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GROWTH, BEHAVIOR, AND MATING OF PHARAOH CUTTLEFISH *(SEPIA PHARAONIS* EHRENBERG) IN CAPTIVITY

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Abstract

The pharaoh cuttlefish (*Sepia pharaonis*) was successfully reared from egg to an average size of 168 mm mantle length and 521 g in 210 days, using simple biological filtration systems. The period of egg incubation was 15 days at a temperature of 27-31˚C. Hatchlings were reared at a stocking density of one animal per liter during the first month; density was reduced as growth proceeded. Food items consisted of live mysids, *Artemia salina*, juvenile fishes, and prawns. Juveniles were gradually acquainted with dead food items such as caridian prawns and small fishes. The present study shows that the pharaoh cuttlefish can be reared in captivity with a survival rate of 41%, using live feeds during the first 50 days. Future commercial scale culture of this species depends on development of artificial feeds and high density culture systems.

Introduction

About 117,278 tons of cephalopods were exploited in India during 2003 (Annam et al., 2004). During 2002-2003, India exported 41,381 tons of frozen cuttlefish valued at US$86.37 million to Japan, the USA, and the European Union (MPEDA, 2003). Cephalopods are unique because they are 85% protein by dry weight (16-21% by wet weight; Lakshmanan and Balachandran, 2000) and considered a delicacy in seafood restaurants.

Recent years have witnessed a significant amount of research interest in cephalopod culture, in developing technology for commercial farming and producing multiple laboratory generations for neurobiology research (Minton et al., 2001). Cephalopods are promising bio-

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medical models and interesting to neurobiologists because of their giant axons (Cole, 1972; Rosenberg, 1973; Lee et al., 1994; Hanley et al., 1998).

Choe and Oshima (1963) and Choe (1966) reared three species of *Sepia*, the squid *Sepioteuthis lessoniana*, and the sepiolid *Euprymna berryi* from egg to adult size. Rayong Brackish Water Fisheries Station conducted pioneering research on the culture of several commercially important cephalopods in Thailand (Nabhitabhata, 1994). In India, the spineless cuttlefish *Sepiella inermis* was successfully bred in captivity (Sivalingam, 1999; Anil, 2003).

Sepia pharaonis was successfully bred in laboratory conditions in Thailand and the USA using sophisticated, temperature controlled recirculation systems (Nabhitabhata, 1994; Minton et al., 2001). In India, Nair et al. (1986) described the hatching and post-hatching behavior of this species. Apart from the above, published information on the rearing of the pharaoh cuttlefish is limited. The present paper provides further information on the incubation, hatching, breeding and reproductive behavior, and growth of the pharaoh cuttlefish cultured in simple biological filtration systems.

Materials and Methods

Collection of eggs. Pharaoh cuttlefish egg clusters, attached to seaweeds and rocks, were collected by skin divers in the Vizhinjam Bay, India, in April. The eggs were immediately transferred to plastic containers filled with sea water (salinity 35‰, temperature 28ºC) and transported to the Vizhinjam Research Centre of the Central Marine Fisheries Research Institute on the southwest coast of India. The egg masses were placed in an incubation tank containing 500 l filtered sea water and acclimated gradually to the temperature and salinity of the water. Aeration was provided through airstones from an air blower. The eggs were kept suspended above the aeration point in a smooth nylon net bag of 10 mm mesh.

Stocking and water quality management. Hatchlings were scooped out of the incubation tank and transferred to 60-l circular plastic tubs with a small *in situ* biological filtration unit. Each tub was stocked with 50 hatchlings of 8 mm mantle length (ML) at the rate of one cuttlefish per liter. After 30 days, juveniles (avg 20 mm ML) were transferred to 500-l fiberglass tanks and the stocking rate was reduced to 100 animals per m^3. Ninety days after hatching, they were transferred to 1-ton fiberglass tanks at a stocking density of 20 animals per m^3. The density was reduced to 10 animals per m^3 when the animals reached a mantle length of 145 mm, 180 days after hatching.

Salinity in the incubation tank was maintained at 34-36‰, temperature ranged 27-31ºC, and pH ranged 7.8-8.2. About 50% of water was exchanged daily. In the hatchling and juvenile containers, salinity ranged 32-36‰, pH 7.6-8.2, and temperature 27-32°C. The pH was adjusted when required by replacing some of the rearing medium.

All rearing containers contained *in situ* biological filters for nitrification of ammonia to nitrite and nitrate. Nitrate accumulation was prevented by daily partial replacement of the water. In the hatchling tubs, the biological filtration unit was cylindrical (10 cm diameter, 18 cm height) and contained a filter bed of coral sand and a 14-cm layer of charcoal. Water was circulated by an airlift and the flow rate was 2 l/min. The daily water exchange rate was 80%. In the 500-l tanks, there was a cylindrical filter (50 cm diameter, 35 cm height) with a 30-cm filter bed and the flow rate was 20 l/min. Water exchange was 60% daily. In the 1-ton tanks, two such filters were provided. Daily water exchange was 30%. The filters were taken out and washed with sea water every month but care was taken that at least one filter with an active bacterial population was present in the tank at all times. The bottom of the tank was covered with sea sand to check whether cuttlefish require a substratum for burying. The tanks were covered with nylon nets to prevent the animals from jumping out.

Twice a week, the ammonia levels in the rearing containers were checked using the phenolhypochlorite method (Solorzano, 1969) and nitrate levels using the Visocolor Eco test kit (Macherey-Nagel, Dueren, Germany). Dissolved oxygen (Winkler, 1888), pH, and salinity (by refractometer) were checked daily.

The ammonia level was kept very low (<0.005 mg/l NH_3-N) and nitrate below 25 mg/ml. Dissolved oxygen ranged 6-7 mg/l.

Feed. During the first 20 days, live 4-6 mm mysids *(Eurobowmanilla simulans)* and *Artemia salina* (6-10 mm) were the primary food items. Shrimp postlarvae (*Penaeus indicus* and *Metapenaeus dobsoni*), caught together with the mysids in low numbers (<1%), and feeds such as shrimp meat suspension, brine shrimp (*A. salina*) nauplii, and rotifers *(Brachionus plicatilis)* were tried as alternate feed items. Afterwards, mysids (4-8 mm), caridian prawns (*Macrobrachium idella*; 20-45 mm), and juvenile fishes (*Mugil* spp. *Liza* spp, *Therapon* sp.; 10-20 mm) were given. All the feeds, except the *Artemia,* were collected from the surf region or estuary using plankton nets or a small dragnet. The collected feed items were sorted and stocked in 500-l fiberglass tanks. The required quantity (*ad libitum*) of feed items was taken with a scoop net and washed before broadcasting in the rearing tanks. In the case of *Artemia,* cysts (OSI Inc., USA) were hatched and the nauplii were fed *Chlorella salina*.

From day 50 onwards, the juvenile cuttlefish were weaned to accept dead items such as whole *Acetes* sp. (30-40 mm), anchovies (45-65 mm), beheaded and eviscerated sardines *(Sardinella longiceps*; 60-120 mm), and carangids *(Decapterus* sp., *Caranx* sp.; 50-100 mm) purchased at the market. The quantity of feed was 7-10% of the body weight of the cuttlefish (estimated every two weeks by sampling) and the ration was adjusted according to feed intake. Feed was given two times a day, once in the morning and once in the evening. Uneaten food and excreta were removed twice daily by siphoning. Total length, ML, head length, and wet weight of live or sacrificed cuttlefish were measured every 30 days.

Behavior was observed and recorded.

Results

Embryo development. Egg clusters contained 107-307 eggs. Embryos were oval (3.5 mm diameter, 5 mm length) and immersed in protective fluid within the egg (23 mm diameter, 10.5 mm length). During incubation, the eggs swelled, the diameters increased by 7-8 mm, and the length by 2 mm. Embryo development was clearly visible through the egg membrane. Fully formed embryos, with arms clinging to the spherical yolk, and ink sacs were

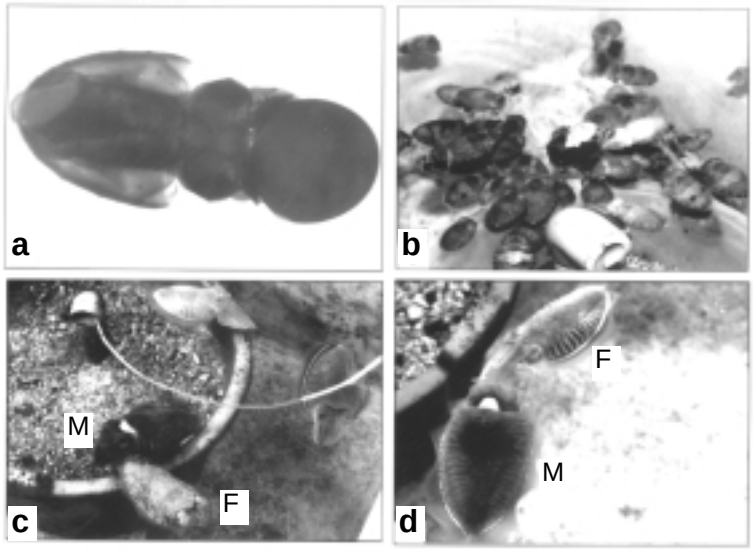

Fig. 1. (a) Embryo (9.5 mm), (b) 50-day juveniles, (c) courting behavior, and (d) mating behavior of *Sepia pharaonis*. M = male, F = female.

visible (Fig. 1a). The embryos jerked and released ink inside the egg capsule when mechanical shocks were given.

Hatching. Egg hatching began on day 12 of incubation. It took seven days to complete the hatching process at 27-31°C. The hatching percentage was 97-99% of the eggs in the cluster. The majority of eggs (80%) hatched on the third to fifth days after hatching began (on days 15-17 of incubation). The hatchlings, which hatch as miniature adults and have no larval stages, averaged 15 mm in total length (8 mm ML). In most cases, the yolk was completely absorbed before hatching. In a few cases, a small amount of yolk remained in the capsule. An external yolk sac was rarely observed and, when present, was shed immediately. Early hatchlings were inactive and tended to remain at the bottom of the container. Chromatophores were distributed throughout the body with a high concentration on the dorsal side.

Feed. The hatchlings were fed small mysids at the rate of 25 per cuttlefish per day, obtained by sieving out larger mysids. During the first two days, the hatchlings were not observed feeding on mysids during day time but there was a noticeable reduction in the live feed available in the rearing containers in the morning. From the third day onwards, the hatchlings actively fed on mysids during the day time by striking them with their tentacles. Other feed items such as meat suspension, brine shrimp nauplii, and rotifers did not attract the hatchlings. During the second week, they were fed mysids of all sizes, shrimp postlarvae, and *Artemia*. They readily accepted the *Artemia*, fed at the rate of 10 per cuttlefish per day. Shrimp postlarvae, though accepted by the cuttlefish, were not given in a significant quantity due to unavailability. From the fourth week onwards, the cuttlefish were fed mostly caridian prawns and juvenile fishes at the rate of 4-6 per animal per day. Young cuttlefish were seen capturing animals that were larger than themselves. Even small cuttlefish exhibited the three stage sequence of attack: fixating prey, positioning itself in attacking position, striking prey with ejected tentacles. From the eighth week onwards, the juvenile cuttlefish were gradually acquainted with dead fish (anchovies) and *Acetes* purchased from the market and the quantity of live feed was slowly reduced.

Growth and survival. Growth and survival are given in Table 1. The weight increase was low during the first 60 days, greater in the third month, and shot up in the sixth and seventh months. The increase in mantle length was highest during the fourth and fifth months. Survival was high during the first four months and 41% at the end of the experiment.

Behavior. Pharaoh cuttlefish exhibited most adult behavioral characteristics (locomotion, capture of prey, ejection of ink, sudden changes of color in reaction to excitement and escape) by the hatchling size of 8 mm ML. These animals frequently changed from pale yellowish brown to dark brown and black. Although the hatchlings stuck to the bottom of the container during the first 4-5 days, they actively swam in water afterwards. During the first three months they aggregated (Fig. 1b). To train the animals to accept dead fish, anchovies (45-50 mm), pierced by sticks, were presented to the animals. Within 2-3 days, most began to accept the feed. Fish pieces were accepted from the hand or taken from the bottom after one week of training.

During the fourth month, some animals buried themselves in the coral sand of the biofilter, changing their body color to match the surroundings. The sea sand on the tank bottoms was never used. Therefore, it was removed because it interfered with cleaning and resulted in fouling of the rearing medium. Failure to remove excess feed from the medium resulted in increased ammonia and nitrate levels and was corrected immediately by replacement of the medium with fresh sea water.

The cuttlefish began to show mating behavior in the fourth month. The intensely tiger-colored male swam parallel to and slightly above the female (Fig. 1c). The male lifted its first two pairs of arms and placed them over the head and arms of the female. The pair swam together with the male above the female and holding her head and arms in his arms. During copulation, the male slowly deflected

Table 1. Growth of cuttlefish *Sepia pharaonis* reared in laboratory.

	Days from hatching							
	0	*30*	*60*	*90*	*120*	*150*	*180*	*210*
Number in tank	250	120	120	32	32	32	24	24
Stocking density (per l)	1/l	0.1/l	0.1/l	20/m^3	20/m^3	20/m^3	10/m^3	10/m^3
Avg wt (g±SD)	0.16±0.01	1.9±0.4	9.2±2.1	34±5.5	85±13.6	135±22.8	325±31.1	521±38.4
Growth rate (g/day)		0.06	0.24	0.82	1.7	1.7	6.3	6.5
Avg total length (mm±SD)	15±0.8	29±3.6	45±7.6	85±12.3	145±19.1	203±33	240±41.2	283±52.2
Avg mantle length (mm±SD)	8±0.13	20±2.3	32±4.6	59±8.6	95±15.3	128±21.6	145±25.4	168±30.2
Growth rate (mm/day)		0.4	0.4	0.9	1.2	1.1	0.6	0.8
Survival rate from day one (%)	100	94	92	90	86	72	53	41

sideward and mating took place in a head to head position (Fig. 1d). The spermatophore was transferred to the seminal receptacle of the female. Mating took about 5-7 min. Males sometimes behaved aggressively towards competing males, resulting in their jumping and ejecting ink up to 3 m from the tank.

Discussion

Nabhitabhata (1994) reported an incubation period of 9-25 days at 28°C for *S. pharaonis*. The present observation of 12-19 days at 27-31°C is slightly less due, perhaps, to the higher temperature in the present study. The direct relation between incubation period and temperature is well known.

As observed by Sivalingam (1999) for *S. inermis*, newly hatched *S. pharaonis* exhibited most adult behaviors. During feeding, hatchlings showed the three stage sequence attack observed by Messenger (1968, 1977) in the case of *Sepia officinalis*.

La Roe (1971) successfully reared *Sepioteuthis sepioidea* by feeding hatchlings *Mysidium columbia*, *Gambusia* spp., and small shrimps. Sivalingam and Pillai (1983) were unable to rear *Sepia aculeata* on fresh plankton containing copepods and decapod larvae. Nair et al. (1986) suggested that, although feeding was poor, mysids are the favorite food of *S. pharaonis* hatchlings and that the lack of a sufficient concentration of mysids within the visual field of the cuttlefish was the main reason for their poor feeding and subsequent mortality within a few days of hatching.

Mysids are preyed upon by hatchlings of other cuttlefishes such as *S. esculenta*, *S. subaculeata*, *Sepiella maindroni*, and *Sepiella inermis* (Choe, 1966; Nabhitabhata, 1994; Sivalingam, 1999; Anil, 2003). Minton et al. (2001) successfully reared S*. pharaonis* using live mysids (*Mysidopsis* spp.) supplemented with small grass shrimp (*Palaemonetes pugio*) and cultured guppies (*Poecilia reticulate*) during the first 30 days. During the present study, *A. salina* were successfully used as a supplement from the second week onwards. Acceptance of *Artemia* can help circumvent total dependence on mysids in culture of

hatchlings. Hanley et al. (1998) reared European cuttlefish *Sepia officinalis* with (a) a mixture of brine shrimp, mysid shrimp, and amphipods (mainly *Gammarus* sp.), or (b) with brine shrimp enriched with fatty acids and amino acids (Super Selco, INVE Inc.). Survival of the cuttlefish fed the mixed food was significantly higher than that of the group fed only enriched brine shrimp.

According to Silas et al. (1986), pharaoh cuttlefish grow to 114.65 mm (average of males and females) by the end of six months, lower than found in the present study. The high growth rate in the present study may be due to better feed availability, low energy expenditure for capture of prey, and restricted movement in culture conditions.

Nabhitabhata (1994) observed the formation of mating pairs after 90 days, similar to the observation made in the present study.

Average survival at seven months (41%) is not low for a commercial scale operation. The future of cephalopod culture depends on the development of techniques for mass culture of mysids for feeding hatchlings (nursery phase), using *A. salina* as a supplement, and providing artificial feed in the growout phase. The success achieved in this study in supplementing the feeding of hatchlings with *Artemia* and weaning them onto dead feeds such as anchovies and sardines which are obtainable in large quantities are steps in this direction.

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