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Laboratory Rearing of the Pharaoh's Cuttlefish, *Sepia Pharaonis* (Erhenberg, 1831) through Multiple Generations in a 'Semi-Closed' Water System

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Abstract

The pharaoh's cuttlefish, *Sepia pharaonis* were cultured in a 'semi-closed' water system through multiple generations. Eggs of *S. pharaonis* landed in overnight crab nets, gill nets and shrimp nets were collected and brought to the laboratory, incubated and the hatchlings were maintained throughout their life cycle. The hatchlings from the eggs collected from the wild were called G_1 generation. Subsequent generations were named as G_2 and G_3 generations. Incubation period for the eggs were 21±3 days, 21±2 days and 21±1 days, respectively for G_1, G_2 and G_3 generations. The hatchling behaviour, as well as feeding and reproductive behaviours were recorded. Brine shrimp and post larvae of *Acetes indicus* were given during the initial days of culture, but switched to dead fish after 40 days. Daily growth rate was noted to be 0.72 mm in G_1, 0.52 mm in G_2 and 0.2 mm in G_3 generations, respectively. Using simple and inexpensive filtration system *Sepia pharaonis* can be reared to marketable sizes in fishermen's backyards and serve as an additional source of income. This technique could also be employed in sea-ranching projects of *S. pharaonis*.

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Introduction

Sepia pharaonis (Erhenberg, 1831) is the dominant cuttlefish species found in the Indian waters (CMFRI 2005). It is generally found in shallow coastal waters not beyond a depth of 100 m (Norman and Reid 2000). The culture of cephalopods is carried out in many parts of the world. But it has not been successfully cultured commercially except for a few places like Japan and Thailand (Sato and Tsuzaki 1984; JSFFA 1985; Nabhitabhata 1995). The failure in culture attempts during the last 50 years are mainly attributed to the small hatching size, unknown dietary habits, active behaviors, susceptibility to skin damage and disease resulting during captivity (Hanlon 1990). The scientific community is forced to depend largely on culture programs to provide information such as early life histories, because it is extremely difficult to make direct and detailed observations of cephalopods in the nature due to their mobility, excellent vision and generally nocturnal habits and hence culturing can be considered as a means by which a number of pertinent problems of basic and applied science can be solved (Boletzky and Hanlon 1983).

In the world scenario, cephalopods do not rank among the major candidate species for aquaculture. However, recently, they are being used extensively as research models in some fields like neuroscience, biology, nutritional biochemistry, oncology, ageing and ethiology (Gilbert et al. 1990; Oestmann et al. 1997). There are detailed studies on cephalopod maintenance and rearing conducted in many parts of the world (Boletzky 1974; Boletzky and Hanlon 1983; Hanlon 1990; Nabhitabhata 1995; Lee et al. 1998; Nabhitabhata and Nilaphat 1999; Minton et al. 2001; Walsh et al. 2002). The aquaculture potential of cephalopods in India has not been studied in detail yet except for a few ones (Sivalingam et al. 1993; Sivalingam 1999; Samuel 2003; Anil 2003; Anil et al. 2005). Studies on the rearing of Pharaoh's cuttlefish (*Sepia pharaonis*, Sepiidae) for multiple generations in India are not available. The present study tries to throw light on the biology, behaviour and culture of the pharaoh's cuttlefish *S. pharaonis* maintained in the laboratory through multiple generations using semi-closed water system.

Materials and Methods

The culture of *S. pharaonis* was conducted in three successive generations for a period of 18 months in the laboratory using 'semi-closed' type of water system. The hatchlings from the eggs collected from the wild were considered as G_1 generation. The subsequent generations were designated as G_2 and G_3 generations.

Collection and incubation of eggs

The eggs of *S. pharaonis* landed as by-catch of the major gears used in the landing centers of Tuticorin were collected (250 eggs) and transferred into plastic containers with fresh seawater. The eggs were transported to the laboratory immediately with supplementary aeration through a portable aerator. Upon reaching the laboratory, 150 eggs were separated from the cluster using a pair of scissors, and the remaining 100 eggs were left as cluster itself. The eggs were rinsed using fresh UV treated filtered seawater and transferred into perforated plastic baskets for incubation. The baskets containing eggs were incubated in fiber reinforced plastic tanks (FRP) of 200 liters capacity till hatching of the eggs. Aeration was provided throughout the incubation period with the help of an air blower.

Stocking of hatchlings and water quality management

Soon after hatching, the hatchlings were transferred carefully into 1 ton FRP tanks. Each tank contained 100 hatchlings. Aeration was provided from the air blower through air stones. Daily water exchange was carried out up to 80%. Water was drawn directly from the sea and stored overnight in an overhead tank. The particulate matter settled during this storage period. The next day the water was filtered through an indigenous filter. This filter was constructed using a layer of fine sand on the top, a layer of charcoal below it, a layer of small pebbles underneath it and finally a layer of larger stones in the bottom-most layer. The emergent water was treated by UV rays before finally allowing into the culture tank. The seawater pH was between 7.7 and 8.2. The temperature was within 28-32ºC and salinity was 35 ppt. Drastic changes in these parameters were overcome by immediate water exchange.

Feeding

The hatchlings were fed with brine shrimp collected from the salt pans of the Tuticorin sub-urban region and with post larvae of *Acetes indicus* (5-10 mm length) collected from the mangroves. A study with the duration of 30 days was conducted to find out the suitability of the two live feeds to the cephalopod hatchlings. Sixty hatchlings were selected and divided into two equal batches. One group was fed with post larvae (PL) of *Acetes indicus* while the other group was fed with brine shrimp (*Artemia* sp.). The differences in survival and growth were noted during the experiment.

After 40 days, the juveniles were trained to accept dead fish as feed. Dead juveniles of anchovies (*Stolephorus* sp.), sardines (*Sardinella* sp.), carangids (*Caranx* sp.) and silver bellies (*Leiognathus* sp.) were collected from the shore seines and sardine nets and given as feed. As the

cephalopods increased in size, bigger fishes of sardines, were given as feed. Live crabs (*Charybdis* sp.) and stomatopods were also tried as feed. Feeding was done twice a day to satiation. As there was no running water or raceway system the excreta and feed remains were siphoned out regularly.

The population data viz., life span, total eggs laid, incubation period, hatching percentage, initial hatchling measurement and survival were taken from the lab reared animals. The growth of the animals in terms of dorsal mantle length (DML) and weight increase (wet) were determined every 10 days. The hatchling behaviours, feeding and reproductive behaviours were also noted at different growth stages.

Results

Eggs obtained from the crab nets, gill nets and shrimp nets placed overnight in the sea were young and newly laid, whereas, the eggs obtained from gears such as trawl nets and push nets operating in the sea grass beds and sea fans were larger and more mature. It was noted that young and recently laid eggs were found to be ideal for transportation than the larger and mature ones. The disturbances and jerking of the container triggered premature hatching of the matured eggs. During incubation of the eggs in the laboratory, it was observed that separating the eggs and incubating them individually yielded a higher hatching percentage whereas eggs remaining in cluster suffered fungal infestation that halted the embryonic development. The incubation period for the eggs was 21±3 days in G_1, 21±2 days in G_2 and 21±1 days in G_3 generations.

The hatching percentage of the G_1 was 80. A total of 27 adult G_1 females laid 1484 eggs of which 33.49% (497 G_2 hatchlings) hatched. A total of 17 G_2 females laid 898 eggs of which 31 hatched (G_3). The hatching percentage was 3.45%. The G_3 generation faced 100% mortality after 20 days of their culture resultant to the damage caused to the seawater intake system and subsequent inability to change water due to the tsunami of 26 December 2004. The G_1, G_2 and G_3 hatchlings soon after hatching measured an average DML of 5.5 ± 1.2, 5.48 ± 1.3 and 5.43 ± 1.1 mm and weighed 0.29 ± 0.02, 0.28 ± 0.03 and 0.26 ± 0.02 g, respectively.

Immediately after hatching, the hatchlings squeezed themselves outside of the perforated baskets and descended to the bottom to start their benthic mode of life. No substrate was provided in the tanks and the hatchlings were found to rest at the bottom in small groups. They were actively moving only during the feeding time and at night time.

During the initial feeding experiment, the hatchlings fed with brine shrimp showed lower growth (Table 1) and survival (Fig. 1). After 30 days, only 5 hatchlings from the group fed with brine shrimp survived while 28 hatchlings survived in the group fed with PL. Student *t*-test showed no significant difference ($P<0.05$) in the growth rate of the paralarvae fed with *A. indicus* and brine shrimp, whereas there was high significant difference ($P<0.05$) in survival between the PL and *Artemia* fed groups. The remaining 140 hatchlings and G_2 and G_3 hatchlings were fed with shrimp post larvae only. Initially 100 hatchlings were maintained at a stocking density of 33 per m^3 and 70-80% of water was changed daily with fresh filtered UV treated seawater.

Juveniles started to accept dead fish after 40 days of culture. Collection of live feed was very tedious and it was necessary to feed the animals with an alternate diet. The juveniles did not show any interest in dead fish pieces initially. But soon they started accepting dead fish fingerlings as a whole. One draw back of the dead fish feed was it had the tendency to spoil the water quality very quickly. To prevent this, feeding was done prior to water exchange. The left over feed were siphoned out immediately. Here also, the water exchange was found to be of prime importance and a slight deterioration in the quality of water tends to trigger mortality. About 14-16 juveniles were maintained per m^3.

Table 1. Growth of *S. pharaonis* hatchlings fed with shrimp mysids and brine shrimp for 30 days

Days of culture	DML of hatchlings fed with shrimp mysids (mm)	DML of hatchlings fed with brine shrimp (mm)
0	5.5 ± 1.2	5.5 ± 1.1
5	6.2 ± 1.3	6.1 ± 1.0
10	7.04 ± 1.1	7 ± 1.2
15	7.8 ± 1.5	7.5 ± 1.2
20	8.55 ± 1.2	8.3 ± 1.1
25	9.9 ± 1.1	9.6 ± 1.0
30	12.87 ± 1.2	12.12 ± 0.88

DML- Dorsal Mantle Length

The *S. pharaonis* showed a great degree of curiosity when new substances such as a net piece or a stone were introduced into the culture tanks. The young hatchlings oriented themselves around the new substrate with the posterior side pointing towards the substrate, giving an impression that they are guarding it (Fig. 2). The juveniles were observed to be constantly browsing their environment. When a net piece was introduced into the tank, they swam around the new 'object' with their first pair of arms pointed upwards in a 'V' shape.

Hunting of the prey involved three steps namely locating, positioning and striking. The prey was located first and then the animal positioned itself with its arms pointing to the prey. Then they chased the prey and finally using the tentacles the prey were struck. While feeding on dead fish the animals mostly struck the fish before it landed at the bottom. After landing, there was no positioning step and the dead fish were just picked from the bottom using the arms. There was no schooling behaviour observed but, from day 60 onwards behaviour called 'follow the leader' was noted. The sudden moving away of one animal in fear triggered the other animals to follow.

Hungry *S. pharaonis* swam along the water column instead of remaining at the bottom which is their normal posture. They swam up to the water surface with the arms pointing downwards and moved up and down. Cannibalism was not observed among *S. pharaonis*, but, as the animals attained maturity, stronger males attacked the weaker ones and occasionally consumed them.

The reproductive behaviour was exhibited from the 110th day onwards in both G_1 and G_2 generations. As the males gained maturity, they exhibited a bright zebra pattern on the entire dorsal region of the body. Hierarchy was observed in adult *S. pharaonis* males where the stronger ones always enjoyed superior access to food and mates. Courtship was noted in the form of adjacent swimming and spreading of the arms of the males over the females. Initially the females never showed any interest. This kind of courtship and caressing lasted for about two weeks. Finally, the male succeeds in finding a mate. The male spreads his arms over the mantle of the female, glides to the head region and comes face to face with the female. They interlock their arms and mate. Mating usually lasts 1-15 minutes. The females laid their eggs onto the net pieces, dead sea-fans and nylon ropes tied and hanged into the tanks within 1-2 days time (Fig. 3). During the egg laying period the males accompanied the females and caressed the mantle region. The dominant male chased other males from the spawning site and flashed the tiger pattern at other cephalopods. The males also fanned and blew water occasionally onto the laid eggs. While mating and egg laying, the adults were least interested in feeding. After egg laying both the males and females were found in an exhausted state and they died within two days. But one male in the G_1 mated with three different females before dying. The G_1 generation of the pharaoh's cuttlefish

started laying eggs for the first time on the 179th day of its laboratory culture. A total of 1484 eggs were laid in 13 batches of which 497 hatchlings hatched out. The G_2 generation females laid eggs for the first time on 178th day. A total of 898 eggs were laid and 31 hatchlings hatched out.

The growth of all the three generations is given in table 2. Two way ANOVA was carried out and it was noted that there was significant difference ($P<0.05$) between the growth parameters, DML and weight increment, in G_1 and G_2 generations. Population data of the three generations of *S. pharaonis* is given in table 3. The G_1 animals were found to show higher growth rate (mm/day) when compared to the subsequent generations. Fifty-nine animals in G_1 reached adulthood and the survival percentage was 29.71%. In G2 generation, a total of 38 animals reached adulthood showing a survival percentage of 7.65%. The spawning output and lifespan was also more for the G_1 animals.

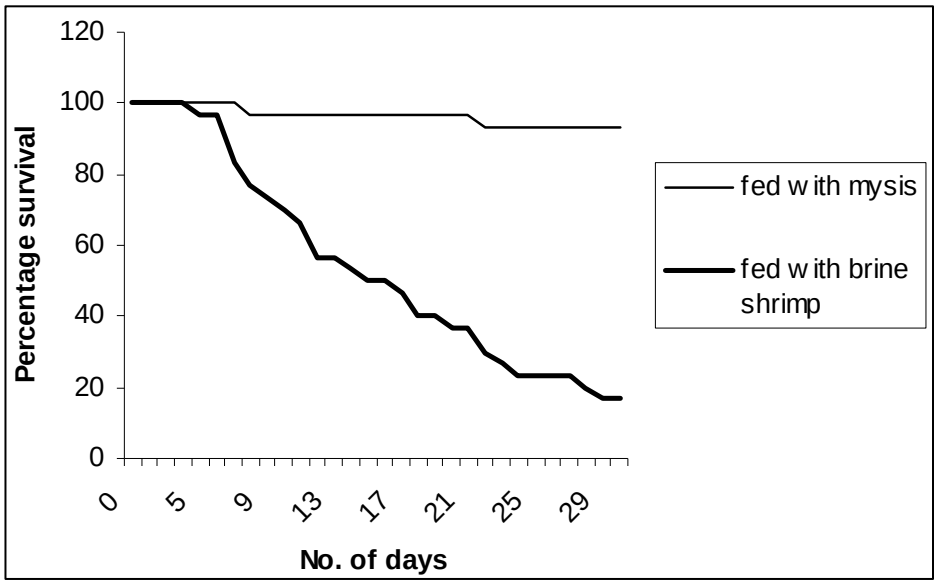

Fig. 1. Survival rate of the *S. pharaonis* hatchlings fed with shrimp mysis and brine shrimp

Discussion

The present study indicates that *S. pharaonis* is hardly enough to be reared in captivity with simple water exchange and filter techniques. The culture of cephalopods become a purely technical and economical consideration, because from the biological point of view the animals need only water of high quality, irrespective of its delivery system (Boletzky and Hanlon 1983). With a coastal location for the culture, the 'semi-closed' system can be cost effective and easy to

maintain and the culture of *S. pharaonis* for multiple generations proves that it easily adapts to this kind of system.

An earlier report states that embryos become increasingly excitable towards the end of embryonic development, but they are maintained at a low activity level by the effect of a tranquillizing compound contained in the perivitellinic fluid (Marthy et al. 1976). In the current study this finding was evidenced while in transport where the mature eggs tend to hatch out prematurely as a result of the slightest of the mechanical disturbances. The premature hatchlings which hatched out subsequently failed to survive. It was noted that younger eggs were better for transportation and incubation.

Egg capsules were incubated in perforated baskets after separating them from the cluster. Suspension of egg capsules as a whole in cluster will prevent the flow of the well-oxygenated seawater in between the eggs (Boletzky and Hanlon 1983). Walsh et al. (2002) and Nabhitabhata and Nilaphat (1999) had maintained the egg capsules in perforated baskets to ensure the circulation of well-oxygenated culture water in between the egg capsules.

In the present study, incubation time was found to be 21±3 days in all the three generations. Room temperature fluctuated between 28-32ºC. Anil et al. (2005) recorded an incubation period of 12-19 days at a slightly lesser temperature of 27-31ºC, where he stated the lesser incubation time could be due to the higher temperature. Earlier an average incubation period of 13.6 days was recorded by Minton et al. (2001) with the temperature between 25-28ºC. In the G_1 the hatching rate was 80% where, the normal hatching rate is about hundred percent in cephalopods. The reason was, out of 250 eggs 150 eggs were separated using a pair of scissors and incubated. The remaining 100 eggs were incubated without separating and it was noted that only half of the eggs developed while the other half were infested by fungi.

One of the major constraints in the cephalopod aquaculture is feeding. *S. pharaonis* hatchlings were found to start feeding actively within a couple of hours after their hatching out from the egg. Hatchlings were fed with shrimp PL and brine shrimp in their initial stages. The young sepiid cuttlefish innately recognized and fed on mysid shrimps (Messenger 1977) and the same was observed in the present study too. In contrast, Nair et al. (1986) had stated that feeding on mysids were poor, which could be due to the lack of sufficient concentration of mysids within the visual field of the cuttlefish. The experiment conducted to find out the suitability of the two feeds revealed that shrimp mysids were by far a better feed than the brine shrimps. An earlier study by Domingues et al. (2001) on *S. officinalis* had affirmed that animals fed with shrimp mysid initially, showed better growth and survival when than the ones fed with *Artemia* sp. initially.

Table 2. Growth of *S. pharaonis* during the laboratory culture

No of days	G_1		G_2		G_3	
	DML (mm±SD)	**Weight (g±SD)**	**DML (mm±SD)**	**Weight (g±SD)**	**DML (mm±SD)**	**Weight (g±SD)**
0	5.5±1.2	0.29±0.02	5.48±1.3	0.28±0.03	5.43±1.1	0.26±0.02
10	7.81±1.4	0.33±0.03	7.04±1.2	0.40±0.02	6.99±1.5	0.45±0.04
20	9.84±1.9	0.84±0.06	8.55±1.7	0.76±0.07	9.21±1.2	0.80±0.09
30	13.74±2.1	1.96±0.56	12.87±2	1.86±0.48		
40	17.34±3.5	2.17±1.1	14.55±2.9	2.00±0.92		
50	25.32±5.3	4.99±1.5	23.63±4.6	4.65±1.6		
60	34.29±8.6	8.44±1.8	30.08±8.1	6.69±1.3		
70	39.18±10.4	10.62±1.7	35.21±10.4	8.51±1.5		
80	44.0±13.1	14.0±1.9	38.93±12.7	9.56±2.4		
90	49.23±15.9	17.56±5.6	45.67±14.9	14.01±4.1		
100	60.05±17.4	47.68±13.2	51.05±16.3	16.99±3.9		
110	57.01±22.3	39.99±10.1	56.23±21.5	38.92±11.3		
120	71.34±23.2	56.18±12.5	63.98±21.8	49.68±10.6		
130	77.84±20.6	71.3±18.4	66.79±21.2	48.98±9.1		
140	76.64±25.8	75.5±19.6	73.24±20.6	60.19±13.2		
150	85.52±24.7	108±22	79.55±15.7	79.85±20.1		
160	101.14±23.9	123±25	88.97±15.6	113.2±22		
170	103.46±22.5	132.5±28	92.98±10.2	115.06±26		
180	113.94±20.4	160.018±32	98.34±0	120.69±0		
190	117.25±22.7	167.5±36				
200	122.89±20.8	201.75±35				
210	125.29±18.9	297.5±43				
220	130.8±17.6	313±45				
230	141.6±16.4	325±47				
240	152.2±15.6	401±36				
250	165.6±15.2	498±38				
260	185.23±10.1	570±35				
270	200.1±0	700±0				

G_1- First generation; G_2 – Second generation; G_3 – Third generation; DML – Dorsal Mantle Length

Table 3. Population data

	G_1	G_2	G_3
Average egg incubation period	21±3 days	21±2 days	21±1 days
Initial number of hatchlings	200	497	31
Hatching percentage	80	33.49	3.45
Initial hatchling measurement	DML = 5.5±1.2 mm weight 0.2932±0.02g	DML = 5.5±1.3 mm weight 0.2754±0.03g	DML = 5.5±1.1 mm weight 0.2585±0.02g
Average growth rate (mm/day)	0.72	0.52	0.2
Life span in days	270	180	20
Survival percentage	29.71	7.65	-
Day of Spawning	179th day	178th day	-
Total eggs laid	1494	898	-

'-' No data due to mortality

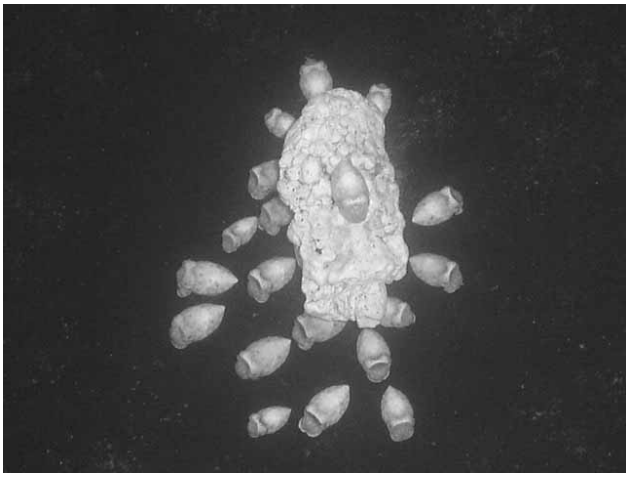

Fig. 2. Hatchlings orienting themselves around a newly immersed stone with posterior pointing the object

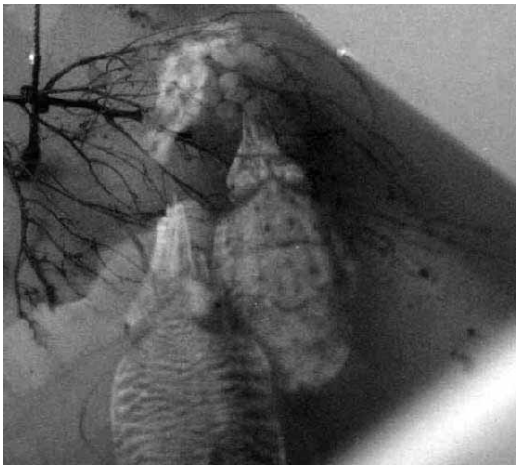

Fig. 3. Egg laying on to the sea-fan branch immersed in the tank

In the present study, the animals accepted dead fish after 40 days of culture. In the culture conducted by Nabhitabhata and Nilaphat (1999), the animals were found to accept dead fish from day 30 onwards.

Feeding in *S. pharaonis* was observed to involve three steps viz., locating of the prey, positioning and striking. Messenger (1968) analyzed the visual attack by cuttlefishes on prawns as a three-stage sequence involving attention, positioning and seizure.

There is little knowledge about the life span of *S. pharaonis*. In the current culture experiment, the maximum lifetime was found to be 270 days in the G_1 generation. The mantle length of the oldest cuttlefish measured 200.1 mm and the body weight was 700 g. The present study could be compared with the work done by Minton et al. (2001), where the oldest cuttlefish had lived 340 days with a mantle length of 300 mm and a weight of 3,045 g. Temperature could be a critical factor in deciding the average life span of cephalopods. The present experiment was carried out at a temperature between 28-32˚C. Minton et al. (2001) had carried out his studies at a temperature range of 25-28˚C. Forsythe et al. (1994) suggested that there should be a general trend of shorter life span with increasing temperature although there was no statistically significant relationship. Domingues et al. (2002) reported a similar observation where the life span and size of the cuttlefish were lesser during the summer months compared to the winter.

In the experiments conducted by Nabhitabhata and Nilaphat (1999) on *S. pharaonis*, the mantle length was measured at 13.94 cm and weighed 275 g in a life span of 210 days. Anil et al. (2005) reported an average mantle length of 168±30.2 mm and a weight of 521±38.4 g after 210 days. In the present study, the mantle length was 125.29 mm and weight was 297.5 g after 210 days of culture. Growth rate was more in the first generation when compared to the other two generations. However, Minton et al. (2001) had recorded maximum size in the G_2 generation. The reason for lesser growth for the subsequent generations in the present culture experiments should be due to the less availability of feed during the culture. The lesser growth in the present study could be attributed to the lesser availability of sufficient amount of feed. Better water quality management could be the reason for increased days of culture of 270 days in the present study, even though the temperature range was more.

No substratum was provided during the entire culture of *S. pharaonis*. It was observed that the absence of substratum did not create any problem during the culture period. The cleaning of the tanks was much easier in the absence of substrata. Forsythe et al. (1994) and Nabhitabhata and Nilaphat (1999) had agreed that no substratum was necessary for the normal growth and survival of *S. officinalis* and *S. pharaonis*, even under high-density conditions.

Conclusion

From the present study, a few aspects of growth requirements, growth and behaviour of *S. pharaonis* are carried out. The present study also undermines that *S. pharaonis* can be grown in artificial conditions without much investment on infrastructure. The *S. pharaonis* eggs are being landed in large quantities in the landing centers of southern Tamilnadu where trawl nets, shrimp nets and crab nets are the main fishing gears. These eggs are abandoned on the shore at the mercy of the sun with the potential danger of desiccation. In most instances, these eggs are lost forever. The fishermen who are having an easy access to these eggs can maintain and culture them till marketable size in their own backyards, which will give them an additional source of income. The usage of a 'semi-closed' water exchange system will enable them to successfully culture *S. pharaonis*. It is also possible to culture *S. pharaonis* and do sea-ranching in an attempt to replenish the wild stock.

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