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Studies on Egg Morphology, Availability and Hatching of Four Species of Cephalopods along Vizhinjam Coast, Kerala

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Abstract:
Availability and characteristics of cephalopod egg mass along the Vizhinjam coast was studied and peak availability of egg mass of squids Uroteuthis (Photololigo) duvaucelii, Sepioteuthis lessoniana, was noticed during August to October, whereas, the peak availability of egg mass of cuttlefish Sepiella inermis, and Sepia pharonis was noticed during December to February. Egg clusters of Uroteuthis (Photololigo) duvaucelii, Sepiella inermis, and Sepia pharonis were obtained from shore seine catches, whereas the egg mass of Sepioteuthis lessoniana was found attached to coconut tspadices and nylon ropes hung from rafts moored at Vizhinjam bay. Eggs of Uroteuthis (Photololigo) duvaucelii were encased in gelatinous, whitish, transparent, club shaped capsules. The egg capsule measured 4.5 - 6cm in length and 1.5-2cm in width, contained 120-130 eggs. It took total of 8 days for complete hatching. Egg cluster of Sepioteuthis lessoniana consisted 50-70 creamy white, slender, finger like capsules. Length of the egg capsules ranged from 5.3-9.2cm, whereas, the width 1.2-2.2cm and contained 4 to 9 eggs organised in a well-spaced single row. Maximum period of incubation was 21 days and hatching period was 17 days. The eggs of Sepia pharonis were creamy white coloured, opaque and lozenge or ovoid in shape. The length of the capsules on the first day was 2 -2.5cm and width was 1.4 -1.8 cm. Total incubation period recorded in case of Sepia pharonis was 22 days and period of hatching was 11 days. The eggs of Sepiella inermis were ovoid, lozenge shaped, with a proximal thin stalk and a conical protruberance at its tip. Eggs of Sepiella inermis were externally covered by a multilayered elastic, gelatinous coating and it was pigmented black. Maximum incubation period of S. inermis was16 days and hatching period was 5 days.

Keywords: *Availability, Hatching, Incubation period, Morphology, Uroteuthis (Photololigo)duvaucelii, Sepioteuthis lessoniana, Sepiella inermis, Sepia pharonis*

1. Introduction

Squids, cuttlefishes, octopuses and chambered nautiluses of class cephalopoda occur in all marine habitats of the world; benthic, cryptic or burrowing on coral reefs, grass flats, and mud and rocks; epibenthic,pelagic and epipelagic in bays, seas and open ocean. They can be found along the depths extends from surface to 5000m (Clyde et al.,1984). They are bilaterally symmetrical, highly organized with well developed head and contains a circumoral crown of mobile appendages that bear suckers(except in nautilus). They show exponential growth during younger stages, but they have short life span. All cephalopods are dioecious (separate sexes) and many exhibit external sexual dimorphism. Most of them are semelparous. In the majority of cephalopods, mating behavior or egg laying is followed by death (Giese et al.,1977).

In the total landings of molluscans (42,769t) along the Kerala coast during 2013, cuttlefishes and squids were the major contributors. Cuttlefish formed 47.8% (20,385t) of the total molluscan landings. *Sepia pharonis* contributed 90.9% of the total cuttlefish catch. Other species were *Sepiella inermis*(3.3%), *Sepia elliptica* (3.1%) and others(1.8%). Squid landings formed 14,389t (37.7%) of the total landings. Catch was represented mainly by *Uroteuthis (Photololigo)duvaucelii* (59.8%), *Uroteuthis(Photololigo)singhalensis* (23.5%), *Uroteuthis(Photololigo)edulis* (20.2%), *Sepioteuthis lessoniana* (14.4%) and others formed 2.3%(CMFRI, 2013).

The exploited cephalopods at Vizhinjam coast included *Sepia pharonis, Uroteuthis (Photololigo) duvaucelii, Uroteuthis(Photololigo)singhalensis, Uroteuthis (Photololigo) edulis,Sepia pharonis, Sepioteuthis lessoniana and Sepiellainermis.*

The present study focuses on the availability, morphology and hatching of eggs of two cuttlefish species (*Sepiella inermis* and *Sepia pharonis)* and two squid species *(Uroteuthis (Photololigo) duvaucelii* and *Sepioteuthis lessoniana*) found along Vizhinjam coast,

Kerala. Egg clusters for the study were collected from the fishing grounds off Vizhinjam coast. Cephalopods lay their eggs on substrates such as sea weeds, twigs, branches, rocks, pieces of ropes, coconut spadix, remains of fishing nets, shells etc. Some are less dependent on solid surfaces for egg laying, and may simply insert their egg into sandy or gravel sediment. A few squids notably Branchioteuthis spp and members of the Enoploteuthidae, are known to release their eggs singly into water (Young *et al*.,1985).

The loliginid genus Sepioteuthis shows some external similarities to cuttlefish, but can be easily distinguished from them by the presence of a gladius in the dorsal mantle rather than a chalky cuttlebone with a posterior calcareous spine. Eggs of squids generally, are encased in a gelatinous matrix and are laid as multi-finger like masses (sometimes called "sea mops") attached to rocks, shells, other substrates in the bottom in shallow waters (inshore squids),or they are extruded as large, singular, sausage shaped masses, that drift in the open sea (oceanic squids)(Clyde *et al.*,1984).Owing to the shape of egg masses of squids, they have been known for centuries as "dead man's fingers"(Kotpal,1978). The fingers each may contain from a few to several hundred eggs, while the sausage shaped egg mass contain tens or even hundreds of thousands of eggs.

Cuttlefishes lay relatively few large grape like eggs that are attached to hard substrates and are usually coloured black or creamy white. The masses of eggs are attached by stalks to a twig of sea weeds or any similar objects and form the characteristic bunches of "sea grapes".The eggs are larger and developing embryos feed on large amount of stored yolk. The young ones that hatch out are like the adults (Kotpal,1978).Cephalopod eggs are very yolkyand hence cleavage is incomplete, so that the typical molluscan spiral cleavage is absent(Clyde *et al*., 1984).Period of embryonic development may vary from a few weeks to several months depending on species and temperature. Being one of the most economically important resource, it is of much importance to study the availability of their egg masses for understanding the biology and prospects of culture.

Eggs of most cephalopod species are morphologically dissimilar. So, it is possible to distinguish each species by analyzing the morphology of eggs. Studies related to seasonal availability of egg mass and egg morphology are scanty. But several studies were conducted by various authors on growth, development and biology of cephalopods (Choe and Oshima,1963;Choe,1966; Nabhitabhata,1994; Sivalingam,1999; Anil, 2003,2005;Minton et al.,2001; Nair *et al*.,1985).

2. Materials and Methods

The present study was conducted along the Vizhinjam Coast ($8^0$22'45"N Latitude and $76^0$59'29"E Longitude), situated on the Southwest coast of Kerala, India. Fortnightly observations were made to study the availability of egg masses in shore seines along the coast. In addition objects like coconut spadix, old net pieces, and nylon ropes were hung from the rafts moored at Vizhinjam bay for studying the availability of egg mass.

The collected eggs were immediately placed in plastic containers containing sea water (Salinity-32ppt-35ppt, temperature-27^0C-30^0C) and transported to hatchery of Vizhinjam research centre of CMFRI for further studies. Continuous aeration was given using battery operated aerators during conveyance. After taking photographs, the egg masses were placed in PVC tanks containing 1000l filtered and aerated sea water for incubation. The incubation tanks were maintained with optimum level of water quality parameters for rearing. Constant aeration was given inaddition to the biological filtration unit provided in the tank. The egg bunches were kept suspended above the aeration point by tying them to a horizontal steel rod with the help of a soft twine. This ensures uniform hatching and oxygenation of egg clusters. Siphoned out 25% of water every day without disturbing the egg clusters and refilled with filtered sea water.

Water quality parameters such as temperature, salinity, pH, ammonia, nitrate and dissolved oxygen were checked regularly in each tanks. Ammonia level was measured by phenolhypochlorite method (Solorzano,1969), Nitrate level using Nitrate testing kits (MERCK) by adopting photometric method (NOVA 60, Spectroquent) and dissolved oxygen was measured by Winklers method (Winkler, 1888). Hatching percentage, developmental and morphological changes of egg clusters were recorded daily.

3. Results

Egg clusters of four species of cephalopods namely Uroteuthis *(Photololigo)duvaucelli, Sepioteuthis lessoniana, Sepiella inermis, and Sepia pharonis* were collected during the study. Peak availability of egg mass of squids *Uroteuthis (Photololigo) duvaucelli, Sepioteuthis lessoniana,* was noticed during August to October, whereas the peak availability of egg mass of cuttlefish *Sepiella inermis and Sepia pharonis* was noticed during December to February. Eggs of *Sepiella inermis and Sepia pharonis* were relatively fewer in number and large as compared to *U(P)duvaucelii* and *Sepioteuthis lessoniana* and were laid as "lozenge"or "grape" like capsules and each capsule has a conical projection at its free end and a stalk at its proximal end. They were individually enclosed in a tough, elastic, protective external gelatinous coating. Eggs of loliginid squids were fingerlike, each finger contained few to several eggs and were ensheathed by mucous or jelly secretion. The proximal slender end of the cluster was found attached to the substratum.

Salinity of the hatching tank was maintained between 33-35ppt, temperature varied from 27^0C-30^0C, pH maintained between 7.7-8.2, Ammonia level was kept very low, 0.01 mg/l. Nitrate levels were kept in a range of 0.1±0.01mg/l. Dissolved oxygen was kept in a range of 5-7mg/l. No of eggs per bunch, period of incubation, period of hatching and hatching percentage *(fig.4)* varied considerably between all the four species. After organogenesis completed, yolk got fully absorbed and larvae hatched out from the gelatinous layers as miniature adults.

3.1. Uroteuthis (Photololigo)duvaucelli,Orbigny,1848

Peak availability of eggs of *Uroteuthis (Photololigo) duvaucelii* was noticeable during August to October. During the present study eggs were obtained from shore seine operations. Eggs were whitish transparent (*Fig.1a & 1b*)and were encased in a translucent gelatinous matrix and found attached to an old nylon fishing net. Narrow slender proximal ends of each capsules were twisted together and formed a bunch or cluster. Distal end was broad, this gives the capsule an elongated, "club" like shape. It was noticed that all the eggs in each capsule were in different developmental stages. Eggs present on the distal free end hatched out early.

Of the two bunches analysed, one bunch had 41no of capsules and other had 47 number of capsules. Length of the capsule varied from 4.5 – 6cm and width varied from 1.5-2cm and each capsule contained 120-130 eggs. Eggs were very small as compared with cuttlefish eggs. Most of the eggs were in their early stage of development, here the egg diameter ranged between 1.7-1.9mm with chorionic covering and embryonic diameter ranged between 1-1.1mm (*fig.1b*). In some of the capsules, eggs were in the early stage of division, cleavage was visible through microscope. Some of the eggs were in the early stages of organogenesis and the organ buds were visible under the microscope, here the embryos were much elongated. The length of the elongated embryos ranged between 1.5-2.2 mm. Chorionic membrane expanded as embryo proceeded to hatching, this membrane protects the embryo from desiccation and damage. The diameter of the chorion measured at this stage varied from 2-2.4mm and it was due to imbibing of water.

A cluster with 41 capsules containing 5066 eggs were kept in a PVC tank of 1000l capacity containing filtered seawater. Continuous aeration was given. Hatching began from the second day and proceeded upto 8 days, producing 3611 hatchlings. Hatching percentage was 71.3%. Most of the hatchings occurred at 6thday (47.63%). Most of the unhatched eggs were found infested with ciliates.

Figure 1a: Egg cluster of U(P)duvaucelii.

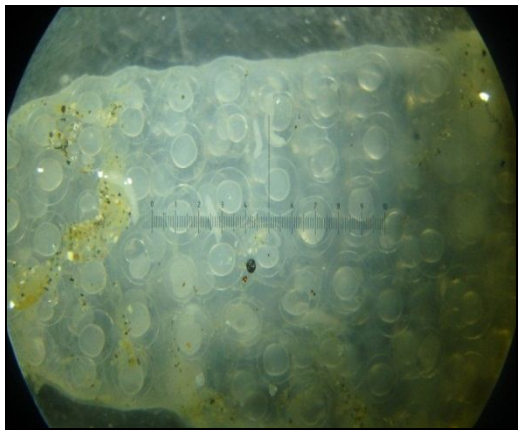

Figure 1b: Microscopic view of one capsule

3.2. Bigfinreef squid or Oval squid(Sepioteuthislessoniana ,Lesson ,1839)

Availability of eggs of *Sepioteuthis lessoniana* were noticed from March to September with peaks in August and September. Egg capsules were creamy white in colour, finger like and slender (*fig.2a*). Clusters of eggs were found attached to coconut spadices and nylon ropes tied in rafts moored in the Vizhinjam Bay. Eggs were found attached as bunches. Each bunch consisted 50-70 healthy capsules. Each capsule was of length 5.3-9.2cm, and width 1.2-2.2cm. Each capsule contained 4 to 9eggs organized in a well spaced single row. Distal end of each capsule was enlarged and more transparent than proximal end. Proximal viscid, slender stalk was entwined together and formed a radiating cluster. Four bunches containing 1485 eggs were kept in 1000 litre PVC tanks for hatching. Hatching started from the fifth day onwards. 86% (1262 nos) of eggs were hatched out and 223 remained unhatched, most of the unhatched eggs were undeveloped and remain whitish in colour (*fig.2b*) inside the capsule throughout hatching period, some eggs were damaged due to parasitic worms. Period of hatching was 17 days, and the maximum incubation period was 21 days. Most of the hatchings occurred on the 10th day (39.1%) of incubation.

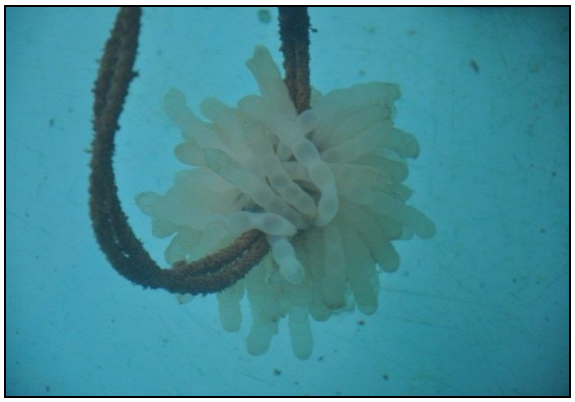

Figure 2a: Egg bunch of Sepioteuthis lessoniana.

Figure 2b: Egg capsules

3.3.Pharoah cuttlefish (Sepia pharonis,Ehrenberg ,1831)

Availability of egg mass of S. pharonis was noticed during post monsoon months (October-April) and peaked during December to February. During the present study egg clusters were collected from a rocky area off Vizhinjam by skin divers. The eggs were creamy white coloured, opaque and lozenge or ovoid in shape with a proximal stalk and a conical projection at its distal round end *(Fig.3a&3b)*. Capsules were singly laid, but their distal stalks connect each other and form the bunch. These stalks were found adhered with the substratum like a piece of fishing net or a twig.

Length of the capsules in the beginning of incubation was 2 -2.5cm and width as 1.4 -1.8 cm. During the incubation period, egg capsules imbibed water and resulted in an increase in length, upto3.5cm and width upto 1.9cm. Embryo could be seen through the multilayered gelatinous protective covering during the last days of incubation. A bunch with 519 capsules were kept in a 1000l PVC tank to study hatching and it was observed that 95% of eggs (493 nos) were hatched out within 22 days. First hatching was noticed at 12thday. Period of hatching was11 days. Peak hatchings were observed on the 16th day (28.2%) day of incubation.

Figure 3a: Egg capsules of S.pharonis

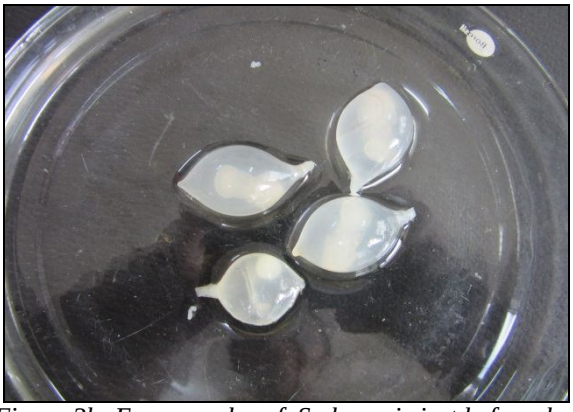

Figure 3b: Egg capsules of S.pharonis just before hatching.

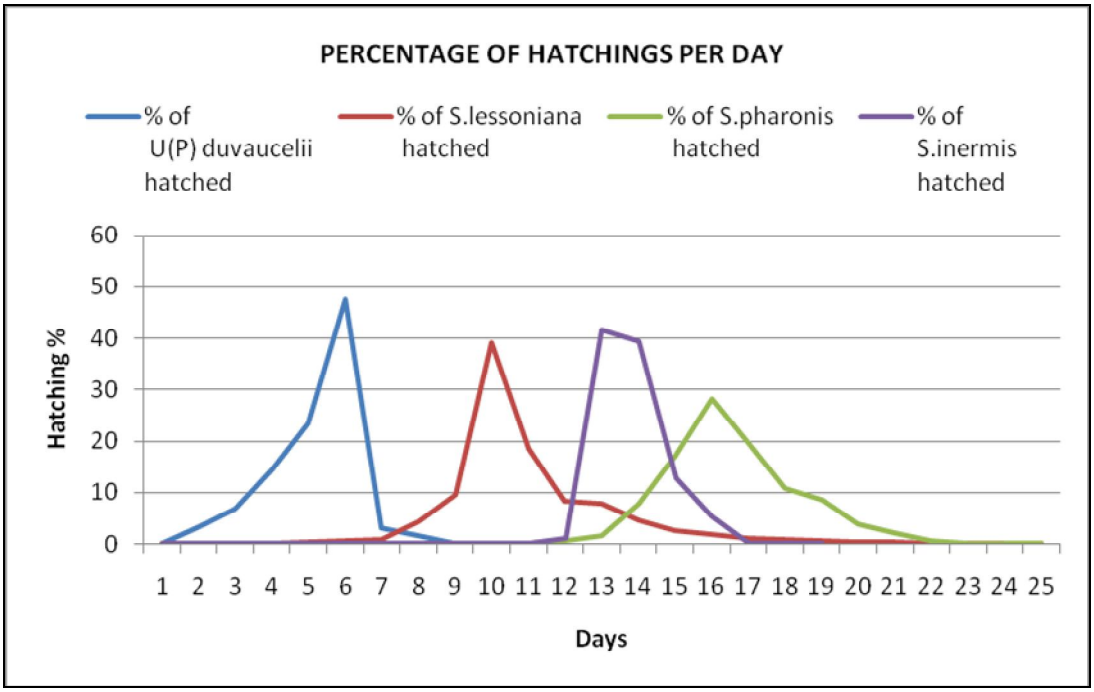

Figure 4: Percentage of hatching of 4 species per day

3.4. Spineless cuttlefish,(Sepiella inermis,Orbigny,1848)

Peak availability of eggs of *Sepiella inermis* was observed at Vizhinjam during the postmonsoon season (December- February). In present study egg capsules *(fig.5a,5b,5c&5d)* were collected from shore seines operated along Vizhinjam coast and were found attached to ropes, twigs or netting. The egg was ovoid, lozenge shaped, with a thin stalk and a conical protuberance at its tip. Eggs were externally covered by a multilayered elastic, gelatinous coating *(fig.5b)* and it was pigmented black. Black pigmentation of eggs gives its characteristic grape like appearance. Long stalk of 192 eggs entwined and formed one cluster, and were kept for hatching on 1000lPVC tank. The eggs of *S.inermis* started hatching from 12th day onwards. Hatching extended for a period of 5 days. Total

incubation period was16 days. Maximum hatching percentage was 98%. Most of the hatchings were noticed on day 13 (41.5%) and day 14(39.2%).

Figure 5a: Egg cluster of S.inermis.

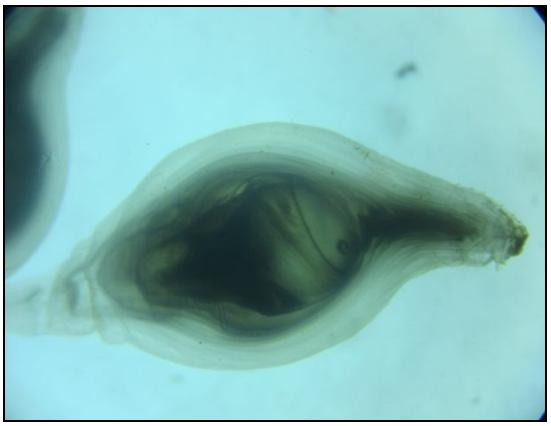

Figure 5b: Egg capsule showing multilayered gelatinous covering

Figure 5c: Egg capsules of S.inermis (1st day)

Figure 5d: Egg capsule just before hatching

4. Discussion

Availabilty of egg mass of four species of cephalopods were observed during the present study. The peak availability of eggs of *Uroteuthis* (*Photololigo) duvaucelii* was noticed during August to October along Vizhinjam coast. Kripa *et al.*(1995) reported the maximum monthly catch of this species in September 1991 as well as in October 1992 and a lower catch were reported during March to May along Kerala coast. This observation may be an indication of migration of this species towards the coastal region for spawning during August to October. Ashokan and Kakati (1991) reported the availability of egg masses of *U (P) duvaucelii* during September at Karwar from shore seine catches. This is in confirmation with the present study. Hatching percentage was 71.3% and most of the hatchings occurred at 6th day during the present study.

Egg clusters of *Sepioteuthis lessoniana* were noticeable from March to September with peak in August and September during the present study. The collected egg bunches consisted 50-70 capsules, having a length ranging from 5.3cm-9.2cm and width of 1.2-2.2cm. Sivalingam *et al.*(1983) in their study observed 26-545 egg capsules in different clusters and length of egg capsule ranged from 3cm-7.5cm in Dhargavalasai on Palk Bay. In the present study egg capsules were in different stages of development with 4 to 9 eggs per capsule. Nabhitabhata (1996) reported that upto 2-11 eggs can be seen in a single capsule, and in most capsules eggs were not in the same stage of development. A similar observation was made in the present study.

In the present study egg mass of *S.pharonis* was collected during January from Vizhinjam coast whereas Anil *etal.*(2005) and Nair *et al.*(1985)had collected egg mass during April and December respectively. On the first day, length and width of the egg capsules were2-2.5cm and 1.4-1.8 cm, this showed a slight variation from the observation of15-20 mm,14-17 mm made by Nair *et al*.(1985). Hatching percentage was observed to be more than 95 and the incubation period 22 days, period of hatching was 11 days and first hatching was noticed from 12thday. These observations are in agreement with the observations made by Nabhitabhata (1994) and Anil *etal*.(2005) regarding *S.pharonis*.

In the present study peak availability of eggs of *Sepiella inermis* was observed at Vizhinjam coast during the postmonsoon seasons. Sivalingam (1999) reported the availability of egg mass of *Sepiella inermis* in September along east coast of India. Kripa *et al*.(1995) reported the availability of *Sepiella inermis* during March-May and August-November with the highest monthly catch in September from Cochin, Kerala coast. Hatching percentage was 98% in the present study. This was almost similar to the observations made by various authors (Anil *et al.*, 2003; Nabhitabhata,1994). Incubation period was 16 days but it shows variation to the incubation period of 13 days reported by Nabhitabhata (1994). Silas *et al*. (1985) reported that egg capsules were commonly seen in trawl and shore

seine catches during periods of intensive spawning. During the present study egg clusters of *U (P) duvaucelli* ,*Sepiella inermis*, and *Sepia pharonis* were observed in shore seine catches. Boyle and Rodhouse (2005) reported that loliginid squids ensheath their eggs with material from the nidamental glands, embedding them into finger like capsule each containing some 90-120 eggs in *Loligo*, but as few as 3-10 in *Sepioteuthis*. The viscid secretion of the nidamental and ink gland provide the tough gelatinous covering of eggs (Kotpal,1978).

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