

## RESEARCH COMMUNICATIONS

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## Embryonic development in *Octopus aegina* Gray, 1849

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**Cephalopods occur in all marine habits of the world. They are extremely important in ecological, biological and biomedical research. The genus *Octopus* consists of more than 100 species and along with *Sepia* sp. and *Sepiella* sp. accounts for about 50% of all described cephalopods. Octopus earns valuable foreign exchange for the country. Knowledge on the embryonic and larval development is essential for hatchery and culture techniques. Development stages of the embryo were sequenced based on morphological characters. During the**

**course of development, the embryo reverses its position two times; the new born resembles adults in form except for its free-swimming mode of life. The gestation period of the embryo was 18 to 20 days at a temperature range of 28–30°C.**

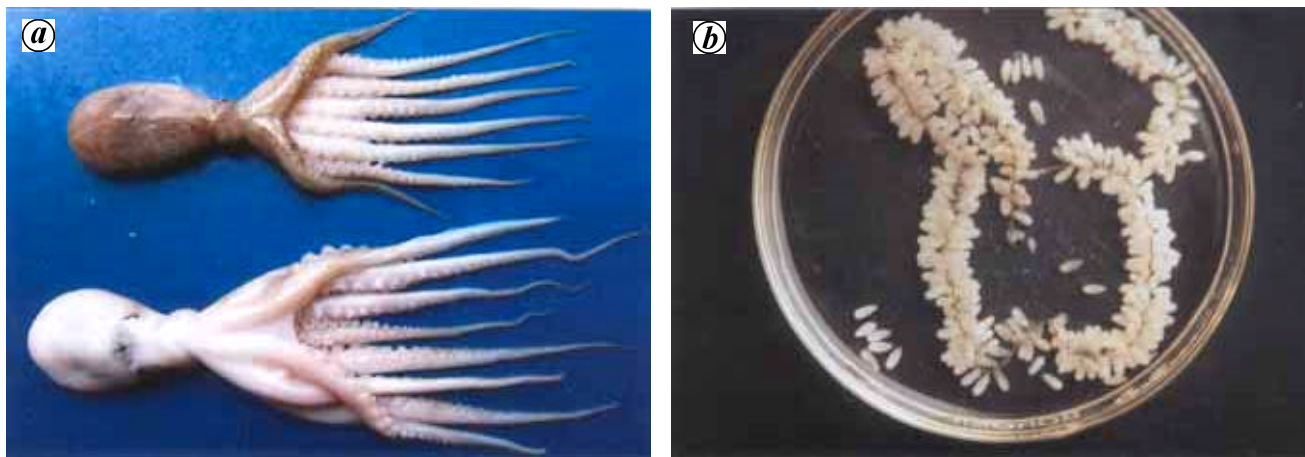
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IN the phylum Mollusca, class Cephalopoda are commercially the most important group, containing the nautilus, cuttlefishes, squids and octopuses. Octopuses are bottom-dwelling species usually limited to the neritic province. The genus *Octopus* consists of more than 100 species, and occurs in all marine habitats of the world. *Octopus vulgaris* is the most important and widely distributed species occurring throughout the tropical and temperate waters of the world<sup>1</sup>. In India, cephalopods started gaining importance with the development of an export market and consequent increase in demand. The annual production of cephalopods was 1,04,354 tonnes, valued<sup>2</sup> at more than Rs 800 crores during 2002–03. The body of the *Octopus* is utilized for food, while the tentacles and ink sac are used for biomedical research. Due to heavy demand, various species of *Octopus* were successfully cultured in pilot scale circulating seawater systems for biomedical research<sup>3</sup>. In India, *Octopus aegina* (Figure 1 a) is caught throughout the year by fishermen, but no information is available with regard to embryonic and larval development of this species.

Life histories of majority of octopuses are still unknown and our knowledge on octopus lifecycle is fragmentary. Some species like *O. cyanea*<sup>4</sup> and *O. vulgaris*<sup>5</sup> were studied under laboratory conditions. Octopus is a dioecious animal and fertilization is internal. The incirrate octopus lays eggs without gelatinous envelope around the egg chorion. In the benthic octopus, a feature that varies between species is the way in which the chorion stalks are glued to a substratum either individually or in small clusters with a common fixation disk or as festoons made from many interwoven stalks that are glued together<sup>6</sup>. Most females spawn only once. Females remain with eggs to brood and groom them throughout the development period, after which the females dies<sup>7</sup>.

The samples were collected from Mandapam region, Palk Bay (lat. 9°45'N; long. 79°13'E) southeast coast of India. Live *O. aegina* caught in trawl nets were collected, transported and maintained in the laboratory. Feeding of octopus was done with small-sized live crabs @ 2–3 crabs per day per animal. When females laid the eggs, a portion of the egg cluster was removed from the brooding female and kept in a separate tank for observing the development. From this egg cluster, 5–10 eggs were taken out daily for noting the development stages using a stereozoom microscope with attached photographic facility. Samples of the eggs at various developmental stages were preserved in glycerine and alcohol (1/1 volume) for further observa-

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**Figure 1.** *a*, Dorsal and ventral view of adult *Octopus aegina*. *b*, Portion of egg string.

tion. The development of embryos was compared with stages of development described by earlier workers<sup>8,9</sup>.

The female octopus released eggs after 2–3 days of mating in the morning hours. The egg capsules were small, rice-like and whitish in colour. The average length of an egg capsule was  $3.18 \pm 0.5$  mm and width was  $1.04 \pm 0.07$  mm ( $n = 46$ ). The stalks of egg capsules were woven together with cement secreted by the oviducal gland during laying (Figure 1 *b*). The number of eggs per cm of a string (festoon) was 29–32. The female performed parental care in octopus, wherein during brooding she continuously cleaned and aerated her eggs with the arms by squirting a jet of water over them. Throughout the brooding period, the female held the festoons close to the body on the aboral side of the broad extensile interbranchial membrane. The female octopus died soon after all the eggs were hatched. In *O. aegina*, the fecundity ranged from 2962 to 8820 in individuals of mantle length ranging from 67 to 85 mm.

The sequential development of the embryo is described below.

**Day 1:** The size of the egg ranged from 3.05 to 3.35 mm in length. The number of eggs present in the string was estimated as 44–48 eggs/1.5 cm of string. Eggs were clear inside with narrow perivitelline space along the periphery (Figure 2 *a*).

**Day 2:** A clear area of cytoplasm lying immediately beneath the micropyle. It continued as a thin layer of cytoplasm surrounding the yolk.

**Day 3:** Clear area of cytoplasm beneath the micropyle increased slightly.

**Day 4:** The extra embryonic ectoderm begins to spread over the yolk (Figure 2 *b*).

**Day 5:** The cap-like yolk sac enlarged steadily until development.

**Day 6:** Embryo turns around in the egg so that the posterior of the animal pore faces toward the stalk instead of toward the micropyle (first inversion).

**Day 7:** Formation of major organ primordia.

**Day 8:** Covering of the yolk with extra embryonic ectoderm almost complete. Rudiments of cephalic organs developed. Rotation of embryo inside the egg case was noticed. This rotation might help exchange oxygen and waste between the inner and outer chorion quickly.

**Day 9:** A pair of orange eyespots appear; rudiments of arms, mantle, funnel, and gills were visible. Pulsation of yolk sac was observed. This helps not only in circulating the blood in the embryonic blood system, but also moves the liquefied yolk in the yolk sac.

**Day 10:** Eyespots well-developed; tentacles, suckers and funnel are developed further and visible. Embryo has taken the shape of an octopus.

**Day 11:** Eyespots visible through egg case; tentacles, suckers and funnel are developed further (Figure 2 *c*).

**Day 12:** Further development of funnel, tentacles and suckers.

**Day 13:** Development of two chromatophores on the ventral head observed. Presence of three suckers on each arm.

**Day 14:** Gills are now tucked inside the mantle. The buccal mass was embedded with dorsal arms.

**Day 15:** Chromatophores appear on the mantle, head and arms (Figure 2 *d*).

**Day 16:** Wriggling movement of mantle inside the egg case was noticed. This is due to the second inversion of the embryo. After this, the embryo is positioned in such a way that posterior mantle tip faces towards the micropyle. When ready to hatch, larvae use the tip of the mantle to beak the capsule wall.

**Day 17:** Embryos well-developed, outer yolk sac reduced considerably.

**Day 18:** Outer yolk sac disappears. Release of few hatchlings was noticed (Figure 3 *a*).

**Day 19:** Release of hatchlings continued.

**Day 20:** Release of hatchlings continued. Almost all eggs were found hatched.



**Figure 2.** *a*, Fertilized eggs. *b*, Four-day-old eggs. *c*, Eleven-day-old eggs. *d*, Fifteen-day-old eggs.

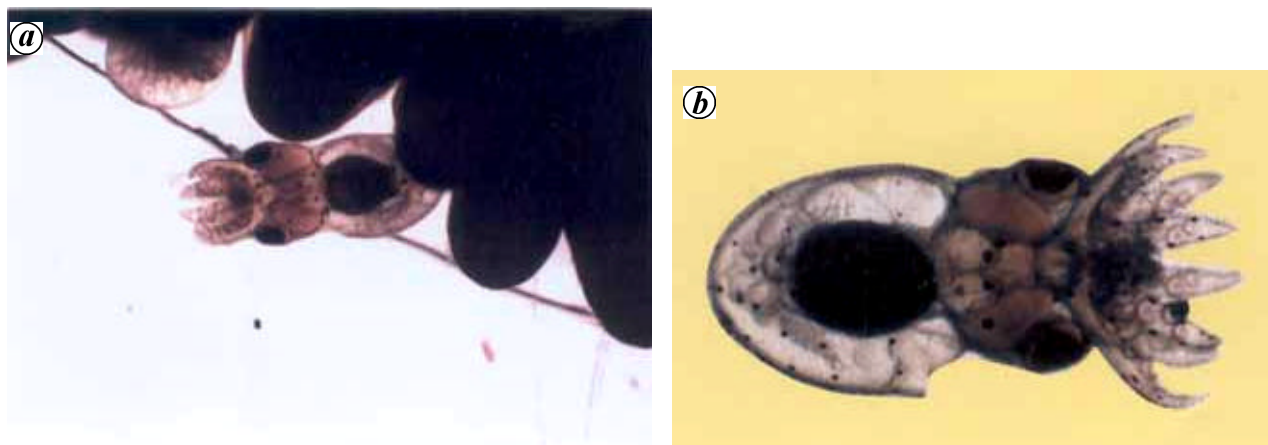
The hatchlings resembled adult octopuses in general body pattern (Figure 3 *b*). The arms were short and stubby. Eyes were large and prominent. The mantle length of the newly hatched octopuses was  $3.07 \pm 0.15$  mm. There are six suckers on the dorsal arms, becoming two rows from 5th and 6th suckers. The number and distribution of tegumental chromatophores in the skin covering the arms, funnel, mantle and head are species-specific. Premature hatching of eggs incubated artificially was noticed. These hatchlings had external yolk sac at the buccal opening which obstructed free movement of larvae, and died after 24 h. Premature hatching of eggs may be the result of inadvertent physical stimulation on the part of the investigators while handling late stage eggs or during the daily maintenance of rearing tanks. Rearing of hatchlings of *O. aegina* was attempted using various feeds; however, none of them survived beyond five days.

The embryonic development of *O. aegina* took 18–20 days at 28°C. Environmental factors like water temperature play an important role in the development of embryo in various octopus species<sup>4,10,11</sup>. Hatching of entire eggs of

egg cluster took three days. The extended hatching time indicated that the spawning of eggs might not be performed in a single day.

Embryonic development of *O. aegina* closely resembled the development of other species of octopus embryos<sup>12–15</sup>, including two reversals, one during earlier stage and the other during final stages of development. Although the reasons for the first inversion are entirely unknown, the second inversion presumably brings the embryo back into an appropriate position for unimpeded escape from the egg<sup>16</sup>.

The duration of embryonic development, size and body proportion of the hatchlings and mode of life have been shown to be influenced by egg size. Based on the egg index (length of egg/length of mantle  $\times$  100), octopus hatchlings can be classified into two types, planktonic and benthic. Species having small eggs usually have planktonic hatchlings, while species having large eggs produce benthic hatchlings. The egg index of *O. aegina* is about 4% and hatchlings lead a planktonic life during early stages, before they settle down for benthic living. During the planktonic phase, growth of the arms was faster than that



**Figure 3.** *a*, Release of hatchlings. *b*, Octopus hatchling.

of the body. When the arms are long enough, the larvae settle for benthic life. It is noted that the species having a planktonic phase in their life cycle have a better distribution than others. This is true in the case of *O. aegina*, which enjoys a wide distribution from Japan to India. The present study revealed that many characters of the life history of *O. aegina* are similar to those of other octopus species studied so far, including brooding behaviour, developmental process for the embryo, etc. More studies are required for the rearing of larvae, especially on the type of feed required for the planktonic phase. This animal is found suitable for captive rearing and perfecting the technology for large-scale rearing will be helpful in increasing production.

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