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Hatchery production of juveniles of pharaoh cuttlefish, *Sepia pharaonis* (Ehrenberg, 1831) from stranded eggs and sea ranching along the Thoothukudi coast

M Kavitha*, I Jagadis, D Linga Prabu, C Kalidas, L Ranjith & S Willington

ICAR-Tuticorin Research Centre of CMFRI, Thoothukudi, Tamil Nadu – 628 008, India

*[E-mail: kavifish@gmail.com]

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Accidentally entangled and stranded egg masses of *Sepia pharaonis* in fishing gears were collected from various landing centres of Thoothukudi district. The stranded egg masses were incubated, hatched and larval rearing was carried out at the Molluscan Fish Hatchery of Tuticorin Research Centre of CMFRI. The average hatching rate of *S. pharaonis* was 95 % for the eggs collected from the fishing nets. The size of day 1 paralarvae of *S. pharaonis* was 5.46±0.06 mm and reached the average size of 40.10±1.15 mm length and 9.6±0.20 g weight on day 60 with the average survival rate of 70 %. Maximum weight gain (%) was noticed between 20 – 40 days. The paralarvae of *S. pharaonis* fed with suitable live feeds until 50 days, after which fed with dead fish and shrimp. The juveniles were transformed as broodstock with an average length of 137.4±8.08 mm and weight of 229.7±21.54 g on 180th day of culture. In the hatchery, the first captive spawning was witnessed on 167th day and the eggs laid by the females ranged from 46 – 118. However, the captive bred egg masses failed to hatch out. Therefore, the hatchery technology needs to be perfected through the development of nutritionally improved broodstock diet to ensure better hatching. During the years 2016 – 2018, altogether 8400 numbers of hatchery reared 60 days old juveniles of *S. pharaonis* were ranched into the fishing grounds off Tuticorin coast. The biological significance of sea ranching activities to replenish the natural stocks requires further research and evaluation.

[Keywords: Broodstock, Hatching rate, Paralarvae, Sea ranching, *Sepia pharaonis*, Survival rate]

Introduction

Fishery of *Sepia pharaonis* (Ehrenberg, 1831) has been increased tremendously due to demand from the local and international market promoting elevated fishing pressure on this high value commodity day by day[1]. The cephalopod catches of India and the state of Tamil Nadu showed an increasing trend since 2012 to 2017 and a fluctuating trend is observed thereafter until 2020 (Fig. S1)[2,3]. Further, the cuttlefish production (in which *S. pharaonis* contributes nearly 60 – 70 %) of Tuticorin, Tamil Nadu alone shows a fluctuating trend during 2012 – 20 (unpublished data; Fig. S2). Several conservative measures can be adopted to increase the fishery production of any species including the hatchery production and sea ranching which may support the steady increase in the fishery. Hatchery production and sea ranching of *S. pharonis* has been practiced since couple of decades back in Thailand[4] and USA[5]. In India, hatchery production of *S. pharonis* was initiated by Sivalingam[6] and perfected by Anil *et al.*[7] and Chacko *et al.*[8] through wild collected egg masses. However, efforts were not done to enhance the wild stock of *S. pharaonis* till date through sea ranching as a measure of improved fishery production.

The observations on *S. pharaonis* fishery and enquiries involving the stakeholders engaged in cephalopod fishery at Thoothukudi coast of Gulf of Mannar, revealed that the egg masses of cephalopod species frequently entangled in trawl and bottom set gill nets accidentally during the fishing operations. The fishers have acquired adequate knowledge and awareness of releasing the entangled egg masses back to the sea through CMFRI's awareness programs. However, on some occasions stray number of egg masses may be brought to the shore unknowingly leading to ultimate annihilation of egg masses. Hence, an attempt has been made to collect the viable eggs that were brought accidentally to the shore, incubate, nursery production and ranching the juveniles in the cephalopod fishing grounds at Sippikulam, Vellappatti and Fishing Harbour area off Tuticorin coast. Attempts were also made to maintain the broodstock of *S. pharaonis* in captivity for breeding, larval rearing and sea ranching for increased production.

Material and Methods

The viable egg masses of *S. pharaonis* that accidentally entangled in fishing gears during the fishing activities and brought ashore unnoticeably were collected from various fish landing centres located at Thoothukudi district. The egg masses were transported in a plastic container containing sea water with adequate aeration to the molluscan hatchery of Tuticorin Research Centre of ICAR-CMFRI, Thoothukudi. In hatchery, the egg masses of *S. pharaonis* were incubated in 1000 L FRP tank with adequate aeration. During incubation, 100 % water exchange was carried out daily.

The hatching started from next day of incubation onwards and it was continued up to 15 days depending on the embryonic stages during collection (Fig. 1). The hatching percentage was calculated by dividing the number of eggs hatched out from the total number of eggs collected multiplied by 100. During nursery rearing, water temperature, salinity, dissolved oxygen, ammonia and nitrate levels were monitored and maintained in the rearing tank on daily basis[9].

The sub-adults of live feed organism and adults of *Artemia salina* were collected from the saltpans. Other live feeds such as mysids, paste shrimp, mysis and post larvae of shrimp, fries and juveniles of different fish species were collected from Karapad backwaters during morning hours and maintained at the hatchery (Table 1). Ranching of the juveniles was carried out in the cephalopod fishing grounds at Sippikulam, Vellappatti and Fishing Harbour areas off Tuticorin coast. The statistical analysis of growth-related data expressed as mean ± SD were performed through MS-Excel 2010 program.

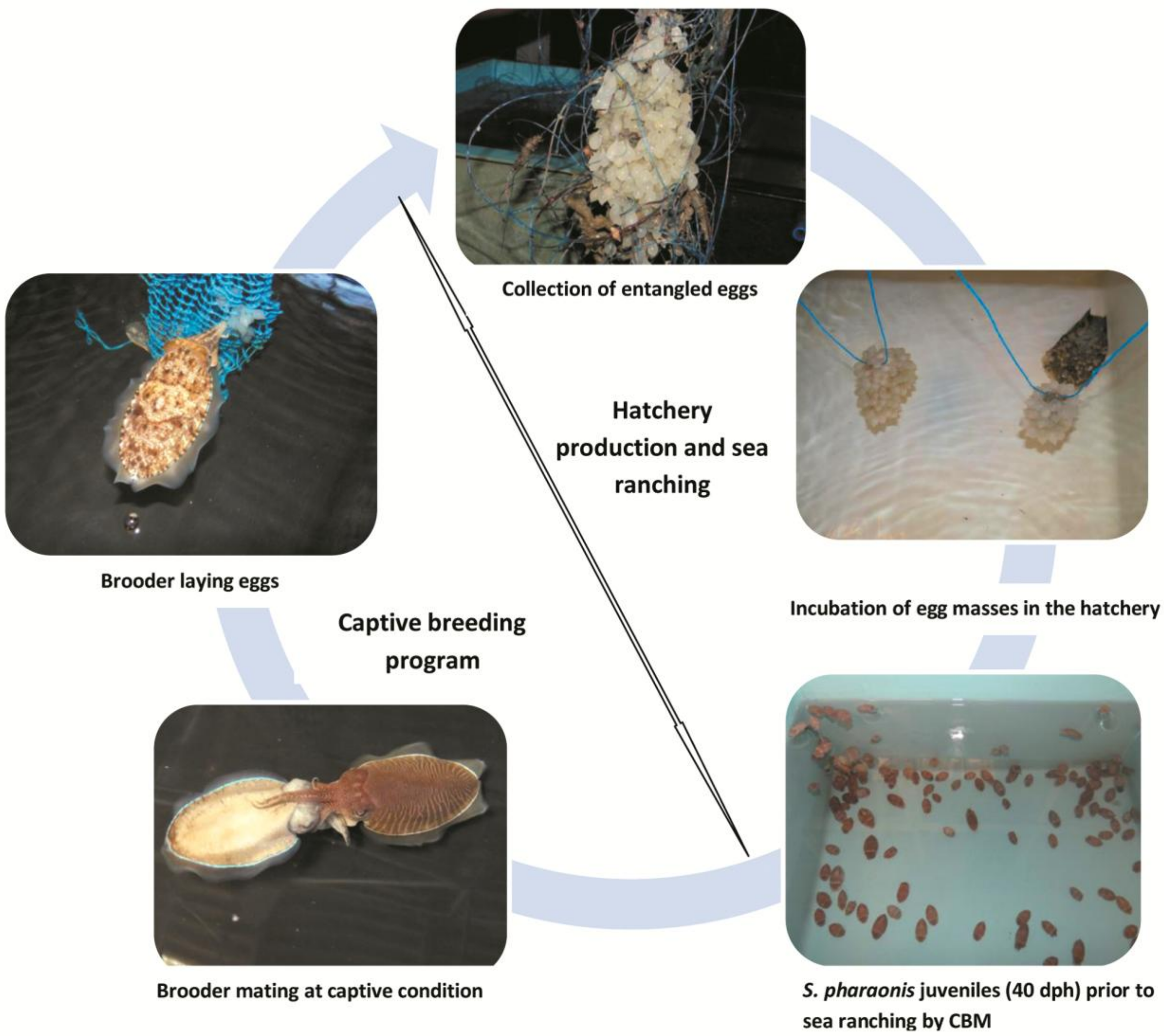

Fig. 1 — Captive breeding and hatchery production of pharaoh cuttlefish *Sepia pharaonis*

Table 1 — Stocking and feeding regime of *Sepia pharaonic*. DPH: Days Post Hatch

DPH	Stocking density of S. *pharaonis* (Ind./m^3)	Feed	Feeding frequency (Per day)
1 – 30	500	Sub adults and adults of *Artemia salina*, live mysids (*Mesopodopsis orientalis* & *Eurobowmaniella simulans*), mysis of Indian white shrimp (*Fenneropeaneus indicus*) and fries of mullet (*Mugil cephalus*),rice fish (*Oryzias dancena*).	Once with adequate numbers
31 – 50	200	Live mysids, paste shrimp (*Acetes* sp.) juvenile fishes of mullet, rice fish, target fish (*Terapon* sp.) and glassfish (*Ambasis ranga*)	Twice (10:00 & 16:00 hrs)
51 – 60	70	Dead sardines, anchovies, carangids and silver bellies	Twice (10:00 & 15:00 hrs)

Table 2 — Growth and survival of *Sepia pharaonis*

DPH (days)	DML (mm)	ADGL (mm/day)	Length gain (%)	Body weight (g)	ADGW (g/day)	Weight gain (%)	Survival (%)
1	5.46±0.06	-	-	0.1±0.006	-	-	-
20	12.13±0.15	0.33±0.007	121.97±3.28	0.24±0.006	0.007±0.00	130.2±12.10	96.0±2.64
40	27.43±0.51	0.76±0.01	126.09±1.38	4.23±0.15	0.2±0.008	1688.6±41.99	89.9±1.80
60	40.10±1.15	0.64±0.04	46.16±2.88	9.6±0.20	0.27±0.016	127.15±11.61	70.2±1.70
80	55.20±1.31	0.74±0.02	37.67±0.72	31.67±0.80	1.10±0.045	230.0±12.78	50.97±3.66

Data represented as mean ± SD; n = 3; DPH: Days Post Hatch; DML: Dorsal Mantle Length; ADGL: Average Daily Growth in Length; ADGW: Average Daily Growth in Weight

Results and Discussion

Nursery rearing

In the juvenile and broodstock rearing tank, the water temperature (27 – 31 °C), salinity (32 – 35 ppt), dissolved oxygen (> 5 mg L^{-1}), pH (7.5 – 8.2), ammonia (< 0.01 mg L^{-1} NH_3-N) and nitrate (< 1.0 mg L^{-1} NO_3-N) level were maintained well within the acceptable range[5]. Egg capsules collected from the wild varied in size and embryonic stages. Eggs became larger and more transparent during the advancement of embryonic development and the embryo could be visible through the gelatinous membrane of egg at the later developmental stages[10]. Average hatching rate of *S. pharaonis* was 95 % for the eggs collected from the fishing nets. Similarly higher hatching percentage of more than 90 % was reported in wild collected eggs of *S. pharaonis* by Nabhitabhata & Nilaphat[11]. Hatchlings of *S. pharaonis* are benthic in nature and hence soon after the hatching, the hatchlings were settled at the bottom of the rearing tank. On day one, larvae did not take feed as small amount of yolk sac was present in the *S. pharaonis* hatchlings which may serve as a food source for one or two days[10]. Greater percentage of hatchlings started feeding since the second day after hatching[8].

S. pharaonis paralarvae are highly predatory in nature[8] and prefer live feed organisms (Table 1) and were fed to apparent satiation with suitable live feeds according to the size requirement by the larvae until 50 days. From 50th day onwards, the young ones were trained and adopted to dead fish and shrimp within 5 days. This is in agreement with the report by Anil *et al*[12]. The transformation of live feed to dead feed is a crucial in the culture of any cephalopods.

Growth and survival

The growth and survival rate of *S. pharaonis* was recorded for a period of 80 days (Table 2). The size of the day 1 paralarvae of *S. pharaonis* (5.46±0.06 mm) was comparatively larger than other cuttlefish larvae[10]. The length and weight of *S. pharaonis* was 12.13±0.15 and 27.43±0.51 mm and 0.24±0.006 and 4.23±0.15 g during 20th and 40th day of post hatching, respectively. On 60th day, the juveniles reached to the average length and weight of 40.10±1.15 mm and 9.6±0.20 g, respectively. Maximum weight gain (%) was noticed between 20 – 40 days and maximum length gain (%) was witnessed during 20 – 40 days and 60 – 80 days of culture. Similar observations were made by Anil *et al.*[12] and Nabhitabhata & Nilaphat[9]. The survival percentage of *S. pharaonis* was higher among other cuttlefish species with the average survival rate of 70 % was achieved until 60 days of rearing. This is in line with Anil *et al.*[12] who recorded higher survival of 92 % on 60th day of rearing. It was inferred that the higher survival rate of *S. pharaonis* was due to acceptance of any live feed organisms and even it can capture the prey of two times bigger than its mantle length as well as comparatively better adaptability to dead feed from live feed[7].

Scope for laboratory breeding

During the course of hatchery rearing, some juveniles were maintained in the hatchery at the stocking density of 30 and 10 individuals at the age of 90 and 120 days, respectively in 1-ton FRP tank (Fig. 1). After 150 days of culture the juveniles were transformed as broodstock. The brooders were reached the average size of 137.4±8.08 mm and 229.7±21.54 g on 180th day of culture (Table 3). The sizes of broodstock were comparable with the broodstock reported by Nabhitabhata & Nilaphat[11]; smaller than Anil *et al*[12] and larger than Nabhitabhata[13]. The animals were started showing mating behaviour on 142nd days of rearing and spontaneous mating took place during the day time. After mating, the first spawning was witnessed on 167th day. The female was started attaching the eggs on the fishing net web kept in the tank for this purpose. Totally 4 females were spawned in batches and died after completion of last batch of spawning. The egg masses laid in the captivity did not hatch out as embryonic development was not progressed few days after spawning. This may be due to the inferior quality of eggs and sperms. Therefore, in future it is necessary that the broodstock diet need to be supplemented with indispensable nutrients such as Vitamin E, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for enhancing hatching percentage and survivorship in the young ones. This is supported by Izquierdo *et al.*[14] stating that many of the deficiencies and problems encountered during the early life stages of newly hatched fish larvae are directly related to the feeding regime along with indispensable nutrient level and duration of the broodstock feeding. According to Luo *et al.*[15] feeding the female broodstock of *Acipenser baeri* with high DHA would improve fecundity, egg hatchability and the overall quality of the larvae. Deficiency of EPA and DHA in the broodstock diet is a reason for decreased fecundity, egg viability, hatching rate and larval survival[16]. Similarly, vitamin E deficiency affects reproductive performance, reduced percentage of fertilized eggs, viability and motility of spermatoids, lower hatching rate and survival of offsprings[14]. However, the major bottlenecks in the captive breeding of *S. pharaonis* are the requirement of new set of brooders for every breeding cycle as this species is semelparous (which dies after complete spawning), having short life span and lower fertility under captive conditions[11].

Table 3 — Broodstock development and spawning details of *S. pharaonis*

DPH (days)	DML (mm)	Body weight (g)
90	56±4.58	31.5±3.17
102	69±6.24	50.1±5.78
110	76±4.58	68.5±4.76
120	98.3±7.63	108.4±17.01
135	110.7±8.14	134.5±12.73
150	118.7±6.5	179.7±17.67
165	125.2±9.29	210.3±18.87
180	137.4±8.08	229.7±21.54
210	144.5±3.51	278.2±25.5
First mating		142nd day of age
First Spawning		167th day of age
Number of eggs/ females		46 – 118
Maximum life span		219 days

The data expressed as mean ± SD; n = 3; DPH: Days Post Hatch; DML: Dorsal Mantle Length

Table 4 — Ranching of *Sepia pharaonis* with the involvement of local fishers

Period	Number of juveniles released	Stakeholders
March 2016	455	Sippikulam
April 2016	550	Sippikulam
August 2016	545	Fishing harbour
September 2016	490	Vellapatti
November 2016	950	Fishing Harbour
February 2017	580	Fishing Harbour
April 2017	830	Sippikulam
July 2017	650	Sippikulam
October 2017	770	Fishing Harbour
December 2017	690	Fishing Harbour
February 2018	800	Sippikulam
April 2018	740	Vellapatti
May 2018	350	Sippikulam

Sea ranching of *S. pharaonis* young ones

Over a period of two years (2016 – 2018), altogether, 8400 numbers of hatchery reared 60 days old juveniles of *S. pharaonis* were ranched into the fishing grounds (Table 4). The sixty days old juveniles were packed in polythene bags with well aerated seawater and transported to the fishing boat. On board, the polythene bags were opened and the juveniles were transferred to the plastic containers with aerated sea water. After reaching the fishing grounds the juveniles were released into the sea.

Conclusion

The fishers are very much aware to release the entangled egg masses of *S. pharaonis* from the fishing gears into its natural habitat immediately after their sighting onboard. The hatchery rearing and sea ranching practice needs to be augmented further in terms of surplus numbers through captive breeding program. The biological significance of sea ranching activities to enhance the natural fish stocks needs to be evaluated in future through adoption of suitable methodology.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at http://nopr.niscpr.res.in/jinfo/ijms/IJMS_51(04)322-326_SupplData.pdf

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Conflict of Interest

All authors of this manuscript are declaring that no conflict of interest is present in this manuscript.

Ethical Statement

The use of *S. pharaonis* for this experiment was carried out according to the guidelines of animal ethical procedures of ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Kochi, by carefully considering the welfare of the fishes.

Author Contributions

MK: Investigation, methodology, writing – original draft preparation; IJ: Conceptualization, supervision; DLP: Data curation and analysis, writing – original draft preparation; CK: Visualization, laboratory analysis; LR: Writing – reviewing and editing MS; and SW: Laboratory assistance and laboratory analysis.

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