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LIFE CYCLE OF CULTURED BOBTAIL SQUID, *EUPRYMNA HYLLEBERGI* NATEEWATHANA, 1997

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ABSTRACT: The bobtail squid, *Euprymna hyllebergi,* was cultured in the laboratory through three generations. Eggs were deposited as single egg capsules, pyramid shape with a calcified chorion. The incubation period was 14.0±1.8 days at 28°C. Hatchlings were temporarily planktonic becoming benthic after 6–8 hrs. Mean mantle length was 2.20±0.04 mm and weight 0.0041±0.0006 g. The squids were fed on larvae and postlarvae of penaeid shrimps, mysids and gobiid fish during the first month after hatching. After one month, squids were trained to accept pieces of fish meat. The squids were solitary in habit and cannibalism was observed in culture tanks. Mating and spawning was observed after 93.9±12.8 days of age. Spawning was more terminal to the life span compared to other cultured sepioid cuttlefish. Average total numbers of eggs per female was 191.3±107.4 capsules. At the age of 100 days, mean mantle length was 22.4±0.6 mm and body weight 5.88±0.17 g. Instantaneous growth rate from hatching to 100 days of age was 2.41±0.46 % by mantle length and 7.51±1.75 % by weight. Growth was similar among the three generations. Life span was average 98.9±13.6 days due to death of both sexes after the last spawning.

INTRODUCTION

The sepiolid bobtail squids of the genus *Euprymna* are small (less than 100 mm mantle length), neritic and strictly nektobenthic species, inhabiting coastal waters of the Indo-Pacific region (Summers 1985, Norman and Lu 1997). *Euprymna* spawn single egg capsules with a leathery outer coat like most other sepiolid eggs (Boletzky 1998) and the embryos possess unique bilobed external yolk sacs (Arnold *et al*. 1972). Like sepiid cuttlefishes, the bobtail squids can completely bury themselves in the substrate (Anderson 1997). The interesting behaviour of *Euprymna* is the capability to retain a "carapace" (Moynihan 1982, 1985) or "coat" of sand or other debris on their back when they emerges from burial to hunt prey (Anderson *et al*. 2002). The sand-coat makes the squid difficult to be detected visually from above and functions presumably to prevent them from being seen by predators (Anderson and Mather 1996; Shears 1988). The stickiness of the coat depends upon secretions of the ectodermal epithelium (Moynihan 1982). The choice between sticky and non-sticky is voluntary and fluctuative or obligate (Moynihan 1982). Shears (1988) suggested that the ability to use a sand-coat for camouflage of *E. scolopes* might have evolved from the initial use of the behaviour for sand consolidation when the squid is buried.

Euprymna is a well-studied group, especially since the symbiotic association between *E. scolopes* and the bioluminescent bacterium *Vibrio fischeri* has been a recent focus as a model system for investigating the process of bacterial colonization of host tissues and its effect on host development (Ruby and Lee 1998). *V. fischer*i and other luminous bacteria form a variety of pathogenic and cooperative associations with marine animals; they are increasingly recognized as causes of invertebrate diseases (Ruby and Lee 1998). Understanding the factors controlling both benign and pathogenic bacterial association and hosts will significantly benefit biotechnological and biomedical sciences (Ruby 1999). Since the process of

bacterial colonization of the squid light organ begins immediately after hatching (Ruby and McFall-Ngai 1992), studies can yield valuable results when the squids (and their luminous bacterial partners) can be cultured (independently) in the laboratory (Nishiguchi *et al*. 1998).

Summers (1985) stated that sepiolid squids are capable of laboratory culture and provide excellent models of experimentation. They are able to tolerate crowding well which is an advantageous feature of adaptability in aquarium conditions. Choe and Ohshima (1963) and Choe (1966a, b) successfully reared *E. berryi* from hatching to about 70 days. The hatchlings were planktonic with approximately 2–3 mm mantle length (ML) with daily growth rate of 2–5% by weight. *Euprymna scolopes* has been studied for embryonic development and hatchlings were reared for 28 days by Arnold *et al.* (1972) and Singley (1983). Some of the reared squids survived up to 202 days without reproducing (Arnold *et al.* 1972). Hanlon *et al.* (1997) succeeded in culturing *E. scolopes* through one life cycle of about four months after hatching. The hatchlings of *E. scolopes* were planktonic of approximately 2 mm ML and daily growth was about 8% by weight. The squid spawned several batches of egg but the hatchlings of the following generation did not survive.

Euprymna hyllebergi was firstly described from the Andaman Sea of Thailand (Indian Ocean) (Nateewathana 1997) (Fig. 1). Occurrence in the Gulf of Thailand (Pacific Ocean) was also recorded (Nateewathana *et al.* 2001). Nilaphat (2001) successfully cultured *E. hyllebergi* to one life cycle of about 3 months after hatching. Bilobed yolk sac was observed during the embryonic development. The hatchlings were planktonic of approximately 2 mm ML and daily growth rate of 6.5% by weight. Total number of eggs per one female was about 300.

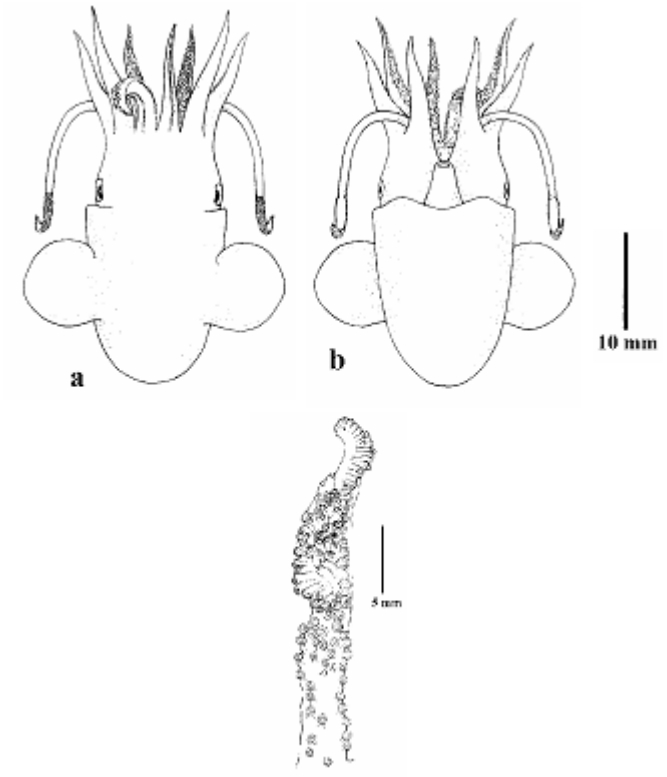

Figure 1. *Euprymna hyllebergi* Nateewathana, 1997 a -dorsal, b -ventral, c -hectocotylus. (Source: Nateewathana 1997)

The life history of *E. hyllebergi* is studied by the Department of Fisheries of Thailand as a research project in Cephalopod Culture Research and Development Programme. The aims of this project are to study the life cycles, behaviour and aquaculture methodology of *E. hyllebergi* supplying basic informations for estimation of the feasibility of small-scale culture of this species as an ornamental animal. Small-scale aquaculture yields higher unit cost of production compared with large-scale culture but it is suitable for developing countries. Supplying live squids as ornamental or home-aquarium species should add more value to the products through higher prices and shorter period of production compared with food production.

MATERIALS AND METHOD

Culture

Broodstocks of bobtail squid, *Euprymna hyllebergi*, were collected live from otter board trawlers and beam trawlers, operating along the coast of Rayong Province in the eastern part of the Gulf of Thailand, South China Sea, Pacific Ocean. The squids were transported to the cephalopod hatchery of Rayong Coastal Fisheries Research and Development Centre and then maintained in 2 m^3 concrete tanks. The squids mated and spawned in the tanks, attaching their egg capsules in clusters to artificial substrata, coral gravel (150x100 mm) and pieces of longitudinal-cut PVC pipe (50 mm diameter, 150 mm length). Substrata with egg clusters were transferred to hatch in fiberglass tanks of 50L capacity with 40L filtered seawater. Two pieces of longitudinal-cut PVC pipe (25 mm diameter, 400 mm length) equipped with aeration devices were placed in each tank, facing in the same direction, to generate artificial current. Tanks were cleaned by siphoning and water was changed about 50% by volume daily. Temperature change was minimized by means of running water around the tank base. Average temperature during the experiment was 28.2±1.6ºC, pH was 8.0±0.4 and salinity was 32.5±1.7 ppt. Hatchlings were raised using the same system.

Hatchlings were fed with live, hatchery-produced penaeid shrimp larvae (*Penaeus merguiensis*, *P. monodon*) of protozoea and mysis stages for 5 days after hatching (Fig. 2). Postlarvae of penaeid shrimps of same species as well as wild mysids (*Mesopodopsis oreintalis*) were fed to the squids from hatching to 40 days. After 30 days, young squids were fed wild palaemonid shrimps (*Palaemon styliferus*) and wild mysids (*Acetes* spp.)

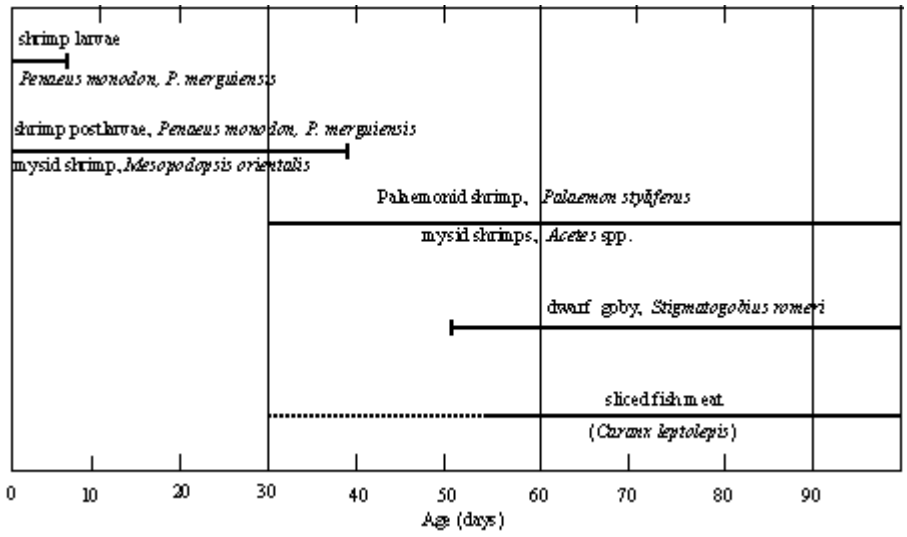

Figure 2. Diagram of feeding of *Euprymna hyllebergi* on various live (——) and dead ( ) food corresponding to the age (days) after hatching.

and after 50 days, wild dwarf gobies (*Stigmatogobius romeri*). After 30 days, the squids were trained to feed on dead fish meat (*Caranx leptolepis*). Size grading was performed every 10 days from 30 days after hatching and culture density was reduced from initial density of 2–6 ind.L^{-1} for at least 25% after each grading.

Behaviour

Aspects of behaviour were observed from live specimens and recorded with drawings, and still and video photography. The total number of the bobtail squids observed in this study was 3,750 individuals.

Feeding and Growth

Feeding rate (% per day) and gross food conversion efficiency (%) was determined every 10 days from 1,850 individuals and calculated according to Choe (1966a, b):

$$FR = [F/ (tW)] \times 100$$
$$GFCE = (W_2\text{-}W_1) /F \times 100$$

where FR is feeding rate (% per day),
GFCE is gross food conversion efficiency (%),
F is total food consumed in wet weight basis (g),
W average weight (g)
W_1 initial weight (g),
W_2 final weight (g),
t is number of days (10 day period).

Growth was determined every 10 days in terms of gain in dorsal mantle length (ML, mm) and wet body weight (W, g). Instantaneous relative growth rate (%) of ML and weight was calculated according to Forsythe (1984) and Forsythe and Van Heukelem (1987) from 1,850 individuals:

$$IGRL = [(\ln ML_2\text{-}\ln ML_1)/ t] \times 100$$
and $$IGRW = [(\ln W_2\text{-}\ln W_1)/ t] \times 100$$

where IGRL is instantaneous relative growth rate in terms of ML (%),
IGRW instantaneous relative growth rate in term of wet body weight (%),
ML_1 initial mantle length (mm),
ML_2 final mantle length (mm).

Growth in terms of the mantle length-weight relationship was expressed by power regression models from 1,506 individuals. Mantle length-age relationship was expressed by exponential regression model in the early growth phase (936 individuals) and quadratic regression model in the following phase (741 individuals). Weight-at-age relationship was determined by exponential regression (936 individuals) and cubic regression models (734 individuals), respectively.

$$W = a_1 ML^{b}_1$$
$$ML = a_2 e^{b2A}$$
$$ML = a_3 + b_3 A + b_4 A^2$$
$$W = a_4 e^{b5A}$$
$$W = a_6 + b_6 A + b_7 A^2 + b_8 A^3$$

where a is the constant elevations,
b the slope,
A the age (days).

RESULTS

Eggs

Egg capsules are single, lacking a stalk, opaque white and droplet shape (Fig. 3). The size of the egg capsule was 4.15±0.41 mm in major axis, 3.34±0.28 mm in minor axis and 0.021±0.002 g in weight. About 2 hrs after being laid, the outer coat turned brown and leathery. The capsules were laid in single layer or piled up into clusters of several layers. After 5 days, the capsule became more transparent and the visible embryo could be observed.

Figure 3. Egg capsule of *Euprymna hyllebergi* (20x), tip at right.

The egg was telolecithal. Eight-cell cleavage observed at 10 hrs after fertilization was asymmetric. Clockwise rotation of the embryo was observed from 76 to 198 hrs (day 3–8). Organogenesis occurred from 98 hrs (day 4). The unique bilobed character of the external yolk sac was observed from 120 hrs (day 5). Chromatophores were observed from 164 hrs (day 6). Four diverticula of the internal yolk sac were observed from 198 hrs (day 8). First hatching occurred at 288 hrs (day 12) (Fig. 4).

The embryonic phase was 12–18 days in length, average 14.0±1.8 days at 27.5±2.0°C. Hatching occurred at night until dawn, mostly between 0400–0700 hrs. 97–99% of eggs hatched at night, 1–3% during the day. Hatching period of the eggs in the same cluster took five days from the first to the last eggs. About 80% of hatching occurred on the third day. Hatching rate was 94.35±5.01% (range 82.32–100.00%), the majority of the remaining eggs were unfertilized.

Behaviour

The living mode of the hatchling was planktonic. The internal yolk sac was still visible through the transparent mantle from hatching to the third day (Fig. 4). The planktonic phase lasted 6–8 hrs. After that, the hatchling adopted a benthic habit but still entered the water column on a regular basis to about 25–30 days. Nocturnal swimming as well as foraging and eating prey in the water column was observed in 80–90% of hatchlings during this period. Then the squid gradually became fully benthic. Most of the squids were observed foraging in the water column but eating on bottom substratum instead of eating in the water column. At night, the squid rose up from the bottom and hovered in the water column. The squid returned to the substrate in the morning around 0700–0800 hrs. Camouflaging by disruptive coloration and inking was functional from hatching.

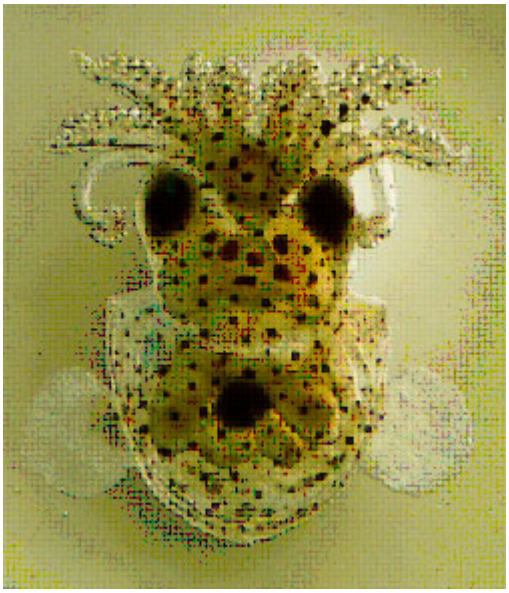

Figure 4. Hatchling of *Euprymna hyllebergi* (dorsal, 17x) with internal yolk sacs remaining inside the mantle cavity.

Squid were solitary in habit. No gregarious behaviour was observed. Cryptic behaviour was exhibited as disruptive coloration on hard substrata (tank bottom) and burrowing in soft substrata (sand). Burrowing was diurnal and could be observed from 5–7 days after hatching. Complete burying (whole body under substratum) and partial (upper part of body exposed) burying were achieved by mantle rocking in association with fin beating and water jetting. The last step of complete burying was when the third arms were swept backwards collecting sand grains on to the head. Angling behaviour, about one-third of the third arm vertically exposed upward out of the substratum and waved, was observed while burying in two specimens. In tanks without soft substratum, the squids rested on the substrate adjacent to coral gravels.

Squid had the ability to adhere a continuous coating of bottom materials to the mantle, to form the so called “sand coat” (Fig. 5). Sand coating was observed during daytime when the squid emerged from the sand. It also had ability to drop the sand coat instantaneously as a unit of similar shape to itself. Sand coating behaviour was observed from 5–7 days after hatching, the same period of the first burrowing capability.

Swimming was observed at night more than during the day. During 1–25 days after hatching, about 80–90% of squids were observed swimming at night, decreasing to 30% after this period. Colour pattern during swimming was a transparent

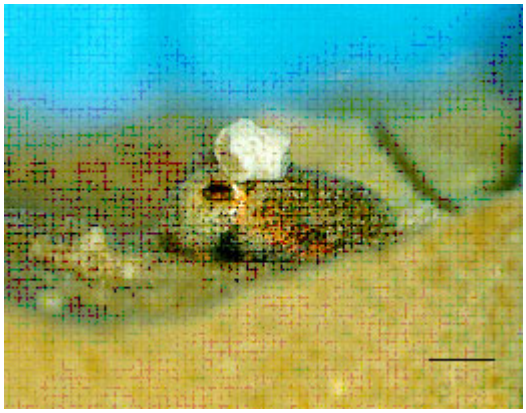

Figure 5. *Euprymna hyllebergi* with sand-coat and coral gravel for camouflaging (bar scale 10 mm).

mantle with small brown spots. If artificial light was turned on at night and the light period was prolonged for more than 10 minutes, the squid sank to the substrate. Swimming was counter-current in direction and its mantle was at the angle of 20–40° to the substrate.

Feeding on live prey was observed only at night, exhibiting nocturnal behaviour. From hatching to 30 days, the squid seized and ate its prey in the water column while hovering. The prey was seized and held using the arms. After 25 days, the squid gradually changed to seizing its prey in the water column and then consuming it on the substrate. The live prey was seized using the tentacles and the dead prey using the arms. Crustaceans were preferred over dwarf gobies. Cannibalism was observed in squids with size difference of more than 50% between 10 to 30 days after hatching.

Feeding and Growth

Hatchlings of bobtail squid grew from 2.20±0.04 mm average mantle length (ML) and 0.004±0.001 g average weight to 7.28±0.20 mm ML and 0.263±0.019 g in 30 days (Fig. 6a, b). At 60 days after hatching, the squid had grown to 16.74±0.78 mm ML and 2.602±0.233 g weight and at 100 days 22.43±0.56 mm ML and 5.878±0.168 g. Instantaneous relative growth rate (IGR) was highest between 10 and 20 days after hatching with an average of 4.83±0.23% in ML

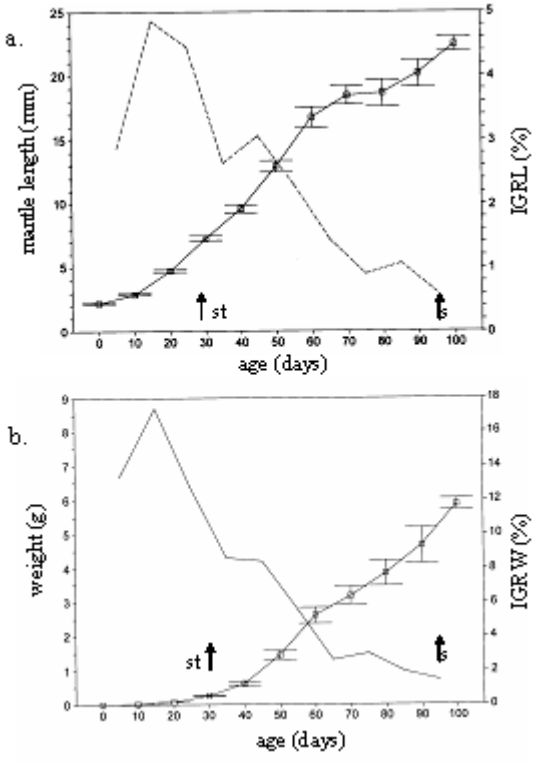

Figure 6. Growth in terms of: a -mantle length (mm), instantaneous relative growth rate (IGRL: %) and age (days) after hatching; b -weight (g), instantaneous relative growth rate (IGRW: %) and age (days) after hatching. Arrows indicate spawning (s) and settling stage (st).

(Fig. 6a) and 17.40±0.67 % in weight (Fig 6b). Average IGR from hatching to 100 days of age was 2.41±0.46 % in ML and 7.51±1.75 % in weight. Food consumption of the bobtail squid averaged 0.193±0.055 $g.d^{-1}$ or 37.12±6.75 %body $weight.d^{-1}$. Food conversion efficiency was highest between 50 and 60 days (Fig. 7), 63.70±5.91% (range 42.83–98.55) with average from hatching to 100 days of 37.22±4.68 % (range 14.20–98.55).

Growth in terms of ML (mm) and weight (g) was not significantly different ($P>0.05$) among the three generations of the bobtail squid (Fig. 8a, b). IGR in ML was highest in the first generation (G_1), 2.50±0.49 % and in weight in the third generation (G_3), 9.11±2.33 % (Tab. 1). Feeding was highest in the first generation (G_1) in term of wet weight, 0.192±0.0535 $BW.d^{-1}$, but in term of percentage

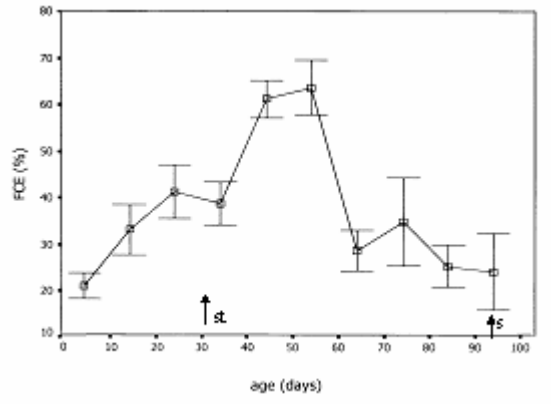

Figure 7. Food conversion efficiency (%) of cultured *Euprymna hyllebergi* during growth (age: days after hatching). Arrows indicate spawning (s) and settling stage (st).

of BW.d^{-1} was in the second generation (G_2), 48.19±22.26 %. Food conversion efficiency was highest in the third generation (G_3), 50.8±12.4 %.

The growth models showed two phases. The early phase was from hatching to 30 days and the second phase was from 30 days to 122 days. The relationship between mantle length (mm) and weight (g) was expressed as power regression models (Fig. 9):

$W = 1.320 \times 10^{-4} ML^{4.124}$

($r^2 = 0.852$, n = 686)

and $W = 1.032 \times 10^{-3} ML^{2.780}$

($r^2 = 0.947$, n = 761)

The relationship between mantle length (mm) and age (days after hatching) was expressed as the exponential model in the early phase and the quadratic regression model in the second phase (Fig. 10):

$ML = 1.988e^{4.205 \times 10^{*}(-2)A}$

($r^2 = 0.887$, n = 936)

and $ML = 0.407A - 1.553 \times 10^{-3}A^2 - 3.648$

($r^2 = 0.767$, n = 741)

The relationship between weight (g) and age (days after hatching) was expressed as the exponential model in the early phase and the cubic regression model in the following phase (Fig. 11):

$W = 2.750 \times 10^{-3} e^{0.153A}$

($r^2 = 0.895$, n = 936)

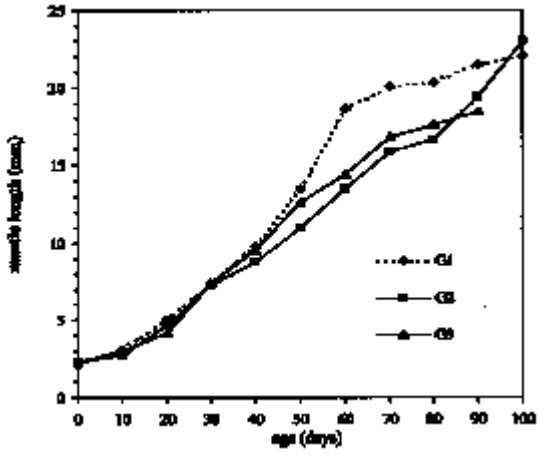

Figure 8a. Growth of three consecutive cultured generations (G1, G2, G3, respectively) of *Euprymna hyllebergi* in terms of mantle length (mm) and age (days) after hatching.

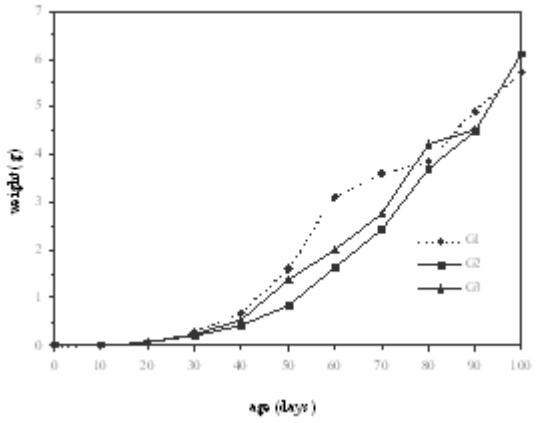

Figure 8b. Growth of three consecutive cultured generations (G1-G3) of *Euprymna hyllebergi* in terms of weight (g) and age (days) after hatching.

and $W = 1.952 - 0.147A + 3.570 \times 10^{-3}A^2 - 1.728 \times 10^{-5}A^3$

($r^2 = 0.805$, n = 734)

Mating and Spawning

Mating was observed from 66 days after hatching. Mating occurred without prior pair formation. Courtship with colour displays and agonistic behaviour was not observed. The male attended the swimming female then approached and grasped her from below in male to female neck

Table 1. Feeding and growth in three cultured generations (G) of *Euprymna hyllebergi*: food consumption (g, %body weight.d^{-1}), FCE: food conversion efficiency (%), IGRL: instantaneous growth rate in length (%), IGRW: instantaneous growth rate in weight (%) and LS: longevity of life span (d).

G		food consumption		FCE	IGRL	IGRW	LS
		(g)	(% body weight.d^{-1})	(%)	(%)	(%)	(d)
1	mean	0.192	24.89	33.91	2.50	7.12	100.4
	SE	0.053	11.48	5.97	0.49	1.65	15.5
2	mean	0.179	48.19	39.52	2.22	7.80	91.5
	SE	0.053	22.26	6.42	0.49	1.93	10.6
3	mean	0.091	38.28	50.82	2.38	9.11	98.5
	SE	0.037	24.03	12.42	0.52	2.33	13.3
1-3	mean	0.193	37.12	37.22	2.41	7.51	98.9
	SE	0.055	6.75	4.68	0.46	1.75	13.6

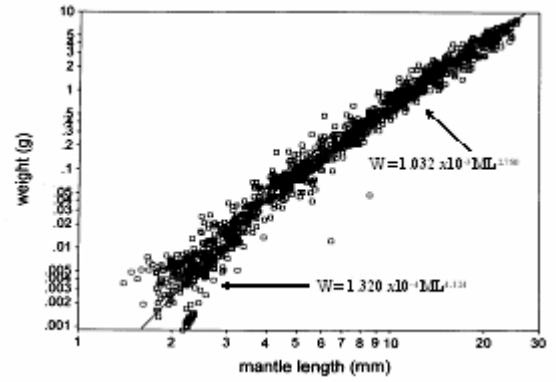

Figure 9. Relationships between mantle length (mm) and weight (g) of *Euprymna hyllebergi*, intercept of the two regressions at 5.5 mm mantle length.

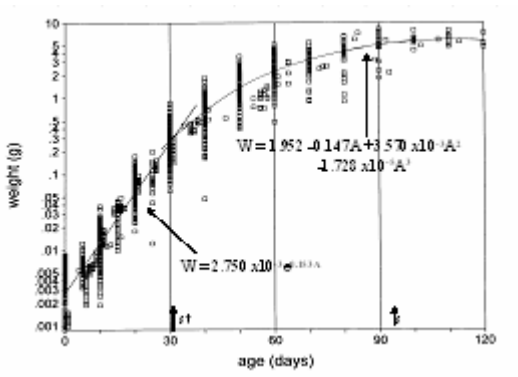

Figure 11. Relationships between weight (g) and age (days) after hatching of *Euprymna hyllebergi.* Arrows indicate spawning (s) and settling stage (st).

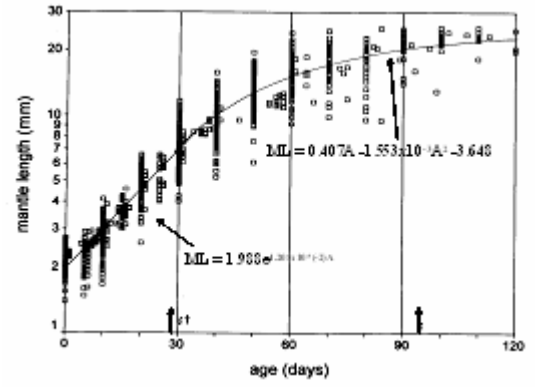

Figure 10. Relationships between mantle length (mm) and age (days) after hatching of *Euprymna hyllebergi.* Arrows indicate spawning (s) and settling stage (st).

position (Fig. 12). The colour pattern of the male was dark brown during mating. The male initially grasped the female at her mantle, using arms II, III and IV, then the grasp shifted to the female neck. The female was pulled down to bottom where copulation took place (Fig. 12). The male hectocotylus was inserted into the female mantle cavity (Fig. 13). The colour pattern of the female was pale brown during copulation. Copulation took 7–10 min and the pair separated after that.

Spawning was observed at dawn, 2–3 days after mating. Prior to spawning, the female investigated substrata for attaching her egg capsules by swimming around, touching the substrata with the tip of her arm cone. At the

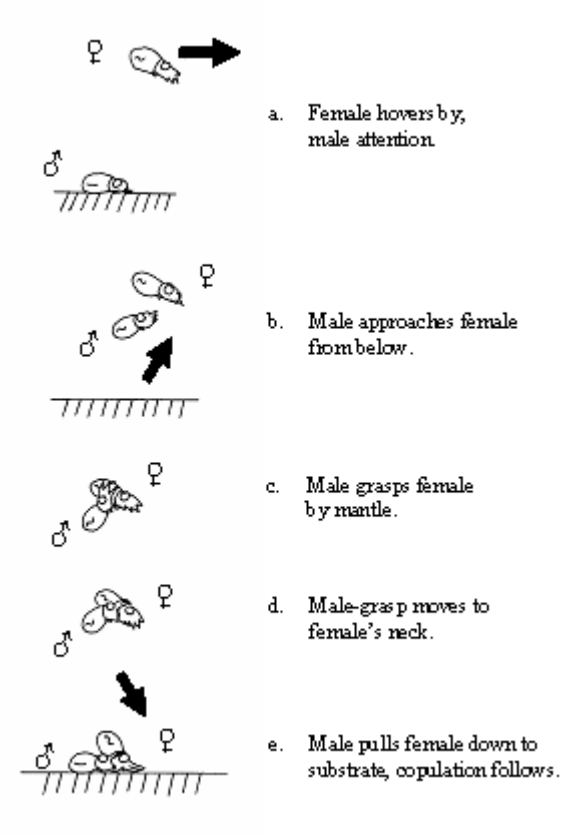

Figure 12. Stages in mating behaviour of *Euprymna hyllebergi*; a) female hovers by, male attention, b) male approaches female from below, c) male grasps female by mantle, d) male-grasp moves to female's neck, e) male pulls female down to substrate, copulation follows.

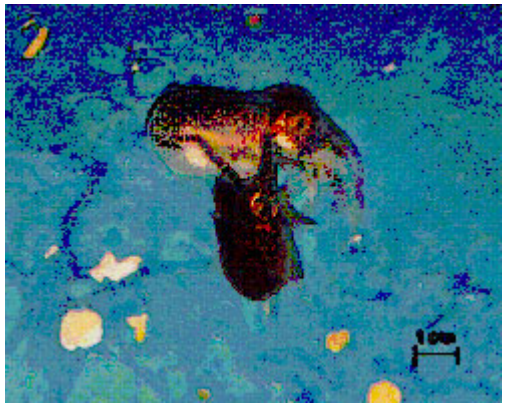

Figure 13. Mating behaviour of *Euprymna hyllebergi*.

Figure 14. Spawning behaviour of *Euprymna hyllebergi*, female attaching egg capsules to coral gravel.

selected site, the female moved towards the substratum from her lying position to attach her egg capsules (Fig. 14). The period of attaching was 40–60 seconds (s) for one capsule. The interval between each capsule attachment lengthened as the number of capsules increased, up to 2–3 minutes. During the interval between egg-laying, the female twisted her mantle to left and right several times and stopped for 10–20 s before attaching another capsule. No subsequent maternal care on the egg capsules was observed. Spawning behaviour was normally cryptic. In cultured tanks, the female attached her egg capsules to the underside of coral (Fig. 15) and on the ceiling of PVC pipe "dens". In tanks without cryptic sites, egg capsules were attached in exposed sites *i.e.* lateral side of coral, tank wall at about 100–150 mm above the bottom. Spawning was continuous and intermittent, egg capsules were laid in only one or several batches in a period of about 1–20 days. Number of egg capsules per batch was not observed. Total number of eggs per female was 108–464 with average of 191.3±107.4 capsules.

After her last spawning, the female swam up into the water column, her arms spread unable to form the regular arm cone. Respiratory movement of the mantle was slower than in normal conditions. Swimming became jerky in nature and her colour pattern turned pale yellow and then transparent. Then the female sank down to the substrate and died within 1–3 hrs.

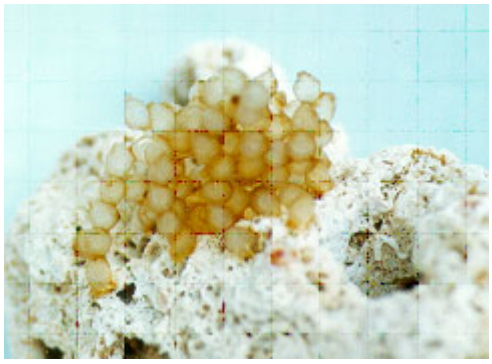

Figure 15. Egg cluster of *Euprymna hyllebergi* attached to underside of coral gravel (bar scale 0.5 cm).

Life Span

Females died within 1–3 hrs after last spawning. The male died within a few days after the female. Life span of the female was 92.7±9.7 days (80–113) and of the male was 108.3±12.6 days (92–122). Maximum life span of the male was 122 days and 113 days in the female. Life span of both sexes combined was an average of 98.9±13.6 days (80–122). Life span of the first generation (G_1) was the longest among the three generations (Table 1), 100.4±15.5 days (80–122), and the second generation (G_2) was the shortest, 91.5±10.6 days (84–99).

DISCUSSION

The shape and size of the egg capsules of *Euprymna hyllebergi* were similar to other species, particularly in the calcareous leather-like coating (Choe 1966a, Boletzky 1998, Arnold *et al.*1972). The embryonic period of *E. hyllebergi* was about 14 days at 27.5ºC, which was shorter than the 20 days in *E. berryi* at 23.5–24.0ºC (Choe 1966a), 20 days in *E. scolopes* at 24ºC (Arnold *et al.* 1972), 21 days in *E. scolopes* at 23ºC (Claes and Dunlap 2000), 18–22 days in *E. scolopes* at 21–23ºC (Hanlon *et al.* 1997) (Table 3), 29 days at 20ºC in *E. tasmanica* (Lu and Dunning, 1998) and 32 days in *E. morsei* at 20ºC (Watanabe 1997). Other sepiolids possess longer embryonic period, *e.g., Sepiola* of about 45 days at 20ºC (at lower temperature), *Sepietta obscura* 45 days at 16–17ºC and *Rossia macrosoma* 90–120 days at 15ºC (Boletzky 1975). Boletzky (1998) reported that the outer coat of sepiolid egg capsules become a rigid shell or a solid protection that allows the developing embryo to become a "sessile organism", as the development covers several months during which the temperature were minimal. The leathery coat of the egg capsule with comparatively shorter embryonic period of *Euprymna* at higher temperature needed further explanation. However, the embryonic period of *E. hyllebergi*, 14.0 days, was comparable to other cultured sepioids that were 12.6 days in *Sepiella inermis* (Nabhitabhata 1997) and 14.3 days in *Sepia pharaonis* (Nabhitabhata and Nilaphat 1999) at 28ºC. The single egg capsule and two weeks of embryonic development (at 28ºC) are common characters in life history of the cultured sepioid cephalopods.

The bilobed external yolk sac is a embryonic character that has also been observed in *E. scolopes* (Arnold *et al.* 1972), *E. morsei* (Watanabe 1997) and *E. hyllebergi* (this study). Hatching rate of more than 80% in *E. berryi* (Choe 1966a) and 70–90% (Hanlon *et al.* 1997) in *E. scolopes* are comparable to 82–100% of *E. hyllebergi* (Table 2). Planktonic mode of hatchling is similar in the three species. The hatchlings were alternatively planktonic and benthic in the first month after hatching in *E. scolopes* (Hanlon *et al.* 1997) as well as in *E. hyllebergi* (this study). Burrowing behaviour of *E scolopes* was observed from 5–6 days after hatching (Singley 1983), similar to what was observed in *E. hyllebergi*. Southern bobtail squid, *E. tasmanica*, is different. Norman (2000) reported that hatchlings of *E. tasmanica* quickly settled to the substrates and buried in the sand (benthic mode?). English (1981) and Lu and Dunning (1998) also reported immediate settlement of hatchlings in *E. tasmanica* but no successful burying was achieved prior to death at three days after hatching.

Mysid shrimps were used in successfully rearing *Euprymna*: *Neomysis japonica* for *E. berryi* (Choe 1966a, b; Choe and Ohshima 1963), *Neomysis*, *Mysidopsis* and *Amisomysis* for *E. scolopes* (Arnold *et al.* 1972; Hanlon *et al.* 1997, Claes and Dunlap 2000) and *Mesopodopsis orientalis* for *E. hyllebergi* (this study). However,

Table 2. Comparison of life cycle and behaviour of cultured bobtail squids. References: a -Choe (1966a,b), Choe and Ohshima (1963); b -Hanlon and Messenger (1996), Hanlon *et al.* (1997), Claes and Dunlap (2000), Moynihan (1982, 1985); c -Lu and Dunning (1998), Norman (2000), Norman and Lu (1997) d -Nilaphat (2001), present study.

aspects	*Euprymna berryi*[a]	*Euprymna scolopes*[b]	*Euprymna tasmanica*[c]	*Euprymna hyllebergi*[d]
Habitat	seagrass (?)	sandy beach	seagrass, sand, mud	mud bottom
Prey	mysids, shrimp larvae	mysids, fish larvae palaemonid shrimp	isopods, amphipods, palaemonid shrimp	shrimp larvae, mysids, palaemonid shrimp, goby
Mating behaviour:				
-age (d)	-	61-115	-	>66
-motion of male to female prior copulation	-	lying-lying (?)	-	lying-swimming
-motion during copulation	-	lying	-	lying
-position	-	male to female neck	male-to-female-neck	male to female neck
-period (min)	-	25-80	-	7-10
Spawning behaviour:				
-age (d)	-	>58	-	66-115 (93.9)
-period of attachment (s)	-	10	-	40–60
-number of eggs per female	-	50-250	100-146	108-464 (191.3)
Egg:				
-capsule type	single	single	single	single
-embryonic phase (d)	20	18-22	29	12-18
-temperature (°C)	23.5-24	21-23	20	26-30 (27.5)
-hatching rate (%)	>80	70-90	-	82-100 (94.4)
Hatchling:				
-mode of living	planktonic	planktonic	benthic (?)	planktonic
-size (ML : mm)	2.4-2.8	1.6-1.9	3	1.4-2.8
Daily growth (% w)	2.34-5.28	7.6-8.4	-	1.50-17.40
Life span:				
-average (d)	-	80	-	99
-maximum (d)	-	139	-	125

live zooplankton, amphipods and postlarval mysids were also used for *E. scolopes* (Hanlon *et al.* 1997, Claes and Dunlap 2000). This is similar to feeding the shrimp larvae of comparable size to E. *hyllebergi* hatchlings for 5 days after hatching. Fishes were used as food in rearing of *E. scolopes* (Arnold *et al.* 1972, Hanlon *et al.* 1997), *E. morsei* (Segawa and Maekawa 2003) and *E. hyllebergi* in the present study but preference of *Euprymna* on crustaceans (mysids, palaemonid and penaeid shrimps) compared to fish was agreed in all studies. The general trend for *Euprymna* culture from Hanlon *et al.* (1997) and the present study is to feed planktonic food to squids before the settling stage, at which time the squids are column feeders, and benthic food after settling stage, at which time the squids are benthic feeders.

The alternative planktonic-benthic mode during the first month after hatching of cultured *E. scolopes* (Hanlon *et al.* 1997) and *E. hyllebergi* in the present study corresponded to behaviour observed in wild *E. scolopes* (Anderson and Mather 1996). Anderson and Mather (1996) reported the majority of the juveniles (d ≤ 10 mm ML) swam in midwater or just under the surface at night while the adults (e ≥ 10 mm ML) sat on the sand surface. The "benthic" mode observed in hatchlings of *E. tasmanica* (Norman 2000) probably corresponds to the mentioned planktonic-benthic mode.

The difference in behaviour between the species was the "angling" behaviour during burrowing which was observed in *E. hyllebergi* but not in *E tasmanica* (Norman and Lu 1997, Norman 2000) or *E. scolopes* (Anderson *et al.*

2002). Anderson *et al.* (2002) suggested that the angling behaviour observed in *Rossia pacifica* might be used while partially buried. Angling behaviour of *E. hyllebergi* should be noted as one of the differences between the species of *Euprymna*, although it was a rare case.

Transition in growth is reflected in the nature of the growth models. The stage where the models shifted to a higher elevation was at about 30 days after hatching, corresponding to the observed settlement stage (Figs. 9–11). Hanlon *et al.* (1997) reported the mantle length-age relationship as ML = 0.102 +0.217A (r^2 = 0.914, n = 49) and the weight-age relationship was W = 3.296 $x10^{-3}$ $e^{(8.373\ x10*-2)A}$ (r^2 = 0.945, n= 34) in cultured *E. scolopes*. These models differ from the present study for *E. hyllebergi*. Growth patterns of *E. scolopes* were simple for the whole life span without the transition stages. The difference may be due to the differences in the numbers of replicates used in model plotting, resulting in different model fitting. The length-weight relationship model of *E. scolopes* was W = 1.5 $x10^{-3}$ $ML^{2.674}$ (n = 41) (Hanlon *et al.* 1997). The slope, 2.674, was similar to 2.780 in *E. hyllebergi* (n = 761) for mantle length of more than 5.5 mm, hence after the settlement stage.

Daily growth in weight, 1.5–17.4%, of *E. hyllebergi* was comparable to other *Euprymna*; 2.3–5.4% in *E. berryi*, from hatching to 67 days (Choe 1966b), and about 8% in *E. scolopes* (Hanlon *et al.* 1997, Claes and Dunlap 2000) (Table 2). Growth variation may be due to differences among species and culture condition. In similar conditions, growth was similar among the three cultured generations. From an aquaculture point of view, the supply of live broodstocks was feasible without any obvious effects of inbreeding, at least through three generations that nearly encompass one year.

Food conversion efficiency (FCE) rose from around 30–40% after hatching to 60–70% during 40–60 days after hatching, potentially relating to the storage of energy for the consequent reproductive period (Fig. 7), since the first mating was observed after 66 days. The FCE appeared stable during reproduction, at about 20–30%.

Mating and spawning of *E. hyllebergi* occurred at a similar age after hatching to *E. scolopes*, around 2–3 months (Singley 1983, Hanlon *et al.* 1997). Position, male to female neck, and behavioural process was also similar. Period of attachment of egg capsules tended to be longer in *E. hyllebergi* compared to *E. scolopes*, although the capsule size of the former species is smaller than that of the latter, 3 versus 4 mm (Table 2). Along with smaller size of the egg capsules, the number of eggs per female of *E. hyllebergi* was greater, 108–464, compared to 100–150 eggs of *E. morsei* (Watanabe 1997), 12–250 eggs of *E. scolopes* (Singley 1983, Hanlon *et al.* 1997), 38–175 eggs of *E. stenodactyla* (Deepak and Patterson 2003) and 100–146 eggs of *E. tasmanica* (Norman and Lu 1997). Mangold (1987) suggested that sepiolid species spawned between 50 and 200 eggs and spawning is intermittent, often irregular and may be extended over several weeks which agrees with the present study in *E. hyllebergi*. The ovary of these species, all of small adult size, can only contain a fraction of the total number of mature ova at any one time (Mangold 1987).

The overall life cycle of the four species, *E. berryi*, *E. scolopes*, *E. tasmanica* and *E. hyllbergi* is similar (Table 2). The common characters were egg capsule characters, embryonic phase of 2–3 weeks, hatching rate of 80% or higher, planktonic hatchlings and mating behaviour. The notable differences for *E. hyllebergi* were habitat, shorter period of mating, longer period of egg-capsule attachment, greater numbers of eggs per female and longer life span. The average life span of *E. hyllebergi* was 99 days which was nearly three weeks longer than the 80 days reported for *E. scolopes* (Hanlon *et al.* 1997).

Compared to other cultured sepioid cephalopods, *E. hyllebergi* is the smallest species and the spawning of *E. hyllebergi* was more terminal both on basis of age (days) after hatching (Fig. 16a) and period of longevity as percentage of life span (Fig. 16b). The spawning time of *Sepia pharaonis* and *Sepiella inermis* was at around 80% of their life span while of *E. hyllbergi* was at 97% of its life span. This might indicate that the strategy of this small species is to spend a comparatively longer proportion of its life in collective energy storage in order to reach maximal reproductive output.

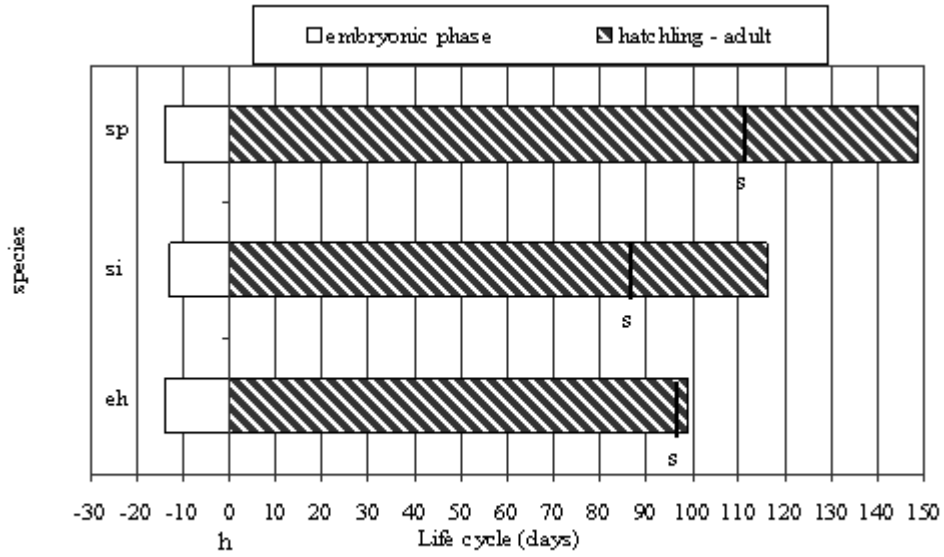

Figure 16a. Comparison of life cycle of cultured cephalopods in age (days). References : sp -*Sepia pharaonis*, (Nabhitabhata and Nilaphat 1999); si -*Sepiella inermis* (Nabhitabhata 1997); eh -*Euprymna hyllebergi* present study; h -hatching, s -spawning.

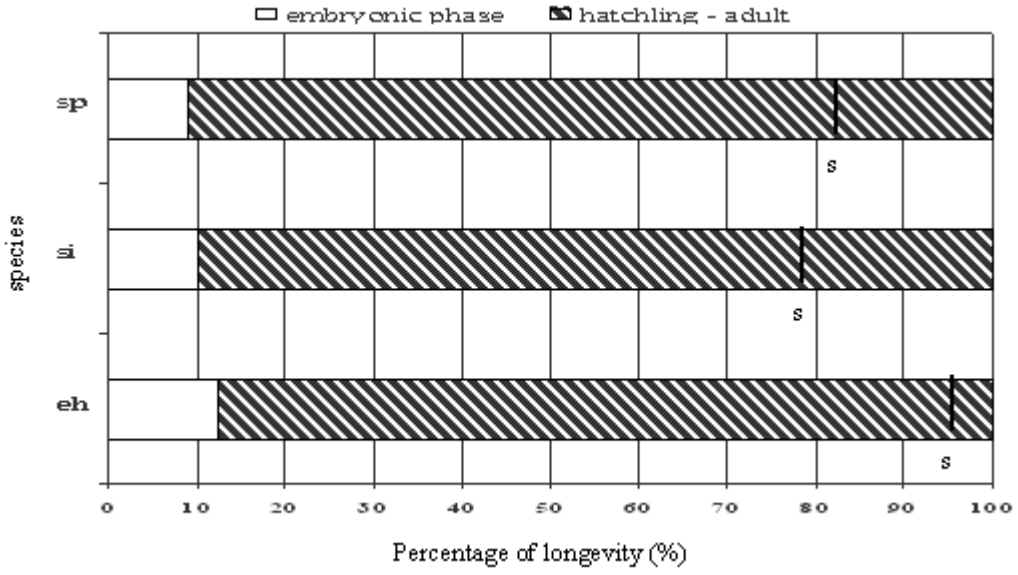

Figure 16b. Life cycle of cultured cephalopods in percentage of longevity (% of life span). References : sp -*Sepia pharaonis*, (Nabhitabhata and Nilaphat 1999); si -*Sepiella inermis* (Nabhitabhata 1997); eh -*Euprymna hyllebergi* present study; s -spawning.

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