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LABORATORY REARING AND BREEDING OF SPINELESS CUTTLEFISH *SEPIELLA INERMIS* ORBIGNY (MOLLUSCA: CEPHALOPODA)

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ABSTRACT

The spineless cuttlefish *Sepiella inermis* was successfully reared from the egg mass collected from wild. They mated under captive conditions and spawned on 86th day at a size of 60 mm mantle length (ML) producing 214 viable eggs. Only live food organisms, consisting of mysids, shrimp post larvae, fish larvae and small fishes and shrimps formed the diet of these animals at different stages. The initial average size of hatchling was 4 mm ML (0.02 g) that increased to 26 mm (3.19 g), 48 mm (16.5 g), 64 mm (46.48 g) and 69 mm (54.67 g) on 30th, 60th, 90th and 110th days respectively. Average survival was 43, 37 and 28% at the end of first, second and third months. With good water quality management and supply of required live-feed in sufficient quantity, the laboratory culture of cuttlefish is distinctly possible.

Key words: Cuttlefish, egg cluster, culture, breeding.

Cephalopods comprising cuttlefish, squids and octopi are an important fishery resource in many areas of the world. They are also fished from seas around India from very early times. In addition to the use as protein rich food material in domestic and international markets, they are extensively used as bait for capturing finfish. Cephalopods are also a unique and well-known experimental model for studying nerve impulse propagation and cell membrane function (Cole, 1972; Rosenberg, 1973).

Traditionally cephalopods were not considered important for aquaculture due to several reasons such as, comparatively low ability to withstand culture conditions, lack of knowledge of their biology, low price and the production from the capture fishery was able to meet the demand for human consumption. Ever since the export of these animals started, there was a steady increase in price of this commodity, which has resulted in an increased interest

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in the culture of these animals. Considering the difficulties of observing young cuttlefishes in nature, laboratory culture can provide much basic information on the biology of these animals. The basic biological data acquired through these culture experiments will also be important for the management of commercially exploited cuttlefishes. Choe and Oshima (1963) and Choe (1966) reared 3 species of sepia, the squid *Sepioteuthis lessoniana*, and the sepiolid *Euprymna berryi* from egg to adult size. One species of squid and two species of cuttlefish were successfully cultured in Thailand (Nabhitabhata, 1978a, b; Nabhitabhata *et al.*, 1984).

In India, Sivalingam and Pillai (1983) conducted some preliminary experiments on hatching and rearing of a squid *S. lessoniana* and cuttlefish *Sepia aculeata* whereas Nair *et al.* (1986) described in detail the hatching and post hatching behavior of pharaoh cuttlefish *Sepia pharaonis*. Hardly any published information is available on the rearing of spineless cuttlefish *Sepiella inermis* except for a brief article by Sivalingam (1999). The present paper gives a detailed account of the incubation, hatching, salinity tolerance, growth and spawning of *S. inermis* cultured in the Karwar Research Centre of Central Marine Fisheries Research Institute located on the west coast of India.

MATERIALS AND METHODS

Egg clusters of *S. inermis* were collected from shore seine operated in the Karwar coast. The collected eggs were immediately transferred to plastic containers filled with seawater and transported to the wet laboratory. Constant aeration was given using battery-operated aerators during transit. On arrival the eggs were acclimated gradually to the temperature and salinity of the water in the incubation tank. The incubation tank was of one-tonne capacity filled with filtered aerated seawater. The salinity of the incubation tank was maintained at 34–36‰ while the temperature ranged between 27–32°C. The egg cluster was tied using a cotton twine and suspended above the aeration point so as to ensure oxygenation and uniform development of eggs.

For salinity tolerance experiments on hatchlings, glass troughs of five-litre capacity filled with seawater of different salinity grades were used. Experimental salinities (5, 10, 15, 17.5, 20, 25 and 30‰) were made up by diluting seawater (35‰) and for salinity greater than 35‰ (40, 42.5 45, 50 and 60‰), stored seawater of salinity 60‰ was diluted and used. All the hatchlings used in the experiments were collected within 24 hours of hatching. Twenty hatchlings were transferred to each trough. Thus the salinity change was immediate and the exposure time was 24 hours. Changes shown by the experimental animals were noted at 0, 6 and 24 hours. At the end of 24 hours active ones were picked up and counted. The moribund animals were examined repeatedly for any sign of life. Finally, the number dead was counted and survival percentage calculated.

For the rearing experiments hatchlings were scooped out and transferred to 100 litre glass aquaria. Each tank was stocked with 50 juveniles (4 mm ML). Water exchange was given at the rate of 80% daily. At the end of 30 days (15–30 mm ML) they were transferred to one-ton tank and the stocking rate was reduced to 30 numbers/tank. Water exchange was given at the rate of 50% daily. At the end of two months the young cuttlefish (38–51 mm ML) (Fig. 4C) were transferred to circular plastic pools of 5 tonne capacity. An *in situ* biological filter was provided in the pool to maintain the water quality and to reduce the water exchange. Water exchange was reduced to 20% on every alternate day. Stocking rate at this stage was 10 animals per pool. The salinity of the seawater ranged between 32–36‰ and the pH was maintained between 7.5 and 8.2. The pH was adjusted by replacing part of the rearing medium whenever required.

The young cuttlefish were fed with various live food organisms several times daily throughout the experiment. Live food organisms such as mysids and shrimp post larvae were the primary food during the first 30 days. Thereafter fish larvae, juveniles of mullets, *Ambassis* sp., clupeids and shrimps were given. All the feed items were collected from the surf region or estuary using plankton net or small dragnet. The feed items so collected were sorted and stocked in glass aquarium tanks or 500 litre capacity plastic pools. The required quantity of feed items was taken using scoop net and washed thoroughly before broadcasting to the rearing tanks. Dead cuttlefish and dead food organisms from the previous feeding were removed twice daily by siphoning. Measurements of mantle length (ML) and wet weight of 10 animals were taken either in live condition or by sacrificing them every 10th day.

RESULTS

The egg cluster contained 357 eggs, which were spherical to oval in shape (9–11 mm diameter) with their free end drawn into a teat-like projection. Each egg capsule was attached at one end to the substratum by a short stalk and the development of embryo in the cluster was different depending on the position of eggs in the cluster; distal eggs usually hatched out first. During incubation slight swelling of egg was noticed and the colour of the eggs changed from black to near transparent (Fig. 4 A). The yolk sac and the movements of the embryo were clearly visible through the egg membrane. Hatching of eggs began on the 6th day of incubation and it took seven days to complete the hatching process. The hatching percentage was 97. Cuttlefish hatch out as miniature adults without any larval stages (Fig. 4 B). Shaking of egg cluster stimulated hatching. The average hatching size was 6 mm in total length (TL) (range 5–7 mm) with 4 mm mantle length (ML) (range 3–5 mm) and external yolk sac was never observed in hatchlings. Early hatchlings were active and exhibited a tendency to concentrate near the corners with more light. This type of

photopositive behavior was observed only for one or two days thereafter they were seen distributed throughout the tank. The young cuttlefish stocked in one-tonne tanks (one month old) showed strong tendency of avoiding light and they were seen partially buried in the sand provided at the bottom. They avoided regions of tank getting direct sunlight and swam away from light source whenever a bright light source was brought near the tank. Chromatophores were seen distributed throughout the body in hatchlings and their concentration was more on the dorsal side. They frequently changed colour from pale white to dark brown and back. The release of ink was observed from the day one itself whenever disturbed.

In the experiment on tolerance to different salinities, in the salinity grades below 10‰ and 60‰ total mortality was observed within one hour. In 60‰ they ejected ink immediately after their release into the medium but in 0‰ salinity death was instantaneous. The results of the salinity tolerance experiments after 6 hours and 24 hours are presented in Figs. 1 and 2.

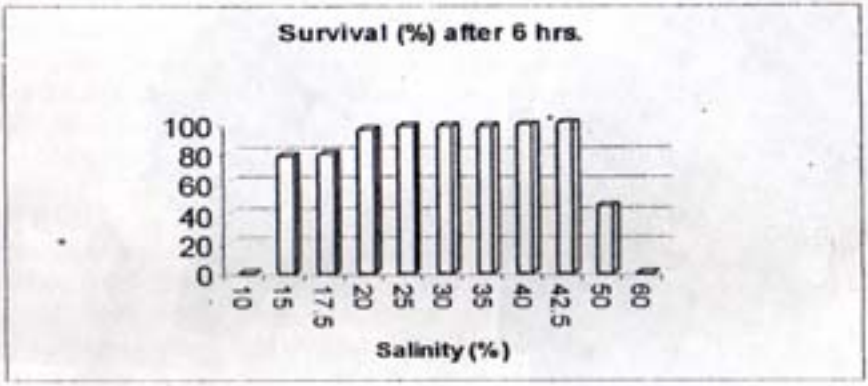

Fig. 1. Survival (percentage) of *S. inermis* hatchlings after 6 hours.

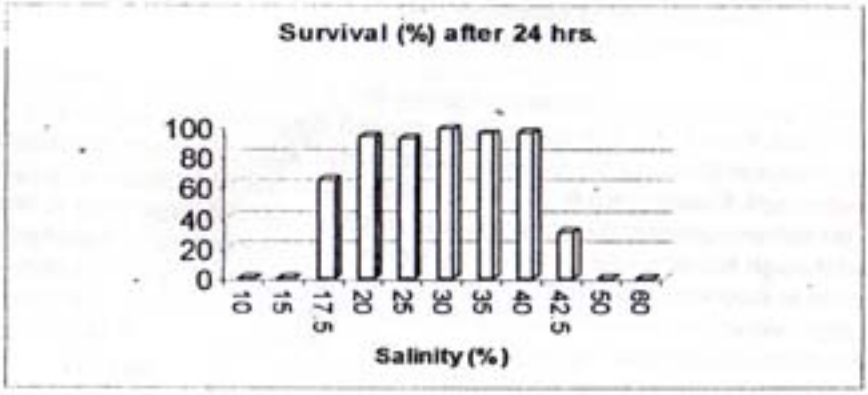

Fig. 2. Survival (percentage) of *S. inermis* hatchlings after 24 hours.

In 10‰, total mortality was noted at the end of 6 hours. At the end of 24 hours total mortality was observed in 15‰ and 50‰ salinities whereas 65% and 30% of the animals survived at 17.5‰ and 42.5‰ respectively. More than 90% of animals were alive in the salinity range 20–40‰ after 24 hours but they were more active in the salinity range 25–35‰ when compared to those in other salinity grades.

In the rearing trials, hatchlings were fed with small mysids of size 4–5 mm obtained by selectively sieving out the larger ones. They actively preyed on mysids by striking them with ejection of tentacles. During second week they were fed with mysids of all sizes and shrimp post larvae and from 4th week onwards they were fed mostly with small fishes and shrimps. They always discarded head and bones of fishes and carapace of shrimps before ingestion. The young cuttlefish were seen capturing animals more than its own size and some times more than one animal were found feeding on a prey simultaneously. Other feed items such as meat suspension, *Artemia* nauplii and *Brachionus* did not attract the attention of these animals.

Growth of the cuttlefish in terms of mantle length (ML) and wet weight (WT) is presented in Table 1.

Table 1: Growth of spineless cuttlefish *S. inermis* reared in laboratory (N=56).

Days	0	10	20	30	40	50	60	70	80	90	100	110
ML (mm)	4	8	13	26	35	42	48	54	59	64	67	69
sd	0.13	0.78	0.98	1.14	1.64	1.72	2.28	2.51	3.36	3.89	4.56	4.71
WT (g)	0.02	0.04	0.62	3.19	4.25	9.85	16.50	25.85	38.82	46.48	52.30	54.67
Sd	.003	.009	0.17	0.47	0.68	1.3	1.93	2.13	2.81	3.45	4.02	4.21

Figure 3 clearly shows that increase in weight was low during the first 40 days but it increased steadily till the 100th day. However, increase in mantle length was almost steady during the corresponding period.

The average growth during the first month was 22 mm in ML and 3.17 g in weight. During the second month average growth in terms of mantle length was almost the same whereas the weight increased by nearly fourfold (13.31 g). During the third month increase in mantle length was reduced to 16 mm and subsequently the rate of growth slowed down. During the corresponding period growth in weight was almost twofold.

First mating was observed on 67th day, the pair was swimming together in head-to-head mating position. Though the animals were in mating position, the transfer of spermatophores could not be observed. On the 86th day eggs were found on the aeration tube close to the stone. There was no mortality

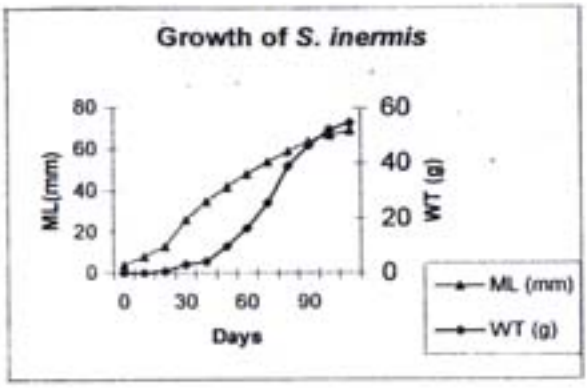

Fig. 3. Growth of *S. inermis*.

immediately after spawning. The aeration tubes along with the eggs (Fig. 4D) were transferred to incubation tank on the 5th day. Hatching of eggs started on 13th day of incubation and it took 7 days to complete the hatching process. A total of 214 hatchlings were obtained from the egg mass. A few embryos remained in the egg capsule itself in atrophied condition. Though the hatchlings were slightly smaller in size (Av. size 5.4 mm TL and 0.34 ML) than those obtained from egg mass collected from the wild, they were healthy and active. The average survival rates for three batches of hatchling were 43, 37 and 28% for 30th, 60th and 90th days respectively and 19.4% survived on 110th day.

DISCUSSION

As observed by Nair *et al.* (1986) in the case of *Sepia pharaonis*, young ones of *Sepiella inermis* have also acquired most of the adult behavior such as locomotion, capture of pray, ejection of ink and sudden change in colour associated with excitement and escape bid. Salinity tolerances shown by the hatchlings throw considerable light on the salinity requirement of the animal. The low resistance to reduced salinity grades makes them unsuitable for rearing in estuarine water. Although they are capable of surviving in low salinity of 17.5‰, they were moribund under these conditions. Under natural conditions this would probably prove fatal, since they are unable to swim, escape from the predator or feed. The preferred salinity range for rearing this cuttlefish is observed to be between 30 and 35‰.

During feeding, even the small cuttlefish *Sepia officinalis* showed the three stage attack sequence of fixating the prey, positioning itself in attacking posi-

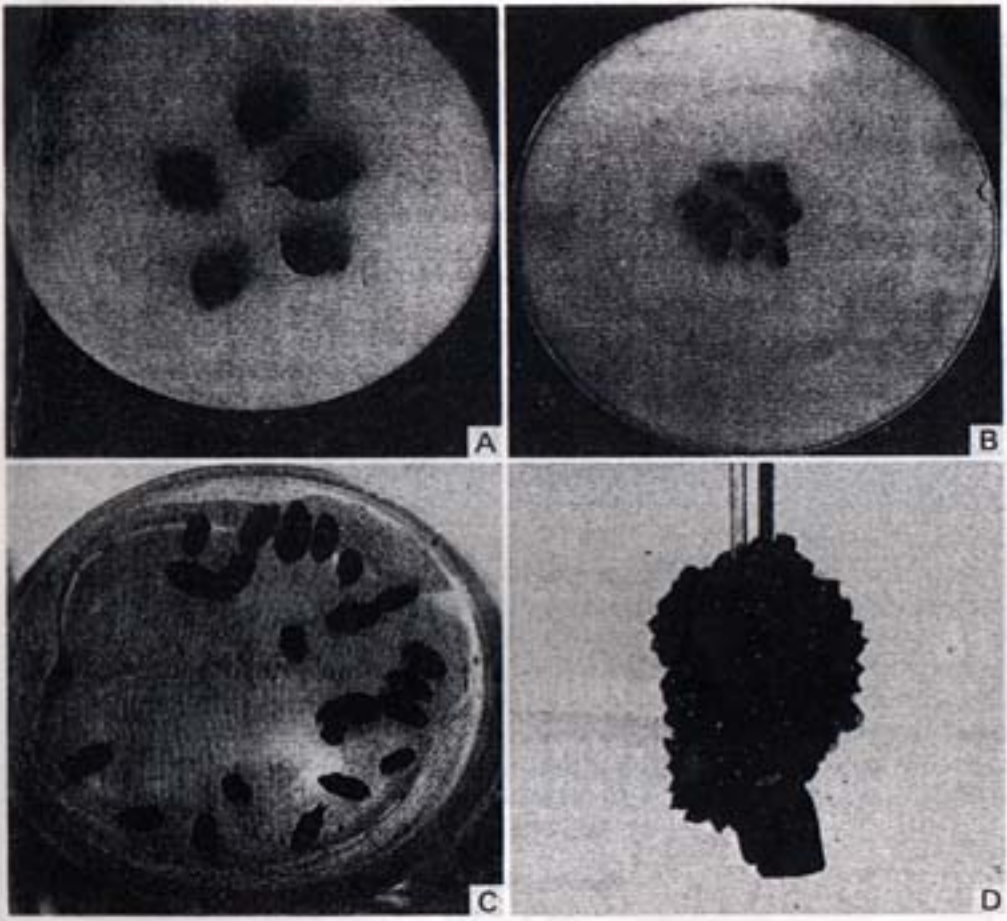

Fig. 4 A. Eggs of *Sepiella inermis* at different stages of incubation. B. Hatchlings (one day old). C. Juveniles (two months old). D. Laboratory produced egg cluster attached to aeration tube.

tion and striking the prey with ejection of tentacles as observed by Messenger (1968; 1977). La Roe (1971) successfully reared *Sepioteuthis sepioidea* by feeding hatchlings with mysid *Mysidium columbia* and older ones with *Gambusia* and small shrimps. Sivalingam and Pillai (1983) could not rear *Sepia aculeata* with fresh plankton containing copepods and decapod larvae. Nair *et al.* (1986) opined that though feeding was poor, mysids were the favorite food of hatchlings of *S. pharaonis*. According to them the lack of availability of mysids in sufficient concentration within the visual field of young was the main reason for the poor feeding and subsequent events leading to death within a few days of hatching. Mysids were also found to be preyed upon by young of other cuttlefishes such

as *Sepia esculenta*, *S. subaculeata* and *Sepiella maindroni* (Choe, 1966). One of the reasons for the present success was the availability of mysids in large numbers from the surf region of the coast. Nabhitabhata (1978b), Nabhitabhata *et al.* (1984) and Sivalingam (1999) also successfully used mysids for rearing hatchlings of cuttlefish.

One of the main bottlenecks in the successful culture of cuttlefish is that they require live feed of proper type, size and quantity. During the present study the culture depended entirely on the feed collected from the wild. They seldom accepted dead food organisms except when the feed was in motion at the time of feed broadcasting. Nabhitabhata (1994) is of the opinion that the adult cuttlefish can be trained to accept prepared feed.

During the present study the average size of the animal increased from 4 mm (0.02 g) to 67 mm ML (52.3 g) in 100 days (27–32°C) whereas it took 120 days in Thailand to reach the size of 68 mm ML (64.8 g) at 28°C (Nabhitabhata, 1994). The faster growth rate observed during the present study may be due to the difference in the strains of the respective populations of India and Thailand as the initial size of hatchling was only 2 mm ML in the case of Thailand strain when compared to 4 mm ML of the Indian strain. Sivalingam (1999) reported a growth of 37.9 g (mean weight) and mean length of 57.2 mm during a period of 75 days, which is close to the present observations.

Study of the progression of model sizes of males and females of this species together at Waltair on the east coast shows that it grows to a size of 33 mm at the end of 6 months, 57 mm at the end of one year and 73 mm at the end of one and a half years whereas at Cochin on the west coast, it grew to 35 mm, 61 mm, and 81 mm during the same period (Silas *et al.*, 1986). The slow growth rate reported by analyzing the progression of modes may be due to reasons such as better feed availability, low energy expenditure for capture of prey and restricted movement in the culture conditions or due to sampling error in the assessment of growth of wild population.

Total number of eggs found in mature ovaries of *Sepiella inermis* of 69–71 mm ML at Mandapam on the east coast of India varied from 470–850 and in the ripe ovaries the ripe eggs formed 37.5–62.6% (Unnithan, 1982). During the present study 60 mm (86th day) female produced an egg cluster containing 114 viable eggs. In the wild, females of this species became sexually mature when they reach 45 mm at Waltair and Porto Novo and 55 mm at Madras (all on east coast of India) whereas at Cochin on the west coast females became mature at 65 mm (Silas *et al.*, 1986). This shows that there is not much variation in the size at maturity of specimens under the wild and captive conditions.

The average survival rate from three batches of hatchling is about 19.4 ± 3.2 %, which is low for a commercial scale of operation. With the use of raceway type of rearing systems and better filtration facilities survival rate can be substantially improved. The future of cephalopod culture depends on the development of mass culture techniques of mysids for hatchlings and artificial feed for the adults.

Unnithan, K.A. (1982). Observations on the biology of cuttlefish *Sepiella inermis* at Mandapam. *Indian J. Fish.*, **29 (1&2):** 101–111.

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