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Ethological studies of the Veined Octopus *Amphioctopus marginatus* (Taki) (Cephalopoda: Octopodidae) in captivity, Kerala, India

V. Sreeja [1] & A. Bijukumar [2]

[1,2] Department of Aquatic Biology & Fisheries, University of Kerala, Thiruvananthapuram, Kerala 695581, India
[1] sreejaaqb@gmail.com, [2] abiju@rediffmail.com (corresponding author)

Abstract: Five Veined Octopus *Amphioctopus marginatus* (Taki), collected from Vizhinjam Bay in the Thiruvananthapuram District of Kerala, India were kept in aquariums to study their behaviour in captivity. Primary and secondary defence mechanisms studied included crypsis, hiding and escape behaviour. Deimatic behaviour was used by captive animals when camouflage failed and they were threatened. Crawling behaviour to escape from the aquarium was observed in all specimens. Stilt walking and bi-pedal locomotion were also observed. As a defence behaviour, *A. marginatus* used aquarium rocks to protect the soft underside of their bodies. *A. marginatus* demonstrated tool use of coconut shells to make protective shelters, carrying the shells for future use. A female specimen also selected a coconut shell for egg laying and performed parental care by continuously cleaning and aerating her eggs with her arms and by squirting jets of water over the eggs.

Keywords: Behaviour, crypsis, India, octopus, tool use.

Octopuses are solitary animals that are considered advanced among invertebrates because of their demonstrated learning abilities (Nixon & Young 2003) and complex nervous system, including a central brain with areas associated with learning and complex personality behaviours (Sinn et al. 2001). The discovery of playful octopuses (Mather & Anderson 1999; Kuba et al. 2003) is an important step towards understanding the phylogenetic origin and function of play, as well as the cognitive abilities of invertebrates. The report on existence of personalities in octopuses (Mather & Anderson 1993) is further proof of their complex behaviour. The octopus is the only invertebrate which has been shown to use tools and is considered as a benchmark for cognitive sophistication (Finn et al. 2009).

Octopuses are dioecious animals with internal fertilization. Breeding occurs seasonally. Mating has been considered opportunistic, indiscriminate and almost devoid of complex behaviour (Hanlon & Messenger 1996). Mating occurs when the male transfers sperm into the body cavity of the female using a specialized hectocotylized arm. Eggs are usually protected by the female. The female usually attaches egg strings to substrates on rocky shores, in a hole, den or sheltered place. During the incubation period the female ventilates and protects the eggs until they hatch. During this time she usually remains sessile, starves and dies. Through the study of captive Veined Octopus *Amphioctopus marginatus*, we aimed to undertake a quantitative analysis of locomotory patterns and characterize: (i) defensive responses under controlled conditions, (ii) tool use behaviour, and (iii) breeding behaviour in captivity.

Materials and Methods

Amphioctopus marginatus (Image 1) is a medium-

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Competing Interest: None.

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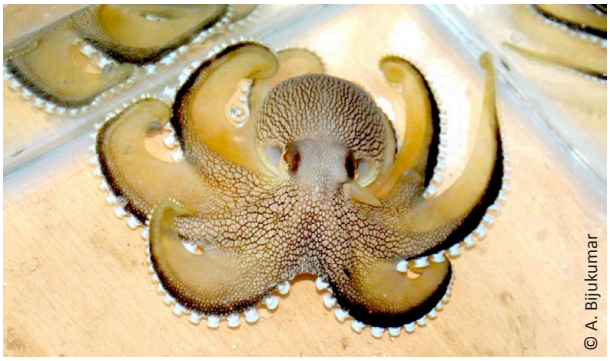

Image 1. *Amphioctopus marginatus* (female) in aquarium tank

sized octopus found in the tropical western Pacific and coastal waters of the Indian Ocean. Two female and three male specimens of *A. marginatus* (ML 110–113 mm; TL 340–345 mm; weight 200–250 g) were collected live from Vizhinjam Bay ($8^0$22'N & $76^0$15'E), Thiruvananthapuram District, Kerala State, India. The specimens were collected with the help of local fishermen from a depth of approximately 2m. The collected octopuses were transported live to the laboratory and kept individually in fully set glass tanks of 120x60x60 cm size. An escape proof plexiglas lid was used to cover the tanks. The substrate used was sea sand with plenty of building materials and rocks so that the octopuses could establish their dens at a place of their choice. The tank water was filtered using protein-skimmers and biological filters. Observations were made for a period of six months. Behavioural responses, locomotory patterns, tool use behaviour and breeding behaviour were recorded in captivity and documented with the help of videos (videos 1–3) and photographs.

Results

Locomotion: Observation on the locomotory patterns of *Amphioctopus marginatus* in captivity showed stilt walking and bipedal locomotion (Images 2, 3). In bipedal locomotion the octopuses used two arms for locomotion and the remaining arms to provide camouflage. In stilt walking the arms were used as rigid limbs. These movements are distinctly different from their normal crawling, which usually involves several arms sprawling around the body, using the suckers to push and pull the animal along.

Defence: *A. marginatus* showed primary and secondary defensive mechanisms in captivity. In their natural environment the defensive behaviour is used for predator avoidance. Primary defensive behaviours shown by the animal in captivity included camouflage

Image 2. *A. marginatus* (male) showing stilt walking

Image 3. *A. marginatus* (male) preparing for bipedal locomotion

Image 4. *A. marginatus* (male) displaying flashing of colours

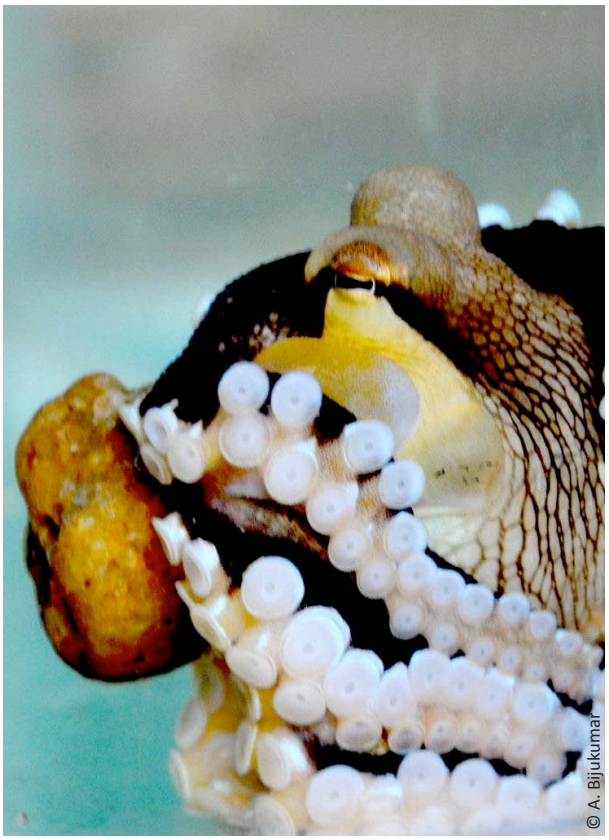

Image 5. *A. marginatus* (female) protecting the soft underside of the body with rock piece

Images 6, 7, 8. *A. marginatus* (female) selecting coconut shell, manipulating the two shell pieces and making a perfect home using the shells

(chromatophore based skin display) and crypsis in motion. Crypsis in motion is the phenomenon whereby an animal's colour pattern, behaviour, or other features help it blend in with the background (Huffard et al. 2005). The main primary defence of the octopuses recorded in captivity was hiding; they hid in small, tight places, made possible by the absence of exoskeleton. The primary defence of escaping from the aquarium was observed in all specimens.

In captivity *A. marginatus* showed secondary defences, while agitated by objects presented to them. The secondary defences exhibited included flight (swimming/burial responses), jet propulsion (fast escape) and flashing of colour (contrasting colours white and dark brown) (Image 4). The most common secondary defence was fast escape. The defensive behavioural study also revealed that *A. marginatus* used aquarium rocks to protect the soft underside of their bodies (Image 5).

Tool use: Observations on the tool use behaviour (advanced cognitive abilities) recorded the use of coconut shells kept in the aquarium by the octopuses as portable armour, protective shelters and homes (Images 6–8). They manipulated two coconut shell pieces to make perfect homes and resided within them as other hiding devices were not made available for them in the aquarium. We observed that they also use coconut shells for egg laying (Images 9,10).

Breeding Behaviour: *A. marginatus* laid eggs as festoons (Image 11) and throughout the brooding period, the female never let go off the egg cluster which

Images 9, 10. *A. marginatus* (female) with eggs laid inside the coconut shell

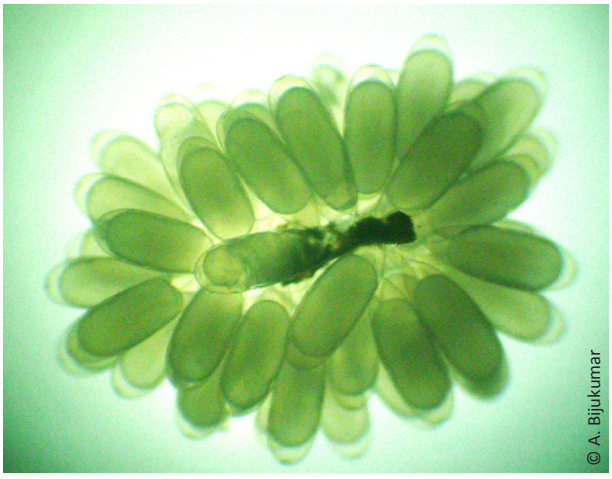

Image 11. Part of egg festoon - a microscopic view

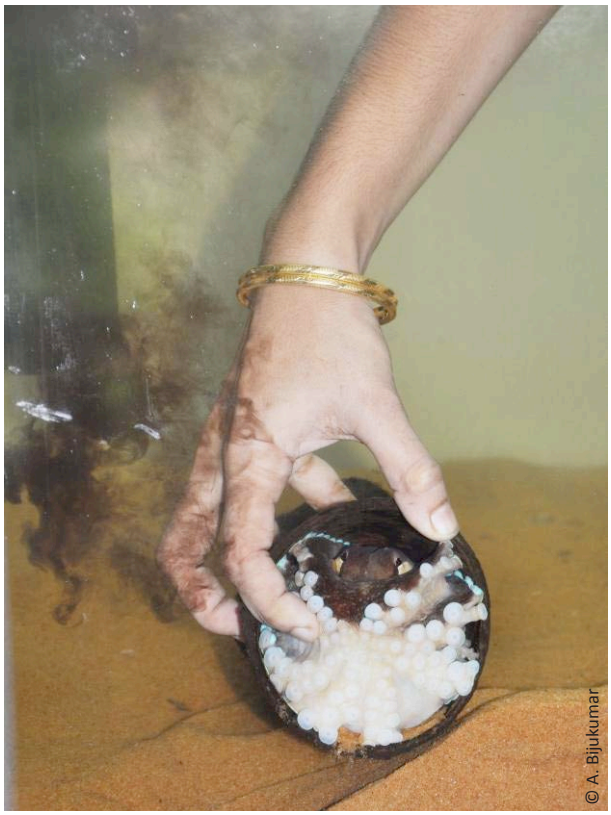

Image 12. *A. marginatus* (female) releasing ink while selected for removing the festoons

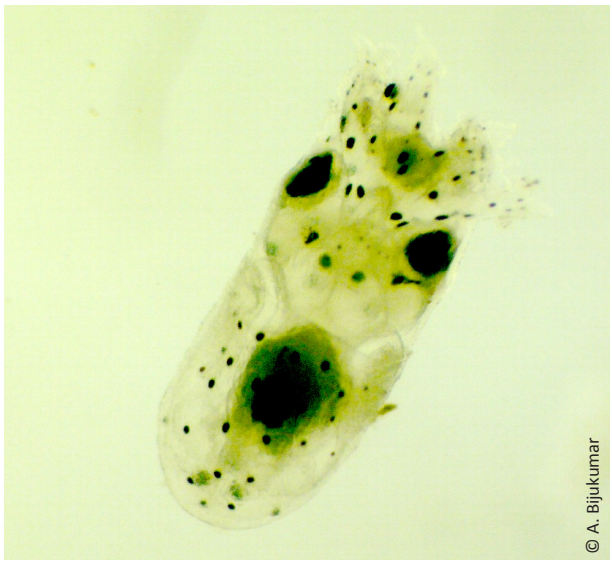

Image 13. Paralarvae hatched out from the egg on 16th day.

was held close to the body on the aboral side. The female became more active and vigilant during brooding and took extreme care to remove anything that came near the spawn. The octopus squatted directly over

the eggs, holding the coconut shell firmly with few suckers and waving free arms across the egg sheet, thus constantly aerating the eggs. Usually, two or more arms were continuously swept over the eggs with slow, radial sweeps and with slow undulating movement of the arms. The octopus also tended to keep particles of debris and micro-organisms from aggregating on the eggs by using the arms and water jets created by the funnel. When the eggs and shell were removed, the octopus was observed to release ink (Image 12). The octopus also attempted to repel approaching objects by darting strong jets of water through the funnel.

On the first day of egg laying the octopus accepted food, whereas it avoided food subsequently. A small part of the egg mass was removed from the coconut shell and kept in separate troughs with filtered and aerated sea water to study the development of the eggs. The brooding octopus died on the eighth day after egg laying. The eggs consisted of an ovoid egg capsule, moderately long stalk and a bulb which attach 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, or festoon, of eggs (Image 11). 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 estimated at around 20,000. The number of eggs present in each string ranged from 33–45/ cm of string. The size of the eggs ranged from 3.3–3.7 mm in length.

Release of a few hatchlings was noticed by the 16th day and the paralarvae were observed to lead a planktonic existence. Hatchlings resembled adult octopuses in general body pattern (Image 13). The larvae were fed with *Artemia nauplii*. Further developmental stages were not obtained in this study.

Discussion

The Coconut or Veined Octopus *Amphioctopus marginatus*, is an under-studied, medium-sized cephalopod common in the coastal waters of India. In natural waters elsewhere in the world, *A. marginatus* is reported to display unusual behaviour, including bipedal walking and gathering and using coconut shells and seashells for shelter (Finn et al. 2009). This study revealed that these behavioural patterns are also exhibited by *A. marginatus* collected in India, when maintained in captivity.

Amphioctopus marginatus achieves bipedal locomotion despite having only a hydrostatic skeleton, although this mode of locomotion has often been thought to require the opposition of muscles against a rigid skeleton (Huffard 2006). *Amphioctopus marginatus* and *Abdopus aculeatus* are the only species of octopods reported to show bipedal behaviour. This behaviour was first recorded in Sulawesi, Indonesia, where the sandy bottom was littered with coconut shells and it was reported to be to mimic a floating coconut (Huffard et al. 2005).

An octopuses' camouflage is aided by certain specialized cells which can change apparent colour, opacity, and reflectiveness of the epidermis; the colour changing ability can also be used to communicate with or warn other octopuses (Wells 1978). *Amphioctopus marginatus* made themselves cryptic by modifying their physical appearance while in captivity.

Crypsis, which can be achieved in a variety of ways, is the primary defence against predators used by most shallow-water octopuses, although there is some evidence that whether they choose to make themselves cryptic by modifying their physical appearance and/ or behaviour may depend on their assessment of the level of threat in their environment (Hanlon 1999). Octopuses are able to modify their appearance and behaviour in such a way that they seem, at least to human observers, to resemble swimming fish, rocks, or other familiar objects in their habitat (Hanlon 1999). In captivity *Amphioctopus marginatus* were observed to use two arms for locomotion and the remaining six arms to provide camouflage.

The coconut shell carrying behaviour is likely to have evolved using large empty bivalve shells prior to the relatively recent supply of the clean and light coconut shell halves discarded by the coastal human communities adjacent to the marine habitat of this species (Finn et al. 2009). The present study shows that *A. marginatus* may also employ this behaviour in captivity in addition to using coconut shells for brooding, reported here for the first time. While examining *A. marginatus* in natural waters Norman (2000) observed that individuals of this species often carry around coconut shell halves, assembling them as a shelter only when needed. Finn et al. (2009) recorded that these shells offer no protection while being carried and, in fact, appear to be a burden as they force the octopus to use a novel and awkward form of locomotion which the authors describe as "stilt-walking". They argued that this is the first known example of tool use by an invertebrate, if part of the definition of a tool is that it provides no benefit until it is used for a specific purpose. They further propose the fact that the shell is carried for future use rather than as part of a specific task which differentiates this behaviour from

other examples of object manipulation by octopuses, such as rocks being used to barricade lair entrances. Furthermore, the necessity to correctly assemble the separate parts (when transporting two shells) in order to create a single functioning tool sets this example of tool use apart from most or all examples previously known for invertebrates.

The embryonic development of *A. marginatus* is recorded for the first time during the present study. 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 (6–10 mm) 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 more widespread distribution than those with benthic young. This is true in the case of *A. marginatus*, which enjoys a wide distribution from Japan to India. While this study revealed that the egg development time of this species is short, 17 days, data on the period of planktonic existence could not be collected during this study.

Octopuses inhabit many diverse regions of the ocean and play a critical role in marine ecosystem functioning. Moreover, they are economically important as a preferred item in marine fisheries export from India. Despite the burgeoning demand for octopuses, not much information is 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 status, because of their increasing exploitation, high economic value and importance as a key element in marine biodiversity.

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Video 1. *Amphioctopus marginatus* showing flashing of colours and jetting of water

Video 2. *Amphioctopus marginatus* making a shelter in captivity using two coconut shell pieces

Video 3. *Amphioctopus marginatus* discarding food while guarding the eggs (also note cleaning of the eggs through movement of arms)