

CMFRI

Course Manual

*Winter School on
Recent Advances in Breeding and Larviculture
of Marine Finfish and Shellfish*

30.12.2008 -19.1.2009

Compiled and Edited by

*Dr. K. Madhu, Senior Scientist and Director,
Winter school*

&

*Dr. Rema Madhu, Senior Scientist and Co-ordinator
Central Marine Fisheries Research Institute*



Central Marine Fisheries Research Institute

(Indian Council of Agricultural Research)

P.B.No.1603, Marine Drive North Extension,

Ernakulam North ,P.O.

Cochin, KERALA – INDIA - 682018



M.K. Anil,

Senior Scientist, Vizhinjam Research Centre of Central Marine Fisheries Research Institute,

Vizhinjam, Kerala - 695 521

E-mail : mkanil65@gmail.com

Introduction

Cephalopods are the largest, most active invertebrates. About 1, 17, 278 tonnes of cephalopods are exploited during 2003 in India (Annam *et al.*, 2004). During 2002-2003 India has exported 41,381 tonnes of frozen cuttlefish and 37838 tonnes of frozen squid valued at US\$ 166.2 million to countries such as Japan, USA and the European Union (Anon, 2003). Cephalopods are unique because they are 85% protein by dry weight (16-21% by wet weight) (Lakshmanan and Balachandran, 2000) and are considered a delicacy in seafood restaurants. Recent years have witnessed a significant amount of research interest in cephalopod culture, in order to develop technology for commercial farming as well as to produce multiple laboratory generations for research in neurobiology (Minton *et al.* 2001). They are highly promising biomedical models because of their giant axons and are of interest to neurobiologists. Squids 4 months old have giant axons larger than 450µm in diameter. Studies have shown that the ultrastructure and physiology of these systems rival the sophistication of their vertebrate counterparts, the vestibular end organs and the vestibulo-oculomotor system. In detail, many parallels exist, e.g., the dynamic response characteristics (gain and phase lag values) of the cephalopod angular acceleration receptor systems are similar to those of the vertebrate semicircular canals, the putative transmitters in the afferent and efferent fiber systems are similar, and the cephalopod brain pathways involved in oculomotor control have vertebrate-like organizations. Thus, these systems are interesting invertebrate models that can substantially contribute to our understanding of the basic principles of morphology, physiology and pathology of these systems in higher vertebrates, including man. Choe and Oshima (1963) and Choe (1966) reared three species of the genus *Sepia*, the squid *Sepioteuthis lessoniana* and the sepiolid *Euprymna berryi* from egg to adult size. Nabhitabhata and co-workers of Rayong Brackish water Fisheries Station have conducted pioneering research work on the culture of several species of commercially important cephalopods in Thailand (Nabhitabhata, 1978a, b, Nabhitabhata *et al.*, 1984 and Nabhitabhata and Nilaphat, 1999). *Sepia pharaonis* was successfully bred under laboratory conditions in Thailand as well as the USA using sophisticated, temperature controlled recirculation systems (Nabhitabhata, 1994, Minton *et al.*, 2001). In India our first major success in Cephalopod Mariculture was realized in 1999 with the cuttlefish *Sepiella inermis* (Sivalingam, 1999) at Tuticiorin Research Centre of CMFRI. Since that time we have worked on Squids *Euroteutis duvaucillii*, *Sepioteuthis lessoniana*, cuttlefish *Sepia pharaonis*, and Octopus *Octopus dulfousii*. However, for the past three years we have focused our efforts on developing the potential of the cuttlefish *Sepia pharaonis* and squid, *Sepioteuthis lessoniana*.

Egg Collection: For the collection of egg capsules different collectors such as old net, coconut spadix and nylon ropes can be hung from a raft or coconut spadices tied together can be submerged at selected points in the breeding season. These egg collectors are recovered using GPS.

Rearing of cephalopods

Cephalopods require high standards of water quality while feeding at high rates and producing copious quantities of ammonia and ink.

Water quality

The water quality criteria for cephalopod culture in both nursing and grow-out phases regardless of species, are as follows:-

Dissolved oxygen: >5 mg/l

Salinity : 30-35 ppt₀
Temperature : 27-32 c
P_H : 7.0-8.5 Ammonia : <0.005 mg/lit
Nitrate : <25 mg/ml.

Food and feeding

The limitation is that the cephalopods are carnivorous and selective feeders; they require live feed with a specific size, shape and movement. Feed without these characteristics will be ignored and the cephalopods will starve to death. The degree of selectivity is higher in the early stages compared to the adults. After a stage they can be trained to accept low value fish. Brine shrimp nauplii, which is used as live feed for most of the cultivated marine fishes and shellfishes, is unfortunately not suitable for cephalopods. But adult brine shrimp can be used as a feed supplement. Mysid shrimp collected from natural waters is used world over to rear cephalopod hatchlings. Experiments conducted in Thailand and India have shown that live prawn postlarvae can be used as feed for Cephalopod but will substantially increase the production cost. In USA the first successful defined diet formulated specifically for cephalopods. The next step will be to utilize these nutritionally defined diets and to investigate the amino acid metabolism of cephalopods. At Karwar Research Centre of CMFRI, spineless cuttlefish *Sepiella inermis* was successfully reared from the egg mass collected from wild. They mated under captivity and spawned on 86th day at a size of 60 mm mantle length producing 214 viable eggs. Only live food organisms, consisting of mysids, shrimp post larvae, juvenile fishes formed the diet of these animals in different stages. The initial average size of hatchling was 4mm ML (0.02g) that increased to on 110th days respectively. Average survival was 43, 37 and 28% at the end of first, second and third months (Anil, 2003). At Vizhinjam Research Centre of CMFRI, Pharaoh cuttlefish (*Sepia pharaonis*) was successfully reared from egg to an average size of 168 mm mantle length (ML) and weight of 521 g in 226 days in the laboratory, using simple biological filtration systems. The period of egg incubation was 15 days at a temperature range of 27-31° C. Food items given were live mysids, *Artemia salina*, juveniles of fishes and prawns. Subsequently, the juveniles were slowly acquainted with food items such as dead caridean prawns and small fishes. Hatchlings were reared at a stocking density of one animal/litre during the first month, and subsequently stocking density was reduced as the growth proceeded. The study shows that the pharaoh cuttlefish can be reared under captivity with a survival rate of 40% with the use of live feed limited to the initial phase of 50 days. (Anil *et al.* 2004).

With the use of cage type of rearing systems in open waters and with better feeding schedules, commercial culture systems with better survival rates and growth can be developed. The future of cephalopod culture depends on the development of mass culture techniques of mysids for feeding hatchlings with *Artemia* as supplement and artificial feed for the adults. The recent success achieved in feeding the young ones with *Artemia* as supplement and acquainting the cuttlefish to food items other than live feed such as anchovies and sardines which can be obtained in large quantities are steps in this direction.

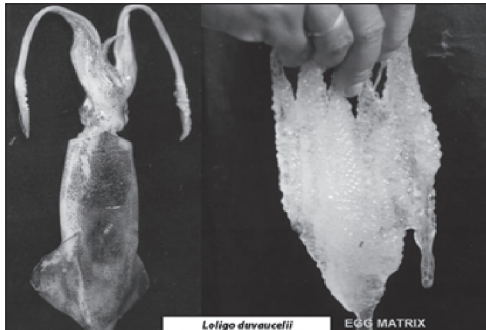
Current problems and future trends

Cuttlefish *S. pharaonis* has several characteristics that make it one of the most promising species for future commercial aquaculture. Among them are: large eggs, which are easily transported and maintained; hatchlings resembling miniature adults in shape, ability to handle relatively large prey (sometimes twice as big as themselves); high survival rates of hatchlings, compared to other species of cephalopods; resistance to crowding, disease and handling, so they can be easily shipped; fast growth and short life cycle, in some geographical regions, allowing more than one generation every year. However, *S. pharaonis* culture shows several problematic factors keeping it out of commercial culture, so they represent bottlenecks. Those are: lower fertility and fecundity under culture conditions; semelparous life history, therefore requiring a new group of breeders for each cycle; hatchlings requiring live food and juveniles and adult stages refusing dry pellets; the species is cannibalistic; production of the live food required is not yet developed, so the cost of food supply is high; and a basic immunological system (Forsythe *et al.* 1987, 1990) which may generate problems in intensive culture.

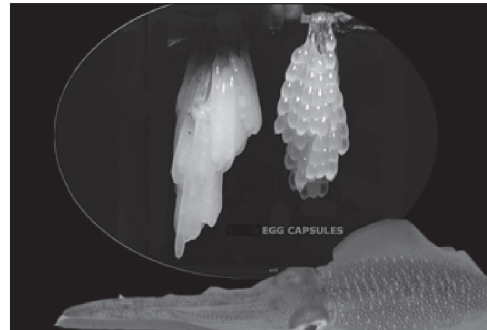


Egg masses of different species of cephalopods

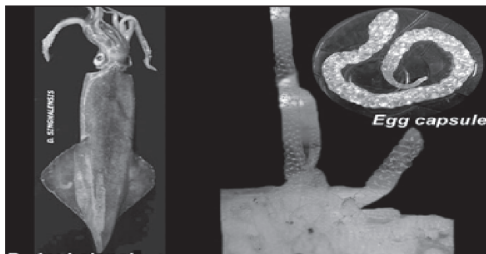
Egg mass of *L. duvaucelii*



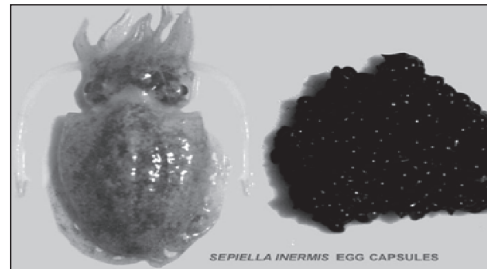
Egg mass of *S. lessoniana*



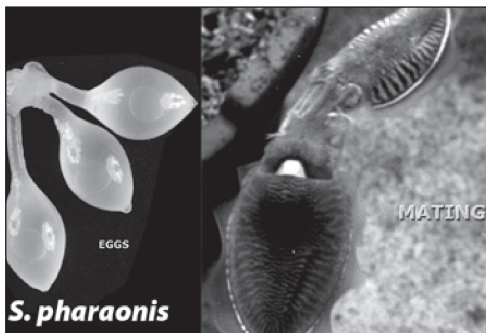
Egg mass of *D. singhalensis*



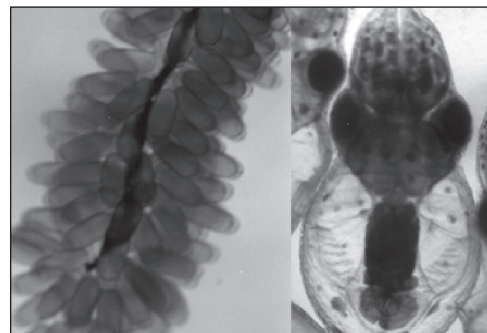
Egg mass of *S. inermis*



Egg mass of *S. pharaonis*



Egg mass of *O. dollfusii*



Coconut spadix



Collected eggs



References

- Anil M.K., 2003. Laboratory rearing and breeding of spineless cuttlefish *Sepiella inermis* Orbigny (Mollusca: Cephalopoda). *J. Aqua. Trop.*, 18 (1): 35-44.
- Anil M.K., Joseph Andrews and C. unnikrishnan, 2005. Growth, behaviour and mating of pharaoh cuttlefish *Sepia pharaonis* Ehrenberg under captivity. *Israeli Journal of Aquaculture*.
- Choe S., 1966. On the eggs, rearing, habits of fry and growth of some cephalopods. *Bull. Mar. Sci.*, 16 (2): 330 – 348.
- Choe S. and Y. Oshima, 1963. Rearing of cuttlefishes and squids. *Nature*, Lond., 297:3-7.
- Choe S., 1966. On the eggs, rearing, habits of fry and growth of some cephalopods. *Bull. Mar. Sci.*, 16 (2): 330 – 348.
- Cole K.S., 1972. Membranes, ions and impulses. Second edition, University of California Press, Berkeley, CA, 569 pp.
- Forsythe JW, Hanlon RT, Lee PG 1987. A synopsis of cephalopod pathology in captivity. *Intern Assoc Aqua Anim Med* 1(4): 130-135.
- Forsythe JW, Hanlon RT, Lee PG 1990. A formulary for treating cephalopod mollusc diseases. In F O Perkins & T C Cheng ed, *Pathology in Marine Science*. San Diego, Academic Press: 51-63.
- Hanley J.S., Shashar N., Smolowitz R., Bullis R.A., Mebane W.N., Gabr H.R. and R.T. Hanlon, 1998. Modified laboratory culture techniques for the European cuttlefish *Sepia officinalis*. *Biol. Bull.* 195: 223-225.
- La Roe E.T., 1971. The culture and maintenance of the loliginid squid *Sepioteuthis sepioidea* and *Doryteuthis plei*. *Mar. Biol.*, 9 (1): 9 –25.
- Lee P.G., Turk P.E., Yang W.T. and R.T. Hanlon, 1994. Biological characteristics and biomedical applications of the squid *Sepioteuthis lessoniana* cultured through multiple generations. *Biol. Bull.* (Wood Hole, Mass.) 186: 328-341.
- Messenger J.B., 1968. The visual attack of the cuttlefish, *Sepia officinalis*. *Anim. Behav.* 16: 342 – 357.
- Messenger J.B., 1977. Prey-capture and learning in the cuttlefish, *Sepia*. *Symp. Zool. Soc. Lond.*, 38: 347-376.
- Minton J.W., Walsh L.S., Lee P.G. and J.W. Forsythe, 2001. First multi-generation culture of the tropical cuttlefish *Sepia pharaonis* Ehreberg, 1831. *Aquaculture International*, 9: 375-392.
- Nabhitabhata J., 1978a. Rearing experiment on economic cephalopod –I: Long-finned squid *Sepioteuthis lessoniana*. Technical paper 1978, Rayong Brackish water Fisheries Station, Brackish water Fisheries Divisions, Department of Fisheries. 41 pp.
- Nabhitabhata J., 1978b. Rearing experiments on economic cephalopod-II: Cuttlefish *Sepia pharaonis* Ehrenberg., Technical paper 1978, *Ibid.* 62 pp.
- Nabhitabhata J., Pitak P. and K. Somnuk, 1984. Culture, growth and behaviour of spineless cuttlefish *Sepiella inermis* Fer. & d'Orb. Technical paper 5/1984, *Ibid.* 48 pp.
- Nabhitabhata J., 1994. The culture of cephalopods. *Fishing chimes*, September, 1994, 12 – 16.
- Nabhitabhata J. and P. Nilaphat, 1999. Life cycle of cultured pharaoh cuttlefish *Sepia pharaonis* Ehrenberg, 1831. Phuket Marine Biological center special Publication, 19: 25-40.
- Nair K.P., Thomas P.A., Gopakumar G., Vincent S.G. and T.A. Omana, (1986). Some observations on the hatching and post-hatching behaviour of the cuttlefish *Sepia pharaonis* Ehreberg. In: (E.G.Silas Ed.) *Cephalopod Bionomics, Fisheries and Resource of the Exclusive Economic Zone of India.*, *Bull. Cent. Mar. Fish. Res. Inst.*, 37: 157 – 159.
- Rosenberg P., 1973. The giant axon of the squid; a useful preparation for neurochemical and pharmacological studies. In: (R. Fried (Ed.), *Methods of Neurochemistry*, Vol.4, 97 – 160, MerceL Dekker, Inc., New York, NY.
- Silas E.G., Sarvesan R., Nair K.P., Sastri Y.A., Sreenivasan P.V., Meiyappan M.M., Vidyasagar K., Rao K.S. and B.N. Rao, 1986. Some aspects of the biology of cuttlefishes. In: (E.G.Silas Ed.) *Cephalopod Bionomics, Fisheries and Resource of the Exclusive Economic Zone of India.*, *Bull. Cent. Mar. Fish. Res. Inst.*, 37: 49 – 70.
- Sivalingam D., 1999. Successful breeding and hatchery experiments of the spineless cuttlefish *Sepiella inermis* at Tuticorin shellfish hatchery. *Mar. Fish. Infor. Serv. T&E Ser.*, No.161: 11 – 13.
- Sivalingam D. and S.K. Pillai, 1983. Preliminary experiments on breeding of Cephalopods. *Proc. Symp. Coastal Aquaculture*, Part 2, *Mar. Biol. Assn. India*: 633 – 635.

