

IN VITRO FERTILIZATION OF BANANA PRAWN *PENAEUS MERGUIENSIS* DE MAN

S. R. SREEKUMARAN NAIR

National Institute of Oceanography, Dona Paula, Goa-403 004.

ABSTRACT

Successful *in vitro* fertilization of the commercially important prawn, *Penaeus merguensis* has been achieved. Suspensions of sperms and ova were mixed and agitated for five minutes. Healthy nauplii were observed on following day and developed to the mysis stage by the tenth day. 42% of the eggs hatched to nauplii and 28.5% reached the mysis stage.

Key-words: Fertilization; penaeid shrimp; *Penaeus merguensis*.

Recent studies on shrimp reproduction have resulted in the techniques necessary to complete the life cycles of many species in captivity (Nurjana and Yang, 1976; Aquacop, 1975; Primavera, 1978). Induced maturation and spawning has been achieved for some species of commercially important penaeid shrimp (Muthu and Lakshminarayana, 1977; Lawrence, Chamberlian and Hutchins 1981; Muthu, Lakshminarayana and Mohamad, 1986). However, there are many uncertainties still to be overcome for complete control on the entire reproductive cycle of shrimps. Repeated spawning, viability of spawns, drop in hatching rate during subsequent spawns and fertilization are part of it. There is still no way to predict that one particular female would spawn on a specific night. This depends on many other factors including mating, full maturity of the female and environmental conditions. Uncertainty of the night of spawning could be overcome by the use of *in vitro* fertilization. Successful *in vitro* fertilization using flagellated sperm have been reported in a number of invertebrates (Costello, Danielson, Eggers, Fox and Hensley, 1957) and vertebrates (Austin, 1972). However, in penaeid shrimps with non-motile sperm, such attempts are few (Clark, Talbot, Neal, Mock and Salser, 1973). The present paper reports successful *in vitro* fertilization with the gametes of prawn *Penaeus merguensis*.

Mature banana prawns, *P. merguensis*, were collected from the sea off Goa by trawl fishing at 25 m depth. Each trawl was restricted to a short duration of 30 minutes. The mature females and males were separated out and kept in fibreglass tanks containing seawater from the same location. Prawns were transported to the aquaculture laboratory (Chatterji, Ingole and Parulekar, 1983) for further work. The whole experiment was conducted in seawater of 33‰ salinity and 8.1 pH.

Sperm ampoules (seminal vesicles) were removed from two sexually mature males (115 and 125 mm total length) and they were minced in filtered seawater to form a thick sperm suspension (50,000 sperm/ml seawater). Ripe (stage 4) females (Chamberlain *et al.*, 1985) of total length 130 and 145 mm were dissected and their ovaries removed and placed in seawater. The teased ovary portions were agitated in seawater to release ova. About 2000 eggs were removed into five 1 lit. Ehrlenmeyer flasks each containing about 150 ml of sperm suspension prepared as before. The flasks were moved rapidly in a gyratory path for nearly 5 minutes. The sperm-egg suspensions thus obtained were removed into round fibre glass tanks carrying 25 l clean seawater which is vigorously aerated. The seawater temperature was around 29.5°C. The tanks were left undisturbed overnight. The control flasks were without sperm suspensions.

Active nauplii at normal appearance were observed swimming in the tanks in the morning after (16 hours) treatment. All 5 replicate tanks carried actively swimming nauplii.

About 42% of the 10,500 eggs used in the experiment hatched out as nauplii and 28.5% reached the mysis III stage at which the experiment was terminated. They were fed mixed diatoms and rotifers during the zoea and mysis stages.

Control egg samples which were given the same treatment except mixing with sperms did not show any development.

Tremendous success has been achieved recently in reproduction of prawns and shrimps under captivity including artificial insemination and interspecific breeding (Sandifer and Smith, 1979; Dobkin and Bailey, 1979; Laubier-Bonichon and Ponticelli, 1981; Lumare, 1981; Muthu and Lakshminarayana, 1984). In the context of trials for better and assured spawnings and better survival of eggs and larvae, the results obtained with *Penaeus merguensis* in the current study are encouraging. The survival rate obtained out of this experiment is not very high and further trials would be needed to perfect the techniques. The technique would find greater application for open thelyca shrimp where spermathecal deposition takes place only just before spawning resulting in poor spawning successes. *In vitro* fertilization would also find application in interspecific and intraspecific breeding trials.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. B. N. Desai, Director, National Institute of Oceanography, Goa for his encouragements and Dr. A. H. Parulekar for his enthusiastic support for prawn culture research.

REFERENCES

- Aquacop, 1975. Maturation and spawning in captivity of penaeid shrimp: *Penaeus merguiensis* de Man, *P. japonicus* Bate, *P. aztecus* Ives, *Metapenaeus ensis* de Hann and *P. semisulcatus* de Haan. *Proceedings of the World Mariculture Society*, 8: 123-132.
- Austin, C. R., 1972. Fertilization. 1.1: *Reproduction in mammals. I. Germ cells and fertilization*, Ed. C. R. Austin and R. V. Short, Cambridge Press, London; 103-133.
- Chamberlain, G. W., M. G. Haby and R. J. Miget. 1985. *Texas shrimp farming Manual*, Texas Agricultural Extension Service Publication, Corpus Christ, Texas, VII, 37pp.
- Chatterji, A., B. S. Ingole and A. H. Parulekar, 1983. Sea-water circulating system in aquaculture laboratory *Mahasagar-Bulletin of the National Institute of Oceanography*, 16: 81-86.
- Clarke, Jr., W. H., P. Talbot, R. A. Neal, C. R. Mock and B. R. Salser, 1973. *In vitro* fertilization with non-motile spermatozoa of the brown shrimp *Penaeus aztecus*. *Marine Biology*, 22: 353-354.
- Costello, D. P., M. E. Danielson, A. Eggers, M. H. Fox and C. Hensley, 1957. *Methods for obtaining and handling marine eggs and embryos*. Lancaster Press, USA, 247pp.
- Dobkin, S. and D. J. Bailey, 1979. Growth rates of *Macrobrachium rosenbergii* in South Florida. 2. Growth of intraspecific hybrids. *Proceedings of the World Mariculture Society* 10: 571-574.
- Laubier-Bonichon, A. and A. Ponticelli, 1981. Artificial laying of spermato-phores on females of the shrimp *Penaeus japonicus* Bate. *World Mariculture Society Meeting*, Venice, Italy, Poster 92.
- Lawrence, A. L., G. W. Chamberlain and D. L. Hutchins, 1981. Shrimp mariculture: an overview. *Texas A & M Sea Grant Publ.*, TAMU-SG-82-503, College Station, Texas, 9pp.
- Lumare, L. 1981. Artificial reproduction in *Penaeus japonicus* Bate on a basis of commercial production of eggs and larvae. *World Mariculture Society Meeting*, Venice, Italy, Poster 96.
- Muthu, M. S. and A. Lakshminarayana, 1977. Induced maturation and spawning of Indian penaeid prawn. *Indian Journal of Fisheries* 24: 172-180.
- Muthu, M. S. and A. Lakshminarayana, 1984. Artificial insemination of *Penaeus monodon*. *Current Science*, 53: 1075-1077.
- Muthu, M. S., A. Lakshminarayana and K. M. Mohamad, 1986. Induced maturation and spawning of *Penaeus indicus* without eyestalk ablation. *Indian Journal of Fisheries*, 33: 246-250.

- Nurjana, M. L. and W. T. Yang, 1976. Induced gonad maturation, spawning and postlarval production of *Penaeus merguensis* de Man. *Bulletin of Shrimp Culture Research Centre*, II, (2): 177-186.
- Primavera, J. H., 1978. Induced maturation and spawning in five-month-old *Penaeus monodon* Fabricius by eyestalk ablation. *Aquaculture*, 13: 355-359.
- Sandifer, P. A. and T. I. J. Smith, 1979. A method for artificial insemination of *Macrobrachium* prawns and its potential use in inheritance and hybridization studies. *Proceedings of the World Mariculture Society*, 10: 403-408.