Successful Use of Cryopreserved Oyster Trochophores as a Live First Feed for Larval Marine Fish and Invertebrates

Brian J. Harvey
MTL Biotech Ltd.
P.O. Box 5760, Station B
Victoria, B.C., Canada

Abstract

Trochophore-stage larvae of the Pacific oyster *Crassostrea gigas* were cryopreserved in bulk and stored in liquid nitrogen for periods up to two years before thawing and feeding to a variety of warmwater and coldwater larval marine fish, as well as to marine shrimps and other invertebrates. The commercial product ("TrochoFeed"), marketed in both pre-thawed and cryopreserved versions, has been used successfully in the early rearing of cultured species including red drum, snook, grouper, and black cod, as well as for numerous warmwater and coldwater aquarium display fish.

This paper describes the nutritional profile of the cryopreserved trochopores and presents a summary of the available growth and mortality data.

Introduction

Trochophore-stage bivalve larvae have frequently been used as a live first feed in marine fish larviculture, particularly with species whose larvae are very small or have small mouths. At approximately 50 µm, the bivalve trochophore would appear to be an ideal first prey item; movement in the water column is sufficient to attract the attention but not so rapid as to make the trochophore impossible for young larvae to catch. For larvae with particularly small mouths, such as siganids and groupers, the fact that trochophores are generally one-third to one-half the size of rotifers makes them physically suitable as feed. Nutritionally, trochophores present an excellent HUFA profile (Whyte 1992).

During the 1970's, trochophore-stage Pacific oyster, *Crassostrea gigas* were frequently used as a first feed for red sea bream, *Pagrus major,* black sea bream, *Acanthopagrus schlegeli,* yellowfin tuna, *Thunnus albacores* and redeye mullet, *Liza hematocheia* (Kuronuma and Fukusho 1987). More recently, Lim (1991) describe using trochophore-stage larvae of the green mussel, *Perna viridis* as a first feed for

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two small-mouthed marine fish larvae of global economic importance: grouper, *Epinephelus tauvina* and snapper, *Lutjanus johnii,* and Ali (1996) describes using oyster trochophores in grouper larviculture in Malaysia.

Lim's paper, which described grouper larviculture in Singapore, illustrates the problems involved in producing adequate supplies of live bivalve trochophores. For one experimental five-day feeding run, the necessary 350-400 million bivalve trochophores require 4200 mussel broodstock (700-800 daily), all of which must be temperature-shocked to induce spawning, followed by eight hours culture before feeding to the larval fish. Thus, although bivalve larvae clearly perform well as a first food item, producing them in sufficient quantities is a significant undertaking in facilities and manpower.

Cryopreservation

Cryopreservation is a practical application of low-temperature biology that provides an indefinite shelf life for cells and some embryos by cooling them to the temperature of liquid nitrogen (-106°C). The damaging effects of intracellular ice crystal formation during cooling and thawing are minimized by addition of non-toxic cryoprotectant chemicals and manipulation of cooling and thawing rates. Re-animation of the frozen material can be done up to decades after freezing, offering a high degree of control over the use to which cryopreserved material is put. In agriculture and aquaculture, the technology is frequently used to offer greater control over production systems by allowing control over timing of artificial fertilizations.

For biological material that must be living to be useful, but can only be produced by labor-intensive, seasonal means, cryopreservation is a powerful tool. Until recently, most practical cryopreservation procedures have been for single cells (mammalian spermatozoa, tissue culture cells, blood cells, and plant cells) or for mammalian embryos. Development of a large-scale cryopreservation protocol for bivalve trochophores was achieved at MTL Biotech Ltd. in 1992; the remainder of this paper describes some preliminary results using the frozen-thawed material ("TrochoFeed") as a starter diet for fish larvae.

Size and Nutritional Profile of Trochopores

A fundamental requirement of a first feed is that the particle size is sufficiently small for fish to ingest. While it is possible to achieve very small particle sizes in artificially prepared diets, the swimming behavior and nutritional components of live food cannot be duplicated.

Bivalve trochophores have traditionally worked well as a starter diet because their size (50-60 μm) is one third to one half that of the average rotifer; even the relatively new SS strain rotifers, developed to provide a particle size smaller than 100 μm are still larger than trochophores, and, moreover, are difficult to culture in Southeast Asian conditions. Cryopreserved trochophores require no culture facilities, an advantage that is self-evident to any larviculturist.

Nutritionally, an analysis of the HUFA profile of Pacific oyster trochophores shows the important 20:5n-3 and 22:6n-3 to be present at 15.6% and 13.5% of total HUFA, respectively; these values are not significantly different from the corresponding HUFA levels in unfertilized eggs (Whyte 1992). These nutrient reserve levels are similar to those encountered in rock scallop, *Crassodoma gigantea* 12 h post-fertilization (Whyte et al. 1990a), and could be expected to increase following manipulation of broodstock nutrition (Whyte et al. 1990b).

Preliminary Test Results

Cryopreserved trochophores of Pacific oyster, *Crassostrea gigas* exhibit characteristic swimming behavior when thawed. This behavior, typical of many planktonic larval stages, results in the slow up-and-down circulation of masses of trochophores in the water column, often referred to as "streaming" or "stringing". This swimming behavior is most vigorous in warm water (25-30°C); at lower temperatures, trochophores swim, but less vigorously.

Warmwater Cultured Species

Quantitative results using the cryopreserved trochophores are presently limited to red drum, *Sciaenops ocellatus,* in studies carried out at the University of Texas (Pickering and Holt 1993). In these studies, cryopreserved trochophores were presented at different densities and in different combinations with rotifers. Based on analysis of gut contents, red drum fry showed a clear preference for trochophores for the first four days of feeding, thereafter, rotifers were selected over trochophores, a result that corifirms size selection of prey items. Equally important, growth rate of fry offered both prey items together was significantly greater than that obtained with either prey item offered alone.

Other warmwater cultured species to which cryopreserved trochophores have been fed successfully include snook, *Centropomus undecimalis* and sea trout, *Cynoscion* spp; in both cases larvae were observed to feed vigorously on the trochophore (C. Neidig, pers. comm.). Limited trials with Nassau grouper in the Bahamas also showed newly feeding larvae to survive better on trochophores than on rotifers during the first two days of feeding (W. Watanabe, pers. comm.).

Coldwater Cultured Species

On the Pacific Coast of British Columbia research aimed at developing culture methods for black cod or sable fish, *Anopoploma fimbria* has resulted in significant advances in obtaining spawning in captivity, but only limited success in larviculture due to small gape of the larval fish. In preliminary trials in 1992, larval sable fish reared at 10°C were shown to feed voraciously on cryopreserved trochophores, and analysis of gut contents showed trochophore in an advanced state of digestion indicating that the prey item has significant food value (I. Whyte, pers. comm.). Quantitative data are being obtained in the breeding year 1993. The suitability of trochophore at the relatively low water temperature of 10°C is interesting; even more remarkable is the observation made at Vancouver Aquarium, of Arctic cod, *Boreogadus saida* larvae feeding on trochopore at 0°C.

Captive Breeding in Aquaria

Many public aquaria in North America have embarked on captive breeding programs for "display" of fish species, often in an attempt to circumvent the environmental destruction that can accompany their capture in the wild. Similar objectives are shared by numerous private tropical fish-supply companies, with a view to supply the public and home aquarium market. Coldwater aquarium species reared successfully using cryopreserved trochophore as a first feed include the flathead sculpin, *Artedius fenestrate* and the tidepool sculpin, *Oligocottus maculatus* (Marliave 1993); warmwater species for which trials of the cryopreserved trochophores are ongoing include clownfish, blue damsel fish, and neon goby.

References

Ali HM. 1994. A review of grouper (*Epinephelus suillus*) fry production research in Malaysia. p. 139-148. In: Proceedings of the Seminar-Workshop on Breeding and Seed Production of Cultured Finfishes in the Philippines. Marte CL, Quinitio GF, Emata AC (Eds.). Tigbauan, Iloilo, Philippines.

Kuronuma K, Fukusho K. 1987. Rearing of marine finfish larvae in Japan. IDRC TS 47e. International Development Research Centre, Ottawa. 109 pp.

Lim LC. 1991. An overview of live feeds productions systems in Singapore. P .203-221. In: Rotifer and Microalgae Culture Systems. Argent Laboratories, Redmund, W.A. 364 pp.

Marliave J. 1993. Vancouver Public Aquarium. Unpublished data.

Pickering I, Holt CJ. 1993. Pacific oyster trochophores as a first food for red drum larvae. Proceedings of the Texas Chapter, American Fisheries Society, in press.

Whyte JNC. 1992. Pacific Biological Station, Nanaimo, B.C. Unpublished data.

Whyte JNC, Bourne N, Hodgson CA. 1990a. Nutritional condition of rock scallop *Crassodoma gigantea* larvae fed mixed algal diets. Aquaculture 86:25-40.

Whyte JNC, Englar JR, Carswell, BL. 1990b. Biochemical composition and energy reserves in *Crassostrea gigas* exposed to different levels of nutrition. Aquaculture 90:157-172.