The storage and use of cryopreserved oyster trochophores as food for larvae. pp. 270-272.

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Oyster trochophores have emerged as a practical first food for extremely small fish larvae, as well as a very nutritious food for larvae of a variety of marine organisms, from fishes to gelatinous zooplankton. Cryopreservation has been the breakthrough which has taken oyster trochophores from being a food only seasonally available in oyster farming areas like Japan to being available on a wide geographic basis and amenable to long-term storage on site. Nonetheless, the initial experience with cryopreserved trochophores is not always successful. The purpose of this talk is to discuss problems which have been apparent in various applications attempted at the Vancouver Aquarium, and possible remedies to those problems. As well, success stories will be related to avoid discouraging others from attempting this promising new approach.

Since the specific materials and methods for cryopreservation of Pacific oyster (Crassostrea gigas) trochophores are proprietary, and since a commercial scale of broodstock maintenance and production is involved, the full description of production methods would not be appropriate for this venue. For example, in order to pack one billion trochophores of high quality, 240 broodstock oysters must be spawned under controlled conditions.

The characteristics of trochophores which differentiate them from the other commonly available live foods, rotifers and brine shrimp /Artemia sp.) nauplii, will be described and the methods used for storing, handling, thawing and feeding of trochophores to fish larvae will be discussed. During 1991 and 1992, MTL. Biotech was shipping pre-thawed, chilled bags of trochophores in seawater, but the logistics of ordering and delivery proved inconvenient, so that shipping is now conducted with trochophores in the frozen state. Frozen trochophores can be stored indefinitely in liquid nitrogen until the very day that live food is required.

Oyster trochophores are extremely small in size, have an ideal nutritional composition, and are slow swimming. Oyster trochophores, at about 50 microns diameter, are about one quarter the size of rotifers and one tenth the size of brine shrimp nauplii. Trochophores have only the marine fatty acids typical of crustacean zooplankton, including 15% 20:5n3 (EPA) and 15% 22:6n3 (DHA), so they are better nutritionally than rotifers or brine shrimp for marine fish larvae. Trochophores are even nutritionally superior to rotifers or brine shrimp with fatty acid supplements, since there are no low-n fatty acids in trochophores. The low-n fatty acids have to be metabolized by the fish larvae but are not normally found in wild larvae.

Broodstock oysters are naturally conditioned, which probably lends variability to the lipid/protein makeup of the eggs. It positively affects the quality, quantity and viability of the trochophores. The trochophores are cryopreserved at about the 15-hour stage, which is ciliated and free-swimming, but without any shell formation and thus low ash content. Trochophores are screened several times prior to freezing. They are counted using a Coulter counter, and the number per ml is adjusted for consistency between straws. Currently, most straws are being packed at 30 million per straw, at 10.5 ml volume. There is also no threat of oysters becoming established as an exotic species via effluent water, since the cryopreservation process ensures that the trochophores will not complete normal embryonic development.

A variety of Northeast Pacific fishes which could not be reared with rotifers and brine shrimp nauplii alone have been successfully reared with use of cryopreserved oyster trochophores as first food. One species which could be reared as larvae in very small numbers without trochophores, the tidepool sculpin (Oligocottus maculosus), was reared on trochophores with a higher survival rate through the larval stage, but also with other signs of superior long-term viability beyond the period of trochophore feeding. The tidepool sculpins displayed larval schooling, a normal feature in the field, for the first time in tank rearing when first feeding had been on trochophores.

For the padded sculpin (Artedius fenestralis), individuals started on trochophores survived through to spawning as adults. In the case of painted greenlings (Oxvlebius pictus), repeated rearing efforts had failed prior to the use of trochophores. Trochophores alone as first food did not yield survival of painted greenlings through the larval stage, but a combination of trochophores and algae paste (to increase turbidity and draw larvae away from tank walls) has yielded survival to the juvenile stage. This successful trial with painted greenlings encompassed a period of mass mortality when larvae were switched from a combination of trochophores and rotifers to rotifers alone. They were switched back to trochophores alone and remained on trochophores through extensive growth, then finally were overlapped with and switched onto brine shrimp nauplii (Selco-soaked). This illustrates the species-specific nature of feeding behavior, since rotifers are a logical intermediate size of live food organism.

With coral reef damselfishes, initiation of feeding on trochophores has been observed, but no demonstrable growth has yet been achieved. Algae paste has been used in combination with trochophores, and a variety of tank designs and flow (aeration) systems have been attempted. One suspicion at this point is that a combination of poor larval fish viability, poor trochophore viability and poor tank design are combining to yield extremely low survival rates. It is clear that excessive densities of trochophores (50-100/mi) with poor aeration can cause abrupt total moralities of damselfish larvae, probably resulting from mortality and necrosis of trochophores.

Frozen trochophores are thawed by quickly removing a straw of trochophores from the storage container and breaking a piece of the straw off into seawater at 30ºC, then returning the straw to storage. The first possible difficulties can arise if the straw is out of the freezer long enough to rise in temperature. If the straw temperature rises from the liquid nitrogen temperature of -196ºC to higher than -100ºC, the remaining trochophores can become reduced in viability, even if they remain in storage at -196ºC until further use. Thus, handling times of over ten seconds can affect the viability of remaining frozen trochophores. This introduces a conflict between the safety measure of wearing cotton gloves to protect skin from frostbite and the expediency of greater dexterity and faster handling with bare hands. Another problem, again related td safety, has to do with straws which may have included air bubbles during the original packing or which drew air in during previous handling. Rapid expansion of gas bubbles can cause parts of straws to explode while being handled, so that use of protective eyewear is essential. Breaking the straw inside the heavy plastic bag provided by the manufacturer reduces this risk and will save trochophore material should it blow up inside the bag.

After thawing, the material should be sieved at once through a 20 micron screen in order to eliminate fluid and fragments of broken trochophores. Viability appears to be decreased if trochophores are held in the order to diluent. Trochophores should remain at 30°C for one hour prior to introducing to tanks at lower temperatures, if they are to be introduced sequentially to tanks at temperatures below 25°C, they should be held in aerated water at 25°C until they are to be fed to the fish larvae. Holding is not affected by aeration, whereas drifts of trochophores which are allowed to settle to the bottom of a bowl will rapidly suffer from anoxia. Similarly, drifts of trochophores in a rearing tank can kill fish larvae, presumably from degraded water quality. It should be stressed that viability of trochophores decreases on a continuum, as does that of fish larvae, so that the combinatory effects of low-viability food presented to low-viability larvae can result in lack of feeding or growth without either the quality of food organisms or cultured larvae being identifiable as the critical component which led to failure.

Trochophores have rings of cilia which result in slow swimming with the body rotating or spinning. Actively swimming trochophores should form visible patches or vertical sheets at the water surface. If they sink to the tank bottom they lose their viability, especially at high densities. The slow swimming is both a benefit and a liability. The slow swimming makes them easy prey for fish larvae, especially in comparison with rotifers, which swim in a spiral path which larvae of Somme species of fish cannot fixate upon. The relatively feeble swimming of the trochophores may not be adequate to keep them in the water column, however, in tanks with a relatively large surface area-to-depth ratio. If a drip-feed system is used, then poorly swimming trochophores will be more continuously available. Swimming ability is not entirely indicative of viability, and the nutritional content is equivalent so long as trochophores remain alive and intact; the most important factors are particle size and fatty acid profile.

Trochophores will have been received in a dry shipper, a container which is pre-chilled with liquid nitrogen. The trochophores can either be held in the dry shipper for a period of up to two weeks between recharging (chilling with liquid nitrogen) or the straws of trochophores can be transferred to a liquid nitrogen freezer for long-term storage. Again, the danger of impairing viability must be considered if storage in a dryshipper is planned. The inconvenience and payroll expense of taking a shipper out for recharging has to considered together with the risk of impaired quality in contrast to the higher initial cost of purchasing a liquid nitrogen freezer. Used freezers are available from artificial insemination centers for the agricultural industry. The price of cryopreserved trochophores, together with the payroll costs of maintaining broodstock fish and obtaining high quality hatches, would seem to mitigate the capital cost of obtaining a liquid nitrogen freezer.

Despite these difficulties, trochophores offer great promise for successful rearing of very small marine fish larvae. Certainly, cryopreservation is a convenient and effective means of obviating all the difficulties of maintaining live cultures, as with algae and rotifers, and many potential difficulties could be listed for incubating, hatching and harvesting brine shrimp nauplii. In any case, considerable dedication to larval marine fish rearing is required to achieve repeated success in the application of cryopreserved trochophores as a food in rearing larval marine fishes. Different AZA institutions will be predisposed to succeed more readily with particular taxa of fish, due to the type of seawater system in use, the availability of good broodstock and previous husbandry experience with those particular taxa. If a number of institutions could become regular producers of even a few marine fish species, then AZA member institutions, through distribution efforts, could focus to a greater extent on display of propagated, rather than wild-caught, marine fishes. The conservation mission of the AZA would seem to call for such dedication to propagation effort as an integral component of the overall conservation effort .