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EMBRYONIC DEVELOPMENT OF *AMPHIOCTOPUS MARGINATUS* (TAKI) (MOLLUSCA: OCTOPODIDAE) IN CAPTIVITY

Sreeja, V.,[1] Anushma, K. and Biju Kumar, A. [2]

1Dept. of Aquatic Biology & Fisheries, University of Kerala, Thiruvananthapuram 695 581, Kerala, India.

E-mail: [2]abiju@rediffmail.com; [1]sreejaaqb@gmail.com

Abstract: Octopuses, represented by over 300 species are the cephalopod molluscs inhabiting primarily tropical waters of the world. They are solitary invertebrates with little documentation of their response behaviour. Octopus is a dioecious animal with internal fertilization. The veined octopus *Amphioctopus marginatus* (Taki) is a medium-sized cephalopod reported to exhibit the unique behaviour of using coconut shells for making their homes and hence often referred to as the 'coconut octopus'. *Amphioctopus marginatus* was collected from the Vizhinjam Bay in Thiruvananthapuram district of Kerala and acclimated to the aquarium conditions for recording their behaviour in captivity. The female specimen in captivity selected coconut shell for egg laying and performed parental care by continuously cleaning and aerating her eggs with her arms and by squirting jet of water over it. For the first time the embryonic development of the species was recorded in captivity. Developmental stages of the embryo was analysed based on the morphological characters. Development of chromatophores and its migration to various body parts and reduction in the size of the yolk sac was recorded stage by stage. *Amphioctopus marginatus* have planktonic hatchlings. The gestation period of the embryo was 17 -19 days at a temperature range of 28 - 30^{0}C and pH 7.8-8.1.

Keywords: Octopus, festoon, chromatophores, embryo, development, captivity

Octopus is a marine cephalopod mollusc inhabiting mostly tropical and subtropical seas around the world, in diverse habitats including coral reefs, pelagic waters and the ocean floor and play a critical role in marine ecosystem functioning. Mating in these primarily bottom dwelling bisexual creatures occurs when the male transfers sperm into the body cavity of the female through a specialized hectocotylized arm. In octopuses eggs accumulate in the coelom in small or large quantities and are released in single spawning; females guard the eggs until they hatch, starve and usually become sensile and die. Hatchlings lead planktonic life initially and then sink to the bottom. Majority of octopuses are semelparous, laying all eggs in one brood at the end of the life cycle. Poor economic viability (Berger, 2010) and absence of standardised technologies for larval rearing notwithstanding, various species of octopods are now cultured on pilot scale for biomedical research (Hanlon and Forsythe, 1985).

The life history of these ubiquitous creatures is characterized by short life spans, rapid growth, reaching relatively large body sizes compared to other invertebrates, early maturity and little overlap in generations. Their growth is influenced by various factors such as water, temperature, food availability, size and species (Osborn, 1995). But researchers recognized the inadequacy of defining stages of embryonic development in time intervals, because development time may be dependent upon various environmental conditions, especially temperature. The octopus species can be identified based on the size of the eggs, as there are large egg and small-egg species of octopuses. Detailed embryonic development has been reported for many large

species of octopus species such as *Octopus joubini,* (Boletzky and Boletzky, 1969; Opresko and Thomas, 1975), *Eledone moschata* (Luis *et al.,* 2004) and *Hapalochlaena maculosa* (Dews, 1959) and for species producing small eggs such as *O. dofleini* (Gabe, 1975), *O. cyanea* (Dews, 1959), *O. tetricus* (Joll, 1976, 1978), *O. bimaculatus* (Ambrose, 1981), *O. burryi* (Forsythe and Hanlon, 1985), *Scaeurgus unicirrhus* (Boletzky, 1977, 1984), *Eledone cirrhosa* (Mangold *et al.,* 1971; Bradbury, 1974).

A female specimen of coconut octopus *Amphioctopus marginatus* (Taki) (Fig. 1) was collected from the Vizhinjam Bay (80^0 22'N; 76^0 57' E), Thiruvananthapuram district, Kerala, from a depth of about 2 m. This animal was transported live to the wet lab and then kept in an aquarium tanks of 120 x 60 x 60 cm size filled with filtered sea water for initiating ethological studies in captivity. The aquarium was covered with fine meshed net and tied at the top to prevent the escape of the octopus. Sea sand was used as the bottom material in the aquarium. The tank water was filtered using protein-skimmers and biological filters. The temperature of water ranged from 26-28^0C and salinity between 33 and 35 ppt. In the aquarium octopus showed hiding behaviour and coconut shells were given as the hiding material, based on previous records of Finn *et al.* (2009). The octopus was given fresh marine fish and crabs as food.

The octopus kept in captivity laid eggs inside the coconut shell (Figs. 2, 3). The behaviour of octopus was recorded during the period.

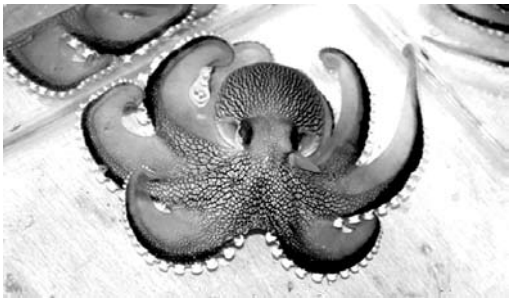


Fig. 1. *Amphioctopus marginatus* (Taki) – Live specimen

A part of the egg cluster was removed from the coconut shell for studying the embryonic development. All the glass troughs were covered by black paper to reduce light from outside. Eggs were collected from the egg mass every day, observed under stereo dissecting microscope in petri dishes, photographed under stereo zoom microscope. The octopus died in the eighth day after egg laying. The observation continued for 17 days till the free swimming larvae emerged. The observation could not be continued because of the mortality of free swimming larvae.

The eggs consist of an ovoid egg capsule, moderately long stalk and a bulb which attach

Fig. 2. *Amphioctopus marginatus* (Taki) – Usage of coconut shell for egg laying

Fig. 3. *Amphioctopus marginatus* (Taki) – A portion of festoon

the egg with the substratum. The eggs were laid by intertwining and cementing the long chorion stalks of the egg capsules together to form a string, a festoon of eggs (Fig. 4). Each festoon is cemented at one end to the substratum. A total of about 22 festoons were collected from the coconut shell after the death of the octopus and the total number of eggs was around 20,000. A part of the festoon was removed in the first day of egg laying, which facilitated observations and data acquisition on eggs at the same stage of embryonic development. Number of eggs present in the string ranged from 33-45 per 1cm of string. The size of the eggs ranged from 3.3 to 3.7 mm in length. Eggs in the early stages of development were white in colour. The successive development of eggs in different days is described below:

Fig. 4. *Amphioctopus marginatus* (Taki) – Festoon inside coconut shell

Day 1 (Fig. 5): Eggs were clear inside with narrow perivitelline space along the periphery. Small micropyle present and attached to the yolk at the animal pole.

Day 2 (Fig. 6): Micropyle length increased, it continued as a thin layer of cytoplasm around the egg yolk. Yolk mass slightly increased compared to the day 1. Clear cytoplasm present.

Day 3 (Fig. 7): Area of cytoplasm increased, micropyle length increased. Yolk mass increased.

Day 4 (Fig. 8): Micropyle length and cytoplasm content increased. Extra embryonic ectoderm began to spread over the yolk. Development of cephalic organs began.

Day 5 (Fig. 9): Micropyle length increased, cap-like yolk sac enlarged steadily and eye development started.

Day 6 (Fig. 10): Embryo turns around the egg. The posterior region of the embryo faces towards the stalk instead towards the micropyle. Development of cephalic region started.

Day 7 (Fig. 11): Cephalic organ development increased. Formation of major organ primordia. Eye region prominent.

Day 8 (Fig. 12): A pair of orange eye spots appeared on the embryo. Mantle development increased. Rotation of embryo inside the egg was noticed. This rotation helps exchange oxygen and waste between the inner and outer chorion. Embryo showed the pulsatory movement of yolk sac and the cephalic region under stereo zoom microscope. Development of arm crown also started. Covering of the yolk with extra embryonic membrane complete.

Day 9 (Fig. 13): Eye spots became clear and the colour of the eye turned reddish orange, rudiments of mantle, arm and funnel become visible. Pulsatory movement of yolk sac observed.

Day 10 (Fig. 14): Development of arm crown, tentacles, suckers and funnel increased.

Day 11 (Fig. 15): Tentacles, funnel and sucker developed. Funnel distinct. Eye spots visible through the egg case.

Day 12 (Fig. 16): Development of two chromatophores on the ventral side of the head. Eyes prominent. Movement of embryo visible under the stereo zoom microscope.

Day 13 (Fig. 17): Eye spots visible clearly as round blackish spots. Few orange chromatophores in the cephalic region turned to reddish or

brownish. Colour change of chromatophores recorded. Presence of suckers noticed on each arm. Pulsatory movement of embryo was noticed.

Day 14 (Fig. 18): Chromatophore distribution extended to the mantle region, blackish chromatophores in the arm crown, cephalic region and orange coloured chromatophores in the mantle region. Size of the chromatophores increased compared to the earlier stages. Development of tentacles and suckers noticed.

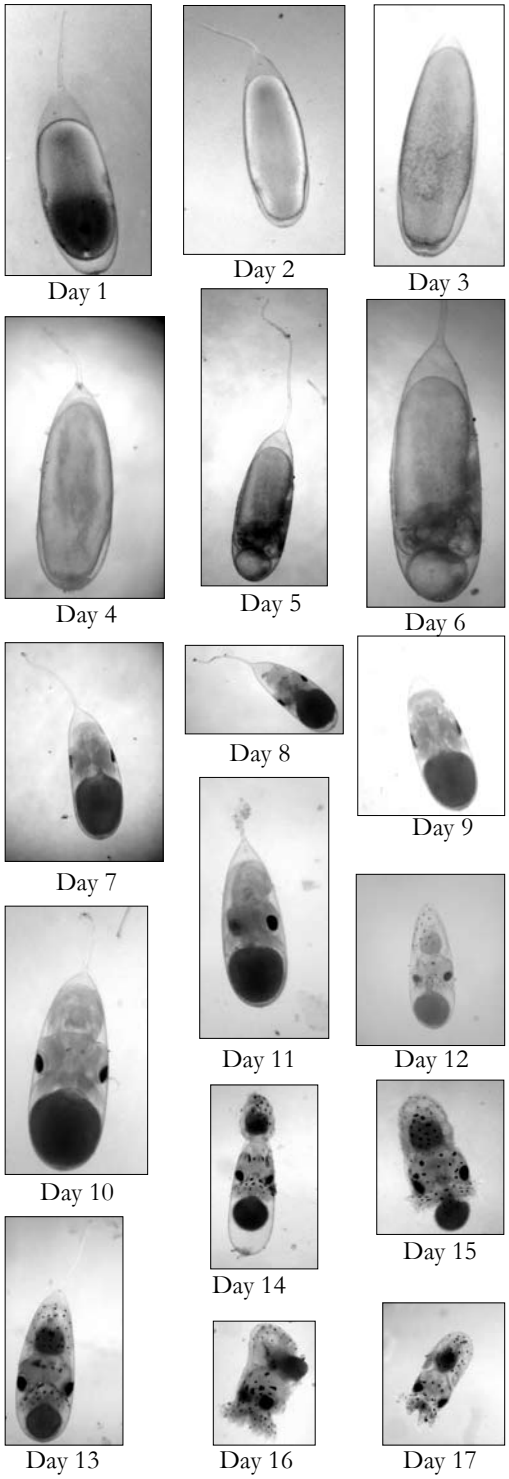
Day 15 (Fig. 19): Wriggling movement of mantle inside the egg case appeared. This is due to the second inversion of the embryo. The embryo is positioned in such a way that posterior mantle tip faces towards the micropyle. When ready to hatch, larvae use the tip of the mantle to break the capsule wall. Development of chromatophores in the ventral head was observed.

Day 16 (Fig.20): Embryo well developed. Outer yolk sac reduced considerably. Size of the chromatophores in the mantle region increased and the mantle turned black. Release of few hatchlings was noticed by the breaking of the capsule wall.

Day 17 (Fig. 21): Release of hatchlings continued. The paralarvae led planktonic existence. Planktonic hatchlings swim actively both forward and backward. Hatchlings resembled adult octopuses in general body pattern. Eyes large and prominent, arms short and stubby. Presence of chromatophores in the funnel region noticed.

The larvae were fed with artemia nauplii. However, further developmental stages were not obtained in the study.

The embryonic development of *A. marginatus* has not so far been recorded. Data on fecundity of octopuses is limited in scientific literature. The available data shows that the octopuses inhabiting benthic, littoral waters fall into three categories: (i) species that produce large eggs (>10mm) and



benthic young; (ii) species that produce small eggs (<6mm) and long duration planktonic young (Boletzky, 1974); and (iii) species that produce medium-sized eggs (6-10mm) and short duration planktonic young. This result showed that *A. marginatus* falls into the second category. 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 *A. marginatus,* which enjoys a wide distribution in Indo-Pacific from Japan to India. However, the hatching time of 17 days duration recorded during the present study was short and the data on the period of planktonic existence could not be collected during the study.

The captive breeding and embryonic development of coconut or veined octopus *A. marginatus* is reported for the first time. Octopuses belonging to suborder Incirrina lay eggs without gelatinous envelope around the egg chorion, while in the benthic octopuses 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 (Boletzky, 1998). *Amphioctopus marginatus* also laid eggs as festoons and the fecundity was around 20,000. Most females spawn only once and the females guard the eggs throughout the development period, after which the females die.

Ignatius and Srinivasan (2006) reported that the embryonic development of *O. aegina* collected from Indian coastal waters was completed in 18–20 days at 28°C. Environmental factors like water temperature play an important role in the development of embryo in various octopus species (Mangold and Boletzky, 1973). The average temperature maintained for rearing *A. marginatus* in this study was about 28^0C and the pH range 7.8- 8.1. Embryonic development of *A. marginatus* resembled the development of other species of octopuses (Joll, 1976; Warnke, 1999; Boletzky *et al.,* 2001) 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.

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 *A. marginatus,* which enjoys a wider distribution from Japan to India. The present study revealed that many characters of the life history of *A. marginatus* are similar to those of other octopus species studied so far, including brooding behaviour, developmental process for the embryo, etc.

Hochberg and Fields (1980) recorded that the life cycle of *Octopus rubescens* is completed annually, with embryonic development time of 6-8 weeks at 14^0C. The temperature of water during the present study was 26-28^0C, which is higher than the temperature maintained for embryonic development in other studies. This indicates that the embryonic development of octopods in Indian coastal waters may be shorter than that of species inhabiting temperate waters. The burgeoning demand for octopuses notwithstanding, much information is not available on the reproduction and embryonic development of octopods inhabiting the coastal waters of India. Research to update present knowledge on the reproductive biology of octopods should be given priority, as the exploitation of octopuses are on the increase during high demand from the market, not to speak of their pivotal role in marine biodiversity. Absence of standardised paralarval rearing system and lack of information on behavioural and nutritional requirements of octopus paralarvae, are the major handicaps in achieving culture of octopuses.

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