branchial hearts. Before entering the branchial heart, the right precava receives three branches; the right mantle vein returning blood from the anterior portion of the mantle; the right postcava collecting blood from the posterior portion of the mantle; and the genital vein. The left precava receives two branches, the left mantle vein and the left postcava. The branchial artery carries blood from each branchial heart to the gills. It courses along the dorsal edge of the filaments. The branchial vein returns blood from each gill to the systemic heart, an asymmetrical organ lying in the mid-line. The openings of these veins into the heart are protected by a pair of semi-lunar valves. The aortae leave the heart. Each is protected by a single semi-lunar valve. The anterior aorta, the larger one, leaves the heart at its anterior projection and runs parallel with the esophagus to the head. The posterior aorta divides immediately into three mantle arteries; the median one which runs anteriorly along the ventral surface of the mantle cavity; and the paired lateral arteries which run obliquely and posteriorly to supply the dorsal surface of the mantle cavity. From the anterior border of the heart a small genital artery runs forward a short distance and then bends dorsally.

EXCRETORY SYSTEM

If the specimen is a female remove the nidamental glands to expose the underlying paired kidneys. These are usually white or pale, except in the case of injected specimens. The intimate connection of the kidney with the venae cavae results in the assumption by the kidney of the color of the injection fluid. The kidneys appear to be simple, triangular structures with the longest extension anterior. The papilla, enclosing the nephridiopore, lies at the tip of each extension and on either side of the rectum. However, the kidney is more complex than it appears, for it is composed of a large bilobed sac which encloses and hides the urinary gland.

DIGESTIVE SYSTEM

Remove the funnel and its muscles and dissect the head along its median line. The buccal mass or bulb is a hard, round, organular organ, on the anterior surface of which are the horny beaks.

Pry them open, and note their interlocking arrangement as well as the odontophore and radula enclosed within. Posterior to the buccal bulb are the paired buccal salivary glands. The esophagus, a thin-walled narrow tube, leads out of the bulb and into the stomach. It enters the liver, passes dorsally, later ventrally, and joins the stomach near the middle of the visceral mass. In the adult squid, the liver is a single, median, coneshaped organ, with its broad base near the collar. The pancreas is a U-shaped organ lying anterior to the stomach. The ducts of the pancreas and liver unite and enter the caecum as a single hepato-pancreatic duct. The stomach is a thick-walled muscular organ, which communicates directly with the caecum, a long sac which may extend to the apex of the visceral mass. It is into this caecum that the single hepato-pancreatico duct leads. Close to the entrance of the esophagus into the stomach is the opening of the intestine, a short tube which runs forward between the lobes of the pancreas. A constriction marks the beginning of the rectum which terminates in the anal opening.

The squid is a carnivorous Mollusk, preying upon small fish which it catches easily. The suckers of the arms hold fast the prey once it is caught, and the radula and jaws devour it.

NERVOUS SYSTEM

On the inner dorsal surface of the mantle at the level of the tip of the gills, lie the large stellate ganglia. From these, several nerves radiate into the mantle, and one, the visceral nerve, connects with the visceral ganglion in the head. With the exception of the stellate, the paired ganglia of the central nervous system are concentrated in the head. Four pairs lie clustered around the esophagus a short distance posterior to the buccal bulb. With a razor cut a median sagittal section through the head. Above the esophagus lies the supra-esophageal ganglion, a single rounded mass which represents the fusion of the pedal and visceral ganglio. Completing an esophageal ring by forming its sides are the paired pleural ganglia. The fibres of the infra-esophageal ganglion extend forward to join with the paired propedal ganglia. Those from the supra-esophageal connect with the small supra-buccal ganglia.

The eye of the squid is a highly modified one, similar to but not identical with that of the vertebrate. Each eye is closely applied to the cephalic cartilages which partly surround it, and thus form an incomplete orbit. The cornea, iris, pupil, and lens have been previously identified. Carefully remove the eye, and as you do so observe the large optic ganglion to which the eye is connected by a short optic nerve. Float the eye in a dish of water. The eye consists of an anterior and posterior chamber which are separated from each other by the lens. The outer wall of the anterior chamber is formed by the broad, transparent cornea. The dark iris, just anterior to the lens, may surround either a circular or reniform pupil. When the eye is exposed to light, a fold of the iris projects across the circular opening, resulting in the reniform or crescentic shape of the pupil. The lens is attached to the ciliary body located behind it. The posterior chamber is filled by a semi-fluid, transparent mass, the vitreous humour. Forming the posterior and lateral walls of this chamber is the retina which

extends forward as far as the ciliary body. The inner layer of the retina contains the rods which are directed anteriorly. The outer layer is composed of ganglionic cells which converge to form the optic nerve. Change of focus is accomplished in the following manner. The muscles of the ciliary body, by changing the pressure on the vitreous humour, indirectly move the lens forward or backward and thus increase or decrease the distance between the lens and the retina. External to the retina is a tough, cartilaginous layer, perhaps comparable to the sclerotic coat of the vertebrate eye.

The olfactory crests have already been noted.

Two statocysts, probably organs of equilibration, lie embedded in the cephalic cartilage below the visceral ganglion. Each is composed of a cavity, lined with epithelium, a portion of which is ciliated. Upon this ciliated area the statolith lies.

SKELETAL SYSTEM

The most conspicuous part of the skeleton is the pen, a transparent chitinous structure which is the internal shell. The pen lies in the mid-dorsal line and extends from the edge of the collar to the apex of the mantle. It articulates at its anterior end with the nuchal cartilage which is embedded in the muscle lying between the visceral mass and the pen. On either side of the base of the funnel lie the infundibular cartilages which articulate with the pallial cartilages embedded in the mantle. In the head is the cephalic cartilage which serves as a protection to the ganglia concentrated there. The cephalic cartilage consists of very irregular plates perforated by foramina for esophagus, blood vessels, and nerves.

REPRODUCTIVE SYSTEM

Remove the left gill and branchial heart. In the mole at the apical end of the visceral mass lies the single large white testis, which opens directly into the coelom by a slit at its anterior end. Near this opening is located a ciliated funnel, the entrance to the vas deferens. The vas deferens is a very slender coiled tube which appears opaque white due to the tightly packed sperms contained within. The tube continues forward between two organs into which the vas deferens eventually leads. On the right is the transparent spermatophoric sac containing packets of sperm called spermatophores. On the left is the somewhat convoluted, thick-walled sac, the spermatophoric organ which receives the sperms brought to it by the vas deferens. Here the sperms receive appropriate secretion and membranes, and are packed into bundles, the spermatophores, which are then transferred to the spermatophoric sac. In this chamber the spermatophores are temorarily stored before being ejected by the muscular penis.

Remove several spermatophores from their sacs and place them in a dish of sea water. If the animal is a fresh specimen, the spermatophores may ejaculate as they are being transferred. Place

a few in a solution of $\frac{1}{4}$ saturated MgCl, for 10 minutes. This treatment will slow down the ejaculation process so that it may be watched.

Each spermatophore consists of an outer transparent tunic which has a cap and a cap thread at the smaller oral end. Within the tunic are three important structures. At the oral end is the ejaculatory organ which has the appearance of a coiled spring. Beyond it is the small cement gland, followed by the large sperm mass. When the cap is broken, the ejaculatory organ turns wrong side out and pulls the cement gland and sperm mass with it. The sperm mass is thus automatically cemented to the object upon which it is thrown. Under usual conditions, this object is the sperm receptacle or horseshoe organ of the buccal membrane of the female. (Drew 1919)

If the specimen is a female, the apical region of the visceral mass will be occupied by the ovary. Through a ciliated funnel which is not readily seen, the eggs pass into the oviduct. In this region the oviduct is a transparent tube tightly packed with eggs and scarcely distinguishable from the ovary. Trace the oviduct as it runs first anteriorly, then posteriorly, and finally anteriorly again to become the thick-walled glandular portion, the oviducal gland. The terminal portion is the short, flared opening of the duct. The horseshoe organ, the nidamental glands, and the accessory nidamental glands have already been described.

During the breeding season the male squid becomes very excited, and displays a courtship behavior which culminates in the deposition of spermatophores within the female (Drew, 1911). He may thrust them into the mantle cavity of the female, or he may place them within the seminal receptacle on her buccal membrane. The eggs may therefore be fertilized either within the cavity, before their extrusion through the funnel; or they may be fertilized after their expulsion as they are held within the arms of the female squid before transference to the place of attachment.

At the time of intrusion the eggs are enclosed in two cases, the single capsule about each egg, and the gelatinous matrix surrounding the capsules. The female holds the fluid, gelatinous mass within her arms, until she finds a suitable spot for attachment. Then she deposits 10-20 elongated egg masses at one central point. These slender masses of semi-fluid substance stiffen in contact with the sea water, so that they soon have the consistency of a stiff jelly, and the appearance of a long finger. Squid egg masses are usually found attached to rocks below the low tide mark. The eggs, which are relatively large, contain an abundance of yolk, and cleavage is therefore meroblastic. A rudimentary trochophore larva, but no veliger, occurs during early development. The young squid hatch within 2-3 weeks. It is thought that two years is the normal life span of the squid, although possibly some live through the third summer.

Squid eggs develop readily in the laboratory if kept in plenty of cool running sea water. They can be examined from time to time, since their development is slow, and successive stages studied. It is very difficult to raise the young squid beyond the point of hatching.

MOLLUSCA
SELECTED REFERENCES

Andrews, E. A. 1940 — The snail, Neritina virginea, in a changing salt pond, Ecology 21: 335-346

Atkins, D. 193 6 — On the ciliary mechanism and interrelationships of lamellibranchs. 1. New observations on sorting mechanisms. Quart. Jour. Mic. Sci.79:181

---------- 1937 — 11. Sorting devices on the gills. Ibid 79:339

---------- 1937 — 111. Types of lamellibranch gills and their food currents. Ibid. 79:375

---------- 1937 — 1V. Cuticular fusion, with special reference to the fourth aperture in certain lamellibranch. Ibid 79:423

Atkins, Daphne 1943 — On the ciliary mechanisms and interrelationships of Lamellibranchs. V111. Notes on gill musculature in the Microciliobranchia. Q. J. Mic. Sci. 84: 187-256

Baldwin, Ernest 1933 — On Cehalopod Phosphagen. J. Exp. Biol. X:222-229

Belding, David L. 1915 (1916) — Report on Mass. Clam Fisheries. Pub. Document #25 50th Annual Rep. Commission. Fish and Game

Benham, W. B. 1942 — 1. The octopodous Mollusca of New Zealand. Trans. Roy. Soc. New Zeal. 72: 226-236

---------- — 11. The octopodous Mollusca of New Zealand. Ibid. 73:53-54

Benham, W. B. 1943 — Octopodous Mollusca of New Zealand. 111 and 1V. 111. Trans. Roy. Soc. NZ. 73, 139-153 1V. Trans. Roy. Soc. N. Z. 73, 255-261

Bockeley, C. 1935 — The chemical composition of the crystalline style and of the gastric shield with some new observations on the occurrence of the style oxidase. Biol. Bull. 68:107

Bøggild, O. B. 1930 — The shell structure of the mollusks. Mem. d'Acad des Sci. et des Lett. de Denmark 2:233-325

Carriker, M. R. 1943 — On the structure and function of the proboscis in the common oyster drill Urosalpinx cinerea. J. Morph. 73: 441-506

Coe, W. R. 1932 — Histological basis of sex changes in the American oyster (Ostrea virginica) Sci. 76:125

Coe, W. R. and Dennis L. Fox 1942 — Biology of the California Sea Mussel (Mytilus Californianus.). Influence of temperature, food supply, sex and age on the rate of growth. J. E. Z. 90:1-30

Coe, W. R. 1944 — Sexual Differences in Mollusca Quart. Rev. Biol. 19, 85-97

Coe, W. R. and Denis, L. F. 1944 — Biology of the California sea mussel (Mytilus Californianus). lll. Environmental conditions and rate of growth. Biol. Bull. 87, 59-72

Coe, W. R. 1945 — Nutrition and growth of the Californian bay mussel (Mytilus edulis diegensis). J. Exp. Zoo 99: 1-14

Coe, W. R. 1946 — A resurgent population of the California bay mussel (Mytilus edulis diegensis). J. Morph. 78: 85-101

Commonwealth of Mass. 1909 — Report on Mollusc fisheries

---------- 1910 — Report on Scallop Fisheries

---------- 1912 — Report on Quahog and Oyster

Daiter, Ralph W. 1943 — Observations of the local movements of Littorina littorea and Thais lapillus. Nautilus 57: 6-8

Elsey, C. R. 1925 — On the structure and function of the mantle and gill of Ostrea gigas (Thunberg) and Ostrea lurida (Carpenter). Trans. Roy. Soc. Canada 29:131

Emerson, A. E. 1943 — Ecology, Evolution, and Society. Am. Nat. 77:97-118

Engle, James B. 1942 — Growth of the oyster drill, Urosalpinx cinerea, feeding on four different food animals. Anat. Rec. 84:505

Everdam, W. J. 1941 — Cleaning shells with aid of blow fly maggots; Nautilus 53: 145-146

Field, I. A. 1922 — Biology and economic value of the sea mussel Mytilus edulis. Bull. Bur. Fish. 38:127

Fox, Denis L. and W. R. Coe 1943 — Biology of the California Sea Mussel (Mytilus californianus) 11. Nutrition, metabolism, growth and calcium deposition J. E. Z. 93: 205-249

Fraeme, M. E. 1943 — Crab-mussel association. Victorian Nat. 59:156

Galtsoff, Paul 1928 — Experimental study of the function of the oyster gills, and its bearing on the problems of oyster culture and sanitary control of the oyster industry. Bull. U. S. Bur. Fish. 44: 1-39

Galtsoff, Paul S. 1934 The biochemistry of the Inveretbrates of the sea. Ecolog. Monogr. 4:481-490

Gould, A. A. and W. C. Binney 1870 Invertebrata of Mass., State Report

Graham, G. 1937 The structure and function of the alimentary canal of Aeolid molluscs, with a discussion of their nematocysts. Trans. Roy. Soc. Edinburgh 59:267

Graham, H. W. and Helen Gay 1945 Season of attachment and growth of sedentary organisms at Oakland, California. Ecol. 26:375-386

Greve, Benj. H. 1928 Natural History of the shipworm, Teredo naualis at Woods Hole. Mass. Biol. Bull. 55:260-282

Greve, B. H. and Jay A. Smith 1936 Sex inversion in Teredo navalis and its relation to sex ratios. Biol. Bull. 70:342-3 43

Gutsell, J. S. 1930 Natural history of the bay scallop. Bull. U. S. Bur. Fish. XLVI, 569-632

Hoyes, F. R. and D. Pelluet 1939 (1940 Inorganic constituents in Molluscan blood and muscle. Rep. W. H. O. I. 19, 20

Hopkins, A. E, 1934 Accessory hearts in the oyster, Science 80:411

Howells, H. H. 1942 The structure and function of the alimentary canal of Aplysia punctata. Q. J. Micr. Sci 83:357-397

Ingram, W. M. 1941 Habits of Land Mollusca. Am. Midl Nat. 25:644-651

Jacot, A. 1924 Marine Mollusca of the Bridgeport, Conn. Region Naut. 38:49, Oct.

Johnson, C. W. 1904 Notes on some Cape Cod Mollusca. Naut. 18:#4, 47 (marine and fresh water).

---------- 1915 Fauna of New England. #13 List of Mollusca Occasion. Papers Boston Soc. Nat. Hist. Vll.

---------- 193 4 List of the marine Mollusca of the Atlantic coast from Labrador to Texas Proc. Boston Soc. Nat. Hist. 40:1-204

Kellogg, J. L. 1903 Feeding habits and growth of Venus mercenaria N. Y. State Bull. 71, Zool.10 (Univ. of N. Y.) 296

---------- 1900 Life history of the common clam. Biol. Lab. U. S. Fish. Com. Woods Hole, Mass. (U. S. Fish Com. Bull. year 1899) pp. 193-202

Kepner, W. A. 1943 — The manipulation of the nematocysts of Pennaria tiarella by Aeolis pilata. J. Morph. 73:297-319.

Krogh, August 1934 — Conditions of life in the ocean. Ecol. Monogr. 4:421-429

LeBour, M. V. 1937 — Eggs and larvae of British Prosbranchs with special reference to those living in plankton. J. Mar. Biol. Assoc. 22, 105-166

Loosanoff, V. L. and J. B. Engle 1943 — Polydora in oysters suspended in the water. Biol. Bull. 85: 69-78

Lysaght, Averil M. 1943 — Incidence of larval trematodes in males and females of the gastropod Littorina neritoides (L) on the Plymouth breakwater. Parasitology 35:17-22

MacGinitie, G. E. 1934 — The egg laying activities of the sea hare, Tethys californicus (Cooper). Biol. Bull 67:300

MacGinitie, G. E. — On the Method of Feeding of Four Pelecypods. Biol. Bull. 80:#1, p. 18

Matthews, S. A. 1928 — The palps of lamellibranchs as autonomous organs J. Exp. Zool. 51, 209-264

Medcof, J. C. and A. W. Needler 1941 — Influence of temperature and salinity on condition of oysters. J. Fish. Res. Board Canada 5:253-257

Moore, H. B. 1936 — The biology of Purpura lapillus. I. Shell variation in relation to environment. Jour. Mar. Biol. Ass'n. 21:61

----------1938 — The biology of Purpura lapillus. III. Jour. Mar. Bio. Ass'n 23:67

Nelson, T. C. 1918 — On the origin, nature, and function of the crystalline style of lamellibranchs. Jour. Morph. 31:53

Nelson, T. C. 1928 — Pelagic dissoconchs of the common mussel, Mytilus edulis, with observations on the larvae of allied genera. Biol. Bull. 55, 180-192

----------1925 — Recent contributions to the knowledge of the crystalline style of lamellibranchs. Biol. Bull. 49:86

Orton, J. H. 1937 — Oyster biology and oyster culture. Edwin Arnold, London

Perry, Louise M. 1940 (1942 second print) — Marine shells of southwest coast of Florida. Bull. Amer. Paleontol. 26:260

Reese, A. M. 1942 — The old starfish-clam question Sc. 96:513-515

Ridgewood, W. G. 1903 On the structure of the gills of the Lamellibranchia. Phil. Trans. Roy. Soc. London B 195:147

Rothschild, M. 1936 Gigantism and variation in Peringia ulvae Pennant 1777, caused by infection with larval trematodes. J. Mar. Biol. Ass'n 2:537

--------- 1941 The effect of trematode parasites on the growth of Littorina. J. Mar. Biol. Ass'n Plymouth 25:69-80

Scharrer, E. 193 5 Uber das Hanstromische Organ Xbei Opiathobranchiern. Pubb. d. Sta. Zool. Napoli 15:132

Scharrer, B. 1941 Endocrines in Invertebrates. Physiol. Rev. 21: No. 3, p. 383

Schecter, Victor 1943 Tolerance of the snail Thais floridans to waters of low salinity. Ecol. 24:493-499

Smith, M. 1937 East Coast marine shells. Edwards Bros. Inc. Ann Arbor, Mich.

Stauber, Leslie A. 1942 The oyster crab, Pinnotheres ostreum, parasitic on the oyster. Anat. Rec. 84:495-496

Stiles, K. A. and Nettie R. Stiles 1943 The pearl, a biological gem. Bios. 14:69-80

Verrill, A. E. 1875 The Collossal Cephalopods of the North Atlantic Am. Nat. 9:21

Verrill, A. E. and S. I. Smith 1874 The invertebrate animals of Vineyard Sound and adjacent waters, U. S. Gov't Print. Office

Wald, G. 1939 (1940) Vitamin A. and Retinene in squid eye tissues. Rep. W. H. O. I. pp. 33

Weymouth, F. W. and H. C. McMillin 1930 Relative growth and mortality of the Pacific razor clam (Siliqua patula, Dixon) and their bearing in the commercial fishery. Bull. U. S. Bur. Fish. 46: 543-567

Weymouth, F. W. and S. H. Thompson 1930 The age and growth of the Pacific cockle (Cardium corbis, Martyn). Bull. U. S. Bur. Fish. 46: 633-641

White, K. M. 1937 Mytilus. Memoirs on typical British plans and animals. Liverpool Mar. Biol. Comm. 31:1-177

Winkley, H. W. 1907 Cape Cod Notes. Naut. 21:74 Nov. (Mar. & Fr.w)

Wood, A. E. and H. E. Wood 1927 A qualitative study of the marine Mollusca of Cape May county, N. J. Naut. 41:8 July

Hurtz, Charles B. 1941 Studies of the mantle of Triodopsis tridentata (Say) Proc. Penn. Acad. Sci. 15:32-35

Yonge, C. M. 1936 The evolution of the swimming habit in the Lamellibranchia. Mem. Mus. Royal d'Hist. Nat. Belgique, Deux, Ser. Fasc. 3:77

---------- 1942 Ciliary currents in the mantle cavity of the Atlantidae Q. J. Micr. Sci. 83: 197-203

Young, J. Z. 1936 The giant nerve fibers and epistellar body of Cephalopods. Quart. Jour. Mic. Sci. 78

---------- 1939 Fused neurons and synaptic contacts in the giant nerve fibers of cephalopods. Phil. tr. Roy. Soc. B. 229-465

Young, R. T. 1942 Spawning season of the Californian mussel, Mytilus californianus. Ecol. 23:490-492

PHYSIOLOGY

Aneloos, Marcel 1942 Sur la regeneration de la tete des Nollusques Gastropodes. Compt. Rendu. Acad. Sci. (Paris) 214:883-884

Allan, J. 1941 New parts for old in the shell fish world Aust. Mus. Mag. Sydney 7:280-285

Andrews, E. A. 1934 Shell repair by the snail Pleurodonte rosstrata Pfr. Biol. Bull. 67:294

---------- 1935 Shell repair by the snail Neritina. Jour. Exp. Zool 70:75

Coe, W. R. 1934 Sexual rhythm in the polecypod mollusk, Teredo Science. 80:192

----------1934 Alternation of sexuality in oysters. Am. Nat. 68:236

---------- 1935 Sexual phases in prosobranch mollusks of the Genus Crepidula. Science 81:570

---------- 1936 Sex Ratios and sex changes in mollusks. Mem. Musee Royal d' Hist. Nat. de Belgique Deux. Ser., Fasc. 3:69

----------1936 Evironment and sex in the oviparous oyster Ostrea virginica Biol. Bull. 71:353

----------1936 Sequence of functional sexual phases in Teredo Biol. Bull. 71:122

---------- 1936 Sexual phases in Crepidula. Jour. Exp. Zool. 72:455

---------- 1936 Conditions influencing change of sex in mollusks of the genus Crepidula. Jour. Exp. Zool. 77:40 1

Coe, W. R. 1943 Development of the primary Gonads and Differentiation of Sexuality in Teredo navalis and other pelecypod Molluscs. Biol. Bull. 84:178

---------- 1943 Sexual Differentiation in Mollusca 1. Pelecypoda. Quart. Rev. Biol. 18:154

Ccle, H. A. 1942 Primary sex phases in Ostrea edulis, Q. J. Micr. Sci 83: 317-356

Drew, G. A. 1911 Sexual activities of the squid, Loligo paelii. 1. Copulation, egglaying, and fertilization. J. Morph. 22:327-359

---------- 1919 11. Spermatophore structure, ejaculation, and formation. J. Morph. 32: 379-43 5

Forbes, Grace S. 1942 The effect of added amounts of vitamin B on the life cycle of Lymnaea columella. Anat. Rec. 84: 526

Galtsoff, P. 1938 a. Physiology of reproduction of Ostrea virginica 1. Spawning reactions of the female and male Biol. Bull. 74:461

---------- 1938 b. 11. Stimulation of spawning in the female oyster Ibid. 75:286

---------- 1940 111. Stimulation of spawning in the male oyster Ibid. 78:117

----------1942 Accummulation of manganese and the sexual cycle in Ostrea virginica. Physiol. Zool. 15:21-215

Grave, B. H. 1942 Sexual cycle of the ship worm Teredo navalis Biol. Bull. 82:438-445

Hanstrom, B. 1939 Hormones in Invertebrates. Clarendon Press.

Hartline, H. K. 1938 The discharge of impulses in the optic nerve of Pecten in response to illumination of the eye. Jour. Cell and Comp. Physiol. 11:465

Holmes, Wm. 1940 Color changes and color patterns of Sepia officinalis L. Proc. Zool. Soc. London. Ser. A. 110:17-35

Hopkins, A. E. 1936 Pulsation of blood vessels in oyster Ostrea lurida and Ostrea gigas. Biol. Bull. 70:413-425

Jennison, M. W. and J. W. M. Bunker 1934 Analysis of movement of cilia from the clam (Mya)

by high speed photography with stroboscopic light. J. Cell. and Comp. Physiol. 5: 189-197

Jones, David T. 1935 Formation of shell in Tiger snail J. Morph. 57:547-565

Jullien, A. and L. Vincent 1938 Sur l'action de l'acetylcholine sur le coeur des mollusques. L' antagonisme curare acetylchlorine. Compt. Rend. Acad. Scie. T.206-209

Lameere, A. 1936 Histoire de la classification des mollusques. Mem. Mus. Royal d' Hist. Nat. de Belgique, Deux. Ser. Fasc. 3:1

Loosanoff, V. 1936 Sexual phases in the quahog. Science 83:287

---------- 1937 Development of the primary gonad and sexual phases in Venus mercenaria Linnaeus.Biol. Bull. 72:389

----------1937 Seasonal gonadal changes of adult clams, Venus mercenaria (L) Bio. Bull. 72:406

----------1942 Seasonal Gonadal changes in the adult oyster, Ostrea virginica. Biol. Bull. 82:195

----------1942 Accummulation and Discharge of sperm by Oysters living at different depths. Biol. Bull. 82:413

Maloeuf, N. S. R. 1937 The energy source of the mussel (Mytilus edulis during oxygen lack. Zeit. f. Verg. P hys. Bd. 25, S. 43

Motley, H. L. 1934 Physiological studies concerning the regulatior of heartbeat in fresh water mussels. Phys. Zool. 7:62

Otis, Arthur B. 1942 Effects of certain drugs and ions on the oyster heard. Physiol. Zocl. 15:418-435

Prosser, C. L. 1940 Acetylcholine and nervous inhibition in the heart of Venus mercenaria. Biol. Bull. 78: No. 1 page 92

Prytherch, H. F. 1929 Investigations of the physical conditions controlling spawning of oysters, and the occurrence, distribution, and setting of oyster larvae in Milford Harbor, Conn. Bull. U. S. Bur. Fish XLIV, 1928 (1929) 429-503

Prytherch, H. F. 1934 Role of copper in the American oyster. Ecol. Monogr. 4:49-107

Robertson, James D. 1941 The function and metabolism of calcium in the invertebrata. Biol. Rev. 16: 106-133

Wait, R. B. 1944 — The action of cetylcholine on the heart of Venus mercenaria. Biol. Bull. 85: 79-85

Walzl, E. M. 1937 — Action of ions on the heart of the oyster (Ostrea virginica) Physiol. Zool. 10:125

---------- 1938 — Response of the oyster heart to electrical stimulation and effect of calcium and potassium on its threshold of inhibition. J. Cell. and Comp. Physiol. 12:237

Wilhelmi, R. 1944 — Serological relationships between the Mollusca and other invertebrates. Biol. Bull. 87,96-105

Young, R. T. 1945 — Stimulation of spawning in the mussel (Mytilus Californianus.) Ecol. 26, 58-69

EMBRYOLOGY

Clark, J. M. 1936 — An experimental study of polyspermy, Biol. Bull. 70:361

Conklin, E. G. 1897 — The Embryology of Crepidula, J. Morph 13:1-226

Crampton, H. E. 1896 — Experimental studies on gastropod development Arch. F. Entwick, Mech. Bd. 3

Drew, G. A. 1897 — Notes on Embryology of Yolida, Ann. Mag. Nat. 7:1

---------- 1899 — Yoldia limatula. Mem. Biol. Lab. Johns Hopkins Univ. 4

---------- 1901 — Life History of Nucula delphinodon ta Q. J. Mier Sci. 44:313-392

---------- 1906 — Habits, Anatomy and Embryology of Giant Scallop, Pecten tenuicostatus. Univ. Maine Studies. Nov.

Koeppern 1909 — N otes on Prof. E. A. Minchin's preparations of early stages in development of Sepia Proc. Roy. Soc. Edin. 18

MacBride, E. W. 1914 — Textbook of Embryology. Vol. 1. Invertebrates Macmillan Co. Ltd. London

Meisenheimer, J. 1901 — Entwicklungsgeschichte von Dreissensia polymorpha. Zeit. f. Wiss. Zool. 69

Morgan, T. H. 1928 — Experimental Embryology. Columbia U. Press. N.Y.

Patten, W. 1885 — The Embryology of Patella. Arb. aus. d. Zool. Inst. der Univ. Wien. Bd. 6

Richards, Aute 1931 — Outline of Comparative Embryology J. Wiley & Sons, Inc.

Roney, H. B. 1943 — The effect of temperature and light on oxygen consumption and rate development of Helisoma Ecol. 24: 218-243

Schecter, V. 1938 — Notes on how to obtain Mactra eggs. Collecting Net 13:203

---------- 1941 — Experimental studies on the eggs of the clam Mactra solidissima J. E. Z. 86: 461-480

Vialleton, 1888 — Reserches sur les premieres phases du develop-ment de la Seiche (Sepia officinalis) Ann. Sci. Nat. Zool. 6

Watase, S. 1891 — Studies on Cephalopods. 1. Cleavage of ovum. J. Morph. 4:247-302

Wilson, E. B. 1904 — Experimental studies on Germinal Localization Germ regions in egg of Dentalium J. E. Z. 1: 1-72

Woodward, T. M. Jr. 1942 — Development of the nurse cells of Littorina irrorata (Say) Trans. Am. Micr. Soc. 61: 361-372

JOURNALS DEVOTED EXCLUSIVELY TO MALACOLOGY.

American - Nautilus Vols. (1888 - present)
American Journal of Conchology Vols. 1-7 (1865-1872)
Johnsonia Vol. 1 (1941-present)
Mullsca. Vol. 1. #1-18 1944-1946
Occasional Papers on Mollusks. Mus. Comp. Zool. Vol. 1 1945 present

English — Journal de Conchyliologie Vols. 1-83 (1850-present)
Bull. Soc. Malac. France Vols. 1-7 (1884-1890)

Italian — Bull. Soc. Malacol. Italiano Vols. 1-20 (1875-1895)

Belgian — Soc. Malac. Belgique Vols. 1-27 (1872-1898)

Japanese — Venus Vols. 1-7 (1928-present)

German — Malakozoologische Blatter Vols. 1-36 (1854-1891)
Deut. Malak. Gesell. Vols. 1-72 (1869-present)

Additions to Molluscan References

1947

Alexander, R. C. 1947 Littorina littorea on the New Jersey coast Nautilus 60: 73-76

Clarke, G. L. 1947 Poison and recovery in barnacles and mussels. Biol. Bull. 92: 73-91

Hartung, E. W. 1947 Cytological and experimental studies on the oocytes of freshwater pulmonates. Biol. Bull. 92: 10-22

Hermitte, L. C. D. 1946 Venomous marine mollusks of the genus Conus Trans. Roy. Soc. Trop. Med. and Hyg. 39:485-512

Von Brand, Theodor 1946 Anaerobiosis in Invertebrates. Biodynamics Monogr. 4: 185-328

Young, R. T. 1946 Spawning and setting season of the mussel, Mytilus californianus. Ecology 27: 354-363

KEY TO PELECYPODA

1. Inequivalve — 2
1. Equivalve — 6

2. Attached to rock — 3
2. Free living, usually burrowing — 5

3. Shell thin; lower valve flat or slightly convave, with hole near beak — 4
3. Shell solid; lower and larger valve convex, and adhering to substrate — Ostrea virginica

4. Shell fragile, translucent, often brightly colored and with a characteristic sheen; aperture oval — Anomia simplex
4. Shell surface roughened by prickly spines; shell a dingy white; aperture circular — A. aculeata

5. Valves swollen, pearly, with radiating fringed wrinkles; length 17 mm. — Lyonsia hyalina
5. Semilunar or saddle-like in shape; right valve flat, left valve slightly convex; shell smooth; length 30mm — Pandora gouldiana

6. Dark in color, umbo at or near end of elongated valves; byssus present (see figs. 7 and 9) — 7
6. Valves white or pastel, or covered with a polished yellow periostracum; no byssus present — 9

7. Umbo at tip end of valve — Mytilus edulis
7. Umbo near, but not at tip end of valve — 8

8. Valves smooth and polished, often partly covered by shaggy beard-like periostracum — Modiolus modiolus
8. Valves with radiating ridges — Brachidontes demissus

9. Valves fragile, swollen; anterior third sculptured with radiating rows of teeth for burrowing or boring — 10
9. Not so — 11

10. Shell widely gaping at both ends; dorsal margin of shell reflected over umbo; posterior end broadly truncate; siphons long and united — Barnea truncata
10. Shell slightly gaping; dorsal margin not reflected; ligament external; siphons long, united only — Petricola pholadiformis

11. Valves with strong radiating ribs — 12
11. Not so — 14

12. Umbo central, flanked by two winged ears; hinge line straight and toothless — Pecten irradians
12. Umbo prominent and oblique; series of small comb-like teeth on hinge line — 13

13. Shell rhomboidal; 32-35 ribs; beaks at anterior 1/3 of series of teeth — Arca transversa
13. Shaggy brown epidermis; 32 ribs — A. campechiensis

14. Shell, D--V dimension at least 3/4 A--P dimension 15
14. Shell, D--V " less than 3/4 A--P dimension 20

15. A--P dimension equals or almost equals D--V dimension 16
15. A--P " greater than D--V dimension 18

16. Shell minute, less than 5 mm.; white to purple in color Gemma gemma
16. Shell more than 5 mm. in length 17

17 Anterior dorsal edge of valves almost perpendicular to hinge; hinge with row of many tiny teeth, interrupted with a central pit for the internal ligament Nucula sp.
17. External ligament present; within shell bright yellow, with purple blotch on posterior edge of each valve; cardinal and lateral teeth Laevicardium mortoni

18. Shell fragile, thin; no lateral teeth, weak cardinals; outer surface covered with thin brown periostracum Macoma baltica
18. Shell thick and heavy, tightly closed; strong cardinals; umbo region prominent and oblique (swollen); ventral edge semicircular in outline (see figs. 8 & 9) 19

19. Heart shaped depression present anterior to umbo; inner ventral margin crenulated; 3 cardinals; no lateral teeth. Fig. 10 Venus mercenaria
19. No heart shaped depression present; margin not credulated Callocardia convexa

20. Periostracum of yellowish brown 21
20. Shell white; if pale salmon or pink, with high polish 25

21. Periostracum highly polished 22
21. Periostracum dull 24

22. Periostracum extending beyond edge of shell, as a velum Solemya velum
22 . No velum present 23

23. Shell elongated, razor-shaped; no teeth Ensis directus
23. Shell boat-shaped; within, a row of minute hinge teeth interrupted by a cup for the internal ligament Yoldia limatula

24. Dorsal and ventral margins straight, and parallel with each other; band of purple running across shell from umbo to ventral border Tagelus divisus
24. Shell triangular, smooth; well developed lateral teeth flanking medium cup for internal ligament Mactra solidissima

25. Shell highly polished; white, salmon, or pink; 1 cm. or less in length Tellina tenera
25. Shell not so; dull and white 26

26. Shell large, 5-9 cm.; ellipsoidal; gaping for protrusion of siphons which are united into one long "neck"
Mya arenaria

26. Shell small, 1-2 cm. 27

27. Shell, thin, oval, up to 25 mm.; hinge with a spatulate process in each valve; interior rib extending from hinge to posterior margin
Cochlodesma leanum

27. Shell 10-14 mm.; ellipsoid 28

28. Valves bent on posterior end; external ligament present; two cardinal teeth in right valve, one in left
Macoma tenta

28. Surface roughened by regular concentric lines; ventral margin contracted in the middle
Corbula contracta

Key to the common mollusca of Woods Hole

I. Shell of 8 calcareous plates in a longitudinal row (1 species, Chaetopleura apiculata) — CLASS: AMPHINEURA
II. Shell univalve (absent in Nudibranchs); head with one or two pairs of tentacles — CLASS: GASTROPODA
III. Shell bivalve — CLASS: PELECYPODA
IV. No external shell; head with long arms — CLASS: CEPHALOPODA

Key to Gastropoda

A. Shell more or less elevated, coiled spire — 1
B. Shell without coiled spire, cap shaped — 22
C. Shell an irregular twisted tube
1 species, Vermetus spirata

1. Aperture rounded or entire, no canal (fig. 2) — 13
1. Aperture forming a canal or rudiment thereof (fig.1.) — 2

2. Shells 5-10 mm. in length; dark brownish red; surface gramular; or if smooth with a series of pale crescentic markings — 3
2. Shells more than 10 mm. in length — 6

3. Six smooth whorls decorated with a revolving series of pale half moons; aperture narrowly elongate and less than ½ length of shell — Mitrella lunata
3. Whorls with a series of revolving tubercles — 4

4. Shell sinistral (fig. 5) — Triphora perversa
4. Shell dextral (fig. 4) — 5

5. Six to eight rounded whorls; aperture oval-with a slight rudiment of siphonal canal — Bittium alternatum
5. Sixteen to seventeen flattened whorls; aperture nearly circular, with short but conspicuous siphonal canal — Cerithiopsis subulata

6. Canal short, less than 1/5 total length of shell — 7
6. Canal elongated, more than 1/5 total length of shell — 11

7. Aperture narrowly elongate, less than ½ length of shell — Anachis avara
7. Aperture oval, equalling or more than ½ length of shell — 8

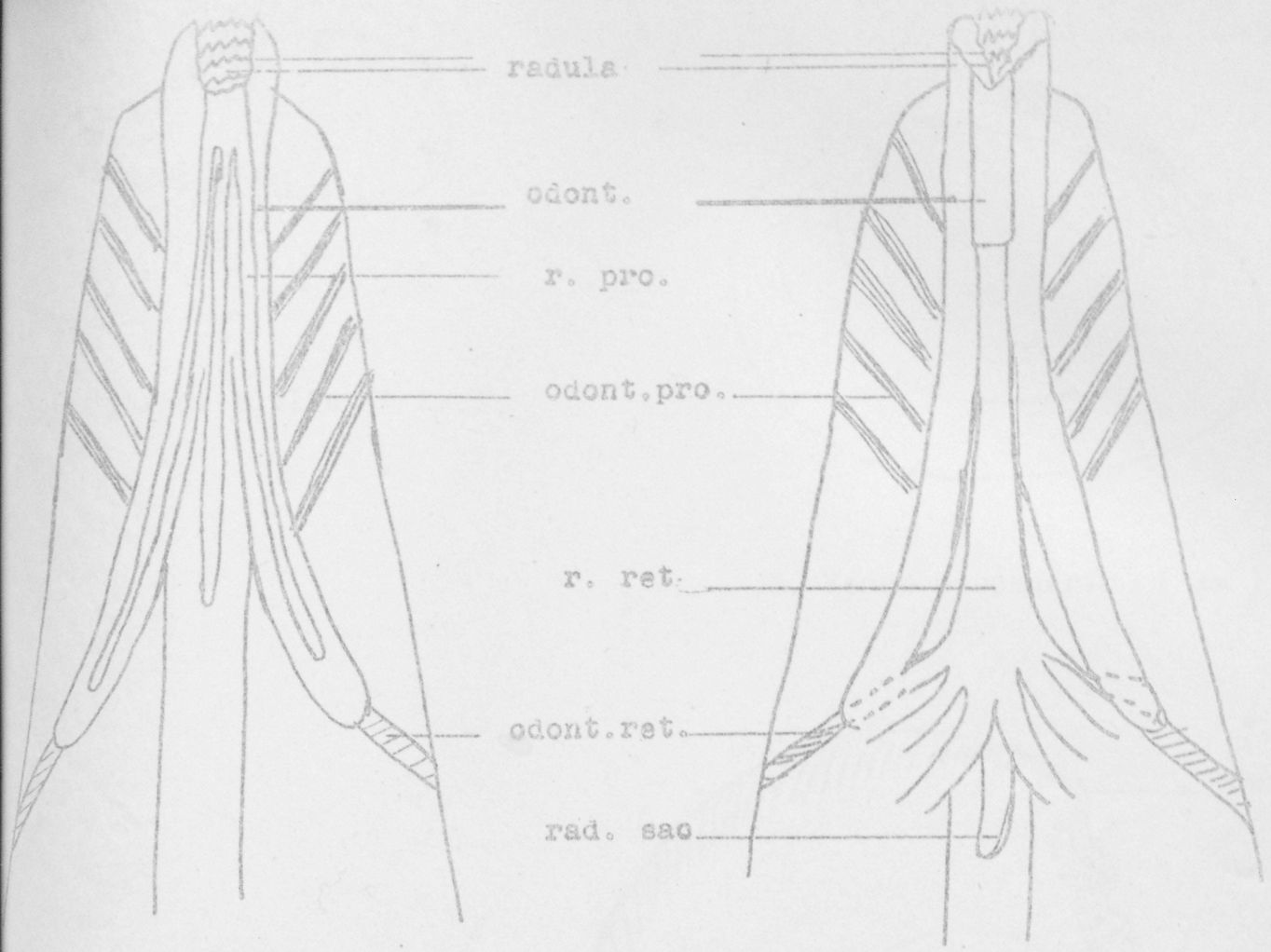
8. Aperture oval, more than ½ length of shell — 9
8. Aperture oval, equalling ½ length of shell; canal truncated and ending in a conspicuous notch (fig. 6) — 10

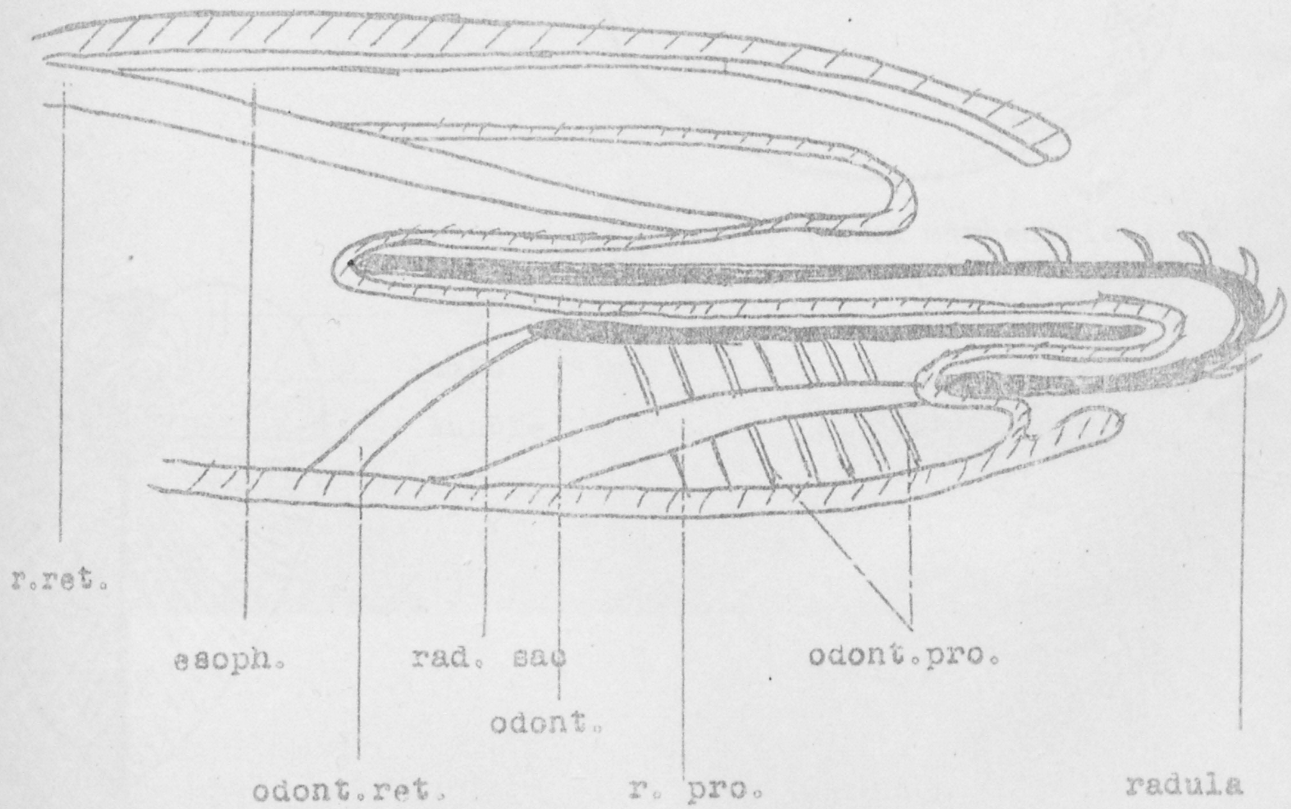
9. Shell fusiform; sutures deeply impressed and roughened by 12 longitudinal ridges; outer lip of aperture not flaring — Urosalpinx cinereus

9. Shell oval; sutures not deeply impressed; outer lip of aperture flaring and thickened — Thais lapillus

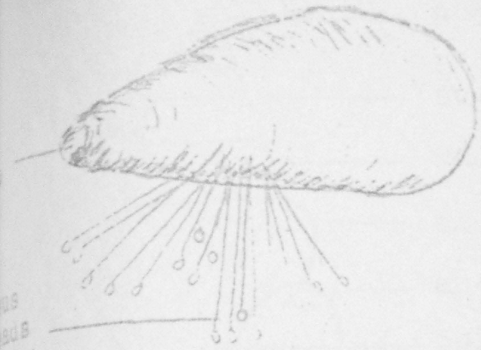
10.	Sutures deeply impressed, with shoulder; surface with waffle-like pattern, greenish white	Nassa trivittata
10.	Sutures not well impressed; dark brown or black; a mud dweller	Nassa obsoleta
11.	Length not more than 20 mm. Five angular whorls with 11 elevate ribs; aperture oval, with outer lip very thickened	Eupleura caudata
11.	Length more than 20 mm.; 6 rounded whorls with no ribs; aperture rounded with outer lip flaring.	12
12.	Sutures extremely deeply impressed, with broad flat shoulders.	Busycon canaliculatum
12.	Sutures lightly impressed, with moderate shoulders bearing a series of teeth at the edge.	B. carica
13.	Spire elevated, at least 7 whorls; shell small (minute) white or greenish	14
13.	Spire relatively low	15
14.	Whorls 12, flattened, inconspicuous sutures; highly polished	Melanella oleacea
14.	Whorls 5 or 6, separated by well defined sutures, with revolving just below, giving appearance of a double suture.	Odostomia bisuturalis
15.	Aperture narrowly elongate, outer lip thin and sharp; inner lip with two folds or plaits extending internally	Melampus bidentatus
15.	Aperture rounded or oval, no folds on columella	16
16.	Umbilicus present or closed by a conspicuous callus fig. 2, and 3	17
16.	Shell without umbilicus, aperture rounded, shell thick, inner lip flattened.	20
17.	Umbilicus forming cresentic groove on surface of columella; aperture not more than 1/2 length of shell.	Lacuna vincta
17.	Umbilicus widely open, or closed by conspicuous callus; aperture more than 1/2 length of shell.	18
18.	Umbilicus open	Polynices heros
18.	Umbilicus closed	19
19.	Dark reddish purple callus	P. duplicata
19.	White callus; shell minute, white, with white operculum	Natica pusillus

20. Spire extremely flattened; whorls scarcely raised above body whorl	Littorina obtusata
20. Spire elevated above body whorl	21
21. Whorls 4 to 5, rounded, with coarse revolving ridges; sutures well impressed; greenish yellow	L. saxitilis
21. Whorls 6 to 7 slightly flattened; apex acute; reddish brown or black, often with spiral stripes	L. littorea
22. Without a shelf on under side	Acmea
22. With a shelf on under side	23
23. Shell white flat, polished inside	Crepidula plana
23. Shell convex, colored	24
24. Shelf concave and white, occupying $\frac{1}{2}$ the aperture	C. fornicata
24. Shelf convex and brown, occupying 1/3 the aperture	C. convexa

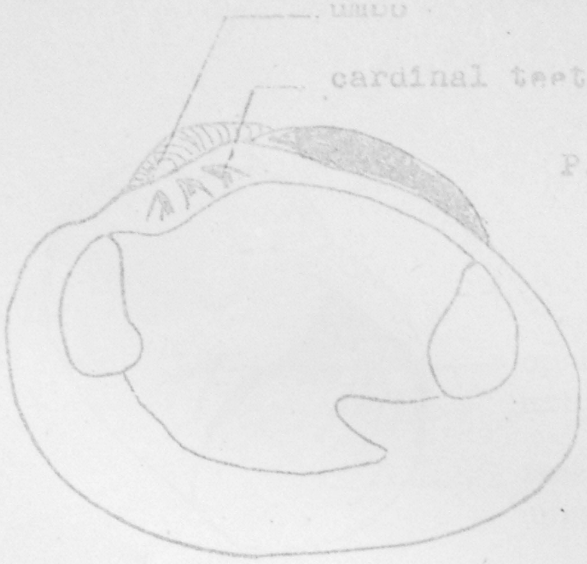




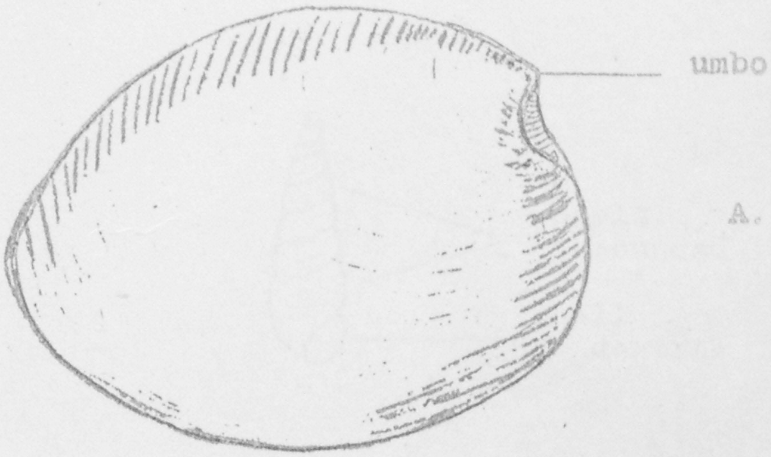
g. 3. Diagrammatic drawings of the porboscis of Busycon. a. ventral
pect; b. dorsal aspect ; modified lateral aspect.



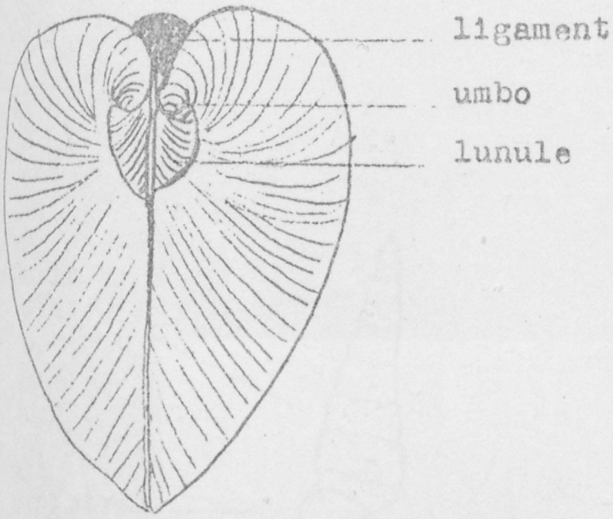
7. Mytilus edulis (1x)



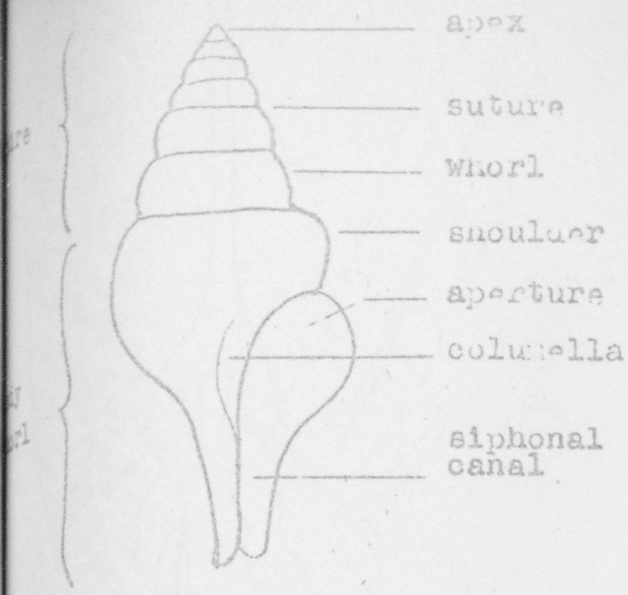
8. Venus mercenaria (1x)



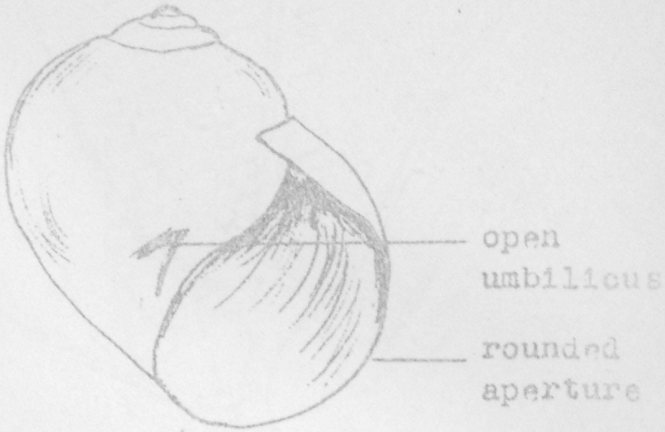
9. Venus mercenaria (1x)



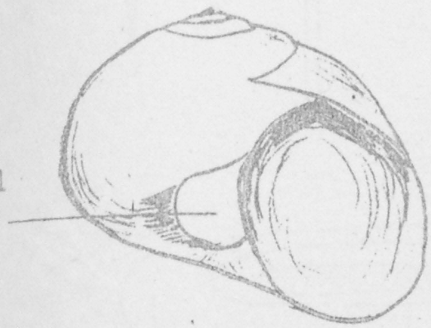
10. Venus mercenaria (1x)



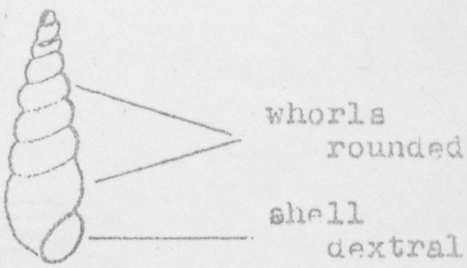
1. Diagram



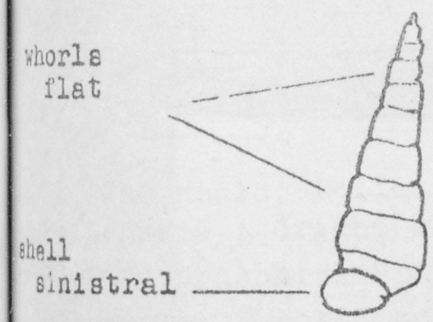
2. Polynices heros (1x)



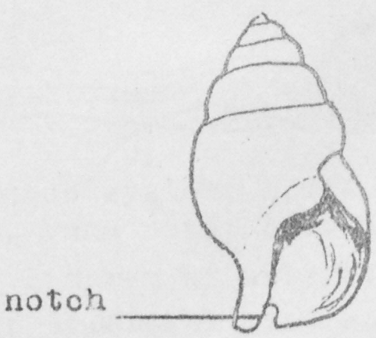
3. Polynices duplicata (1x)



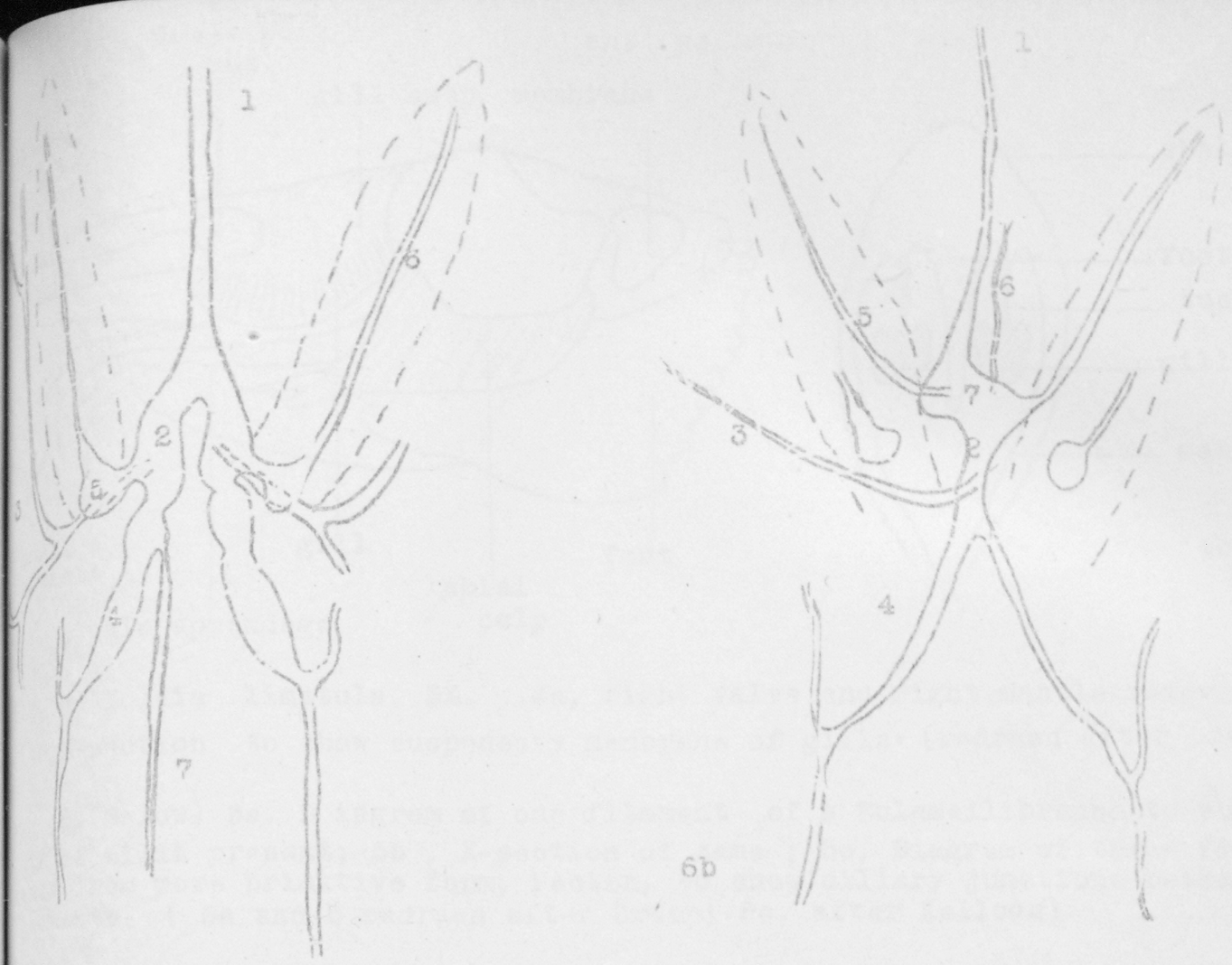
4. Turbonilla (3x)

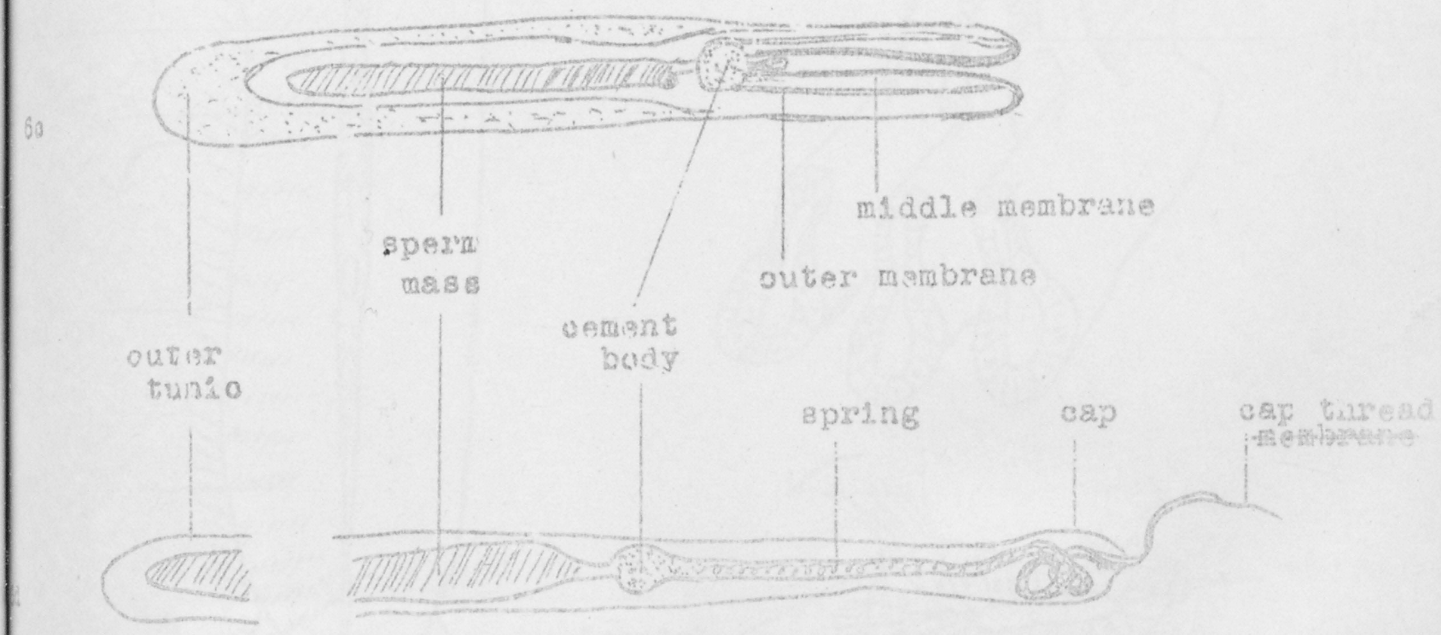


5. Triphora perversa (4x)

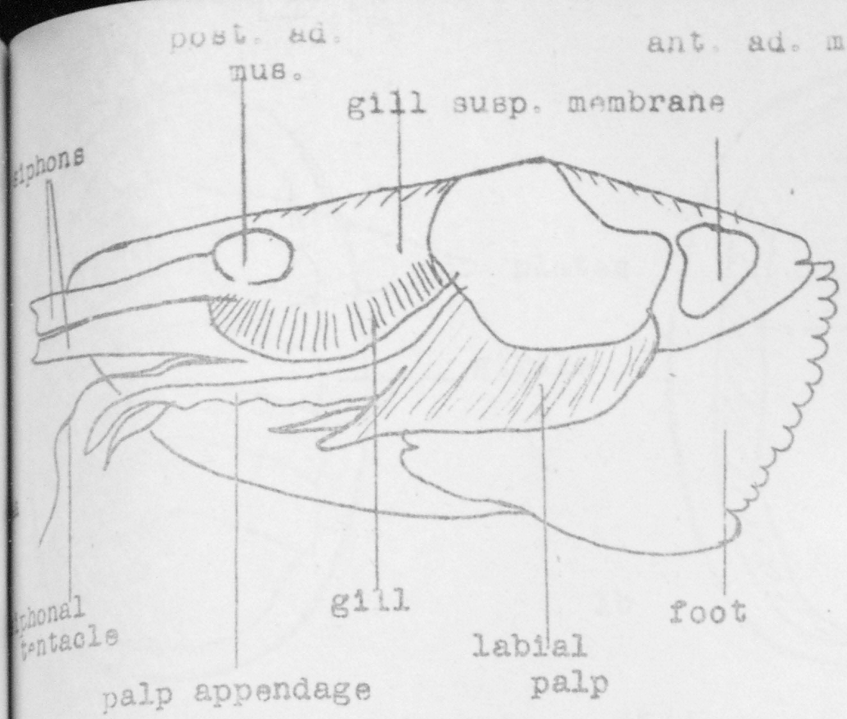


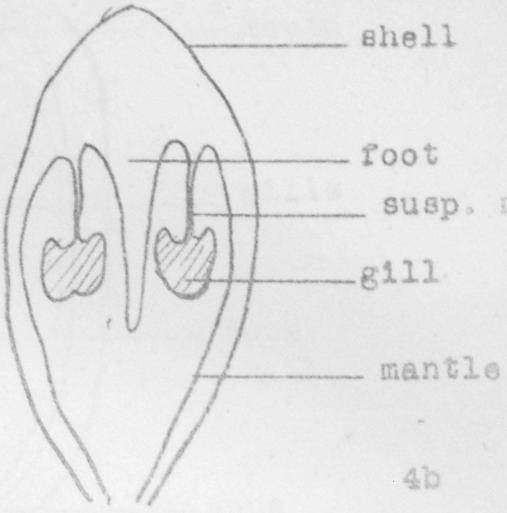
6. Nassa obsoleta (2x)





g. 6. The squid, Loligo . 6a, diagram of venous system; 1,ant. vena cava; right precava ; 3right mantle ; 4,right post. vena cava; 5,right branchial art;6,left branchial art.; 7,genital. 6b, diagram of arterial system: , ant. aorta; 2,post. aorta; 3,median mantle; 4,lateral mantle; 5,branchial n; 6,genital;7,systematic heart. 6c, spermatophore, before ejaculation. , spermatophore after ejaculation. 20X (6c and d redrawn after Drew)

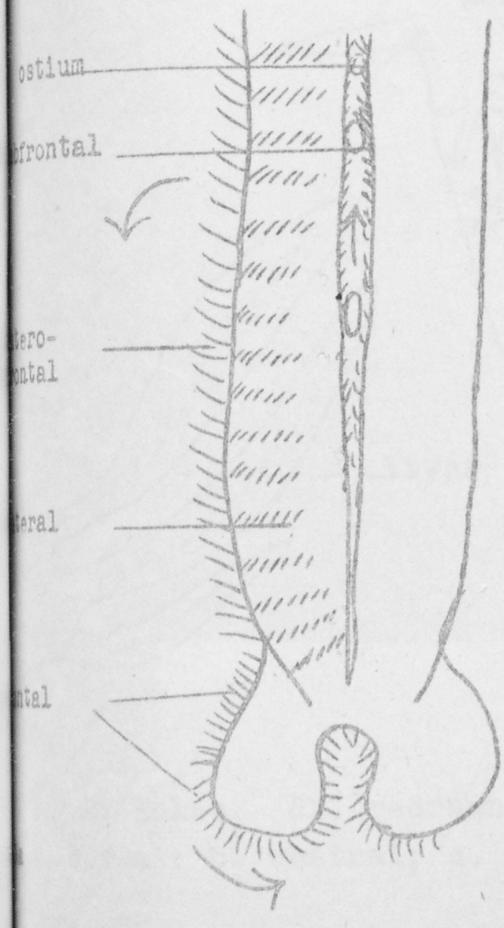


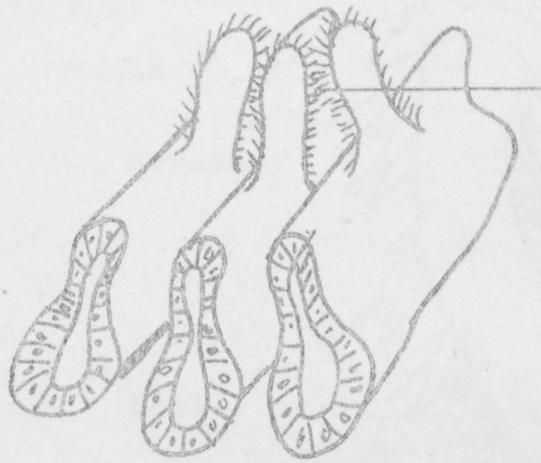


4b

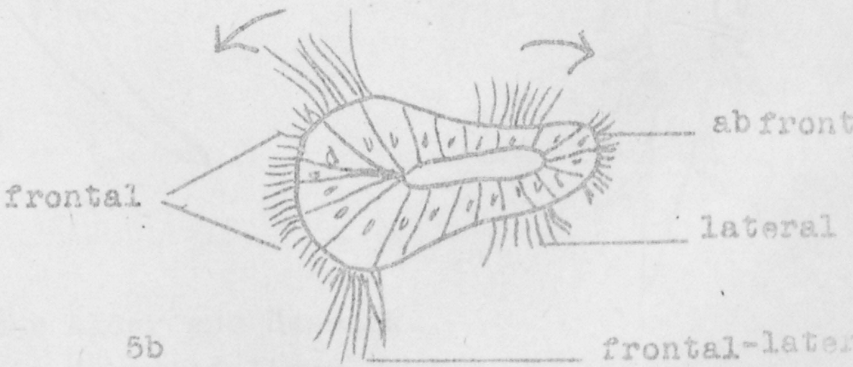
Fig. 4. Yoldia limatula. 3X. 4a, right valve and right mantle removed. 4b, X-section to show suspensory membrane of gills. (redrawn after Drew)

Fig. 5. (Below) 5a. D iagram of one filament of a Eulamellibranch to show sets of cilia present; 5b , X-section of same ; 5c, Diagram of three filaments from more primitive form, Pecten, to show ciliary junctions between filaments. (5a and b redrawn after Orton; 5a, after Kellogg)





5c



5b

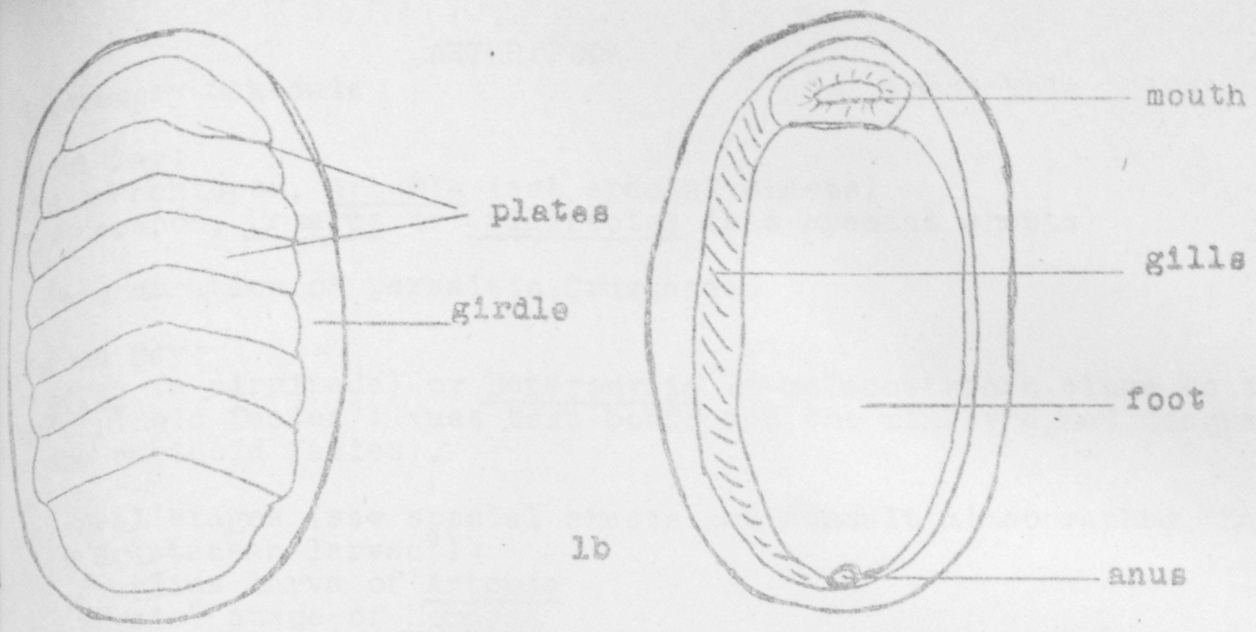


Fig. 1. Chaetopleura apiculata . 3X
a. dorsal view; b. ventral view, foot slightly reflected to show gills.

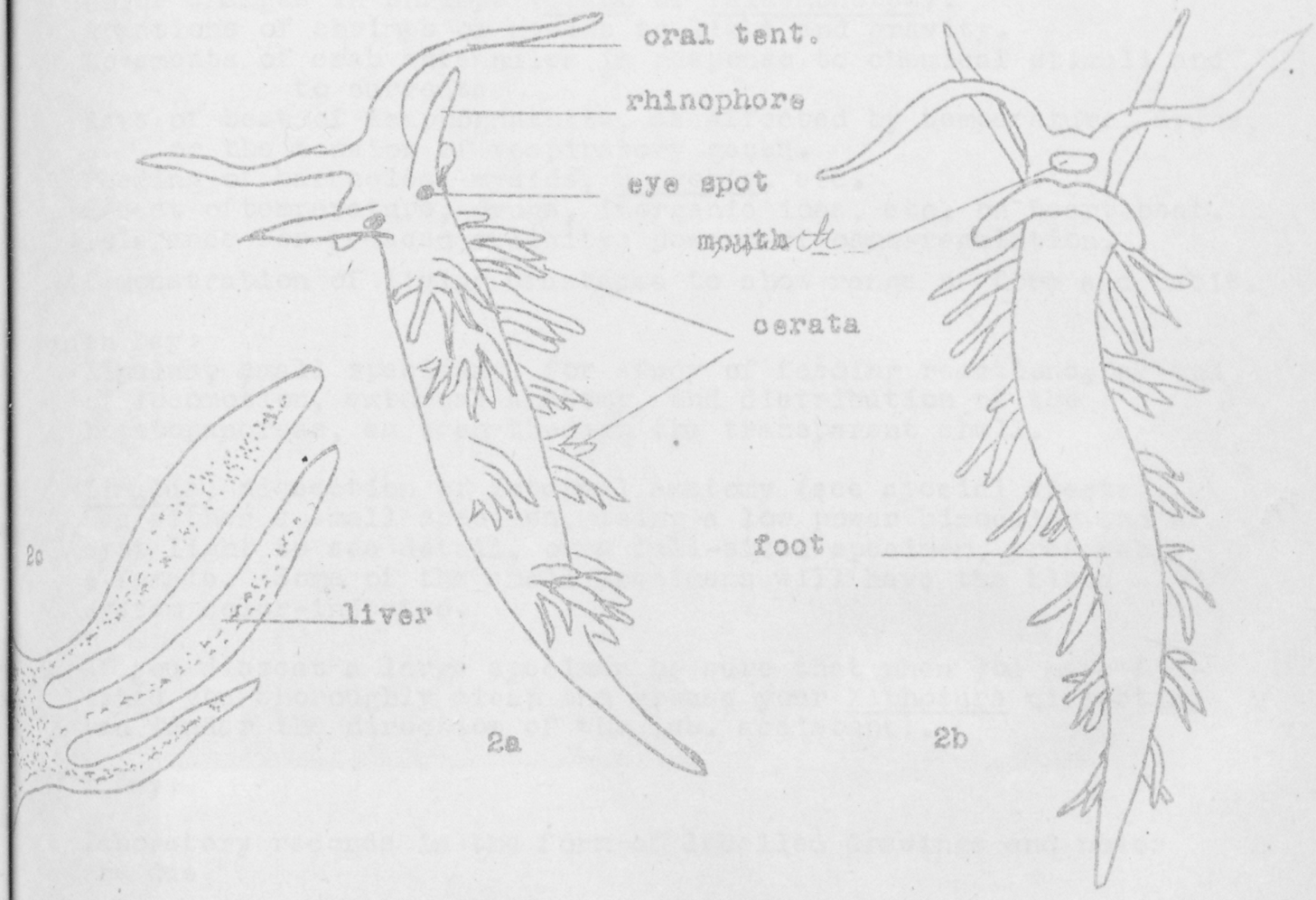


Fig.2. Eolis. 5X. redrawn after Alder and Hancock.
a. dorsal; b. ventral; c. cerata, showing liver.

ARTHROPODA

Laboratory Schedule Woods Hole 1949

First Day:
A branchiopod, Artemia (see special sheets)
A decapod, Homarus or Callinectes (see special sheets)

Demonstration of parasitic Crustacea.

Second Day:
Lepas (a cirripede) or Heteromysis (a malacostracan close to the "caridoid facies") (use text-books and the mimeographed diagram of the caridoid facies).

Larval stages (see special sheets and consult mimeographed "Key to Crustacean larvae"):
Nauplius larva of Artemia
"Mysis" stage of Homarus
Zooea larva (of Polyonyx macrocheles or some other crab)

Third Day:
Experimental observations, such as any of the following:
Autotomy in Uca (see special sheets).
Color changes in shrimps (Crago or Palaemonetes).
Reactions of shrimps or mysids to light and gravity.
Movements of crab antennules in response to chemical stimuli and to currents
Rate of best of scaphognathite, as affected by temperature, drugs, or the tension of respiratory gases.
Feeding of barnacles, mysids, Upogebia, etc.
Effect of temperature, drugs, inorganic ions, etc. on heart beat.
Tolerance for reduced salinity: powers of osmo-regulation.

Demonstration of living Crustacea to show range of form and habit.

Fourth Day:
Limulus, small specimens, for study of feeding reactions, method of locomotion, external anatomy, and distribution of the hepatopancreas, as seen through the transparent shell.

Limulus, dissection of internal anatomy (see special sheets). Use either a small specimen, using a low power binocular and a spot light to see detail, or a full-sized specimen, preferably a female. Some of the small specimens will have the blood system color-injected.

If you dissect a large specimen be sure that when you have finished you thoroughly clean and grease your Xiphosura dissecting pan (under the direction of the lab. assistant).

Fifth Day:

Laboratory records in the form of labelled drawings and notes are due

DIAGRAM OF THE "CARIDOID FACIES", HYPOTHETICAL ANCESTRAL TYPE FOR MALACOSTRACA

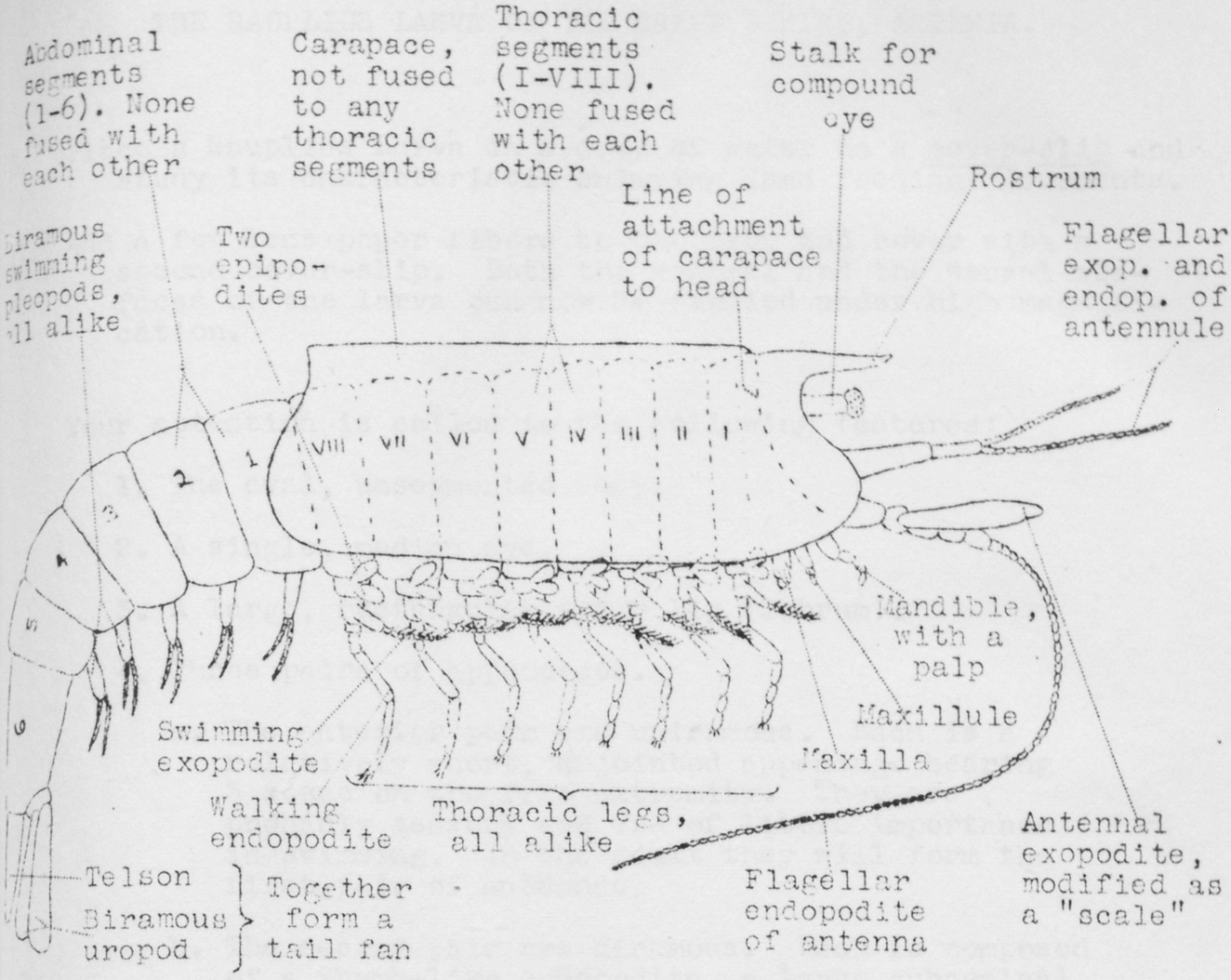
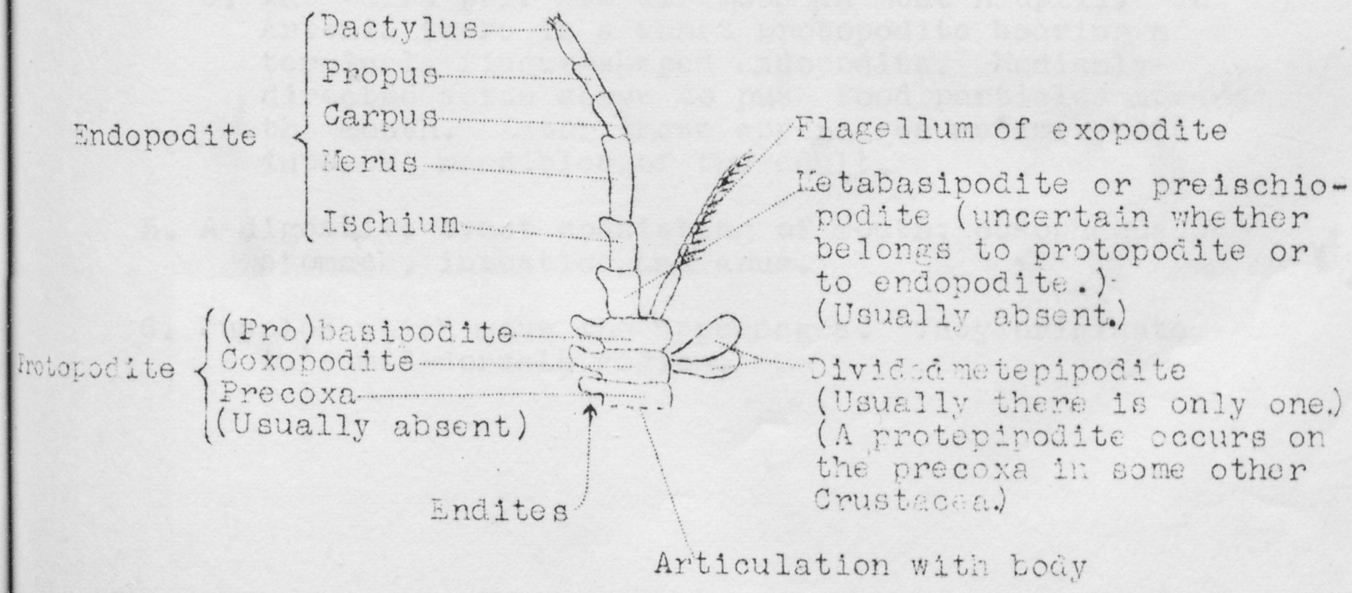


DIAGRAM OF A PRIMITIVE MALACOSTRACAN THORACIC APPENDAGE



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 first pair of antennae.

   b. The second pair are biramous. Each is composed of a thumb-like endopodite, a large subconical expodite 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 la rva. They will form the second pair on 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. Medianly 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 a mid-dorsale region.

THE "MYSIS" 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 these 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 one.

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 postero-dorsal 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 Nephropaidae, to which the lobster belongs, but also Penaeidea, Caridea, Stenopidea, Scyllaridea, and Thalassinidea, have "Mysis" or "schizopod" larvae. These larvae are so named because of their resemblance to members of the Mysidacea (of which Mysis 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, have an elongate heart extending through much of both the abdomen and thorax (best seen in living specimens), and do not have a hepatopancreas: most of them have natatory exopodites on the last seven pairs of thoracic appendages. Euphausiacea, Caridea, and schizopod larvae, being members of the Eucarida, have the carapace fused dorsally with all thoracic segments, have a shortened heart located in the thorax, and have a large hepatopancreas. 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 modfied as scaphognathites. Caridea and schizopod larvae reveal themselves as Decapoda by having the first three pairs of thoracic appendages modified as maxillipeds (or in larvae often only the first two pairs); the natatory exopodios are absent on at least the first two pairs; gills are covered by the carapace; and the maxillae are modified as scaphognathites. Caridea can be recognized by the wide pleura on the second abdominal segment overlapping the pleurae of the first and third abdominal segments. In lobster larvae the third, as well as the second, "walking legs" are minutely chelate, in contrast to the schizopod larvae of caridea and of Thalassinidea. The first "walking legs" have larger chelae, in contrast to the schizopod larvae of Penaeidea. In the schizopod stage of Stenopus the fifth walking legs are enormously enlarged, while in Schllaridea the whole body of the larva is leaf-like and very transparent, making them quite unlike any other larvae (the special term "phyllosoma" larva usually being applied).

If you can find specimens or good figures of several of the adults and larvae mentioned above, note their close general resemblance to each other, and try to spot the distinguishing characteristics to which reference has been made.

REFERENCES

* Gurney, R. 1942
* Heldt, Jeanne H. 1938-9
Herrick, F. H. 1911. Natural history of the American lobster. Bull. U. S. Bur. Fish. 29 (1909): 149-408, 42 fig., 20 pl. (See esp. fig. 34-42 and pl 28 and 31)
* Kükenthal, W. and Krumbach, T. 1926-7
Templeman, W. 1936. Fourth stage larvae of Homarus americanus intermediate in form between normal third and fourth stages. J. biol. Board Canada, 2: 349-354, 1 fig.

* See Arthropoda, Selected Bibliography, for full reference.

ZOOEA LARVA OF POLYONYX OR OF SOME OTHER CRAB

The term zooea is given to larvae of decapod crustacea which swim by means of the exopodites on specially modified maxillipeds, the remaining thoracic appendages beig 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 protozooea, and a later stage, which has abdominal pleopods and well-developed rudiments of the posterior thoracic appendages, is called a metazooea.

The zooeal 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 zooeae in text-books. But the variety of types of zooeae 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 zooea 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 on account of which it is classed as an anomuran, family Porcellanidae. Its zooea 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 zooea, sensu strictu. But in the metazooea the third maxillipeds, though smaller, are also natatory, a distinction from the Brachyura, in which the third maxillipeds are never natatory.

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

On the zooeae 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 metazooea
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 a metazooea

If the zoeae are alive, try to observe how the maxillipeds are used in swimming, 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. Consult Hyman (1922) for notes regarding feeding and other activities.

REFERENCES

Aikawa, H. 1929-30; 1933; 1937. (Three papers on brachyuran larvae) Rec. Oceanogr. Wks. Jap., 2:17-35; 5:124-254; 9:87-162 Many figs.

* Faxon, W. 1879
* Foxon, G. E. H. 1934
* Gurney, R. 1942
* Heldt, Jeanne H. 1938-9
* Hyman, O. W. 1922

* See Arthropoda, Selected Bibliography, for full reference

AUTOTOMY IN UCA.

Suggestions are made below for a variety of experiments and observations that can be made in regard to autotomy in Uca. No one person could do all these in a single day, so select items that appeal to you most and that you feel competent to do. Several of the experiments are of the sort that should be done on a large number of specimens, so as to obtain results that are statistically significant. In order to be able to do this in the time available, and so as not to exhaust the supply of Uca, you should find other students planning to do the same experiment, and then work together as a group.

Effective stimuli.

Plan and carry out a series of experiments to determine the most effective stimuli for autotomy.
Methods that can be tried include:

Cutting off part of the leg with sharp scissors.
Crushing the leg with blunt forceps or small pliers, both in the air and under water.
Pulling the leg, or attaching a weight to it.
Applying heat to the leg with a hot wire or even by flame.
Mechanical stimulation of the leg nerve by insertion of a fine pin at one of the joints. Before doing this, try to learn the exact location of the nerve by dissection and staining with methylene blue.
Electrical stimulation, by electrodes either on the outside of the leg or directly on the nerve.
Chemical stimulation by partly or totally immersing the animal in alcohol, in histological fixatives, or in other chemicals.
Electrical or mechanical stimulation of the ventral ganglion, or of the nerve leading from this to the leg. This will require partial dissection or hemisection of the animal, after you have made a preliminary anatomical study.

Effect of permitting the leg to press against an external support.

Determine the relative ease with which autotomy occurs when, during application of a standard effective stimulus, the leg:

a) projects freely from the body, not in contact with any external support.
b) can press against a thin rod held ventral to the meros.
c) can press against such a rod held dorsal to the meros.
d) has the rod held anterior to the meros
e) has the rod held posterior to the meros.

Relative effects of the same stimulus applied in different regions, or to different types of individuals.

Determine the frequency and the rapidity with which autotomy is produced by the application of a standard stimulus to different segments of the leg.
Investigate whether there is any difference in the response given by the large chelipeds, small chelipeds, and other walking legs.

Similarly compare responses in the two sexes and, if specimens are available, in recently molted individuals and in egg-bearing females.

Frequency of response when successive legs are stimulated.

On a number of animals, apply a standard effective stimulus to each of the ten legs in random sequence, stimulating each leg only once, and keeping a record to show the frequency with which autotomy occurs on the first leg stimulated, second leg stimulated, etc.

Effect of drugs.

On a number of animals, stimulate the ten legs in random sequence as before, after having injected a drug. Compare the results with those obtained on controls injected with crustacean Ringer. Inject through the soft inter-segmental membrane, such as at the dorsal junction of thorax and abdomen. If available, acetylcholine, eserine, and atropine might be tried. For probable effective doses, consult Welsh and Haskin (1939).

Response after loss of blood

Determine the relative frequency of autotomy in specimens which have suffered a maximum loss of blood. Probably the animals can best be bled by making a wide cut in the dorsal membrane between thorax and abdomen, holding the animals in such a way as not to favor autotomy while making the cut.

Structure of the autotomized stump.

Examine the stump left after autotomy, and compare carefully with intact legs. The fracture plane appears to be at the point where basis and ischium are fused together, but there is reason to believe that it actually is in the proximal end of the ischium. Observe carefully the diaphragm which covers most of the exposed end of the stump, and try to make out the structure of the valve which prevents loss of blood through the hole in the center. Note, too, the thickened rim of the exoskeleton at the end of the autotomized stump. Is it equally thickened all the way around? On the basis of your experiments in which the leg was allowed to press against an external support, at which point of the circumference do you think the break occurring in autotomy first starts? Is this correlated in any way with the structure seen in the autotomized stump?

Strength or weakness of the fracture plane at which autotomy occurs.

On an animal which has recently died, determine just where the leg breaks when it is twisted, pulled straight out, and pulled at various angles.

Mechanism of autotomy.

Autotomy is a reflex action, involving a special muscle, the anterior levator of the basis, or "autotomizer muscle". This inserts by a tendon on the exoskeleton of the basis, near the

antero-dorsal segment of its proximal border. From this insertion the muscle extends through the coxa some distance into the body, fanning out in a dorso-ventral plane. Three other muscles insert on the proximal border of the basis, namely the posterior levator posterior depressor, and anterior depressor, inserting postero-dorsally, postero-ventrally, and antero-ventrally respectively. Two other prominent muscles are the protractor and the retractor of the coxa, inserting anteriorly and posteriorly on the proximal rim of the coxa. If by careful dissection you can distinguish the six muscles here innumerated, you may try to cause autotomy artificially on a dead animal, by pulling on the tendon of the autotomizer muscle with a good pair of forceps, perhaps at the same time holding a thin rod suitably braced against the meros. Best results probably will be obtained by removing only just enough of the exoskeleton to permit the tendon of the autotomizer muscle to be easily grasped and pulled by the forceps. Does it seem possible to apply the force as effectively as when the muscle contracts in the living animal?

After an especially neat dissection it may prove possible to cut each of the three muscles in the basis other than the autotomizer muscle, and then demonstrate that autotomy still occurs in response to a suitable external stimulus. The reverse experiment, in which only the autotomizer muscle is cut, ought also to be done as a control.

References

Welsh, J. H., & Haskin, H. H. 1939. Chemical mediation in crustaceans. III. Acetylcholine and autotomy in Petrolisthes armatus (Gibbes). Biol. Bull. 76: 405-415, 5 fig

Wood, Florence D., & Wood, H. E., II. 1932 Autotomy in decapod Crustacea. J. exp. Zool. 62: 1-55 . 3 pl.

TABLE OF CLASSIFICATION OF THE CRUSTACEA

CLASSES	SUBCLASSES	ORDERS	SUBORDERS	DIVISIONS	TRIBES	SUB-TRIBES	Super- FAMILIES
Branchiopoda		Anostraca	Lipostraca				
			Euanostraca				
		Notostraca					
		Onychura (=Diplostraca)	Conchostraca				
			Cladocera	Calyptomera	Ctenopoda Anomopoda Onychopoda	(Division) Eucladocera	
				Gymnomera	Haplopoda		
Ostracoda		Myodocopa					
		Podocopa					
Branchiura							
Copepoda		Mystacocarida					
		Gymnoplea					
		Podoplea	Harpacticoida				
			Cyclopoida				
			Notodelphyoida				
			Monstrilloida				
			Caligoida				
			Lernaeopodoida				
Cirripedia		Thoracica	Pedunculata				
		Acrothoracica	Operculata				
		Ascothoracica					
		Apoda					
		Rhizocephala					
Malacostraca	Leptostraca	Nebaliacea					
	Syncarida	Anaspidacea					
	Peracarida	Mysidacea					
		Cumacea					
		Thermosbaenacea	Oniscoidea				
		Tanaidacea	Asellota				
		Isopoda	Phreatoicidea				
			Flabellifera				
			Valvifera				
			Epicaridea				
		Amphipoda	Gammaridea				
			Hyperidea				
			Ingolfiellidea				
			Caprellidea				
	Eucarida	Euphausiacea					
		Decapoda	Natantia	Penaeidea	(Suborder) Macrura		
				Stenopidea			
				Caridea			
			Reptantia	Astacura	Eryonidea Scyllaridea		
				Palinura			
				Anomura	Thallassinidea Galatheidea Hippidea Paguridea		
				Brachyura	Dromiacea Oxystomata Brachygnatha	Oxyrhyncha Brachyrhyncha	Cyclometopa Catometopa
	Hoplocarida	Stomatopoda					

KEY TO THE CLASSES OF CRUSTACEA

A. Trunk segments number 14 or 15.
Trunk tagmata: a thorax of 8 and abdomen of 6 or 7 segments.
Abdomen usually bears limbs.
Median eye vestigial or absent in adult.
Gastric mill present, and often highly developed.
Excretory gland of adult is often[1] the antennal.
Nauplius rare.

Malacostraca

B. Trunk segments do not number 14 or 15.
Trunk tagmata, various but never those of Malacostraca.
Abdomen rarely bears limbs.
Median eye usually present in adult.
Gastric mill rarely present and then ill developed.
Excretory gland of adult always the maxillary.[2]
Nauplius generally present.

(Entomostraca).

1. Trunk limbs not distinctly biramous.
Compound eyes generally present.
Always free throughout life.

a) Postoral limbs to at least 6 pairs[3] are present as such.
Antennules usually, and antennae sometimes, small and purely sensory.
Shell if bivalve usually does not cover head.[4]
Mandibular palp absent, or vestigeal.[5]
Maxillae reduced or absent

Branchiopoda.

b) Postoral limbs recognisable as such are not more than 5 pairs, tho' penis possibly represents a 6th pair.
Antennules and antennae large, and generally used as organs of locomotion or prehension or both.
Shell bivalve and covers head.
Mandible has a palp.

Ostracoda.

2. Trunk limbs distinctly biramous (exc. Mystacocarida and some parasites)
Paired eyes lacking in adult, exc. Branchiura.
Often temporarily or permanently attached

---

1. Amphipoda, "Schizopoda", Decapoda (and a trace in Leptostraca, Tanaidacea and Isopoda, which have maxillary glands)
2. Small and accompanied by s mall antennary gland in Ostracoda. A trace of antennary gland also in some Branchiopoda, such as Artemia, Daphnia, Simosa.
3. E.g. Lathonura (Lyncodaphnidae), which has only 4 pairs trunk limbs, plus mandibles and maxillules (no maxillae).
4. But does so in many Conchostraca.
5. Polyartemia

KEY TO THE CLASSES OF CRUSTACEA (Continued)

a) Compound eyes present in adult
Some thoracic limbs have flabellar appendage to exopodite
Carapace present as lateral horizontal lobes

Branchiura.

b) Compound eyes lacking in adult
No limb has a flabellar appendage
Carapace absent, or present as mantle.

i. No carapace
Abdomen not greatly reduced (exc. some parasites)
Sexes separate in nearly all cases[1].
Female generally carries eggs attached to oviducts.
Median eye usually retained in adult.

Copepoda.

ii. A mantle-like carapace almost always present
Abdomen vestigial in non-parasitic forms
Hermaphroditic in most cases
Eggs not attached to oviducts after laying
Median eye vestigial or lost in adult

Cirripedia.

1. Xenocoeloma is hermaphroditic.

KEY TO THE ORDERS OF BRANCHIOPODA

1. Carapace absent.
   Anostraca

11. Carapace present.
   A. Carapace forming a dorsal shield. Antennae vestigial. Trunk limbs 40-63 pairs. Furcal rami multiarticulate. (Development with metamorphosis).
   Notostraca

   B. Carapace compressed. Antennae biramous (exc. female Holopedium), natatory. Trunk limbs not more than 27 pairs. Furcal rami claw-like.
   Onychura = Diplostraca

KEY TO THE SUBORDERS OF ANOSTRACA

1. Antennae biramous, alike in sexes. Trunk limbs in two series, a phyllopod and a "biramous". 1st. thoracic segment fused to head. Fossil from Devonian period.
   Lipostraca

11. Antennae uniramous, prehensile in male, reduced in female. Trunk limbs alike, phyllopod. 1st thoracic segment free.
   Euanostraca

KEY TO THE SUBORDERS OF ONYCHURA

1. Carapace usually encloses head and body.[1] Trunk limbs 10-27 pairs. Development usually with metamorphosis.
   Conchostraca

11. Carapace leaves head free. Trunk limbs 4-6. Development without metamorphosis (exc. from winter eggs of Leptodora).
   Cladocera

[1] Not head completely in Lynceus.

KEY TO THE DIVISIONS OF CLADOCERA

1. Carapace completely enclosing body and limbs
Calyptomera

11. Carapace not enclosing body and limbs
Gymnomera

or

1. Terminal claws of abreptor directed downwards or curved forward[1]. Limbless part of trunk short and not distinctly segmented. Relatively few setae on antennae. No metanauplius.
Eucladocera

11. Terminal claws of abreptor directed backwards. Limbless part of trunk long and clearly segmented. Numerous swimming setae on both endopodite and exopodite of antennae. A metanauplius hatches from winter eggs.
Haplopoda

KEY TO THE ORDERS OF OSTRACODA

1. Candal furca with lamellar rami armed with spines.
Myodocopa

11. Candal furca with styliform or vestigeal rami.
Podocopa

[1] Probably lost in Bythotrephes and allied genera.

KEY TO THE ORDERS AND SUBORDERS OF COPEPODA

1. All thoracic and abdominal somites free, not fused to each other nor to the head. Large labrum in adult. Second to fifth pairs of thoracic limbs reduced to simple lamelliform plates in the larvae as well as in the adult; sixth pair absent. Urosome and metasome cylindrical, not differing in width. Free living in sand.

Mystacocarida

11. At least first thoracic somite fused with head, and usually some trunk somites fused with each other. Labrum small in adult. Some or all of the second to sixth pairs of thoracic limbs developed as biramous swimming legs, at least in the later larval stages.

A. Sixth thoracic somite moveably articulated with genital somite, and firmly connected with fifth. Sixth pair of thoracic limbs of male well developed and unsymmetrical, one of them usually modified for pairing. Animals free-living and planktonic.

Calanoida (Gymnoplea)

B. Sixth thoracic somite firmly united with genital somite and usually moveably articulated with fifth. Sixth pair of thoracic limbs in both sexes reduced or absent. Animals free or parasitic, usually not planktonic.

(Podoplea).

1. Body retains segmentation in adult of male, and usually in both sexes. Male neither a pigmy nor attached to female. Power of swimming usually retained.

a. Without free articulation between 4th and 5th thoracic somites. Adult free-feeding, commensal, or parasitic; sometimes loses power of swimming.

(i) eggs carried in external masses, ventrally or laterally. Mostly free; a few free-parasitic.

(a) Urosome about as wide as metasome and both more or less cylindrical in most cases. Basal segment of 5th leg (6th thor. limb) with inner expansion. One or a pair of egg-masses.

Harpacticoida

(b) Metasome much wider than urosome and depressed. Basal segment of 5th leg without inner expansion. A pair of lateral egg-masses.

Cyclopoida

(ii) Eggs carried in dorsal brood-pouch. Adults commensal in ascidians, etc.

Notodelphyoida

b. With free articulation between 4th and 5th thoracic somites. Adult nearly always retains power of swimming, but is not free-feeding.

(i) No Ant. 11 or mouthparts in adult. Animals parasitic during development and of free pelagic habits as adults

Monstrilloida.

KEY TO THE ORDERS AND SUBORDERS OF COPEPODA (continued)

(ii) Adults retain Ant. II and mouthparts. Animals free during development and parasitic as adults.
Caligoida

2. Body loses segmentation in adult (not always completely in male.) Male a pigmy attached to female. Power of swimming lost completely in adult.
Lernaeopodoida

KEY TO THE ORDERS OF CIRRIPEDIA

1. Alimentary canal and limbs and other traces of segmentation remain. No roots (exc. Anelasma).
A. A mantle and trunk limbs.
1. No abdomen (exc. sometimes a caudal fork). Mantle does not contain diverticula of alimentary canal. Body attached preorally.
(a) Six pairs of thoracic limbs.
Thoracica

(b) Thoracic limbs reduced in number.
Acrothoracica

2. An abdomen. Mantle contains diverticula of alimentary canal. No preoral attachment. Parasitic.
Ascothoracica

B. No mantle. No trunk limbs. Parasitic. (Antennule present. Abdomen probably present).
Apoda

II. No alimentary canal. No limbs or other traces of segmentation. Roots present. Parasitic.
Rhizocephala.

KEY TO THE SUBORDERS OF THORACICA

1. Peduncle and capitulum distinct. Outer plates of shell, when present, not forming a "wall".
Pedunculata

II. No peduncle. Scuta and terga forming a movable operculum. Outer plates of shell coalesced to form a "wall".
Operculata

KEY TO SUBCLASSES OF MALACOSTRACA

1. Abdomen of seven segments and a telson bearing a pair of moveably articulated furcal rami. An adductor muscle of the carapace. Thoracic limbs all similar, more or less foliaceous.

Leptostraca

11. Abdomen of six segments (or fewer by coalescence) and a telson which bears no furcal rami. No adductor muscle. Thoracic limbs rarely all similar, pediform. (Eumalacostraca)

i. No moveable ophthalmic or antennulary "somites". Thoracic limbs have not three-jointed protopodites. A branched gill is not present on any pleopod.

A. Carapace, if present, leaves at least 4 thoracic somites free. Precoxa of antennae often present. Thoracic limbs flexed between 5th and 6th actual joints. Heart generally elongate.

1. Mandible has no lacinia mobilis. Oostegites absent. First thoracic somite may be free, second always so.

Syncarida

2. Mandible has no lacinia mobilis. Oostegites on some or all thoracic limbs. At least 1 thoracic somite fused to head. (parasites may lose these features in adult stage).

Peracarida.

B. Carapace always present and fused with all thoracic somites. Precoxa of antennae never present. Thoracic limbs flexed between 4th and 5th actual joints. Heart short.

Eucarida

ii. Moveable ophthalmic and antennulary "somites" present. Last 3 pairs thoracic limbs have 3-jointed protopodites. A branched gill stands on exopodite of each pleopod. (Carapace fused with two to four thoracic somites. Antennae lack precoxa. No oostegites or lacinia mobilis. Heart long).

Hoplocarida

KEY TO THE ORDERS OF THE PERACARIDA

1. A distinct carapace present, fusing in almost all with two, and in most with more than two, thoracic segments, and overhanging at the sides to form a gill-chamber. Antenna usually has two flagella.

A. Carapace long, leaving few[1] or none of the thoracic segments uncovered dorsally. Eyes, when present, moveable. Exopodite of antennae developed, usually as a broad scale. Uropods broad, forming with telson a tail fan. Young leave brood pouch with all the limbs of an adult.

Mysidacea

B. Carapace short, leaving most of the thoracid segments uncovered. Eyes, when present, immoveable. Exopodite of antennae small or absent. Uropods narrow, not forming a tail fan. Young leave brood pouch without the last thoracic limb.

1. Carapace fairly large, fused with 3 or 4 thorocic segments and produced in front into two plates which form a "pseudorostrum". Sixth abdominal segment not fused with telson. Three maxillipeds. No.mandibular palp.

Cumacea (= Sympoda)

2. Carapace very small, fused with two thoracic segments only. No pseudorostrum. Sixth abdominal segment and telson fused. One pair maxillipeds. A mandibular palp.

a. Thoracic limbs 7 and 8 absent; the others have exopodites. No chelae. Uropods flat, no multiarticulate. Antennal peduncle three-jointed.

Thermosbaenacea

b. Thoracic limbs all present; with exopodites[2]; Second pair chelate. Uropods cylindrical, rami may be multiarticulate. Antennal peduncle two-jointed.

Tanaidacea (= Anisopoda)

---

1 Up to three in number.

2 Minute vestiges of exops. on th. limbs 2 and 3 in Apseudes.

KEY TO THE ORDERS OF PERACARIDA (continued)

11. No distinct carapace. Rarely more than one, never more than two, thoracic segments fused to head[1]. Antenna often has only one flagellum.

A. Body generally depressed. Telson rarely defined from last abdominal segment. Abdominal limbs k-5 when present generally have the form of respiratory lamellae and are never divided into two sets. Young almost always leave the brood pouch without the last pair of legs.

Isopoda

B. Body generally compressed. Telson usually distinct from last abdominal segment. Abdominal limbs 1-5 when present, never lamellar but divided into two sets. Young leave brood pouch with all the limbs of adult.

Amphipoda

---

[1] Two fused in a few Isopoda (q.v.) and in Caprellidea among Amphipoda.

KEY TO THE SUBORDERS OF ISOPODA

1. Rarely parasitic. If so, then with female not greatly modified.

A. Terrestrial forms, with antennules minute, and mandibles palpless.
(Abdominal segments rarely fused. Uropods subterminal).
Oniscoidea

B. Aquatic forms, with antennules not minute, and mandibles bearing palp, except in a comparatively few instances.
1. First pair abdominal limbs modified in male; this pair[1] absent in female. Antennal peduncle 6-jointed.
(All abdominal segments fused (except Stenasellus) Uropods subtemminal, styliform).
Asellota

2. First pair abdominal limbs not modified in male; this pair present in female. Antennal peduncle almost always 5-jointed.[2]
a. Body more or less compressed. Coxopodites of thoracic limbs small. Uropods subterminal, with styliform rami. (Abdominal segments free).
Phreatoicidea

b. Body more or less depressed. Coxopodites of thoracic limbs expanded into coxal plates. Uropods lateral; their rami not styliform.
i. Uropods biramous, lamellar, forming a caudal fan. Mouthparts often suctorial (Abdominal segments free or more or less fused).
Flabellifera

ii. Uropods have exopod vestigial or absent, endopod opercular, folded inwards over pleopods. Mouthparts normal. (Abdominal segments more or less fused).
Valvifera.

11. Parasitic forms, with female greatly modified. (Late larval stage has abdominal segments free and uropods terminal).
Epicaridea

---

[1] It was formerly thought to be the 2nd pair which is absent.

[2] By loss of precoxa.

KEY TO THE SUBORDERS OF AMPHIPODA

1. Head coalesced with 2nd thoracic segment. 3rd and 4th legs usually vestigial or absent. Abdominal segments generally a ll fused, abdominal appendages vestigial[1].

Caprellidea

11. Head not coalesced with 2nd thoracic segment. All thoracic legs present. Abdominal segments generally distinct, with well developed appendages.

A. Eyes lacking, but articulated eye-lobes present. First three, and last, pairs of abdominal limbs vestigial

Ingolfiellidea

B. Eyes present[2], much or little developed, but without eye lobes. All abdominal limbs usually well developed.

i. Palp of maxilliped with 2 - 4 segments; eyes rarely very large. Rarely pelagic.

Gammaridea

ii. Palp of maxilliped absent; eyes generally very large. Pelagic

Hyperidea

KEY TO THE ORDERS OF EUCARIDA

1. No statocysts. No maxillipeds. Maxilla not modified as a "scaphognathite".

Euphausiacea

11. Statocysts in the basal segment of the antennules.[3] Three pairs of maxillipeds. Maxilla modified as a "scaphognathite".

Decapoda

---

1 Cercops has stout 2-segmented limbs on abdominal segments 4&5.

2 Subterranean Gammaridea (Niphargus, etc.) are blind.

3 Absent in a few Caridea (Pandalus, Hippolyte, Virbius, Lysmata)

KEY TO THE SUBORDERS AND DIVISIONS OF DECAPODA

I. Body usually compressed, with long, compressed rostrum. Antennal scale usually large. Legs mobile, having basis free from ischium, and only one fixed point in carpopropodal articulation. Pleopods all present, large, and natatorial. Male opening arthrodial (Natantia).

A. 3rd legs chelate (unless much reduced). 3rd maxillipeds 7-jointed. Dendro- or trichobranchiate.

i. 3rd legs much larger than 1st or 2nd. Trichobranchiate.
Stenopidea

ii. 3rd legs not greatly different from 1st or 2nd. Dendrobranchiate.
Penaeidea.

B. 3rd legs no chelate. 3rd maxillipeds 4-6 jointed. Phyllobranchiate.
Caridea

II. Body not compressed, usually depressed: rostrum, if present, short and depressed. Antennal scale small or absent. Legs strong, with basis and ischium usually fused, and two fixed points in carpo-propodal articulation. Pleopods often reduced or absent, not natatorial. Male opening coxal or sternal. (Reptantia).

A. 3rd legs like the first (either chelate or simple). Abdomen macrurous. Gills numerous, trichobranchiate.

i. Carapace fused at sides with epistome. Rostrum small or absent (exc. Palinurellus). Body often depressed.
Palinura

ii. Carapace free from epistome. Rostrum of fair size. Body subcylindrical
Astacura

B. 3rd legs unlike first, never chelate. Abdomen rarely macrurous. Gills usually few, nearly always phyllobranchiate.

i. Carapace free from epistome. Uropods nearly always present. 3rd maxillipeds usually narrow.
Anomura

ii. Carapace fused at sides with epistome. Uropods absent (vestiges in Dromia). 3rd maxillipeds broad.
Brachyura

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.
Copepodoid larvae ("Cyclops" stages).

B. A carapace and paired eyes.

1. Thorax cirripede-like, abdomen 4-segmented, a bivalve shell.
"Cypris" larvae.

2. Thorax and abdomen malacostracan, carapace not bivalve. (Malacostracan larvae).

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.
Protozooea.

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.
Zooea.

(2) Limbs behind 3rd maxilliped present and if biramous well developed. Abdominal limbs all present.
(i) Limbs behind 3rd maxilliped uniramous. body Zooea-like.
Metazooea.

(ii) Well developed biramous limbs on most or all thoracic segments. Body not Zooea-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.

ARTHROPODA SELECTED BIBLIOGRAPH
WOODS HOLE

(References to be found in Drew and in Pratt are not included here. For further references, prior to 1926, consult Kükenthal & Krumbach.)

GENERAL

Beutler, Ruth. 1939. Vergleichende Betrachtungen über den Zuckergehalt des menschlichen und tierischen Blutes. Ergebn. Biol. 17: 1-104. 14 fig.

Brown, F. A. 1944. Hormones in the Crustacea. Their sources and activities. Quart. Rev. Biol. 19: 32-46 and 118-143.

Calman, W. T. 1909. Crustacea; in Lankester's "Treatise on Zoology". 1-346. 194 fig. London. (Esceptionally good on morphology and classification.)

Calman, W. T. 1911. The Life of Crustacea. 1-289. 32 pl., 85 fig. London. (Natural history at its best. Very easy reading.)

Cambridge Natural History, Vol. 4. 1909. Crustacea and Arachnida. 1-566. 287 fig. London. (Consult this especially for arachnids.)

Dennell, R. 1947. The occurrence and significance of phenolic hardening in the newly formed cuticle of Crustacea Decapoda. Prof. Roy. Soc. London (B), 134: 485-503. 2 fig.

Edwards, G. A. 1946. The influence of temperature upon the oxygen consumption of several arthropods. J. cell. comp. Physiol. 27: 53-64. 4 fig. (Emerita and Talorchestia at Nobska in winter, etc.)

Florkin, M., & Duchateau, G. 1943. Les formes du systeme enzymatique de l' uricolyse at l' evolution du catabolisme purique chez les animaux. Arch. internat. Physiol. 53: 267-307.

Fredericq, H. 1947. Les nerfs cardiorégulateurs des invertébres et la théorie des mediateurs chimiques. Biol. Rev. 22

Hansen, H. J. 1925. Studies of Arthropoda. 11. 1-176 (Very detailed study of segmentation of the appendages.)

Hurst, H. 1940. Permeability of insect cuticle. Nature, 145:462-463.

Hurst, H. 1941. Insect cuticle as an asymmetrical membrane. Nature, 147: 388-389.

Kukenthal, W., & Krumbach, T. 1926-7. Handbuch der Zoologie. Bd. 111, Erste Hälfte. Tardigrada. Pentastomida. Myzostomida. Arthropoda: Allegemeines. Crustacea. 1-1158. 1172 figs. Berlin. (The outstanding general reference book for the morphology, physiology, and taxonomy of these groups.)

Ludwig, D. 1945. The effects of atmospheric humidity on animal life. Physiol. Zool., 18: 103-135.

Maloeuf, N. S. R. 1938. Physiology of the alimentary tract of arthropoda. Riv. Biol., 24: 1-60. 3 fig.

Maloeuf, N. S. R. 1938. Physiology of excretion among the Arthropoda. Physiol. Rev., 18: 28-58. 3 fig.

Maloeuf, N. S. R. 1938. Secretions from ectodermal glands of arthropoda. Quart. Rev. Biol., 13: 169-195. 4 fig.

Maluf, N. S. R. 1939. Physiology of the arthropodan circulatory mechanisms. J. New York entomol. Soc., 47: 227-286, 8 fig.

Maluf, N. S. R. 1939. The bood of arthropoda. Quart. Rev. Biol., 14: 149-191. 1 fig.

*Page, L. 1933-4. Peches planktoniques a la lumiere effectuees a Banyula-sur-Mer et a Concarneau. 111. Crustacea. Arch. Zool. exp. gon., 76: 105-248. 14. fig.

Prosser, C. L. 1942. An analysis of the action of the action of acetylcholine on hearts, particularly in arthropoda. Biol. Bull. 83: 145-164. 3 fig.

Prosser, C. L. 1946. The physiology of nervous systems of invertebrate animals. Physiol. Rev., 26: 337-382.

Scharrer, E., and Scharrer, Berta 1945. Neurosecretion. Physiol. Rev. 25: 171-181. 2 fig.

Snodgrass, R. E. 1938. Evolution of the Annelida, Onychophora, and Arthropoda. Smithson. misc. Collec., 97: 1-159. 54 fig. (Excellent on embryology.)

Welsh, J. H., and Schallek, W. 1946. Arthropod nervous systems: a review of their structure and function. Physiol. Rev., 26:447-478.

Wigglesworth, V. B. 1939. The Principles of Insect Physiology. 1-434. 316 fig. London. (By far the best text on any kind of invertebrate physiology).

BRANCHIOPODA

Borg, F. 1935. Zur Kenntnis der Cladoceren-Gattung Anchistropus. Zool. Bidrag Uppsala, 15: 289-330. 2 pl., 13 fig.

Erikson, S. 1934-5. Studien über die Fangapparate der Branchiopoden nebst einigen phylogenetischen Bemerkungen. Zool. Bidrag Uppsala, 15: 21-287, 1 pl., 46. fig.

Lochhead, J. H. 1941. Artemia, the "brine shrimp". Turtox News, 19: 41-45. 1 fig.

Rammner, W. 1930. Phyllopoda. Die Tierwelt der Nord-und Ostsee (Grimpe u. Wagler), Lief. XVIII, Teil X.a., 1-32. 25 fig.

Sars, G. O. 1896. Fauna Norvegiae. Vol. 1. Descriptions of the Norvegian species at present known belonging to the sub-orders Phyllocarida and Phyllopoda). 1-140. 20 pl.

Scourfield, D. J. 1926. On a new type of crustacean from the Old Red Sandstone (Rhynie chert bed, Aberdeenshire) - Lepidocaris rhyniensis, gen. et sp. nov. Phil. Trans. roy. Soc. Lond., B 415, 153-187. 3 pl. 51 fig.

OSTRACODA

Cannon, H. G. 1931. On the anatomy of a marine ostracod, Cypridina (Doloria) levis Skogsberg, Discovery Reports, Cambridge, 2: 435-481. 2 pl. 12 fig.

COPEPODA

Clarke, G. L. 1939. The relation between diatoms and copepods as a factor in the productivity of the sea. Quart. Rev. Biol. 14: 60-64.

Lowe, Esther. 1935-6. On the anatomy of a marine copepod, Calanus finmarchicus (Gunnerus). Trans. roy. Soc. Edinb., 58: 561-603. 12 fig.

Lowndes, A. G. 1935. The swimming and feeding of certain calanoid copepods. Proc. zool. Soc. Lond., (1935) 687-715, 2 pl.

Pennak, R. W., & Zinn, D. J. 1943. Mystacocarida, a new order of Crustacea from intertidal beaches in Massachusetts and Connecticut. Smithson. misc. Collec., 103: 1-11. 2 pl.

BRANCHIURA

Herter, K. 1927. Reizphysiologische Untersuchungen an der Karpfenlaus (Argulus foliaceua). Z. vergl. Physiol., 5:283-370. 48 fig.

CIRRIPEDIA

Day, J. F. 1935. The life-history of Sacculina. Quart. J. micr. Sci. (N.S.) 77: 549-483. 11 fig.

Kuhnert, Liselotte, 1934-5. Beitrag zur Entwicklungsgeschichte von Alcippe lampas Hancock. Z. Morph. Okol., 29: 45-78. 24 fig.

McDougall, K. D. 1943. Sessile marine invertebrates at Beaufort, North Carolina. A study of settlement, growth, and seasonal fluctuations among pile-dwelling organisms. Ecol. Monogr., 13:321-374 19 fig. (Or: Bull. Duke Univ. mar Sta., 1). (Good review of zonation in barnacles.)

Moore, H. B. 1935-6. The biology of Balanus balancoides. IV. Relation to environmental factors. J. mar. biol. Assoc., (N. S.)20: 279-307. 11 fig.

LEPTOSTRAGA

Cannon, H. G. 1927-8. On the feeding mechanism of Nebalia bipes. Trans. roy. Soc. Edinb., 55: 355-369. 7 fig.

Manton, Sidnie, M. 1934. On the embryology of the crustacean Nebalia bipes. Phil. Trans. roy. Soc. Lond., B, 223; 136-238. 7 pl.17 fig.

MYSIDACEA

Cannon, H. G., & Manton, Sidnie M. 1927-8. On the feeding mechanism of a mysid crustacean, Hemimysis lamornae. Trans. roy. Soc. Edinb., 55: 219-253. 4 pl. 16 fig.

Nair, K. Bhaskaran. 1939. The reproduction, cogenesis and development of Mesopodopsis orientalis Tatt. Proc. Ind. Acad. sci. Sec. B. 9: 175-223. 10 pl. 41 fig.

CUMACEA

Dennell, R. 1934-6. The feeding mechanism of the cumacean crustacean Diastylis bradyi. Trans. roy. Soc. Edinb. 58: 125-142. 9 fig.

Foxon, G. E. H. 1936. Notes on the natural history of certain sand-dwelling Cumacea. Ann. Mag. nat. Hist., (10)17: 377-393. 7 fig.

TANAIDACEA

Dennell, R. 1937-9. On the feeding mechanism of Apseudes talpa, and the evolution of the Peracaridan feeding mechanisms. Trans. roy. Soc. Edinb., 59: 57-78. 9 fig.

ISOPODA

Monod, T. 1926. Les Gnathidae. Essai monographique. (Morphologie, biologie, systematique). Mem. Soc. Sc. Natur. Maroc, Rabat, 13: 1-668. 1 pl. 277 fig.

Suneson, S. 1947. Colour change and chromatophore activators in Idothea. Lunds. Univ. Arsskr.,(n.F.) Avd. 2,43 (5): 1-34 8 fig.

AMPHIPODA

Beadle, L. C., & Cragg, J. B. 1940. Studies on adaptation to Salinity in Gammarus spp. 1. Regulation of blood and tissues and the problem of adaptation to fresh water. J. exp. Biol., 17: 153-163. 2 fig.

Dennell, R. 1933-4. The habits and feeding mechanism of the amphipod Haustorius arenarius Slabber. J. Linn. Soc. Lond. (Zool.) 38: 363-338. 13 fig.

Goodhart, C. B. 1939. Notes on the bionomics of the tube-building amphipod, Leptocheirus pilosus Zaddach. J. mar. biol. Assoc. (n.S) 23: 311-25. 3 fig.

Hart, T. J. 1930. Preliminary notes on the bionomics of the amphipod, Corophium volutator Pallas. J. mar biol. Assoc., (n.S.) 16: 761-789. 7 fig.

Holmes S. J. 1901. Observations on the habits and natural history of Amphithoe longimana Smith. Biol. Bull., 2: 165-193. 2 fig.

Lowenstein, O. 1935. The respiratory rate of Gammarus chevreuxi in relation to differences in salinity. J. exp. Biol., 12: 217-221.

Watkins, E. E. 1941. The yearly life cycle of the amphipod, Corophium voluntator. J. animal Ecol., 10: 77-93, 3 fig.

SYNCARIDA

Manton, Sidnie, M. 1930. Notes on the habits and feeding mechanisms of Anaspides and Paranaspides (Crustacea, Syncarida). Proc. zool. Soc. Lond., (1930), 791-800. 4 pl. 1. fig. (Beautiful colored paintings of the animals in their natural habitat.)

DECAPODA

Barnes, T. C. 1932. Responses in the isolated limbs of Crustacea and associated nervous discharges. Amer. J. Physiol., 99: 321-331.2fig.

Blaschko, H., Cattell, M. & Kahn, J. L. 1931. On the nature of the two types of response in the neuromuscular system of the crustacean claw. J. Physiol., 73: 25-35.

Bloch, F. 1935-7. Contribution a l'etude des gametes et de la fecondation chez les Crustaces Decapodes. Trav. Stat. zool. Wimereux, 12: 181-279. 168 fig.

Bohn, G. 1902. Des mecanismes respiratoires chez les crustaces decapods. Bull. sci. France Belg., 36: 178-551. 209 fig.

Borradaile, L. A. 1922-4. On the mouth-parts of the shore crab. J. Linn. Soc. Lond. (Zool.), 35: 115-42. 2 pl.

Brock, F. 1930. Das Verhalten der ersten Antennen von Brachyuren und Anomuren in Bezug auf das umgebende Medium. Z. vergl. Physiol., 11: 774-790. 5 fig.

Brown, F. A. Jr. 1940. The crustacean sinus gland and chromatophore activation. Physiol. Zool., 13:343-55. 10 fig. (Figures the glands in seven species.)

Brown, F. A. 1946. The source and activity of Crago-darkening hormone (CDH). Physiol. Zool., 19:215-223. 4 fig.

Brown, F. A. Jr., & Wulff, V. J. 1941. Chromatophore types in Crago and their endocrine control. J. coll. comp. Physiol., 18: 339-353. 4 fig.

v.Buddenbrock, W. 1914. Uber die Orientierung der Krebse im Raum. Zool. Jahrb. (allg. Zool.), 34: 479-514.

Crane, Jocelyn. 1943. Display, breeding and relationships of fiddler crabs (Brachyura, genus Uca) in the northeastern United States. Zoologica (N. Y.), 28: 217-223. 1 fig.

Cole, W. H. 1941-2. A perfusing solution for the lobster (Homarus) heart and the effects of its constituent ions on the heart. J. gen. Physiol., 25: 1-6.

Drach, P., 1939. Mue et cycle d'intermue chez les Crustaces Decapodes. Ann. Inst. Oceanog. Monaco, (n.S) 19: 103-391. 6 pl. 13 fig.

Faxon, W. 1879. On some young stages in the development of Hippa, Porcellana, and Pinnixa. Bull. Mus. comp. Zool., Harvard, 5:253-268. 5 pl.

Foxon, G. E. H. 1934. Notes on the swimming methods and habits of certain crustacean larvae. J. mar. biol. Assoc.,(n.S.) 19: 829-49 7 fig.

Gurney, R., 1939. Bibliography of the larvae of Decapod Crustacea. Ray Society Monograph (1937), No. 125. London. 1-123.

Gurney, R. 1942. Larvae of Decapod Crustacea. Ray Society Monograph (1941), No. 129, 1-306. 122 fig.

van Harreveld, A., & Wiersma, C. A. G. 1939. The function of the quintuple innervation of a crustacean muscle. J. exp. Biol., 16: 121-133. 5 fig.

V. Heerdt, P. F., & Krijgsman, B. J. 1939-40. Die Regulierung der Atmung bei Eriocheir sinensis Milne Edwards. Z. vergl. Physiol., 27: 29-40. 6 fig.

Heldt, Jeanne P. 1938-9. La reproduction chez les Crustaces Decapodes de la familla des Peneides. Ann Inst. Oceanog. Monaco, (n.S.) 18: 31-206. 131 fig.

Hodgkin, A. L. 1938-9. The subthreshold potentials in a crustacean nerve fibre. Proc. roy. Soc. Lond. (B), 126: 87-121 23 fig.

Holmes, W. 1942-6. The giant myelinated nerve fibres of the prawn. Phil. Trans. roy. Soc. Lond., 231B: 293-311. 48 fig.

Hyman, O. W. 1922. Adventures in the life of a fidler crab. Rep. Smithson. Inst. Wash. (1920), 443-459. 6 pl.

Jackson, H. G. 1913. Lupagurus. Liverpool mar. biol. Comm. Mem., 21: 1-88. 6 pl.

Jones, L. L. 1941. Osmotic regulation in several crabs of the Pacific Coast of North America. J. Cell. comp. Physiol., 18:79-92. 2 fig.

Knowlton, F. P. 1942. Observations on the dual contraction of crustacean muscle. Biol. Bull., 82: 207-214.

Kropp, B., & Crozier, W. J. 1928-9. Geotropic orientation in Arthropoda. III. The fiddler crab Uca. J. gen. Physiol., 12: 111-122 6 fig.

Kuffler, S. W., and Katz, B. 1946. Inhibition at the nerve muscle junction in Crustacea. J. Neurophysiol., 9: 337-346. 7 fig.

Kyer, D. L. 1942. The influence of the sinus glands on gastrolith formation in the crayfish. Biol. Bull., 82: 68-78.

MacGinitio, G. E. 1930. The natural history of the mud-shrimp, Upogebia pugettonsis. Ann. Mag. nat. Hist., (10) 6: 36-44. 3 pl. 1fig.

MacGinitie, G. E. 1938. Movements and mating habits of the sand crab, Emerita analoga. Amer. midland Nat., 19: 471-481. 9 fig.

Maluf, N. S. R. 1939. On the anatomy of the kidney of the crayfish and on the absorption of chlorid from freshwater by this animal. Zool. Jb., Abt. allg. Zool. Physiol. Tiere, 59: 515-534. 19 fig.

Maluf, R. 1941. Experimental cytological evidence for an outward secretion of water by the nophric tubule of the crayfish. Biol. Bull. 81: 127-133. 1 pl.

Maluf, R. 1941. Micturition in the crayfish, and further observations on the anatomy of the nephron of this animal. Biol. Bull., 81: 134-148.

Maluf, N. S. R. 1941. Secretion of inulin, xylose and dyes and its bearing on the manner of urine-formation by the kidney of the crayfish. Biol. Bull., 81: 235-260. 7 fig.

Mansour-Bek, J. J. 1932. Die proteolytischen Enzyme von Maja squinado Latr. Z. verg. Physiol., 17: 153-208. 14 fig.

Morgan, T. H. 1924. The artificial induction of symmetrical claws in male fiddler crabs. Amer. Nat., 58: 289-295. 2 fig.

Nicol, Edith A. T. 1932-33. The feeding habits of the Galatheidea. J. mar. biol. Assoc., (n.S.) 18: 87-106.

Orton, J. H. 1927. On the mode of feeding of the hermit crab, Eupagurus bernhardus, and some other Decapoda. J. mar. biol. Assoc. (n.S) 14: 909-921.

Panikkar, N. K. 1941. Osmoregulation is some palaemenid prawns. J. mar. biol. Assoc. (n.S.) 25: 317-359. 8 fig.

Pantin, C. F. A. 1936. On the excitation of crustacean muscle. 11. Neuromuscular facilitation. 111. Quick and slow responses. 1V. Inhibition. J. Exp. Biol. 13: 111-130. 7 fig. 148-158, 4 fig; 159-169, 5 fig.

Parrot, J.-L. 1941. Recherches sur la transmission chimique de l'influx nerveux chez les crustacés. Libération d' une substance active sur l'intestin de Maia squinado par l'excitation des nerfs cardio-inhibiteurs. C.r. Soc. Biol., 135: 929-933. 2 fig.

Pearse, A. S., Humm, H. J; and Wharton, G. W. 1942. Ecology of sand beaches at Beaufort, N. C. Ecol. Monogr., 12: 135-190. 2 4 fig (Wharton covers life cycle, habits, and ecol. of Emerita talpoida)(

Pearson, J. 1908. Cancer. (The adible crab). Liverpool mar. Biol. Comm. Mem. No. 16., 1-2)9. 13 pl. 13 fig. or Proc. Trans Liverpool biol. Soc. 22 (1907-1918), 11. Trans., 291-499. 13 pl. 13 fig.

Perkins, E. B. 1928. Color changes in crustaceans, especially in Palaemonetes. J. Exp. Zool., 50: 71-105. 3 pl.

Regan, Sister Mary L. 1944. Histo-chemical observations on glycogen in the liver of the blue crab, Callinectes sapidus Rathbun. Publ. Chesapeake biol. Lab., 62: 1-14. 4 fig.

Reinhard, E. G., and v. Brand, T. 1944. The fat content of Pagurus parasitized by Peltogaster and its relation to theories of sacculinization. Physiol. Zool. 17: 31-41. 2 pl.

Roberts, T. W. 1944. Light, eyestalk chemical, and certain other factors as regulators of community activity for the crayfish, Cambarus virilis Hagen. Ecol. Monogr., 14: 359-392. 21 fig.

Robertson, J. D. 1937-8. Some features of the calcium metabolism of the shore crab (Carcinus maenas Pennant). Proc. roy. Soc. Lond. B. 124: 162-82.

Schallek, W. 1942. Some mechanisms controlling locomotor activity in the crayfish. J. exp. Zool. 91: 155-167.

Schijfsma, K. 1935. Observations on Hydractinia echinata (Flem.) and Eupagurus bernhardus (L.). Arch. néarl. Zool. 1: 201-314.

Smith, R. I. 1947. The action of electrical stimulation and of certain drugs on cardiac nerves of the crab, Cancer irrorotus. Biol. Bull. 93:72-88. 2 fig.

Stauber, L. A. 1945. Finnotheres ostreum, parasitic on the American oyster, Ostrea (Gryphaea) virginica. Biol.Bull., 88:269-291. 4 pl.

Volz. P. 1838. Studien über das "Knallen" der Alpheiden. Nach Untersuchungen an Alpheus dentipes Guérin und Synalpheus laevimanus (Heller). Z. Morph. Ökol. Tiere, 34: 272-316. 17 fig.

Webb, D. A. 1940. Ionic regulation in carcinus maenas. Proc. roy. Soc. Lond. (B), 129: 107-136. 7 fig.

Wells, W. W. 1940. Ecological studies on the Pinnotherid crabs of Puget Sound. Bull. Univ. Wash. Seattle, Gen. Ser., 2:19-50 1 fig.

Welsh, J. H. 1939. Chemical mediation in crustaceans. 11. The action of acetylcholine and adrenalin on the isolated heart of Panulirus argus. Physiol. Zool., 12:231-237. 5 fig.

Welsh, J. H. 1941. The sinus glands and 24-hour cycles of retinal pigment migration in the crayfish. J. exp. Zool., 86: 35-49. 3 fig. (Figures sinus gland and its nerve supply.)

Wiersma, C. A. G. 1941. The efferent innervation of muscle. Biol. Sympos. 3:259-289.

Wiersma, C. A. G. 1941. The inhibitory nerve supply of the leg muscles of different decaped crustaceans. J. comp. Neurol., 74: 63-79. 2 fig.

Wiersma, C. A. G., & Wright, E. B. 1947. The nature of the action potentials of crustacean muscles. J. exp. Biol., 23:205-212. 2 pl.

Younge, C. M. 1924. Studies on the comparative physiology of digestion. 11. The mechanism of feeding, digestion and assimilation in in Nephrops norvegicus. Brit. J. exp. Biol., 1: 343-389. 18 fig.

MARINE INSECTS

Imms, A. D. 1906. Anuridæ. Liverpool mar. biol. Comm. Memm., No. 13, or Proc. Trans. Liverpool biol. Soc., 20 (1905-1906), 11 Trans., 353-451. 7 pl. 4 fig. (Also lists other marine insects).

ARACHNIDA

Cole, L. J. 1901. Notes on the habits of pycnogonids. Biol. Bull., 2: 195-207. 5 fig.

Cole, W. H. 1923, Circus movements of Limulus and the tropism theory. J. gen. Physiol., 5: 417-426. 3 fig.

Fage, L. 1932-3. Pêches planctoniques a la lumière effectuées a Banyuls-sur-Mer et a Concarneau. 11. Pycnogonides, Arch. Zool. exp. gén., 74: 249-61. 1 fig.

Gerard, R. W., & Hartline, H. K. 1934. Respiration due to natural nerve impulses. A method for measuring respiration. J. cell. comp. Physiol., 4: 141-160. 3 fig. (Limulus)

Hartline, H. K. 1934. Intensity and duration in the excitation of single photoreceptor units. J. cell. comp. Physiol., 5:229-47. (Limulus)

Helfer, H., & Schlottke, E. 1935. Pantopoda. Bronn's Klassen u. Ordnungen des Tierreichs, Bd. 5, Abt. 1V, Buch 2: 1-314.

Schlottke, E. 1935. Biologische, physiologische und histologische Untersuchungen über die Verdauung von Limulus. Z. vergl. Physiol. 22: 359-413. 28 fig.

Versluys, J., and Demoll, R. 1922-3. Das Limulus-Problem. Die Verwandschaftsbeziehungen der Merostomen und Arachnoideen unter sich und mit anderen Arthropoden. Ergebn. Fortschr. Zool., 5: 67-388. 123 fig.

ARTEMIA, THE BRINE SHRIMP

Please report to J.K.Lochhead any mis-statements found in this outline.

Artemia is not a true shrimp, but belongs to a quite different, and primitive order of crustaceans. It is able to live in a notably wide range of salinities, from a salt concentration not much above that of fresh water, up to one over three times that of sea water. Normally, however, Artemia is only found in highly saline lakes and pools, probably because it falls an easy prey to the predators present in other environments. The distribution is world wide, mostly in the warmer and more arid regions. Dried eggs, from which laboratory cultures can be started, are on sale by biological supply companies. The closely related fresh water "fairy shrimps", such as Eubranchipus, occur seasonally in small, usually temporary ponds, and differ from Artemia only in minor anatomical features.

Living specimens are best for study. These should be watched in the culture jar, and can be mounted whole under a cover glass, or between two thin slides if both sides are to be studied on a single specimen. Chloroform or ether (a few drops to 10 ml. of water) may be used, if desired, as a narcotant or to kill the specimens. Dissection of appendages or other parts should be done with mounted needles, under a binocular.

Body plan

The body appears divided into three main regions, but because of difficulty in defining thorax and abdomen in a manner permitting comparisons with other crustaceans, it seems best to speak only of a head and trunk. The trunk has eleven limb-bearing somites, followed by two genital somites and six further, limbless somites. Attached to the last somite is a caudal furca, which is used as a rudder and for making sudden spurts through the water. It is, however, greatly reduced in polyploid specimens reared in strong brines.

Exoskeleton

The whole body is covered by an extremely thin exoskeleton, which nevertheless is remarkably impermeable to salts, water, and many other substances. This exoskeleton is molted about every 4-6 days, throughout the adult life of several months.

Paired appendages

The head bears the five pairs of appendages typical for Crustacea.

The antennules are slender and tipped with a few chemo-sensory(?) setae, or "aesthetascs". The antennae are much thicker, do not bear setae and are modified in adult males as large claspers. The large mandibles are without palps, and act like a pair of mill wheels, rolling food into the mouth. Each of the much smaller maxillules bears a sheaf of setae, all curving medio-anteriorly, and lying one above the other in a single, almost vertical plane. The maxillae are greatly reduced, being discernable with difficulty as small bumps, each bearing only three setae, located between the maxillules and the bases of the first trunk limbs. To see them and the maxillules, it is necessary to lift forward the large upper lip or "labrum".

The trunk bears eleven pairs of leaf-like appendages, or "phyllopodia". A typical one of these, from near the middle of the series, should be dissected off as close to the body as possible, then carefully spread out in a drop of water and mounted under a cover-glass. The different parts arise from a central "corm". Along the median border there is a series of five "endites", of which especially the first two bear a long, regular fringe of filter setae, serving an important role in the process of filter feeding. Distally the endites are succeeded by a large "telopodite" (see Ericsson, 1934-5), bearing strong, scraping setae, used in feeding on bottom detritus. Lateral to the telopodite is the highly movable "exopodite", of special importance in swimming; it is fringed with long, feathered, paddle setae. Proximal to it, on the lateral border, is the fleshy "metipipodite", serving probably as a gill and perhaps also in the osmo-regulation of salts. More proximal still is a very thin flap, the "protepipodite".

Having determined the various parts on the flattened limb, their arrangement on the intact animal should be studied. It will be seen that the endites, telopodite, and epipodites all are directed backwards, forming valvular flaps closing off the space between their own limb and the limb next behind. Alternate expansion and contraction of the inter-limb spaces, as the limbs beat forward and back, is largely responsible for the current of water which flows toward the animal, bringing food particles and oxygen. The food particles enter a wide median space, between the two walls of filter setae, from which they pass to the median, ventral food grove, in which they are swept forwards to the mouth.

Digestive system

A short, vertical fore-gut, or esophagus, leads up from the mouth, to open at right angles into the long mid-gut or stomach-intestine. In front of the point of entrance of the esophagus, the mid gut expands into a pair of pouches. Secretion of digestive enzymes and absorption of food products probably occurs both here and along the length of the mid-gut, which also plays an important part in osmo-regulation. Posteriorly the mid-gut joins the short hind-gut or rectum, provided with dilator muscles. This opens at the anus, between the furcal rami. Fore-gut and hind-gut both are provided with a thin exoskeletal lining.

Circulatory system

The heart is a long tube, dorsal to the gut, extending the length of the trunk. Blood enters it posteriorly and through valved lateral slits, or "ostia", of which there is one pair in each of the posterior 14-15 somites. At the anterior end blood leaves the heart, entering the hemocoelic spaces of the head, and then circulating through the hemocoel of the trunk and appendages. Though there are no definite blood vessels, the blood nevertheless follows a more or less constant route. Movements of the trunk appendages undoubtedly aid in the circulation. Frequently the blood is colored red by a dissolved pigment closely related to hemoglobin. A single type of blood cell is present, behaving as amoebocytes when adhering to tissue surfaces, and originating from blood-cell-forming organs located near the base of each trunk limb (best seen in frontal sections).

Excretory system

Excretion probably is performed mainly by the pair of "maxillary glands", which form rounded prominences on the side of the body just behind the mandibles. Each consists of a blind, coelomic end-sac, followed by a coiled tubule which opens on the maxilla. The corresponding "antennary glands" of the nauplius larva, are claimed by some recent investigators to be entirely absent in the adult, but a rudiment of the end-sac does seem to persist, its cells selectively taking up certain pigments. Uptake of pigment granules and other foreign particles also is performed by large, "phagocytic storage cells", located in many parts of the body, and perhaps most easily seen in the antennae. In well-fed individuals these same cells also store fat and glycogen.

Nervous system

The cerebral ganglion, circum-esophageal connectives, and two parallel chains of ventral ganglia, connected in ladder-like fashion by cross-commissures, can best be studied in frontal sections. The cerebral ganglion receives nerves from the eyes and antennules. Nerves from the antennae join the circum-esophageal connectives. A pair of ventral ganglia occurs in each somite, from the mandibular to the second genital, posterior to which there is only a slender pair of ventral nerves.

Sense organs

The stalked compound eyes, each composed of numerous "ommatidia", are conspicuous. The smaller, median "nauplius eye", is also easily found. It is formed of three hemispherical cups, and is located just in front of the cerebral ganglion. The aesthetascs on the antennules have already been noted. Other presumed sense organs, including probably organs of taste on the mouth parts, have been described, but are difficult to find.

Striking responses to light are easy to demonstrate on living specimens. Most obvious is a dorso-ventral orientation, in which the ventral surface is kept constantly directed towards the source of light. Less frequent is an antero-posterior orientation, expressed as either negative or positive phototaxis. One method to demonstrate this is to keep specimens two to three hours in the dark in water made alkaline with sodium hydroxide, then to shine a foucussed beam of light through the aquarium. For several minutes the animals will then display marked negative phototaxis.

Reproduction

In the male the testes are long and tubular, located in the limbless part of the trunk. Sperm are produced in the more posterior parts, while the anterior part secretes a mucilaginous fluid. From the anterior end of each testis a coiled glandular duct leads ventroposteriorly to one of the two penes which project from the genital somites. The antennae are modified as enormous claspers, shaped so as to fit tightly around the female just in front of the ovisac. Clasping persists a number of days, the animals swimming around while thus united as a pair. Copulation occurs after each molt of the female. In some stocks of Artemia males are absent, reproduction then being parthenogenetic.

In the female the ovaries are located much like the testes of the male. Anteriorly each is connected by a short duct to a "lateral pouch", situated in the dorsal part of the prominent "ovisac". Here the ova undergo maturation. When filled with ova the lateral pouches are round and easily seen, but at other times they may be collapsed and more difficult to distinguish. Each opens into the median central part of the ovisac. Here the eggs are fertilized, acquire a shell, and undergo some embryonic development. Groups of shell glands, appearing like clusters of grapes, open into the median ovisac, and muscles are present by which the eggs can be kept moving. Shortly before a molt, either thick-shelled eggs or free-swimming nauplii are discharged from the ovisac, depending on whether the shell gland secretion was abundant or scanty.

REFERENCES

Barnon, H. G. 1933. On the feeding mechanism of the Branchiopoda... etc. Phil. Trans. roy. Soc. Lond., (B) 222: 267-352.

Eriksson, S. 1934-5. Studien über die Fangapparate der Branchiopoden ....etc. Zool. Bidrag Uppsala, 15: 21-287.

Heath, H. 1924. The external development of certain phyllopods. J. Morphol., 38: 453-483.

Lochhead, J. H. 1941. Artemia, the "brine shrimp". Turtox News, 19: 41-45.

Lochhead, J. H., and Lochhead, Margaret S. 1941. Studies on the blood and related tissues in Artemia (Crustacea Anostraca), J. Morphol., 68: 593-632.

Warren, H. S. 1930. The central nervous system of the adult Artemia. Trans. Amer. micr. Soc., 49: 189-209.

THE CRAYFISH

Please report to J.H.Lochhead any mis-statements found in this outline.

Many species of crayfish exist, belonging to two families, several sub-families, and over thirteen genera. Preserved specimens sold for dissection in North America often, but by no means always, belong to the species Procambarus clarkii, a native of fresh-water marshes in the Gulf states and lower Mississipi valley. Living specimens studied in the laboratory are apt to belong to any large species locally obtainable. The present account is sufficiently general to apply to any species, and can also be used for the lobster (Homarus), which belongs to a third family placed in the same division as the two crayfish families. The habitats favored by crayfish vary with the species. Most live under water, in streams, lakes, or swamps, but some live on moist land, where they construct burrows surmounted by the wellknown "crayfish chimneys". A variety of both plant and animal food is taken by most species.

Preserved specimens, with the blood system color-injected, are most often used for laboratory study. But there are advantages to be gained from the use of living specimens. Not only do they permit observation of vital activities, but many of the internal organs show up better in the fresh state than after preservation. Chloroform can be added to the water as a narcotant, or the specimens can be bled prior to dissection by cutting off the limbs with scissors. On specimens not bled in this way, the blood system can be color-injected, as will be described later.

Body plan

Head and thorax are fused together to form a "cephalothorax", the probable line of fusion being marked by a "cervical groove". A sharp "rostrum" projects forward from the head between the eyes. Covering the thorax is a hard "carapace", fused dorsally to all its somites, and extending down on each side to form a gill cover, or "branchiostegite". It should be noted that this is a hollow outgrowth of the body, having outer and inner epidermal walls, with a blood space between. Division of the thorax into somites is revealed ventrally by a series of triangular "sternal plates" lying between the bases of the limbs. The abdomen is made up of six somites, plus a terminal "telson"; peg and socket joints between the somites permit ventral flexure. In the exoskeleton of each abdominal somite a dorsal "tergum" and ventral "sternum" are distinguished, joined together on each side by a lateral "pleuron".

Exoskeleton and hypodermis

The whole body is covered by an exoskeleton, secreted by a very thin, darkly pigmented epidermis (here often termed the "hypodermis"). If a small portion of the hypodermis is mounted under the microscope, it will be seen to contain numerous chromatophores. These appear stellate in shape, with fine, branched processes when the contained pigment is dispersed, appear smaller and more rounded when the pigment is concentrated. The movements of the pigment are regulated by hormones, although in adult animals the color changes thus produced are barely visible through the thick exoskeleton. The exoskeleton is hardened by calcium salts, except at the flexible joints and in the period just after a molt. Molting of adult crayfish occurs, in at least some species, only twice a year. Many species are restricted to waters with a calcium content above a certain minimum.

Appendages

The appendages vary much in appearance, but are thought nearly all to be derived from a single basic type. Reference to the figure given under enimysis will show the parts of this basic, "biramous" appendage. In the simplest case there is a "protopodite", divided into two segments, the "coxa", and "basis", from the last of which arises a median "endopodite" and a more lateral "exopodite". In addition, on the appendages of the head and thorax, the coxa may bear a lateral "epipodite", and both the coxa and basis may be produced into median lobes or "endites". On the thoracic appendages the endopodite usually is divided into five segments, the"ischium", "merus","carpus", "propus", and "dactylus" ("can bad indigestion make crayfish penitent drinkers?").

Because of the way some of the appendages overlap, it is best to study them in succession from behind forwards. They are numbered, however, from before backwards. To see the basal parts of most of those on the head and thorax, the branchiostegite and the ventro-posterior covering of the head should be removed from one side. This can be done by cutting with scissors from the dorso-posterior margin of the branchiostegite, along a line high on the side of the thorax, then curving ventrally a short distance in front of the cervical groove. The small portion of exoskeleton of the head thus included has to be carefully freed from underlying tissues with the aid of a sharp scalpel. As far as possible the appendages should be studied in situ on the ani-

cel. If it is necessary to remove an appendage for closer study, this is best done by cutting with a sharp scalpel as close to the body as possible, while pulling the appendage with forceps held in the other hand. Afterwards the corresponding limb of the other side can be studied *in situ*. Observations of limb movements on living specimens ought also to be made.

The most posterior appendages are the "uropods". On these the endopodites and exopodites are modified as flat, rounded blades. When expanded, the two uropods, together with the telson, form a wide "tail fan", by means of which the animal can dart backwards. Further forward are the "pleopods" or "swimmerets" of the first five abdominal somites. The third, fourth, and fifth pleopods are simple, biramous, best developed in the female, in which sex they serve to carry the eggs; the attaching cement for this purpose is said to be secreted by "tegumental glands" in the ex- and endopodites, and sometimes adjacent regions. In both sexes the pleopods beat so as to cause a current of water, but the functional significance of this is uncertain. The second pleopods resemble the succeeding three pairs in the female, but in the male are modified as stiff copulatory appendages. The first pleopods are much reduced in the female (in certain species sometimes absent), while in the male they again are modified as stiff, copulatory appendages. (In crayfishes of the southern hemisphere the first pleopods are absent in both sexes).

On the thorax the last five pairs of appendages are called "pereiopods". Exopodites are absent on all of these. Each of the first three pairs terminates in a "chela", so constructed that the dactylus can bite against a prolongation of the propus. The term "cheliped" is appropriate for these three pairs of appendages, but usually is restricted to the large first pair. This pair comes into use chiefly as weapons of defense or in predatory feeding, while the two smaller pairs are used for cleaning the body and for picking up small particles of food which they then pass to the third maxillipeds. The large first pereiopod, as compared with succeeding pairs, has undergone some torsion, bringing the dactylus into a median rather than a lateral position. A clear concept of this torsion can be gained by determining the axis of flexure at each joint of the first two pereiopods, and indicating these axes by means of pins appropriately inserted. Incidentally it will be found that the line of flexure differs at every joint, a feature which gives the limbs a wide range of mobility. The large

Chelipeds further differ from the other pereiopods in that the basis and ischium are firmly fused together (though occasionally this condition is observed on the other pereiopods too). Perhaps associated with this fusion is the fact that it is especially the large chelipeds which can be cast off, or "autotomized", when the limb is wounded or firmly held. The break on such occasions always occurs at a specific fracture-plane in the basi-ischium, possibly representing the plane of fusion between the two segments, though more probably lying in the ischium just distal to this. Fracture-planes just distal to the joint between basis and ischium, occur also on the remaining pereiopods, though autotomy of these limbs is relatively rare. Crayfish have been reported to cast these appendages only when in (or recently removed from) their natural habitat, while lobsters seem unable to cast them without an assisting pull applied distally. After the autotomy of any limb, loss of blood is minimized by a diaphragm and valve, which may be seen closing the end of the stump. Other features of interest to observe on the pereiopods include special setae and spines on the dactylus and propus of the fifth, or fourth and fifth legs, used by the female to clean the abdomen and pleopods before egg-laying; also, in certain male crayfish, large spiny projections from the ischia of the third, and sometimes an adjacent pair of pereipods, used to lock under the legs of the female during mating. The male and female genital openings also should be noted, located on the median-posterior border of the coxa of the fifth or of the third pair of pereiopods respectively.

The three anterior thoracic appendages are called "maxillipeds". Of these, the third pair forms a protective cover for the other mouth parts; it also helps to close off a part of the channel in which the respiratory current flows; its endopodite assists in tearing and manipulating the food, and its exopodite, together with those of the other two maxillipeds, often beats in such a way as to give further impetus to the emerging respiratory current; in the lobster the coxa of this appendage is produced into two small endites. The second maxilliped resembles the third, but has a much smaller endopodite; there are no endites. The first maxilliped has a much reduced endopodite, a large endite on both coxa and basis, and a leaf-like epipodite folded longitudinally to form a groove into which fits the scaphognathite of the second maxilla.

Each thoracic appendage, except the first and the last, bears a gill, attached to the coxa. Further details concerning these will be

riven under the respiratory system.

The head bears the five appendages typical for Crustacea. On he maxilla, the exopodite and epipodite are fused into a leaf-like lap, the "scaphognathite" or "gill bailer", important in respiration; he endopodite is small and unsegmented; the coxa and the basis each ears a prominent two-lobed endite. The maxillule is small and curves closely around the mandible; it lacks an exopodite and epipodite, has small endopodite, and has relatively large endites attached to coxa nd basis. The mandible has a small, three-segmented "palp", of which probably the two distal segments represent the endopodite, and a much larger biting portion, probably the coxa; not much actual chewing is done by the mandibles, which serve rather either to roll food into the outh, or to hold it firmly while fragments are torn off by the third maxillipeds, On the antenna there is a coxa, a basis, a scale-like xopodite, and an endopodite consisting of three large segments plus long, terminal flagellum; the most proximal segment of the endopodite ppears to be divided longitudinally, permitting greater flexibility of ovement; a noticeable mound on the coxa contains the opening from the reen gland; the left and right exopodites can be spread out to act as orizontal rudders, especially during a downward dive through the water. The antennule consists of three basal segments, followed by two small lagella; these are not thought of as being exopodite and endopodite, since the primitive crustacean antennule was uniramous.

Respiratory system

On each side of the thorax a gill chamber is present, bounded xternally by the branchiostegite, internally by the body wall, and to large extent closed ventrally by the appendages. Small chinks between the appendages open into the chamber ventrally, but the only considerable openings are one at the ventro-posterior angle of the branchiostegite and one through a channel which leads out near the anterior nd of the scaphognathite. The rapid beat of the scaphognathite can e observed in a living specimen if this organ is exposed by cutting ut a narrow strip of the branchiostegite just posterior and parallel o the ventral end of the cervical groove. A strong current of water s driven forward in the channel leading out from the gill chamber, y the alternate beating of the posterior (epipodite) and anterior exopodite) portions of the scaphognathite. Water enters the gill hamber mainly through the posterior opening, as can be demonstrated

by introducing from a pipette a few drops of carmine suspension near this opening. Once every few minutes the scaphognathite reverses its beat for a few strokes, so as to blow loose any particles of silt or other debris, which may have settled on the gills.

The gills are of the type known as "trichobranchs", each consisting of numerous filaments arranged around a central axis, somewhat like a bottle brush. Three series of gills may be distinguished, those arising from the coxae of the limbs being called "podobranchs", those arising from the membranous joints which attach the coxae to the body being called arthrobranchs, and those arising higher on the side of the body, being called "pleurobranchs". Each thoracic appendage except the first and the last, bears a single podobranch. The axes of these gills perhaps represent modified epipodites, since in most crayfishes of the southern hemisphere a few gill filaments occur on the epipodite of the first maxilliped. However, in crayfishes of the northern hemisphere, and in lobsters, each podobranch (except the last one in Cambarinae) is accompanied by a leaf-like lamella (the "mastigobranch") which at least in appearance more closely resembles an epipodite. In crayfishes this lamella is longitudinally pleated and is fused along most of its length with the axis of the gill. In lobsters the lamella and gill are separate. Each gill-bearing appendage, except the second maxilliped, also bears a tuft of hair-like "coxal setae", sometimes termed a "setobranch"; these tufts help to close off the gaps between appendages which lead up into the gill chamber. Just proximal to the coxa of each thoracic appendage, except the first two and the last, two arthrobranchs arise (except in some crayfish of the southern hemisphere); the more anterior one in each case arises slightly ventral to the more posterior one. In addition, there is a single (anterior) arthrobranch proximal to the second maxilliped, except in the lobster. One pleurobranch occurs on each of the last four thoracic somites of the lobster, but in most crayfish the first three of these four gills are either rudimentary or absent, and in Cambarinae no pleurobranchs are present.

Circulatory system

Parts of this system are among the first items to be met with in an internal dissection. Thus it seems desirable to describe the circulatory system now, although some parts will not be seen until later. The internal dissection is best done under water, particularly when dissecting very soft structures, such as the hepatopancreas and gonads.

If a living specimen is being used, the circulatory system may be color-injected. This is best done by boring a hole mid-dorsally in the carapace, half-way between the posterior border and the cervical groove, and using a syringe needle just large enough to fill the hole. This method usually fills the pericardial sinus and branchio-cardiac sinuses, as well as the heart and arteries. If it is desired to avoid having the heart surrounded by the color-mass, it is possible to inject the heart directly, using a very fine needle, after first having cut a small window through the carapace and dorsal wall of the pericardial sinus.

To see the heart and neighboring arteries, it is necessary to remove the carapace and the dorsal exoskeleton of the head. For this, suitable lateral scissors-cuts should be made and the exoskeleton then carefully loosened from underlying tissues with a sharp scalpel, starting at the posterior margin of the carapace. Considerable care is necessary not to damage certain arteries and muscles located close under the hypodermis. The dorsal exoskeleton of the abdomen should be removed in the same way.

The heart lies in a space called the "pericardial sinus". It is suspended by three elastic ligaments on each side, and is stretched laterally by these during diastole. Blood enters the heart from the pericardial sinus through valved slits, termed "ostia", of which there is a dorsal, a lateral, and a ventral pair. Thin-walled arteries, whose connection with the heart is very easily broken, leave the heart anteriorly and posteriorly. An internal valve is located at the start of each. From the anterior dorsal margin of the heart a slender "ophthalmic artery" runs forward in the mid-line, close under the hypodermis, to supply the brain and eyestalks, as well as parts of the stomach. A large "antennary artery" on each side starts close to the origin of the ophthalmic and runs latero-anteriorly, to supply the

hepato-pancreas, gonad, adductor muscle of the mandible, stomach and its muscles, other thoracic muscles, branchiostegite, body wall, eyestalk, antennule, antenna, and antennal gland. From the ventro-anterior margin of the heart a pair of "hepatic arteries" originates, each running a course parallel to the more dorsal antennary artery, and supplying the hepato-pancreas. Ventro-posteriorly the heart tapers into a thin-walled bulb from which two arteries take their origin. The "dorsal abdominal artery" runs backwards, dorsal to the intestine, giving off a pair of lateral segmental arteries in each abdominal somite, to supply the intestine, posterior parts of the hepato-pancreas and gonad, abdominal muscles, pleopods, uropods, and telson. The "sternal artery" runs vertically downwards from the posterior bulb of the heart, supplying the genital ducts with minute branches, then passing either to left or right of the intestine and piercing the ventral nerve cord, below which it divides into a small, "ventral abdominal artery" and a larger, "ventral thoracic artery". The ventral abdominal supplies the nerve cord, the last two pairs of pereiopods, and a small portion of the ventral part of the abdomen. The ventral thoracic supplies the nerve cord, first three pairs of pereiopods, the mouth parts, and portions of the antennal glands.

All of the arteries branch repeatedly, the fine, ultimate branches sometimes being termed capillaries, because they often have a diameter as small or even smaller than that of a mammalian capillary. However, such a use of the term seems inadvisable, since we have no proof of physiological exchanges through the walls of these vessels, nor do they connect with venules as do true capillaries in a closed blood system. Instead, they open into tissue spaces, in which the blood can bathe the tissue cells directly. Some of these spaces are no more than the chinks between cells. These, however, are continuous with wider spaces, termed sinuses, as well as with the primary body cavity, or "hemocoel". In the sinuses and hemocoel the blood follows a more or less definite course of

circulation, eventually reaching the "sternal sinus" in the thorax, where it surrounds the ventral nerve cord and ventral thoracic artery. From here the blood enters the gills, passing up the outer side of each gill in an "afferent branchial sinus", then descending on the inner side of the gill in an "efferent branchial sinus." A small branch from the afferent sinus leads out to a superficial plexus in each gill filament, from where the blood is returned to the efferent sinus by a second small channel. From the gills the blood on each side of the body passes to the pericardial sinus through six "branchio-cardiac sinuses" (five in the lobster). These run dorsally in the body wall, which here is very thin and membranous, being protected by the overlying gills and branchiostegite. From the pericardial sinus the blood enters the heart, as already described.

The nervous supply to the heart will be described under the nervous system, but if only a single specimen is available and it is desired to see these nerves, they should be looked for before the dissection has progressed too far.

The blood has a slightly bluish tint, imparted to it by a respiratory pigment, "hemocyanin", dissolved in the plasma. Three types of blood cells are present: 1. "Explosive corpuscles", which initiate blood-clotting by an explosive cytolysis. 2. "Thigmocytes", which phagocytize foreign particles and which further contribute to blood-clotting by throwing out long, fine processes. 3. "Amoebocytes", which are much less phagocytic than the thigmocytes, but which display considerable amoeboid activity, as can be observed in a gill filament of a living animal. Blood allowed to flow out under water usually coagulates rapidly, the clot having the consistency of a soft gel. The blood cells are derived, at least in part, from a blood-cell-forming organ on the dorsal surface of the cardiac stomach, as well as from small nodules in the connective tissue in various parts of the body. The organ on the

stomach is thin, semi-transparent, formed of discontinuous lobules in the crayfish, more solid and continuous in the lobster.

Muscular system

Muscles will be considered now, because many of them have to be cut during the dissection of other structures. Only the most important ones will be mentioned. Some will not be seen until later in the dissection, but are nevertheless included here.

Most noticeable are the muscles of the abdomen. Of these, the relatively slender extensors originate dorso-laterally in the thorax, a short distance behind the cervical groove, and run back through the pericardial sinus, to insert on the six terga of the abdominal somites. Also starting in the thorax, the much more powerful flexor muscles originate near its floor, along most of the length of the upper surface of the endophragmal shelf; passing into the abdomen the fibers become arranged in groups and follow a complexly twisted course, inserting on the six sterna and in the telson.

Prominent on each side of the stomach is a large "adductor mandibularis" muscle. This originates dorsally by a large head attached to the exoskeleton, which quickly tapers to a strong tendon running anteroventrally, to insert about mid-way along the posterior border of the mandible. Contraction of the two adductors rotates the mandibles in such a way that they bite together.

Connecting the stomach with the exoskeleton are a number of paired muscles. Largest of these are three pairs chiefly responsible for the action of the teeth in the gastric mill (q.v.), namely the "anterior", "posterior", and "postero-lateral gastric muscles". These are all dorsal in position. A number of pairs of much smaller "dilator muscles" also run between the stomach and various parts of the exoskeleton. In the wall of the stomach itself numbers of small "intrinsic muscles" run

between various ossicles. One recent author claims that these serve chiefly to bring about movements whereby the teeth and other internal parts of the gastric mill are kept clean.

Under the endophragmal shelf will be found the proximal muscles of the pereiopods. Most of these have their origin on parts of the endophragmal skeleton (g.v). Two small muscles insert on the proximal rim of each coxa, four insert on the proximal rim of the basis of the large chelipeds, and two insert on the proximal rim of the basis of each of the other pereiopods. It is of interest to note that the muscle used in autotomy is the "levator anterior basis" in the case of the large chelipeds, or simply the "levator basis" in the case of the other pereiopods. This muscle can best be found by cutting a window in the dorsoanterior part of the coxa, enlarging the window if necessary after the muscle has been seen. The autotomizer muscle in the chelipeds inserts by two separate tendons.

The more distal muscles of the pereiopods both originate and insert within the appendage. Some of them have provided material for important studies of neuro-muscular physiology, but space precludes a discussion of them here.

Reproductive system.

To disclose the gonad it is necessary to remove the heart and the tough, membranous floor of the pericardial sinus. In the female care is necessary not to damage the thin-walled oviducts which lie immediately below the pericardial floor and which may be confused with the transverse bands of fibers in that floor.

In the crayfish there is a single, Y-shaped gonad, very small in immature specimens, becoming much larger at maturity, particularly in the female. The paired lobes of the Y extend forwards on either side of the stomach, while the unpaired lobe may extend back a short distance

into the abdomen. In a male the testis often resembles the hepatopancreas (g.v.) in its whitish color and soft consistency, and thus rather easily becomes indistinguishable if at all squashed during dissection. In some crayfish the three distal extremities of the testis have the form of little, bulbous swellings, of which the posterior one sometimes is found twisted below the intestine.

In the lobster the ovary is H-shaped, the short cross-bar being placed just in front of the pericardial sinus. The paired longitudinal parts may extend two-thirds the length of the animal. In fresh specimens the color of the ovary provides interesting evidence regarding the reproductive history of the individual. For four or five years the ovary slowly matures, going through color phases of bright yellow, flesh color, salmon, light olive green, and a rich dark green. Copulation then occurs (immediately following a molt), and one or two months later the eggs are laid. The ovary now is greyish-white with green flecks (the remains of eggs that failed to be discharged). After five or six weeks the ovary turns light green and the flecks become orange. The eggs which were laid are retained on the swimmerets for about eleven months, when the larvae hatch. Shortly after that there is another molt and copulation again occurs. Meanwhile the shade of green of the ovary steadily darkens, until again the eggs are laid, two years after the first laying. The ovary is again greyish-white for a few weeks, but this time has both orange flecks and green flecks (remains of ova which failed to be discharged at the first and second layings respectively).

The testis of the lobster is H-shaped like the ovary, or the cross-bar may be lacking so that there are separate paired testes.

The oviducts are paired and start below the pericardial sinus, each passing outwards to the body wall and then running downwards just inside this, to open on the inner aspect of the coxa of the third pereiopod. Each duct is wide and ribbon-like as it leaves the ovary, but quickly

tapers to a small cylindrical tube. Often the ducts are overlooked because they are thin-walled and transparent. But at the time of egg-laying the walls actually become quite glandular, contributing an albuminous or mucilaginous secretion.

The vasa deferentia are paired and leave the gonad at the same level as do the oviducts. In the crayfish each vas deferens is a small, relatively thick-walled tube, often pinkish in color, which coils frequently on its way to the external aperture at the tip of a flexible papilla on the inner aspect of the fifth pereiopod. Usually the coils are especially numerous in the upper part of the duct, and care is needed not to be deceived as to the point where the duct leaves the testis. The walls of the duct secrete a gelatinous material which surrounds the sperm as it passes out. In the lobster the duct first runs outwards from the testis to the body wall, then curves ventrally and thickens along its dorso-posterior margin. This thickened part of the duct is glandular and coils in an S-shaped fashion. It is followed by a small swelling, representing a sphincter, and this in turn by a thinner "ductus ejaculatorius".

A "seminal receptacle" is present between the bases of the fourth and fifth pairs of pereiopods in the female of the lobster and of certain crayfish genera. This is not an internal structure, but is simply a small pocket in the exoskeleton. Frequently it is termed the "annulus". It is thought to have evolved independently in the lobster and in crayfish respectively.

During copulation the male holds the female on her back by means of his chelipeds and other pereiopods, aided in some crayfishes by the spiny projections on certain of the ischia. The copulatory pleopods, which normally lie forwards tightly pressed against the ventral surface of the thorax, are raised to about a forty-five degree angle, and then are held in that position by one of the fifth pereiopods carefully

crossed under the body. The flexible papillae on which the vasa deferentia open, fit into the grooves of the first pleopods, and issuing sperm flows along these grooves, surrounded by a gelatinous secretion. If there is a seminal receptacle, the tips of the pleopods are inserted into this and the sperm passes in, being securely retained there by the gelatinous secretion which hardens into a plug. Where there is no seminal receptacle, the sperm is deposited in pockets, termed "spermatophores", on various parts of the female's body, but particularly between the bases of the last two pairs of pereiopods. In crayfishes copulation occurs when both male and female have completely hardened exoskeletons, but in the lobster copulation is always shortly after a female molt, when her exoskeleton is soft or at most of a leathery consistency.

Egg-laying occurs quite some time after copulation -- 10-45 days later in the case of some crayfish without any seminal receptacle, a few days to several months later in the case of crayfish with a seminal receptacle, and up to a year or more later in the case of the lobster. The sperm remain viable all this time, and indeed have been found to survive at least two years in the receptacle of the lobster. During egg-laying the female lies on her back, with the abdomen curled forwards so tightly that the space in which the pleopods occur is completely enclosed except for an opening in front. The eggs emerge from the oviducts in a mucilaginous secretion and are carried back into the temporary brood chamber by a current of water caused by the beating of the second and perhaps other pairs of pleopods. The ova are fertilized by sperm released from the receptacle or from spermatophores, and are attached to the pleopods by a secretion which hardens into a cement. After some weeks or months, during which the developing embryos are continually supplied with fresh water by movements of the pleopods, the young hatch, as schizopod larvae in the case of the lobster, or as

miniatures of the adult in the case of a crayfish. Newly hatched crayfish remain attached to the maternal pleopods for a further short period of time.

Digestive system.

The digestive tract is divided into fore-gut, mid-gut, and hind-gut. The internal epithelium of the first and the last of these is ectodermal and secretes an exoskeletal lining which is cast at each molt.

All parts of the digestive tract should first be studied in situ in the animal. To facilitate this the body wall of the cephalothorax should be cut away on one side, if this has not already been done. Care has to be taken not to break the delicate connections of the hepatopancreas and intestine behind the pyloric stomach. After a general study has been completed, the different portions of the digestive tract may be removed from the body for study of parts not otherwise visible. However, this should not be done until certain parts of the nervous system (q.v.) have been studied, unless a second specimen is to be available. The stomach and the hind-gut should be slit open mid-ventrally and their internal structure observed under water. A small part of the hepatopancreas should be mounted for study under the microscope.

From the mouth a short, vertical "esophagus" runs up to open into the floor of the "cardiac stomach". The opening is guarded by a median anterior and a pair of lateral valves, all beset with setae pointing into the stomach. On the anterior wall of the cardiac stomach a large oval area is present on each side which before each molt secretes a calcareous disc known as a "gastrolith". Immediately after the molt the two gastroliths, being no longer protected by the overlying exoskeletal lining, are dissolved in the stomach. Probably the calcium

salts thus released are absorbed and used for prompt hardening of certain essential structures such as the mandibles, tips of the chelae, and teeth of the "gastric mill".

It is as the site of the gastric mill that the cardiac stomach is chiefly important. The grinding elements of this mill are three teeth, one dorsal and two lateral, much more used for chewing the food than are the mandibles or other mouth parts. The mechanism for moving the teeth and other parts of the cardiac stomach is very complex, involving some thirteen pairs of muscles and a framework inside the stomach-wall composed of about twenty-eight distinct plates, ossicles and bars. Small setae on many parts of the stomach-lining point towards the region where the three teeth bite together.

In the cardiac stomach the food not only is ground up, but also is digested by enzymes from the hepatopancreas, which arrive probably via route "3" described in the next paragraph. Only after the food has been reduced to fine proportions or is in solution, can it pass into the next division of the stomach. Larger particles have to be rejected through the esophagus.

A constriction marks the boundary between the cardiac stomach and the much smaller "pyloric stomach". Internal folds and ridges of the stomach-lining, beset with numerous setae, form a complex system of filters and channels, through which small particles are admitted from the cardiac stomach into the pyloric stomach, and thence either into the intestine or into the left or right hepatopancreas. Three main routes are distinguished: 1. A route starting just behind the dorsal tooth and running in a dorsal groove of the pyloric stomach, termed the "mid-gut filter", which presently divides into left and right channels opening dorsally into the mid-gut. 2. A pair of routes starting below the lateral teeth, curving laterally around a median ventral projection termed the "cardio-pyloric valve", then coming together behind this to run

back medianly between a pair of plates which form a "muscular press", about mid-way between the upper and lower limits of the pyloric stomach.

3. A route starting behind the esophagus, in a ventral groove of the cardiac stomach, then diverging ventro-laterally around the cardio-pyloric valve, to enter the "gland filters", which lie within a pair of prominent ventral swellings of the pyloric stomach; materials in solution, or particles small enough to pass between the tiny setae in the gland filters (spaced about 10μ apart), pass on into channels leading to the openings of the left and right divisions of the hepatopancreas; particles too large to passthrough the gland filters can pass dorsally by converging channels which join route "2" in front of its entrance into the mid-gut.

The walls of the pyloric stomach are stiffened internally by some nine plates and bars, complexly articulated with one another. Five or six muscles serve to regulate the size of the various channels and to squeeze particles through some of the filters.

What appears to be the posterior part of the pyloric stomach is the beginning of the mid-gut. This lacks an exoskeletal lining and possesses a cuboidal rather than a columnar type of epithelium. Its junction with the pyloric stomach slants ventro-anteriorly. Into it a very delicate and easily broken hepatopancreatic duct opens on each side, just dorso-posterior to the swelling of the pyloric stomach which houses the gland filter. Four valvular flaps, dorsal, lateral, and ventral, project into the mid-gut from the pyloric stomach. Dorsally a short mid-gut caecum is directed dorso-anteriorly, pressed so closely against the pyloric stomach that it frequently is overlooked. Just posterior to this a slight, transverse ridge can usually be seen in crayfishes, marking the end of the mid-gut and the beginning of the hind-gut. The latter runs back as the "intestine" almost to the telson, where it enlarges slightly as the "rectum". The anus opens on the ven-

ral surface of the telson. The internal surface of the hind-gut is thrown into six longitudinal folds and is covered throughout with an exoskeletal lining. In the lobster the situation is rather different. Here the mid-gut includes the whole intestine, which runs back to the last abdominal somite, where it meets the hind-gut modified as a relatively large rectum; a posterior mid-gut caecum is given off dorsally just before the junction with the rectum, and runs back most of the length of the sixth abdominal somite. In larval lobsters the mid-gut is said to be short and the hind-gut long, as in crayfishes.

The hepatopancreas is divided into left and right divisions, although these are united under the pyloric stomach. The two divisions represent outgrowths from the mid-gut. The whole organ is invested by a very thin membrane. When this is broken the tiny terminal tubules which make up the bulk of the organ may be seen to fray out under water. The total internal surface offered by these tubules is very large, since they are extremely numerous and the two divisions of the hepatopancreas extend nearly the whole length of the cephalothorax and into part of the abdomen. The color of the hepatopancreas varies in fresh specimens from whitish, through yellowish, to various shades of green, depending on the season and the stage of the molt cycle.

Histologically the tubules and ducts of the hepatopancreas show four types of cells. Most numerous are columnar cells with a striated border, important in absorption and in storage of reserve food substances. Less abundant are vacuolated, often pear-shaped cells, which secrete the digestive enzymes. Still less frequent are certain tiny cells near the basement membrane, which are thought to replace spent gland cells. Outside the basement membrane both circular and longitudinal muscle fibers are said to occur, but of such minute size as to be almost undetectable in ordinary sections.

The functional importance of the hepatopancreas is unusually

aried. It is the chief site of absorption, the absorptive surface of he rest of the mid-gut being very small. It secretes all the diges- ive enzymes. Its cells store reserve food substances in quantities arying with the seasons and with the stages of the molt cycle. Pro- ably it acts to regulate the amount of sugar and possibly other con- tituents in the blood. It is the chief organ of excretion for dialy- able dyes injected in excess into the blood. Its cells contain a com- lete set of enzymes for breaking purines down through uric acid and rea to ammonia.

Excretion and osmo-regulation.

The principal organs for excretion are the "antennary glands", requently referred to as the "green glands". These are located, one n each side, anterior to the ventral portion of the cardiac stomach. n the crayfish each consists of a ventral, glandular part, connected ostero-dorsally with a more dorsal bladder, which drains anteriorly hro a duct leading ventrally to the opening on the ventral surface of he antennal coxa. The glandular part is of a firm consistency, shaped ike a plano-convex lens, with the flattened surface directed upwards. he bladder lies on top of this and is apt to be overlooked, since it sually is collapsed and its walls are very thin and membranous. When ull, in a living animal, the bladder may be considerably larger than he glandular part, pressing posteriorly against the stomach, hepato- ancreas, and other organs.

Internally the glandular part has a complex structure, difficult o make out even in sections. Essentially the organ begins blindly with a "coelomosac", located dorso-anteriorly, with complexly infolded walls. his opens anteriorly into the "labyrinth", made up of a maze of chan- els occupying the peripheral parts of the organ. A long and very much onvoluted "tubule" follows, which occupies most of the central portion f the organ and leads finally to the opening into the bladder.

The cells of all three of these sections of the glandular part of the nephron are secretory in appearance. Branches from the antennal and ventral thoracic arteries provide an unusually copious blood supply, their minute terminal branches discharging into tiny hemocoelic spaces which surround all the ramifications of the gland. The bladder is non-secretory and receives from the antennal artery a much more restricted arterial blood supply.

It is reported that no valves or sphincters occur between any of the internal parts of the gland. Only at the external orifice is a valvular arrangement present. Here a soft operculum covers most of the opening in the papilla on the antennal coxa. The actual excretory opening is only a thin, crescentic slit near the anterior border of this operculum, the concavity of the crescent being directed forwards. Any attempt to inject or inflate the bladder will fail, unless the needle or tube is aimed accurately at the crescentic slit rather than at the operculum as a whole.

In the antennal gland of the lobster certain important differences have to be noted. The coelomosac occupies most of the dorsal aspect of the glandular part, with the labyrinth surrounding it peripherally and extending under it ventrally. A tubule is absent, probably correlated with the fact that the lobster secretes a scanty, isotonic urine, in contrast to the copious, hypotonic urine secreted by the freshwater crayfish. From the antero-median portion of the labyrinth a white projection extends ventro-posteriorly, containing a number of parallel tubes which open into the duct from the bladder not far from its external orifice. There is no direct opening into the bladder, and thus the bladder can be filled only by the backing up of urine through its duct. This duct runs downward from the bladder in front of the glandular part of the organ, then turns and runs ventro-posteriorly to the external opening, located in the center of the operculum.

Besides the antennal glands, the hepatopancreas and the gills are of importance in excretion or osmo-regulation. The roles of the hepatopancreas in excretion have already been mentioned. At the gills a passive exchange of ions and water molecules occurs between the blood and the surrounding water, and there is evidence also that the gills are able to take in certain ions against the concentration gradient. When dyes which are non-dialysable or of large molecular size are injected into the blood, they are accumulated by certain cells in the gills, as well as by the cells of the coelomosacs in the antennal glands. (The injection of dyes into the hemocoel can provide a striking demonstration of the separate identity of the parts of the antennal glands. Suitable for the purpose in a large crayfish would be 0.25 ml. each of 0.5% aqueous solutions of cyanol and Congo red. Examination 1½-2½ hours after injection should show the lumen of the labyrinth to be a vivid blue, and the cells of the coelomosac a bright red.)

Endoskeleton

The exoskeleton not only is continued internally as linings for the fore- and hind-gut, but also projects internally as "apodemes", secreted by in-pocketings of the epidermis. Collectively, these apodemes form the "endoskeleton". Some of them take the form of tendons for the attachment of certain muscles. Others are in the form of hard, skeletal parts. Of these the most conspicuous are those in the floor of the thorax, which form the "endophragmal shelf". This is a lattice-work made up of curiously shaped apodemes, of which two pairs project internally at each junction between somites. The median pair forms an arch over the sternal sinus, in which the nerve cord and the ventral thoracic artery lie. Each member of the lateral pair is in the shape of a Y when viewed from above, with the stem of the Y directed outwards and the arms of the Y meeting the tops of successive endosternal arches.

functions of the endophragman shelf are to afford protection for the ventral nerve cord, to contribute one component in the joint for the articulation of each of the pereiopods, and to provide attachment for a number of muscles, particularly those of the appendages below, and the large flexor abdominis above.

Prior to each molt certain parts of the endoskeleton are resorbed, to make the molt mechanically possible. All other parts of the endoskeleton are cast at the molt along with the exoskeleton to which they remain attached.

For those who wish to study in detail the endoskeleton and the ossicles of the stomach, three types of preparation can be employed. Boiling in 5% KOH to remove all non-skeletal tissues is the method of preparation most often used. This has the disadvantage, however, of removing the cuticulin from the skeleton, leaving only the underlying chitin. A perfect cast skin is superior in this respect, but lacks a few connecting parts of the endoskeleton. If a really complete specimen is desired, this can be obtained by prolonged tryptic digestion, followed by soaking in any suitable fat solvent.

Nervous system

The "cerebral" or "supra-oesophageal" ganglion lies in the midline, just ventral to a point between the eyestalks. From this a number of nerves radiate out, of which four pairs are larger than the others, namely: 1. The nerves to the antennules. 2. The optic nerves from the eyes, each expanded within the eyestalk into a series of four optic ganglia; these latter will be seen when the dissection for the sinus gland is carried out, as described under "endocrine glands". 3. Tegumentary nerves from epidermal sense organs on the dorsal surface of the head. 4. The nerves to the antennae, with small branches to the excretory bladders.

Larger than any of these nerves are two "circum-esophageal connectives", which run from the cerebral ganglion to the "sub-esophageal" ganglion, located behind the esophagus underneath the anterior end of the endophragmal shelf. A slight swelling on each connective as it passes to one side of the esophagus marks the position of the "connective ganglion". Two minute nerves leave this ganglion, belonging to the "stomatogastric system". One of these passes forwards and medianly, to meet its fellow from the other side under the stomach. Here a minute median branch is received from the cerebral ganglion, and another branch passes over the anterior surface of the stomach towards its dorsal surface. Other branches of the stomatogastric system, too minute to be easily seen, ramify over the surface of the stomach and esophagus, pass outwards to certain areas of the integument, and extend back dorsally as far as the pericardial sinus.

If the esophagus is gently pulled forwards, a slender "tritocerebral commissure" may be seen, running just behind the esophagus from one connective to the other.

To see the regulatory nerves of the heart, which issue from the subesophageal ganglion, a special method of preparations has to be used. First, the esophagus should be cut, just above the connectives. Then the stomach and the hepatopancreas should be carefully pulled forwards and removed. The adductor muscles of the mandibles also should be removed. This should reveal the "cardiac inhibitors", a pair of nerves which emerge from a squarish median hole in the endophragmal shelf, just behind the esophagus and just in front of the most anterior point of origin of the flexor abdominis muscles. Each of these nerves passes outwards and backwards, crossing the extensor abdominis muscle to the body wall, inside of which it climbs dorso-posteriorly to the pericardial sinus, where it breaks up into an internal plexus. The pair of "cardiac accelerators" emerges through the second median hole in the

endophragmal shelf, which is much larger and more rounded than the squarish, first hole. This hole, however, is covered by the flexor abdominis muscle, the anterior origin of which should be carefully cut, at least on one side, and the muscle cautiously bent back. Each accelerator nerve follows a course parallel to the more anterior inhibitor, and eventually joins the same pericardial plexus.

Details of the pericardial plexus can be made out only with great difficulty. It is known, however, that it connects dorso-laterally with the heart. Impulses arriving via either accelerator or inhibitor fibers serve only to modify the beat of the heart. Normally this beat is maintained at a steady rate by a spontaneous, rhythmic discharge of impulses from a ganglionic group of scattered nerve cells on the inner dorsal surface of the heart. There are about sixteen such cells in the crayfish, nine in the lobster. They can be seen by slitting open the heart mid-ventrally, pinning it out flat, and staining for about ten hours in a refrigerator with 0.005% methylene blue in physiological saline, acidulated to pH 4.0-5.0 with hydrochloric acid.

Since the sub-esophageal ganglion and succeeding thoracic ganglia lie under the endophragmal shelf, the beginner may find it easiest to identify the ventral nerve cord first of all in the abdomen, and then work forwards. To do this, the left and right flexor muscles in the abdomen should be separated with the fingers, aided slightly with a scalpel. Looking down between these muscles in the mid-line, the ventral nerve cord will then be seen, lying just above the slender ventral abdominal artery. In each of the first five abdominal somites the cord is slightly expanded as a ganglion, each of which gives rise to three pairs of lateral nerves, the third pair appearing to come off slightly behind the ganglion. In the sixth abdominal somite a terminal ganglion is present which supplies the telson as well as the last somite.

On following the nerve cord forwards into the thorax it will be found to dip down into the sternal sinus. Here it can be traced further forwards to the sub-esophageal ganglion by gradually cutting or chipping away the endophragmal shelf. The two most posterior thoracic ganglia are very close together, just behind the point where the sternal artery comes down to pass between the two halves of the nerve cord. In front of this, three distinct ganglia are present, one in each of the somites bearing the first three pairs of pereiopods. In the somite bearing the third maxillipeds a ganglion is present indistinctly divided from the sub-esophageal ganglion. This latter represents the fused ganglia of the somites bearing the mandibles, maxillules, maxillae, and first two pairs of maxillipeds.

The ventral nerve cord may now be lifted out from the animal and placed in water in a petri-dish, for study under the binocular microscope. Roots of the lateral nerves will show up clearly, and it will be seen that the cord and its ganglia really are paired, though the left and right divisions are for the most part rather completely fused. In the connectives between ganglia only nerve fibers are present, while in the ganglia nerve cells also are present. Among the nerve fibers it should be possible to distinguish four especially large ones, located near the mid-line on the dorsal surface. These are the "giant fibers", which conduct impulses more rapidly than do the other fibers. Their stimulation brings about the "escape reflex", in which the antennae, pereiopods, and pleopods are thrust forwards, while the flexor muscles of the abdomen contract strongly to produce a powerful flip of the tail. If not seen in a whole mount, the giant fibers can be located in a histological cross-section.

The innervation of the appendages will not be described here, but certain unique features about the nerves in the pereiopods deserve special mention. Each of these nerves contains the usual number of sensory

fibers (upwards of 10,000), but contains extremely few motor fibers and is further remarkable in containing a few special inhibitory fibers. Determinations of the number of efferent fibers in the nerve as it passes through the ischium have indicated about twelve motor and three inhibitory fibers. These have to supply the great number of muscle fibers in seven different muscles, which they do by repeated branching. Moreover, each of the fibers in a single muscle may be supplied by up to four motor nerve fibers and sometimes by two inhibitory nerve fibers. Naturally the efferent nerve fibers in the main nerve are all much larger than are the sensory fibers, a fact which makes possible their isolation for physiological experimentation.

Sense organs

A pair of stalked "compound eyes" is present, each composed of upwards of 10,000 photoreceptor units called "ommatidia". Under a binocular microscope the exoskeleton of the eye can be seen to be modified into many corneal lenses or facets, mostly square in shape, each being the outermost portion of a single ommatidium. The inner parts of these ommatidia, and their connections with the optic nerve, can be made out in a general way in an eye bisected longitudinally with a sharp scalpel, or in a histological preparation, but to ascertain the whole structure would require a more prolonged microscopical study.

Each ommatidium is surrounded along its whole length by a sleeve of cells containing black pigment and near its inner end by some cells which contain a reflecting pigment. In a light-adapted eye the black pigment is distributed along most of the length of the cylindrical sleeve, so that light entering the ommatidium is not able to pass into neighboring ommatidia. In a dark-adapted eye the black pigment is concentrated at the two ends of the sleeve, leaving the reflecting pigment exposed, and permitting light to pass from one ommatidium to another.

These differences in the distribution of black pigment are easily seen in eyes split open longitudinally after the animals have been killed by immersion in water at 80°C. for 10-15 seconds. The two different states also can be detected in intact animals by briefly illuminating the eye, in the dark, with a bright beam of light. If the eye is light-adapted it will appear black, especially towards the periphery. If the eye is dark-adapted it will still appear black around the periphery, but in a large circular area in the center will be a brilliant orange-red. This color results from the fact that the exposed reflecting pigment near the inner end of each ommatidium can reflect the light back through the central "rhabdome", containing "visual red".

Distal to the rhabdome, between it and the corneal facet lies the "crystalline cone", which plays the principal role in the optics of the ommatidium.

Even after loss of the eyes, crayfishes still are able to avoid light. The photo-receptor concerned in this reaction has been shown to lie in the sixth abdominal ganglion, although the actual receptor cells have not been demonstrated. Possession of such a caudal photo-receptor is perhaps of value to the crayfish when backing into a supposedly dark crevice; a second opening into the crevice would admit light, which might act as a stimulus to the crayfish warning it of the unsafe nature of its retreat.

Identified as probable chemo-receptors are certain small, blunt-tipped setae, with thin walls, and without lateral setules, known usually as "aesthetascs". These occur on some of the mouth parts, and more particularly on the ventral surface of the more distal segments of the outer flagella of the antennules. In this latter position the setae are in small tufts of three or four setae, two tufts to each segment, and each seta has a characterictis node about half-way along its length. Chemo-sensitivity has been demonstrated on various other parts of the

body, but the nature of the receptors in such locations remains uncertain.

Setae which respond to touch, and perhaps sometimes to water currents, are abundant on various parts of the body. These setae are small, pointed, usually feathered with setules, and at the base each has a thin-walled, bulb-like swelling of the exoskeleton, permitting free movement of the seta in any direction.

A specialized use of such setae is met with in the "statocysts", one of which is located in the proximal segment of each antennule. Each of these statocysts consists of a sac, opening to the outside, within which are numerous sand grains and the sensory setae. The external opening is a minute slit, concealed by a tuft of setae, located on the dorsal surface of the proximal segment, about one-third of the distance from the anterior end. In large specimens a fine pin can be pushed through the opening into the membranous sac, which occupies most of the proximal two-thirds of the segment. To see this sac it is necessary to cut away the exoskeleton on the two sides of the segment, using a sharp scalpel. The sac should then be cut open dorso-laterally, to disclose the sand grains and the rows of minute sensory setae, arranged in a horse-shoe shaped fashion on the floor of the sac, and in a small tuft projecting down from the dorsal surface. A binocular microscope is required to see the details. Most or all of the sand grains are cemented to the setae, though many of the delicate strands of cement are apt to become broken during the dissection. Like all other sensory setae the ones here are supplied by nerve fibers, which in this case come from a small branch of the antennulary nerve, entering the sac posteriorly. If a crayfish commences to molt in captivity it may be possible to repeat Preidl's classical experiment, by placing the animal in filtered water with a supply of minute iron filings on the bottom. The lining of the membranous sac, with its contained sand grains, is cast at the molt,

and after the molt the animal rubs its antennules on the bottom to refill the sac. If iron filings, instead of sand grains, enter the sac, it becomes possible to induce the animal to respond to the direction of a magnet instead of to the direction of gravity.

Proprioceptors presumably must be present to make possible the maintenance of posture and of muscle tonus, particularly in the pereiopods. The only sense receptors of this type for which we have any clear evidence are some so-called "movement receptors", located in the membranous joints between successive segments of the legs. These respond to movement, but not to tension or pressure. They can be demonstrated physiologically, but are little known morphologically.

Endocrine glands

Internal regulation in the crayfish or lobster is partly nervous, but depends also on a variety of hormones. Activities affected by these hormones include dispersion and concentration of the pigment in chromatophores, migration of retinal pigment in the eyes, frequency of molting, deposition of the gastroliths, amount of blood sugar, and the rate of heart beat. Hormones influencing all these activities are secreted by the "sinus glands", one of which is located in each eyestalk. In addition at least two chromatophorotropic hormones are secreted by unidentified cells in certain parts of the central nervous system.

The sinus glands are extremely difficult to locate in preserved specimens. In a fresh specimen they are more easily found, because of a characteristic bluish color. If, in such a specimen, the exoskeleton, the hypodermis, and the dorsal retractor muscle, are carefully removed from the dorsal surface of the eyestalk, as far out as the base of the eye, the sinus gland will then be seen, lying on the postero-dorsal surface of the second and third optic ganglia. The gland is roughly stellate in shape, with blunt lobes. It can be seen more clearly after re-

moval of the thin sheath which covers it and the optic ganglia. This may also reveal another gland which may turn out to be endocrine in nature, namely the "x-organ". In the crayfish this is very small and is located some distance proximal to the sinus gland. In the lobster the x-organ is larger, shaped like a bunch of grapes, and located on the anterior surface of the optic ganglia, just opposite the sinus gland.

Selected references

Baumann, H. 1919-21. Das Gefässsystem von Astacus...etc. Z.wiss.Zool., 118: 246-312. 35 fig.

Bock, F. 1925. Die Respirationsorgane von Potamobius astacus...etc. Z.wiss.Zool., 124: 51-117. 27 fig.

Herrick, F.H. 1911. Natural history of the American lobster. Bull. U.S. Bur. Fish. (1909) 29: 149-408. 42 fig., 20 pl.

Huxley, T.H. 1880. The Crayfish. An Introduction to the Study of Zoology. xiv + 371. 81 fig. and fpc. (Reprinted 1884, as "An Introduction to the Study of Zoology, illustrated by the crayfish"). Internat. Scientific Ser., Vol. 28, Appleton, N.Y.

Keim, W. 1915. Das Nervensystem von Astacus...etc. Z.wiss.Zool., 113: 485-545. 28 fig.

Kaluf, R. 1941. Micturition in the crayfish and further observations on the anatomy of the nephron of this animal. Biol. Bull.,81: 134-148. 3pl. (See also his 1939 paper which he cites).

Penn, G.H.Jr. 1943. A study of the life history of the Louisiana red-crawfish, Cambarus clarkii Girard. Ecol., 24: 1-18. 4 fig.

Schmidt, W. 1915. Die Muskulatur von Astacus...etc. Z.wiss.Zool., 113: 165-251. 26 fig.

Welsh, J.H. 1941. The sinus glands and 24-hour cycles of retinal pigment migration in the crayfish. J.exp.Zool., 86: 35-49. 3 fig.

Wiersma, C.A.G. and Novitski, E. 1942. The mechanism of the nervous regulation of the crayfish heart. J.exp.Biol., 19: 255-265. 1 fig., 1 pl.

Yonge, C.M. 1924. Studies on the comparative physiology of digestion: II. The mechanism of feeding, digestion and assimilation in Nephrops norvegicus. (Brit.) J. exp. Biol., 1: 343-389. 18 fig.

Zeitschrift für Wissentschaftliche Zoologie, 116, 1916; 121, 1924; 123, 1924; 126, 1925; 141, 1932 (well illustrated papers on different parts of the anatomy of Astacus, from the Marburg Univ. Dept. of Zoology, additional to the four cited above).

CALLINECTES

Please report to J. H. Lochhead any mis-statements found in this outline.

Among the decapod Crustacea the crabs have departed most from the primitive "caridoid facies". They are thus of special interest as highly modified forms which have proved very successful. Callinectes is one of the largest of the crabs on the eastern coast of North America, and various species are abundant from Massachusetts to southern Brazil. Some species are found also on the west coast of Africa and on the Pacific coast from southern California to Ecuador. In C. sapidus, the well known "blue crab" of eastern North America, males commonly reach a width of about 17 centimeters (measured from the tips of the lateral spines), while the females are somewhat smaller. This species sometimes penetrates from the ocean into fresh-water, and indeed seems to be most abundant in brackish water, as for example in Chesapeake Bay. The females, however, always return to waters of relatively high salinity prior to the hatching of larvae from their eggs. In Chesapeake Bay, and perhaps in some other localities, this involves a migration of several hundred miles, by the young crabs travelling to the waters of low salinity where mating occurs, and by the mature females returning to the salt waters suitable for larval development. The adults are able to feed on a variety of living plants and animals, but their principal food seems to be dead animals. In common with most other members of the family Portunidae, the last pair of pereiopods are modified as paddles by means of which the animals can swim very rapidly. Indeed, it has been claimed (?) that one portunid is capable of catching mackerel in the open ocean.

With the large chelae the animals can strike with lightening rapidity, in contrast to the slower crayfish or lobster. The bite, however, seldom does more than draw blood. Painful bites can be avoided if the animal is picked up by the base of one of the flippers, held between the thumb and finger.

The account to be given here will deal mainly with those features in which the crab differs notably from the crayfish or lobster. It is assumed that one of the latter will already have been studied by the student. In most features the account will be applicable to almost any other brachyuran crab.

Body plan.

The cephalothorax is greatly depressed and is much expanded laterally. The pereiopods are thus borne wide apart, and the last five thoracic sterna on the ventral surface are very broad. The first three thoracic sterna, and the last two of the head, become progressively narrower as one passes forward, the three most anterior ones being fused into a minute triangle. All of the other sterna are fused, but the lines of fusion remain distinct. Smaller "episterna" are present between the outer ends of the last five sterna and the coxae of the pereopods, and a minute pair of episterna also can be distinguished on the somite bearing the third maxillipeds.

A little more than half way back on the dorsal surface of the cephalothorax, a shallow, transverse groove extends a few millimeters to either side of the mid-line, then abruptly turns latero-anteriorly.

This is said to be the cervical groove. The short, pointed rostrum is situated slightly below the dorsal anterior border of the cephalothorax.

The abdomen is much reduced and is bent under the thorax. If it is straightened out, however, one can distinguish the six somites and the telson, although the first somite is over-lapped dorsally by the thorax, and the third to fifth somites are fused in the male. The somites have calcified terga and pleura, and membranous sterna. Three shapes of abdomen are met with, those of the male, immature female, and mature female respectively. In the first two of these the abdomen fits into a depression on the ventral surface of the thorax and is held there by a pair of tubercles on the fifth thoracic sternum, fitting into grooves near the tip of the sixth abdominal somite.

Paired appendages

Uropods are absent. The same is true of the third to fifth pleopods in the male, and of the first pleopods in the female. The remaining pleopods in the female are relatively small and lack large setae until the mature stage is reached. In this stage the eggs become attached to specially long setae on the endopodites. The first copulatory pleopods of the male are remarkably long and slender.

In the pereiopods the segments are named as in the crayfish. In all pairs the basis and ischium are fused. All pairs autotomize readily, the fracture plane probably being just distal to the proximal end of the ischium. The male genital openings are on flexible papillae on the last coxae, as in the crayfish, but the female openings are in the sixth thoracic sternum, near the mid-line. The chelipeds are jointed in an interesting manner, permitting them to be folded close against the body, from which position they can be thrust forward very suddenly, as already noted.

The maxillipeds, maxillae, maxillules, and mandibules are all constructed on essentially the same plan as in the crayfish. The various parts can easily be identified by a comparison with the latter animal, although there often are notable differences in shape and relative proportions. The greatest difference lies probably in the epipodites of the three pairs of maxillipeds, which in the crab are very long, fringed with feathered setae, and project back into the gill chambers. They will be seen later when the respiratory system is studied. Use of the appendages in feeding is in the main essentials much as in the crayfish. The terminal three segments of the third maxillipeds form a flexible palp, used to clean the eyes, antennules, antennae, and portions of the other mouth parts.

The antennae are attached just median to the eyes. Each has a large basal segment fused to the head, a flattened immovable process at the side, which may represent the exopodite and an endopodite composed of two small segments followed by a moderately long flagellum. A movable, calcified operculum on the basal segment, just above the prominent ridge in front of the mouth parts, covers the opening of the antennary gland. The antennules are placed median to the antennae. Each consists of a three-segmented basal portion, tipped by two tiny flagella. The most proximal segment is very broad and is recessed into a pit on one side of the rostrum. The two succeeding segments can be folded back on the proximal one.

During much of the time the antennules flick jerkily in the direction of any approaching current of water. The currents are perceived by touch receptors on both flagella of the antennules, on the antennae, and perhaps on other neighboring structures. Correlated with this activity of the antennules the exopodites of the maxillipeds beat rapidly so as to draw a current of water across the front of the head, first from one direction, then from another. Thus the animal is able to test out its surroundings, and can move in the appropriate direction when an attractive chemical stimulus is thereby discovered. The presumption is that the chemo-receptors are on the antennules, but this is not fully established.

Exoskeleton and hypodermis

The exoskeleton is of the same type as in the crayfish. It is molted as long as growth continues, but at least in the female there normally is no further molting after the animal has become mature. The animals apparently rarely live more than three years.

For a dissection of the internal parts the student should begin by removing the exoskeleton from most of the dorsal surface, trying at the same time not to remove the richly pigmented hypodermis. With scissors a cut can be made all the way around the cephalothorax, about a millimeter or so from the periphery. The dorsal exoskeleton then can be pried up, starting at the anterior margin, using a scalpel to free it progressively from the underlying hypodermis. Just in front of the cervical groove two ligaments attach to the exoskeleton about three millimeters apart, one on either side of the mid-line. These have to be cut. Behind them there are whisps of muscle attaching to the exoskeleton along two diverging lines, one on either side, which run latero-posteriorly from the positions of the ligaments. These and a few other small muscles attaching to the exoskeleton also have to be cut.

In the hypodermis, red, black, and white chromatophores can readily be distinguished under a binocular microscope. The melanophores are reported to be remarkably rich in riboflavin.

Muscular system

Certain muscles will be among the first items seen, so this system will be described first. But no dissection should yet be done which might damage other structures. Each of the two tendons already mentioned represents the common origin of the "dorsal pyloric" and "internal posterior gastric" muscles. After the hypodermis has been cautiously removed, these two paired muscles can be followed forwards to their insertions on the stomach. Just lateral to the same two tendons, the "internal adductors of the mandibles" arise, each by a small head which quickly tapers to a long tendon running ventro-anteriorly to the posterior border of the mandible. The whisps of muscle that were noted along a line running latero-posteriorly, will be found to connect the carapace with the top of an inward projection of the white, calcified body wall. Below this projection, the levator coxae muscle of the fifth pereiopod will be found, with the other large proximal muscles of that important appendage lying further ventrally. Further forward, where the tips of the gills converge to an apex, there is a second inward projection of the body wall, on which and in front of which more strands of muscle insert from the carapace. A short distance in front

of this the head of the small "external abductor of the mandible" will be seen, surrounded by lobes of the hepatopancreas. This muscle quickly tapers to a ligament, which runs antero-ventrally to insert near the tip of a conspicuous white apodeme, projecting dorso-latero-posteriorly from the mandible. Also attaching to the tip of this apodeme is the large "external adductor of the mandible", which arises by two heads, slightly lateral to the position of the eyes, underneath the overlying hepatopancreas.

Lateral to the inner, dorsal limits of the body wall, the carapace extends far out to the side as the branchiostegite. As in the crayfish this is a hollow outgrowth from the body, but here the internal cavity is much more extensive, especially towards the front. In it there are found extensions of the gonads, hepatopancreas, and bladder, as well as certain muscles. After removal of the exoskeleton, the outer, dorsal surface of the branchiostegite is represented only by the hypodermis. This hypodermis is attached to the thin inner surface of the branchiostegite, which forms the roof of the gill chamber, by numerous, regularly spaced, little columns of connective tissue. In addition, there are a number of wider columns of muscle tissue, arranged in about three rows, running approximately antero-posteriorly. Contraction of these "dorso-ventral" muscles would raise the roof of the gill chamber, but the functional significance of this is unknown.

The abdomen is provided with flexor and extensor muscles, running from somite to somite as in the crayfish. As might be expected, however, they are much reduced, and they have no fibers arising further forward than the seventh thoracic somite.

In the pereiopods four muscles insert on the basis, not only in the chelipeds as in the lobster, but in the more posterior pairs as well. The "levator anterior basis", inserting by a strong tendon on the antero-dorsal edge of the proximal rim of the basis, is the muscle used in autotomy.

The complex set of muscles inserting on the cardiac and pyloric stomachs is in its main essentials as in the crayfish.

Circulatory system

The circulatory system is surprisingly similar to that of the crayfish, the main difference being in the way venous blood is conducted to the gills. This difference probably is correlated with the fact that the thoracic appendages of the crab are attached far out from the mid-line.

The heart is broad and fills most of the pericardial sinus. Its three pairs of ostia are antero-dorsal, lateral, and dorso-posterior in position. It is suspended by five pairs of elastic ligaments, plus a single, median posterior ligament. The arteries which leave the heart, with an internal valve at the base of each, are the same as in the crayfish. Their distribution to the various organs is in essentials much as in the crayfish. Perhaps the major difference worthy of note is the presence of a pair of branches from the superior abdominal, which supply the levator coxae muscles of the more posterior pairs of pereiopods, the muscles running from the carapace to the top of the body wall, and the hind-gut caecum (to be described later). The antennal artery on each side pierces the head of the internal adductor mandibularis muscle, just after leaving the heart.

The sternal sinus, into which venous blood comes from most parts of the body, is incompletely divided into left and right portions, and it extends up to the floor of the pericardial sinus, surrounding not only the nerve cord and ventral thoracic artery, but also the thoracic portion of the hind gut. From its dorso-lateral margins, just below the ventro-lateral limits of the pericardial sinus, five channels on each side, termed "branchial sinuses", run ventro-laterally, to open into the "infra-branchial sinus". This runs longitudinally, just above the dorso-lateral margins of the thoracic coxae, from the level of the second maxilliped as far back as the fifth pereiopod. Blood returning from these last seven thoracic appendages does not go to the sternal sinus, but instead enters the infra-branchial sinus directly. From here nine afferent branchials enter the gills, each passing up the outer side of its gill. An efferent branchial comes down the inside of each gill, and five branchio-cardiac sinuses on each side run up in the body wall to enter the pericardial sinus by three openings.

From the postero-lateral corner of the pericardial sinus, a tough, membranous pouch on each side projects into the posterior part of the branchial chamber. The function of this is unknown.

The blood contains hemocyanin, the same three types of blood cells as in the crayfish, and clots in a similar fashion. A blood-cell-forming organ is located on the dorsal part of the stomach.

Reproductive system

The ovary has two lateral divisions, connected by a cross-bar just behind the pyloric stomach. From this cross-bar each lateral division runs both forwards and backwards, above the hepatopancreas. The anterior portion curves laterally, skirting the cardiac stomach, and in the fully developed ovary continues out behind the anterior margin of the cephalothorax to a point just posterior to the lateral spine. The posterior portion runs rather directly backwards, and when fully grown may extend back into the first abdominal somite.

A short distance behind the cross bar, under the pericardial sinus, there is a pair of short oviducts, entirely concealed within the ovary. The duct on each side connects with an oval-shaped "seminal receptacle", which it enters near the anterior, median, dorsal border. The receptacle extends downwards almost to the sternal floor of the thorax. A short vagina leads from its postero-ventral border to the opening on the sixth thoracic sternum. Both the vagina and the ventral part of the receptacle have an exoskeletal lining.

The appearance and size of the ovary and of the seminal receptacle varies greatly with the stage of development. In immature specimens the ovary is thin and white, and its arms may be very short. The seminal receptacles are white and not larger than about the size of the pyloric stomach. Immediately after copulation, which occurs just after the final molt, the ovary is still small and white, but the seminal receptacles are enormously distended, sometimes being as large as the heart, and the gelatinous "sperm plug" which they contain makes them pink in color. A few weeks later, the receptacles are much smaller and are again white, the sperm plug which accompanied the sperm apparently having been absorbed. The ovaries now have started to grow and are becoming orange in color. Their growth continues for several months, their arms first reaching full length and then broadening, until, shortly

before egg-laying, they almost completely cover the hepatopancreas. The ova which they contain are large and crowded with orange yolk.

Fertilization of the ova evidently occurs at or shortly before egg-laying, either in the oviducts or in the seminal receptacles. Examination of the inside of a receptacle reveals a mass of white sperm stored on a median ventral ridge.

After laying, the eggs are attached to setae on the endopodites of the pleopods, and are carried for a few weeks, until the zoeae hatch. The mass of attached eggs, or "sponge", is at first a light orange, but steadily darkens as embryonic development progresses, until it is almost black.

The ovary at this time may be somewhat smaller than before, but remains bright orange because of numerous mature ova which it still contains. After liberation of the zoeae, the fact that one batch of eggs has already been laid can readily be detected only from the remnants of egg shells or attaching stalks, which a careful search will reveal on the endopoditic setae of the more anterior pleopods. More indirect evidence that eggs have been laid is provided by the presence in the gills of mature specimens of the nemertean parasite, Carcinonemertes, otherwise found in the egg sponge.

Somewhat later, probably usually in the same summer, a second batch of eggs is laid, and now the ovary appears collapsed and is grey or brownish in color, with few or no orange areas. Enough sperm is present, from the single copulation, to fertilize even further batches of eggs, but from this point on the ovary degenerates, and two batches of eggs thus seems to be the normal limit.

In the mature male, the testis has two narrow, rather convoluted arms, running a course closely similar to that of the latero-anterior arms of the ovary. These are connected by a cross-bridge posterior to the pyloric stomach, shortly behind which the testis connects on each side with the much coiled vas deferens. The degree to which this is coiled varies greatly with the stage of development. A short, glandular section, near the upper end, secretes the thin capsules for the spermatophores, and is thought to secrete at least a part of the material for the sperm plug. This is followed by a long greatly swollen region in which spermatophores and a gelatinous fluid are stored. A thinner terminal part of the duct leads to the opening on a papilla on the last thoracic coxa. This papilla is inserted into the groove of the first pleopod. Tegumental glands of the pleopod, which open into this groove, perhaps contribute further material for the sperm plug. A part of the second pleopod fits into the groove and is said to act as a piston, pushing the sperm along towards the vagina. The spermatophores break up, probably by mechanical action while passing along the groove.

For about three days prior to a female's final molt, she usually is carried around by a male, which clasps her, back uppermost, against his ventral surface by means of his three pairs of walking legs. Evidently the males can distinguish when the female is approaching this particular molt, or else only such females submit to being carried.

After the female has molted she turns over on her back, extends her abdomen, and copulation occurs. This persists for perhaps as much as a day or two, the male moving around while holding the female in place with his walking legs.

Digestive system

The esophagus, cardiac stomach, pyloric stomach, and intestine, are essentially as in the crayfish. The mid-gut is under a centimeter long. The terminal part of the hind gut is only slightly dilated, hardly deserving to be distinguished as a rectum. The hepatopancreas is divided into left and right divisions, each of which extends far out around the anterior margin of the cephalothorax, with many small lobes, besides extending deep into the floor of the cephalothorax, and running posteriorly below the pericardial sinus, sometimes as far back as the fourth abdominal somite.

In place of the single, very small mid-gut caecum met with in the crayfish, there is a pair of quite long, though very narrow, caeca, arising just behind the pyloric stomach. The two come up vertically together under the ophthalmic artery, just posterior to and together with the dorsal pyloric muscle. Between this muscle and the internal posterior gastric, each caecum curves latero-anteriorly and runs out under the gonad (if present), close to the wall of the stomach. Presently it turns more laterally, passes ventral to the gonad and antennary artery, turns again to run antero-laterally outside and ventral to the artery, and finally turns laterally once more to end in coils just dorsal to the first large lobe of the hepatopancreas that is visible in dorsal view. In color the mid-gut caeca are a translucent white, often with contained air bubbles in specimens that are being dissected. Their epithelial cells are like those of the rest of the mid-gut, absorptive in function, with a striated border.

Behind the heart, another much coiled caecum will be seen, mostly dorsal to the intestine. From these coils a straight part of the caecum runs back, usually on the right side of the intestine, and finally opens into the intestine where this shows an annular thickening in the second abdominal somite. Unlike the median posterior caecum of the lobster, this is not a branch of the mid-gut, but is a hind-gut caecum, with an exoskeletal lining like that of the rest of the hind-gut. It has an especially rich blood supply, but its function appears to be unknown.

Excretory system

The antennary glands are on the ventral surface of the head, back of a position between the antenna and the eye stalks, and partly concealed by the overhanging stomach. Each is a roughly square organ, pale greenish or yellowish in color, and often appearing as though composed of tiny, coiled tubes. Its microscopic structure is essentially as in the lobster, the corsal central part being occupied by the much lobed coelomosac, which opens ventro-anteriorly into the still more lobed labyrinth occupying the periphery and ventral parts of the organ. A tubule, such as occurs in the crayfish, is absent. A horse-shoe shaped opening at the inner posterior corner of the gland, not often seen, opens upwards from the labyrinth into the bladder. The bladder is a remarkable organ, of very large size, yet likely to be overlooked because its walls are thin and transparent and if seen at all are likely to be mistaken for connective tissue. In a fresh specimen it can be rendered more opaque and thus more easily seen, if as the dissection progresses it is flooded locally with 70% alcohol acidified with nitric acid in the proportion of three parts per hundred. From the "main vesicle" of the bladder, located above the gland a number of lobes ex-

tend out in various directions. Most prominent are an "epigastric lobe", rising at the front and sides of the stomach towards the dorsal surface, and a "hepatic lobe" which passes posteriorly under the insertion of the external adductor of the mandible and then turns laterally between the muscle and the hepatopancreas. Smaller lobes push their way between the hepatopancreas and the stomach, and around the oesophagus. The cells of all these lobes of the bladder when viewed in sections are secretory in appearance.

The main vesicle of the bladder opens to the exterior by a duct, starting above the middle of the dorsal anterior margin of the gland, and passing forwards below the ventral one of two transverse endophragmal struts seen just behind the antenna. The duct opens on a thin membrane, corresponding to the operculum of the crayfish, but here covered over by a different kind of operculum, which is calcified and is thought to be the modified antennal coxa. This operculum can be raised and lowered by special muscles. The actual opening of the duct is under the ventral, medial corner of the operculum, but it is so minute that any attempt to inject into the duct is almost certain to puncture the thin, surrounding membrane.

The urine of the crab is isotonic with the blood, in conformity with the absence of a tubule in the antennal gland. When in brackish or fresh-water, the crab excretes a copious isotonic urine, and makes good the salt loss by an active and selective uptake of ions by the gills.

Certain injected substances are actively removed from the blood by cells of the hepatopancreas, and by special cells in the gills, as in the crayfish. The excretory activity of the antennal glands is not wholly passive, for it is known that they can concentrate at least some substances into the urine.

Respiratory system

Study of the respiratory system has been delayed to this point, because of the way branches of the reproductive, digestive, and excretory systems extend into the cavity of the branchiostegite.

Along its lateral margin, the outer wall of the branchiostegite turns very sharply downwards and inwards, to meet the body wall just above the insertions of the thoracic appendages. The inner wall, forming the outer wall of the gill chamber, curves much more gradually. Above the appendages, the branchiostegite fits so tightly against the body wall that at first sight the two appear to be fused together. However, if the branchiostegite is strongly pressed upwards, while the specimen is held up to the light, a thin slit all around the lower margin of the branchiostegite will be disclosed. This slit normally is tightly shut, and a fringe of fine setae on the rim of the branchiostegite further increases the closeness of fit. Only above the base of the cheliped is an opening left, leading posteriorly into the hypobranchial part of the gill chamber. This opening is very large when the chelipeds are raised and extended forwards. When the chelae are held in the normal position, folded against the body, the opening is narrowed to a wide slit, which becomes further narrowed by a flange on the base of the epipodite of the third maxilliped whenever the two maxillipeds are opposed in the mid line. A fringe of setae on the basiischium of the cheliped filters part of the water which enters the slit.

Water which enters through the above opening finds itself in the hypobranchial division of the gill chamber. It then passes upwards, through the gills, into the hyper-branchial division, whence it flows forwards into the exhalent passage. This opens above the specially shaped distal end of the endopodite of the first maxilliped. Even when the two third maxillipeds are tightly closed over the other mouth parts, a pair of small anterior openings remain, through which the exhalent currents can escape. The lateral edge of each exhalent passage is sealed by the exopodite and endopodite of the first maxilliped, by the exopodite of the second maxilliped, and by the exopodite of the third maxilliped with its lateral fringe of setae. The flattened base of the epipodite of the first maxilliped helps to separate the hypobranchial space from the exhalent passage. The effective beat of the scaphognathite is normally from below upwards, and this is thought to be a further factor ensuring the correct circulation of the water. As in the crayfish, the emerging current of water may be given further impetus, or deflected to either side, by the beating of the exopodites on the maxillipeds.

At periodic intervals the beat of the scaphognathite is reversed, to aid in cleaning the gills. Such reversal is also made use of in water of low oxygen content, for taking in surface oxygenated water or even air through the anteriorly placed exhalent openings. When a partially submerged animal thus takes in air, this emerges as bubbles through the large exhalent openings.

If the thin roof of the gill chamber has not already been removed, this should now be done. The shape of the gill chamber should be observed, and its relations to the body wall, branchiostegite, inhalent openings, and exhalent passage should be fully understood. It probably will prove necessary gradually to cut away the anterior exoskeleton as far medially as the antennae.

Overlying the gills is the long epipodite of the first maxilliped, fringed with feathered setae. The shorter but similar epipodites of the second and third maxillipeds lie below the gills. By sweeping up and down the length of the gills these epipodites help to keep the gills clean. The first is moved largely by its own independent muscles. The other two for the most part share the movements of the coxae of their respective appendages.

The gills are not trichobranchs, like those of the crayfish, but are "phyllobranchs". Each consists of a central axis bearing an anterior and a posterior row of closely set, leaf-like lamellae (except on the arthrobranch above the second maxilliped, which bears a posterior row only).

There are eight gills on each side, distributed on the somites of the different appendages as follows: second maxilliped, one podo- and one arthrobranch; third maxilliped, two arthrobranchs; first pereiopod (or cheliped), two arthrobranchs; second and third pereiopods, one pleurobranch each.

Endophragmal skeleton

Parts of the endophragmal skeleton will be met with in an ordinary dissection. For a more complete study it can be prepared by any of the methods outlined under the description of the crayfish. It is, however, extremely complex, and a description of it here does not seem worth

hile, particularly as there is no endophragmal shelf to conceal the entral nerves and arteries.

ervous system

The nerves in a fresh specimen are rather transparent, and are etter seen if the specimen is first submerged for a while in 70% alcool. Sometimes local application of methylene blue or toluidin blue o a fresh specimen helps to bring out details.

The general plan of the nervous system is the same as in the crayish, the one great difference being the fusion of all the ventral anglia in the cephalothorax into a single mass. The cerebral ganglion ies immediately behind the rostrum. It can be better seen after reoval from just above it of a movable, transverse apodeme, which runs etween the two eyestalks. Nerves that can be seen connecting on each ide with the cerebral ganglion are the antennulary, optic, oculo-motor, egumentary, and antennary. Posteriorly there is a double connection ith the stomatogastric system.

The circum-esophageal connectives are long, over two thirds of this ngthbeing behind the esophagus. The trito-cerebral commisure, conective ganglion, and connections with the stomatogastric system are ssentially as in the crayfish.

The fused ventral ganglion lies near the floor of the cephalohorax, in the fourth and fifth thoracic somites. Its dissection from he dorsal surface is difficult, because of the way it is covered dorally and at the side by a complex arrangement of small muscles, tough embranes, and lobes of the hepatopancreas. For most purposes a dissection from the ventral surface will prove adequate. This can be done y cutting out a broad central strip from the sternal plates, and reoving some of the proximal muscles of the pereiopods, above which the anglion lies. A conspicuous hole near the center of the ganglion is or the descending sternal artery. From the periphery of the ganglion, erves radiate out to supply the successive appendages, from the mandible to the fifth pereiopod. The nerves for the first five of these ppendages are very small. Those for the mandible and maxillule are ften fused together. The nerve for the third maxilliped is somewhat larger, nd that for the cheliped is largest of all. Posteriorly a small double erve passes back in the mid-line into the abdomen. From the dorsal urface of the ganglion, at the same level as the nerves to the third axillipeds, arise a pair of fairly large "cutaneous" nerves. Each of hese runs latero-anteriorly, just behind the nerve to the second maxiliped, then curves upwards inside the body wall, to supply the lining f the carapace and the roof of the gill chamber.

Accompanying each cutaneous nerve, along its posterior border, is small cardiac inhibitor nerve. This presently leaves the cutaneous erve and turns abruptly posteriorly, to run back inside the body wall o the pericardial plexus. Two other small nerves, the cardiac ccelerators, arise on each side a short distance behind the cardiac nhibitor, at about the level of the nerves to the chelipeds. The anerior of these two runs outwards dorsal to and between the nerves for he third maxilliped and the cheliped. After passing upwards it then urves posteriorly and runs back ventral and parallel to the cardiac nhibitor, to join the pericardial plexus. The more posterior accelertor nerve runs out dorsal to the nerve for the cheliped, curves upards through a lobe of the hepatopancreas, then passes back at a con-

siderably deeper level than the other two, to join the pericardial plexus from below.

Parts of the pericardial plexus lie over the openings from the branchio-cardiac sinuses into the pericardial sinus. Connection of the pericardial plexus with the heart is made dorso-laterally. The intrinsic ganglion inside the dorsal wall of the heart contains about nine nerve cells.

Sense organs

The compound eyes are constructed on fundamentally the same plan as in the crayfish. For protection from mechanical injury they can be laid back into sockets in the front of the head.

The statocysts of the megalops larva function like those of the crayfish. But in the adult crab they do not contain sand grains or other otoliths, partly because the dorsal slit leading into the statocyst is entirely closed, except immediately after a molt. The line marking the position of this slit is easily seen, however, running transversely across the outer two thirds of the dorsal surface of the relatively very broad first antennulary segment. If the dorsal exoskeleton of this segment is carefully cut away, the sac of the statocyst will be disclosed. Three swellings project into this sac, each bearing specialized setae. Those on the small postero-median swelling are the ones which bore sand grains in the megalops larva. The setae on the large lateral swelling are of uncertain function. But a vertical row of setae on the antero-median swelling are of special interest because they perhaps react to movements of the liquid in the statocyst. At least this would seem to be possible from their structure. They are extremely thin, relatively long, fringed with setules only near their tips, and are very movably attached at the base. Observation shows that very slight movements of the liquid in the sac are sufficient to cause these setae to sway.

Tactile setae like those of the crayfish occur on many parts of the body. The receptors of Callinectes have not been closely studied, but in related crabs various other types of sensory setae have been found and have been tentatively identified as touch and contact receptors. The tips of the pereiopods in those crabs have been shown to be chemo-sensory, as also some region which is bathed by the respiratory current. The chemo-receptors in this case seem to be cylindrical protoplasmic processes from single nerve fibers, lodged in pores in the exoskeleton. There is some dispute as to whether the aesthetascs on the outer flagellum of the antennules are chemo-sensory. Setae on both flagella of the antennules, and on the antennae, appear to be current receptors. Proprioceptive movement receptors in the joints of the limbs have been demonstrated physiologically in Callinectes, as described under the crayfish.

Endocrine glands

The sinus gland can be seen after carefully cutting away the exoskeleton and hypodermis from the dorsal surface of the eyestalk. It is a pear-shaped organ, lying in line with the notch in the proximal border of the eye. At least one chromatophorotropic hormone is contained in unidentified cells in various parts of the central nervous system.

Selected references

Borradaile, L. A. 1922. On the mouth-parts of the shore crab. J. Linn. Soc. London (Zool.), 35 : 115-142. 2 pl.

Churchill, E. P. Jr. 1919. Life history of the blue crab. Bull. U.S. Bur. Fish., 36 : 91-128. 2 fig., 8 pl.

Pearson, J. 1908. Cancer. Livpl. mar. biol. Comm. Mem. 16 : i-viii, 1-209. 13 fig., 13 pl.

Smith, R. I. 1947. The action of electrical stimulation and of certain drugs on cardiac nerves of the crab, Cancer irroratus. Biol. Bull., 93 : 72-88. 2 fig.

LIMULUS

please report to J. H. Lochhead any mis-statements found in this outline.

Limulus is popularly known as the king crab, horse-shoe crab, or horse-foot crab, but it is not really a crab at all, being a member of an isolated class of the arachnids, known as the Xiphosura. It is abundant on the eastern coast of North America, from Maine to Mexico. Attempts to introduce it to Europe and to the California coast have succeeded only temporarily. Two closely related genera, embracing four species, occur in Asiatic waters, from Japan to India.

The adults of Limulus live for the most part in water of a few fathoms depth, where they shovel through the mud, probably feeding mostly on worms. Laboratory observations indicate that they are most active at night. In the spring and early summer they briefly visit the shore for breeding, coming in on a high tide. The eggs are laid in a hollow scooped out in the sand, usually at or just below the average high water mark, and the accompanying male then deposits sperm on top of them. The mass of eggs is then quickly covered over with sand. In the late summer tiny young specimens of Limulus sometimes are abundant on sand or mud flats exposed at low tide.

It has been calculated from measurements of a large number of specimens, that males of the Japanese species become mature at the thirteenth post-larval instar, the females one instar later. The mature females of all species are considerably larger than the males. There is evidence that at least the males do not molt any more after reaching maturity. Eggs, larvae, young, and adults all are remarkably hardy, and can be shipped alive for long distances. The adults can live for days out of water, and will survive for weeks without food.

The bizarre appearance, large size, and isolated taxonomic position of Limulus have long excited the interest of zoologists. The closest relatives of the Xiphosura appear to be found in the extinct Gigantostraca (= Eurypterida), with which they often are allied in the class Merostomata. This is the only exclusively aquatic class of arachnids, and one possible theory holds that it has a terrestrial origin. At one time the view was vigorously upheld that Limulus lies close to the ancestral tree of the vertebrates, this view being advanced in two entirely conflicting theories by Gaskell and by Patton. Patton always used the terms "haemal" and "neural" in place of "dorsal" and "ventral", because of his belief that the functionally ventral surface in Limulus is represented by the functionally dorsal surface in a vertebrate. The larva of Limulus has been said to resemble the extinct trilobites, but this resemblance would appear to be purely superficial. However, certain features of the earlier embryology do seem to suggest a trilobite relationship.

The body is much depressed, and is covered dorsally by a tough exoskeleton. It is divided into a "prosoma" or "cephalothorax", bearing the appendages round the mouth, a "mesosoma" or "preabdomen", bearing the operculum and the gill flaps, a "metasoma" or "postabdomen", which is without appendages, and a long spine-like telson. The mesosoma and metasoma are fused together into an "opisthosoma". This is jointed both with the prosoma and with the telson. Powerful muscles can flex or extend the body at each of these joints.

The body is hollowed out below, so that when the animal is resting on a flat surface the limbs are entirely concealed by the overhanging carapace.

The mouth is mid-ventral in position, between the bases of the walking legs. The anus is located ventrally, on the proximal end of the telson.

paired appendages, operculum, and gill flaps

A pair of small chelate appendages, termed "chelicerae", is located in front of the mouth. Each has three segments. The next pair of appendages are much larger and are termed "pedipalps". Each has six segments and is chelate except in mature males. A masticatory process, or "gnathobase", beset with movable spines, is present on the basal segment, pointing towards the mouth. In structure and in number of segments these two appendages are closely comparable to the corresponding pairs in the scorpion. The following three pairs of appendages are closely like the pedipalps (and so differ somewhat from the condition in the scorpion). The sixth pair of appendages are constructed like the others, but differ in two particulars. On the outside of the basal segment of each of these appendages there is a movable spatulate process, said to be used in cleaning the gills. And the appendages are not chelate, but instead each has four large, blunt, flattened spines, movably articulated with the distal end of the penultimate segment, besides a movable spine on the preceding segment and two much smaller spines at the tip of the terminal segment. Just behind and partly between the gnathobases of the sixth pair of appendages, a pair of blunt, movable processes is present, bearing a median row of short spines. These are called "chilaria", and it has been shown that they are the appendages of a rudimentary pre-genital somite.

The second to fifth pairs of appendages are used for walking, and their chelae pass food up to the gnathobases. The gnathobases work in an alternating fashion, which "cards" the food into fragments and gradually pushes it into the mouth. The chelicerae in front, and the chilaria behind, probably help to keep fragments of food from escaping. The sixth pair of appendages are used to push against sand or mud, or to push this out from under the carapace. Each time one of these appendages is extended, the four large, movable spines automatically are spread out by the resistance of the sand or mud, a load of which is then carried outwards and backwards, or else the animal gets pushed forward. The method by which Limulus plows forward through the surface of the substratum involves also the use of the telson and an alternate arching and straightening of the back. The back is arched up, the sixth legs drawn forwards, and the telson thrust into the substratum. Then the body is straightened again, and the sixth legs push backwards, against the firm anchorage provided by the telson.

At the molt leading to maturity, the pedipalps of the male change from a chelate to a sub-chelate condition. The immovable finger becomes much reduced, while the distal segment becomes thick and hook-like. When the time comes to go inshore for breeding, the male rides on the back of the female, using these claspers to hold on to the edge of her opisthosoma, with a most tenacious grip. In the Asiatic genera the third pair of appendages also become modified as claspers, and in one of these genera the claspers are relatively little modified, retaining a chelate condition. Autotomy of the prosomatic appendages does not occur.

On the opisthosoma six flap-like structures are present. The first of these does not bear gills and is termed the "genital operculum". It acts as a protective cover to the gills when these are laid back against the body. Paired genital openings are present on its posterior surface, close on either side of the mid-line, slightly less than half way towards the distal extremity. Each of the succeeding five flaps bears on each side a so-called "gill-book", containing a large number of lamellar gills, stacked one on top of the other. There is some resemblance between these external gill-books, and the internal "lung-books" of scorpions and other arachnids. According to one theory the flaps on the opisthosoma of Limulus represent paired appendages which have fused together in the mid-line, and each of which has an endopodite and an exopodite reminiscent of the Crustacea. The lung-books of terrestrial arachnids have, by this theory, been derived by an in-sinking of gill-books. A more recent theory holds that Limulus is descended from terrestrial arachnids, the gill-books thus having been derived from lung-books, and the flaps of the opisthosoma representing much modified sternal plates which now hang down free from the body. Both theories, however, suffer from the fact that the lamellae of gill-books are outfoldings, whereas those of lung-books are infoldings.

In the living animal all six of the flaps on the opisthosoma beat to and fro most of the time. Usually the beat is rather leisurely and serves only to aerate the gills. At times, however, the beat may become powerful, and it then is the means by which the animal can swim. This the smaller animals do quite frequently, and the larger ones swim when they come inshore to breed. Apparently when swimming the animals usually lie on the back. The beating of the flaps on the opisthosoma probably also is of use during burrowing, creating a current carrying out mud or sand posteriorly.

Each time that the flaps move forward the gill lamellae fill with blood, while each time the flaps move backward the blood drains out from these lamellae.

Exoskeleton and hypodermis

The exoskeleton is uncalcified, but is made leathery by a high content of scleroprotein. One worker claims to have found mucous glands which pour out mucus onto the surface of the exoskeleton when the animal is strongly stimulated. Chromatophores are absent from the underlying hypodermis.

Moulting is accomplished through a slit which appears nearly all around the anterior and lateral margins of the prosoma.

For internal dissection the dorsal exoskeleton has to be removed. In the case of small specimens this is easily done, but on large specimens it can be a laborious task. The following method is essentially the same as one that was first described by E. C. Cole. First of all the animal is bled, by cutting off the legs with tin-snips or with bone scissors, or by slitting the thin membranes at the points where the legs join the body. As much blood as possible should thus be drained out, since coagulated blood internally will hamper the dissection. The loss of blood also rapidly kills the animal. Next a median dorsal cut should be made through the exoskeleton of the prosoma, from the anterior to the posterior margin. This can be done with a hack saw, bone scissors, or even an old-fashioned can opener.

Cole recommends a hack saw, the teeth of which have been filed on a slight bevel and slightly set. Care has to be taken not to cut deeply at the posterior end, where part of the heart is located. Next a horizontal cut should be made all around the edge of the prosoma, about one centimeter above the free margin. For this the can opener is good, or the hack-saw can be used. Transverse dorsal cuts, from the lateral margins inwards as far as the ligamentous joint between prosoma and opisthosoma, are the next to be made. Then two cuts running the full length of the opisthosoma must be made, each one parallel to and about one centimeter outside of one of the rows of six depressions visible on the dorsal surface. The ligament at the joint between the opisthosoma and the prosoma next has to be cut with a sharp scalpel, restricting the depth of the cut to about five millimeters. The dorsal membrane at the base of the telson also has to be cut. If it is desired, cuts can be made around each of the lateral eyes, and around the median eyes (located just in front of the most anterior median spine on the prosoma), so that the arteries and nerves from these sense organs will not be broken.

Portions of the dorsal exoskeleton on the opisthosoma and on the prosoma can now be pried up, starting at the posterior margin in each case. Numerous muscle attachments will be met with, particularly in the prosoma, and these should be cut with a scalpel as close to the exoskeleton as possible. A sharp scalpel, or small bone scissors, should be slipped under the integument of the opisthosoma, to cut the strong apodemes, or "entapophyses", one of which projects downwards from each of the twelve depressions already noted. In doing this, care has to be taken not to damage the heart, which here lies not far below the surface. At the posterior margin of the prosoma, two especially strong entapophyses, in line with those in the opisthosoma, have to be cut with a saw or with bone scissors.

If a large specimen is chosen for dissection it might as well be as large as possible, which means that a female will be preferred. On the other hand there are some advantages to be gained from using a small specimen, say about 8 to 10 cm. long, exclusive of the telson. Observations on the activities of the living animal are readily made with such specimens, which do not require an especially large aquarium. Injection of a suitable color mass into the heart of a small specimen is a simple matter, the needle being inserted through the ligament at the posterior border of the prosoma. Many of the arteries then show up through the semi-transparent exoskeleton. Removal of the dorsal exoskeleton can be accomplished easily, with ordinary scalpel and scissors. Among the internal organs it should be possible to locate most of the major structures without too much difficulty, especially if a spotlight and a binocular microscope are used. But, of course, if a detailed dissection is desired, particularly of the nervous, muscular, or reproductive systems, there can be no substitute for a large specimen.

Muscular system

The dorsal origins of a considerable number of muscles will be seen as soon as the dorsal integument has been removed. The more ventral insertions of most of these, as well as some other deeper placed muscles, will not be met with until later in the dissection. But their description will be given here. Only those muscles will be included that are most likely to be seen, or which have special functional interest.

Two pairs of extensors for the telson will readily be found, as well as one pair of flexors.

The internal extensors of the opisthosoma arise from the dorsal integument of the prosoma, above the heart. A pair of external extensors arise from the large entapophyses at the posterior margin of the prosoma. Both pairs insert medianly on the anterior dorsal border of the abdominal integument.

There are three large pairs of flexors for the opisthosoma, two of which arise dorsally. These two will be seen just outside the pericardial sinus, opposite the first and second ostia of the heart respectively. These run together on each side, and insert on a series of six tendinous apodemes that project inwards from the "stigmata", which can be seen as transverse slits on the proximal posterior surfaces of the abdominal appendages. Besides flexing the abdomen these muscles make an important contribution to the movement of the gill flaps. The third pair of abdominal flexors arise from the posterior dorsal side of the endocranium (to be met with later), and pass backwards close to the mid-line, to insert on the ventral integument behind the last abdominal appendage. These muscles also insert, in the opisthosoma, on the six entapophyses of each side, and on the six median endochondrites (to be described later). They also receive on each side four small slips of muscle, arising ventrally at the level of each of the first four gill flaps.

The endocranium, which lies dorsal to the ventral nerve mass, between the bases of the prosomatic appendages, is suspended by a number of muscles from the dorsal integument. Those that will be seen on one side are as follows. One especially large muscle arises lateral to the crop, about in line with the two dorsally attached abdominal flexors of that side. Just medio-posterior to this muscle a much smaller muscle arises. Another small muscle arises beside the anterior suspensory ligament of the heart. A fourth, slightly larger muscle, arises between and median to, the two abdominal flexors, just outside the pericardial sinus. Fifth and sixth muscles in this series arise from the large entapophysis near the posterior border of the prosoma. The function of these various muscles is to counteract the pull on the endocranium of various other muscles, such as the ventral abdominal flexors already described, and some of the numerous muscles running to the prosomatic appendages. It also has been suggested that by compressing the prosoma these muscles may aid in the expulsion of genital products. In this they may be aided by numerous small strands of muscle which pass between the dorsal and ventral integuments in the lateral parts of the prosoma.

In the opisthosoma, seven small dorso-ventral muscles are present on each side. The first arises from the anterior border of the abdominal carapace, while each of the remaining six arises just antero-median to one of the entapophyses. The first five pass downwards between the pericaridal sinus and the collateral artery, while the last two pass outside this artery. All seven of these muscles run ventro-medianly, each of the first six inserting on the outer border of one of the endochondrites, and the last one inserting on the ventral integument behind the last gill flap. One function of these muscles seems to be to compress the opisthosoma, and the first six also serve to oppose the pull exerted on the endochondrites by small "internal branchial" muscles, which arise from the outer borders of the endochondrites and run ventro-laterally into the corresponding mesosomatic appendage.

Muscles running to the base of each of the prosomatic appendages are divided into two sets, those that have their origin on the ventrolateral margin of the endocranium, and those that originate on the dorsal integument. The chelicerae form an exception in not receiving any muscles from the endocranium; each is supplied by four small muscles arising on the dorsal integument. Each of the remaining five prosomatic appendages receives four muscles from the endocranium (five in the case of the sixth appendage) and five muscles from the dorsal integument (four in the cases of the second and sixth appendages). The chilaria each receive four muscles from the endocranium and one from the dorsal integument, plus another which inserts just lateral to the chilarium. The origins of the muscles running from the dorsal integument to the third to sixth prosomatic appendages lie lateral to the other body muscles, forming rather conspicuous large blocks of muscle. In an ordinary dissection the division into a number of separate muscles running to each appendage will not be distinguished.

Each of the abdominal appendages receives on each side the small internal branchial muscle already mentioned, and two much larger muscles which respectively pull the appendage forwards, or abduct it, and pull it back towards the body, or adduct it. All of these muscles actually run into the appendage, in contrast to the proximal muscles of the prosomatic appendages. The first abductor (for the operculum) arises from the large entapophysis of the prosoma and from the dorsal integument in front of this. The second abductor (for the first gill flap) also arises from the entapophysis of the prosoma. The third to sixth abductors arise from the first four abdominal entapophyses. The first adductor arises near the anterior border of the dorsal abdominal integument, slightly lateral to the line of entapophyses. The second to sixth adductors arise just lateral to the first five abdominal entapophyses.

A pair of flattened dorso-ventral muscles serve to open the anus. A slender pair of ventral longitudinal muscles serve to draw the anus forward, and by elongating the slit probably aid the sphincter muscle which surrounds the hind gut.

Strong circular muscles surround the crop and gizzard. Muscles run from the ventral integument to the crop and esophagus, and from the endocranium to the esophagus near the mouth.

Of functional interest, and of interest, too, because similar muscles are found in the scorpion, are a series of eight slender, rather transparent muscles on each side, which run ventrally from the floor of the pericardial sinus. Presumably these pull the pericardial floor downward.

Reproductive system

In mature females the ovary consists of an intricate reticulum of tubes, with small follicles on the walls of many of them, which extend into all parts of the body not occupied by other organs, with the exception of the appendages, telson, and more lateral parts of the opisthosoma. The hepatopancreas is equally extensive, and its tubules, together with some loose connective tissue, seem to the student to be inextricably mingled with the branches of the ovary. If the specimen has not laid its eggs, details of the ovarial tubes will be much obscured by the enormous number of eggs, which tend to clump together in

small groups, greatly distending the very thin, transparent walls of the tubes. Earlier stages of oogenesis occur more particularly in the lateral parts of the prosoma, outside the row of limb muscles. The oviducts are large muscular tubes, each of which starts median and posterior to the block of muscles supplying the sixth prosomatic appendage, and passes postero-ventrally to its opening on the genital operculum.

In the mature male the testis is very similar in structure and extent to the ovary of the female. With a hand lens it may be possible to see the globular sperm sacs in which the earlier stages of spermatogenesis occur, attached to the ducts in grape-like clusters. The vasa deferentia are located in the same position as are the oviducts of the female. The spermatozoa are provided with a long, vibratile tail.

The male genital openings on the operculum are round and each is located on a well defined papilla. In the female each opening is a transverse slit and there is no definite papilla. These differences can be seen even in the smallest specimens and afford a simple means of distinguishing the sexes, prior to the appearance of the male claspers.

The spawning habits of Limulus and the use of the male claspers have been described above. Reports that certain of the Asiatic species carry the eggs on the abdominal appendages, instead of laying them in the sand, have been shown to be erroneous.

Circulatory system

About one-half of the elongated, dorsal heart lies in the prosoma, and the other half in the opisthosoma. The whole organ is surrounded by a thin-walled pericardial sinus. This is invaded by a certain amount of areolar connective tissue, and its dorsal wall is indistinguishable from the epidermis. In the opisthosoma, five branchio-cardiac canals enter the pericardial sinus on each side, coming up dorso-medianly from the gills, just in front of each of the first five abdominal entapophyses. The first one receives a canal from the operculum just as it passes behind the dorsal part of the opercular adductor muscle.

The heart is suspended from the walls of the pericardial sinus by nine pairs of elastic ligaments. The first pair arise near the front of the heart and are exceptional in that they pass latero-antero-dorsally through the pericardial wall to attach to the dorsal integument. The remaining eight pairs occur just opposite the eight pairs of ostia, and pass laterally out to the pericardial wall, the last five on each side attaching inside the upper ends of the branchio-cardiac canals. The ostia are paired, transverse slits on the dorsal surface of the heart, through which blood enters the heart. Each ostium is guarded internally by two semi-lunar valves. Three longitudinal nerves on the dorsal surface of the heart will be more fully described under the nervous system.

The arteries which leave the heart are four pairs of "lateral arteries", one pair of "aortae", and a median "frontal artery". The lateral arteries are placed opposite the first four pairs of ostia, passing outwards below the suspensory ligaments. Two semi-lunar valves occur inside the proximal portion of each. Just outside the pericardial wall, the lateral arteries of each side run into a longitudinal "collateral artery". The two large aortae, and the much smaller ...

frontal artery, leave from the anterior end of the heart. Backflow of blood from these three arteries is prevented by one large semi-lunar valve on the dorsal wall of the heart. Just in front of this, at the level of the anterior suspensory ligaments, a pair of rudimentary ostia can be detected on the inner surface of the heart, but their slits do not penetrate the heart wall.

The collateral arteries run the full length of the heart, on either side, each giving off something like fourteen lateral branches which supply adjacent muscles and other tissues. Anteriorly each collateral ends in a few small branches just lateral to the beginning of the aorta. Posteriorly the two collaterals come together, so close behind the heart that students often make the mistake of supposing that an actual connection with the heart exists at this point. From the point of junction a large "superior abdominal artery" passes backwards to enter the telson. Medianly each collateral artery gives off a series of branches which pass ventro-medianly to the stomach-intestine. The more anterior of these "intestinal arteries" are hidden under the lateral arteries. More posteriorly, four intestinals are visible on each side, under the last four suspensory ligaments. It requires close inspection to see that these are not additional lateral arteries coming from the heart, but that instead they curve ventral to the heart and supply the stomach-intestine.

About opposite the second lateral artery, the collateral of each side gives off an especially large lateral branch. This passes outwards and backwards to near the postero-lateral angle of the prosoma, where it curves forward as the "anterior marginal artery". Before this curve is reached, about half-way out from the collateral artery, two important branches, anterior and posterior, are given off. The posterior branch runs back into the opisthosoma, where from its position it is known as the "posterior marginal". This anastomoses with a branch which leaves the superior abdominal near the posterior end of the opisthosoma. The anterior branch passes forward lateral to the prosomatic limb muscles, and is known as the "hepatic artery". Near the lateral eye this anastomoses with the "lateral eye artery", which comes up from below at a point lateral to the muscles of the second prosomatic appendage.

The frontal artery is sometimes absent, and sometimes arises from one of the aortae instead of between the two. When present it passes anteriorly above the gizzard and crop, to divide further forward into left and right anterior marginal arteries, which anastomose with the arteries of the same name already described.

All of the arteries thus far described can be seen in dorsal view. Further dissection of the vascular system should be delayed until after the digestive and endoskeletal systems have been studied. But the description of the vascular system will be completed here.

The two aortae curve downward and then backward, behind the crop. At the most anterior part of the curvature each gives off a branch which supplies the gizzard, crop, and esophagus. Ventrally the aortae pass back on either side of the esophagus, and so disappear below the endocranium. After the endocranium has been removed, it will be seen that the two aortae come together immediately behind the esophagus where this curves downward to the mouth. Just in front of the junction, three or four transverse arterial commissures cross the posterior or dorsal surface of the esophagus, between the two aortae. If

the esophagus is lifted up, it will be seen that the junction of the aortae is part of a "vascular ring", which encircles the short vertical part of the esophagus just above the mouth. Numerous arteries radiate out from this ring in all directions, and a large "ventral artery" passes backwards from it in the mid-line.

The vascular ring and associated arteries are very remarkable in that enclosed within them are the principal ganglia and nerves of Limulus. In an uninjected specimen the student would be apt to suppose that he is looking only at the nervous system, since the nerves are white and somewhat opaque, whereas the arterial walls are thin and transparent. In an injected specimen it will be seen that the dorsal nerves, which radiate out to supply more especially the lateral parts of the prosoma, are not enclosed by arterial walls except just where they come out from the vascular ring. Practically all the other nerves, however, including the ventral nerve cord and its branches, are enclosed within arteries. The arteries thus can be given the same names as the nerves, and a description of either one will serve for the other.

Most conspicuous are the large arteries which radiate out from the ventral edge of the vascular ring, to supply the six pairs of prosomatic appendages. A much smaller opercular artery, and a still smaller chilarial artery, leave the ring ventro-posteriorly and run back for some distance parallel with the large ventral artery. Each of the second to sixth prosomatic appendages also receives two or three smaller arteries which arise just dorsal to the main artery.

The anterior part of the vascular ring forms a rounded swelling, enclosing the cerebral ganglia. From the dorsal surface of this swelling, a relatively large artery with a bulbous, swollen base, arises on each side, and passes forward median to the cheliceral artery. Further forward this artery curves outward, upwards, and finally backwards, passing under the lateral eye and then anastomosing with the hepatic artery as already described. A much smaller, "stomodaeal" artery, also with a bulbous swelling at the base, arises on each side of the cerebral part of the vascular ring, from its dorso-latero-posterior border, in contact with the esophagus. This artery passes forward along the ventro-lateral surface of the esophagus, supplying the esophagus, crop, and gizzard, and then anastomoses with the aorta where this starts to bend upwards behind the crop. From the anterior border of the cerebral part of the vascular ring, three small arteries can be seen running forwards, median to the lateral eye arteries. Two of these arise from the ring slightly ventrally, and supply the so-called olfactory organ, located mid-ventrally, in front of the chelicerae. The median artery of the three arises slightly dorsally, a little off-center, runs forward below the esophagus, curves upwards in front of and slightly to the right of the crop, and finally reaches the median eyes on the dorsal surface.

The large ventral artery gives off branches to the five gill flaps, the abdominal muscles, and the stomach-intestine. Near the posterior end of the opisthosoma a branch passes dorsally on each side to anastomose with the superior abdominal artery. Nerves for the telson emerge from branches of the ventral artery just behind the anus, the arteries not continuing any further.

All of the arteries finally discharge into hemocoelic spaces, as in other arthropods. But Limulus is perhaps unique in its possession

of minutely branched collecting channels which drain the blood from the lateral parts of the prosoma. The system of collecting channels is not likely to be seen in an ordinary dissection, but will be described here to complete the functional picture. Ventrally one main collecting channel runs nearly the full length of the body on each side. In the prosoma it lies median to the outer row of large limb muscles, and for part of its course is close to the stolon of the coxal gland (described under the excretory system). In the opisthosoma it lies just lateral to the ventral abdominal flexor. Three large branches enter it laterally in the prosoma, each in turn greatly branched, so that the drainage of the lateral parts of the prosoma is perhaps as complete as that provided by the lymphatics in a vertebrate. Drainage of other parts of the body, and of the appendages, is through wider and less clearly defined hemocoelic spaces, which gain access to the main collecting channel on each side through ventral and lateral openings. Opposite each of the gill flaps, an afferent branchial channel leaves each of the main channels ventrally and passes to the inner border of the gills. From the lateral side of the gills the blood is returned to the pericardial sinus by a branchio-cardiac canal, as has already been seen. The operculum, also, seems to have on each side an afferent branchial channel and a branchio-cardiac canal.

According to Milne-Edwards the openings from the main collecting channel into the afferent branchials are associated with the tendinous spodemes on which the dorsal abdominal flexors insert. Contraction of these muscles, he states, results in closing of the openings, while dilation of the openings is brought about by contraction of the small lateral slips of muscle which join the ventral abdominal flexors.

The blood of Limulus contains hemocyanin and a single type of white blood cell. When observed in one of the gill lamellae placed under a microscope, these cells are seen to be spindle-shaped, large, and without amoeboid activity. As soon, however, as they come in contact with any foreign body, as when blood is withdrawn from the animal, they become amoeboid, agglutinate with one another, partially disintegrate, and may throw out long fibrous processes. The agglutinated mass of blood cells forms a soft, gelatinous clot, around which there may later occur a slight secondary clotting of the surrounding fluid.

Digestive system

The esophagus passes upwards from the mouth, through the vascular ring, then runs forwards to the more swollen crop. This curves upwards and backwards, merging into the gizzard without any noticeable change in external appearance. A marked constriction marks the boundary between the gizzard and the stomach-intestine, which is a wide tube running to near the posterior end of the opisthosoma. A short rectum leads to the anus. On each side of the stomach-intestine two ducts from the hepatopancreas enter, one usually in front of and the other slightly behind the level of the mouth. The very much branched hepatopancreas is co-extensive with the gonad, as has already been described.

If the digestive tract is slit open longitudinally, it will be seen that all except the stomach-intestine has an exoskeletal lining. The lining of the esophagus is longitudinally folded. That of the crop is wrinkled, permitting wide distension of this organ. The gizzard is much less distensible, and its lining is thrown into longitudinal ridges studded with somewhat flattened teeth. From the gizzard

a conical valve projects into the stomach-intestine, its exoskeletal lining thrown into about thirteen longitudinal ridges which project at the free end.

The inner wall of the stomach-intestine is considerably folded, and in the most anterior part, overlapped by the conical valve, bears numerous close-set papillae. Its columnar, glandular epithelial cells have a striated border. In the hepatopancreas there are absorptive cells with a striated border, and glandular cells which secrete the digestive enzymes. All parts of the gut, including the tubules of the hepatopancreas, are provided with both circular and longitudinal muscle fibers.

Food is ground up in the gizzard and admitted to the stomach-intestine through the conical valve. Coarse fragments are ejected through the mouth. Digestion occurs in the stomach-intestine, by means of enzymes from the hepatopancreas, except for the splitting of dipeptides, which occurs intracellularly in the hepatopancreas after absorption has occurred. A Japanese worker reports a second set of enzymes active in acid medium in the fore-gut, but where these would come from is not clear. The stomach-intestine secretes a mucilaginous coating for the fecal pellets, which may be up to 10 cm. long. Storage of fat, glycogen, and protein, is in the connective tissue cells between the tubules of the hepatopancreas, rather than in the cells of the hepatopancreas itself. Excretion, of calcium phosphate and perhaps of other substances, is performed by the absorptive cells of the hepatopancreas.

Endoskeleton

Three distinct classes of structures make up the endoskeleton, differing in their chemical composition, histology and embryology.

First of all there are a number of infoldings of the exoskeleton. Most noticeable of these are the six pairs of dorsal entapophyses in the opisthosoma, plus the single large pair at the posterior margin of the prosoma, and ventrally the six pairs of tendinous apodemes, which project inwards from the stigmata visible on the proximal posterior surfaces of the operculum and gill flaps.

Secondly there are the six median endochondrites and the endocranium. The endocranium is a structure of complex shape, located for the most part above the vascular ring, but with a posterior portion which encircles the ventral artery. The endochondrites are small, transverse bars, located below the ventral artery, one at the base of each of the abdominal flaps. Both the endocranium and the endochondrites develop from the ends of embryonic muscles. Histologically and chemically they have been found to show some resemblance to vertebrate fibroid cartilage.

Lastly there are seven pairs of so-called "branchial cartilages", and on each side a series of longitudinal cartilaginous bars connecting the ventral ends of the abdominal entapophyses. Each of the first pair of branchial cartilages runs from the posterior margin of the endocranium into one of the chilaria. The remaining six pairs run from the ventral ends of the abdominal entapophyses into the operculum and the gill flaps. Those that enter the gill flaps can be found just medial to the gills. Embryologically these cartilages develop from

mesoderm of the somite walls. Histologically, and perhaps chemically, they resemble the hyaline cartilage of some of the lower vertebrates.

The entapophyses, tendinous apodemes, endochondrites, endocranium, and branchial cartilages, all serve for the attachment of muscles, some of which were mentioned earlier.

Nervous system

If an uninjected specimen is used for the dissection of the nervous system, application of Schaudinn's fluid will be found to whiten and harden the nerves. After a study from the dorsal surface has been completed, as much as possible of the nerve ring and attached nerves should be removed to a petri dish for more detailed study, especially of the ventral surface.

A considerable part of the nervous system has, in effect, already been covered in the description of the circulatory system, since many of the nerves run inside corresponding arteries. Thus there is no need to repeat a description for the neural ring, with its three or four post-oral commissures and anterior cerebral swelling, nor for the nerves to the operculum and chilaria, the several nerves to each of the prosomatic appendages, the stomodaeal nerves, the olfactory nerves, and the nerves to the median and lateral eyes. The nerve ring is, of course, made up of fused ganglia, but details of its structure are not easily made out in an ordinary dissection.

Some of the nerves not adequately covered in the description of the circulatory system are the following. Emerging through the dorsal surface of the vascular ring there are eight dorsal nerves on each side, which do not run within arteries. The first of these is a very small nerve, which arises just latero-posterior to the root of the lateral eye nerve. Although small, this nerve has a lengthy course. It starts by following the lateral eye nerve, to which it at first is lateral, becoming median as the two curve posteriorly. Passing median and ventral to the lateral eye it continues back in the ventral epidermis, curves postero-medianly around the base of the sixth prosomatic appendage, then curves slightly laterally again near the adductor muscle for the first gill flap, and finally ends in the anterior part of the metasoma. It appears to supply ventral sense organs in the opisthosoma. The second dorsal nerve is very much larger, being actually the largest of the dorsal nerves, approximately equalling the size of the lateral eye nerve. It arises dorsal to the roots of the nerves to the pedipalp, close to the stomodaeal nerve, then passes forward just lateral to the first dorsal nerve. At the point where this and the lateral eye nerve curve laterally it divides into three main branches, which continue forwards, one to the dorsal and two to the ventral epidermis. Each of the third, fourth, fifth and sixth dorsal nerves arises dorsal to the nerves for the corresponding appendage, and runs out in front of that appendage, to supply the lateral parts of the epidermis, usually with three main branches, one dorsal and two ventral. The fifth and sixth of these nerves at first run back above the nerves to their corresponding appendages, then turn sharply laterally. The seventh and eighth dorsal nerves arise close together, between the origin of the sixth nerve and the start of the ventral nerve cord. They branch like the more anterior dorsal nerves, to supply the lateral epidermis in the posterior part of the prosoma and anterior part of the opisthosoma respectively.

On the ventral surface of the nerve ring, a pre-oral transverse commissure will be found, at the posterior border of the cerebral ganglia. From it three tiny nerves pass to the anterior lip of the mouth.

The ventral nerve cord is actually double, as in all arthropods, though this can be seen only after removal of the arterial sheath. Opposite each of the first four gill flaps it is enlarged as a ganglion, from which an anterior dorsal nerve passes outwards to the lateral epidermis and into one of the marginal spines, and a posterior ventral nerve passes into the corresponding gill flap. In addition two small nerves arise from each ganglion close to the dorsal nerve, passing dorso-laterally to supply the ventral abdominal muscles and the stomach-intestine. Intestinal branches also arise from the sixth, seventh, and eighth dorsal nerves, near where these curve laterally. A network of anastomoses connects together the various intestinal nerves and also the nerves to the ventral abdominal muscles.

Behind the fourth abdominal ganglion, the ventral nerve cord appears to fray out on each side into something like eight nerves, which supply the last gill flap, the stomach-intestine, the rectum, the more posterior abdominal musculature, the epidermis and last two marginal spines, the telson, and the posterior parts of the heart and pericardium.

A series of segmental cardiac nerves arise from the sixth to the thirteenth dorsal nerves, inclusive, just lateral to the ventral abdominal flexor. From the base of each of these, excepting the first two, or from the adjacent part of the corresponding dorsal nerve, connection is made with a lateral longitudinal nerve, which runs forward onto the ventral surfaces of the two dorsal abdominal flexors. A further connection with this nerve is received from the fourteenth dorsal nerve. In the opisthosoma the lateral longitudinal nerve should be looked for just lateral to the tendinous apodemes and median to the branchial cartilages.

The segmental cardiac nerve arising from the sixth dorsal nerve passes up to the wall of the pericardial sinus, on which it branches at a level mid-way between the first two pairs of ostia. No connection has been found from this nerve to the heart. The next two of the segmental cardiac nerves join together to form a somewhat larger nerve, which reaches the pericardial sinus at a level between the second and third pairs of ostia. This nerve, and each of the succeeding five segmental cardiac nerves, sends branches to the dorsal epidermis, connects with a longitudinal nerve running in the wall of the pericardial sinus, and also connects with the median dorsal nerve or ganglion on the surface of the heart. From this, in turn, minute nerves pass out to a plexus on the dorsal surface of the heart, with some of the strands on each side forming a more definite lateral nerve. Both the two lateral nerves and the median ganglion run nearly the full length of the heart.

The beat of the heart is maintained by a rhythmic discharge of impulses from the dorsal cardiac ganglion. Normally the pace-maker for the beat occurs in this ganglion at the level of the fifth and sixth pairs of ostia, which is where the greatest number of nerve cell bodies are located. The beat is subject to regulation by impulses arriving through the segmental cardiac nerves. Of these, the one

which takes its origin from the seventh and eighth dorsal nerves is an inhibitor nerve. Those which originate from the ninth, tenth, and eleventh dorsal nerves, and which join the cardiac ganglion at about the level of the fourth, fifth, and sixth pairs of ostia, are augmentor nerves. Presumably the last two segmental cardiac nerves also are augmentory, but this point appears not to have been determined.

After removal from the body of the animal, the Limulus heart will continue to beat for hours, if merely moistened with sea water, provided that some nervous connection is left between the dorsal cardiac ganglion and the heart muscle.

Sense organs

The most conspicuous sense organs are the large lateral eyes. The exoskeletal covering over each of these is divided into numerous, relatively large facets, corresponding with an internal organization into separate receptor units. Each unit has about 10-15 receptor cells, and a conical lens formed by an ingrowth of the exoskeleton. There is no curtain of surrounding pigment cells, no crystalline cone, and there are no tapetal cells. The structure of these receptor units is so different from that of the ommatidia in the compound eyes of insects and Crustacea, that their homology has been doubted. The differences, however, may well be the result of degeneration, associated with the burrowing and nocturnal habits of Limulus.

The median eyes are located just in front of the most anterior of a row of small spines on the dorsal surface of the prosoma. Normally there are two, close together on either side of the median line. Each is a hemispherical cup, with a nearly spherical lens formed from an ingrowth of the exoskeleton, behind which is a columnar epidermis and a layer of receptor cells which tend to be arranged in groups. The two nerves from these cups soon unite together, and in the angle between them there is a mass of tissue which when examined histologically proves to be a degenerate eye, or rather the rudiments of two eyes which have fused together in the mid-line. Occasionally adult specimens are found with this posterior double eye in a functional condition, provided with a lens like the pair of eyes just in front.

On the ventral surface, a median, wart-like elevation of the exoskeleton, about 5-8 mm. wide, can be seen just in front of the "elbows" of the chelicerae. A distinctive oval area on each side of the wart will be noted. This is the structure supplied by the two so-called "olfactory" nerves. Its internal structure changes remarkably during development. In the larva there is a median, pigmented "frontal organ", of doubtful significance, on either side of which is a fully functional ventral eye, supplied by one of the so-called olfactory nerves. Actually the fibers of these nerves originate, even in the adult, from the same ganglionic centers within the cerebral ganglia as do the fibers of the lateral eye nerves. Each of the ventral eyes has an exoskeletal lens, a cup of receptor cells, and a backing of pigment cells around this cup. During further development the frontal organ largely breaks down, the pigment cells become arranged in scattered groups, and apparently the photoreceptor cells become transformed into bipolar and multipolar sensory neurons, the distal fibers of which extend into tiny sensory setae on the surface of the organ!. There are also numerous vertical pores through the exoskeleton, smaller than those leading to the setae, and quite different from the pores found in other parts of the exoskeleton. Pos-

sibly these pores serve for the escape of a glandular secretion, but the most recent worker has been unable to find gland cells. Whether this organ in the adult condition is actually olfactory, as has been surmized, has not been determined. The possession of ventral eyes by the larva probably can be correlated with the larval habit of swimming on the back, contrasting with the more usual burrowing habits of the adult.

The movable spines on the gnathobases of the second to fifth prosomatic appendages appear to contain taste receptors. When they are touched with appropriate food, reflex chewing movements of the limb which is thus stimulated, and bending and snapping movements of the chelicerae, are initiated. Below each of the spines is a group of bipolar sensory neurons, the distal fibers of which extend into the cavity of the spine. Taste receptors of some sort may also be present in the chelae of the first five pairs of appendages, which have been reported to open when touched with food.

The external spatulate process of the sixth prosomatic appendage receives an unusually large nerve, the fibers of which have been reported to be connected with an enormous number of receptor cells located underneath the anterior surface of the process. Since water going to the gills passes over this anterior surface, it has been surmized that the function of these sense receptors is to test in some way the quality of the water in the respiratory current.

On the jointed distal processes on either side of the mid-line of the gill flaps, a small number of blister-like elevations are present. The exoskeleton of these has been reported to be perforated by peculiar pores, with underlying receptor cells. But what sense, if any, is here involved has not been determined. On other parts of the gill flaps sensory setae have been reported, likewise of unknown function.

Limulus has been claimed to be remarkably sensitive to slight increases of temperature. The margins of the gill chamber, the margins of the carapace, and the gill flaps, are said to be the most sensitive areas, with other areas of the body only slightly less sensitive. The presumed temperature receptors have not been found.

Excretory system

In the ventral part of the prosoma a complicated "coxal gland" is present on each side, which from its structure has been presumed to be excretory. Each includes four glandular lobes, segmentally arranged, within which is a maze of fine ducts, partly intracellular. The ducts of the first three lobes open into a labyrinth of slightly larger ducts in a longitudinal stolon which connects the inner ends of the lobes. The ducts of the fourth lobe, and of the stolon, open into a coelomosac at the base of the fourth lobe. This in turn opens into a long, wide, and very much convoluted tube, which finally leads to the exterior near the base of the fifth prosomatic appendage. Except for a short length of the excretory tube near the external aperture, all parts of the coxal gland are mesodermal in origin.

The four lobes of the gland are brick-red in color, and are located near the bases of the second to fifth prosomatic appendages. Each is an elongated lobe which starts a short distance lateral to the nerve ring and runs outwards into the basal part of the corres-

leading appendage, just dorsal to the smaller nerves supplying that part of the appendage, and between anterior and posterior muscles from the endocranium to the appendage. Each lobe has a copious arterial blood supply. The first lobe often is considerably smaller than the others. The stolon connects the inner ends of the lobes, running at right angles to them, at a level dorsal to the nerves of the appendages and ventral to the dorsal nerves which pass outwards between the appendages. It widens posteriorly where it meets the fourth lobe, this being where the coelomosac is present. The duct leading away from this passes latero-dorso-posteriorly, above the fourth lobe, coils a few times posterior and dorsal to the outer third of this lobe, then runs forward, with many coils, dorsal to the outer thirds of the third and second lobes. In front of the second lobe, or dorsal to the first lobe, the duct widens considerably, turns medianly, and then runs back above the middle portions of the second and third lobes, close to the lateral border of the endocranium. Above the fourth lobe it turns ventro-medianly, passing through part of the lobe on its way to the external apperture. This is located on the articular membrane proximal and posterior to the basal segment of the fifth prosomatic appendage. When the folds in this membrane are smoothed out by stretching, the position of the opening will be seen to be indicated by a small light area surrounded by a ring of darker grey, located in the membrane, near to the mid-point of the posterior proximal border of the appendage. In young specimens the position of the opening may be very hard to see.

Of the internal parts of the coxal gland only the lobes and the stolon are at all likely to be seen in an ordinary dissection. The duct is very transparent, and probably will be seen only if a successful injection is achieved through the external apperture.

The excretory activity of the hepatopancreas has already been mentioned.

Selected references

Benham, W. B. S. 1885. On the testis of Limulus. Trans. Linn. Soc. Lond. (Zool.) (2) 2 : 363-366. 1 pl.

Gerhardt, U. 1935. 2. Ordnung der Merostoma: Xiphosura...etc. Kükenthal u. Krumbach Hdb. der Zool., Bd. 3, Halfte 2, Lief. 8 : 47-96. 45 fig.

Hanström, B. 1926. Das Nervensystem und die Sinnesorgane von Limulus polyphemus. Lunds Univ. Arsskr. (n.F.) (2) 22 (5) : 1-79 31 fig.

Milne-Edwards, A. 1873. Recherches sur l'anatomie des limules. Ann. Sci. nat. (Zool.) (5) 17 (4) : 1-67. 12 pl. (Especially on circulatory system).

Manson, J. P. 1898-9. The ovarian egg of Limulus...etc. J. Morphol., 15 : 111-220. 4 pl. (Includes anatomy of female reproductive system).

Patten, W. & Hazen, Annah P. 1900. The development of the coxal gland, branchial cartilages, and genital ducts of Limulus polyphemus. J. Morphol., 16 : 459-502. 3 fig., 7 pl. (Includes anatomy of adult coxal gland.)

Patten, W. & Redenbaugh, W. A. 1899-1900. Studies on Limulus. II. The nervous system of Limulus polyphemus, with observations upon the general anatomy. J. Morphol., 16 : 91-200. 18 fig., 5 pl. (Most valuable single paper on anatomy, especially of nerves, heart, arteries, and muscles.)

Schlottke, E. 1935. Biologische, physiologische und histologische Untersuchungen über die Verdauung von Limulus. Z. vergl. Physiol., 22 : 359-413. 28 fig. (Digestive system).

Schulze, P. 1937. Trilobita, Xiphosura, Acarina. Eine morphologische Untersuchung über Plangleichheit...etc. Z. Morph. Ökol. Tiere, 32 : 181-226. 39 fig.

Versluys, J. & Demoll, R. 1922-3. Das Limulus-Problem. Die Verwandschaftsbeziehungen der Merostomen und Arachnoideen...etc. Ergebn. Fortschr. Zool., 5 : 67-388. 123 fig.

ECHINODERMATA

Phylum Echinodermata

Enterocoelous secondarily radially symmetrical animals with a water vascular system.

Subphylum 1. Eleutherozoa

Free-swimming forms

Class 1. Asteroidea

Ambulacral grooves contain tube feet. Typically pentagonal body. Arms contain genital organs and pyloric caeca, not sharply marked off from the disk.

Order 1. Phanerozonia

Prominent marginal plates, simple pedicellariae if present. Ctenodiscus, Luidia, Astropecten

Order 2. Spinulosa

Marginal plates inconspicuous, simple pedicellariae if present. Henricia, Solaster, Crossaster

Order 3. Forcipulata

Marginal plates inconspicuous, crossed and straight pedicellariae present Asterias

Class 2. Ophiuroidea

Slender arms sharply defined from the central disk. No ambulacral grooves. Genital organs confined to disk.

Order 1. Euryalae

Disk with prominent ribs from base of arms to center of disk. Arms movable vertically and horizontally. Arms simple or branched. Gorgonocephalus

Order 2. Ophiurae

Disk without ribs, arms move horizontally but usually not vertically. Arms simple.

Ophiopholis, Ophioderma, Ophiura

Class 3. Echinoidea

Globular or disk-shaped shell or test composed of close fitting plates. Covered with spines.

Order 1. Centrechinoidea

Anal opening usually polar. Test globular. Gills may be present on peristome.

Arbacia, Strongylocentrotus

Order 2. Clypeastroidea

Anal opening not polar. Test somewhat flattened. Dental apparatus well developed. Echinarachnius

Order 3. Spatangoidea

Neither mouth or anus polar, dental apparatus undeveloped. Moira

Class Holothuroidea

Skeletal plates reduced in number and embedded in the body wall. Cylindrical body elongated along oral-aboral axis. Tentacles surround the mouth.

Order 1. Dendrochirota

Water vascular system well developed, dendritic tentacles, well developed retractor muscles.

Cucumaria, Thyone

Order 2. Molpadonia

Tentacles small in a wheel around the mouth. Tube feet missing. Posterior end of body may be elongated. Caudina

Order 3. Apoda

Tube feet and respiratory trees missing. Feathered, digitate or simple tentacles present.

Leptosynapta, Epitomapta

Subphylum 2. Pelmatozoa

Stalked forms attached during part of their lives.

Class Crinoidea

Arms generally branched and with pinnules. Aboral pole sometimes surrounded by cirri. Open ambulacral grooves. Spines, pedicellariae, and madreporic plate missing.

Antedon, Heliometra, Hathrometra

THE SEA STAR, ASTERIAS FORBESI

Asterias forbesi, which is the common sea star on most of the Eastern seaboard, ranges from Maine to the Gulf of Mexico and may be found from low water to a depth of 160 feet. It is very similar to Asterias vulgaris, a more northern species, and also resembles closely the well-known Asterias rubens from English shores. Specimens may be found singly or in large aggregations on rocky or shelly bottoms. They have been studied extensively because of the damage done to oyster beds.

It is very desirable to have living specimens to demonstrate external structure and activities of the animal. Dissection may also be carried out on a living form after anesthetizing with 15 ml of 10 % magnesium chloride. Unless anesthetized or killed in fresh water, autotomy will occur while dissecting. Alcohol is superior to formalin as a preserving fluid. The water vascular system may be injected with a contrast color if desired. The skeletal system may be studied from a dry specimen. Individual calcareous plates will be separated but the form of the animal is lost if treated with 30 % potassium hydroxide. Dry specimens may be prepared by killing in 70% alcohol, boiling in water and then permitting to dry. Decalcified stained sections of the ray will be required for study of the skeletal and nervous systems.

External characteristics. This species is known for great variability in color with cream, pink, orange, and purple being common. The madreporic plate is bright red in life. Colors fade in preserved specimens to a yellow or brown.

The separation of the five rays from the central disc is poorly marked off. Four to seven or eight rays are also known but they are probably the result of regeneration after injury. The mouth is situated in the center of the disc on the oral or actinal surface. It is guarded by five groups of spines or mouth papillae. Radiating out from the mouth are ambulacral grooves in each arm which are filled with tube feet. Each of these extensible structures terminates in a suction disc. The tube feet in each arm are located in four rows except at the tip of the arm where a single terminal tentacle is found. Through the action of internal muscles the grooves may be deepened and narrowed. The presence of a double row of spines also serves for protection of the delicate tube feet. Each tube foot is able to exert

considerable traction. If an animal attached to a piece of glass is suddenly lifted up, many of the tube feet will break in two leaving the sucker end behind. Movement is brought about by coordination of many tube feet more by a pushing than a pulling movement. This may be best demonstrated by watching an animal on a sandy bottom or through the bottom of a glass dish.

The aboral or abactinal surface is marked by the presence of the asymetrical subpentagonal madreporic plate. With magnification numerous closely set grooves radiating out from the center may be seen. The plate may be used as a land mark to designate the various rays. The two rays adjacent to it are known as the bivium while the other three constitute the trivium. If the madreporic plate is placed away from the observer the arm to the right is numbered I, the one to the left II, while III, IV, and V are found by following around in counter-clockwise fashion. The anus is located in the interradius between IV and V but is seldom found externally.

The aboral surface is studded with irregularly arranged blunt spines. Examine this surface with magnification. Attached to these spines and between them are large numbers of pincher-like pedicellariae. Draw a thread across some of them and note the movements. Remove a few, mount on a slide and examine under the compound microscope. Two kinds may be distinguished in this species. The crossed pedicellariae are crossed like a pair of scissors. They are smaller than the uncrossed or major pedicellariae. Minute sets of muscles operate to open and close the jaws.

In living specimens membranous sacs, the gills, dermal papullae or dermal branchiae, will be seen to extend and withdraw. There are thought to be chiefly respiratory in function. Inject about 5 ml of

carmine suspension in sea water into the animal. The carmine particles may be seen circulating in the gills due to ciliary action. Set aside this animal for examination after eight hours. Much of the carmine will be found within phagocytic cells from the body fluid which are migrating to the outside through the thin-walled gills. Pick off some gills and examine under the compound microscope. From such demonstration it has been concluded by some workers that these cells are excretory. Examine a few drops of fluid from the coelomic cavity under the microscope. The cells, some of which also contain carmine particles, tend to agglutinate on the slide as a part of the clotting process.

The entire external surface of the body including spines, pedicellariae, tube feet, and gills are ciliated. Various functional activities have been ascribed to the direction of movement.

Skeleton. The skeleton is composed of a large number of tube-shaped calcareous ossicles lodged in a leathery perisoma. The plate arrangement in the rays may be seen in a dry specimen. Cut a cross section of one ray and remove the aboral surface of another on such a specimen. The ambulacral groove is bounded on the two sides by a series of closely fitting aboral plates which unite at a peak in the center. From the inside they form the ambulacral ridge. Each of these flattened plates is channeled by a half-pore which with the corresponding half-pore of the next plate makes a channel through which the tube foot and its ampulla connect. These pores alternate in position, the first one being close and the second further from the ambulacral ridge. Below these ossicles is a single row of spine bearing adambulacral ossicles while the aboral surface consists of a framework of smaller ossicles.

Dissection. Cut up both sides of the arms of the trivium on the aboral aurface just above the lateral edge from the tip to the central

disc. Loos en the underlying organs from the suspending mesenteries and lift up the free flaps. Half of the central disc next to the trivium should be cut away taking care not to damage organs attached to the anus in the disc between rays IV and V and the madreporic plate. Cut the tough skeleton and mesentery in the two interradial areas between the rays.

Digestive System. The mouth leads into a narrow esophagus which expands into the sac-like cardiac stomach. The cardiac stomach may be everted through the mouth and may often be found in this condition. It is retracted by paired retractor muscles found on the ambulacral ridge of each arm. Aborally the cardiac stomach is divided by a constriction from the pyloric portion which is the part seen on top in the dissection. The large double brownish glands located in the rays are the hepatic caeca or gastric glands. Ducts on the aboral surface of each portion unite to empty into the pyloric stomach. These glands secrete powerful proteolytic enzymes. Being hollow and ciliated they also assist in circulation of digested food.

The pyloric stomach narrows into the cone-shaped intestine which is connected to the anus. A short distance before the anus the intestine is joined by the expanded rectal sac which varies in the number of lobules composing it.

Reproductive System. A pair of gonads is present in each arm lateral to the hepatic caeca. They are simply branched, much-lobulated sacs which lie free in the perivisceral cavity except at the point of attachment at the proximal end of the ray in the aboral body wall. Ovaries are organge w hile te stes are gray, both kinds of gonads being more prominent before spawning. Fertilization and development are external, large streams of eggs and sperms being released early in summ

Water Vascular System. The stone canal is a roughened tube made up of calcareous rings leading from the madreporic plate to the ring canal which surrounds the mouth. The stone canal is almost completely enveloped by a sac-like covering which makes up the axial sinus. Behind the stone canal and within the axial sinus is the glandular axial organ. The function of the axial sinus and organ has been much disputed. Locate the union of the stone canal with the ring canal. Nine small vesicles, Tiedmann bodies, project inward from the ring canal in the positions corresponding to the entrance of the stone canal into the ring canal. The Tiedmann bodies probably produce the amoeboid cells found in the water vascular system. Five radial canals concealed under the ambulacral ridge go from the ring canal into each of the arms. Each tube foot is supplied with liquid from the radial canal by a transverse canal which may be seen by spreading out the tube feet on an injected specimen. The transverse canal joins the tube foot just at its aboral surface orally to the pore canal. The tube foot is also joined by a bulb-like organ, the ampulla. The ampullae contain reserve fluid which by means of circular muscles may force fluid into the tube foot extending them. As the longitudinal muscles of the tube foot contract the fluid is again restored to the ampulla. A valve-like arrangement at the junction of the transverse canal with the tube foot and its ampulla is said to prevent fluid from being forced back into the radial canal.

Coelom. The spacious body cavity is known as the coelom in the adult. It is lined with ciliated epithelium which maintains a very active circulation of albuminous body fluid which contains amoebocytes. Other derivatives of the embryonic coelom persist as a system of tenuous

tubes in the adult which has been known as the perihaemal system. These include the axial sinus, the inner and outer perihaemal canal near the nerve ring, five radial canals aboral to the radial nerves, a pentagonal aboral perihaemal sinus seen on the aboral disc together with strands connecting with the gonads, and two gastric haemal tufts which lie attached to the stomach on one side. Within these spaces are the axial organ and thin strands of haemal tissue. Most of these canals may be seen only in sectioned material. The function of this system has been much discussed. It has been called a blood vascular system because of pulsations seen in the axial organ, an excretory system, the site for origin of genital cells, and simply a glandular system. Because of the uncertain function of these canals they should be regarded as coelomic derivatives and the terms haemal and perihaemal should be used with caution.

Nervous System. The highly developed nervous system consists of a plexus of nerve cells and processes which underlie both the ectodermal and the endodermal derivaties of the body. Since these cells do not respond to the usual nerve stains, demonstration of the entire system is difficult. By spreading apart the two rows of tube feet the oral radial nerve trunk may be seen. This joins a nerve ring in the mouth area. Destruction of selected portions of the nerve ring or the radial nerves impares coordination of major portions of the animal.

Other portions of the nervous system may be distinguished by study of a prepared slide of an arm cross section. Between the tube feet will be seen the V-shaped radial nerve with a mass of supporting cells, their bases near the outside and filamentous processes ending near the basal membrane. Tiny dots, the nerve fibrils traverse the

intersticies. The nerve thins out toward the edges but a layer may be seen to be continuous into the tube feet between the muscular and the epithelial layers. Around the sucker at the base is a nerve ring. The nerve encloses a cavity, the perihaemal radial canal which is separated in the center by the radial haemal strand. Between the perihaemal canal and the radial nerves is the deep nervous system or Lang's nerve. Although anatomical demonstration of minute divisions of the nervous system has not been achieved, a high degree of coordination is exhibited by such isolated portions as individual pedicellariae indicating an intricate nervous system.

Development. Because of the clear structure and ease with which fertilization can be controlled, echinoderm eggs have been used extensively for embryological and physiological studies. The sea star, sand dollar and sea urchin are most commonly employed. A brief description of the methods used in fertilization of all three forms is included here. The larval forms are described in embryology textbooks.

Ripe gonads are found in Asterias and Echinarachnius in the spring and early summer months. In sea urchins the season is later and gravid gonads may be found up until early fall. Since sexes cannot be distinguished externally and since the gonads may be shrunken and empty if the animal has already spawned it will be necessary to open several animals. Instruments should be washed in fresh water after opening each animal to prevent undesirable contamination with sperm.

Sea stars are opened by cutting along the aboral margin of a single arm and the gonads removed. When a satisfactory specimen has been obtained the animal with the other arms may be saved in a sperm-

free awarium for future work. Ovaries are orange while testes are gray. Urchins are cut in half around the equator. The gonads which hang by mesenteries in the upper half are red in gravid females and gray in males. With sand dollars, cut across the animal with heavy scissors thus exposing the gonads. Ovaries are purple.

Remove the ovaries from the femal and suspend them in double cheese cloth over a finger bowl containing sea water. After sufficient numbers have shed discard the cheese cloth. Using a pipette for this bowl only, obtain about 200 eggs in about 50 ml. of sea water. Wash once by decanting surface water after the eggs have settled. Examine to see if a large proportion of the eggs have intact germinal vesicles(sea urchins only). If so, set aside until mature.

To obtain normal development the sperm should be very dilute or polyspermy will produce abnormal embryos. Add one drop of "dry" sperm to 25 ml. of sea water, mix and add about 6 drops of this suspension to the eggs in about 50 ml. of water. Agitate to mix in the sperm. After five minutes pour off the water and wash several times. Examine at intervals. The rate of development depends upon available oxygen and the temperature so that developmental rate may vary considerably from the table. Keep the eggs cool for normal development.

Approximate Developmental Rate of Echinoderm Larvae Maintained at Room Temperature.

	Asterias forbesi	Arbacia punctulata	Echinarachnius Parma
Polar body formation	3/4 - 1 hour		1 ½ hours
Two cell	2-3	1½	2 3/4
Four cell	3	2	3
Eight cell	4	2 3/4	3¼
Sixteen cell	5	6 ¼	6¼
Early blastula	7	14	10
Late blastula	10	23½	21
Late gastrula	18-21	28	38
Bipennaria	24-30	Pluteus 48	Pluteus 48

Later development depends upon a satisfactory supply of food such as certain species of diatoms,

THE SEA URCHIN
Arbacia Punctulata

Arbacia punctulata, the purple urchin, ranges from Cape Cod to Florida and has been reported off the coast of Cuba and Yucatan from the littoral zone to 740 feet. In some localities it may be seen it its natural habitat in tide pools while in other areas it must be obtained by dredging. Although it is commonly known as the purple urchin it varies somewhat in color from brick red, dull red, purplish brown to almost black. Urchins live together in aggregations which move about slowly by means of spines and tube feet on shelly or rocky bottoms.

The larger green urchin, Strongylocentrotus dröbranchiensis, is found abundantly north of Cape Cod. Although it is readily distinguished from Arbacia by its color, smaller spines and various specialized features of the test and lantern, the following description may be used with minor modifications if Strongylocentrotus is substituted for Arbacia.

Three kinds of preparations may be used to advantage in study. External features are best studied on living animals but specimens preserved in alcohol may be substituted. A dry test with organic matter removed will be needed for study of the plates. Boiling in 10% potassium hydroxide will loosen the organic matter and further cleaning can be completed with a tooth brush. A preserved specimen with the skeleton softened in 2% nitric acid for 24 to 48 hours will show many delicate internal structures. If only the aboral surface is softened for dissection in the acid the oral skeleton may assist in holding the animal together for study.

External Structures. Examine the living or preserved specimen. The internal organs are enclosed in a globular test which is a skeleton made of many interlocking calcareous plates. The test is somewhat

compressed so that two poles are distinctly recognizable. It is flattened at the oral end which is readily identified by the presence of five protruding teeth. Four plates at the opposite pole surround the anus, this area being known as the periproct.

Examine the oral region under magnification noting the collar-shaped lip surrounding the teeth. A circular ring of muscles in the lip draws together the mouth opening like a purse string. Surrounding the lip is the ciliated peristome, a membranous covering which extends to the tim of the calcareous test. Perforating the peristome outside the lip are five pairs of large oral tube feet which have also been called oral or ambulacral suckers, paillae or tentacles. Two other modifications of tube feet are present. Just outside the peristome are five double rows of ambulacral tube feet, their prominent suckers being held open by embedded calcareous plates. These form meridianal rows entending in the direction of the aboral surface. Near the equator tube feet are replaced by tentacles which are similar except that suckers are missing. Both ambulacral tube feet and tentacles may be extended beyond the spines in living specimens. On the outer edge of the periproct in the interambulacral area will be found five pairs of finger-like, branching, peristomal gills which appear to be respiratory in function. If the ambulacral tube feet are carefully removed at the edge of the peristome a small bean-shaped sphaeridium will be found in the center between each double row of tube feet at the base of the first ambulacral spines. This sense organ, a modified spine, is sensitive to gravitational stimuli. Experimental removal slows the righting reaction.

Three types of pedicellariae have been described from Arbacia. On the peristome are numerous ophiocephalous pedicellariae. The three

blades expanding at the tip curved inward giving the whole head a serpent-like appearance. The supporting calcareous plate in the stalk comes almost to the head. Triphyllous pedicellariae are found in among the spines along with ophiocephalous forms. Their leaf-like valves are smaller and more slender and the stalk is short with the calcareous plate not reaching to the head. Tridentate pedicellariae with large tooth-shaped valves are less abundant.

Primary spines are the only type found on Arbacia. Secondary and tertiary or miliary spines which are reduced in size are found on Strongylocentrotus. In Arbacia the spines which all bear minute longitudinal fluting are shortest in the aboral areas and longest at the equator. Those close to the peristome are often spatulate (spatula form) with a wider tip than base. Occasional blunt spines are found in this area. Each spine has on its proximal end a hollow socket which fits into a corresponding tubercle on the test. Remove a spine and examine it and the tubercle under magnification. The circular attachment of the spine to the tubercle consists of two rings of muscle as well as an outer epidermal covering. The innermost muscular ring is used in fixation of the spine. It remains fixed when pressure is applied to the spine. The outer ring moves the spine in locomotion. The entire test, spines, pedicellariae and sphaeridiae as well as the coelomic cavitites within are covered with ciliated epithelium.

Test. Examination of a clean test will show the interlocking calcareous plates which are used extensively in echinoid taxonomy. The description here given applies only for Arbacia. The four suranal plates usually fall out in such a preparation. Five meridionally arranged ambulacral rays point from the peristomal region upwards to

the periproct. Each possesses two double rows of perforations through which the tube feet connect with the water vascular system inside the test. Between these ambulacral areas are five double rows of interambulacral plates. The ambulacral areas correspond to the ambulacral grooves while the interambulacral areas correspond to the sides of the rays of the sea star. The sea urchin thus consists of a sea star-like structure in which the five arms have been pinned upward covering all of the aboral surface except the periproctal region. The periproct is therefore homologous to the entire aboral surface of the sea star while the remainder of the sea urchin surface is homologous to the oral surface of the sea star.

In the periproct around the hole left by the four suranal plates is a ring of 10 plates. Occasional variants in this number may be found. The larger triangular plates at the end of the interambulacral areas are called genital, gonadal or basal plates. They each contain a pore through which the reproductive products are expelled. One of these, the madreporic plate, is larger than the others and contains numerous minute perforations. Between the genital plates at the tip of the ambulacral area are the smaller ocular or radial plates. They are perforated by a small pore through which the terminal tentacle projects.

The body of the test consists of ten rows of interambulacral plates and ten rows of ambulacral plates. The double row of interambulacral plates is separated by a prominent zigzag suture. Plates at the equator are the longest and may have as many as four conves, highly polished, tubercles for spine attachment. The width becomes progressively less in the periproctal region where only one spine per plate is found.

A similar progressive narrowing in the peristomal region occurs. The double row of ambulacral plates which are narrowest at the periproct becomes increasingly larger toward the peristome so that they are about equal to the adambulacral plates in this region. Each ambulacral plate bears a single tubercle nearest the center of the ambulacral region and a set of double pores toward the outside. There are usually three of these double sets of pores. In the pair the holes slant so that the outer faces are closer together than the inner openings. Examination of the inner side shows that each of these ambulacral plates is compound resulting from the fusion of several little plates, each pair of pores representing an individual plate. The first pair of ambulacral plates nearest the peristome is detached from the test. They may be demonstrate on the inner surface of the peristome where they support the oral tube feet. Five pairs of prominent projections, the auricles, are seen inside the peristomal edge.

Internal Structure. To examine the internal anatomy make an equatorial cut after the spines have been removed using heavy scissors or better a fine saw. Before separation of the two halves carefully separate the mesenteries holding loops of the stomach and intestine to the walls and locate the non-calcareous madreporic canal. The canal may be identified in a mesentery connecting the esophagus as it leaves the lantern with the aboral region. It leads from the madreporic plate to the ring canal closely surrounding the esophagus as it emerges from the lantern. The axial sinus is a bulbular attachment on the madreporic canal. After demonstrating these structures the mesentery and the madreporic canal may be cut and the two halves separated.

If a second specimen is available soak the aboral surface in 2% nitric acid. Cut a circle around the outside of the periproct with a pair of fine scissors and then make five meridional cuts through the interambulacras areas. Dissections should be carried out with the specimen completely submerged in water.

Digestive System. A complex structure of calcareous plates and muscles known as Aristotle's lantern surrounds the mouth and forward end of the esophagus. It serves to crush and grind the onmiorous diet of the urchin which includes algae, small crustacea, sponges and detritu. A detailed description of this intricate mechanism is given below.

Follow the ribbon-like digestive tract from the esophagus as it leaves the lantern, through the almost complete clockwise turn of the stomach (cardiac stomach of some workers), then to a counterclockwise loop of the intestine (pyloric stomach) to the rectum which turns sharpl toward the anus in the middle of the periproct. As the esophagus leaves the lantern area it gives off a smooth, hollow, ciliated tube, the siphon. The siphon parallels the stomach on the inner side and again rejoins the digestive tract. Since currents of water pass from the esophagus to the intestine through the siphon it is thought to act as a supplementary respiratory and flushing organ. The stomach is festoone on the outer edge and attached by delicate strands of mesentery to the outer test wall in the interambulacral areas. Mesenteries are absent in the ambulacral areas and on the siphon side. Fecal pellets may often be seen within the intestine. These are ejected from the anus and handed along by pedicellariae until they are over the edge of the test.

The Ambulacral System. The perforated madreporic plate, the madreporic canal, and the ring canal which rests upon the lantern membranes just outside the esophagus have already been described. With a strong light and some magnification five grape-shaped bodies, the polian vesicles, may be located connected to the ring canal in the position corresponding to the Tiedman's bodies in Asterias. Between them the radial canals go under the rotulae where them may be traced as the lantern is dissected. Without dissection they again may be found passing between the auricles to the ambulacral areas. From the radial canal a transverse canal leads into each tube foot through the inner pore canal. Just before entering the inner pore canal a branch leads to the ampulla. The outer pore canal also connected with the tube foot leads to the ampulla by another duct. This double connection with the ciliated ampulla makes possible better circulation between it and the tube foot than is found in the sea star. The radial canal continues aborally giving off transverse canals until the terminal tentacle is reached.

The Gonads. The five interambulacral aboral areas of the test are filled with reproductive organs in gravid specimens. The gonads are a reddish-brown in the female and a gray color in the male. After shedding the genital pores and a ring-shaped coelomic sinus, the genital rachis, may be seen. The sexes do not differ externally.

Aristotle's Lantern. The lantern is an intricate mechanism used in chewing food materials and sometimes for locomotion. Examine first the parts in a dry specimen. Five V-shaped jaws or pyramids hold the teeth in place. Each limb of the V is a separate plate known as an alveolus or half-pyramid. In an isolated pyramid break apart the two alveoli

along the median suture and notice the grooved dental slide in which the tooth is held. The lateral surface of the alveolus is greatly roughened by horizontal corrugated ridges which make an effective sawing surface. The outer edge of this same surface serves for attachment of a short but powerful interpyramidal (comminator) muscle closing the jaws and used in chewing. The tooth is pointed at the oral tip and tapers off into a long, curved membranous extension, missing in dry s pecimens, which curves inward at the aboral end. The aboral end of each tooth is enveloped in a large, delicate, balloon-shaped dental capsule seen only in recently collected living specimens. Perched on the aboral end of each alveolus is a small epiphysis one end of which curves inward towards the tooth. It may be readily broken loose at the line of suture revealing a pitted surface on the end of the alveolus. In Strongylocentrotus the two epiphyses join and act as additional support for the tooth. The cavity between the upper ends of the alveoli, the foramen magnum, is comparatively large in both species. Radiating outward from the esophagus are five, fine, conspicuous curved rods, the compasses or radii. Each compass is made up of an inner portion, and an outer portion which has a rounded tip in Arbacia but a bifurcated one in Strongylocentrotus. Beneath each compass and jointed to it at the inner end is a heavier rod-shaped plate, the rotula or brace. The outer end of the rotula joins the alveoli in the interpyramidal area. There are thus 40 pieces in the skeletal apparatus of the lantern consisting of 5 teeth, 10 alveoli, 10 epiphyses, 5 rotulae and 10 compass parts.

On a preserved specimen two delicate muscle strands are inserted on the expanded tips of each compass. These compass depressors or

radial compass muscles take origin from the interambulacral area of the edge of the test. A group of circular compass muscles (compass elevators connect each compass with the next one. The entire lantern is covered with a membrane known as the lantern membrane. Beneath it is a fluid filled cavity, the lantern coelom. It is connected with the dermal branchiae found on the peristome. Elevation of the compasses by the combined action of the circular compass muscles causes this lantern membrane to be lifted and the lantern coelom is enlarged with consequent withdrawing of the gills. The combined action of the compass depressors decreases the volume of the cavity expanding the gills. The compass depressors are probably also involved individually in pulling the lantern to an upright position.

Protractor muscles originate on the aboral end of each alveolus and are inserted in the interambulacral area. They serve to extend the mouth parts. Retractor muscles which open the jaws take origin from the lower outer face of the alveolus and are inserted on the auricles of the test. By pulling upon the rotula with a pair of forceps it can be demonstrated that the rotula is held firmly in articulation with the epiphysis by an external and an internal pair of rotula muscles. These appear to bind the lantern together and assist in rocking movements. There are thus 60 muscles connected with the lantern itself. These consist of 10 compass depressors, 5 compass elevators, 10 protractors, 10 retractors, 5 interpyramidal, 10 internal rotula and 10 external rotula muscles.

Nervous System. With a good light and some magnification the major nerve cords may be demonstrated. The radial nerve may be readily located as it passes through the base of the auricles under the radial

canal. It appears as a double, whitish cord. Carefully cut away the alveoli on one side with scissors as the radial nerve is traced inward to reveal the union with the pentagon-shaped peripharyngeal nerve ring. Histological preparations are required to demonstrate the branches feeding each tube foot and connections to the lantern muscles.

THE SAND DOLLAR, ECHINARACHNIUS PARMA

Echinarachnius parma, the common sand dollar, ranges from Labrador to New Jersey on the Atlantic coast and in the Pacific Ocean from Vancouver to Japan. It is common in isolated areas on sandy bottoms from low water to 5328 feet. Living specimens vary in color from a flesh-red to deep brownish-red. When injured or preserved the color changes to greenish-brown. Although they are of little economic importance, young specimens frequently furnish food for haddock.

This species is to be studied after the sea urchin for comparison with the latter. Fundamental differences in structure are correlated with adaptation to a sandy rather than a rocky bottom. A dry test and a living or alcolhol-preserved specimen will be required.

External Structure. The test is extremely flattened, the mouth opening central on the oral surface, while the anus is near the margin of the test. It is usually on the aboral surface in young specimens, later marginal and on the oral surface in older specimens. The madreporic plate is centrally located on the aboral surface surrounded by four genital pores. A fifth genital pore and gonad are mis sing as the space usually occupied by them is utilized by the posterior portion of the intestine.

Both surfaces are covered with two kinds of minute spines. Short spines with an enlarged tip are covered with cilia while the longer ones

have cilia missing on the end. The longest spines are found in the interradial areas near the peristome and at the margin of the disc. Ambulacral areas are marked by indistinct grooves on the oral surface. These give off two side branches near the margin. On the aboral surface bi-lobed, highly ovoid, respiratory tube feet are located in five double rows. These double rows flare apart in the middle giving a petal-like appearance to the ambulacral zone which is best seen on the dried test. Very numerous minute locomotor tube feet are found both in the ambulacral and interambulacral areas of both surfaces. These feet with pink-tipped suckers are used effectively in feeding on sand which is encrusted with microorganisms, and in combination with the spines, are used in locomotion through the sand. Among the spines will be found a single type of two-jawed pedicellaria.

Dissection and Internal Anatomy. Dissection may best be carried out by breaking into the aboral surface and picking or cutting away the plates with heavy forceps or a small saw. The test may be softened by soaking overnight in 2% nitric acid. In the margin of the disc numerous supporting pillars connect the dorsal and ventral surfaces giving additional strength and partially dividing the interior.

Immediately under the aboral wall are the extensive gonads which extend into all available spaces in sexually mature individuals. Fertilization is treated briefly under the sea star. Carefully remove these to reveal the ribbon-shaped digestive system which has coils similar to the sea urchin except for the marginal anus. A siphon is found on the internal side.

The madreporic canal is easily broken loose from the madreporic plate. Attached to it is the prominent axial sinus containing the dark

axial organ. Radiating outwards from the lantern are the darkened radial canals. They branch into extremely fine transverse canals which supply the numerous tube feet.

The Lantern. Although the lantern is well developed it is very different from the lantern of Arbacia. It has a greater diameter in a horizontal plane with a star-shaped cross section while Arbacia has a lantern shaped like an inverted cone which is circular in cross section. In the Clypeastroids the lantern is said to be procumbent with the five unequal jaws holding the teeth in a plane nearly parallel with the oral test wall. Each jaw consists of two alveoli with fluted wings which hold the blade-shaped tooth in the dental slide between them. The wings of the alveoli project sharply outward giving the entire jaw an arrow-head shape. The alveoli from adjoining jaws come together to form the points of the star. Resting between these alveolar tips near the point are three small calcareous bars. The center bar is the rotula while the other two are the small epiphyses. Compasses are missing. Well developed, paired, retractor muscles are inserted on the lower surface of the jaw suture near the tooth while the origin is on an auricle. A weakly - developed protractor muscle is attached higher up on the jaw with its origin also on the auricle between the retractors. Very strong inter-pyramidal muscles are visible on the oral surface. These appear to work as antagonists to the retractors giving the lantern a well-developed grinding apparatus. The teeth, however, are unable to extend as far outside the mouth opening as the sea urchin because of their procumbent position and because of the lack of a flexible peristome.

THE SEA CUCUMBER, *THYONE BRIAREUS*

Thyone briareus is found in limited area in the intertidal zone down to 60 feet. It ranges from Vineyard Sound south to the Gulf of Mexico. It lives on either a sandy or muddy bottom, usually being completely buried except for the posterior end. The anterior end is occasionally protruded in feeding. Since it is seldom possible to view this species in its natural habitat, feeding and respiratory movements may best be studied in an aquarium with a sandy bottom. By contractions of circular and longitudinal muscles animals so placed gradually work themselves down until only the two ends are left protruding.

Musculature must be relaxed for successful dissection. Injection of 5 to 10 ml. of 10% magnesium chloride will accomplish this after an hour. Tentacles then may be gradually worked out at the anterior end. Preserved specimens must be killed with the tentacles extended. Color fluids may be used to inject the respiratory trees through the cloaca or the water vascular system through the Polian vesicle.

Autotomy and regeneration. The radical autotomy for which the holothurians are noted rarely occurs in nature with *Thyone* even under conditions of extreme fouling. However, it may be readily induced by various chemical stimuli. Most specimens autotomize if placed in a solution of 0.08 % ammonium hydroxide made up in sea water. After the animal has remained in the solution for about a minute, violent circular muscular contractions usually occur. If these movements do not begin spontaneously, hold the animal up by the posterior end for a few seconds. The entire anterior end of the animal with the tentac

calcareous ring, and the digestive system is expelled. These structures are regenerated in miniature in two or three weeks.

External Morphology. Thyone briareus varies in color from dirty olive to almost black. The tentacles have a grayish appearance. The tube feet or podia are rather evenly distributed over the entire surface of the body although on some specimens it is possible to group them into five ambulacral areas. They often have a pinkish tinge due to the presence of hemoglobin in the water vascular system. Suckers are well-developed on the ventral surface but are often lacking on the podia near the dorsal radii.

The secondarily acquired radial symmetry is somewhat obscured by a superimposed bilateral symmetry associated with the elongated structure and the burrowing habits of this group. The mouth may be considered anterior and the cloaca posterior. The concave surface which is uppermost when the animal is held by both ends is dorsal and the convex lower surface is ventral. In relaxed specimens radial orientation may be established by ridges formed by longitudinal muscle bands located in the radii. On either side of the mid-dorsal line are two of these bands, the right dorsal radius and the left dorsal radius. Another, the ventral radius, is found in the mid-ventral position. The other two radii spaced between the forsals and the ventral radius are the left ventral radius and the right ventral radius. The five interradii are the dorsal, right dorsal, left dorsal, right ventral and left ventral interradii.

The cloacal aperature is built around a calcareous framework describe below. Five groups of sensory papillae surround the opening. A genital papilla is sometimes present between the bases of the tentacles in the mid-dorsal region. It is said to be found only in males.

Dissection. Make a longitudinal cut into the cloacal cavity in the right ventral interradius. Cut just below the right ventral radius, if i can be located externally, to protect the intestinal mesentery in the middle of the interradius. The specimen may then be pinned down under water in a dissecting pan.

Digestive System. The mouth opening is surrounded by a lip leading into a pharynx which penetrates the calcareous ring. After a slight constriction the pharynx joins a short esophagus. This in turn leads to the muscular stomach. The stomach joins the coiled intestine. In lengt the intestine exceeds by many times the length of the animal. Three maj divisions may be distinguished. The firs t portion attached to the stom progresses forward with attachment to the left dorsal interradius. The third division is attached to the right ventral interradius and is conne to the muscular cloaca by a short rectum.

During the early evening hours the anterior end is sometimes extend and the eight large tentacles and the two small ventral tentacles are expanded into long delicate strands as they take part in feeding movements. The tentacles are waved about in the water to pick up food particles and then are alternately drawn into the mouth, the food particles scraped off and the particles rejected. Analysis of the digestive system contents shows the food to be protozoa, small worms, crustacea, diatoms and algae fragments along with detritus from muddy bottoms.

Respiratory System. Respiration is accomplished by means of a pumping device, the cloaca, which brings about an exchange of fluid in t highly branced respiratory trees within the coelom. Red corpuscles containing haemoglobin within the water vascular canals also assist in oxygen transport.

The two finely branched respiratory trees which extend from the cloaca to the anterior end are suspended by delicate mesenteries. The cloacal wall contains a ring of circular muscles while radiating muscles connect the cloaca with the body wall. Contraction of the circular muscles causes water to be expelled through the cloacal aperature if it is open or forced into the respiratory tree if closed. Rhythmic closing and opening of the aperature may be readily observed on living animals, the speed of the process being dependent upon the oxygen content of the water. The contraction of the radiating cloacal muscles causes the cloacal chamber to enlarge and water to be sucked in. Occasionally the musculature of the enitre body may contract causing a forceful spouting.

Reproductive System. Sexes are separate and fertilization and development are external. The male and female reproductive organs can be distinguished only by microscope preparations. Each type of gonad consists of a series of fine filaments on either side of and attached to the dorsal mesentery. These filaments converge into a genital duct which conducts the eggs or sperm cells forward to the genital papilla.

Water Vascular System. In living specimens the water vascular system stands out clearly because it contains red hemaglobin-containing cells. To see these cells and the ciliary activity within the system scrape off a few of the filamentous red ampullae and examine under the microscope. In favorable specimens the ring ring canal may be seen just below the calcareous ring. From it hang one to three Polian vesicles. The internal madreporic plate is a calcareous mass hanging by a slender madreporic canal which is in turn attached to the ring canal. From the area of the ring canal radial canals run forward each giving off a pair

of transverse canals which serve two of the oral tentacles. Ten ampullae near the calcareous ring contain reserve fluid for these tentacles. The radial canals then bend backwards and toward the posterior end. They are imbedded between the paired longitudinal muscle bands. Radial canals give off numerous long transverse canals which connect with tube feet and ampullae.

Muscular System. Five paired bands of longitudinal muscles extend the length of the animal in the radial zones. Joined to these at the anterior end are the powerful retractors attached to the calcareous ring These retractors serve to pull in the entire anterior end of the animal. Equally strong but more diffuse circular muscles line the body wall under the circular muscles. These muscles not only make possible worm-like movements, but also come into play in spouting and in autotomy.

Nervous System. The circular and radial nerves correspond in relative position with those in the starfish. They are difficult to distinguish in gross dissections but may be seen in histological preparations.

Calcareous Plates. In all holothurians the calcareous skeleton is much more dispersed than in the other classes of echinoderms. It consis of isolated calcareous plates distributed in various parts of the body. These are considered of major significance in the taxonomy of the group. Isolated plates may best be seen after boiling the animal in strong potassium hydroxide. In Thyone calcareous deposits are found in the tentacles, the podia, the madreporite, the calcareous ring, and the anal ring, while skin "tables" are confined to the two ends. These tables consist of plates perforated by 8 holes. At right angles to these plates are four spires which taper to sharp teeth at the end opposite the

attachment to the plate. The spires are connected with one or two cross rods. The calcareous ring consists of 5 radial and 5 interradial plates. The radial plates are notched anteriorly for passage of the radial nerves while posteriorly they have two long posterior projections. The cloacal aperature is supported by a ring of 5 plates each with a posterior projection, the anal tooth. The podia contain terminal plates with many perforations in the suckers.

THE BRITTLE STAR, OPHIODERMA BREVISPINA

Ophioderma brevispina is an olive green to almost black brittle star found in shallow water down to a depth of 930 feet. It is widely distributed having been reported from Cape Cod to Brazil. The more southern forms are sometimes regarded as a different subspecies named olivacea. Specimens are often common among sea weeds where they hide during the day time and feed at night.

Brittle stars may best be studied by comparison with the sea star. Since they are not very suitable for gross dissection some parts of the anatomy have been worked out only after a study of serial sections. The following description applies to structures visable with the aid of a dissecting microscope. A single living or alcohol-preserved specimen will suffice.

External Form. It will be noted that pedicellariae, ambulacral grooves, dermal gills, and suckers upon the tube feet are missing. The tube feet, called tentacles in this group, are largely sensory in functi but they are capable of passing a particle of food along the rays to the mouth. The flexible arms are sharply marked off from the pentagonal disc. The arms resemble the vertebral column in the tail of a vertebrat

in structure and function. Internally they consist of a column of calcareous vertebrae each one being connected to the next by two pairs of muscles.

By well coordinated activity of four arms the animal is able to mov about swiftly. The fifth unused arm may either lead or follow behind. The temporary anterior and posterior pairs of arms cooperate in alternat bearing the weight of the animal and shoving the body forward, then thru ing themselves forward. If an attachment point is available an arm may become coiled around it and pull the body forward.

Dermal Skeleton. The more than 1600 species of described ophiuroid are distinguished largely by the arrangement of very constant dermal pla which form characteristic patterns. The more typical of these plates ar present in Ophioderma brevispina although this species is atypical in that all of the aboral and part of the oral disc surface is covered with uniform granules. These granules which range from 100 to 180 per square millimeter hide some of the plates.

The arms, which in this species are almost circular in cross sectio except for flattening on the oral surface, have two lateral, one upper-a and one under-arm plate. The upper-arm plates which are broadly in contact with each other are oblong and nearly twice as wide as long. Near the tip they become more rounded on the sides with the outer edge being more curved and the inner edge more narrow. Near the lateral edge of the third upper-arm plate are about 10 little scales. The under-arm plates are nearly square with rounded corners. They are usually slightl longer than wide and are broadly in contact with each other. The first under-arm plate is much wider than long and has rounded sides. Although the second plate is much longer in contrast it is not quite as long as th

third. Near the lateral margins of each plate are the pores through whi the tentacles extend. Just proximal to this pore are two short spines known as the tentacle scales. These should not be confused with the longer arm spines of which there are 7 or 8. The inner tentacle scale i nearly half the length of the under-arm plate while the outer is about half as long and covers the base of the lowest arm spine. The arm spine are constant enough in size, number and position to be of diagnostic importance. In this species they are appressed to the arm, about equal in size but with the under spine shortest, and all relatively short. Th species name, brevispina, is derived from this latter characteristic.

On the aboral surface the granules extend out on the sides of the arm so that the first three upper-arm plates form a narrow ridge running toward the center of the disc. If the granules are scraped from the disc surface, two oval plates will be found lateral to the attachment of each arm. These are the radial shields which are often of taxonomic importance.

The largest plates on the oral surface, the oral shields, are in the interradial areas. These are oval and longer than wide in this species. One oral shield which is slightly larger and more irregular in shape than the others is the madreporic plate. The minute pores communicating with the madreporic canal are difficult to locate. Lying on both sides of each oral shield are two triangular aboral plates with rounded corners. Except for these 15 plates the oral surface is granulated in this species. Pointing forward from the oral shields are the five jaws. On the inner tip of each jaw inside the mouth cavity is a row of 5 blunt teeth. Unless the mouth is well opened these will not be seen since they are arranged one under the other. Although the teeth

may be turned down to enlarge the mouth opening they are not used in mastication. Tooth papillae which are sometimes present near the teeth are missing in this species. The edge of the jaw is fringed by a row of oral papillae. These are located on the surface of the jaw in contrast to the teeth which are inside the mouth. There are about seven on each side of the jaw, the one next to the arm plate being the smallest while the second is the widest and longest of all.

Genital bursae. Distal to the oral shields is a pair of prominent grooves known as the genital bursae. A second pair is found on the edge of the disc next to the arms. This genus thus has 10 pairs of these bursae, a characteristic distinguishing it from other genera in which the outer 5 pairs are missing. The genital products are discharged by the gonads into these bursae. In some viviparous species such as Amphipholis squamata these bursae act as brood pouches. In Ophioderma squamata as with most species development is external with the reproductive products being discharged into the water. The bursae open and close rhythmically in a movement which has been interpreted as a respiratory activity.

Dissection. The only practical dissection may be made by removing the aboral disc. This view shows the gonads and the sac-like stomach. Hepatic caeca, intestine, and anus are missing. Neither the gonads nor the digestive system extends into the arms. All the teeth may now be seen. The vertebrae of the proximal portion of the arm together with the muscular attachments show clearly.

The water vascular system differs from that of the sea star only in minor respects. Ampullae are missing as are also Tiedmann's bodies while the madreporic plate and canal are aboral. The nervous system is highly

developed with ganglionic enlargements in the arms which supply each group of intervertebral muscles with nervous connections.

Pull off an arm and examine the cross section. Locate four muscles an upper pair and a lower pair. Simultaneous contraction of the right lateral muscles or the left lateral pair brings about side motion. Contraction of the upper muscles raises the arm while the lower muscles draw the arm orally. However, movement in a vertical position is more limited than lateral movement due to mechanical limitations imposed by the structure of the vertebrae. Ther vertebral articulations consist of a complicated system of knobs and grooves. These may be demonstrated by boiling an arm in potassium hydroxide and microscopic examination of the plates. In the Euryalids the vertebrae are shaped like an hour glass permitting more vertical movement than is found in the Ophiurida.

Physiological, Behavior and Embryological Studies.

Students who have made extensive morphological studies in this group may investigate other problems. It is suggested: (1) The literat should be reviewed before starting such a study. The bibliography on t following sheets will suggest papers leading into the literature. (2) Organize the experiments in a systematic manner. (3) Outline the problem and the procedure to be followed with the instructor. (4) Keep a careful record of results. (5) Analyze and criticize the experimenta procedure and the results.

Suggested Topics.

1. Direction of ciliary currents on any species of echinoderm.

2. Experiments in movement and behavior before and after destruction of the nerve ring or radial nerves.

3. Clotting mechanisms.

4. Cellular constituents in the coelomic fluid.

5. Righting reactions in any species.

6. Respiration in Thyone.

7. Regeneration in Thyone.

8. Autotomy in Asterias, Ophioderma, Thyone, Leptosynapta.

9. Normal embryology of Asterias, Echinarachnius, Arbacia. (See page 10-11) and the bibliography.

10. Direction of locomotion of any form.

11. Feeding of Asterias, Thyone.

12. Digestive enzymes of any form.

13. Habit formation.

BIBLIOGRAPHY

Crozier, W. J., 1916. The rhythmic pulsations of the cloaca of holothurians. Jour. Exp. Zool. 20: 297.

Crozier, W. J., 1921. The orientation of a holothurian by light. Amer. Jour. Physiol. 37:

Donellon, J. A., 1938. An experimental study of clot formation in the perivesceral fluid of Arbacia. Physio. Zool. 11: 389-398.

Fujiwara, T., 1935. On the poisonous podicellaria of Toropneustes pileolus (Lamark). Annotations Zool. Jap. 15: 62-69.

Galtsoff, Paul S., and Loosanoff, Victor L., 1939. Natural history and method of controlling the starfish (Asterias forbesi, Desor). Bul. Bur. Fish. 49(31): 75-132.

Gemmill, J. F., 1912. The Locomotor function of the lantern in Echin with observations on other allied lantern activities. Proc. Roy. Soc. Lond. B 85: 84-109.

Glaser, O., 1907. Movement and problem solving in Ophiura brevispina Jour. Exp. Zool. 4: 203.

Holmes, S. J., 1912. Phototaxis in the sea-urchin, Arbacia punctulat Jour. Anim. Behavior 2: 126-136.

Hopkins, A. E., 1926. On the physiology of the central nervous syste in the starfish Asterias tenuispina. Jour. Exp. Zool. 46: 263-275.

Irving, L., 1924. Ciliary currents in the starfish. Jour. Exp. Zool 41: 115-124

Irving, L., 1926. Regulation of the hydrogen ion concentration and i relation to metabolism and respiration in the starfish. Jour. Gen Physiol. 10: 345-358.

Jennings, H. S., 1907. Behavior of the starfish Asterias forreri de Loriol. Univ. Calif. Publ. Zool. 4. 53-185.

Kille, F. R., 1935. Regeneration in Thyone briareus Leseur following induced autotomy. Biol. Bul. 69: 82-108.

Kille, F. R., 1939. Regeneration of gonad tubules following extirpat. in the sea-cucumber Thyone briareus (Leseure). Biol. Bul. 76: 70-7

Kindred, J. E., 1924. The cellular elements in the Perivisceral fluid of echinoderms. Biol. Bul. 46: 229-251.

Lutz, B. R., 1930. The effect of low oxygen tension on the pulsation of the isolated holothurian cloaca. Biol. Bul. 58: 74-84.

Moore, A. R., 1920. On the righting movements of the starfish. Biol. Bul. 19: 235-239.

Moore, A. R., 1910. On the nervous mechanism of the righting movement of the starfish. Amer. Jour. Physiol. 27: 207-211.

Moore, A. R., 1920. Stereotropism as a function of neuromuscular organization. Jour. Gen. Physiol. 2: 319-324.

Paine, V. L., 1926. Adhesion of the tube feet in starfishes. Jour. Exp. Zool. 45: 361-366.

Parker, C. H., 1922. The geotropism of the sea-urchin Centrechinus. Biol. Bul. 43: 374-383.

Parker, G. H., 1927. Locomotion and righting movements in echinoderms especially in Echinarachnius. Amer. Jour. Psychol. 39: 167-180.

Parker, G. H., and van Alstyne, M., 1932. Locomotor organs of Echinarachnius parma. Biol. Bul. 62: 195-200.

Pearse, A. A., 1908. Observations on the behavior of the holothurian, Thyone briareus (Leseur). Biol. Bul. 15: 259-288.

Smith, J. E., 1937b. The structure and function of the tube feet in certain echinoderms. Jour. of the Marine Biol. Assoc. 22: 345-357
Smith, J. E., 1945. The role of the nervous system in some activities of the starfish. Biol. Reviews 20: 29-43.
Stier. T. J., 1933. Diurnal changes in activities and geotropism in Thyone briareus. Biol. Bul. 64: 326-332.
Van der Hoyde, H. C., 1922. Haemoglobin in Thyone briareus Lesueur. Biol. Bul. 42: 95-98.
Van der Hoyde, H. C., 1922. On the physiology of digestion, respirat and excretion in echinoderms. Den Holder, C. deBoer Jr. 1-111.
Weese, A. O. 1926. The food and digestive processes of Strongylocentrotus dröbachiensis. Publ. Puget Sound Biol. Sta. 5: 165-179.

III. Embryology (Normal)

Field, G. N., 1892. The larva of Asterias vulgaris. Quart. Jour. Mi Sci. 34: 105-128.
Gemmill, J. F., 1914. The development and certain points in the adul structure of the starfish Asterias rubens, L. Phil. Trans, Roy. Soc. Lond. B. 205: 213-294.
Goto, S., 1898. The metamorphosis of Asterias pallida with special reference to the fate of the body cavities. Jour. Col. Sci. Imp. U Tokyo. 239-278.
Hörstadius, Svon., 1936. Determination in the early development of t sea urchin. Collecting net (Woods Hole). 11(9-10): 1-11.
Hörstadius, Svon., 1939. The mechanics of sea urchin development stu died by operative methods. Biol. Rev. 14: 132-179.
Korshelt, E., 1936. Vergleichende Entwicklungsgeschichte der Tiere. I Jena Fischer, 537.
MacBride, E. W., 1914. Textbook of embryology 1. London. Macmillan. 692 p.
Mortensen, Th., 1931-1938. Contributions to the study of the develop ment and larval forms of echinoderms. K. Dansk. vidonsk. Solsk. Skrifter (9) 4-7: I-IV.

TERMINAL INSTRUCTIONS SHEET

Please fill out the following data (LEGIBLY??) and place it on instructor's desk as soon as you can. Thank you.

1. _____
   Your last name First name Middle Name

2. _____
   Campus address (dormitory and room #)

3. _____
   Approx. date (day) & time of day (a.m. or p.m.) of vacating above

4. _____
   Day or time of leaving Woods Hole By train? (Yes or no)

5. Address at which you can be reached should any phone calls, mail, etc. come for you after you have left your campus or Woods Hole add ress; _____ _____ _____ _____

6. Have you checked in all borrowed apparatus?_____

7. Have you turned in your key?_____

8. Have you filled out a post office forwarding address card & left it with the instructors or on instructor's desk?_____

9. Have you obtained all the back drawings coming to you from the previous instructors (pre-Protochordates?)......_____

10. By what time must your Protochordate set of drawings & data be graded or checked in order for you to be able to get them before leaving?. . . . . _____

11. Has all your written work been turned in which you intended to turn in, so that the instructors will not be holding back your other drawings, etc. waiting for the rest of the assignment?_____

12. Do you plan to ask for a CERTIFICATE for the course?_____

13. Will you thoroughly clean out your desk, drawers, wall cabinets, top of your desk, your share of the Eel Pond immersed slides, finger bowls, watch glasses, etc. -- not to mention any packing mass (excelsior, waste paper, string, packing litter, etc.) for which you are responsible? If so, we shall all be exceedingly grateful to you. If not _____**!!©©&&&, etc.

PROTOCHORDATES

LABORATORY INSTRUCTIONS

A. General instructions

1. All records and finished drawings are due at 4 p. m. of the second day. They will be returned later in the evening.
2. The type of records and the number and kind of drawings to make are left to the discretion of the student. If you have previously done one of the forms thoroughly you may substitute another for it.
3. Drawings are suggested at the end of the exercise on each separate animal.
4. At the end of your last lab. exercise dispose properly of your living or dead specimens, wash the slides or glassware which contained the colonies and specimens, dry them thoroughly and place where assistant directs. Clear your table, inside and out, making sure that it is left in better shape than when it was assigned to you.
5. Refer to the lab. bulletin board for further announcements.

B. Forms to be studied in laboratory:

1. UROCHORDATA or TUNICATA
   a. Simple ascidians
      1. Molgula
      2. Styela
      3. Ciona -- if available
   b. Composite ascidians
      b'.---Parietal budding; ascidiozooid body short, compact, and not divided into regions:---
      4. Botryllus
      b".-- Budding stolonial; heart, alimentary canal and reproductive systems are placed behind the branchial sac, making a body of 2 or 3 regions:---
      5. Didemnum
      6. Amaroucium
      7. Perophora
   c. Thaliaceans --- pelagic
      8. Salpa, if available
2. HEMICHORDATA or ENTEROPNEUSTA
      9. Dolichoglossus

C. Tentative plan of lab. work:---

1. First day---morning---simple ascidians #1, #2, #3
   afternoon--Botryllus #4
2. Second day--morning---#5,#6, Didemnum, Amaroucium
   afternoon-#7,8,9, Perophora, Salpa, Dolichoglossus

SIMPLE ASCIDIANS

A. Pla ce Styela and Molgula in a bowl of sea water. Allow to remain undisturbed for a short time, until siphons begin to function normally. If Ciona is available it should be studied in the same manner as the 2 above.

B. Touch very gently with your dissecting needle each siphon and

selected areas of the test or body covering to determine the sensitivity of the animal in that area. Do the siphons contract separately or together?

C. Place a little powdered carmine or dry Chinese Laundry ink near each siphon of a relaxed, functioning animal, being careful not to disturb either siphon. Which siphon is for intake and which for out-go? Study each siphon carefully to see if there is any anatomical difference between them. Later you may dissect them after you have made the following studies. Tentacles of the Molgulidae are supposed to be much branched and in Styela unbranched and finger-like.

D. Styela's test is too thick but Molgula's is thin enough to observe the following:

1. Endostyle - a ciliated groove which looks like a white thread along the mid-ventral line of the branchial basket or pharynx. With this line and the siphons as guides, determine the dorsal, ventral, anterior, posterior, right and left sides of the animal.
2. Heart ---- a tubular organ on the right side, visible chiefly because of its pulsation. The blood flow reverses after a number of beats. The heart will be difficult to see at first.
3. Viscera---- see later under various organs.

E. Remove test or tunic by cutting thru it with fine scissors then carefully strip it from the animal. Be careful not to injure the body wall at this dissection.

F. Examine a small piece of the test under either the dissecting or the compound scope, or both. Do you find blood vessels and cells?

G. The muscle fibers are especially conspicuous around the siphons. There they are arranged in sphincters. How are the muscle fibers arranged elsewhere in the mantle?

H. DIGESTIVE SYSTEM

The large saccular p harynx is fused ventrally with the mantle, in the region of the endostyle. The incurrent siphon opens into it (the pharynx) with a circlet of tentacles (see paragraph C above).

Later in the exercise (after you have cut into the mantle) it would be good to remove a small piece of pharynx for closer study of the stigmata or perforations in the branchial sac of pharynx Compare those of Styela and Molgula. The stigmata are used as diagnostic features. In Molgula the stigmata are not all the same size, are less regularly placed and are curved. The beat of the cilia can be seen in dissected specimens.

H. The endostyle is a conspicuous ciliated groove along the midventral pharyngeal wall. At some distance to either side of it, can be seen the blood vessels. Anteriorly, the endostyle is continuous with the peri-pharyngeal ciliated bands, which encircle the oral end of pharynx. The endostyle is similar in all Tunicates (Herdman

1882) so is not diagnostic.

The peri-pharyngeal bands are continuous ventrally with the endostyle and dorsally with the dorsal lamina.

The dorsal lamina extends backward along the mid-dorsal line of the pharynx and opposite the endostyle, and ends at the opening of the esophagus.

The pharyngeal wall has longitudinal folds in addition to the endostyle and the dorsal lamina in both Styela and Molgula. Are there any processes or projections from these longitudinal folds?

A very short esophagus connects the pharynx and stomach.

The stomach is covered or hidden largely by a brown mass of several non-descript organs or glandular structures collectively called the "liver". In the Molgulidae the "liver" is a well-developed lobed mass. In the Cynthiidae (Styela) it coats the stomach.

With the stomach the intestine forms a transversely directed loop on the left side. The intestine is provided with a longitudinal fold, the typhlosole. The intestine ends as the anus, which opens into the atrial chamber.

Do you have a fairly clear conception of the mode of operation of the digestive tract, the purpose of the stigmata, the atrial cavity, the digestive glands or "liver", etc? Does the intestine seem free or attached?

I. ATRIUM

The space between the enteron and the mantle is the atrial cavity or peribranchial chamber. Into it open the intestine, the stigmata and the gonoducts.

J. REPRODUCTIVE SYSTEM

In the Molgulidae the ovaries and testes are united into 2 usually ovate, hermaphrodite genital masses, a right and a left. The right mass is in the center of the right side, anterior to the heart. Its length is transversely placed and the gonoducts are at the dorsal end. The left gonad lies in a corresponding position on the left side, and in front of the intestinel loops. The gonads are light in color, the testes being somewhat lighter than the ovaries. Each gonoduct consist of 2 tubes, the oviduct and the vas deferens. The gonads are adherent to the innder side of the mantle.

In Styela 1 or 2 long sausage-like masses are formed, each of which consists of a number of small ovaria and testes stuck on or in the mantle.

K. SEX CELLS AND LARVAE

Molgula citrina is viviparous but M. manhattensis is not. Styela lays eggs late afternoon or by sundown while Molgula does so early in the morning.

Early developmental stages of Molgula may be obtained in this way:

1. Remove test
2. Under the binocular dissecting scope identify ovary by eggs within
3. Remove eggs from hollow ovary with dissecting needle.
4. Wash eggs fairly free of body fluid with several changes of sea water after you have removed animal from the dish. Eggs of Molgula species range in diameter from 0.11 to 0.21 mm.
5. Dissect out testes from another animal. Place in small dish with a very small amount of water.
6. Cut it up finely then add more water.
7. Take a few drops of this suspension and place in dish containing eggs.
8. After a time wash carefully in changes of sea water
9. Development is rapid in Molgula. The tadpole stage should be formed in about 24 hours. Styela develops into fully organized free-swimming larvae in 10 hours.

L. EXCRETORY SYSTEM

The renal organ is a conspicuous sausage-shaped or sac-like organ lying on the inner side of the mantle, on the right side of the body, posterior to the genital mass of that side and separated from the peribranchial cavity by the lining membrane. It contains a brownish fluid and usually some solid matter--wastes in the form of concretions. It is ductless.

M. CIRCULATORY SYSTEM

Look for a translucent pulsating organ, the heart, which is enclosed in a pericardium and which has blood vessels at each end. It is simple, fusiform and periodically changes the direction of the blood flow. Study under binocular. Numerous vessels can be found in the branchial region, around the stigmata. Consult some text or Herdman 1882 Challenger Report (XVLL, p.52) for details regarding circulatory system if interested.

Count the number of heart contractions which send the blood in 1 direction, then count the number which send blood in the reverse direction. Compare notes with your neighbors. Record on drawing sheet somewhere.

N. NERVOUS SYSTEM

A cerebral ganglion is located between the two siphons in the mantle. It is almost completely surrounded by the neural gland. Nerves connect the ganglion with the two siphons. They can be seen. The hypophysis, a tube leading from the neutral gland, opens as the dorsal tubercle, a small volute-shaped lump at the anterior end of the dorsal lamina.

O. Suggested drawings or sketches of simple ascidians.

Fig. 1. Draw a comparative, external view of Molgula, Styela, and possibly Ciona to show the nature of the test, the arrangement and detail of the siphons and the relative size of the specimens. Indicate under each figure the size which the animal might attain (data obtainable in Pratt, etc.).

Fig. 2. Microscopic view of test or tunic showing vessels, cells, fibers, matrix, etc.

Fig. 3. Tentacles of Molgula and Styela.

Fig. 4. Microscopic view of inner surface of branchial wall to show the stigmata, cilia, longitudinal folds, interstigmatal vessels, etc.

Fig. 5 Egg or embryo of simple ascidian.

Fig. 6 Internal anatomy of a simple ascidian, or such organs as you think need drawing.

COMPOSITE ASCIDIANS

Botryllus

A. MATERIAL AVAILABLE FOR STUDY:

1. Adult colonies of Botryllus
2. Young Botryllus colonies of various ages, growing on slides which had been suspended in Eel Pond for definite periods of time---see board for exact number of days.
3. Larvae liberated that morning, if possible to obtain.

B. YOUNG COLONIES

1. Obtain Eel-Pond-immersed slides on which are growing young Botryllus colonies. Place the slides in a shallow dish (Petri, or culture dish) with a little sea water, enough to cover the colonies comfortably, so that colonies can be studied alive and functioning. The slides will contain numerous other forms in addition to the Protochordates--- Bryozoa, Protozoa, worms, etc. If too much debris has accumulated, scrape off some of it, leaving only the Protochordate colonies and study them. Not all colonies on the same slide will be the same age. Why?

2. Study the colonies from as many angles as possible, from the sides and from the top and under sides.

3. The colony or individual is encased in a transparent test. Is it perfectly transparent or does it have any structures in its matrix?

4. Around the young Botryllus zooid is a number of transparent leaf-like ampullae, all joined to a connecting vessel. Inside each ampulla are visible circulating blood cells. Does the course of the blood flow reverse as in Molgula? How many ampullae?

5. Can you identify the incurrent and excurrent siphons on the zooid? The incurrent siphon is the entrance to the branchial chamber, and it has a circlet of tentacles on its inner surface. Touch siphons gently to see how sensitive they are.

6. Compare the pharyngeal slits and stigmata of individuals of different ages. How are they different---that is as to number and as to direction?

7. Note the fairly prominent endostyle (ventral) and the epibranchial groove (dorsal).

8. Identify other organs also such as neural gland and ganglion, digestive tract, mantle, heart, etc.

9. Note the distribution of the pigment in the young and older colonies. Is there any difference in color?

10. At what point do the buds seem to arise or is there any definite system? Do buds arise consecutively or simultaneously? What is the largest number of buds from a particular aged colony at your table and at neighboring tables?

11. Do you understand how the Botryllus individuals came to be on the slide or what stage it was which attached to the slide?

C. MATURE OR ADULT COLONIES OF BOTRYLLUS

1. Select 2 small Botryllus masses, 1 in which the individuals look thin and clean and fairly young and the other in which the zooids seem thick, mossy and matted together. The former colony will probably not liberate larvae but the latter being sexually mature, may. Botryllus schlosseri is viviparous. More about the larvae later.

2. Note the character which makes this form a "composite" or compound ascidian,---the common tunic or test, in which numerous colonies or systems are imbedded. The test is soft, transparent, and has numerous ampullae and vessels in the vicinity of the colonies or systems of zooids. Examine some test material on a slide under the compound scope. Note any cells, fibers, vessels, etc., which are there.

3. How many individuals or zooids are there in a system or colony? Compare with neighbor's results. Note the common cloacal opening, or cavity. From that region radiate rows of pigmented material toward the mouths of the zooids. What are the colors of these pigmented areas, matrix or test, zooid proper, etc.?

4. Touch the cloacal opening's edge gently with dissecting needle. Observe reaction of the colony. Touch 1 mouth. Does colony react in same manner?

5. Identify as many of the internal organs of the zooids as you can, using Molgula as a guide. Find the annular blood vessel with its many ampullae around the colony.

D. IMMATURE AND LARVAL STAGES OF BOTRYLLUS

1. Segmenting eggs can be obtained only by dissection of sexually mature zooids and by removal from the brood chamber in the atrium. Eggs are 0.42 mm. in diameter

2. If sexually mature colonies are left in fingerbowls for a few hours or overnight tadpole larvae may be found at the bottom of the dish, some attached, some free-swimming. You may be able to get some from the containers in which the colonies are. Use a pipette. The time interval between release of larvae and attachment of metamorphosed larvae ranges from 10 minutes to 12 hours. Most metamorphose within 2 hours after release.

3. Examine a living larva under the scope in a small quantity of water or on a slide. (See Zinkin, 1938, and Lohman, 1911, for sketches of larvae).

E. SUGGESTED DRAWINGS OF BOTRYLLUS

Fig. 7. A low power habit sketch of one or two mature Botryllus colonies, preferably in color. Indicate scale of actual size.
Fig. 8 Side view of a young (week old or so) zooid from the Eel Pond slide material.
Fig. 9. Top view of young zooid or very young colony from Eel Pond slide material.
Fig. 10 Segmenting eggs of Botryllus.
Fig. 11 Tadpole larva of Botryllus, if found.

DIDEMNUM

A. SUPPLIES ON HAND:
1. Colony of Didemnum per student.

B. ADULT COLONY
1. Didemnum forms a high soft but firm white or pinkish crust on rocks, wharves or similar places of attachment. Observe the colony surface thru a dissecting scope, then with a compound scope, (with surface illumination).

2. Look for the openings beneath which are the zooids. The systems are complicated, irregular, inconspicuous or sometimes some are lacking, (Herdman 1886, p. 255). Peal off a small portion of the colony and look at it from both sides.

3. Look for small calcareous stellate or burr-like spicules which densely cover the surface of the colony. They are diagnostic for the various species. Tease some out.

C. SUGGESTED SKETCHES OF DIDEMNUM:

Fig. 12. A habit sketch of encrusting Didemnum mass.
Fig. 13. A Didemnum spicule, enlarged.
Fig. 14. An area of Didemnum, showing openings, spicules, matrix, etc., as seen under the microscope.

Amaroucium

A. SUPPLIES ON HAND:
1. Amaroucium pellucidum--adult colonies, sand encrusted.
2. Amaroucium constellatum-adult colonies, orange-red, fleshy.

3. Amaroucium constellatum-tadpole stages.
4. " " -young colonies, several days old (exact age will be stated on dishes) either on watchglasses or slides for each student.

B. ADULT STAGES

1. Compare the grouping of the individuals in a colony with Botryllus. Is there any regularity in the number of a group connected with a common cloacal cavity? What is the color of the matrix, zooids, and other areas?

2. Cut a very thin vertical slice from an Amaroucium corm. It should be 2 or 3 mm. thick and 2 or 3 inches long, if possible. Study with microscope, trying to identify parts of the zooids. Estimate or measure the length of the zooids. Note that all parts of the zooid are not colored the same.

3. Take another corm or lobe of Amaroucium, squeeze from the center or base outward, to force out the polypides into a fingerbowl of sea water. Tadpoles (if colony is mature) also will be forced out into the bowl. They are small but readily visible with the naked eye because of their swift swimming. More about the larvae later.

4. Transfer any entire adults which have been squeezed out into a smaller container or on a slide and identify the following parts:
   a. Note the coloration of the three body regions (thorax, abdomen, postabdomen).
   b. The thorax contains the pharynx, nerve ganglion and part of the esophagus, anus, cloaca.
   c. The abdomen contains the rest of the esophagus, stomach, Intestine, epicardiac tube.
   d. The postabdomen contains the gonads, heart, epicardium and sometimes buds.
   e. Oral and atrial openings.
   f. The pharynx has peripharyngeal bands, stigmata, endostyle..
   g. The esophagus is short and narrow.
   h. The stomach is orange-brown, saccular, corrugated with folds breaking up into knobs (an Amaroucium characteristic)
   i. The intestine curves upward toward the pharynx.
   j. If the postabdomen is complete there will be a red tip on it. The rest of it will be light in color. The heart is U-shaped and near the tip. Its pulsation rate is slow. Record the rate. The epicardium is hollow and connected with the pharynx.
   k. Do you find any buds which have been formed by budding from the epicardium and segmentation of the postabdomen.
   l. The atrium serves as a brood pouch for embryos in all stages of development. Embryos can readily be seen in most mature or ripe ascidiozooids.

C. LARVAL STAGES

1. Observe as many embryonic stages as you can find from the squeezed out mater. Look for curled up tadpole larvae.

2. Remove any swimming larvae with a pipette to a slide and study under the compound scope. If some larvae are set aside in a bowl of sea water they may metamorphose and attach within a short time.

3. Identify the following parts of a tadpole larva:
   a. Two body divisions: body proper, and tail.
   b. Thick transparent test, with vesicular cells at some stage .
   c. The 2 siphons, oral and atrial in older larvae.
   d. The adhesive organs or papillae in younger larvae. How many are there? What is their shape?
   e. The notochord extending into the tail region.
   f. The Tail muscles.
   g. The visceral mass which includes the pharynx with a few gill slits, the endostyle, stomach, intestine and yolk mass.
   h. The cerebral vesicle with the eye spot and otolith.

4. If a tadpole larva has attached and begun its metamorphosis a change will be evident in its external appearance and position. The test swells, becomes more vesicular or covered with large vesicular cells and the animal inside begins to slowly rotate to a different position. The tail disappears.

D. SUGGESTED SKETCHES OF AMAROUCIUM

Fig. 15. A habit sketch of a lobed mass of Amaroucium pellucidum, in natural color, with scale for size.
Fig. 16. A colored habit sketch of A. constellatum.
Fig. 17. Detailed view of an Amaroucium zooid showing all body divisions and structures, in color.
Fig. 18. Intermediate, egg or cleavage stages of Amaroucium:
Fig. 19. Tadpole larva of Amaroucium, either in pre-swimming, swimming or attached stages of development.

Perophora viridis Verrill 1871

A. MATERIAL AVAILABLE:
1. Adult colonies of Perophora, the green tunicate.
2. Young, recently started colonies derived from attached tadpoles, if possible.

B. MODE OF BUDDING OR BRANCHING.
1. Handle material carefully to avoid crushing. It can be easily studies in a small watchglass with sea water. Take a small fresh-looking fragment of an adult colony, separate its parts and stolons gently with dissecting needles so that you can study the growth pattern. Are the Perophora individuals arranged in a single row along the stolon?
2. Do the stolons branch profusely or are they single linear threads?
3. Are the stolons adherent or free?
4. Do the branches seem to come off at right angles from the stolon
5. Do the buds arise at very regular intervals from the stolon or does there seem to be little regularity in this respect?
6. Is the stolon hollow? How can you tell?
7. Does it seem to be longitudinally divided into 2 compartments? How can you tell? Study carefully under the microscope.

8. Is the partition complete all the way up and down the stolon?
9. Is there free circulation of blood from individual to stolon or is the stolon cut off by transverse septa from individuals?
10. Does all the blood seem to be going in the same direction in the stolons or does it seem to be going to both directions in the same stolon? Explain.
11. Count the number of heart beats in 1 phase, then the number in the opposite phase. Take several readings. Compare notes with neighbors so that you can get a maximum-minimum range for each phase.
12. Count the time in seconds or minutes for the duration of each phase. Compare figures with those of your neighbors, for minimum-maximum range.
13. Study a stolon or two containing buds of different ages and degrees of development. How do buds change as they mature? Pay especial attention to the orientation of inner organs and also to the shape of the bud.
14. Are all the buds on a stolon of the same age or is there a definite sequence of development? Are the buds at the tip younger than near the base? Are there very young buds between older buds? Try to identify internal organs of the zooids which are like small Molgulae.
15. Is the heart beat synchronized along the entire stolon, ie. are all individuals on the same stolon in the same phase and with the same approximate number of beats per unit of time?

C. RECENTLY ATTACHED MATERIAL

1. Examine watch glasses which have been immersed in a battery jars which contained Perophora material. Examine them in a finger bowl of sea water for any grayish or greenish patches which might be young attached Perophora. Is the attached individual single or a colony yet? If transparent enough you may be able to make out its general anatomy.

D. TADPOLES

1. If any tadpoles are available compare them with those of previously studied forms. Perophora viridis is viviparous and breeds in August and September. Tadpoles with egg membranes will swim inside half an hour after being dissected out of the parent. Then, they will attach to slides is some instances. In 19 hours tail and notochord of larva will have disappeared and branchial stigmata and siphons should be evident.

E. REGENERATION

1. Deviney 1934, p. 202, cut small pieces of stolon ($\frac{1}{2}$ to 2 mm. long) placed them in bowls of sea water. Inside 24 hours some new growth had taken place and a new tunic layer was secreted at the ends. You may try the same experiment if you wish.

F. EXPERIMENTS

1. Waterman (1942) studied the effect of various concentrations of certain drugs on Perophora heart beat. Drugs which had a depressing effect on the heart beat (in the concentrations used) were atropine, strychnine and colchicine.

G. SUGGESTED SKETCHES OF PEROPHORA:

Fig. 20. Make a sketch, using lines and dots or balls to show the

Fig. 21. Show in detail one zooid and a portion of its stolon.

HEMICHORDATA

Dolichoglossus (Balanoglossus)

A. MATERIAL AVAILABLE
1. One Dolichoglossus per student.

B. STUDY DIRECTIONS:
1. D. kowalevskii ranges from Massachusetts Bay to Beaufort, N.C. in the intertidal zone and possibly slightly lower, burrowing in clean sand. It occurs in clean sand flats. Its burrows are marked with coiled "ropes" of sand cemented together or held together by material from the body of the worm. These "castings" represent the sand which has passed thru the alimentary canal of the animal.

2. Dolichoglossus measures 15 mm. or longer. How long is your specimen? Is it complete?

3. Does the animal have an unpleasant odor? This is supposed to be characteristic.

4. Note the body divisions. Can you recognize the proboscis, collar and trunk? The conical proboscis should be yellowish-white to pinkish-yellow; the collor should be darker or even a brilliant orange-red (especially in the males), with a white ring posteriorly; the trunk may range from orange-yellow to greenish-yellow.

5. The mouth is ventrally located at the base of the proboscis; it is concealed by the free anterior ends of the collar and is always open. Place some carmine in the water and watch its course and its behavior.

6. The action and function of the proboscis as regards burrowing is discussed in most texts. It might be well to review it.

7. Are there any cilia covering the body? The carmine particles should be observed with this in mind.

8. Can you find the following regions of the trunk:
a. An anterior branchial region bearing dorsally 2 rows of a variable number of transverse gill slits. These increase in number as the animal matures and ages. How many a re there in your specimen? In your neighbor's specimens?

b. A genital region, consisting of a latero-dorsal fold of body wall on each side, each fold contains reproductive organs. In the male they are ywllow, in the female gray.

c. A posterior abdominal region which is smaller in diameter than the rest of the body. Are there any prominences or structures evident on it?

9. Place a worm in a wide-mouth bottle which contains an inch or two of sand to see if it will burrow, to see what changes take place in the collar and proboscis and any other reactions.

C. SUGGESTED DRAWINGS:

Fig. 22. A habit sketch of Dolichoglossus, in color.
Fig. 23. Detail of gill slit.

D. READ CLEAN-UP INSTRUCTIONS AT THE BEGINNING OF THE PROTOCHORDATE LABORATORY INSTRUCTIONS. Also, look at any demonstrations on instructor's desk.

Field Identification

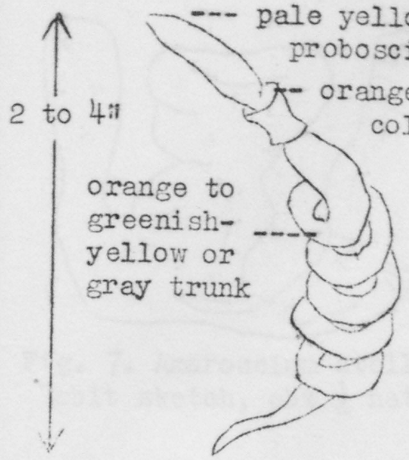


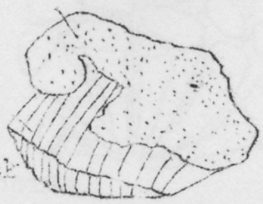
Fig. 1. Saccoglossus or Dolichoglossus



Fig. 2. Sand casting. $\frac{1}{2}$" tall, figure about $\frac{1}{2}$ nat. size.

Saccoglossus body soft, smooth, wormlike. Usually tightly coiled and not swimming. Iodine odor. Found in sand. Burrows marked by castings.

Didemnum crust



rock

Fig. 3. Didemnum

Didemnum is a colonial ascidian which forms an irregularly shaped crust (white, yellowish or peach-colored) over rocks, algae & other submerged objects. Crust is leathery about 1/8th " or less thick; peals off rocks and substrate easily in a firm sheet; minute white dots embedded in crust are spicules. Spicules are key characters for various species.

---

rock



colony

Fig. 4. Botryllus schlosseri

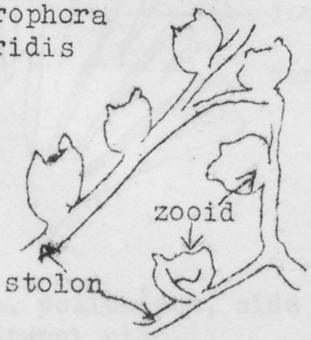
Botryllus is another colonial ascidian. It forms a soft, slimy gelatinoid film (test) ranging in thickness from 1/16" up. The film contains numerous small star-shaped clustars of zooids of variable color: purple, brown, green, yellow, white & combinations of these. Colonies grow over pilings, rocks, seaweed & other submerged objects, giving these surfaces a soft, slimy feel. Test is transparent and feels watery

---

Fig. 5. Habit sketch of Perophora viridis



Fig. 6. Detail of Perophora.



Perophora colony is green, resembling soft, tiny clusters of green grapes. Attached to piling, rocks, algae. Forms a soft, thick green tufted covering. Stolonate, with zooids arising individually along the stolons. New growth bright yellowish green; old growth dark sage green.

Fig. 5 is $\frac{1}{2}$ nat. size
Fig. 6 is about 2x nat. size

Field Identification (continued)



Fig. 7. Amaroucium stellatum
Habit sketch, abt $\frac{1}{4}$ nat. size

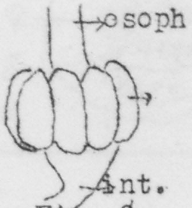


Fig. 8. Stomach folds of A. stellatum

Amaroucium stellatum is a colonial ascidian consisting of flat, tough straplike fleshy blades, resembling long thick slabs of fatty bacon. Blades may be 2' long, 6" high & 1" thick. They are smooth, VERY FIRM to touch (like rubber) & are usually whitish to flesh or pale salmon color but may sometimes be bluish or sea-green. Shape irregular, size variable. Stomach folds or glands of zooid large in size and few in number.

Fig. 9. A. constellatum
Habit sketch, abt $\frac{1}{2}$ nat. size.

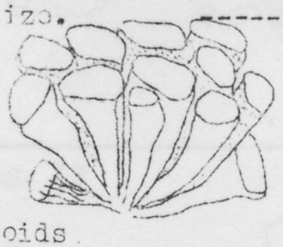
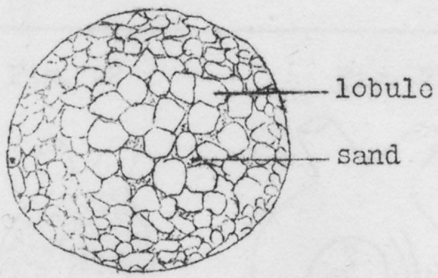


Fig. 10. Numerous stomach folds



A. constellatum forms soft, gelatinous yellowish to orange-red lobulated colonies which in branching and size resemble a head of cauliflower. Test or jelly translucent & of yellowish color, containing numerous parallel zooids. Zooids colored bright yellow and red, 14-17 mm. long, abt 1 mm. thick. Zooids pop out when colony is squeezed.

Fig. 11. A. pellucidum, top view of colony, 1/3 to $\frac{1}{2}$ nat. size.



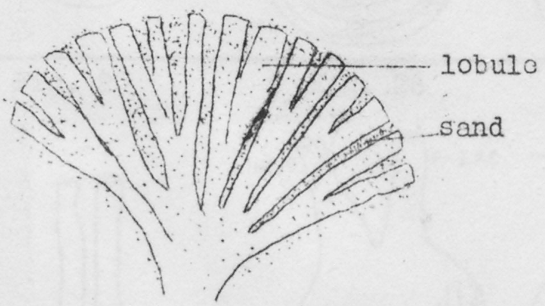


Fig. 12. A. pellucidum, side view of colony abt $\frac{1}{2}$ natural size.

Amaroucium pellucidum is hemispherical to globose, heavily sand encrusted, brittle and VERY FIRM to touch; shows numerous tall narrow lobules closely cemented together with sand. Color is that of sand encrusting the colony, usually a muddy gray in our localities. Colonies grow on shells & rocks & are generally obtained by dredging rather than by shallow water collecting. Stomach folds as in A. constellatum; larvae of the 2 spp. are different, hence one is not a variety of the other.

Field Identification (continued)

Fig. 13 Fig. 14 Fig. 15

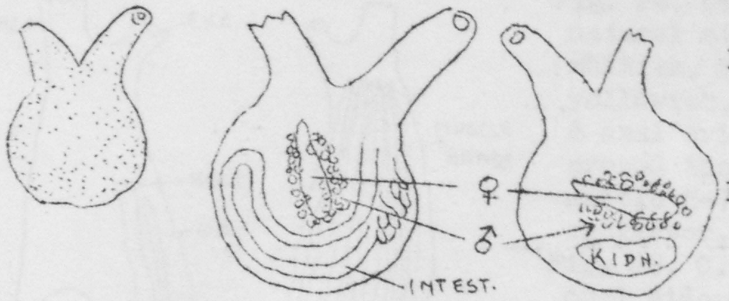


Fig. 13. Molgula manhattensis, exter. view, nat. size. Siphons arise close together.

Fig. 14. M. manhattensis, left side, showing relation of gonads to intestine. Gonads should be more vertical than drawn.

Fig. 15. M. manhattebsis, from right side, showing gonad horizontal and above kidney. Gonad position is a key character.

Fig. 16 Fig. 17 Fig. 18

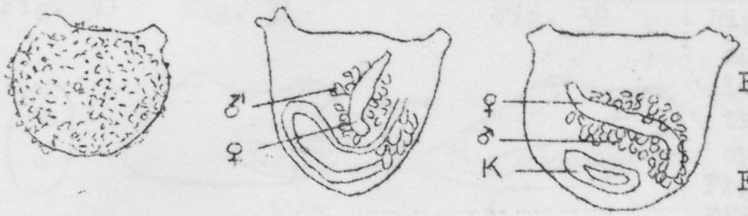


Fig. 16. M. arenata, external, nat. size, contracted. Flattened from side to side like disc, 7mm. thick; 20mm. in diameter. Apertures widely separated. Test evenly and closely covered with sand grains.

Fig. 17. M. arenata, test removed, left side showing vertical left ovary with bent lower end.

Fig. 18. Same, right side, showing angular downward bend in horizontal right ovary.

Fig. 19 Fig. 20 Fig. 21

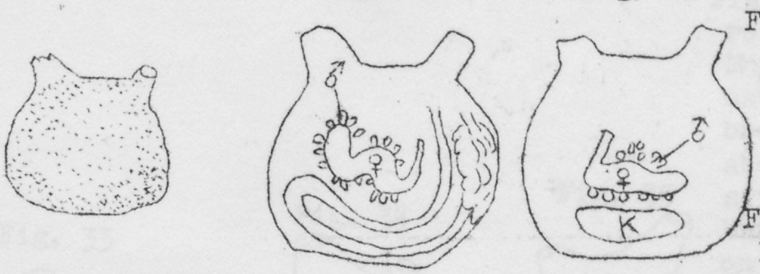


Fig. 19. Molgula citrina, externals.

Fig. 20. M. citrina from left side, showing W-curved female gonad in horizontal position.

Fig. 21. Same, right side, showing L-shaped ovary.

Fig. 22 Fig. 23 Fig. 24

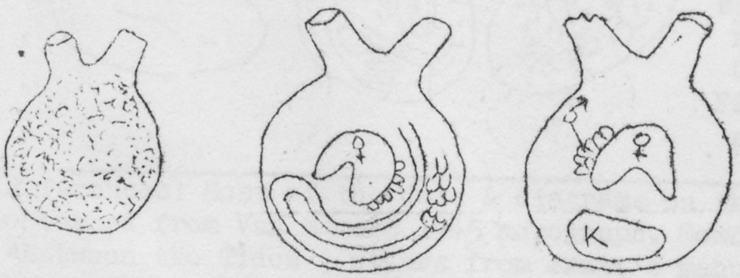


Fig. 22. Molgula complanata, external. Sand and shell.

Fig. 23. M. complanata, left side, test removed to show inverted U-shaped ovary.

Fig. 24. Same, right side, test removed, to show similar ovary.

Fig. 25 Fig. 26 Fig. 27

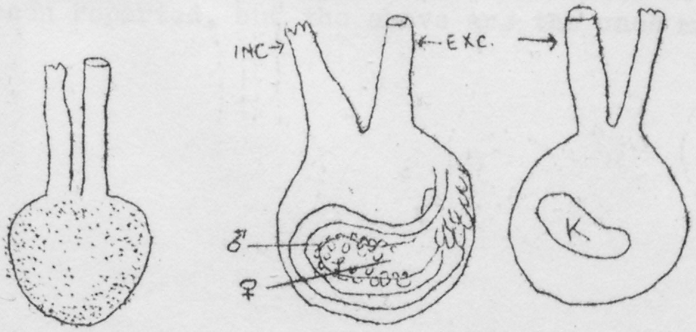


Fig. 25. Bostrichobranchus pilularis, external view.

Fig. 26. B. pilularis, left side, test removed, to show gonad in intestinal loop.

Fig. 27. Same, right side, to show complete absence of right gonads.

Field Identification (concluded)

Fig. 28 Fig. 29

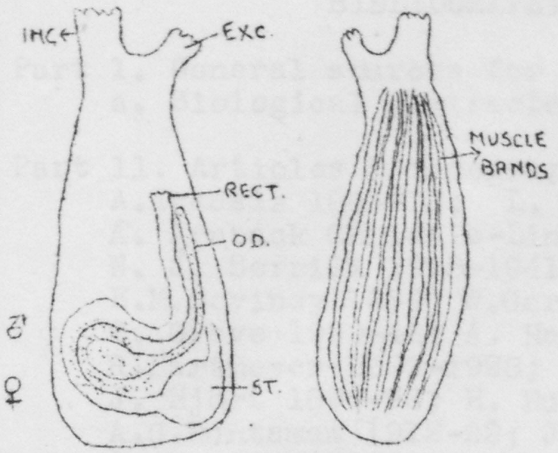


Fig. 28. Ciona intestinalis, left side, about natural size. Ovary in intestinal loop. Test whitish, more or less transparent; mantle pale yellowish, showing easily thru test. Branchial & anal orifices with 6-8 red or orange spots around the margin of each. Muscle bands of mantle 5-7 on each side show thru test, key character.

Fig. 29. C. intestinalis showing 7 muscle bands on 1 side of mantle. Body is very soft, flexible & elongated. Siphons short and near together.

Fig. 30 Fig. 31 Fig. 32

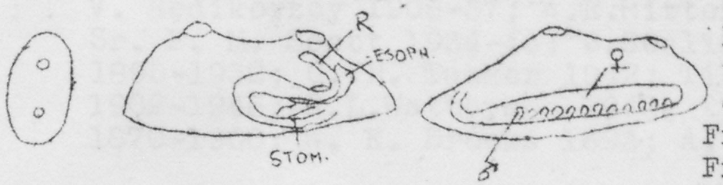


Fig. 30. Dendrodoa carnea, top view of zooid showing elliptical shape, 11mm. long, pink to bright red in color. Test thin, parchmentlike, smooth to naked eye, but finely wrinkled under magnif.

Fig. 31. D. carnea, left side, no gonads

Fig. 32. Same, right side, ovary horizontal with one end hooked.

Fig. 33 Fig. 34 Fig. 35

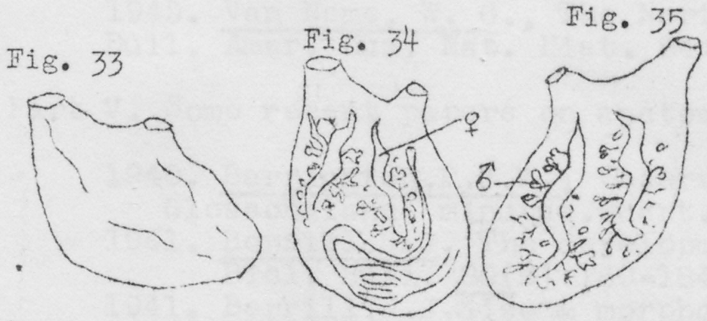


Fig. 33. Styela partita, habit sketch, rough body surface like old leather. May have algae, bryozoans, etc. growing on it. Yellowish posteriorly, growing brown, purplish or red anteriorly, esp. about the apertures. Test thicker near siphons, more or less fibrous externally but smooth internally. 2 sets of gonads on each side. Ovaries sinuously curved and elongated; testes small and irreg. branched. Gonads seen on test removal; easiest means of identification.

Fig. 34. S. partita, left side, test removed to show mantle and its content. Gonads somewhat vertical.

Fig. 35. S. partita, right side, test removed.

References: Most of the data & diagrams on the 4 pp. of Field Identification were obtained from Van Name's 1945 monograph. Some were also obtained from Crowder's "Between the Tides", others from Pratt's manual.

A number of other spp. of protochordates occur at Woods Hole, or at least have been reported, but the above are the ones most commonly found.

BIBLIOGRAPHY of Protochordates

Part 1. General sources for protochordate information:
a. Biological Abstracts b. Zoological Record

Part 11. Articles & monographs by the following workers:
A.Agassiz 1866-73; L. Agassiz 1850; J. Alder 1863, 1905-1912; A. Arnbäck Christie-Linde 1921-1938; W. Bateson 1884-1886; N. J. Berrill 1928-1941; T. Bullock 1940-45; E. G. Conklin 1905-33; E.M.Devincy 1934; W.Garstang 1891-1928; W. C. George 1930-39; C. Grave 1920-44; A. Hancock 1905-1912; H. Harant 1925-33; R.Hartmeyer 1899-1928; W.A.Herdman 1880-1923; W.A. Hilton 1914; J. Hjort 1894-96; H. Huitfeldt-Kaas 1896; G. W. Hunter 1898-1903; A.G.Huntsman 1912-22; J. Huus 1927-37; G. Lefevre 1895-1898; C. Maurice 1888; M.M.Metcalf 1892-1919; W. Michaelsen 1898-1934; T. H. Morgan 1888-1945; J.H.Orton 1913-1914; A. Oka 1906-35; V. Redikorzey 1906-37; W.E.Ritter 1893-1917; S. M. Rose 1939; Sr. F. M. Scott 1934-46; O.Seelinger 1882-1907; C. P. Sluiter 1895-1932; G. H. Tucker 1942; Ti-Chow Tung 1932-41; W.G.Van Name 1902-1945; R.L.Watterson 1945; L.N.Zinkin 1938; A.E. Verrill 1870-1900; W. K. Brooks 1893; A. J. Waterman 1942.

Part 111. Especially good compiled bibliographies:

1894. Amphioxus and the ancestry of vertebrates. Macmillan & Co. N.Y.--By A. Willey. (excellent for Amphioxus refs.)
1913. Hopkinson, J., A. bibliography of the Tunicata, 1469-1910. Ray Soc., London, 288 pp. (Call #596.02 H77)

Part 1V. Most recent taxonomic work of mongraphic size:
1945. Van Name, W. G., The North and South American Ascidians. Bull. Amer. Mus. Nat. Hist. 84:1-476.

Part V. Some recent papers on anatomy, behavior, embryol., physiol.etc.

1940. Barrington,E.J.W., Observations on feeding and digestion in Glossobalanus minutus.Quart.J.Mic.Scl.,82(2):227-260. 19 figs.
1941. Berrill,N.J. The development of the bud in Botryllus. Biol. Bull. 80(2):169-184. April.
1941. Berrill,N.J.Size & morphogenesis in the bud of Botryllus. ibid. pp. 185-193.
1940. Bullock,T.H. The functional organization of the nervous system of Enteropneusta. BIOL BULL.79:91-113. Aug.
1944. Bullock,T.H. The giant nerve fiber system in Balanoglossids. J. Comp.Neur.80 (3):355-367. June (good for n.s. diagr.)
1945. Bullock,T.H., The anatomical organization of the nervous system of Enteropneusta. Quart.J.Mic.Sci.86(1):55-112.7 pl.
1943. Devanesen,D.W. & P.I.Chacko Balanoglossids as food of fish. Proc. Indian Sci. Congress 29(3):155-
1941. Gorbman,A. The identity of an iodine-storing tissue in an Ascidian. SCIENCE 94(2434):Aug.22
1944. Grave,C. The larva of Styela(Cynthia) partita:structure, activities & duration of life. Jour. Morph 75:173-188.
1941. Hilton,W.A. The nervous system & sense organs: LXX1X. Tunicata. Jour.ENTOM&ZOOL.(Claremont, Cal.) 33(3):44-52.
1941. Hilton,W.A. The n.s. & sense orgs. XC. Amphioxus (Branchiostoma). ibid. 33 : 64-74. 3 figs.

1943. Hilton,W.A. The blood of Amphioxus (Branchiostoma)ibid 35:31-32.
1943. MacDougall, K.D. Sessile marine invertebrates of Beaufort, North Carolina, etc. ECOL.MONOGR. 13(3):321-374. 19 figs.
1942 Morgan, T.H. Cross-and self-fertilization in the Ascidian Styela. BIOL. BULL. 82(1): 161-171.
1942. Morgan,T.H.Cross-and self-fertilization in the Ascidian Molgula manhattensis. ibid. 82(1):172-177.
1942. Morgan,T.H. The genetic and the physiological problems of self-sterility in Ciona. V. The genetic problem. J. EXPT. ZOOL 90(2): 199-228.
1944. Morgan,T.H. The genetic & the physiol. probs. of self-ster. in Ciona.VI. Theoretical discussion of genetic data. ibid.95:37-59.
1944. Morgan,T.H. Some further data on self-fertilization in Ciona. JOUR. EXPT. ZOOL. 97:
1945. Morgan,T.H. The conditions that lead to normal or abnormal development of Ciona. BIOL. BULL 88:
1945. Morgan,T.H.Normal and abnormal development of the eggs of Ciona. JOURN.EXPT. OOL 100 (3): Dec.
1940. Plough, H. H. and. N. Jones. Ecteinascidia tortugensis, sp.n.with a review of the Perophoridae(Ascidiacea)of the Tortugas. PAP.TORTUGASLAB., CARN. INSTN. 32: 47-60. 5 pl.
1945. Scott, Sister F.M. The developmental history of Amaroecium constellatum. I.Early Embryonic development. BIOL.BULL.88(2): 126-138. April.
1946. Scott, Sister F.M. The developmental hist. of A. constellatum. II. Organogenesis of the larval action system. ibid, 91:
1940. Spence,J and O.W.Richards, Native cellulose in the Ascidian Phallusia nigra. PAP. TORTUGAS LAB. CARN. INSTN. 32:163-167
1942. Tucker, G. H. The histology of the gonads and development of the egg envelopes of an ascidian (Styela plicata Lesueur). JOURN. MORPHOL. 70 (1): 81-108. 5 pl.
1941. Tung, T.-C., Ku S. and Y. Tung. The development of the ascidian egg centrifuged before fertilization. BIOL.BULL.80(2):153-168.
1942. WatermanA.J. The action of drugs on the compound ascidian Perophora viridis, as indicated by the activity of the intact heart. PHYSIOL.ZOOL. (Chicago)15: 61-74. 5 figs.
1945. Watterson,R.L. Asexual reproduction in the colonial tunicate Botryllus schlosseri (Pallas)Savigny, with special reference to the developmental history of the intersiphonal bands of pigment cells. BIOL. BULL. 88(1): 71-104.
1942. Wilhelmi, R.U. The application of the precipitin technique to theories concerning the origin of the vertebrates. BIOL. BULL. 82(2): 179-189.
1941. Chin,T.G. Studies on the biology of the Amoy Amphioxus, Branchiostoma belcheri Gray. PHILIPP. JOUR.SCI. (Manila) 75 (4):369-424. 5 pl. 18 figs.

Marine Biological Laboratory: Outlines for Invertebrate Course--1949