Journal of the Indian Fisheries Association
27, 2000, 19-25

ARTIFICIAL INSEMINATION IN PENAEID SHRIMPS

RAVINDRA PAWAR* AND M. IFTEKHAR MOHIUDDIN

Central Institute of Fisheries Education
(CIFE) Deemed University
Versova, Mumbai- 400 061

ABSTRACT

Artificial Insemination (AI) is a tool for genetic manipulation in the shrimp stocks. It is seen as one of the means for propagating shrimp culture to new areas by controlled reproduction. Attempts at artificial insemination in the dominant closed-thelycum penaeid shrimps species of the area *viz. Metapenaeus affinis* and *Metapenaeus brevicornis* were induced in wild adult stocks collected off Mumbai coast. Female specimens were subjected to unilateral eyestalk ablation by pinching so as to induce moulting and maturation. AI was performed two days after moulting on these females when the cuticle was still soft and flexible. Moulting also ensured rejection of initial spermatophores, if present. Response of males to electrical stimulation for spermatophore expulsion was spontaneous. Use of tissue glue for spermatophore retention was found to be unnecessary. Latency period ranged between 10-16 days, while spawning occurred within 10-12 days of spermatophore transfer. Three partial spawning were recorded *viz.,* two in *Metapenaeus affinis* and one in *Metapenaeus brevicornis* with an average spawning and hatching rates of 30% and 72.3% respectively. Average survival from first nauplius (N1) to one-day old post-larva (PL1) was a meager 3.43%. Use of AI in genetic manipulation of shrimp stocks for aquacultural purposes is indicated.

Keywords : Artificial insemination, electro-ejaculation, penaeid shrimp, (glue) α–cyanoacrylate

INTRODUCTION

Crustacean genetic engineering or genetic manipulation techniques are of a recent origin with most of the work being carried out on the applied aquacultural aspects, including broodstock development, heritability, and hybridization. The primary techniques for stock improvement are stock selection, selective breeding hybridization and sex control. Artificial Insemination (AI), basically is a tool employed for genetic improvements in the phenotype and genotype characters, growth rates, disease resistance, and a number of other characters. Problems such as fragmentary nature of knowledge, and difficulty in synchronization of mating behaviour of some commercially important decapods can be successfully tackled through AI – the key to successful breeding programme. AI can thus be useful in increasing production

* Present address: College of Fisheries (Konkan Krishi Vidyapeeth), Ratnagiri- 415 629.

from a maturation laboratory and thereby assist in closing the life cycle of such species. This is believed to be helpful in the domestication efforts for shrimp stocks and also in propagating them to new areas by controlled reproduction.

The technique of AI first patented by Persyn (1977) in open-thelycum species has undergone several modifications (Sandifer & Smith, 1979; Sandifer & Lynn, 1980). Later, the technique was extended to closed-thelycum species by Lumare (1981), Ponticelli (1981), Muthu & Laxminarayan (1984), Lin & Ting (1986), Lin & Hanyu (1990) etc. The technique of AI is now routinely used in several leading laboratories to accomplish fertilization in adverse biotic and abiotic conditions (AQUACOP 1983). Yet failures in reproduction are quite common due to factors such as local environmental conditions and species availability. The present work was, therefore, initiated with a view to properly understand such factors by undertaking AI experiments on two of the most commonly occurring penaeid shrimps species of the area viz. *Metapenaeus affinis* (H. Milne Edwards, 1837) and *Metapenaeus brevicornis* (H. Milne Edwards, 1837) both of which possess a 'closed-thelycum'.

MATERIAL AND METHODS

Penaeid shrimps are heterosexuals wherein both the sexes can be distinguished externally. Since AI directly deals with the manipulation of the genital organs, it is worthwhile understanding their basic morphology, which has a direct bearing on the manner in which AI is performed on them. Males are characterized by the presence of "petasma" on the first pleopod and a genital opening on the coxa of the fifth (last) pereiopod or the walking leg. Internally, the male reproductive system consists of a paired testis, vas deferens, and terminal ampoules located in the cardiac region dorsal to the hepatopancreas. The testes are translucent and are comprised of six lobes, each connected in the inner margins leading to a long coiled vas deferens, which terminate as ampoules at the base of the coxa of the fifth pereiopods. Each ampoule contains spermatophore, which are sacs enclosing spermatozoa in a viscous, slightly greyish or milky medium.

Females are characterized by the presence of a raised platform called "thelycum" situated ventrally between the fourth and fifth pereiopods and a genital opening on the coxa of the third picking leg. Thelycum are of two types: (a) 'Closed' – in which the pocket-like opening is covered by an anterior and a pair of lateral plates. In species with this type, the general period between mating and spawning is about one month. (b) 'Open' – in which there is only a depression surrounded by bristles instead of the pocket (plates). In species with this type, the mating takes place barely a few hours before spawning. Internally, the female reproductive system consists of two bilaterally symmetrical and partly fused ovaries, extending through almost the entire length of the abdomen. The oviducts originate at the anterior tips and terminate in gonopores at the base of the third picking legs.

Live individuals of both *Metapenaeus affinis* and *Metapenaeus brevicornis* were procured from the trawl catches of the

MFV "*Narmada*" of the Central Institute of Fisheries Education, Mumbai. Males and females of both species were held separately, specieswise, in glass aquarium tanks of 54-litre capacity filled 2/3 rd with seawater. Diffused aeration with carborundum stone (one per tank) was provided. Large, healthy, and active specimens were used for further experimentation. Daily activities for healthy maintenance included:

1. cleaning of the tank bottoms
2. 100% water exchange with fresh seawater
3. recording of important physico-chemical data at regular time intervals **(Table 1)**
4. feeding the individuals with boiled clam meat, minced meat of prawns or squids and/or pelleted feed (occasionally) @ 4-6% of body weight in 3-4 rations per day.

Table 1: Physico-chemical parameters of experimental medium.

Sr. No.	Parameter	Range
1.	Temperature (°C)	26 - 30
2.	Salinity (ppt)	31 - 32
3.	Dissolved Oxygen (mg/l)	7.5 - 8.5
4.	Free Carbon-dioxide (mg/l)	Nil
5.	pH	8.0 - 8.5
6.	Total Alkalinity (mg/l)	135 - 151
7.	Phosphate (mg/l)	0.31 - 0.35
8.	Nitrate (mg/l)	3.0 - 3.6
9.	Nitrite (mg/l)	0.12 – 0.16
10.	Ammonia (mg/l)	Nil

Females of both species (mature and immature) were initially subjected to unilateral eyestalk ablation by pinching so as to initiate moulting and ovarian maturation. It was necessary for the females to moult for the rejection of spermatophores, if any retained from earlier mating. AI was then performed on the moulted females (Total Length 110 - 145 mm) when their cuticle was still flexible employing the following methods:

1. Electro-ejaculation and transplantation:

In this method, the spermatophores were first obtained by employing mild AC current of 4.5 volts, at the terminal ampoule (coxa of the fifth pereiopod of males). Spermatophores thus elicited were received on a sterilized spatula. The actual process of insemination included – holding of the newly moulted female on a slab of moist rubber foam with her ventral side up, and placing and pushing (inserting) the spermatophores inside the crescent-shaped, soft lateral plates of the thelycum. All these actions were performed as quickly and as gently as possible. The operated females were then allowed convalescence in continuously aerated good quality seawater.

2. Electro-ejaculation and transplantation using tissue glue:

In this method, the same procedure as that of Method 1 was followed. The only difference was the application of a tissue glue (commercially available as α cyanoacrylate) on the thelycal plates for retention/attachment of spermatophores. Generally, use of such glues is recommended in open-thelycum species,

which are devoid of the spermatophore-retaining thelycal plates. Although, closed-thelycum species were used in the present study, tissue glue was used in some instances so as to observe its utility in such species.

In all, a total of seven attempts at AI were carried out on *Metapenaeus affinis* while only three could be made on *Metapenaeus brevicornis*. The number of spermatophores transplanted (per female) as also the sizes of the recipient females were recorded **(Table 2)**.

RESULTS

Almost all males subjected to a current of 4.5 volts AC for 1 - 3 seconds invariably expelled both spermatophores excepting two in which the contents of only one terminal ampoule only could be extruded **(Table 3)**. A single spermatophore was observed to average 5.2 to 5.8 mg. Post-stimulus survival of the males could not be recorded as it was not possible to keep them in isolation for follow-up action.

Females responded to unilateral eyestalk ablation by moulting within 10 - 16 days. All inseminated females were found to retain the spermatophores successfully. Spawning was recorded after 10 - 12 days of sperm transfer. Out of the ten attempts, successful spawning was obtained in three females while in one case abnormal spawning was recorded. All the three spawning, though, were partial with a hatching rate of 66.0 - 81.0% (average 72.3%) with an average survival rate of 3.43% **(Table 4)**.

Table 3: Details of spermatophore expulsion by males of *Metapenaeus affinis* and *M. brevicornis*.

Species	Particulars	Response Level					Total Number
		BC	**OC**	**BP**	**OP**	**N**	
M. affinis	Number	6	1	-	-	-	7
	Percentage (%)	85.7	14.3	-	-	-	
	Cumulative (%)	85.7	100	-	-	-	
M.brevicornis	Number	3	-	-	-	-	3
	Percentage (%)	100	-	-	-	-	
	Cumulative (%)	100	-	-	-	-	

BC = contents of both ampoules expelled, OC = contents of one ampoule expelled, BP = contents of both ampoules partially expelled, OP = contents of one ampoule partially expelled, N = no response.

Table 2: Details of Spermatophore implantation in the females of *Metapenaeus affinis* (Tag no. 1 – 7) and *M. brevicornis* (Tag no. 8 – 10)

Tag No.	Body Weight (g)	Total Length (mm)	Date of Moulting	Date of Insemination	Spermatophores Implanted (no.)	Remarks
1.	15.0	122	29/1/94	31/1/94	One	Sperm mass held intact. Mortality recorded after 3 weeks. No spawning recorded.
2.	15.0	126	20/1/94	22/1/94	One	Female recorded dead on the second day of insemination.
3.	16.5	130	15/1/94	17/1/94	One	Appendages found damaged due to (accidental) excessive glue application. No spawning recorded up to 1 month.
4.	17.2	140	19/2/94	21/2/94	Two	Sperm mass held intact. No spawning recorded despite full ovarian development.
5.	20.1	130	6/3/94	8/3/94	One	Partial spawning with few viable eggs recorded.
6.	26.0	141	2/3/94	4/3/94	Two	Partial spawning with viable eggs recorded.
7.	28.0	145	8/3/94	10/3/94	Two	Abnormal spawning with uneven eggs recorded. Female found dead after 3 weeks.
8.	12.5	110	3/4/94	5/4/94	Two	Partial spawning with few viable eggs recorded.
9.	13.2	111	20/2/94	22/2/94	One	Sperm mass held intact. No spawning recorded despite full ovarian development.
10.	14.5	114	10/2/94	12/2/94	Two	Sperm mass held intact. Female found dead after 2 weeks.

Table 4: Spawning and larval developmental details in the artificially inseminated females of *Metapenaeus affinis* (Tag No. 5 – 7) and *M. brevicornis* (Tag No. 8).

Tag No.	Body Weight (g)	Total Length (mm)	Date of Insemination	Spawning			Days from N1 to PL1	PL1 obtained	
				Date	No. of eggs	Hatching rate (%)		Number	Survival (%)
5	20.1	130	8/3/94	21/3/94	3000	66	11	98	3.3
6	26.0	141	4/3/94	16/3/94	5400	70	12	223	4.1
7	28.0	145	10/3/94	21/3/94	a	Nil	Nil	Nil	Nil
8	12.5	110	5/4/94	18/4/94	3600	81	11	106	29

a: abnormal spawning with uneven eggs.

DISCUSSION

Response of males to electrical stimulus for spermatophore expulsion was found to be very spontaneous indicating their suitability for AI purpose. Though their post-stimulus survival was not recorded, apparently no mortality was observed in such males within two days of spermatophore expulsion. However, mortality was recorded in males where multiple stimuli and/or higher voltages were used in a single attempt to elicit the spermatophores.

Observations on spermatophore retention revealed the use of tissue glue (α–cyanoacrylate) in present to be superfluous in closed-thelycum species. Muthu & Laxminarayan (1984) and Lin & Hanyu (1990) also obtained successful results without such glues in similar species. On the contrary, accidental excessive application of α-cyanoacrylate was found to damage the appendages of the specimen.

Muthu & Laxminarayan (1984) also recorded low spawning and hatching rates as obtained in the present study. This can be partially attributed to the decline in the sperm quality under laboratory conditions as observed by Leung-Trujillo & Lawrence (1987). It has, however, been observed that higher hatching rates can be obtained by directly implanting vas deferens rather than spermatophores since, sperms present in the vas deferens tend to be more viable than those present in the spermatophores (Lin & Hanyu, 1990).

From the foregoing account it is evident that although AI has great significance for inter- and intra-specific hybridization, the

technique needs further refinement in order to match the success rates achieved by natural mating, spawning, and hatching.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the suggestions and co-operation extended by the Late Dr. D.R. Jalihal, College of Fisheries, Ratnagiri.

REFERENCES

AQUACOP 1983. Constitution of broodstock, maturation, spawning, and hatching systems for penaeid shrimps in the Centre Oceanologique du Pacifique. In: J.P. McVey and J.R. Moore (eds.) *CRC Handbook of Mariculture. Volume 1: Crustacean Aquaculture*. CRC Press, Boca Raton, FL, USA, 105-121.

Leung-Trujillo, J.R. and Lawrence, A.L. 1987. Observations on the decline in the sperm quality of *Penaeus setiferus* under laboratory conditions. *Aquaculture* **65**: 363-370.

Lin, M-N. and Hanyu, I. 1990. Improvements on artificial insemination in gravid females of close-thelycum *Penaeus penicillatus*. In: R. Hirano and I. Hanyu (eds.) *The Second Asian Fisheries Forum*, Asian Fisheries Society, Manila, Philippines, 627 pp.

Lin, M-N. and Ting, Y-Y. 1986. Spermatophore transplantation and artificial fertilization in grass shrimp. *Bull. Jap. Soc. Fish.* **52:** 585-589.

Lumare, F. 1981. Artificial reproduction of *Penaeus japonicus* Bate as a basis for the mass production of eggs and larvae. *J. World Maricul. Soc.* **12**: 335-344.

Muthu, M.S. and Laxminarayan, A. 1984. Artificial insemination of *Penaeus monodon*. *Curr. Sci.* **53:** 1075-1077.

Persyn, H.O. 1977. Artificial insemination of shrimp. *U.S. Patent 4031855*. 28 June.

***Ponticelli, A. 1981**. Tentativi di fecondazione artificiale in *Penaeus japonicus* Bate tramite inserimento manuale delle spermatofore nel thelicum. *Rivisti. Ital. Piscicolt. E Ittiopatol.* **16**: 125-129.

Sandifer, P.A. and Lynn, J.W. 1980. Artificial insemination of caridean shrimp. In: H. Clark Jr. and T.S. Adams (eds.) *Advances in invertebrate reproduction. Elsevier,* North Holland. 271-288 pp.

Sandifer, P.A. and Smith, T.I.J. 1979. A method for artificial insemination of *Macrobrachium* prawns and its potential use in inheritance and hybridization studies. *Proc. World Maricul. Soc.* **10:** 403-418.

* Not referred to in original