Sustain Fish

Proceedings of the International symposium on
"Improved sustainability of fish production systems
and appropriate technologies for utilization"
held during 16-18 March, 2005
Cochin, India

Editors

B. Madhusoodana Kurup
K. Ravindran

School of Industrial Fisheries
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
Cochin, India

2006

Sustain Fish

Proceedings of the International symposium on Improved sustainability of fish production systems and appropriate technologies for utilization held during 16-18 March, 2005 at Cochin, India

Editors

B. Madhusoodana Kurup
K. Ravindran

Live feed production for marine aquaculture-status, problems and prospects

G. Gopakumar, Boby Ignatius

Regional Centre of Central Marine Fisheries Research Institute
Maraine Fisheries, P. O., Mandapam Camp – 623 520, India
e-mail: *ggopakumar@eth.net*

Abstract

Micro algal culture is of fundamental importance in hatcheries of marine mollusks, shrimp and fish. A continuous, stable and reliable supply of nutritionally adequate rotifers is the key to the flourishing culture of marine finfish in various parts of the world. Fish larvae are described with two or three types of organisms during the initial 10 – 30·days of exogenous feeding. These include rotifiers of the species Brachionus rotundiformis *and* B. plicatilis *and the brine shrimp,* Artemia, nauplii. *The current pressing need for a very small live feed is rather difficult to meet although several super small geneticstrains have been found and cultured.* Artemia *is inevitable in the hatchery production of seabreams, seabass, groupers wolffish, cod, turbot, halibut, flounder, milkfish, sturgeon, catfish, whitefish, marine ornamental fish and several commercially important crustaceans such as shrimps, crabs and lobsters. Nauplii in instar I and II stages are the most widely used forms of* Artemia *in aquaculture. Recently copepods are gaining importance as live feed.*

Keywords: *Live feed, Larviculture, Microalgae, Rotifer, Copepod, Artemia*

1. Introduction

The world marine aquaculture production has reached 13.1 t (FAO, 2000) and has been growing by about 0.9 t per year in recent years. The major expansion of marine finfish and crustacean aquaculture since the 1980s around the world can be attributed to the development of standard mass production techniques of live feeds. The hatchery production of juveniles of marine finfish is achieved globally by the use of rotifers and *Artemia.* Microalgae are also routinely used in the 'greenwater technique' employed for marine finfish larviculture. Most marine finfishes have altricial larvae and when yolk sac is exhausted, they remain in an undeveloped state. The digestive system is rudimentary, lacking a stomach and much of the protein digestion takes place in the hindgut epithelial cells. Altricial larvae cannot digest formulated feeds and hence live feed is vital for their survival. Live feeds are able to swim in water column and are thus constantly available to the larvae. The movement of live feed in water stimulates larval feeding responses. Live feed organisms with a thin exoskeleton and high water content may be more palatable to the larvae when compared to the hard formulated diets (Stottrup and Mc Evoy, 2003).

2. Micro algae

Micro algae play a critical role in the marine aquaculture of mollusks, shrimps and fish. Nearly 16 genera of microalgae are commonly employed for aquaculture purposes. They are generally free living, pelagic and in the nanoplankton range (2-20 µm). Typically culture units consist of vertical cylindrical tanks (100 – 1,000 l) made of transparent plastic, which are flat or conical at the bottom. Fluorescent tubes placed around the tank of culture provide artificial lighting. Injection of compressed air at the bottom of each tank produces bubbles that rise to this surface at a rate of 0.1 and 3.0 volume per culture volume and per hour. Rectangular tanks (0.5 – 1.0 m depth) are also used with surface lighting. These tanks are easier to clean than transparent vertical cylindrical tanks.

Precautions for maintaining culture asepsis and to prevent contamination are very much needed. All fluids and surface that come into contact with the culture must be sterilized. Natural water used for the culture should be free of pollution and stable in quality. A sand filter reduces the sizes of suspended particles to 10-20 µm, thereby eliminating most of the zooplankton. Additional filtration by cartridges or sieves reduces particle size to 1 µm. Chemical sterilization such as chlorination – dechlorination and by activated carbon can also be practiced. Natural filtered water is enriched by the addition of mineral salts required for photosynthesis, *i.e.* metabolisable nitrogen, phosphorus and trace elements including iron and silicon for diatoms. A chelating agent EDTA is often added to prevent precipitation of ferric hydroxide. Vitamins such as thiamine or biotin should be added with due caution because of their rapid degradation of heat. These salts constitute the enrichment media, the most commonly used being f/2 medium (Gillard and Ryther, 1962) and the medium of Conway (Walne, 1966). Temperature is often controlled between 18 and 25 °C, but this should be fine tuned to each species.

Batch cultures are generally run according to production cycles of 3-7 days. Once illuminated tanks have been cleaned and filled with filtered sterilized water, enrichment medium is added and aeration is provided, and an inoculum is introduced. The algal strains are provided in few milliliters of culture in a test tube. Starting from this sample successive volumes of increasing size are inoculated in order to prepare the biomass required to reach inoculum concentration in the production tanks. The cultures obtained in hatcheries seldom exceed a density of 6×10^6 cells ml^{-1} at the end of 5 days. The costs of producing microalgae in hatcheries include labour (90 %) amortisation (6 %), energy (3 %) and miscellaneous expenses (1 %). In Industrial facilities specialized in the production of microalgae and exploiting

production system in controlled conditions such as photobioreactrors, the cost of production can be reduced considerably.

In continuous cultures the labour consuming operations are eliminated. The semi continuous cultures consist of collecting only part of the culture volume and replenishing with fresh medium. The residual volume of culture then constitutes the inoculum of a new production cycle. In continuous cultures, microalgae are harvested continually and constant nutrient replenishment is provided. A substantial reduction in labour is achieved in continuous cultures.

The productivity of microalgal systems used in aquaculture hatcheries is 10 – fold lower than that of photobioreactors, which is in turn 10 fold lower than that of fermentation techniques. But aquaculture operators are reluctant to take up these technologies mainly because of the significant investment involved. The technological advances in the production of artificial diets could make this leap unnecessary. In shrimp aquaculture, it is proved that microalgae can be replaced by microencapsulated feed, and it may be possible that the same practice could be extended to larval rearing of small larvae fish and most mollusks (Muller – Feuga 2000); an example is the success of live concentrated *Chlorella* produced by fermentation in Japan as feed for rotifers. In Japan, algal culture rooms have disappeared from most fish hatcheries. It is likely that microalgae for fish aquaculture will be produced in the near future by specialized companies implementing high technology.

Microalgae have been shown to play a significant role in larviculture of marine finfish. When phytoplankton was included in larval rearing tanks, the survival, growth and food conversion index of many marine finfish species were better than in clear water condition. The green water technique (larviculture in an endogenous bloom of phytoplankton and rotifers) and the 'pseudo green water technique' (larviculture in a tank supplemented daily with exogenous phytoplankton and rotifers) have much commercial application in marine finfish larviculture (Divanach and Kentouri, 2000). Micro algae can also influence live feed and larval microbiology. It has been found that exudates of some algal species can either enhance or inhibit the feeding activity of copepods in cultures (Van Alstyne, 1986). These substances are also involved in the settlement of micro flora required in the gut of fish larvae to prevent intestinal opportunistic bacteria from causing disease. Bacteria associated with live feed can be transmitted to larval fish during feeding. (Benavente and Gatesoupe, 1988). As live prey actively ingest bacteria, it is possible to introduce favourable bacteria as probiotic. In the 'green water technique' of larviculture micro algae

contribute to maintaining the nutritional quality of live food and also positively influence on the settlement of a healthy intestinal micro flora in fish larvae (Skjermo and Vadstein 1993). Micro algae can also possibly influence the endotrophic stages (egg and pre–larvae) and early exotrophic stages. Micro algal background has an important effect on the timing and intensity of first zooplanktonic feeding. Micro algae also play a role in intestinal transit and gut repletion. Improvement in the survival at first feeding is the main result of larviculture with micro algae. Improvement in growth efficiency during rotifer period is another result of micro algal background in larval tanks. Early enhancement of digestive and assimilative functions improves the survival and growth of fish larvae and favours the transition to exotrophy. The use of micro algae in tanks increases the production of pancreatic and intestinal digestive enzymes and improves the quality of gut flora. Even after the endo-exotrophic phase, micro algae have a positive effect on larviculture and may increase the resistance of larvae to further stressing or adaptive conditions. The indirect effects of micro algae on larvae are mainly related to water quality, luminosity, the bacteriology of water and the quality and accessibility of rotifers. It is thus evident that strategic use of micro algae in hatcheries during the very early life of marine fish improves the success of first feeding, a prerequisite for efficient survival, growth and quality in fish larviculture.

3. Rotifers

Rotifers have been used as live feed for cultured marine fish, since four decades. It is well known that a continuous, stable and reliable supply of nutritionally adequate rotifers is the key to the larviculture of marine finfish. Rotifers of the species *Brachiounus rotundiformis and B. plicatilis* are almost indispensable for larval rearing of most marine finfish (Gopakumar and Jayaprakas, 2001; 2003; 2004).

The success of rotifer cultivation is dependent on selecting the most suitable rotifer species or strain for local culture conditions, maintaining water quality in culture tanks and choosing the most appropriate culture technique. Size, the type of reproduction and reproductive rates are species or strain specific. Culture temperatures, salinities, type of food and its quality - all influence the type of reproduction and its rates. Mass production of rotifers is achieved by encouraging rotifers to reproduce asexually, since sexual reproduction results in males and resting eggs. The amount of food that has to be supplied daily to each tank depends on the reproductive rate of rotifers. Usually 1-4 g of baker's yeast is supplied per million rotifers per day.

The optimal range of pH for culturing rotifers is 7.5 – 8.5 (Fulks and Main, 1991) and the pH affects the percentage of unionized ammonia in the water. The pH of cultures play an important role since the toxicity of NH_3-N is a function of pH, temperature and salinity. The optimal level for ammonia is <1 mg l^{-1} and the acceptable level of ammonia and nitrite levels is 6-10 mg l^{-1}. Rotifer cultures require aeration and the dissolved oxygen level should be maintained above 4 ppm (Fulks and Main, 1991). Surplus food is one of the major factors for the deterioration of water quality. This can be avoided by dividing the daily food ration into four to six meals a day or by continuous feeding using a peristaltic pump.

Stock cultures of rotifers are maintained for long periods which facilitate their availability to mass culture wherever they are needed. Natural seawater should be filtered through a 0.2 μm membrane filter and heat sterilized at 100 °C at atmospheric pressure for 30 min to avoid fermentation of insoluble precipitates. The cool sterile seawater can be employed for stock culture. Erlenmeyer flasks (100 ml in volume) or 50 ml sterile disposable tubes can be used for culture. Each heat sterilized flask is filled with 10 – 20 ml sterile sea water and 40 – 60 rotifers are added. Usually, a salinity of 30 ppt is suitable for most strains. A drop of concentrated algae is added to each culture and the flasks or tubes are incubated at temperatures ranging from 20 – 35 °C. The cultures are fed *ad libitum* every 2 days with concentrated algae. Cultures are renewed every 7-10 days. Culture of *Nannochloropsis sp* was found to be the most convenient source of food for rotifer cultures. As in the case of microalgae, mass cultures are done by batch, semi continuous and continuous culture methods. Evaluating the physiological state of rotifer culture is very important in hatcheries since larval production depends on a predictable and reliable supply of rotifers. Six parameters *viz* egg ratio, swimming velocity, ingestion rate, viscosity, enzyme activity and diseases are employed for assessing the state of health of rotifer cultures.

The nutritional quality of rotifers is improved by enrichment, in which rotifers are collected or harvested from culture tanks into containers where they are kept at very high densities and incubated for 8 – 20 h with enrichment dietary components like HUFA. In addition to nutritional enrichment, rotifers can be enriched with antibiotics.(Verpraet *et al.*, 1992) or with probiotic bacteria (Markridis *et al.*, 1999, 2000). The nutritional value of rotifers depends on their dry weight, caloric value and chemical composition (Lubzens *et.al.*, 1989). The number of rotifers consumed by the larvae determines the quantity of food reaching their gut. In red sea bream, the number

of rotifers consumed daily increases with size or age of the larva, 55 – 72 rotifers per 3.9 mm length larvae to 4,700 per 11.4 mm length larva (Fukusho, 1989).

Various methods of storing rotifers have been studied. Frozen rotifers are not usually adequate as feed because of leaching of nutrients. Live *B .plicatilis* can be stored at 4 °C at relatively high densities for at least one month (Lubzens *et al.,* 1990). Rotifers can be kept at -1 °C without feeding or water exchange for about 2 weeks (Lubzens *et al.,* 1995). *B. rotundiformis* strains are less tolerant to 4 °C than *B .plicatilis* rotifer strains and the strains known as SS type are most susceptible and showed lowest survival. Amictic eggs of rotifers can be preserved by cryopreservation in liquid nitrogen after they have been impregnated with cryoprotective agents like dimethyl sulfoxide (DMSO) (Hadani *et al.,* 1992). This method ensures full preservation of genetic traits of importance to aquaculture. Cryopreservation is not a suitable method for preservation of large numbers of rotifers for direct use as feed.

Artificially produced rotifer eggs have been tried as an alternative to daily production of rotifers. The production of these eggs can be manipulated by environmental factors, such as salinity, food quality and quantity, rotifer culture density, exchange of culture media and temperature and varies between *B. plicatilis* and *B. rotundiformis*. The cost of producing resting eggs is very high and therefore not yet been extensively adapted in hatcheries.

4. Copepods

Copepods are a major component of the natural diet of marine fish larvae. The advantages of copepods over rotifers are that copepods have wide range of body sizes both within and between species. The early stage nauplii and copepodites can be extremely useful as initial prey for species that have very small larvae with small mouth gape at first feeding.

In extensive methods copepods are collected from nature and inoculated into outdoor tanks to mass produce for fish larval rearing. The larvae are then transferred at densities of 0.01 to 0.32 l^{-1}. Additional prey may be added during the larval rearing when necessary to maintain prey densities in the range of 200 – 500 l^{-1}. By this method from 1986 to1994 a total of around 2 million juvenile cod was produced (Svasand *et al.,* 1998). Disadvantages of this system include the inability to control production and thus food levels and predators. Lack of food results in differential growth in fish larvae.

Outdoor production of copepods in ponds or large tanks of 350 – 5,000 m³ is carried out in Europe and Asia for cod, grouper and flatfish. Filtered seawater by using filters of around 20 – 40 µm is generally used in these systems.   Phytoplankton bloom can be induced by application of commercial fertilizers. Filtering devices that allow for selective sieving are used to collect primarily nauplii (80 – 250 µm) and copepodite stages (80 – 600 µm) to inoculate the rearing tanks.

In Asia, a mesh size of 400 – 600 µm was used to inoculate outdoor tanks for grouper rearing with copepodites and adult stages 3 days before stocking the newly hatched fish larvae at densities of 5 m⁻³ (Toledo *et al.*, 1999). In this system, using wild harvested copepods, an average survival of 3.4 % at harvest correspond to an average production of 0.17 grouper *Epinephelus coioides* juveniles" m⁻³. Regular monitoring of densities of live prey in these outdoor systems is important for the successful rearing of marine fish larvae.   An advantage of outdoor ponds over the extensive systems that rely on the local production of zooplankton is the possibility of culturing the zooplankton over one generation before using them as food.   Moreover, feeding wild plankton directly to the fish increases the risk of infections.

The most frequently cultured calanoid species belong to the genera found in coastal waters, such as those of genera *Acartia, Centropages, Eurytemora* and *Temora.* These copepods are small, with relatively short generation time and a wide thermal and salinity tolerance and are easily adaptable to laboratory conditions.   Most calanoids require phytoplankton although Turk *et al.*, (1982) demonstrated that it was possible to culture *Acartia tonsa* on rice bran.

Aeration is required to maintain phytoplankton in suspension and to create small turbulence which helps to distribute copepods within the culture tanks. Most calanoids require large volumes and the adult density rarely exceeds 100 l⁻¹. Successful hatch culture of the calanoid *Acartia* sp was achieved in 1,000 l polyethylene tanks, 1.3 m in diameter with a conical base. The tanks are emptied after the 8 day hatch cycle and cleaned and a new batch culture was started (Schipp *et al.*, 1999). Contamination of copepod culture by bacterial blooms, ciliate infection, other copepods or rotifers may pose a problem. In commercial facilities, contamination by rotifers is most likely to cause the collapse of copepod culture, since the rotifers with their higher reproductive rate would quickly out-compete the copepods. Hence these cultures should be strictly kept apart.

Ciliates are utilized by copepods and in periods of low phytoplankton concentration constitute the major dietary source. Ciliates are often

an indication of overfeeding and if ciliates are noted in cultures it is advisable to empty the culture using a 60 or 80 μm mesh, which retains the adult copepods, but allows the ciliates to be washed out.

Harpacticoid copepods have several advantages for culturing (Stottrup, 2003). They include (i) High tolerance to a wide range of environmental conditions. (ii) Ability to feed on a wide range of live or inert diets. (iii) High reproduction capacity. (iv) Relatively short life cycles. (v) Ability to be cultured in high densities. (vi) Requirement for surface area rather than volume (vii) Planktonic naupliar stages. (viii) Can be used as tank cleaners in rotifer cultures, other copepod culture or larval tanks.

Filtered seawater can be used for harpacticoid culture and most feeds are acceptable to many harpacticoid species. Algae which quickly sediment are also good feed because bacteria colonise these cells, and the mixture of algae and bacteria form a good dietary combination for harpacticoids. Photoperiod influences offspring production and sex ratio. A photoperiod of 12 L / 12 D was shown to be most favourable for offspring production. Many harpacticoids have wide thermal and salinity tolerances. Ciliates and rotifers in the culture tanks compete for food and may lead to crash of copepod culture.

5. Artemia

Comprehensive literature reviews on the use of *Artemia* as live food in fin fish and larviculture has been published by Leger *et al.*, (1986); Sorgeloos *et al.*, (1998 ; 2001). *Artemia* is widely used in the mass culture of different sea bream species, sea bass species, wolf fish, cod, turbot, halibut, flounder species, milkfish, surgeon and many shrimps, prawn, crabs and lobsters. Nauplii in instar I and II stages are the most widely used forms of Artemia in aquaculture. They are the earliest and easiest live feed, being obtained directly from the cysts.

Most marine fish larvae cannot synthesise DHA, EPA or Arachidonic acid from shorter chain precursors and they must be provided in the larval diet, hence artemia is enriched for enhancing the nutritional value for using as a live feed. Although artemia is often an inferior food source for fish larvae compared with wild zooplankton, the ability to produce any amount of biomass within 24 h, and the constant improvement of enrichment products ensure its continued use in marine fish larviculture. It is quite possible that artemia will gradually replaced by formulated diets; it is obvious that the use of nauplii will continue in hatcheries at least for a few more years.

6. Prospects

It is quite obvious that at the present status of technology, live feeds cannot be dispensed in marine larviculture. The major challenge for the future is in the development of commercial artificial feeds for larvae. The immediate research thrust should be on technologies for high density and stable production of high quality live feeds. The identification of causes for sudden crashes in culture and remedial measures for the same requires priority attention. Genetic manipulations for the production of super small strains of rotifers and the commercial production of rotifer cysts are also major areas for intensified research. The production of high density microalgae in photobioreactors and by fermentation techniques are potential areas for future development. The copepod culture techniques, which is the key to the success of rearing of altricial larvae of marine finfish still remains to be standardized. Hence this should receive more focused research than the better studied traditional live feeds.

Reference

Benavente, P.G., Gatesoupe, F.J., 1988. Bacteria associated with cultured rotifers and artemia are detrimental to larval turbot, Scophthalmus maximus. Aqcult.Eng. 7, 289-293.

Divanach, P., Kentouri, M., 2000. Hatchery techniques for specific diversification in Mediterranean finfish larviculture. CIHEM – FAO publication 47, 75 – 87.

FAO 2000. The state of world fisheries and aquaculture, 2000. http://www.fao.org/DOCREP / 003/ X8002E/X8002EOO.HTM

Fukusho, K., 1989. Biology and mass production of the rotifer Brachionus plicatilis (2). Int.J.Aquacult.Fish.Technol. 1, 292-299.

Fulks, F., Main, K.L., 1991. Rotifer and microalgae culture systems. Proc. US – Asia Workshop, Oceanic Institute, Honolulu. HI, 364.

Guillard, R.R.L., Ryther, J.H., 1962. Studies on marine Planktonic diatoms.I. Cyclotella nana Hustedt and Detonula confervacae (Cleve) Gran. Can.J. Microbiol. 8, 229-239.

Gopakumar, G., Jayaprakas, V., 2001. Rotifers as live feeds for larviculture of marine fishes – a research review. In: Menon, N.G., Pillai, P.P., (Eds.), 2001. Perspectives in Mariculture, The Marine Biological Association of India, Cochin. 35 – 66.

Gopakumar, G., Jayaprakas, V., 2003. Community structure and succession of brackish water rotifers in relation to ecological parameters. J.Mar.Biol.Assn. India. 45 (1), 20-30.

Gopakumar, G., Jayaprakas, V., 2004. Life table parameters of Brachionus plicatilis and B. rotiundiformis in relation to salinity and temperature. J.Mar.Biol.Assn. India. 46(1), 21-31.

Hadani, A., Beddig, S., Lubzens, E., 1992. Factors affecting survival of cryopreserved rotifers (Brachionus plicatilis O.F. Muller). (By Moav, B., Hilge, V., Rosenthal, H., (Ed.)), In: Progress in Aquaculture Research. European Aquaculture Society, Special Publication No.17, Ostend. 253-267.

Leger, P., Bengtson, D.A., Simpson, K.L., et al., 1986. The use of nutritional value of artemia as a food source. Oceanogr. Mar. Biol. Annu. Rev. 24, 521-623.

Lubzens, E., Kolodny, G., Perry, B., Galai, N., Sheshinski, R., Wax, Y., 1990. Factors affecting survival of rotifers (*Brachionus plicatilis*) culture. Aquaculture 133, 295 – 309.

Lubzens, E., Rankevich, D., Kolodny, G., Gibson, O., Cohen, A., Khayat, M., 1995. Physiological adaptations in the survival of rotifers (*Brachionus plicatilis* O.F. Muller) at low temperatures. Hydrobiologia 313/314, 175 – 183.

Lubzens, E., Tandler, A., Minkoff, G., 1989. Rotifers as food in aquaculture. Hydrobiologia 186/187, 387-400.

Markridis, P., Bergh, O., Fiellheim, A.J., Skjermo, J., Vadstein, O., 1999. Microbial control of live food cultures. In: Laird, L., Reinertsen, H., (Ed.) *Towards Predictable Quality. Aquaculture Europe.* European Aquaculture Society, Special Publication No.27, Ostend. 155 – 157

Markridis, P., Fiellheim, A.J., Skjermo, J., Vadstein, O., 2000. Control of bacteria flora of *Brachionus plicatilis* and *Artemia franciscana* by incubation in bacterial suspensions. Aquaculture 185, 207-218.

Muller – Feuga, A., 2000. The role of microalgae in aquaculture: situation and trends. J.Appl.Phycol. 12, 527-534.

Schipp, G.R., Bosmans, J.M.P., Marshall, A.J., 1999. A method for hatchery cultivation of tropical calanoid copepods, Acartia spp. Aquaculture 174, 81-88.

Skjermo, J., Vadstein, O., 1993. The effect of microalgae on skin and gut microbial flora halibut larvae. In: By Reinerstsen, H., Dalhe, L.A., Jorgensen, L., Tvinnerein, K., (Eds.), *Proceedings from International Conference on Fish farming Technology*, Trondheim. August, 61 – 67.

Sorgeloos, P., Coutteau, P., Dhert, P., Merchie, G., Lavens, P., 1998. Use of brine shrimp *Artemai spp.* In larval crustacean nutrition: a review. Rev. Fish. Sci. 6, 55-68.

Sorgeloos, P., Dhert, P., Candreva, P., 2001. Use of brine shrimp *Artemia spp.* in marine fish larviculture. Aquaculture 200, 147-159.

Stottrup, J.G., 2003. Production and nutritional value of copepods. In: Stottrup, J.G., McEvoy, L.A., (Eds.), *Live Feeds in Marine Aquaculture.* 145-205.

Stottrup, J.G., McEvoy, L.A., 2003. *Live Feeds in Marine Aquaculture.* Blackwell Publishing Company, London.

Svasand, T., Kristiansen, T., Pedrsen, T., Salvanes, A.G.V., Engelsen, R., Nodtvedt, M., 1998. Havbeite med torsk – artsrapport. Norges forskningsrad. 78.

Toledo, J.D., Golez, M.S., Doi, M., Ohno, A., 1999. Use of copepod nauplii during early feeding stage of grouper Epinephelus coioides. Fish.Sci. 65, 390-397.

Turk, P.E., Krejci, M.E., Yang, W.T., 1982. A laboratory method for the culture of *Acartia tonsa* (Crustacea: Copepoda) using rice bran. J. Agricult. Aquat. Sci. 3, 25- 27.

Van Alstyne, K.L., 1986. Effects of phytoplankton taste and smell on feeding behavior of the copepod *Centropages hamatus*. Mar.Ecol.Prog.Ser. 34, 187 – 190.

Verpraet, R., Chair, M., Leger, P., Nelis, H., Sorgeloos, P., DeLenheer, A., 1992. Live food mediated drug delivery as a tool for disease treatment in larviculture - The enrichment of therapeutics in rotifers and artemia nauplii. Aquacult.Eng. 11, 133-139.

Walne, P.R., 1966. Experiments in the large scale culture of the larvae of *Ostrea edulis*. Fish. Invest. 2, 25-53.