Shrimp embryo cryobanking is now possible

For developing marine shrimp industry all over the world through aquaculture technology, there are two major constraints that we come across. One is the non-availability of sufficient shrimp spawners to produce seed at the desired time. Even in the event of availability of spawners, their maintenance and management becomes extremely difficult and expensive. Therefore, to ease this problem there is an urgent need to evolve a suitable technology for cryobanking of viable gametes, so that shrimp production can be made sustainable according to the need. The second constraint that is encountered by the industry is the large-scale mortality of juvenile shrimps during transportation from the hatchery site to remote rural areas where shrimp farming is being done. Sometime casualties can also be due to infection, disease or pollution. This problem could be relieved to some extent by developing a technology for storage of quality eggs, embryos and nauplii, in a viable but dormant condition. Hence, there is an urgent need to devise the technique of preservation of viable embryos and larvae under low freezing temperatures to ensure the interest and confidence of shrimp industries.

Although sperm cryopreservation has been carried out successfully in a number of commercially important aquatic species, particularly in some teleost fish and also shell fish, the technology is still not at the stage of advanced commercial application that is seen in domestic mammals. The first successful attempt at the cryopreservation of embryos of sea urchin was reported by Asahina and Takahashi. Later Zell and Erdahl and Graham have reported preliminary attempts to freeze the eggs of rainbow trout. Studies have been carried out to cryopreserve the embryos of Japanese medaka Oryzias latipes, rainbow trout Oncorhynchus mykiss and zebra fish Brachydanio rerio. In recent years a few attempts have also been made
to cryopreserve the embryos and nauplii of shrimp *Penaeus indicus*. From a review of the literature it appears that studies on the cryopreservation of embryos and larvae of fish and shellfish are still in a rudimentary phase and much remains to be done.

The reasons given for such meagre success in this area are that in higher animals, due to the large size of the eggs and embryos, there will be interference in the penetration of the cryoprotectants and thus prevention of uniform cooling during the cryopreservation process. Sometimes the large yolk present in the eggs and embryos tends to develop crystals while freezing and damage the egg structures. It has been also stated that the chromosomes in the egg are more vulnerable to damage than those in the sperms; so also the loss of membrane integrity both in the sperm and the egg is a critical damaging factor incurred during the freezing/thawing process. More recent evidence has shown that certain key enzymes in the cells get altered/broken down on freezing. In shrimps, although the size of the eggs and embryos is small, the eggs naturally absorb water soon after their release in order to get swollen and activated for fertilization. After fertilization, a strong hatching envelope (protective extra-cellular matrix) forms around the egg. The presence of water and the thick protective envelope surrounding the eggs makes the freezing of viable eggs and embryos problematic. Subramoniam and Newton were the first to successfully preserve nauplii of *P. indicus*. They held them at −30°C using liquid nitrogen and reported survival of 82% for nauplii frozen at −30°C and 63% at −40°C. They further reported that the toxicity response of the various cryoprotectants (glycerol, ethylene glycol, methanol, DMSO and formamide) to nauplii was similar. But while cryopreserving the embryos using these cryoprotectants they found that glycerol was more toxic to the morulae stage embryos. They could not achieve any success with regard to the cryopreservation of embryos. Diwan and Kandasamy reported some success in freezing viable embryos and larvae of shrimp *P. semisulcatus*. It is mentioned that 40–50% of the embryos preserved in concentrations of 5–20% of a mixture of DMSO and glycerol and −10°C hatched out successfully to nauplii after 1–6 h preservation. Even the revival rate of frozen-thawed nauplii has been reported to be 50% in similar conditions. Arun and Subramoniam while studying the effect of freezing rates on the survival of penaeid prawn larvae, reported that using the DMSO, ethane diol mixture at concentrations of 15 : 25 (v/v), for very high rates of freezing after 60 min of equilibration has yielded larvae of high morphological intactness and indicated the possibilities for use of this mixture in future vitrification studies. The latest developments in cryopreservation of crustacean gametes and embryos have been reviewed recently by Diwan.

Zhang et al. while working on cryopreservation of zebra fish embryos, studied the toxicity response of different cryoprotectants such as methanol, DMSO, glycerol ethylenediol and sucrose on different stages on embryonic development. They found that DMSO and ethylenediole are more toxic to fish embryos of heart beat stage. They obtained the best embryo survival rate at −10°C and the least at −30°C. Ice formation within the egg was found to be the main factor affecting the survival of the embryos. When DMSO and glycerol were used independently they proved to be more toxic to the embryos and larvae of *P. semisulcatus*. Similarly, higher concentrations of cryoprotectants have been reported to be unsuitable for cryopreservation studies at −10°C (ref. 14). The newer technique of glass embryos that has been used with some success to preserve eggs and embryos in human, cattle and sheep has to be tried for preserving shrimp embryos also. The technique involves freezing of embryos in a solution with high

---

**Figure 2.** Developing embryos of shrimp.
glycerol and sucrose content. However, further investigation is warranted to refine the technique, once the exact nature of the damage to the embryos and nauplii by various factors during cryopreservation is known.


NEWS

Ecofriendly microbes as agents in insect pest control*

Pesticides have been the weapon against all insect pests and have contributed unprecedented effects in nature. The growing concern about their ill-effects has necessitated a change in our strategies to manage insect pests in an ecofriendly manner. Search for alternatives that would leave no negative effects on the environment prompted scientists to look for biopesticides such as microbial pesticides. India with a large agrarian society and with agriculture as the prime occupation needs to concentrate on this aspect. Papers presented by scientists from all over the country in a national symposium on microbials in insect pest management, that was organized to discuss the developments in India have stressed this aspect. Research papers were drawn on different areas such as identification and development of new microbial agents for pest management, mass production and utilization, microbial management for education, employment, economics and manpower development, etc. Some of the desirable attributes of microbial control agents and nematodes are: Rapid spread, power of search for host, persistence, safety and acceptability, control to sub-economic level, predictable control, virulence, easy production, low cost, good storage and easy application.

While admitting the success of Bacillus thuringiensis (Bt) in insect pest management this conference discussed the part played by various other microbes, viz. virus, bacteria, fungi and protozoans. A survey for the indigenous microbial pathogens resulted in the isolation of Pandora delphacis, Beauveria bassiana, Entomophthora aulicae, E. grylli, Fusarium sp., Metarhizium anisopliae, M. flavoviridae, Verticillium lecanii and isolates of NPV, from different insect pests like Helopeltis theivora, Buzura suppressaria, Oligonychus coffeae, Nephotettix virescens, Oxya velax, etc. (Table 1).

Most of the organisms tested for field insect pest control caused more than 50% mortality.

Results on the combinational studies revealed possible synergism of M. anisopliae and optimum level of synthetic insecticides against Spodoptera litura. Bt and Neem Azal were found to be more effective on S. litura whereas Bt alone was effective on Plutella xylostella. Additive and synergistic interactions of nematodes (Steinernema carpocapsae) and Bt showed considerable effect on root grub (Holothele serrata).

Field application tests proved the superiority of wettable powder formulations where 66% mortality of rice green leafhopper (Nephotettix virescens) was recorded. Wettable powder, oil and freeze dried formulation showed differential

*Based on the National Symposium on Microbials in Insect Pest Management conducted at Entomology Research Institute, Loyola College, Chennai during 24–25 February 2000.

<table>
<thead>
<tr>
<th>Table 1. Micro-organisms isolated from various sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-organism</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Verticillium lecanii</td>
</tr>
<tr>
<td>Beauveria bassiana</td>
</tr>
<tr>
<td>NPV</td>
</tr>
<tr>
<td>Pandora delphacis, Beauveria bassiana, Entomophthora aulicae, Fusarium, Metarhizium flavoviridae</td>
</tr>
<tr>
<td>Soil</td>
</tr>
</tbody>
</table>

- A. D. Diwan

Fish Genetics and Biotechnology Division, Central Institute of Fisheries Education (ICAR, Deemed University), Versova, Mumbai 400 061, India (e-mail: cife@x400nicgw.nic.in)