ON THE REPRODUCTION OF OLIVELLA

by Eveline and Ernesto Marcus
(with 1 plate)

Thanks to the Oceanographic Institute of the University of São Paulo (Professor Wladimir Besnard) we could observe the reproduction of **Olivella** whose female organs are the most peculiar of our formerly studied olivids. The "Northern Base" (Dr. Edmundo Nonato) of the mentioned Institute put its installations at our disposal in the most generous way.

In the fine sand of the Enseada near Ubatuba (23°27' S. 45°6' W) **O. verreauxii** (Ducros 1857) lives in the zone of the neap tides. On october 31st, 1958 there were about 30 snails per sq. m. on the surface of the sand or a little under it at low tide-time. As the population is dense and the animals crawl rapidly, about 10 cm. a minute at 24° C., the mates meet easily. We maintained about 300 snails in an enamel tray of 60 × 40 cm. with 1-2 cm. of sand and equal height of water without aerification. They were fed with living young **Donax hanleyanus** and crushed larger ones and observed for 9 days nearly without losses. One or other snail which crawled out of the tray during the night, and was found on the dry in the morning, recovered after a short time in water. The accumulation of the animals evidently favoured copulation, because joining began in the afternoon, about 1 hour after the installation of the snails.

The position of the mates of **Olivella** during the act of coupling (Fig. 2) is not recorded from other prosobranchs in the compendia of Simroth (1904), Meisenheimer (1921), Ankel (1936) and Fischer (1950); in some tectibranchs the slug that functions as male is situated behind the female.

The male stretches its propodium forward like a snout (Fig. 1, o) and grasps the uppermost whorl of the female's shell in its median propodial furrow (Fig. 2). We remember that

only in males the ventral propodial furrow is distinct. The anterior pedal mucous gland does not seem to take part in the attachment, to judge from the fact that the mates separate easily after copulation, and threads or traces of mucus with adhering sediments were not left on the shell of the female. A reaction of the female when the tip of the shell was seized by the male was not observed; both snails continue to crawl at the same rate of motion. Evidently the male recognizes the female by chemoreceptors, perhaps the propodial sensorial cells, before it puts forth its propodium; this movement was never undertaken tentatively but always followed by seizing the shell of the snail crawling in front. As in the previously counted samples the females outnumbered the males greatly, so that there was no competition among the latter. While the couple crawls, the penis (Fig. 2, p) is protruded, extended forward on the right side, and enters the mantle cavity of the female. Sometimes a male was seen attached to a feeding female. Rarely the male curved its penis to the left and did not succeed to enter the mantle cavity; usually an initial curving to the left was corrected quickly.

When the male begins to ejaculate, the female stops moving. Both may be on the surface or half burried in the sand, or only the female is partially concealed and the male remains visible. The male jerks vividly so that the ejaculations can be counted; we observed up to 16 effected with intervals of a few seconds. The white pellets of sperm are seen passing through the transparent penis. Sometimes the ejaculating male twitches so violently that the female is turned around without loss of the penial and propodial contact. After the first 3 hours of observation, during which copulation was frequent, the males seemed to be rather tired, to judge from the long duration, up to 10 minutes, of the pre-copulatory attachment.

Females preserved immediately after copulation, dissected or sectioned, showed pellets of sperm with prostatic secretion between the leaflets of the ctenidium. While studying the female organs we had already found sperm in the gill of snails preserved immediately after capture. Therefore we conclude

that the penis does not enter the opening of the capsule gland nor that of the internal vesicle (Marcus 1959, f. 44, iv), but only the mantle cavity. **O. verreauxii** does not have any sperm-receiving organ in the outer part of its female system. The receptaculum seminis (ibid, rn) lies far inward between albumen and capsule glands. It contains sperm in the sectioned females that were preserved immediately after copulation. Problably these sperms do not come from the last but from previous copulations. This is indicated by the stage of the internal vesicle which is in the phase of oviposition to be described in the following. Though spermatozoa were not seen in the sperm channel of the capsule gland in females preserved immediately after copulation or during and after deposition of egg capsules, they probably make their way actively from the gill to the receptaculum.

Paired mates separated as softly as they join; the male withdraws the penis and loosens his propodium from the female's shell. The male moves away immediately, while the female frequently rests for a short time, perhaps due to a momentary difficulty of respiration. When the eggs are excluded from the opening of the mantle cavity they are already fertilized, and each (Fig. 3, ei) is encased in a capsule (k). The capsule passes through the ciliated furrow on the right side that only females have into the ventral pedal gland as in **Nassa mutabilis** (Ankel 1929, f. 1). There the capsule is moulded for about 3 minutes. With help of a mirror one can see how the spherical capsule is kneaded rapidly in the cavity of the gland (Fig. 3).

The internal vesicle contributes to the formation of the capsule with its contents and with secretion. The faecal particles stored in the vesicle which were described in our first study of olivids appear in a fine layer on the outer surface of the deposited capsule (Fig. 5). These particles are spread so sparsely that they cannot afford any mechanical protection to the capsule nor conceal the embryo which is white and completely visible through the transparent capsule. Hence the internal vesicle of **O. verreauxii** cannot be called a "reinforcement sac" as the similar appendage of the neritids, nor

do its contents constitute an "armature" (Andrews 1937, p. 531). Capsules brought from the beach on a shell were quite clean without sand-grains sticking to them, and to those obtained in the laboratory only occasionally a few grains adhered. In egg-laying females the cells of the internal vesicle emit a secretion of pink staining spherules. This secretion forms a layer between the epithelium and the faecal particles. The quantity of the latter diminishes measurably during oviposition. In a female which had laid 10 eggs the internal vesicle had one quarter of the volume of others preserved after copulation before depositing eggs.

In our opinion the faecal particles and the secretion of the internal vesicle supply the egg capsule with a mark. We think that the secretion fastens the particles to the capsule when this leaves the pallial oviduct or capsule gland. The face that receives the particles will be the upper part or lid of the deposited capsule. The latter does not have its definitive form when it passes from the opening of the mantle cavity through the furrow on the right side to the moulding gland. But it has a finely granular and a smooth sticky face. Thus differentiated it is attached to the substratum with the sticky face and pressed into the moulding gland with the face containing the particles.

The folds of the ventral pedal gland shape the ridges of the capsule which is soft when it enters the gland. As Ankel (1929, p. 224) exposed, the gland functions as a mould and its secretion probably hardens the capsule (p. 230). Each capsule is attached separately and with irregular distances from the others (Fig. 4) to living or empty **Donax**, to the shells of other Bivalvia (Veneridae), to **Bulla striata** or to the glass dish. One female isolated after copulation produced 6 egg-capsules in 3 hours, another 13 in 6 hours.

The egg capsule (Fig. 5), a vitreous hemisphere of conchiolin, consists of a right and a left half, firmly coalesced in a median suture as in all capsules of prosobranchs (Ankel 1936, p. 169). In many capsules the median suture corresponds to the greater diameter. It extends like a meridian over the capsule and corresponds to the sperm channel which separates the

two halves of the capsule gland. Functionally more important is the future opening of the capsule, a circular, latitudinal layer which divides the capsule into an upper opercular and a lower part (Fig. 6). The first is 0,18, the second 0,12 mm. high. The capsule is broadest at the bottom, viz. about 0,8 mm. The wall of the bottom, Ankel's foot plate (1937, p. 77), is 5 micra thick, that of the upper part 20-40 micra, and the lumen 0,5-0,6 mm. wide. The latter contains one egg or embryo without "nurse eggs" but floating in albuminous liquid evidently furnished by the albumen gland.

The wall of the capsule (Fig. 6) is composed of the same layers as in **Nucella lapillus** (Ankel 1937, p. 79), an innermost fibrous one, a homogeneous layer, one with longitudinal and one with radial fibres. The innermost and the homogeneous layers are contiguous, while they are separated from one another in **Nucella**. As both stain with light-green and the homogeneous layer is thick, we suppose that they derive from the acidophilous secretion of the greater, inner region of the capsule gland. Certainly they correspond to Ankel's inner pellicle and inner layer. These layers can be peeled off from the outer ones. These are the middel and the outer layer of Ankel's terminology. The longitudinal fibres of the first, which is firm and thin, and the radial fibres of the second are recognizable in clarified empty capsules. Middle and outer layer are basophilous. Therefore their origin might be traced from the basophilous secretion of the glands in the smaller, outer region of the capsule gland. Also the cement which attaches the capsule to the substratum may be a product of this region. The faecal particles from the internal vesicle are embedded in the outer layer of the operculum.

The opercular border is probably produced by the blue staining glands which are disposed in two antero-posterior stripes opposite to one another in the middel of the inner region of the capsule gland. The limiting layer between lid and lower part of the capsule is gradually dissolved and the entire lid falls off (Fig. 4) when the veliger hatches. The surface of the operculum is sculptured with about 5 micra high raised

lines which are 20-30 micra distant from one another (Fig. 4-6). They run more or less parallely to the median suture and their course is wrinkled by the radial fibres of the outer layer disposed transversely to the ridges. The latter are united at their ends generally forming a right angle with the suture. But if the capsule had lain obliquely in the moulding pedal gland, the direction of the ridges is not correlated with that of the median suture. This suture continues over the lower part of the capsule (Fig. 6). Here the ridges are circular, parallel to those of the lid, but straight not wrinkled, though radial fibres are present also here in the outer layer of the wall. The bottom of the capsule is smooth and consists of inner fibrous layer, homogeneous layer, and cement.

We were not interested in the cleavage and the early development in the present study. The eggs are rich in yolk as those of other Stenoglossa. Hence the micromeres occupy a limited and concentrated area over the macromeres (cf. Pelseneer 1906, p. 24). The two and four blastomeres resulting from the first and second cleavage respectively are of equal size. Thus segmentation corresponds to the **Crepidula**-type; a yolk sac (Ankel 1936, p. 182) or polar lobe (Korschelt 1936, p. 869) does not occur.

In order to observe the embryos and obtain the larvae the egg capsules were isolated in small petri dishes. The temperature will not have deviated much from the natural condition, where it is colder at night and warmer when low tides coincide with sunny days. The oxygen supply in the dishes however differed widely from the thoroughly aerified superficial layer of the sand in the zone of the neap tides where the snails live. Therefore our data concerning the duration of the embryo's life in the capsule and that between hatching and metamorphosis only illustrate the plasticity of these processes and cannot be considered averages. The embryonal development lasted 8-9 days. The embryo began to rotate on the 3rd day and had a small bipartite velum on the 4th. Some veligers metamorphosized 2 hours after hatching (Fig. 14), others had a free living stage of about a week. To judge from its behaviour in our

dishes the veliger is not pelagic, but swims at the bottom. It lives upon its yolk (Fig. 13, y) and evidently does not feed.

The newly hatched veliger (Fig. 9) has a quite colourless shell (Fig. 7; 9, x) without any sculpture as it occurs in **Buccinum** (Dakin 1912, f. 64; Portmann 1925, f. 7) and many other prosobranchs (Vestergaard 1935, f. 2, 5, 7A; Thorson 1946, f. 104 A, 130 C, 137 C-F; Rasmussen 1951, f. 8, and others). The width of the shell is 0,4 mm., its height 0,32 mm. The large velar area bears two short tentacles and black eye spots (Fig. 11, z); the cells of the velum (v) contain brown pigment. The statocysts (Fig. 13, t) lie in the region behind the velum. Coloured larval kidneys are not developed nor could we find such organs (Portmann 1930) in the sections. The nuchal sinus (Pelseneer 1906, p. 135) or larval heart (Dakin, l. c., f. 64, Pul) is recognizable (Fig. 9, e) dorsally to the velum at the mantle border (Fig. 13, mi). Its muscle fibres appear in the sections, but the organ that visibly beats in the veliger is the definitive heart (Fig. 9, h) which lies beside the kidney (Fig. 13, n). Also the organs of the pallial cavity (l), a voluminous osphradium (r), a ctenidium (b) with 6 or more leaflets, and a hypobranchial gland are developed. The ganglia of the central nervous system (c, d) are already connected with one another, even the roots of the visceral loop (su, u) are united with the pleural ganglia. The latter do not lie in the plane of the section drawn in Fig. 13. The propodial ganglia (ro) begin to emit nerves into the foot, whose propodium (o) is delimited by a constriction and provided with an anterior gland (g). Three days after hatching the parapodial flaps (Fig. 11, q) are distinct. The stomach (Fig. 13, so) and the two sacs of the digestive gland are in open communication with the yolk (y) which in the living veliger conceals the columellar muscle (w). The fore-gut (i) and the intestine are simple tubes.

RESUMO

Olivella verreauxii (Ducros 1857) foi criada no laboratório da Base Norte (Dr. Edmundo Nonato) do Instituto Oceanográfi-

co. Cópula, ovipostura, e larva foram descritas. Os espermatozóides ejaculados foram encontrados entre os folhetos branquiais, de onde sobem para o receptáculo seminal. Os grumos fecais armazenados na vesícula interna da fêmea são grudados no opérculo da cápsula ovular por secreção da vesícula. Está, destarte, marcada a face dirigida para a água por finos grânulos e diferenciada da face lisa cimentada ao substrato.

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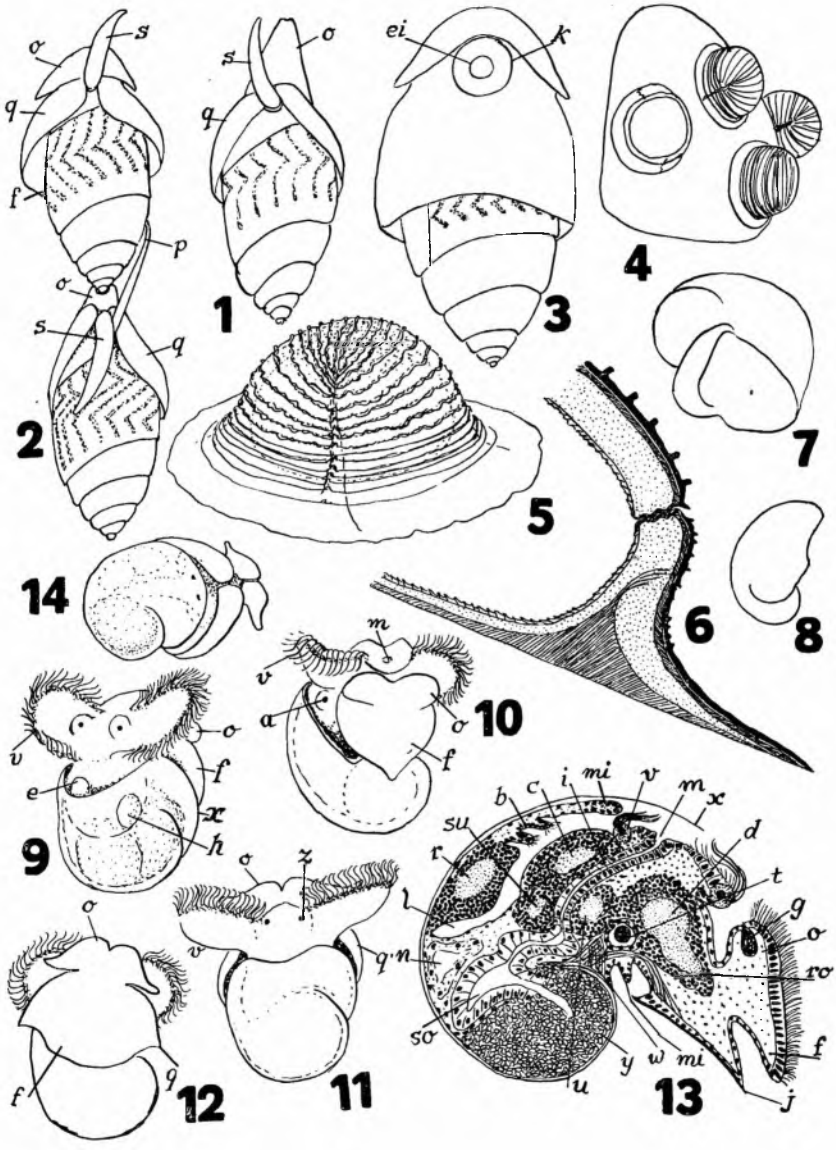
PLATE

PLATE 1

Olivella verreauxii

Fig. 1 — Male scenting female.
Fig. 2 — Mating couple.
Fig. 3 — Female moulding capsule.
Fig. 4 — Shell of **Donax** with 3 capsules and an empty lower part.
Fig. 5 — Egg capsule.
Fig. 6 — Section of same.
Fig. 7 — Shell of veliger.
Fig. 8 — Operculum of same.
Fig. 9 — Newly hatched veliger, dorsal view.
Fig. 10 — Ventral view of same.
Fig. 11 — Three days old veliger, dorsal view.
Fig. 12 — Ventral view of same.
Fig. 13 — Sagittal section of veliger.
Fig. 14 — Recently metamorphosized snail.

a — anus. b — ctenidium. c — cerebral ganglion. d — pedal ganglion. e — larval heart. ei — egg. f — foot. g — foot gland. h — definitive heart. i — fore-gut. j — operculum. k — egg capsule. l — mantle cavity. m — mouth. mi — mantle border. n — kidney. o — propodium. p — penis. q — parapodium. ro — propodial ganglion. s — siphon. so — stomach. su — supra-intestinal ganglion. t — statocyst. u — subintestinal ganglion. v — velum. w — columellar muscle. x — shell. y — yolk. z — eye.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