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Rotifer as live feed for larviculture of marine fishes - a research review

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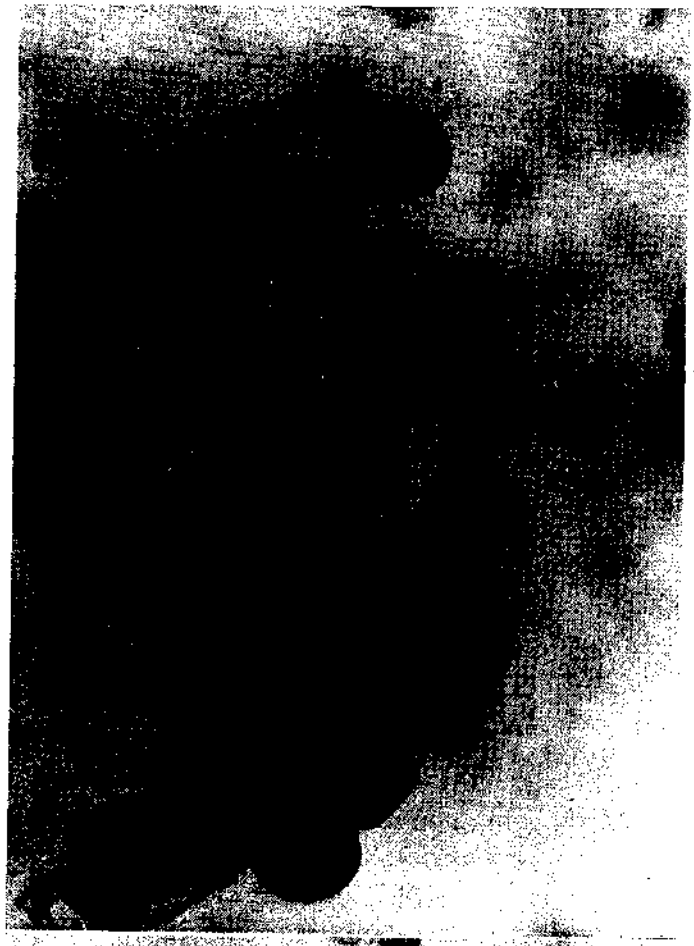

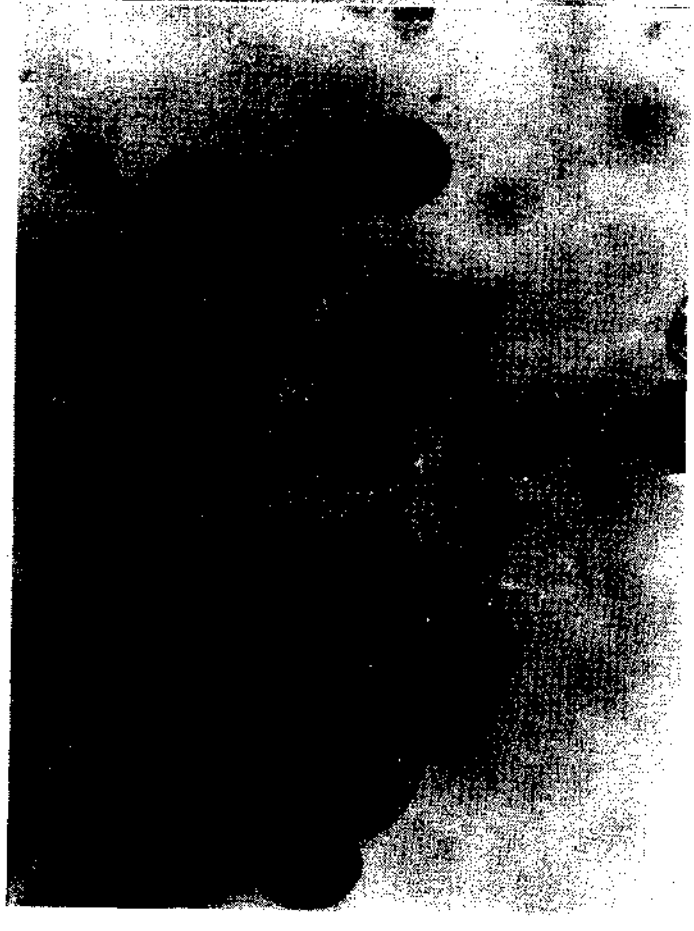

ABSTRACT

The most critical factor in the commercial farming of fish and shellfish is the dependable availability of healthy fry produced in hatcheries. Most of the fishes having aquaculture potential have larvae with very limited yolk reserves and the transition stage from endogenous to exogenous feeding is very critical, often resulting in mass mortalities. The recent success in the consistent supply of many marine and brackish water fish seeds can be attributed to the mass production technologies of high quality live feeds. Research has resulted in the worldwide use of the brackish water rotifers Brachionus plicatilis *and* B.

rotundiformis *as successful diets for the developing larvae. This paper reviews the present status of rotifer research on the different aspects of rotifer culture such as strain selection, mass culture techniques, nutritional quality of rotifer feed and the general requirements for mass culture namely dissolved oxygen, pH, temperature, salinity and unionized ammonia. The salient aspects of rotifer biology relevant to the culture of rotifers and the role of rotifers in the ecosystem are reviewed. The recent research findings on monitoring, harvesting, storage, enrichment, feeding strategies and problems associated with rotifer culture are also reviewed in this paper.*

Introduction

In the last few decades, considerable progress has been achieved in the industrial farming of several species of fish and shellfish. It is well established that the most critical factor in the commercial farming of fish and shellfish is the dependable availability of healthy fry produced in hatcheries. Inconsistent supply of seed was the major constraint for the development of aquaculture of many marine and brackishwater fish and shellfish. One of the major reasons for the improvements in the success of fish and crustacean culture in the 1970s and 1980s was the development of reliable hatchery techniques for the mass production of quality fry and fingerlings. This breakthrough was achieved mainly by the production of adequate quantities of high quality live feeds (Sorgeloos and Leger, 1992)

Most marine fish with aquaculture potential have larvae with limited yolk reserves, small mouths and primitive digestive systems. Nutrition at this early larval stage is very critical and live food production techniques and feeding strategies need to be developed before commercial level production. Over the past few decades adequate feeding strategies have been developed for several fish and crustacean larvae, resulting in the world-wide use of various species of microalgae (approximate size range 50-200μm), the rotifers *Brachionus plicatilis* and *B. rotundiformis* (50-200μm) and the brineshrimp *Artemia* (420-8000μm) (Lubzens, 1987; Lubzens *et al.*, 1989; Dhert and Sorgeloos, 1994; Lavens *et al.*, 1995).

Rotifers

Rotifers are aquatic microscopic invertebrates comprising about 2000 species of unsegmented, bilaterally symmetrical pseudocoelomates. They are commonly referred to as 'wheel animalcules' as their disc like anterior end (corona) bears resemblance to a pair of revolving wheels due to the synchronized beating of their coronal cilia. The rotifers show worldwide distribution and the majority of them inhabit freshwater and some genera also occur in brackishwater and marine habitats. They exhibit fascinating strategies of reproduction, population dynamics, spatial and vertical distribution and survival. Many species are notable for their ecotypic and cyclomorphic variations.

The length range of rotifers is generally 100-1000µm, although the largest species may surpass 2000µm. The body is elongated or saccate, sometimes cylindrical, worm like, or even spherical. The majority of rotifers in natural conditions are females. Males are definitely known for relatively few species; they are much smaller than females, degenerate and seldom live for more than two or three days. In general the body consists of three regions- the head including corona, body or trunk and foot. The head is not well delimited and carries several organs- the corona, the mouth opening and several sensory organs and appendages. The ciliated corona serves as locomotory and food collecting organ. The foot extends from the body ventrally or more commonly terminally.

Internal organization

Rotifers possess a spacious pseudocoelom and muscles, nerves, digestive, reproductive and protonephridian organs are found within this cavity. Respiratory and circulatory systems are absent.

The muscular system consists of both smooth and striated muscles occurring in small bands of longitudinal and circular fibres inserted at various points on the integument or between the integument and the viscera. Contractions of these muscles cause a swift beat of appendages resulting in a rapid explosive movement ('jump'). The nervous system of rotifers is simple consisting of a large cerebral ganglion which is located dorsally below the corona. A paired protonephridial system, comprising of two parallel tubules and flame cells maintain the internal osmotic

pressure and remove toxic metabolites and nitrogenous wastes, mainly ammonia.

Gonads are paired in class Bdelloidea and class Seisonidea. In bdelloid males are entirely unknown and reproduction is always asexual. In the third class Monogononta, only one gonad is present. Males in this class have not been found for a large number of species, eventhough it is generally assumed that all the monogononts are capable of producing males under proper conditions. The appearance of males is usually limited to a few days or a week in one reproductive season and during the rest of the time reproduction is parthenogenetic. The reproductive organs of female rotifers consist of ovary, vitellarium and follicular layer (Amsellem and Ricci, 1982). At birth the total number of oocytes are already present in the ovary. Male rotifers are always smaller than females. Usually the digestive organs of males are rudimentary or entirely absent. The single testis is large and saccate with about 50 mature sperms floating freely within. A ciliated vas deferens leads from tne testis to the penis, and one or rarely two prostate glands discharge into it. Rotifers are usually oviparous, they release their eggs outside the body where the embryo develop. Many planktonic rotifers carry their eggs attached to the mother by a thin thread (*Brachionus, Polyarthra*), others attach them to a substratum (*Asplanchnopus, Epiphanes*) or release them into the plankton (*Notholca, Ploesoma*).

The class Seisonidea reproduces exclusively bisexually, class Bdelloidea reproduces entirely by asexual parthenogenesis and class Monogononta reproduces by a mixture of these two extremes- cyclical parthenogenesis (Fig. 1). Parthenogenesis dominates the monogonont life cycle where reproduction occurs in the absence of males (amictic phase). Under certain environmental conditions (temperature, crowding, food quality and quantity changes) males may be produced and sexual reproduction takes place (mictic phase). Mictic and amictic females are morphologically indistinguishable. Amictic females are diploid and produce diploid (amictic) eggs. They develop mitotically into females (Birky and Gilbert, 1971; Gilbert, 1983). Sexual (mictic) reproduction can be initiated concurrently with amictic egg production in any season, in response to certain environmental factors which are poorly understood.

Environmental stimulus for mixis has been described for a few species of *Asplanchna*, *Brachionus* and *Notommata* (Gilbert, 1980; Pourriot and Snell, 1983; Snell and Boyer, 1988). Dietary tocopherol (Vitamin E) controls the shift from amictic to mictic reproduction in most *Asplanchana* species (Gilbert, 1977). Population density is widely attributed as a stimulus for mictic female production in *Brachionus* (Gilbert, 1977; Pourriot and Snell, 1983, Snell and Boyer, 1988; Carmona *et al.*, 1994). Genetic factors also play an important role in determining sensitivity of strains to mictic stimuli (Snell and Hoff, 1985; Lubzens *et al.*, 1985).

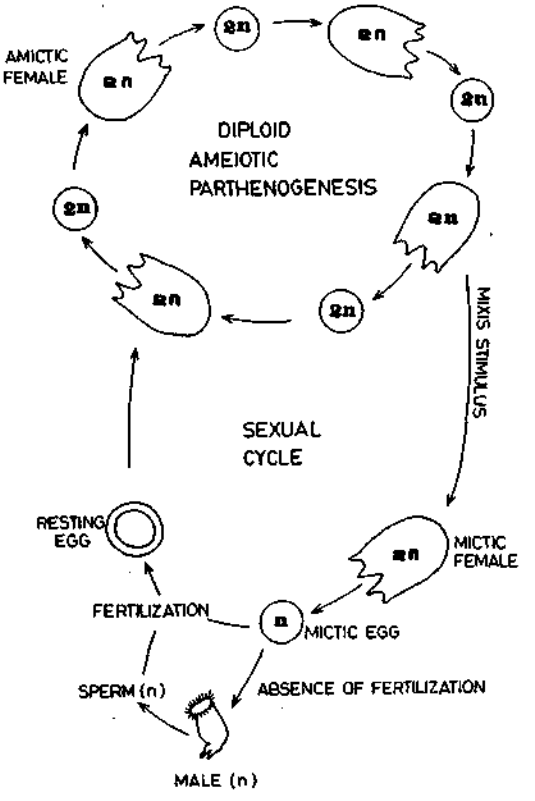

Fig. 1. Life Cycle of a Brachionid

When sufficient intensity of mictic stimulus has been received, amictic females begin producing mictic as well as amictic daughters. The proportion of mictic daughters and duration of their production depends on the strength of the mictic stimulus (Snell and Boyer, 1988). Mictic females produce male eggs which are smaller and more numerous than amictic eggs. A male must inseminate a mictic female within four hours of birth for fertilization (Snell and Childress, 1987). Fertilized mictic female produces a diapausing embryo called a resting egg. Mictic females produce haploid eggs through meiosis. If unfertilized these develop into haploid males. Males live only for 2-5 days because they do not feed (Snell, 1977, King and Miracle, 1980).

Resting eggs are diploid and possess thick, often sculptured walls that are characteristic of the species. This dormant stage is very resistant to harsh environmental conditions (Gilbert, 1974) and may be dispersed over wider areas by the wind, water and migrating animals like water birds. After a period of dormancy resting eggs respond to species specific environmental conditions and hatch releasing diploid amictic females that enter into the asexual phase of the life cycle. The stimuli that induce hatching include change in light, temperature and salinity (Pourriot and Snell, 1983). Formation of resting eggs promotes survival and dispersal and can be considered adaptive in unpredictable environments. Resting eggs have been known to hatch even after twenty years of dormancy (Nipkow, 1961). Because of this capacity for extended dormancy, resting eggs could accumulate in sediments. In the studies conducted at limited sites, the density of resting eggs ranged from 100 to 400 eggs/cm^2 (Snell *et al.*, 1983). These workers found that the highest densities occurred on the sediment surface. Appearance of floating resting eggs of *B. plicatilis* is also reported (Hagiwara, 1996).

Planktonic rotifers swim constantly. Swimming is accomplished with the aid of coronal cilia and may or may not be combined with the creation of feeding currents. Swimming speed is crucial in food acquisition and mate finding. Speed is temperature dependent and varies with the age of the planktonic species. All free swimming non-predatory and sessile species use the coronal cilia to create a water current passing by

the mouth where food selection may take place or all particles of an appropriate size class can be ingested non-selectively. *Brachionus* move small food particles into the buccal region through a process of filter or suspension feeding.

In many aquatic food webs, rotifers serve as important species indicating the extent of exposure to toxicants and quantifying toxic effects. Using rotifer models, toxicity is being investigated on several levels from communities to molecular basis. Changes in the structure of rotifer assemblages are being used as indicators of water quality.

The saline rotifers – *B. plicatilis* and *B. rotundiformis*

B. plicatilis, an euryhaline rotifer, is an important and essential food source in the early part of the commercial rearing of many marine fish and a few shrimp species. Two morphotypes – S (small) and L (large) are distinguished based on morphological and physiological differences (Fu *et al.*, 1991a,b; Rumengan *et al.*, 1991; Fu *et al.*, 1993). These strains could be selectively employed for feeding fish larvae depending on the mouth size of the larvae. The two strains coexist in wild stock, with one of the strains becoming dominant due to environmental conditions, particularly water temperature (Fukusho, 1989a,b). Recent studies on morphology, karyotype, genetics including allozyme constitution and reproductive behaviour of 'S' and 'L' type *B. plicatilis* showed that these types are best treated as different species. A reexamination of existing available names revealed *B. plicatilis* O.F. Muller 1786 and *B. rotundiformis* Tschugunoff 1921 as the correct names for the 'L' and 'S' type respectively (Segers, 1995, Gomez and Serra, 1995a,b; Hagiwara *et al.*, 1995; Natesan *et al.*, 1996). There are various geographical strains, cyclomorphic forms and ecotypes of these two types in the different habitats.

B. plicatilis can reproduce either by mictic or amictic reproduction. The mictic patterns of *B. plicatilis* was recently investigated by Gomez and Serra (1995b). Aquaculturists promote only amictic reproduction because the rate of amictic reproduction is faster than mictic reproduction; males which are only produced during mixis are inferior nutritionally due to the lack of a functional digestive system and the onset of mixis can cause culture collapse (Meragelman *et al.*', 1985). A

model evaluating the contribution of environmental factors to the production of resting eggs in *B. plicatilis* was developed by Lubzens *et al.* (1993). Pozuelo and Lubian (1993) reported that mixis is a strain dependent component of general reproduction response. However, some clones are known to be exclusively amictic (Meragelman *et al.*, 1985). Depending on conditions, an amictic female may produce 20 or more eggs during her seven to ten day of life time (Hoff and Snell, 1989). She carries all her eggs attached to the posterior portion of her body until they hatch. S and L type *B. plicatilis* have an identical pattern of fecundity (Hirayama and Rumengan, 1993). Some life history characteristics of *B. plicatilis* that were fed a variety of algal diets were investigated by Korstad *et al.* (1989a,b). Carmona *et al.* (1993) reported that mixis can be initiated in *B. plicatilis* by pre-conditioning of culture medium by crowding.

B. plicatilis and B. rotundiformis as live feed

B. plicatilis and *B. rotundiformis* cultures have now become an indispensable aspect of many marine finfish hatcheries. *B. plicatilis* and *B. rotundiformis* are excellent first feeds for fish larvae due to their small size, ability to be cultured at high densities, high reproductive rate with parthenogenetic mode of reproduction, slow swimming speed and habit of staying suspended in the water column, ability to tolerate wide range of salinities, ability to be easily enriched with fatty acids, antibiotics etc. and hence can be used to transfer these substances to the larvae. There are other rotifer species that possess some or all these characteristics, but *B. plicatilis* and *B. rotundiformis* are widely used in mariculture because they are able to thrive in a wide range of salinities.

Culture of B. plicatilis and B. rotundiformis

Takashi Ito (Ito 1955, 1957a,b, 1960) was the first to discover that *B. plicatilis* is an excellent food for the larvae of the marine fish *Placoglossus altivelis*. By 1970s *B. plicatilis* has become widely accepted as the best food in the early stages of larval rearing of marine fin fish. The method to produce the rotifer by feeding marine *Chlorella* was developed, but the quantity generated was not sufficient to meet the entire demand for feeding larvae and juveniles. In the 1970s baker's yeast was

introduced as a food organism for rotifers and the foundation of the current mass production system utilizing a combined feeding programme of marine microalgae and baker's yeast was established.

Now rotifers are mass-produced in hatcheries all over the world. Theilacker and Mc Master (1971) found that *B. plicatilis* was an excellent food source for larval anchovies (*Engraulis mordax*). Arnold and Holt (1991) while describing the various methods for the culture of *B. plicatilis* in Texas emphasized the importance of keeping culture containers and water clean, controlling contaminants such as ciliates and bacteria, harvesting daily to maintain the culture in growth phase and adding some algae daily. Orhun *et al.*, (1991) described a practical approach to high density production of *B. plicatilis* at California using two low cost tanks, partial automation of harvesting, water exchanges and waste removal. Hirayama and Satuito (1991) recommended that nutritional improvement of baker's yeast for growth of *B. plicatilis* can be done by rearing yeast in a medium rich in organic nutrients which can incorporate essential lipids into the cells. The selection of optimum phytoplankton species for rotifer culture during cold and warm seasons and their nutritional value for marine finfish larvae was investigated by Hur (1991). While suggesting improvements in the design of mass culture system of *B. plicatilis*, Snell (1991) recommended three thrust areas for research – (a) the identification of culture instability and sudden crashes (b) role of nitrogen excretion and unionized ammonia toxicity and (c) early detection of stress in mass cultures by monitoring reproductive traits, swimming ability and enzyme inhibition.

Strain selection : *B. plicatilis* is widely distributed in brackishwater ponds and lakes. There are lot of strain variations. The reproductive rate, size, optimum culture conditions and frequency of mixis vary among different strains (Lubzens *et al.*, 1989; Lubzens, 1987; Fukusho 1989a; Meragelman *et al.*, 1985; James and Abu Rezeq, 1989c; Fushimi, 1989; Mustahal *et al.*, 1991). In a culture system amictic reproductive rate should be maximized while frequency of mixis should be minimized. Size is an important factor because different target species and developmental stages within species have different optimum food sizes. Great care should be taken when selecting a strain to be cultured, since different strains may perform better at different temperatures and salinities, local conditions as well as those required by the target species.

Mass culture methods

A wide variety of culture systems have been employed which can be categorized into four basic methods viz., batch culture, semi-continuous culture, feedback culture and continuous culture (Lubzens, 1987).

Batch culture: In this method, all the rotifers are harvested when the density of rotifers reaches the desired level. It can be done in outdoor or indoor tanks. Fukusho (1989a) describes a typical Japanese hatchery using 100 m^3 tanks for rotifer culture and a diet of microalgae, usually *Nannochloropsis* and baker's yeast. After a growth period of a few weeks, rotifer densities of about 100/ml are reached and the mass culture is then harvested for several days. Six 100 m^3 tanks hold a standing crop of rotifers from which 1-2 billion can be harvested daily. Using this system, 600 m^3 of water must be managed to produce about one billion rotifers per day. Batch culture of rotifers is the most reliable method but also the least efficient in terms of labour and facilities needed to culture a given number of rotifers (Trotta, 1981; Fushimi, 1989).

Semi-continuous culture: Here, a given population is allowed to grow until it reaches certain population density. Then it is partially harvested and fresh medium is added. The growth and harvest procedures are repeated several times before the water quality mandates that the tank be drained and cleaned. This method can also be practiced in both indoor the outdoor tanks. According the Lubzens (1987) semi-continuous rotifer culture employs vessels ranging from a few hundred liters to 200,000 liters. Relatively high densities can be obtained in the smaller volume cultures. Build up of waste products like uneaten food and contamination are the problems in semi-continuous systems. This makes them less reliable than batch cultures.

Feedback culture: Accumulation of high levels of unused food and excretory products occurs in high density rotifer cultures. These are removed into a decomposer tank. The decomposed matter is employed as fertilizer for algal cultures, which are used to feed rotifer cultures (Hirata, 1979).

Continuous culture: These are delicately balanced systems in which the culture organisms are harvested continually and receive constant

nutrient replenishment. Continuous cultures are the most efficient ways to produce a consistent supply of high quality algae and rotifers. Since continuous culture apparatuses must be maintained under strictly defined conditions, they are always closed and indoors. This limits their size and may add to the cost of operations. The most advanced design of continuous culture is the chemostat which has been applied to aquaculture by James and Abu-Rezeq (1989a,b). In this approach, algae and yeast are supplied continuously at a predetermined rate. The culture is diluted by a certain volume each day and this volume is harvested to obtain rotifer biomass. Production from 1 m^3 chemostat is sufficient to meet the rotifer needs of most small and medium sized hatcheries. Chemostat mass cultures have yielded the greatest rotifer biomass production per unit of effort thus far recorded in aquaculture.

General requirements for culture

Nutritional quality and rotifer feeds : The type of feed used for culturing rotifers can have a significant effect on the cost of operations and on the nutritional value of rotifers (Carnic *et al.*, 1993). When choosing a feed one must consider both the requirements of the rotifer as well as the needs of the target species. *B. plicatilis* and *B. rotundiformis* have broad nutritional requirements. These animals ingest many types of feed including bacteria, so long as it is of appropriate particle size. The rotifers require Vitamin B_{12} (Yu *et al.*, 1989; Maruyama and Hirayama, 1993) and Vitamin A (Fukusho, 1989a). In contrast, the fish larvae require long chain 'highly unsaturated fatty acids' (HUFAs) on the n-3 series, mainly 18:3 n-3, 20:5 n-3 and 22:6 n-3 (Watanabe *et al.*, 1983a). Since many marine fishes cannot synthesize these fatty acids, feeds must contain high level HUFAs. The nutritional quality of rotifers is primarily determined by the type of feed given to the rotifers. Other factors to consider when selecting a feed type include the stability of culture required as well as the availability and cost of purchasing or producing the feed.

Many species of algae are used as rotifer diet according to availability under local conditions and the exact nutritional requirements of the rotifers and the target species. The most commonly used species are *Nannochloropsis oculata, Tetraselmis tetrathele, T. suecice, Isochrysis*

galbana and *Chlorella vulgaris* (Hirata, 1989; Hirayama *et al.*, 1989). Species high in n-3 HUFAs such as *Nannochloropsis* spp. are regarded as very good feeds. The main drawback in using phytoplankton is the huge amount of labour, time and facilities that must be developed for producing the large quantities needed to feed rotifers. Alternatively, marine yeast (*Candida* sp.), baker's yeast (*Saccharomyces cerevisiae*) and caked yeast (*Rhodotorula* sp.) have all been successfully used for rearing rotifers. But yeast has no nutritional value and they lack the much needed HUFAs (Walford and Lim, 1992). Problems encountered with the use of yeast include more frequent rotifer culture crashes and poor survival in target species that have high HUFA requirement (Fukusho, 1989a; Hirayama and Funamoto, 1981). The latter problem is solved by giving rotifers a mixed feed of algae and baker's yeast or by feeding rotifers with algae high in HUFAs for a few hours or days prior to harvest. The use of bacteria as feed for *B. plicatilis* is also investigated, which revealed that addition of Vitamin B_{12} producing bacteria can greatly enhance the growth of cultured *B. plicatilis* (Yu *et al.*, 1989). Photosynthetic bacteria along with baker's yeast have also been used to feed semi-continuously cultured rotifers (Fushimi, 1989; Gatesoupe *et al.*, 1989; Fukusho, 1989a). An overall better quality of rotifers cultivated with algae was noted than that cultivated with yeast and oil (Oie *et al.*, 1994). Relatively high reproductive rates were found in three strains of rotifers fed with frozen *Nannochloropsis* biomass (Lubzens *et al.*, 1995). The study revealed that application of frozen *Nannochloropsis* biomass may promote easier management in the production of lipid enriched rotifers. Attempts to culture *B. plicatilis* with microencapsulated diets were also made (Teshima *et al.*, 1981).

The amount of food supplied and the frequency of feeding can also affect the nutritional quality and growth rate of rotifers. Ingestion rates are correlated with the size of the particles offered and their concentration (Fukusho, 1989a). The absolute quantity of feed provided/rotifer/day is the most important parameter. The algal density measured in cells per ml will vary greatly with the cell size of the particular algae being used. As many as 20 million cells per ml of *N. oculata* (2μm) may be required at the start of the culture. Feeding rates are estimated to be 100,000 – 150,000 *N. oculata* cells / rotifer / day. The daily ration will

also depend on temperature (Nagata, 1989) and salinity (Lubzens, 1987). When temperature and algal concentration interacts, a great influence on filtration rate and marginal influence on ingestion rate of *B. plicatilis* was noted (Acosta Jimeno and Perez Enriques, 1995). Feeding frequency is an important factor affecting rotifer quality and growth rate (Meragelman *et al.*, 1985; Lubzens, 1987; Lubzens *et al.*, 1989). Lebedeva and Orienko (1995) found that *B. plicatilis* feeding rate was largely determined by temperature and food concentration. Most of the nutritional value of rotifers comes from their gut contents, partially digested and highly concentrated phytoplankton, yeast, bacteria etc, not from their own tissues. Hence if rotifers are deprived of food prior to harvest, their nutritional quality will be poor.

A recent breakthrough in production of an artificial diet (Culture Selco, Artemia Systems NV, Belgium) which completely replaces algae and at the same time eliminates the need of an extra enrichment period for enhancement of the rotifer's dietary value (Nyonje and Radull, 1991; Lavens *et al.*, 1995). This dry product needs to be suspended in water prior to feeding. Provided it is continuously aerated and cold stored, the food suspension of Culture Selco can be used in automatic feeding for as long as 48 hr. Under the standard feeding protocol developed a doubling time for different rotifer strains was generally obtained every three days. Under optimal conditions, doubling time of the population may be even expected every 24hr (Lavens *et al.*, 1994). Excellent results in using this diet as an alternative to the traditional mixture of algae and yeast were also obtained on a commercial scale in different bream and bass hatcheries (Komis *et al.*, 1991). This artificial diet gives constant and predictable levels of n-3 HUFA enrichment which cannot be reached by any other micro algal enrichment procedures. Rotifers grown on Culture Selco have constant levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of 6 mg per gram DW and 4 mg per gram DW respectively. Recent investigations on gilthead seabream larviculture (Mourente *et al.*, 1993) demonstrated that during first feeding the best growth rate was achieved with a diet of Culture Selco reared rotifers, which combined a high n-3 HUFA content with a high DHA : EPA ratio.

Dissolved Oxygen: The optimum Dissolved Oxygen (DO) required depends on temperature, food type and rotifer density. If microalgae are

used as sole feed, the amount of aeration that must be provided is lower than that if yeast is being used. This is because given sufficient light algae produce oxygen, while yeast and associated bacteria consume it. Fukusho (1989a) reported that at 20 °C both L and S type rotifers consume 7.07×10^{-5} ml oxygen/day. The rate increases to 10.04×10^{-5} ml/day at 25 °C and to 16.48×10^{-5} ml/day at 30 °C. Hirata and Yamasaki (1987) studied the relationship between oxygen consumption and food availability in *B. plicatilis* and found it to range from 1 – 7 ml/ind/hr. and was seen to increase with increased feeding. The relative swimming speed of *B. plicatilis* was decreased when the concentration of oxygen was very low. Rotifers spend more time in very low concentration of oxygen by slowing and by turning more (Reale *et al.*, 1993). Fushimi (1989) states that 60-100 liters of air/min/m^3 must be provided to the yeast fed rotifer cultures of rotifer density 1000 ind/ml. Blowers, air stones, airlift pumps and PVC piping into which holes have been drilled can be used for aeration.

Light: Rotifers are cultured indoors either with constant or part time illumination. Hoff and Snell (1989) recommended a light : dark cycle of 18 : 6 hours . According to Fukusho (1989a) for *B. plicatilis* the beneficial effect of light may be indirect by stimulating growth of photosynthetic bacteria and microalgae in the rearing tanks.

pH: *B. plicatilis* tolerates a wide range of pH (5-10), the optimum pH range for culture is reported to be 5-9 by Fukusho (1989a) and 7.5 to 8.5 by Hoff and Snell (1989). The optimum may vary depending on the type of feed (Furukawa and Hidaka, 1973). Yu and Hirayama (1986) stated that pH indirectly influences rotifer population by its effect on the amount of unionized ammonia in the culture water. Fushimi (1989) reports an example of rotifer mass culture in which the pH was maintained at 8.0 to 8.2 with hydrochloric acid and sodium hydroxide.

Temperature: The optimum temperature will depend on the strain being cultured (Snell and Carrillo, 1984, Fukusho, 1989a). Theilacker and Mc Master (1971) stated that maximum reproduction occurred between 30 and 34 °C. In general the recommended temperature is between 20 and 30 °C.

Salinity: *B. plicatilis* and *B. rotundiformis* are known for their wide range of salinity tolerance. According to Hoff and Snell (1989) salinities rang-

ing from 1-60 ppt may be tolerated by *B. plicatilis*, but 10-20ppt will give the best growth. Salinity may have a large effect on reproductive rate. Different strains and clones have different salinity optima (Lubzens, 1987). A culturist must also take into consideration the salinity at which the target species will be grown. For example, rotifers cultured at 20 ppt should be acclimatized for a day at 30 ppt before being fed to fish larvae in 40ppt seawater (Lubzens, 1987). Otherwise rotifers will be stressed and they stop swimming. Rotifer filtration rates may also vary with salinity and are reduced at high salinities. James and Abu Rezeq (1990) found that n-3 HUFA content in L-type *B. plicatilis* was highest for those cultured in 30 ppt sea water while 15-20 ppt water was correlated with higher n-3 HUFA content in S-type rotifers tested. Lorica lengths for both the strains were significantly greater at 5 ppt than at 30 ppt. Rapid shifts in salinity and temperature may result in immobilized, non-swimming rotifers. Only slight changes in mobility were observed when rotifers were exposed to changes in temperature from 20°C –30°C and to an increase in salinity from 20×10^{-3} to 30×10^{-3}. When salinity was reduced to 15×10^{-3} and 5×10^{-3} the proportion of mobile rotifers was considerably reduced (Oeie and Olsen, 1993).

Unionized ammonia: Hirata and Nagata (1982) showed that *B. plicatilis* raised on *N. oculata* excrete ammonia, urea and phosphates. Yu and Hirayama (1986) found that unionized ammonia levels can be one of the restrictive factors affecting increase of the rotifers in mass production. Hoff and Snell (1989) recommend that free ammonia concentration should not exceed 1 mg/litre.

Filtration of culture water: Debris that accumulates during high density culture of rotifers can be detrimental both to the health of the rotifers and to the larvae that feed on rotifers (Fushimi, 1989). Removing this debris can enhance water quality in the rotifer and larval rearing tanks and also reduces pathogenic bacteria clogging of nets during harvesting. Fushimi (1989) describes two general types of filtering equipment used in high density batch cultures. In one type a filter is inserted directly into tank – eg. filtering mats which are placed on bottom of the tanks and washed daily. The other consists of a separate filtering tank attached to the main culturing tank.

Monitoring: Parameters such as pH, D.O., temperature, salinity, food

density and ammonia concentration should be monitored and kept within pre-determined levels. Most culturists monitor their rotifers at least once a day. The usual method is to remove a fixed quantity of culture water and observe it under a microscope. The number of rotifers, their activity and the presence of any contaminants like protozoa are noted. Snell *et al.*, (1987) proposed two techniques for determining whether rotifers were under stress. The first is to test the swimming activity of a single rotifer in a 1 ml chamber. A grid with 1mm squares is placed under the chamber and the number of squares entered is recorded for 30 seconds. The second technique is to count the number of eggs carried by each female. Decreases in swimming activity and egg ratios indicated that the rotifers were being stressed. Korsted *et al.*(1995) used swimming speed and egg ratio as predictors of the status of rotifer cultures. Yufera *et al.*(1993) developed a mathematical model in order to obtain a reliable estimate of dry mass of *B. plicatilis* from two single easily determined parameters, the egg female ratio and the mean lorica length.

Harvesting: Harvesting is done by passing the culture water through fine nylon or silk netting. In Japan 80-100μm mesh size net is used to harvest L-type rotifer, 50-70μm mesh size for S-type rotifers. Mechanized means of harvesting are also being tested in Japan (Fushimi, 1989).

Storage: Storage is necessary for maintenance of stock cultures, short term preservation of live harvested rotifers and long term preservations of dead harvested rotifers. Stocks of rotifers could be stored at 3°C for a month without special attention which enabled the recovery of cultures when necessary (Ortega *et al.*, 1995). Alternatively, adult rotifers can be maintained at a low temperature which discourage rapid population growth (Coves *et al.*, 1990). The possibility of cryopreservation is also being studied (Lubzens, 1987); Lubzens *et al.*, 1989; Toledo and Kurokura, 1990; Lubzens *et al.*, 1992). The utility of frozen rotifers for feeding larvae is also investigated (Fontaine and Revera, 1980; Foscarini, 1988).

Enrichment of rotifers

After finding out the importance of n-3 HUFA in live feeds, various methods have been tried to improve the nutritional qualities of rotifers by feeding them with various kinds of microdiets, microencapsulated diets, a new type of baker's yeast and emulsified lipids rich in n-3 HUFA

together with fat soluble vitamins (Watanabe and Ali, 1985). Among them the direct method in which emulsified lipids are used and the indirect method using newly developed yeast are the most popular.

Indirect method : A new kind of yeast has been developed for rotifers to improve upon the nutritional value of rotifers cultured on baker's yeast (Imada *et al.*, 1979). This new type of yeast (n-yeast) was produced by adding fish oil or cuttlefish liver oil as a supplement to the culture medium of baker's yeast, resulting in a high content of lipid and n-3 HUFA. The incorporation of n-3 HUFA from n-yeast reached a maximum around 12 hour of feeding. Rotifers grown on n-yeast had a superior food value for larval fish (Kitajima *et al.*, 1980a, b).

Direct method : The rotifers can also be enriched by n-3 HUFA rich emulsions directly (Ostrowski and Divakaran, 1990). Here the lipids containing n-3 HUFA are homogenised with small amount of raw egg yolk and water and the resulting emulsion is fed directly to the rotifers (Watanabe and Ali, 1985). Rotifers took up lipids very easily and the concentration of n-3 HUFA reached the maximum between six and twelve hours of feeding. This is the easiest means of enriching rotifers, but it can cause clumping of the rotifers and degradation of the larval rearing tank water quality (Hoff and Snell, 1989). Rodriguez *et al.* (1996) investigated the improvement of nutritional value of rotifers by varying the type and concentration of oil and enrichment period. They obtained the highest lipid levels when triglycerols (TAG) were used. Increasing the enrichment period rather than the amount of oil present in the medium was found to be most efficient in increasing the n-3 HUFAs in rotifers. Nichols *et al.*(1996) studied the enrichment of *B. plicatilis* by feeding an Antarctic bacterium containing polyunsaturated fatty acids, The bacterial strain with the ability to produce EPA was shown to be a potential alternative enrichment food for *B. plicatilis*. A simple two step culture using *Chlorella vulgaris* and *Isochrysis galbana* for DHA enrichment was suggested by Takeyama *et al.*(1996).

Problems associated with rotifer culture

Prevention of a 'crash' or rapid production decrease is a vital aspect of rotifer culture. Culture crashes occur frequently in modern hatcheries due to water quality problems, accumulation of waste products and

unionized ammonia. Nutritional deficiencies such as lack of Vitamin B_{12}, other Vitamins or free amino acids (Fyhn, 1989) and toxins produced by bacteria are also attributed as probable causes of culture crashes. Fushimi (1989) reported that crashes are especially common in yeast – fed cultures. Fushimi (1989) suggested that declining water temperatures may sometimes be responsible for sudden population decreases in rotifers.

Feeding *B. plicatilis* to fish larvae

B. plicatilis was successfully employed for the mass raising of commercially important fishes like sole (*Solea solea*) (Howell, 1973), sea bass (*Dicentrarchus labrax*) (Barnabe, 1974), grey mullet (*Mugil cephalus*) (Nash *et al.*, 1974), red seabream (*Pagrus major*)(Fujita, 1979), milkfish (*Chanos chanos*)(Liao *et al.*, 1979; Juario *et al.*, 1984), turbot (*Scophthalmus maximus*) (Kuhlmann *et al.*, 1981; Olsen and Minck, 1983; Witt *et al.*, 1984) flounder (*Paralichthys olivaceus*) (Fukusho *et al.*, 1985) and gilthead seabream (*Sparus aurata*). Rotifers can be used as supplementary feed for penaeid mysids up to PL4 (Kongkeo, 1991). In China, *B. plicatilis* is employed for larval rearing of crabs namely *Charybdis japonica*, *Portunus trituberculatus* and *Scylla serrata* (Chen and Long, 1991).

Lubezens (1987) listed five requirements of *B. plicatilis* for optimization of growth and survival of fish larvae – (a) size (b) distribution and concentration of rotifers in the larval tanks (c) total amount available (d) digestibility and absorption and (e) nutritional quality. Since the size of the prey eaten is a function of larval mouth width, the *B. plicatilis* strain selected should be of the required size suitable for the mouth size of the target larvae. The number of rotifers required depends on the size of the rotifer strain and the duration it is supplied in the fish larval tanks. Generally rotifers are given to the larvae for seven to thirty days after exogenous feeding has begun. It is necessary to supply more rotifers than the fish will eat, the number will depend on the predatory ability of the larvae being cultured (Fukusho, 1989a, b). This is because the rotifers must be maintained at a density high enough to allow the fish to feed efficiently (Theilacker and Mc Master, 1971; Hoff and Snell, 1989). But high rotifer concentrations could cause the fish to ingest beyond the limit to assimilate (Lubzens *et al.* 1995). In turbot larvae (*Scopthalmus maximus*) it was found that the highest feeding rate was obtained with 10 rotifer ml^{-1} on 4th and 5th days (Olmedo *et al.*; 1995).

Disease control of microbial infections through live food in larviculture of fish and shellfish is also getting relevance today. Presently these infections are treated or prevented by dissolving high doses of broad spectrum antibiotics in the culture water. The major constraint of this method is the uncontrolled use of high quantities of expensive drugs and concern over their subsequent discharge into the environment and possible development of resistant bacteria (Brown, 1989). The oral delivery of the drug can be achieved through live feed. It has recently been demonstrated on a laboratory scale that live food enrichment technique through bioencapsulation in *Brachionus* and *Artemia* may be an excellent tool for the prophylactic and therapeutic treatment of larvae with drugs as well as vaccines (Verpract *et al.*, 1992).

The Indian scenario

The Indian scenario of rotifer research in general and of *B. plicatilis* and *B. rotundiformis* in particular is still in is its infancy. Almost the entire quantum of work done on rotifers in India is pertaining to taxonomy and ecology of freshwater rotifers, mainly from North India. Sharma (1991) gave a detailed resume of the present state of Indian work on rotifers. Sharma and Michael (1980) gave a synopsis of taxonomic studies on Indian rotifers. Our knowledge on freshwater rotifers from Kerala is mainly due to Nayar and Nair (1969) and Nair (1972). The information on brackishwater rotifers of Kerala are mainly due to the works on general brackishwater plankton ecology by Abdual Aziz (1978), Nair *et al.*, (1984), Nair *et al.*, (1985), Shibu (1991), Bijoy (1991), Harikrihsnan (1993), Bijoy and Abdul Aziz (1994), Anuradha (1996) and George (1996).

The only experimental studies done on rotifers in India are the investigations on the production of mictic and amictic females in *B. patulus* and the combined effect of food and temperature on life table parameters and population dynamics of *B. patulus* (Rao and Sarma, 1986; Sarma and Rao, 1991). The information on the culture of *B. plicatilis* from India is restricted to the reports by Muthu (1982), Santhanam and Velayudhan (1991) and Rafiuddin and Neelakantan (1990). Gopakumar (1998) made a detailed study on the brackishwater rotifers of Kerala with special reference of *B. plicatilis*. The ecological studies covered were the community structure and succession of rotifers in relation to environmental parameters, diel variation and distribution pattern of rotifers. The mor-

phometric characterization of five clones of *B. plicatilis* and size composition were reported. The experimental studies were on the reproductive potential of five strains of *B. plicatilis* under different salinities, feed types, feed concentrations and temperatures and on the life table parameters and patterns of growth and multiplication of two strains. Results of different culture methods and larval feeding experiments were also investigated in the study.

Discussion

It is quite evident that larviculture nutrition, particularly first feeding by the early larval stages is the major bottleneck for the industrial upscaling of aquaculture of fish and shellfish. Hence the feeding of larval fish still continues to be an active field of research of vital importance in all aquaculturally advanced nations. It is felt that paucity of information on the local strains of *B. plicatilis* and *B. rotundiformis* which are recognised as indispensable live feeds for marine larviculture is the major bottleneck in the progress of marine finfish hatchery production in India.

Strain variation of *B. plicatilis* and *B. rotundiformis* is one of the major parameters which require detailed investigations. The size as well as optimum conditions for best multiplication will vary in the different strains and it has a direct impact on the larval feeding. An extremely small strain (SS strain) has been isolated and its morphology and reproduction has been examined by aquaculturists.

The reproductive pattern of rotifers in relation to various external stimuli is an area of vital significance. If the factors inducing mictic reproduction are clearly identified, the mass production of resting eggs which can be stored in dry condition for ready use can be achieved. Experimental studies on reproductive potential under varying interacting parameters like salinity, temperature, feed type and feed concentration are crucial for the optimization of mass culture of different strains. The mass culture methods and conditions suited for mass production of different strains have to be evaluated. Investigations on nutritional quality and enrichment studies on cultured rotifers are of paramount significance for successful larviculture programmes. Finally the larval feeding strategies for the different species of fish and shellfish have to be standardized in terms of size of rotifers, concentration of rotifers in the

rearing tanks, nutritional quality and feeding protocols.

Improvements in increasing the quality, quantity and reliability of mass production of local strains of *B. plicatilis* and *B. rotundiformis*, understanding the causes of decline of populations, developing techniques for stable yield at low cost, nutritional improvement by enrichment procedures, manipulation of reproductive strategies, genetic improvement of the strains, cryopreservation and development of feeding protocols for the fish larvae of the different species will substantially enhance the hatchery production of seed of many commercially important fishes of India, having aquaculture potential.

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