On a Method of Rearing Larvae of Polyzoa.

By

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COLONIES of *Bowerbankia pustulosa*, collected in July and August, 1911, in and near Plymouth Sound, contained a great many ovicells and produced numerous larvae, when kept in a flat glass trough and put under circulation during the night. The small yellowish larvae have a decidedly positive phototropism. They gather on the surface of the water on that side which is turned towards the light, from which position they sink to the bottom of the glass. Their movements slacken gradually in the course of a few hours, and at last are limited to revolving in a small circle until they fasten on the sides of the glass. Here they complete their metamorphosis, and in a few days the primary polypides are expanding their ciliated tentacles. The result was better when the just-hatched larvae were brought into a jar sterilised by hot water and filled with so-called outside water, i.e. water from beyond the Breakwater. This jar was fitted with a glass stand carrying a number of cover-glasses. When the larvae settled on these, it was easy to make total preparations of them without detaching them.

But this method is not sufficient when sections of a just-settled larva or a young primary zooecium are to be made without injuring it by its separation from the substratum. In this case the larvae must be induced to fasten to a material which can easily be cut with the object. For this purpose egg-shells proved useful. They were washed with water, then kept in alcohol of 70 per cent. for about a day, and the coagulated albumen was mechanically removed. The alcohol was afterwards extracted by sea-water. Prepared in this way, the egg-shells were filled with outside water, charged with a number of swimming larvae and kept cool by running water underneath an inverted bell-glass. As long as the larvae were still active, the water was renewed with a pipette twice a day. When the metamorphosis was accomplished and the young polypide began to come out of its cell, it was necessary to add some well-adapted food. I made use of two cultures of algae in sterilised water, kept in stock in the laboratory and kindly placed at my disposal: *Pleurococcus mucosus* and *Nitzschia*

closterium forma minuta. In other cases the egg-shells were put into a larger jar fed with water from the tanks by means of a siphon.

When the animals are in the stage required, they can be fixed in a very simple manner. To the younger stages the fixing fluid was added directly after having poured out the water. If the individuals were further developed and were to be fixed with expanded polypides, they were paralysed by some crystals of menthol floating upon the surface of the water for some six hours and killed by a pipetteful of the fixative, squirted out directly upon them in order to prevent them from collapsing when the water was removed. As most of the fixatives contain some percentages of acid more or less, the innermost layer of the calcareous shell is dissolved and so the inner skin is spontaneously detached. When a mixture of a hundred parts of a saturated solution of corrosive sublimate and five parts of glacial acetic acid was used, the inner skin was lifted up by bubbles of carbonic acid in a few minutes, so that it is easy to detach the membrane after having washed the objects in the shells, and cut the latter into small pieces. The pieces of membrane with the attached polypides are then treated in the usual way. The membrane becomes very transparent in xylol or cedarwood oil, and the object cannot be lost in the paraffin. The egg-skin serves as a means of orienting the object too, and can be sectionised so excellently that it is not in the slightest degree an impediment.

I employed this method for the larvae of several species of Polyzoa and a Tunicate of the family of Didemnidae. It is especially useful, of course, when the larvae are of a dark colour, as, for instance, those of *Bugula neritina* Oken (= *Cellularia neritina* Pallas), which contrast with the white ground most excellently. These larvae settle in numbers just below the edge, or even on the free surface of the water taking advantage of the surface-tension.