MOLLUSCA

(GASTROPODA)

Crepidula fornicata and C. plana

The greenish-brown, boat-shaped C. fornicata adults pile one on top of another to form "chains" of individuals; the colony is attached to a stone, shell or other solid object by the bottom limpet. This species can be collected at Vineyard Haven Harbor, Mass. Another species common to the Woods Hole area, C. plana, is found within whelk or moon snail shells inhabited by large hermit crabs, and can be obtained at Cotuit. This species has a flat, whitish shell and is considerably smaller than C. fornicata.

Both species are potentially protandric hermaphrodites. The active males are small, whereas the mature females are the large, older individuals; all sizes and sexual conditions can be found in any colony.

C. fornicata breeds from mid-June until mid-August. C. plana has a longer season; it breeds through the first week in September (Bumpus, 1898). Sexual activity is reduced to a minimum at sea water temperatures below 15 to 16°C (Gould, 1950).

A. Care of Adults: These limpets may be kept indefinitely in aquaria or fingerbowls provided with a current of flowing, unfiltered sea water. C. plana will readily attach itself to glass when it is removed from the hermit crab shell. Unless they are already in the adult female phase, these animals eventually differentiate into males and females.

B. Procuring Fertilized Ova: The animals can be detached with a heavy knife. If eggs have been deposited, they are found in a transparent capsule, attached to the substrate or to the foot of the female. Mature females which have not yet deposited eggs may be isolated in glass dishes supplied with running sea water. After a few hours (preferably in the early morning), pour off the water and examine the ventral surfaces of the females through the glass. In this way females can be found in the process of oviposition, and the first stages of development can be obtained. Transfer the capsule to a Syracuse dish of sea water and tease it open with needles to release the eggs (Conklin, 1937).

C. Preparation of Cultures: Eggs removed from the mantle cavity of the female do not develop normally for more than one or two days. However, a complete series of embryos can be obtained by selecting a number of capsules. The young stages appear bright yellow through the capsule, while older embryos are brown.
D. *Methods of Observation*: Details of the living eggs and larvae are more distinct against a dark background. Swimming larvae can be mounted on vaselined slides or on slides with supported coverslips. They are usually quite active, but can be tangled in a few shreds of lens paper or lightly anaesthetized with a dilute solution of chloral hydrate. Due to the opacity of the living eggs, the details of maturation, fusion of the germ nuclei, and cleavage are best studied from prepared whole-mounts.

E. *Preparation of Slides:*

*Sections*: In preparing sections it is advisable to fix, embed, and section eggs while they are still in the capsules.

*Whole mounts: Fixation*. Obtain decapsulated eggs as outlined above. After freeing the eggs, agitate them by gentle rotary rinsing with a pipette, in order to wash them and concentrate them in the center of the dish. Change the water two or three times. Concentrate the eggs and, using a pipette, transfer them to a vial three-quarters filled with Kleinenberg's picro-sulfuric fixative. Fix the eggs for 15 minutes.

Remove the fixative using a pipette of small diameter, and then fill the vial with 70% alcohol. Wash in 70% alcohol until the eggs are white; it is advisable to avoid a prolonged washing, since the stain employed is best when it does not penetrate the macromeres. The latter should therefore be left slightly acid. Thus, the eggs are removed from 70% alcohol immediately after the last wash which removes no picric acid from them, hydrated in 50% and 35% alcohols and washed thoroughly in two or three changes of water.

*Staining*. After washing with water, fill the vial with undiluted Mayer's haemalum, and stain for 5 to 10 minutes. For the polar body stages, a staining time of 5 to 7 minutes is usually sufficient. After staining, wash thoroughly in water, dehydrate, and clear in xylol. Remove the xylol used in clearing and replace it with a small amount of thin damar.

*Mounting*. Coverslips must be supported. For this purpose it is convenient to use paper squares the size of 7/8-inch coverslips. A hole is punched in the center of each square with a paper punch. In mounting, the squares are cleared in xylol, and fixed to the center of the slides by adding three or four drops of thin damar before the xylol evaporates. When the paper mounts have dried, the eggs are removed from the vial, in which they are stored, by the use of a pipette drawn out to a long taper and having a small diameter at its tip. The eggs are allowed to settle toward the tip of the pipette, and one drop of the egg-damar suspension is placed in the center depression of each paper mount. The damar is allowed to dry to the point of formation of a thin film, in order that the eggs may remain dispersed and with the macromere quartet adjacent to the slide when mounted. Apply thick damar to the edge of the paper mount, immerse a #0 coverslip in xylol and apply it to the slide over the paper mount.

*An alternative method:*

1. Fix for 30 to 120 minutes in Mayer's picro-sulfuric fixative.

2. Wash in 35%, 50%, and 70% alcohols; leave in the latter until the yellow color ceases to come out.
3. 50% and 35% alcohols, to water; 5 minutes in each.

4. Stain in Conklin's haematoxylin (one part Delafield's haematoxylin in four or five volumes of distilled water, to which is added one drop of the picro-sulfuric fixative for each ten cc. of the diluted stain): 5 to 10 minutes.

5. Wash in water, dehydrate 5 minutes in each alcohol; 10 minutes in 95% alcohol; two changes of absolute alcohol; xylol.

6. Mount in thick balsam or damar with supported coverslips.

NORMAL DEVELOPMENT

A. The Ovum: The unsegmented ovum of both species is nearly spherical. It contains a small quantity of yolk granules which are more concentrated, and larger, at the vegetal pole. There is no egg membrane. The ovum of C. fornicata measures approximately 182 microns in diameter, that of C. plana about 136 microns. The eggs are deposited in transparent capsules (about 240 eggs per capsule for C. fornicata, and between 64 and 176 per capsule for C. plana), before maturation begins.

B. Cleavage: Two polar bodies are extruded and remain attached for some time. The pronuclei associate, but do not fuse, and the separate maternal and paternal portions of the zygote nucleus remain distinct at least until the 69-cell stage. Crepidula thus illustrates clearly the condition known as gonomery.

Cleavage is spiral and regular, and similar in all four quadrants as far as the 24-cell stage. Gastrulation is by epiboly, which is not accompanied by invagination. The mouth appears near the mid-ventral surface soon after the blastopore closes at this point (Conklin, 1897). Further details and illustrations of these early stages can be found in two papers by Conklin (1897, 1902).

C. Rate of Development: Development proceeds slowly. Not less than four hours elapse between fertilization and first cleavage. In both species, hatching of the fully-formed veliger takes about four weeks. The free-swimming period of C. fornicata probably lasts about two to three weeks (Conklin, 1897).

D. Later Stages of Development and Metamorphosis: There is no typical trochophore stage; the gastrula transforms directly into a veliger larva. The veliger has a bilobed, ciliated velum which develops purple pigment along its margin. The mouth and foot, containing a pair of statocysts, are on the ventral side. The head vesicle, the pair of pigmented eyes with lenses, the heart and oesophagus are dorsal. The digestive tract is well developed; stomach, liver and intestine can be seen. The anus lies on the right side, and the external kidneys are lateral to the foot. At metamorphosis, the head vesicle decreases rapidly in size, the velum is largely, if not entirely, absorbed, the foot becomes enlarged, and the shell, which during the veliger stage was of the spiral type, takes on the form characteristic of the adult. Consult the paper by Conklin (1897) for further details and illustrations of the larval stages of these species. A good description of organogenesis in C. adunca can be found in a paper by Moritz (1939).

BUMPUS, H. C., 1898. The breeding of animals at Woods Holl during the months of June, July and


