A BRIEF NOTE ON EXTERNAL OBSERVATIONS OF THE DEVELOPMENT OF HALOCYNTHIA AURANTIUM (PALLAS)

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A BRIEF NOTE ON EXTERNAL OBSERVATIONS OF THE DEVELOPMENT OF *HALOCYNTHIA AURANTIUM* (PALLAS)[1)]

By

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There are many studies published on the development and the metamorphosis of ascidians, which were known to produce various types of eggs and larvae, and show different types of metamorphosis. Among the many works, Hirai (1941–1968) has reported on the development and metamorphosis of *Halocynthia roretzi*. However, comparative studies are few, and thus the present work was undertaken to compare the development and metamorphosis of *Halocynthia aurantium* with that of *H. roretzi*.

The author is indebted to Prof. Eturo Hirai, the Director of the Marine Biological Station of Asamushi, Tôhoku University, for his suggestions during this work. Thanks are also due to Masato Taga of the Otaru Aquarium, Otaru, Hokkaido, for his help in collecting the material.

MATERIAL AND METHOD

The animals used for the study were collected from Otaru Bay, Hokkaido, in the early March of 1967, transferred to a box packed with ice and then brought to the laboratory at Asamushi. They were kept in glass tanks supplied with running sea water. The gametes were obtained by natural spawning. Each animal was kept in a separate glass tank during spawning. Two hours after spawning the sperms from one tank were added to another tank to ensure cross fertilization. After insemination the fertilized eggs were kept at 8°C in large Petri dishes.

OBSERVATIONS

Spawning. As in *H. roretzi*, the eggs and sperms of *H. aurantium* were released between 10.00 a.m. and noon. Several days' spawning was usually followed by

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three days or more without release of the gametes and then spawning continued again. Spawning began in early March and continued until mid-April.

The egg and early development. The egg is transparent and slightly reddish yellow in color. The egg cells is about 250μ in diameter and enveloped with columnar follicle cells outside the chorion. Before insemination the peri-vitelline space was narrow and the follicle membrane had a diameter of approximately 350μ. After insemination the follicle membrane expanded gradually and reached the maximum diameter after about 20 minutes. The first polar body was extruded about 30 minutes after insemination, following it the second polar body appeared. About two and a half hours after insemination the first cleavage was observed. Approximately one cleavage occurred in an hour in the stage of early development. Consequently about 48 hours after insemination the larvae hatched out of the chorion. The development with time is shown in Table I and Plate VI and VII.

Table 1
Development after insemination (sea water temperature 8°C)

Stage	Time after insemination (hr)	Stage	Time after insemination (hr)
1st polar body	30′	Morula	8
2nd polar body	1.30′	Blastula	12
2-cell stage	2.30′	Gastrula	16
4-cell stage	3.30′	Neurula	24
8-cell stage	4.30′	Tail bud	30
16-cell stage	3.30′	Tail elongation	40
32-cell stage	6.30′	Hatching	48

Larva. The newly-hatched larva was 1.6 mm long and had a rounded body and straight tail. At the anterior tip of the body were three stout adhesive papillae. The larval sense orangs consisting of an otolith enclosed in a brain vesicle, and an ocellus posterior to it, lay dorsally and slightly displaced to the right. At this stage the notochord consisted of rectangular cells and a vertical fin extended along the tail. The body and the adhesive papillae gradually became more slender and the cells of the notochord obscure.

Tail resorption. To initiate tail resorption the larvae were immersed for three minutes in a solution of one drop of 0.1 per cent nile blue solution in 20 ml. of sea water (Hirai), and then transferred to clean sea water. When treated with this solution soon after hatching no tail resorption occurred. Treatment of the larvae six hours after hatching caused the adhesive papillae to withdraw, but tail resorption was not observed. In fully developed larvae, 12 hours or more after hatching, tail resorption was completed within 20 minutes after treatment (Plate

VII Figs. 5–14). In these cases the larvae stopped swimming immediately after immersion in the sea water containing nile blue. The adhesive papillae disappeared and the rupture of the notochord in the trunk was observed about three minutes after immersion. Soon after the rupture of the notochord, the cells anterior to the brain vesicle migrated into the tunic around the trunk. When the tail resorption had proceeded considerably, the muscle cells near the trunk contracted into the posterior of the trunk, but the distal parts of the tail remained unchanged. This constriction of the muscles proceeded posteriorly along the tail with time. Finally the tail end was pulled into the body. After tail resorption the larvae became short along its original antero-posterior axis.

Metamorphosis after tail resorption. A day after tail resorption the larvae became more shortened and many cells were observed in the tunic. Several ampullae had formed at the anterior end of the body, and shortening continued till three days after tail resorption (Plate VIII, Fig. 1–3). About three or four days after tail resorption the tunic became thorny. The otolith and ocellus were observed as a black spot, or sometimes separated from each other until completion of the small ascidians. From two to four days after tail resorption the relative position of the black spot in the body moved gradually from the lower side to the upper side. Namely a day after tail resorption the black spot was near the ampullae but it moved upward from the second to fourth day under the ectoderm, and took its final position at about the fifth day. After five to six days transparent lumps appeared beneath the ectoderm, the central part of the body was dark, and the thorns of the tunic grew conspicuously. On the seventh day contraction of the body was observed, and the branchial and atrial apertures were recognized faintly. They opened completely on the eighth day. At this stage gill slits were observed, and they became apparent and the endostyle was observed from the nineth day. From ten days after the body became transparent, a beating heart was observed at the lowest side of the body. About 12 days after tail resorption the small ascidians were completed.

CONSIDERATION

In 1941 Hirai described the early development of *Halocynthia roretzi*, and recently several studies on the tail resorption were reported (Hirai ,1963 and 1964, Numakunai *et al.*, 1964). When *H. aurantium* is compared with *H. roretzi*, the spawning time, the early development, tail resorption and the metamorphosis after tail resorption resemble each other, but the characteristics of the adults are different from each other especially in their tunic. The egg and larva of the present species were smaller and more transparent than *H. roretzi*. On the early development the present species developed more slowly than the latter, but whether this is an intrinsic difference or caused by a difference of temperature during the deve-

lopment dependent on the difference of the spawning season is not known. The possibility of tail resorption after hatching showed the same tendency in both species, in other words, in the present species the potency to metamorphose differentiated within 12 hours after hatching like that of *H. roretzi* (Hirai, 1963). That is to say, the larvae soon after hatching showed no change when treated to metamorphose, and six hours after hatching disappearance of the adhesive papillae without immediate tail shortening was observed. The tails of these larvae shortened very slowly, several hours or more were needed for complete tail resorption, though the tail resorption was completed within 20 minutes in the full developed larvae. During the tail resorption of the larvae 12 hours or more after hatching cell migration into the tunic was observed. These cells were thought to be of the same type as the cells in *H. roretzi*, which were stained densely with Gomori's AF-staining method (Numakunai *et al.*, 1964). In *H. roretzi* the most active part concerned with tail resorption was considered to be the posterior region of the trunk (Numakunai, 1967). In the present observation the muscles in the anterior part of the tail were arranged in fan shape, and this moved gradually to

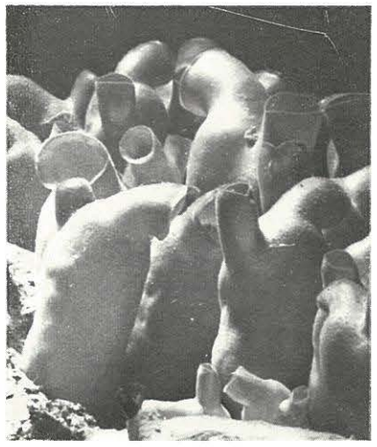

Fig. 1. Adults of *H. aurantium* with smooth tunic, ca. ×1/3

the tail end. This may suggest that the posterior part of the trunk is the most active area in relation to the tail shortening as in *H. roretzi*. After tail resorption the tunic became thorny like the tunic of young specimens of *H. roretzi*, though the adult animals of the present species had a smooth tunic (Fig. 1). In both species the young animal about two weeks after tail resorption showed the same characteristics, and it was very difficult to distinguish from each other. When the difference between them became recognisable was not determined in the present observation.

SUMMARY

The normal development of *Halocynthia aurantium* was observed.

1. The process of the development is shown in the plates.
2. The egg was about 250μ in diameter, the follicle membrane 350μ. During the 20 minutes after insemination the peri-vitelline space increased in size.
3. The larvae hatched out 48 hours after insemination, and were 1.6 mm long. They have three adhesive papillae at the tip of the trunk, an otolith in the brain vesicle and an ocellus posterior to it.
4. The larvae soon after hatching had thick adhesive papillae, a relatively round body and a notochord consisting of rectangular cells and showed no tail resorption after treatment with nile blue. With time after hatching the adhesive papillae became thin, the trunk slender and the notochord obscure. The larvae 12 hours or more after hatching showed complete tail resorption within 20 minutes after treatment.
5. After tail resorption four or five ampullae grew at the original anterior part of the trunk, and the body was flattened. The otolith and ocellus moved upward and took their final position about five days after tail resorption. The tunic became thorny and the siphon apertures opened eight days after and the gill was apparent ten days later. About 12 days after tail resorption the young ascidian was completed.
6. Very similar patterns of development were observed in *Halocynthia roretzi* and *H. aurantium*.

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EXPLANATION OF PLATE VI.

Figures were magnified about ×80.

Fig. 1. Egg before insemination.
Fig. 2. Extrusion of 1st polar body (30 minutes after insemination).
Fig. 3. Extrusion of 2nd polar body (one and a half hour after insemination).
Fig. 4. 2-cell stage with two equal blastomers (two and a half hours after insemination).
Fig. 5. 4-cell stage viewed from the animal pole, with equal blastomoeres (three and a half hours after insemination).
Fig. 6. 8-cell stage, the blastomeres of the animal half were smaller than that of the vegetal half (four and a half hours after insemination).
Fig. 7. 16-cell stage (five and a half hours after insemination).
Fig. 8. 32-cell stage (six and a half hours after insemination).
Fig. 9. Blasula (12 hours after insemination).
Fig. 10. Gastrula (16 hours after inseminarion).
Fig. 11. Neurula (24 hours after insemination).
Fig. 12. Tail bud (30 hours after insemination).

EXPLANATION OF PLATE VII

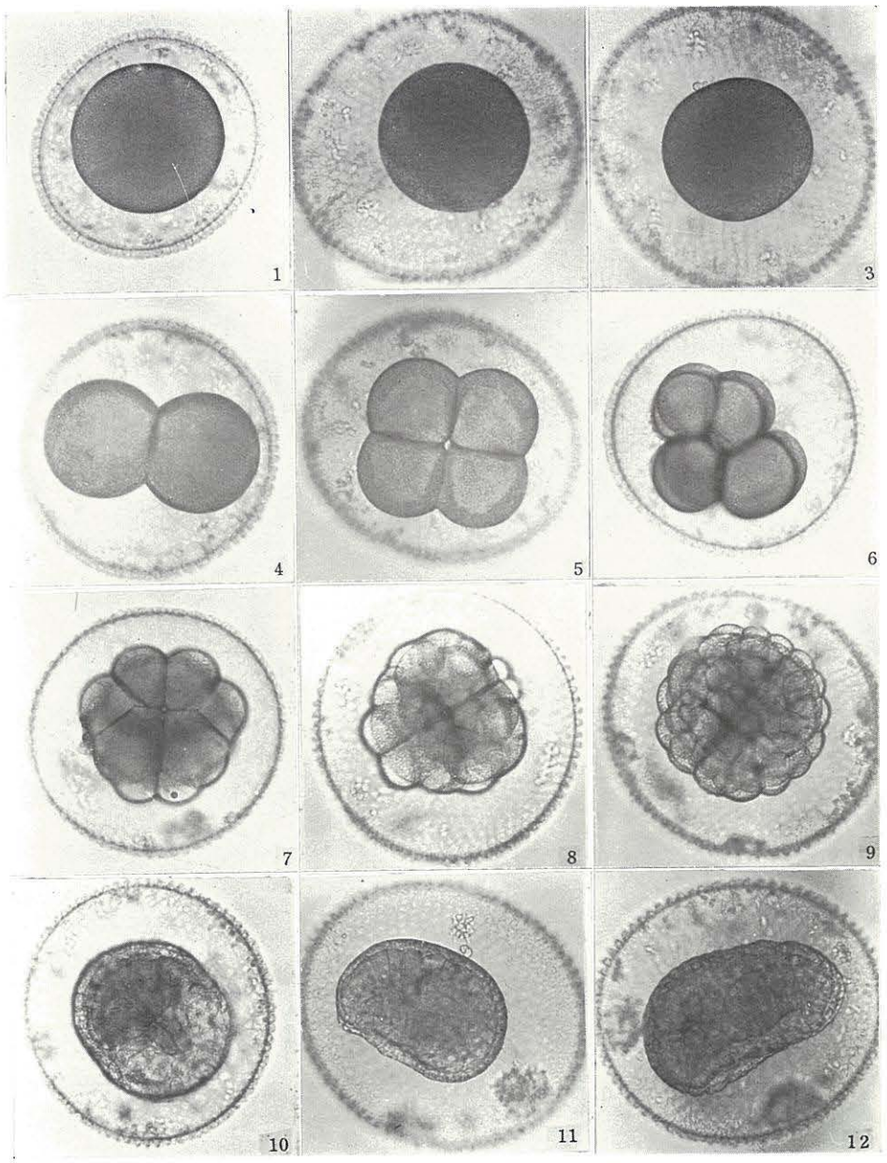
Figs. 1–4 were magnified about ×80, Figs. 5–14 about ×40.

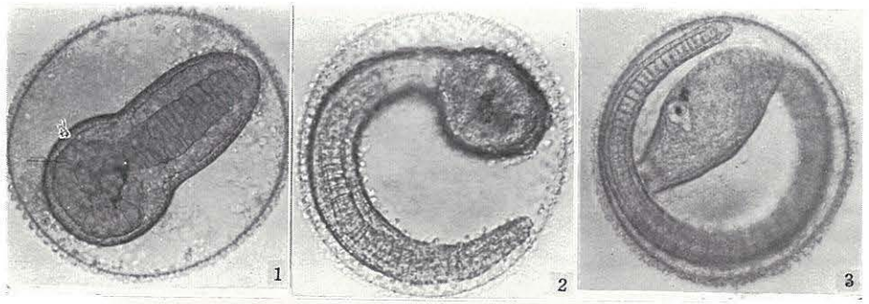
Fig. 1. Larva with short tail (35 hours after insemination).
Fig. 2. Larva during tail elongation (40 hours after insemination).
Fig. 3. Larva before hatching (46 hours after hatching).
Fig. 4. Hatching larva (48 hours after insemination).
Fig. 5–14. Larva during tail resorption. These photographs were taken at intervals of two minutes. Fig. 5 shows a larva immediately after immersion in the sea water containing nile blue.

EXPLANATION OF PLATE VIII

These figures were mangified about ×80.

Fig. 1. A day after tail resorption.
Fig. 2. Two days after tail resorption, flattening of the body.
Fig. 3. Three days after tail resorption, sensory organ became visible at the upper area.
Fig. 4. Four days after tail resorption, appearance of spine-like structure in tunic.
Fig. 5. Five days after tail resorption.
Fig. 6. Seven days after tail resorption,
Fig. 7. Eight days after tail resorption, opening of apertures, gill slits became visible.
Fig. 8. Eleven days after tail resorption.
Fig. 9. Twelve days after tail resorption, completion of a young adults.

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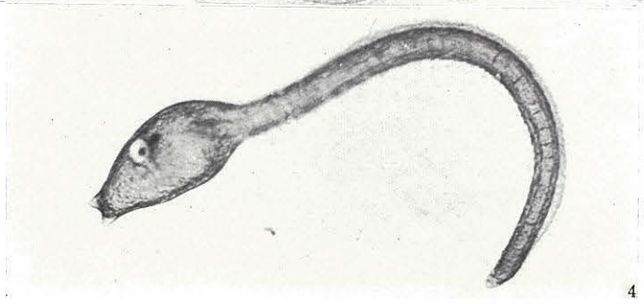
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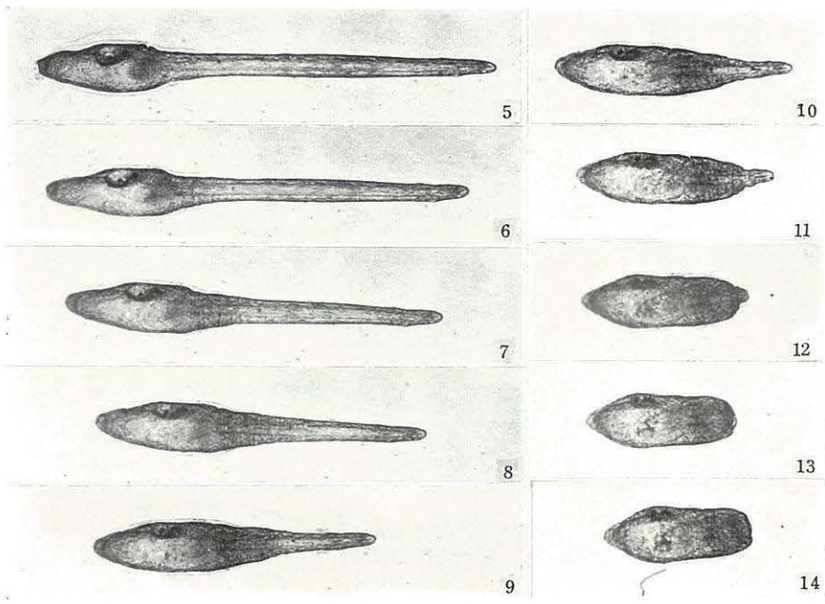
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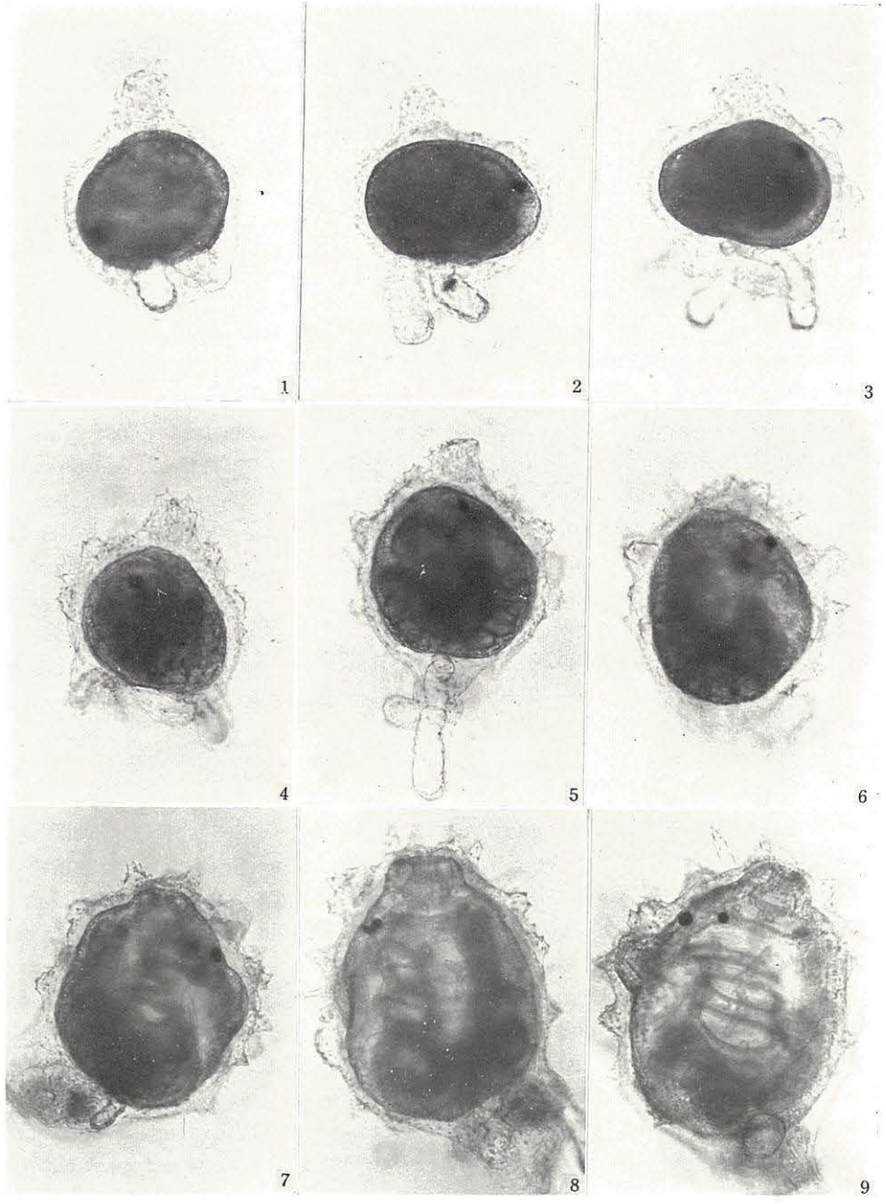
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