ARTIFICIAL INSEMINATION OF *PENAEUS MONODON*

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ABSTRACT

Viable nauplii of the tiger prawn *Penaeus monodon* have been produced by artificial insemination. A technique of implanting electroejaculated spermatophores from the males into the thelycum of newly moulted, eyeablated females is described. The implanted spermatophores are retained by the female till the next moult. Three females were artificially inseminated 10 times and every time they matured and spawned. One of the spawnings yielded healthy normal larvae which were reared to postlarval stage in the laboratory.

INTRODUCTION

ARTIFICIAL insemination (AI) in the freshwater caridean prawns has been fairly successful[1,2]. A similar technique of AI has been patented[3] in the U.S.A. for the American species of *Penaeus* with open thelycum. Among penaeid prawns with closed thelycum AI has been tried in the case of *Penaeus japonicus*[4,5]. These methods are not applicable to the commercially important species of marine prawns in India as they differ from the species referred to above in the shape of the thelycum and the structure of the spermatophore. Prawn breeding work at the Marine Prawn Hatchery Laboratory of the Central Marine Fisheries Research Institute, Cochin, has resulted in the development of an AI technique for the giant tiger prawn *Penaeus monodon*. The method is described in this paper.

MATERIALS AND METHODS

Adult specimens of *P. monodon* were collected from the sea as well as from the brackishwater ponds at Narakkal. Three immature adult females were subjected to unilateral eyestalk ablation using an electrocautery apparatus and were kept individually in circular plastic lined pools containing 250 l of filtered seawater. The pools were aerated with oil free air from an air blower. Everyday about 90 % of the water in the pools, was siphoned out along with the sediments and faecal strands and replaced with fresh filtered seawater without disturbing the prawns. The pools were covered with black cloth to avoid stress to the animals. Three mature males which were not eyeablated were kept in a 10 m^3 plastic lined pool fitted with a subgraval biological filter. The prawns were fed *ad libitum* with clam meat. The temperature in the pools varied from 28.5°C to 30.5°C, the salinity from 32.0 to 33.5 ppt and the pH 8.1–8.2.

The daily change of seawater in the 250 l pools stimulated frequent moulting of females at intervals of 10–15 days. Whenever the female moulted the spermatophores stored in the thelycum were lost along with the moulted cuticle. Moulting took place around midnight and the female was artificially inseminated with spermatophores from a male around 6 o'clock in the morning when the newly formed cuticle was still flexible. The spermatophores were extracted without injuring the male by electroejaculation, a method described in detail by Sandifer and Lynn[2]. For insemination the moulted female was caught from the pool with a soft hand net and held with the left hand, ventral side up, on a slab of rubber foam kept in a trough of filtered seawater. The pair of spermatophores electroejaculated from a male were received on a sterilised spatula held in the right hand and quickly inserted into the thelycum of the female. The female was allowed to recover in the trough of seawater which was continuously aerated. The trough was gently lowered into the 250 l of seawater in the plastic pool and the female was allowed to swim out. The implanted spermatophores were retained inside the thelycum of the female.

Every morning the bottom water in the pool was sampled to see if spawning had taken place. When eggs were seen in the samples the female was transferred to another pool and the eggs were allowed to develop undisturbed. An estimate of the number of eggs spawned was made by counting the eggs in aliquot samples.

RESULTS

The details regarding the dates of moulting, insemination and spawning and the number of eggs laid by the three females are given in table 1. All the females were implanted with a fresh set of spermatophores after each moult. Every implantation was followed by

Table 1 *Details of experiments in artificial insemination of Penaeus monodon*

Specimen No.	1	2	3
Source	Sea	Sea	Pond
Total length of female mm	264	237	221
Carapace length mm	70	58	57
Date of eyestalk ablation	9 2.84	9.2.84	18.3.84
Date of 1st moult and implantation	23/24.2.84	16/17.2.84	20/21.3.84
Date of 1st spawning	9.3.84	26.2.84	28.3.84
(No. of eggs)	(75,600)	(86,800)	(648,400)
Date of 2nd moult and implantation	11/12.3.84	2/3.3.84	30/31.3.84
Date of 2nd spawning	20.3.84	12.3.84	8.4.84
(No. of eggs)	(74,200)	(81,500)	(580,600)
Date of 3rd moult and implantation	21/22.3.84	17/18.3.84	10/11.4.84
Date of 3rd spawning	29.3.84	28.3.84	20.4 84
(No. of eggs)	(71,600)	(72,200)	(90,600)
Date of 4th moult and implantation	1.4.84	–	–
Date of 4th spawning	10.4.84		
(No. of eggs)	(69,600)*		

* 1686 nos. nauplii hatched out.

a spawning 7–15 days later. The 264 mm long female moulted and spawned 4 times, the other two females each moulted and spawned 3 times. Totally there were 10 spawnings which yielded 1,930,800 eggs. However only the 4th spawning of the 264 mm female on 10 April 1984 resulted in the production of 1686 viable nauplii. The eggs in the other nine spawnings did not undergo normal development.

Although the hatching rate was low (2.4 %) in the successful implantation, the very fact that at least some of the eggs developed into normal, healthy nauplii shows that the AI was successful. Since the prawn had earlier moulted 4 times the eggs should have been fertilized only by the sperms from the implanted spermatophores.

Some of these nauplii were collected from the 250 l pool for further rearing in 3 large beakers. In each beaker 100 nauplii were kept in 5 l of seawater and reared on a diet of mixed phytoplankton cultured separately. The larvae were healthy and normal and metamorphosed into postlarvae on 19 April 1984. The number of postlarvae (PL1) obtained was only 37. But this low rate of survival of the larvae was due to frequent current failures leading to stoppage of aeration for extended periods.

DISCUSSION

Although only one out of the 10 artificial insemi-nations was successful, there is no ambiguity about the source of the sperms that fertilized the eggs in the successful experiment, as the spermatophores were implanted after the female had moulted. The sperms from any natural impregnation undergone by the female in the sea would have been lost along with the moult.

Lumare[5] implanted the spermatophores into the thelycum of *P. japonicus* females with fully developed gonads during the intermoult stage. In 22 implantations 994,460 eggs were produced and the average hatching rate was 3.3 %. But, eventhough the females were said to be free of spermatophores, the possibility of some residual sperms remaining inside the seminal receptacle of the thelycum is not ruled out in Lumare's experiments.

In *P. monodon* non-viable eggs are frequently produced even by naturally impregnated spawners collected from the sea. So the low percentage of success in these implantation experiments is not surprising. However, efforts are beings continued to improve the fertilization rate in artificially inseminated *P. monodon.*

The present work has opened new vistas in selective breeding and genetic manipulation of commercially very valuable species of penaeid prawns such as *P. monodon* for aquaculture purposes. The technique will be invaluable for future work in hybridization of penaeid prawns.

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