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DEVELOPMENT OF OCTOPUS VULGARIS, LAMARK FROM EGYPTIAN MEDITERRANEAN WATERS

BY

R. RIAD*, W. F. WADIE AND Y. HALIM.

* National Institute of Oceanography & Fisheries, Alexandria, Egypt.

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ABSTRACT

*A female **Octopus vulgaris** was captured alive on the 1st of July, 1996 and kept in a large glass tank. Ten days later several thousand eggs were laid by this female and their development was followed. The brooding period lasted for about 30 days at 27°C - 28°C water temperature and salinity of 37‰.*

Early cleavage stages, blastodisc formation and gastrulation could not be detected. Stage IV (according to Naef, 1928 definitions) was the first to be observed and stage XX (larvae after hatching) was the last stage recorded. Stages V, VI, XI, and XII were also confused and nondetectable. Hatchlings did not feed and survived for 3 days. The female parent died on 22 August, two weeks after hatching of all embryos.

INTRODUCTION

According to Wells (1978) a male ***Octopus vulgaris*** of 500- 700 gm. that has not recently mated will normally carry about 50 spermatophores and each may contain about 10 gms of sperm. In mating, the spermatophore held in the muscular penis is transferred [illegible], located on the web between the 3rd and 4th right arms, into which spermatophores are placed by the penis and funnel. The guide leads into a groove running along the posterior margin of the hectocotylus. The hectocotylus tip is inserted into the mantle cavity of the

female. What happens afterwards has never been observed, but if the pair is separated a few minutes after the passage of the first spermatophore, the spermatophore is found lodged, filament and forward, in one of the oviducts. It then discharges the sperms into the oviduct. The egg is presumably fertilized as it passes through the oviduct gland. Development begins at once with the formation of a germinal disc of clear cytoplasm at the apical end of the egg, beneath the terminal micropyle. The first cleavage furrow appears 9 - 14 hours later, depending upon temperature, and the process continues for the next two days to form a discoblastula of some 1200 cells, capping one end of the yolk (Naef, 1928).

Cephalopods, unlike other molluscs, show no signs of spiral cleavage and it is a moot question whether the eggs are to be regarded as mosaic or regulative, the normal pattern of embryogenesis is rigid, but it seems that the fate of specific cells is not (Arnold, 1971). Gastrulation begins with the formation of a syncytial yolk epithelium as nuclei from the edge of the blastodisc migrate a way to form a thin layer of clear cytoplasm that eventually envelops the yolk and grows under the blastodisc (Wells, 1978).

The study of embryonic development of ***Octopus vulgaris*** was the subject of study for several authors. Naef (1928), studied the embryonic development of ***Octopus vulgaris*** in great detail. The post-embryonic development was followed by Itami ***et al.*** (1963), the only authors who have been able to rear ***Octopus vulgaris*** from hatching through the planktonic phases to settling and beyond. A summary of many aspects of the life cycle of ***Octopus vulgaris*** can be found in Portmann (1933), Wells (1978), working at Banyuls, found eggs in various phases of development and concluded that they had been developing for about 8 days. Growth and reproduction were carried out in an attempt to interpret the field data gathered over more than two decades (Mangold & Boletsky, 1973 ; Mangold, 1983).

The mating behavior of ***Octopus vulgaris*** has been described by Racovitza (1894), and the egg-laying by Heldt (1948). Lee (1875), observed the mating, laying and hatching of eggs of ***Octopus vulgaris*** in the Brighton Aquarium. Vevers (1961), observed the laying and hatching of ***Octopus vulgaris*** eggs in aquaria. Nixon & Mangold (1996) described the early life of ***Octopus vulgaris*** in the plankton and settlement.

MATERIAL AND METHODS

The female ***Octopus vulgaris*** was captured alive and in good condition on 1st July, 1996 kept and followed up in a large glass tank that was continuously supplied with fresh sea water. Ten days later, this female laid several thousand of eggs which are believed to have been deposited at least 2 days earlier due to the vast number of eggs observed. Wells (1978), stated that egg laying may take a week or more and that atypical egg clutch may embrace about 150.000 egg.

Embryonic development was studied on eggs deposited during the subsequent days to determine and detect any structural change that may occur. During the experiment water temperature was 27°C-28°C and the salinity about 37‰. Samples were preserved in 5% neutral formalin. Photography alone proved to be insufficient to illustrate most structure and hence drawings were made using a Zoom stereoscopic microscope provided with a special camera lucida drawing tube .

RESULTS

Behavior of the female :-

The female ***Octopus vulgaris*** attached the eggs to the side glass wall near the bottom. They were in the form of strings or festoons each about 13-15 cm. in length. Each cm. of a festoon comprised about 80-100 egg. The egg of ***Octopus vulgaris*** (Plate. 2 a & Plate 9 a) is elongated 2.5 by 1 mm., surrounded only by the chorion which has been formed by the follicle cells of the ovary. The chorion is drawn out into a stalk by which the eggs are intermoven to form a string. The strings are attached to a substrate by means of a sticky secretion (Frosch & Marthy, 1975).

After laying eggs, the female oriented herself with the main part of her body lying below the eggs, then gathering stones and shells around herself, attach to the side of the tank, bending its arms over the body in such a way that the suckers were facing upwards. Only one eye was visible at any time. The female stays to brood the eggs and rarely leaves them. She stopped feeding and its respiratory rate was noticed to be markedly increased. The armtips of the female are passed repeatedly between the egg strings and jets of water directed through

them from her funnel. Egg hatching began on the 2nd of August 1996, lasted for about 7 days, giving a brooding period of about a month. The female ***Octopus vulgaris*** died on 22 August. The death of females after egg hatching is a phenomenon recorded by other authors. According to Wells (1978), shortly after the eggs hatch, the female dies and it does seen to be generally true that octopuses, of whatever species, die after spawning whether in captivity or in the sea. Before death the female had become extremely weak and her body and tentacles appeared very emaciated. After death its weight 980 gm., a total length of 93 cm., mantle length 13.1 cm., its empty stomach 1.20 gm., and spent gonad weight 21.49 gm.

Early embryonic stages after Naef (1928)

In the present study, we were not able to detect the early embryonic stages that include cleavage, blastodisc formation and gastrulation. The eggs examined were at a more advanced stage. The early embryonic stages will be described after Naef (1928). They are shown in plate 1, which illustrates the stages of development during 3.5 days after spawning.

The first cleavage, 9 hours after spawning, Plate (1 d) divides the egg into 2 equal blastomeres. The second cleavage, 13 hours after spawning, Plate (1 e) results in the formation of four more or less equal blastomeres. The two posterior ones were described by Naef (1928) to be sometimes slightly smaller than the anterior. The author added that the transverse cleavage then proceeds slightly oblique from the center posteriorly. The 3rd cleavage, 15 hours after spawning, Plate (1 f) consisting of 8 blastomeres which are not equally alike, surrounding a more or less wide space that may last till the end of cleavage and then begins to close, and sometimes persists till the formation of the germ plate. Plate (1 i) : exhibits the 16 blastomere stage, 19 hours after spawning, in which there are 4 central micromeres and 12 macromeres around. Plate (1 j): is nearly the same (16 blastomeres stage) but with more apparent, latter defined and larger blastomeres. Plate (1 k) 22 hours after spawning, illustrates the embryo with 23 blastomeres. Naef (1928) mentioned that in this stage a rapid division occurred within the central four small blastomeres, resulting in 8 small central blastomeres, just before the division of the macromeres and formation of the 32 blastomere stage (Plate 1 l). The latter stage appears twenty four hours after spawning. In this stage the net yield is 4 additional small blastomeres, the 8 small central blastomeres and 20 macromeres surrounding them. Soon after, the

central space becomes markedly enlarged as a result of the upward and downward movement of the micromeres (plate 1 m), and the final shape is in the form of a large central space surrounded by a macromere crown and embracing inside the free micromeres. After that and onwards, 28 hours after spawning, cleavage begins to be irregular and the embryo Plate (1 n) appears as a crown of peripheral macromeres that surrounds irregularly arranged micromeres inside ; forming an embryo that consists of 66 blasntomeres (Naef, 1928). Irregular cleavage proceeds, and 36 hours after spawning *(plate 1 o)*, the developing ***Octopus vulgaris*** embryo now consists of 360 blastomeres with a considerably marked smaller central space. Forty hours after spawning the blastoderm stage is attained and defined by Naef (1928) to be ***stage I***, consisting of 1200 cells in which the central pore is markedly diminished and nearly closed *(plate 1 p)*.

Stage I - II , plate. I q 2 days after spawning:

It is an intermediate phase between both of the two ones in which endomesoderm formation starts. The blastodisc rim does not appear as a plane closed circle but as 12 slightly curved attached peripheries. This structure is in turn externally surrounded by a paler peripheral rim to which the yolk cells adhere.

Stage II, pate 1 r, 2½ days after spawning:

It is an advanced stage in endomesoderm formation.

Stage II-III, plate 1 s, 3 days after spawning:

Exhibits a transitory from between both.

Stage III, plate 1 t, 3 ½ days after spawning:

The animal gastrula, characterized by enlargement of the blastodisc size (Naef, 1928).

Description of stage IV to XX as observed in the present work

Stage IV, plate 2 b, 4 days after spawning:

This is the 1st stage that has been detected in the present study. In this stage the dim thin yolk membrane is easily distinguishable from the embryos body that is lighter in colour and separated from the rest of the yolk by a posterior edge or septum, with the aid of which the embryo is capable to reverse its position inside the egg membrane. Through microscopical examination of the posterior part of the egg (that could not be traced thought camera Lucida or photography; due to the difficulty in orienting the egg in the proper position) a dark area in the middle of the embryo could be detected which is through to be, according to Naef (1928), a dense mesoderm accumulation to form the mantle.

Unfortunately, we were not able to detect stages V and V1 due to the vast number of eggs existing, as the female did not deposit all the eggs at one and the same time.

Stage VII, plate 2, c - d & plate 9 b, 7 days after spawning:

From the two figures, c & d, the 1st inversion of the embryo is clearly obvious as the embryo changes its position inside the egg membrane directing its mantle towards the stalk *(plate 2 d)*. The reason of this inversion is still unknown. At this stage the eyes are first distinguished as pale orange spots, more obvious from the dorsal side. Besides, the mantle border begins to appear as a circular swollen area, *(plate 2 c & d)*. It is worth noting that arm buds begin to appear on both body sides.

Stage VIII, plate 3, a & b, 8 days:

Some advanced features are exhibited. In this stage the eye fold and mouth rim are more prominent. Besides, the arm buds appear more protruded and slightly elevated, more or less nearly equal in size. The mantle rim is much elevated rendering the organ more clear and markedly defined than in the previous stage.

Stage IX, plate 3, c & d, 9 days:

At this stage the embryo was noticed to perform slight contractions. The development of the arm buds proceeds, and they now appear as broad swollen

more or less oval structures arranged altogether beside each other, being markedly separated by shallow depressions in-between, and still possessing quite smooth surfaces.

Stage X, plate 4, a - b & plate 9 c, 10 days:

This stage is characterized by more differentiation of the optic vesicle and marked pulsation of the outer yolk sac.

The arm buds, which were previously separated, begin now to acquire a special definitive shape. The base of the 2nd and 3rd arms begin to surround the eyes as the 2nd is directed towards the mouth while the third one towards the funnel, to which it is attached by a small fold (funnel tube fold). The posterior mouth rim extends forward and the gills, which could not be detected in earlier stages, are now rather visible. The mantle enlarges becoming more or less ring-shape appears more clearly from the dorsal view. Still in this stage the direct contact between the head and mantle rim persists.

It is to be noted that stages XI & XII are missing in the present study, as they were unfortunately not detectable among the vast egg numbers.

Stage XIII plate4, c - d & plate 9 d, 13 days:

It shows marked morphological changes in the arms shape. The arms appear more tapering, with 3 larval suckers on each. The suckers differ in size, getting smaller towards the free tapering end. The mouth occupies a considerable separate area between the arms of each body side. In this stage, it is notable that the funnel is markedly well formed and fully developed and the mantle cavity narrows due to the growth of its rim. The gills appear as small gill plates. At this stage the systematic heart and the branchial hearts begin to pulsate slowly and irregularly.

Stage XIV, plate 5, a & b, 15 days:

No marked morphological difference exists with the previous stage XIII, apart from a general body size enlargement and a more mantle growth till its rim now partially covers the funnel sac. It is also noticeable that the mouth opening is now markedly deeper and more apparent.

Stage XV, plate 5, c - d & plate 10 a, 17 days:

A notable constriction of yolk in the cephalic region is observed. For the first time the chromatophores are well recognized. At the beginning they were yellowish, turned orange about one stage later, and finally became red. Suckers are more prominent and much more developed. As a result of further mantle growth, its rim now completely covers the funnel sac. The iris and the eye-lid could be detected.

Stage XVI, plate 6, a & b, 19 days:

A marked decrease of the yolk mass has occurred, the chromatophores spreading over various body parts and a general enlargement of the body size is observed. At this stage the eye-lid is more differentiated than before and could be easily detected. The mantle is much enlarged and acquires a sac-like shape.

Stage XVII & XVIII, plate 6, c & d, 21 days:

The embryo undergoes a second reversion in which it brings back once more its yolk to adhere to the stalk of the egg. Presumably, this brings the embryo back into an appropriate position for an un-impeded escape from the egg chorion (Boletzky, 1971). A remarkable withdraw for the primary eye lid is noticed. Progressive growth occurred to mantle sac which became remarkably larger than before giving the embryo a more or less true larval shape. The chromatophores now appear more clear on the skin, specially on ventral surface and bottom of mantle cavity, being easily visible through it.

Stage XIX & XX, plate 7, a - b & plate 10 c, 23 days:

Represents a fully formed ***Octopus vulgaris*** larva without an apparent yolk sac. These larvae hatched slowly, plate 7 a & plate 10 b, from their chorions and immediately acquired the ability of active free swimming. They appear to the observer performing repeated upward jumps, for short distance each time, and then passively sink towards the bottom of the container, but never settling on it. At this stage, the arms are remarkably short, each possessing three typical suckers and the free pyramidal tapering ends are thought to be the animal sense organs. The mouth occupies a central location surrounded by the arms. The two primary eye-lid folds approach to each other leaving in-between a longitudinal oval aperture (eye opening) which was noticed to narrow and widen.

The hatchling of ***Octopus vulgaris*** has a total length of nearly 3 mm. and a mantle length of about 2 mm. The mantle is twice as long as the arms.

The Juveniles, plate 8 a, b, c & plate 10 d:

The Juveniles were kept in large glass jars, at a water temperature of 27°C - 28°C, but unfortunately all rearing trials failed though an attempt to feed them on Artemia was carried out. These Juveniles had short arms bearing 3 primary suckers and their eyes are large with clear pupil. All hatchlings died after 3 days. The majority of the larvae lived, presumably on the yolk in their guts, for eight to nine days (Vevers, 1961).According to Nixon & Mangold (1996), ***Octopus vulgaris*** enters the plankton and remains there for perhaps eight weeks.

DISCUSSION

This is the first study to be made dealing with developmental stages of ***Octopus vulgaris*** from Egyptian water. In the present study mating could not be observed as the female had already mated before being captured. Vevers (1961), stated that the period between mating and the laying of first eggs was forty-seven days in aquaria at 23°C temperature. According to Mangold (1983), egg masses are found in nature over the whole depth range of the species, from the surface to about 100 m., but most eggs are probably laid in shallow waters. In the present work the material studied was found as blotches or strings attached to the side glass wall of the tank near the bottom. In the present study the early embryonic development is described according to Naef (1928), who distinguished 20 developmental stages, stage I being blastula formation, while stage XX corresponds to hatching. Early developmental stages and blastodisc formation were passed over as they were extremely hard to be detected among such a vast number of eggs which causes much confusion; stage IV was the 1st to be recognized. It is to be noted that stages V,VI, XI and XII are also missing as they were unfortunately non detectable due to the same reason.

Brooding period lasted for about 30 days at 27°C-28°C. Lee (1875), stated that the incubation period for the same species was 50 days at 12.2°C water temperature. Mangold & Boletzky (1973), recorded that its embryonic development at 13°C takes as long as 125 days. According to Vevers (1961), ***Octopus vulgaris*** brooding period was remarkably consistent 39 - 42 days where water temperature was 22°C-32°C. Mangold (1983) emphasized that the duration of embryonic development in all cephalopods is dependent and much

related to water temperature. This advocates that temperature is of paramount importance in controlling breeding of marine animals. specially when we bear in mind that Mangold-Wirz (1963), recorded that development of ***Octopus vulgaris*** stops when water temperature drops below 10°C, but the embryos survive and continue their development as soon as the temperature increases again.

During the present study, chromatophores were first well recognized at stage XV. They were yellowish, then turned orange about one stage later, and finally became red. Naef (1928), stated that the chromatophores are formed at stage XVII on the skin, specially on the ventral surface and on the base of the dorsal mantle cavity. Mangold ***et al.*** (1971), advocated that the first chromatophores in ***Eledone cirrosa*** appear at stages XIV to XV.

In the present study, the eyes were first distinguished as pale orange spots at stage VII together with the arm buds. The eye fold and mouth rim became prominent in stage VIII. In stage X the gill and funnel could be detected. Iris and eye-lid begin to appear in stage XV. According to Naef (1928), the eye and arm buds begin to appear at stage VI, the mouth and gill could be detected in stage VII, funnel appears at the first time in stage VIII, iris in stage XIII and eye-lid can be detected in stage XV.

Vevers (1961), stated that the majority of the larvae lived, presumably on the yolk in their guts. for eight to nine days. In the present study, the hatchlings were without an apparent yolk and could survive for only 3 days without feeding. According to Mangold ***et al.*** (1971), the internal yolk sac strongly increases in size in ***Eledone cirrosa***, but the rate of resorption evidently being very high, it never becomes as large in relation to the body as in ***Octopus vulgaris*** it is not entirely resorbed by the time of hatching.

It is worth to note that in the present work development took about 23 days at 27°C-28°C water temperature, while Naef reported that it lasted for 28 days at 22°C water temperature. This is obviously to be attributed to the marked difference in water temperature.

Octopus vulgaris has only once been reared from hatclings through the planktonic phase until it settled to the benthic mode of life, common to all adult Octopodidae (Itami ***et al.***, 1963). Subsequent trials failed although the animals

survived for more than a month, but growth ceased after 3 - 4 weeks (Mangold & Boletzky, 1973). Itami ***et al.*** (1963), kept their hatchlings at a mean water temperature of 24.7°C (range 22 - 27°C), the animals settled on the bottom of the tank after 33 - 40 days when their average total length was 11.5 mm. and the mantle length was 6.3 mm. In the present study, the hatchlings were reared in large glass jar with water temperature of 27-28°C, but unfortunately all trails failed after 3 days inspite of an attempt to feed them on Artemia. At the time of hatching the arms were relatively short and had 3 primary suckers in the present study.

Boletzky (1987), stated that the possession of short arms with a limited number of suckers is a common feature of the advanced embryos and hatchlings of all pelagic incirrates of many species among the benthic Octopodidae.

Trials were made in laboratory during the present study in order to obtain the missed embryonic stages (from 2 blastomere stage up to gastrula), in addition stages V, VI, XI, and XII through rearing adult males and females in large aquaria. All these attempts failed and no egg clusters could be obtained.

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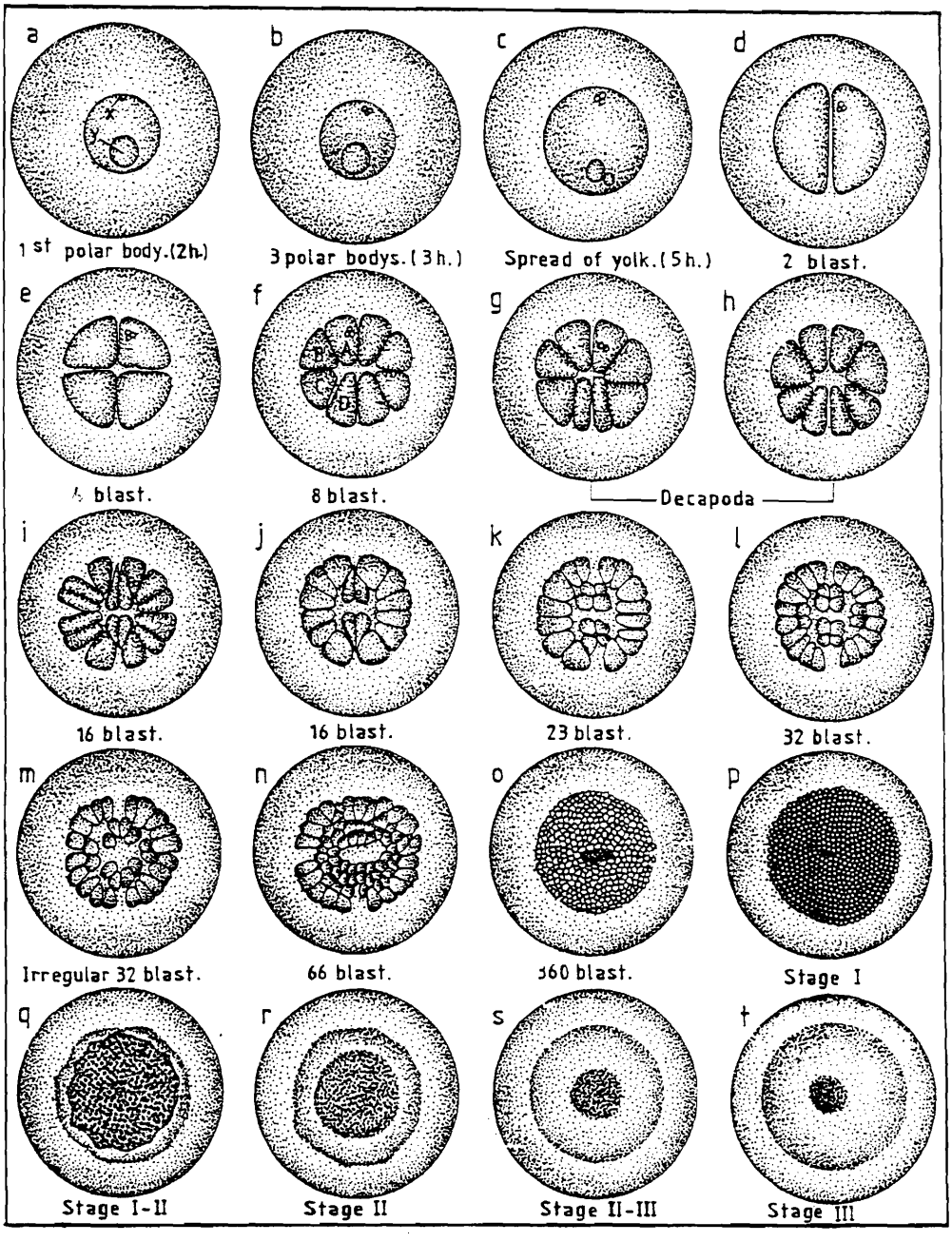


Plate 1 (a-t). Early development of Octopus vulgaris from onset of fertilization up to Gastrula formation, redrawn after Naef (1928).

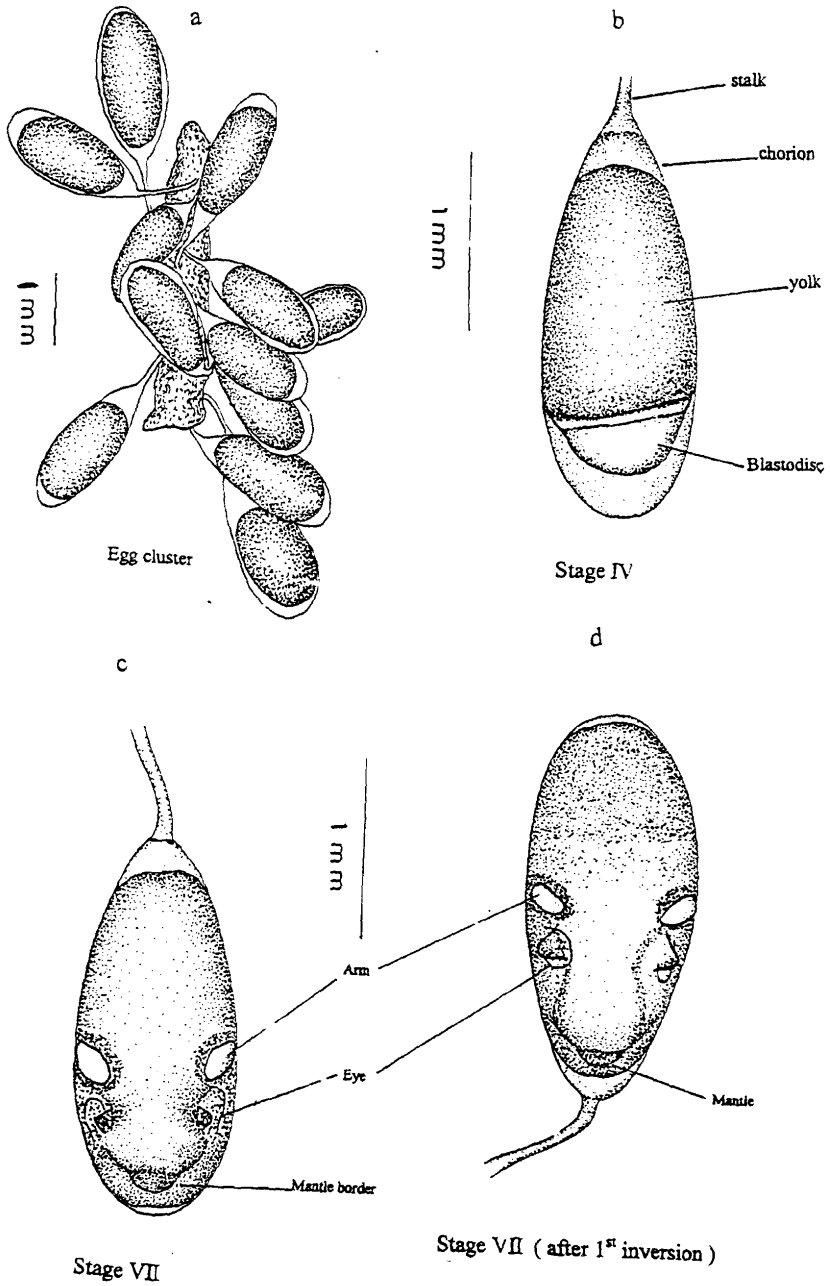


Plate 2 (a - d) Development of Octopus vulgaris

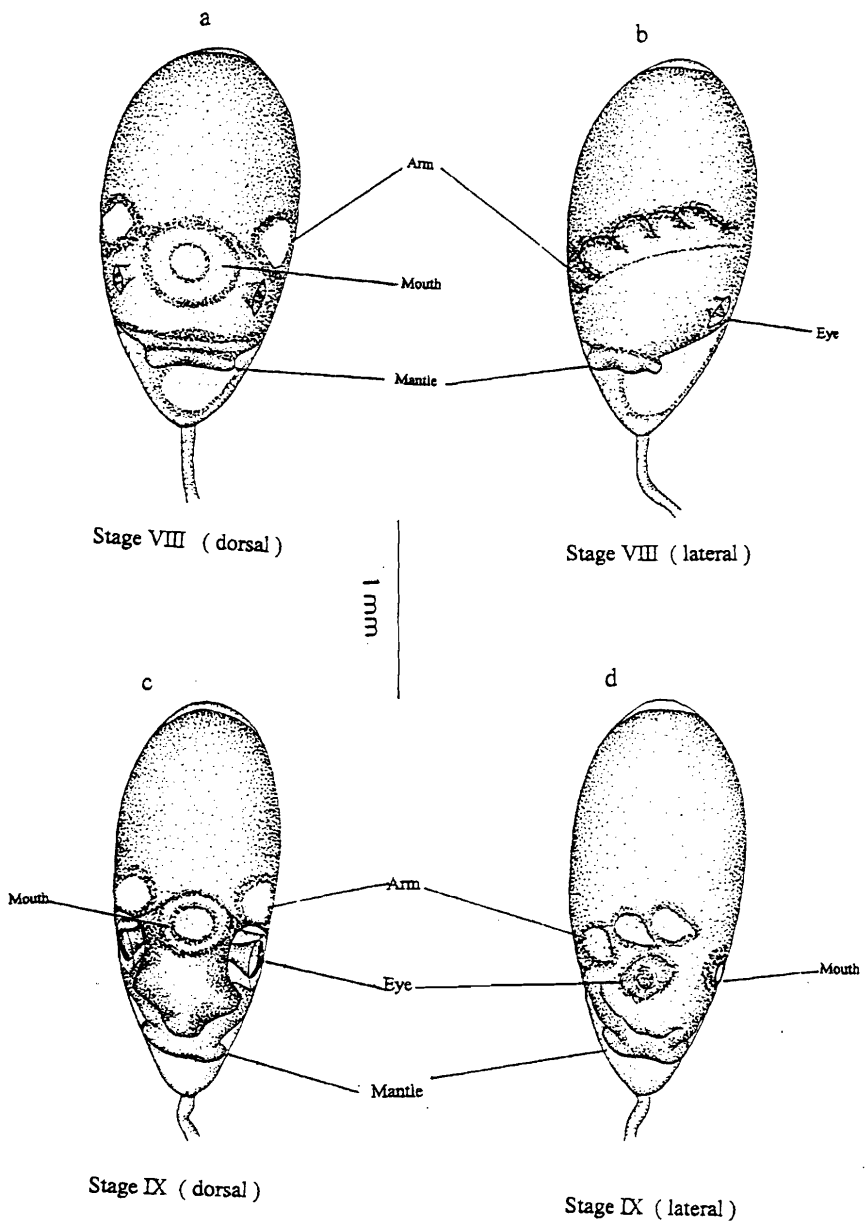

a
b
Arm
Mouth
Eye
Mantle
Stage VIII (dorsal)
Stage VIII (lateral)
1 mm
c
d
Mouth
Arm
Eye
Mouth
Mantle
Stage IX (dorsal)
Stage IX (lateral)

Plate 3 (a - d)

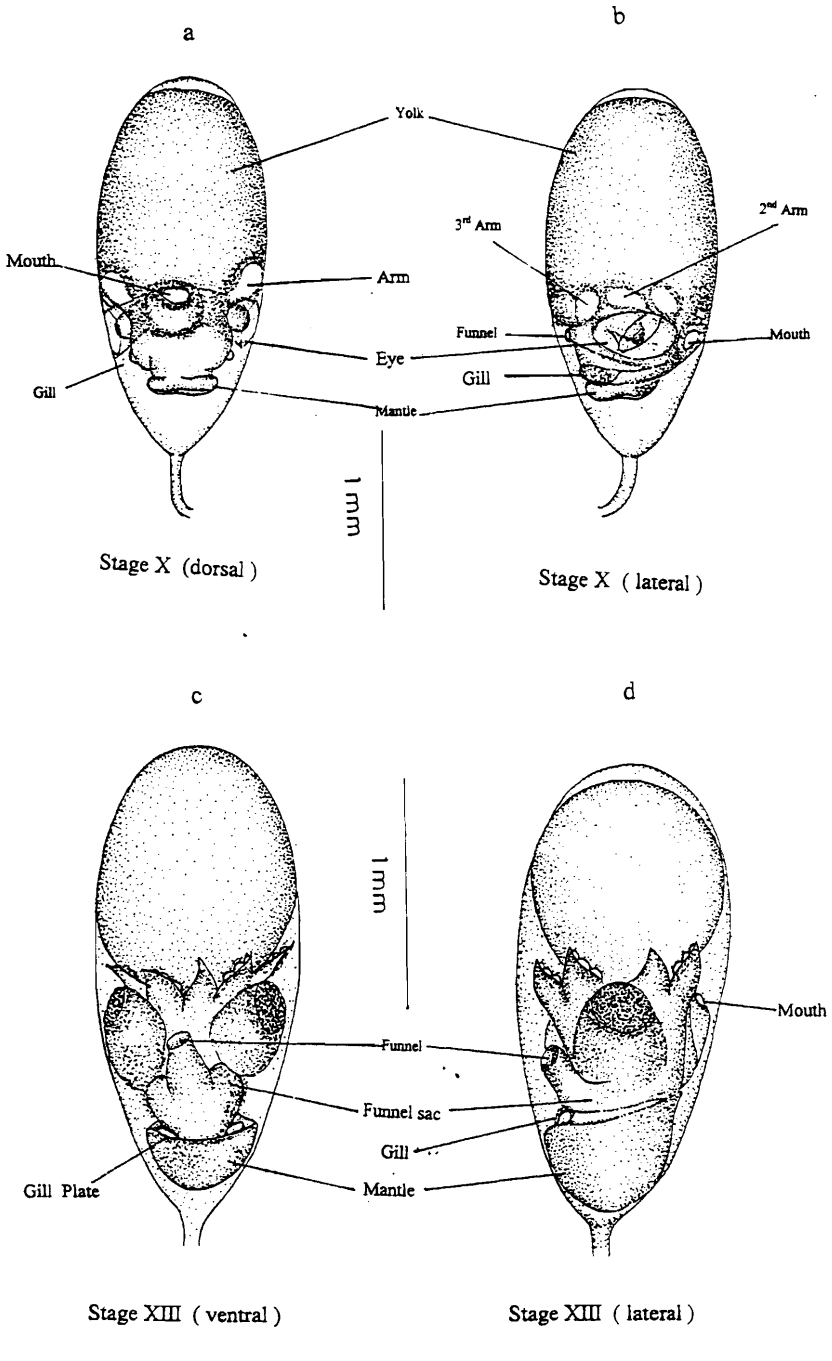

a
b
Yolk
Mouth
Arm
Eye
Gill
Mantle
3rd Arm
2nd Arm
Funnel
Mouth
Gill
1 mm
Stage X (dorsal)
Stage X (lateral)
c
d
1 mm
Funnel
Funnel sac
Gill
Gill Plate
Mantle
Mouth
Stage XIII (ventral)
Stage XIII (lateral)

Plate 4 (a - d)

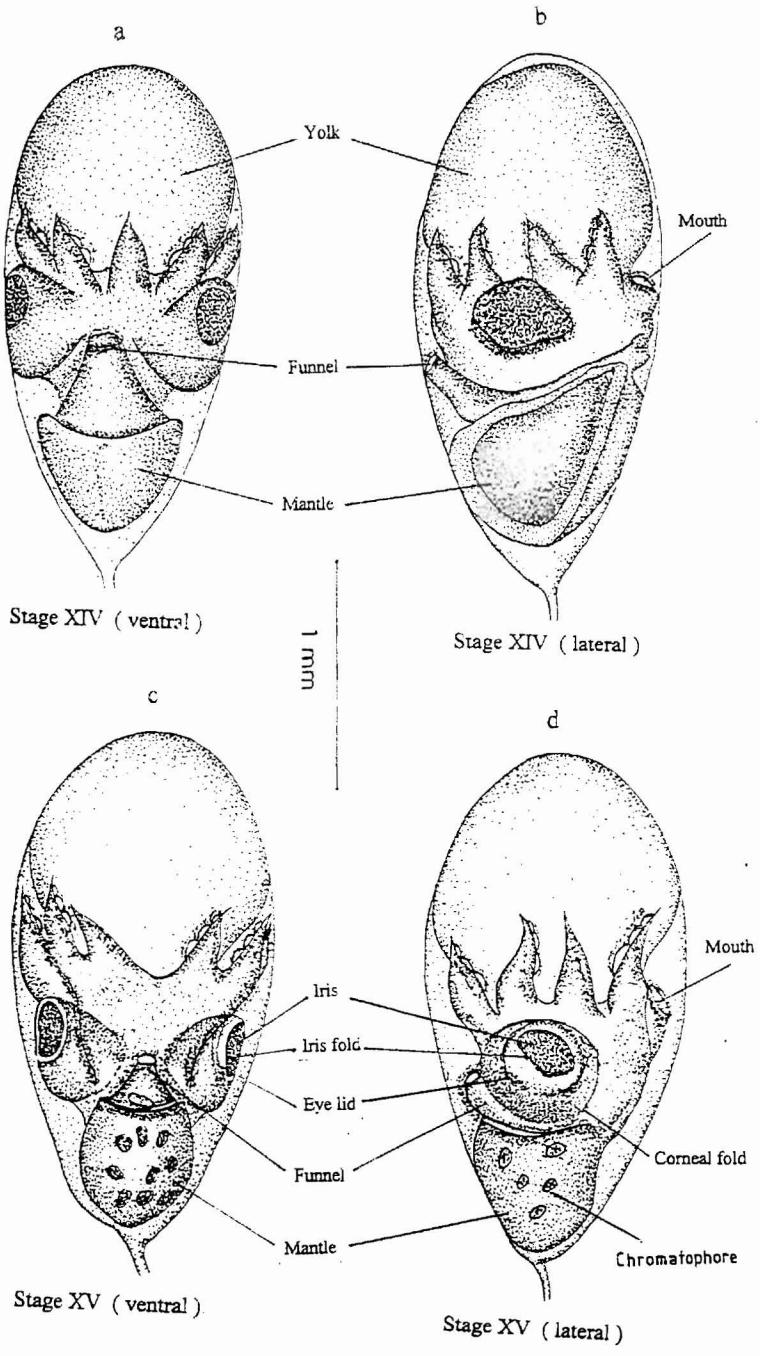

a
b
Yolk
Mouth
Funnel
Mantle
Stage XIV (ventral)
Stage XIV (lateral)
1 mm
c
d
Mouth
Iris
Iris fold
Eye lid
Funnel
Corneal fold
Mantle
Chromatophore
Stage XV (ventral)
Stage XV (lateral)

Plate 5 (a - d)

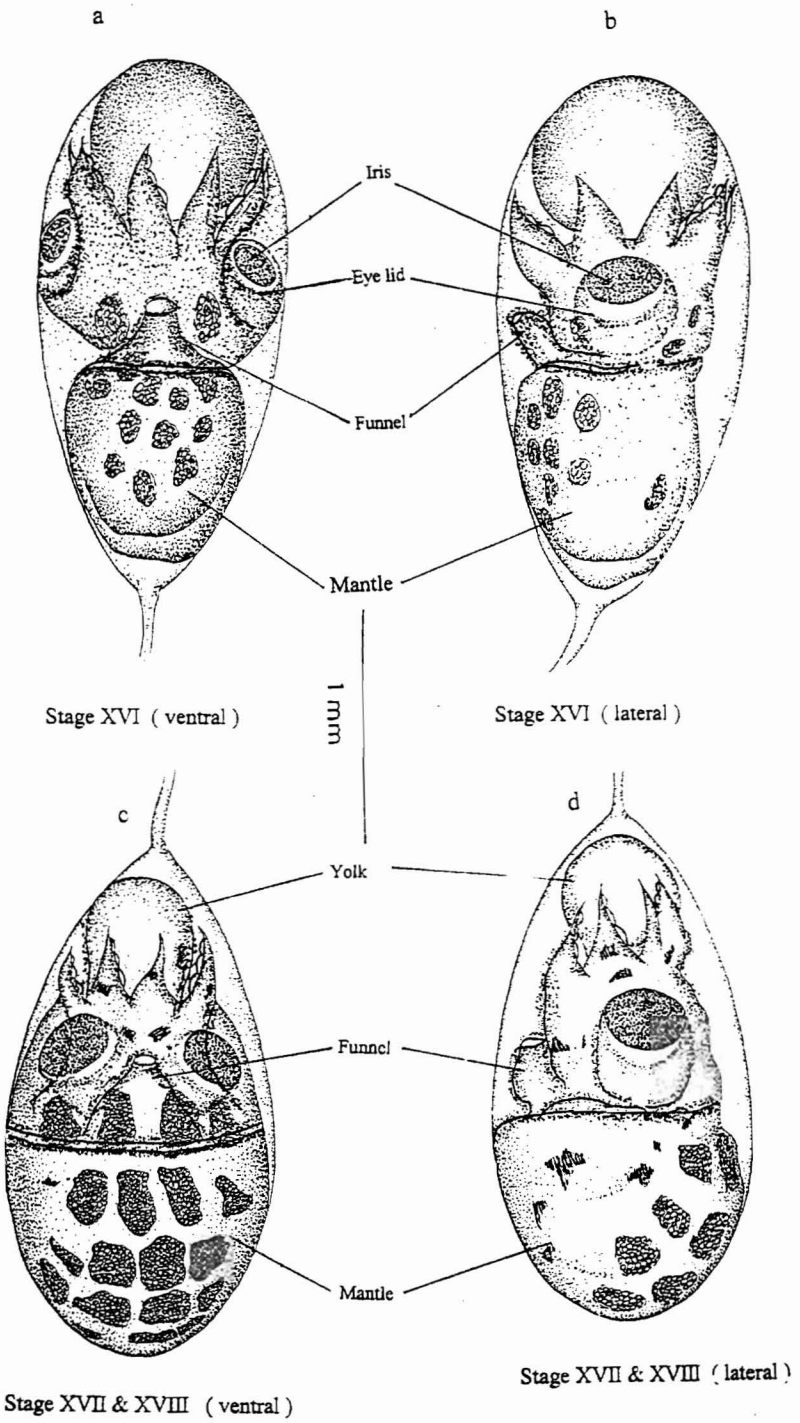
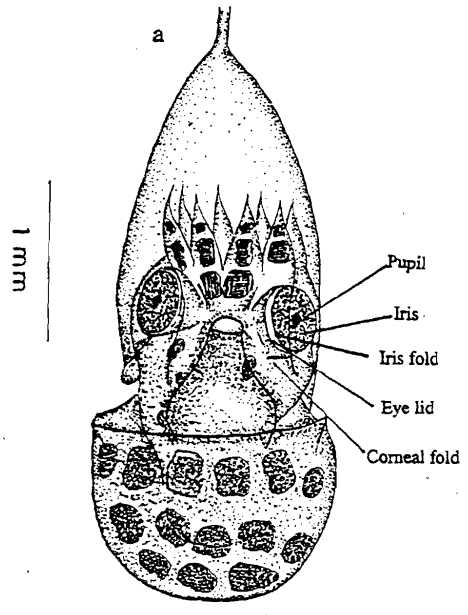
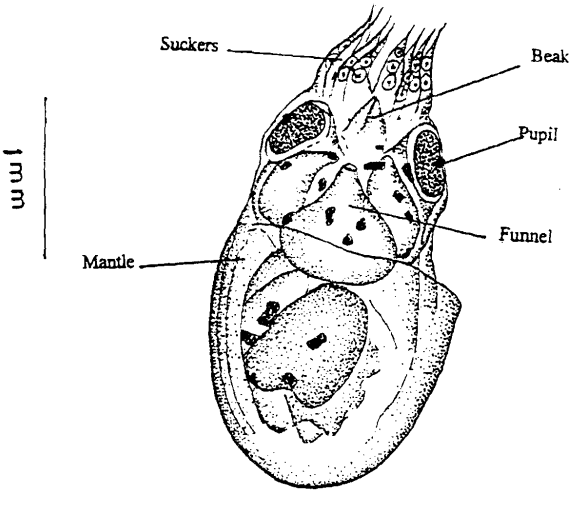

a
b
Iris
Eye lid
Funnel
Mantle
1 mm
Stage XVI (ventral)
Stage XVI (lateral)
c
d
Yolk
Funnel
Mantle
Stage XVII & XVIII (ventral)
Stage XVII & XVIII (lateral)

Plate 6 (a - d)



Moment Of hatching



Stage XIX & XX

Free swimming juvenile immediately after hatching

Plate 7 (a & b) hatching of Octopus vulgaris

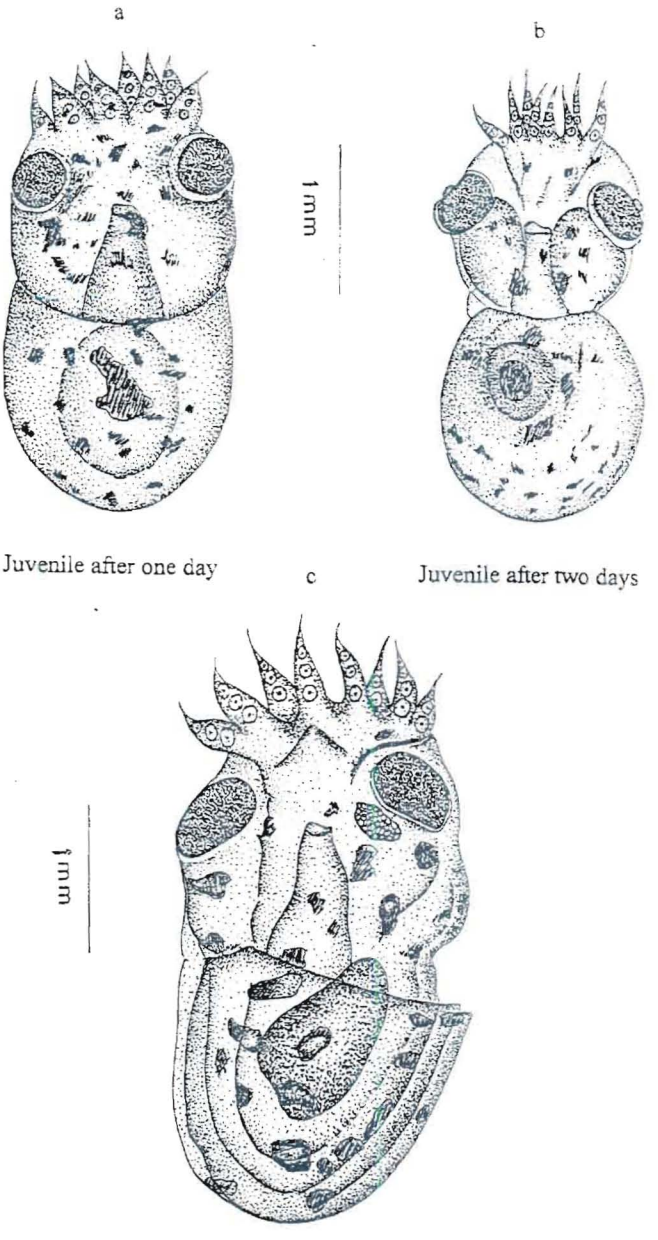
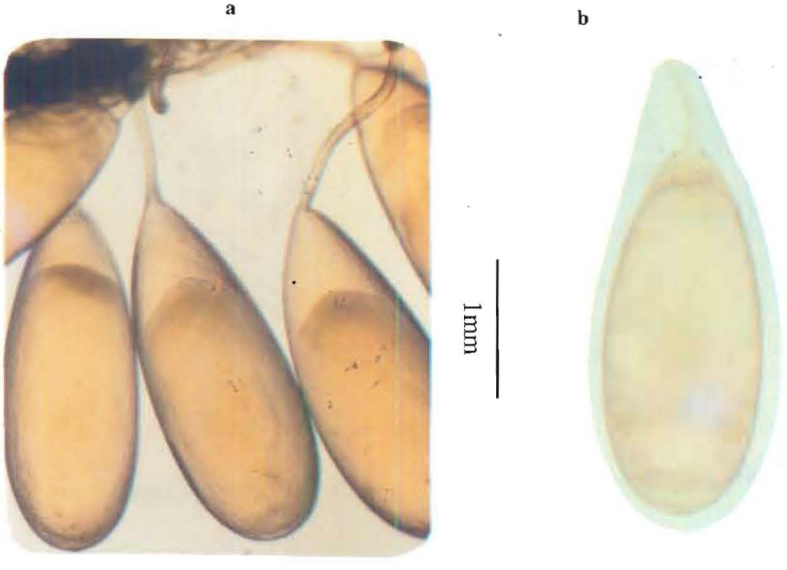


Plate 8 (a - c) juveniles of Octopus vulgaris



Egg cluster

Stage VII

Stage X (dorsal)

Stage XIII (ventral)

Plate 9 (a-d)

Plate 10

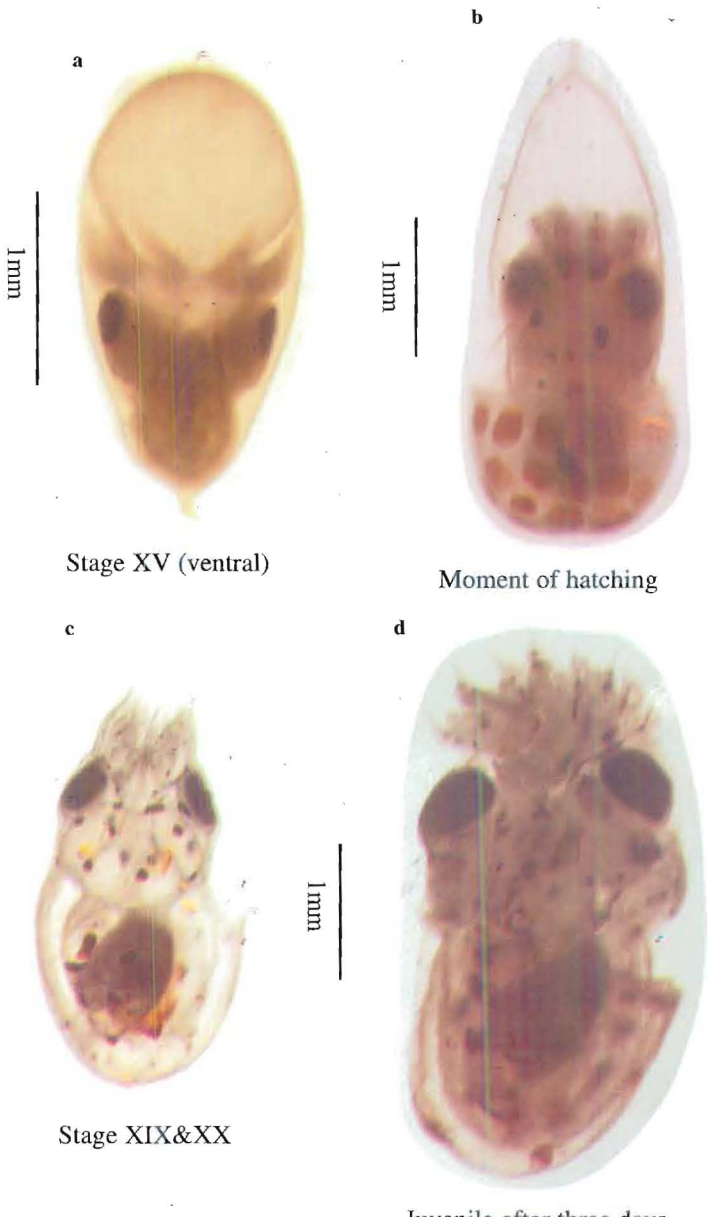


Plate 10 (a-d)