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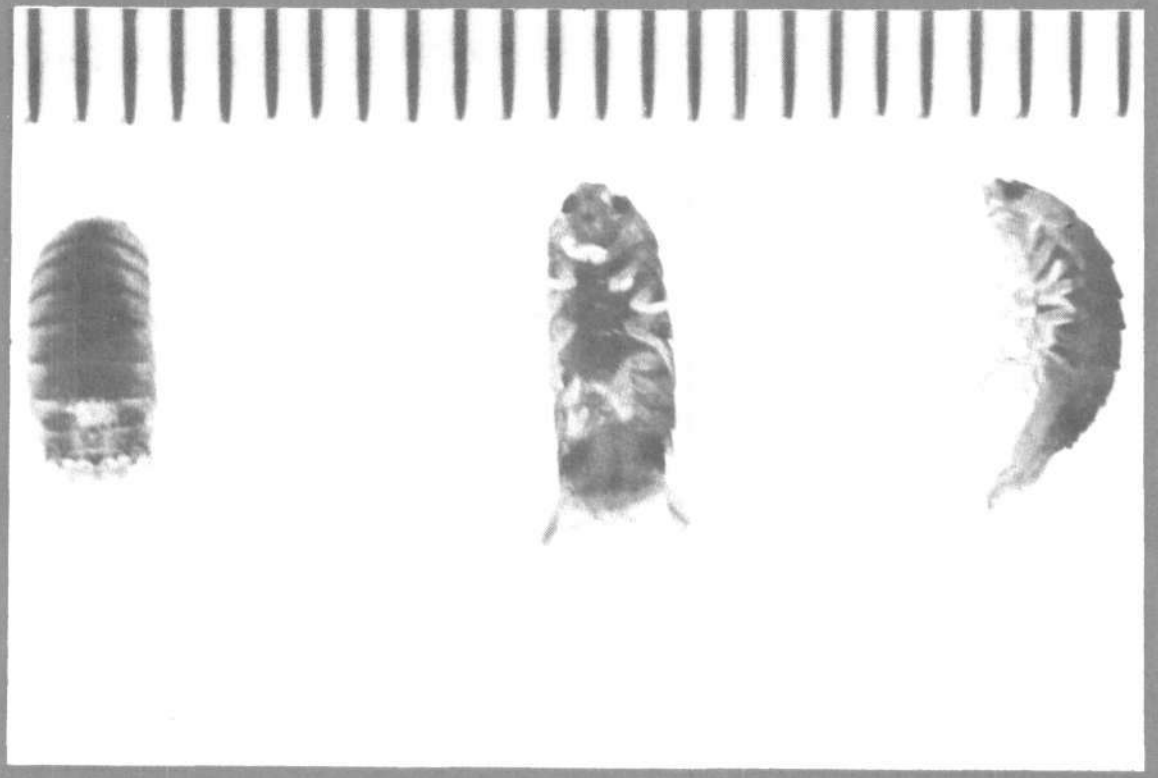

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CRYOBANKING POTENTIALS OF MARINE SHRIMP GAMETES

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Cryobanking of the viable gametes is the potential tool in biotechnological application to improve animal production as per requirement. This technique has been successfully applied in animal husbandary and cattle industry. There is no theoretical reason why the technique should not be applied to marine shellfishes to boost aquaculture industry.

For developing marine shrimp industry through aquaculture technology, one of the major constraints is non-availability of sufficient seed and spawners to produce seed at the desired time. Even in the event of availability of spawners, their maintenance and management become difficult and expensive. Therefore, to ease this problem there is an urgent need to evolve a suitable technology for cryopreservation and cryobanking of viable gametes (sperms and eggs) so that production of shrimp can be made sustainable as per the need.

Cryopreservation of gametes of aquatic animals in contrast to the situation in other vertebrates particularly mammals has met with a very limited success. Sperm cryopreservation has been successful in a number of commercially important aquatic species particularly some teleost fishes. However, the reproductibility of the cryopreserved sperm in general is still poor and the technology involved requires further refinement. Sperm cryopreservation in aquatic animals is not at the stage of advanced commercial application as seen in domestic mammals. This may partly be due to the problems related to the need for relatively large volume of sperms to fertilize the large number of eggs produced by aquatic animals.

In gamete preservation, eggs are fundamentally more difficult to freeze successfully than sperms. The reason mentioned is that due to the large size of eggs there will be some interference in the penetration of cryoprotectants and uniform cooling during cryopreservation process. Sometimes the eggs with large yolk sac tend to develop crystals which damage the egg as it freezes. It has been also stated that the chromosomes in the eggs are more vulnerable to damage than those in sperm, so also the loss of membrane integrity both in sperm and egg is a critical damaging

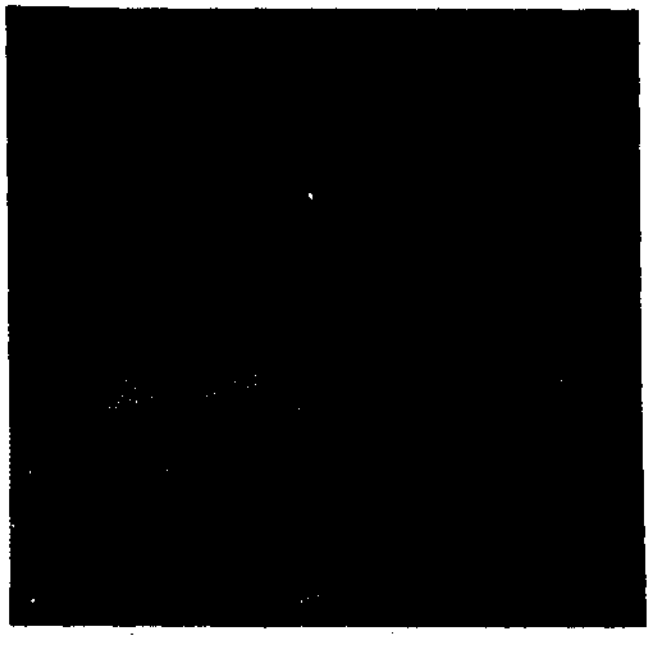

Fig. 1. Unistellate spermatozoan of *P. indicus* by SEM x 2500.

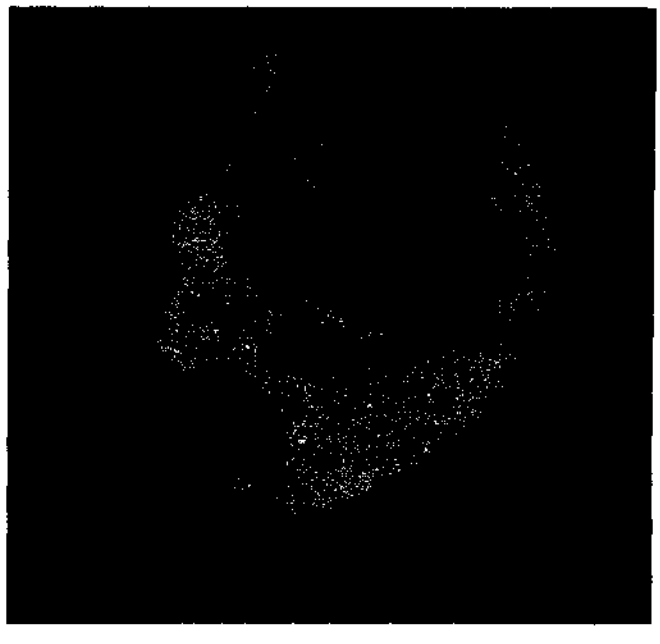

Fig. 2. Unistellate spermatozoan of *P. monodon* by SEM x 2500.

TABLE 1. *Cryopreservation of sperms of decapod custaceans*

Species tectant	Cryopro-rature	Tempe-vation period	Preser-tage of survival	Percen-of testing viability	Method	References
Limulus polyphemus	Glycerol	—74°C	50 days	64	Eosin dye exclusion	Behlmer & Brown, 1984
Macrobrachium rosenbergi	Glycerol	—196°C	31 days	53	Fertility	Chow *et al.*, 1985
Sicyonia ingentis	Trehalose + DMSO	—196°C	2 months	60-70	Acrosome reaction	Anchordogy *et al.*, 1987
Sicyonia ingentis	Trehalose Sucrose, Proline, Glycerol & DMSO	—196°C	1month	56	Acrosome reaction	Anchordogy *et al.*, 1988
Scylla serrata	Glycerol, Trehalose, DMSO, DMSO + Trehalose	—196°C —79°C —4°C	30 days	95 & 89	Eosin dye exclusion	Jeyalectumie & T. Subro-moniam, 1989
Macrobrachium idella	Ringers solution	6°C	96 Hrs	—	Larval production	Joshi & Diwan, 1992
Penaeus indicus *P. monodon*	Glycerol, Trehalose, DMSO, DMSO + Trehalose DMSO + Glycerol	—196°C —35°C	1 week	80 & 76	Acrosome reaction	Laboratory observations (Diwan, Shoji and Nandakumar)

factor incurred during freeze/thaw process. More recent evidence has shown that certain key enzymes in the cells get altered/broken down on freezing.

Very few attempts have been made on cryopreservation of sperms in decapod crustaceans in general and marine shrimps in particular. Initial attempts involved in this technology were how to extrude the spermatophore mechanically through live animals. The electroejaculation technique of extruding spermatophore from male prawn once it was devised, many workers have diversified their research on artificial insemination followed by cryopreservation studies. Initial attempts in this direction were made by Sandifer and Lynn in 1981 on palaemonid prawn viz *Macrobrachium rosenbergti*. Later such studies were extended to penaeid prawns, lobsters and crabs. Not much has been done so far on cryopreservation of sperms of marine shrimp. In recent years Wallis Clark and his associates have made extensive studies on sperm activation, sperm egg interaction and cryopreservation of sperms in the shrimp *Sicyonia ingentis*. They could succeed in preserving viable sperms in liquid nitrogen for a period of one month or so. Similarly in a horseshoe crab, *Limulus polyphemus*, Behlmer and Brown could maintain the viable sperms for a period of 50 days at —74°C temperature. Of late, Subramoniam and his co-workers were able to preserve sperms of a mud crab *Scylla serrata* for a period of 30 days at —196°C. In *M. rosenbergi* and *M. idella* viability of cryopreserved spermatophores was demonstrated by fertilizing the normal eggs and larval production.

CMFRI has been doing the work on cryopreservation of gametes of fishes and shellfishes for past few years. The Institute has succeeded in developing a gene bank for certain

cultivable marine fishes. Efforts are now being made continuously on cryopreservation and cryobanking of penaeid gametes. The viable spermatozoa of *P. indicus* and *P. monodon* have been successfully preserved in the Institute's laboratory at —196°C for a period of 15 days to begin with. The cryoprotectants used were DMSO, Glycerol and Trehalose mixed in different combinations. The percentage of viable sperms after freeze thawing was assessed by induction of acrosome formation which was found to be considerably high (60 to 80%) in both the species. Further efforts are on the way for improving this technology not only for cryopreservation of sperms but also eggs, embryos and larvae of some important marine shellfishes. If proper breakthroughs are made in this sector then the aquaculture would acquire a prestigeous status in our country.