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Review

Culture of the freshwater rotifer, *Brachionus calyciflorus*, and its application in fish larviculture technology

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The bottle neck of most inland freshwater aquaculturists is in obtaining adequate number of fingerlings, due to their high mortality at early life stages. Their successful production is hindered by many factors including adequate supply of food at early larval stages which require live food in good quality and quantity. This paper attempts to review the principles and procedures involved in the culture of the freshwater rotifer, *Brachionus calyciflorus* as starter food for most freshwater fish fry. There are several strains of different sizes of this rotifer, thus making them suitable for fry of a variety of sizes. This rotifer can be isolated, continuously produced by batch culture and 'feed back' culture systems. It can be fortified with diets containing highly unsaturated fatty acids (HUFA) for high survival and overall high growth and performance in several fish species including endangered and some problematic species. In spite of attempts to replace rotifer with more accessible formulated diets they will probably maintain their role as food organism for fish larvae of various species.

Key words: Rotifer, *Brachionus calyciflorus*, HUFA, larvae, food.

INTRODUCTION

Rotifers are valuable live food for the culture of the larvae of most fish species. Several characteristics of rotifers including their very small size, relatively slow motility have contributed to their usefulness as good prey for active larvae (Lubzens et al., 1989). In addition, they have the habit of staying suspended in the water column, high reproductive rate and high density cultures. They can tolerate temperatures of between 15 and 31°C. The optimal pH is 6-8 at 25°C (Ludwig, 1993). *Brachionus calyciflorus* is of course the commonly cultured freshwater rotifer for both freshwater fish species and shrimps (Figure 1). The body of this rotifer is covered by a cuticle, bilateral symmetrical and sexual dimorphism exist in the species. In addition, the body is composed of four regions; head with corona, neck, body, and foot. The foot is appendage that extends from the body ventrally possessing two toes. With the remarkable developments in larval rearing technology of freshwater fish species, demand for rotifer is further increasing. It is important to know the biology of the rotifers so as to be familiar with the manipulative strategies to stimulate their growth and multiplication. The corona, which is a ciliated organ on the head capture food, and a specialized pharynx (mastax) with hard jaws (trophy). The trunk contains a fluid filled stomach; and further down is a long tail like foot which is utilize for anchor. Also there are numerous cilia-like projections surrounding the head. The function of these cilia is to circulate water, food, and nutrients toward the mouth opening. When the cilia detect a meal (usually phytoplankton in the 3-12 µm range), the trunk contracts to pull the mouth opening towards the food, the corona then surrounds the food item and if the food items are the correct size, the particles are crushed and then passed into the stomach. This food response is repeated over and over with seconds, and this is how the energy demands of this creature are met (Fukusho, 1989).

A single rotifer can become thousands of rotifer in a few days. Its primary mode of reproduction is called parthenogenesis, which is a form of asexual reproduction. Usually when environmental conditions are suitable, female rotifers produce up to 7 eggs simultaneously, without any genetic input from a male rotifer. These eggs are genetically identical, and hatch to form new 'daughter' rotifers within 12 h. By 18 h post

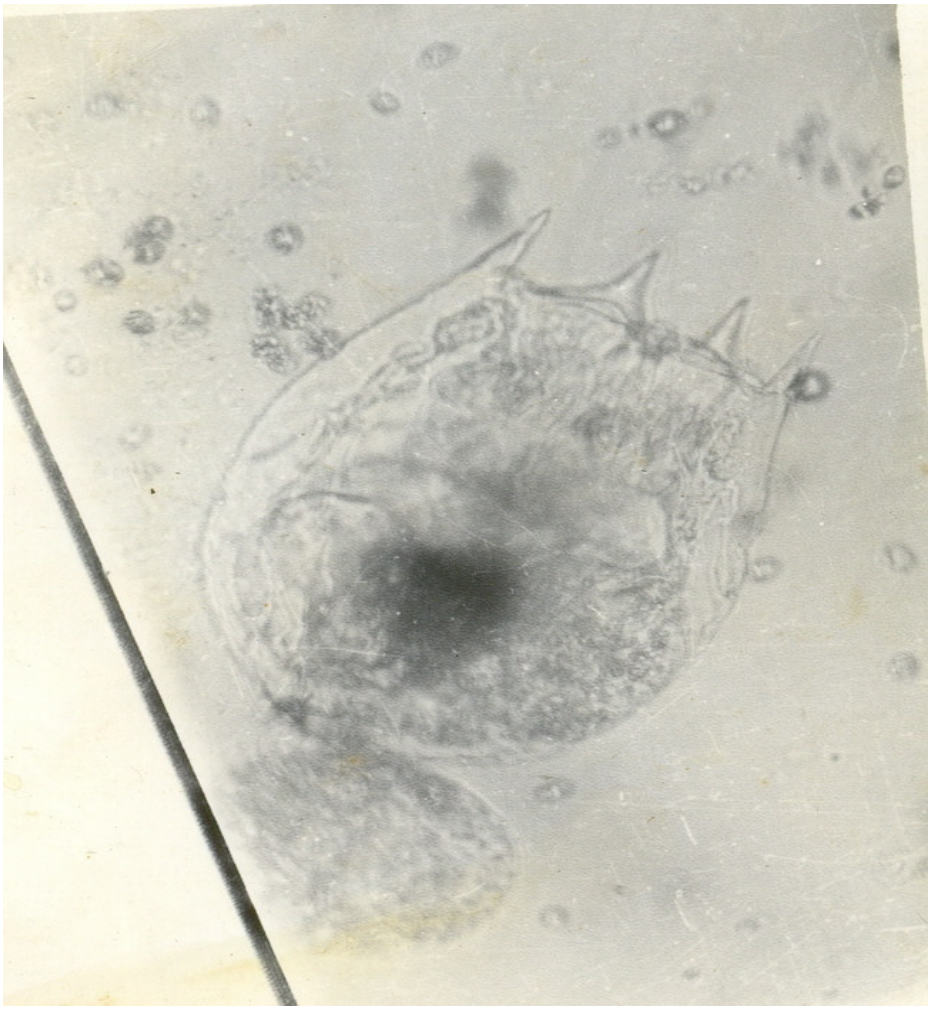

Figure 1. The rotifer, *Brachionus calyciflorus* with parthenogenesis eggs (photograph by Arimoro F.O.).

hatching, the daughter rotifers begin to reproduce themselves, and egg production is maintained for up to a week or more.

PREPARING STOCK CULTURE OF THE FRESHWATER ROTIFER *BRACHIONUS CALYCIFLORUS*

For starting and initiating the rotifer culture, mixed population of zooplankton comprising of rotifers, copepods and cladocerans are collected from the wild, research institutes, fishing holding tanks or commercial hatcheries. To collect rotifers one usually needs a net constructed of fine, soft, sheer fabric of plain weave. These nets work best in open waters but in areas where vegetation abounds masses of submerged plants can be removed and washed out in pans or buckets of clear pond water before using the net. Larger animals can be removed with coarse screens also. To achieve pure culture of the rotifer, *B. calyciflorus*, Arimoro and Ofojekwu (2004) recommended the use of 'Basudine' an organophosphoric acid ester applied at the rate of 1.5 mgl^{-1}. This concentration was arrived at through series of toxicity experiments to determine the safe concentration for the rotifer by Agbon et al., 2002. At this concentration, crustacean including copepods and cladocerans, aquatic insects including mosquito larvae will fail to survive thereby allowing the rotifers to multiply in the absence of predators. Several other chemicals have been found to be effective for the control of parasitic copepods and other crustacean pests (Moore et al., 1984; Burtle and Morrison 1987; Ludwig 1993). The include Trichlorfon, an organophosphate parasiticide that inhibits cholinesterase, is very toxic to cladocerans and slightly toxic to free-swimming copepods and their nauplii, but does not kill rotifers at dosages (0.25 mgl^{-1}) active ingredient. Opuszynski et al. (1984) were able to change copepod-dominated ponds to rotifer-dominated ponds by applying a dose of trichlorfon at 1 mgl^{-1}.

Fenthion, another organophosphate cholinesterase inhibitor is also toxic to crustaceans and effective in the control of parasitic copepod (Moore et al., 1984). Diflubenzuron (Dimilin), a chlorinated diphenyl compound (Thompson, 1989), inhibits chitin formation, preventing the development of arthropods beyond the larval stage. Rotifers and fish are not affected by diflubenzuron at concentrations that are lethal to cladocerans and copepods (Thompson, 1989).

The freshwater rotifer *B. calyciflorus* is isolated from other rotifers; usually it is the most abundant rotifer in most hatchery operations (Arimoro and Ofojekwu, 2004) and in inland Nigerian Rivers (Ovie, 1997). The innoculum water containing them is completely renewed with 7.5 mgl^{-1} Furazolidone (a disinfectant), 10 mgl^{-1} Oxytetracycline, 30 mgl^{-1} Sarafloxacin, or 30 mgl^{-1} Linco-spectin. Stock cultures should be kept in closed vials in an isolated room to prevent contamination with bacteria and ciliates. The cultures should be aerated and exposed to fluorescent light tubes and generally maintained on algal concentrate. Care should be taken not to over heat the cultures.

Culture vessels and water

There are quite a number of culture vessels for rotifer production. The volume of the vessel to be used depends largely on the magnitude of feeding to be carried out. Whatever size vessel that is used, a note of when it was started is important. The smaller the container the sooner it has to be renewed to avoid crashes due to the build up of ammonia. For large scale feeding programmes, concrete tanks are recommended normally measuring 5 m x 4 m x 1.5 m, with a capacity of 25,000 L at full capacity. On the other hand, for small scale feeding, several plastic tanks with a diameter of 16.6 cm and height 11.0 cm can be used (Arimoro and Ofojekwu, 2004). A circular tank of diameter 94 cm and height 120 cm with approximately 300 L is currently being used for the rotifer culture at the University of Jos (Arimoro and Ofojekwu, 2004; Fenqpi, 1996) recommended the construction of earthen rectangular ponds with suitable size of about $1,000^2$ M of the surface area and about 1 M of the water depth for use in large scale production of rotifers. Tanks should not be filled to the capacity so as to ensure adequate light penetration and ease of manual stirring if mechanical aerators are not available. It is advisable to use dechlorinated tap water for rotifer culture, rain water or screened pond water to avoid contamination.

Nutrient source

Algae, baker's yeast, ground shrimp meal, flour, rice bran, frozen algae, formulated diets are some of the food sources that have been exploited for the cultivation of rotifers (Hagiwara,1989; Fukusho,1989; Fulks and Main, 1991; Lubzens, 1995; Arimoro and Ofojekwu, 2004). For formulated diets, shrimp meal and rice bran, Fukusho (1989) advised that the food should be run through a 100 µm sieve to obtain a suspended feed usually the colour of coffee with cream. Research is currently exploring alternative and cheap food source for rotifers. Algae are plants that require large amount of plant fertilizers such as nitrates, phosphates, and iron for growth. Chicken droppings with small quantity of inorganic fertilizer (NPK) have been reported to produce favourable growth of algae for rotifer culture. To achieve unialgal culture repeated sub culturing technique can be applied or the use of chemicals will suffice. Freshwater *Chlorella*, *Scenesdesmus* are important useful algae for the culture of the freshwater rotifer and can be isolated as thus.

Stable tea' is sometimes used as a medium for culturing rotifers. It is prepared by boiling one-half pints of fresh horse manure in a quart of water for 1 h and then straining the mixture. Then two quarts of rain or spring water is added and the resulting mixture is left standing (uncovered) for two days. This can be inoculated with green water and will be ready for the introduction of rotifers in about a week or 10 days.

Culture methods

Batch culture: At the exponential growth phase of algae, they are inoculated with the freshwater rotifer, *B. calyciflorus*. Optimal temperature of culture is usually 20-30^{o}C with a pH of 8.0. Phytoplankton or any of the food substitute mentioned above are added to the container. Cultures can be started by adding a minimum of 10–20 rotifers/ml to minimize the possibility of a crash.

Continuous culture: In continuous rotifer culture, a larger container is used. Rotifers are added at the rate of 10–20 rotifers/ml to the container, and phytoplanktons are added to keep the culture a slightly green colour. The rotifers multiply and a portion of their population is removed daily to avoid pollution of the water body. There is also a continuous culture system by Hirata and Yamasaki (1983) with slight modification by Arimoro (2005 in press). This culture system ensures that sediments such as faeces and excess food are fermented in a bucket for 1-2 weeks. This is used for the cultivation of algae again. The algae produced from these 'subsistence nutrients' are fed back to the rotifers in the form of 'recycled diets'. In this way, continuous, steady cultures of algae and rotifers are maintained.

Mass production of the freshwater rotifer, *B. calyciflorus* is possible by the use of the appropriate alga band supplemented with baker's yeast. The amount of baker's yeast fed on a daily basis is about 1g $million^{-1}$ of rotifers. Although baker's yeast has a small particle size

(5–7 µm) and a high protein content and acceptable as diet for *Brachionus*. It is not advisable to be used alone for rotifer culture. Hirayama et al. (1989) and Lubzens et al. (1989) opined that, rotifers raised on yeast alone lack the essential fatty acids and vitamins to sustain the larval requirements of the predator organisms. Furthermore, the first trials to replace the complete natural rotifer diet by baker's yeast were characterized by varying success and the occurrence of sudden collapses of cultures (Hirayama et al., 1989). Most probably the reason for these crashes was explained by the poor digestibility of the yeast, which requires the presence of bacteria for digestion.

MAINTENANCE AND MANAGEMENT OF ROTIFER CULTURE

It is important that the alga tanks/ponds should be renewed weekly. Fermented bean cake and chicken manure should be added when the bloom begins to diminish. Also to maintain a culture from a heavy use to another, it is necessary to cut back the amount of feed use, and to keep an eye on the population. If the population rises, then it will be advisable to harvest a few. To get bloom, an entirely new culture can be started with a reasonable amount of feed/algae and subsequently harvested when it becomes concentrated with rotifers. It is important to note that feed rates should be based on the actual density of rotifers in the system and care should be taken not to overfeed. In the batch feeding, the culture tank should be clear of algae before the next feeding to avoid excess algae accumulation. Any alga or food that is not consumed within 48 h will degrade, increasing the level of ammonia and this reduces the dissolved oxygen level in the water.

Harvesting of Rotifers

When rotifers reach their peak in the plastic vessels, ponds or small-scale culture; it is advisable to harvest them to avoid sudden crash. A hand net of mesh size (50 µm) is recommended for this exercise. For small scale cultures, the entire culture volume is filtered through the net and the rotifers collected in the plankton net bucket, is emptied into a plastic or any suitable container for onward transfer to the fry holding tanks. For large scale cultures in earthen or concrete ponds, the net is towed from one end of the pond to the other to concentrate the rotifers. Each tow is emptied into a bowl. Several tows are made in this way to obtain a considerable quantity of rotifers for fry culture. Harvest by the use of a rotating drum filter may be an efficient method for the concentration of rotifers, since the drum filters can be used at any time, will efficiently concentrate particulate matter, is self cleaning, keeps the rotifers wet, and can be set up in a portable or stationery configuration (Ludwig and Lochmann, 2000).

APPLICATION IN FISH LARVICULTURE TECHNOLOGY

There are considerable reports on feeding successes using the rotifer, *Brachionus* as live food for several marine fish species (Villegas et al., 1990; Reitan et al., 1993; Ottera 1993, Craig et al., 1994; Castell et al., 2003). Also high growth rate and survival of some freshwater fish species have been reported by several researchers. Among the documented information are the works of Lim and Wong (1997). The rotifer *B. calyciflorus* used in their study were produced by batch culture using *Chlorella* species as feed. They reported an overall survival rate of larvae fed on the rotifers in door tanks ranging between 65.1 and 74.5% in freshwater ornamental fish larviculture. They also reported that the use of rotifers would enable freshwater fish larviculturist to improve larval performance and increase yield. Similarly, Awaiss and Kestemont (1998) observed that the best growth, survival and biochemical composition were evident in *Clarias gariepinus* fry fed the freshwater rotifer, *B. calyciflorus* during the first week of larval feeding. Arimoro and Ofojekwu (2003/2004) obtained over 80% survival in the toothed carp, *Aphyosemion gardneri* larvae raised on the freshwater rotifer, *B. calyciflorus* from the time they were 5 days old through to 32 days of age.

Shiri et al. (2003) successfully cultured the Burbot (*Lota lota*) larvae, an endangered freshwater fish species in the Western Europe with the freshwater rotifer, *B. calyciflorus*. They obtained a survival rate of 69.2% in the fry fed rotifer in green water condition containing algae. They also advocated that rotifers should be maintained in green water condition as this will help to ensure that they remain nutritious and relevant to the fry. Furthermore, Arimoro (2005 in press) reported high specific growth rate and survival in the African catfish *Clarias anguillaris* larvae fed this rotifer.

Enriched rotifers fed to fry enhance their growth and survival rates (Craig et al., 1994; Rimmer et al., 1994; Cho et al., 2001; Castell et al., 2003; Mercier et al., 2004). According to Watanabe et al. (1983), rotifer can be enriched using 7 ml and 15 ml of cod liver oil per 40l and 100l respectively, for 12 h every night with oxygen levels kept at 4- 7 mgl^{-1} all through the period. On the other hand, Lubzens et al. (1989) maintained that in order to ensure adequate amounts of essential lipids, rotifers must be enriched with either an appropriate alga or emulsified fresh oil. Furthermore, Cruz et al. (1999) reported best larval growth and survival by enriching rotifers with powersh-fish oil, containing a high level of Highly Unsaturated Fatty Acid (HUFA) 22; 6_{n-3} in the red porgy (*Pagrus pagrus*) larvae. According to Aragao et al.

2004), feeding rotifers in enriched algae will increase the free Amino Acid (FAA) content of the rotifers. They enriched the rotifers for 24 hrs in a microalga medium. Enrichment of rotifer with HUFA before using them as live food appears to increase growth and survival of a variety of fish larvae (Lubzens et al., 2001).

The establishment of hatcheries and inducement of spawning by hormone injection greatly hold promises for enhanced propagation of most fish species whose larvae can be maintained by feeding with rotifers. Techniques that will be developed for all year round availability of most freshwater species must co-opt the technology of feeding with the freshwater rotifer at least for the first few days of larval culture to enhance its growth and chances of survival. Most fish larvae are small in size and are very slow swimmers and so require rotifers, which are also slow swimmers for food.

The optimum feeding rates for most fish larvae on rotifers have been determined. Ludwig (1994) reported that $20ml^{-1}$ of rotifers added to the tank culture of the sunshine bass, *Morone* spp was ideal for growth and survival of the larvae. He obtained a 48% survival rate by 27 days of culture. Estimates suggest that one red sea bream larva requires 12,000-15,000 rotifers over 25 days until it reaches 10 mm in length (Kafuku and Ikenoue, 1983).

CONCLUSION

The freshwater rotifer, *B. calyciflorus* is an ideal live food for the first few days culture of most fish larvae because of its numerous characteristics; small size, slow morbidity and easy catchability by the larvae. It is also imported to enrich this rotifer for all round best performance in the larva. The culture of the freshwater rotifer, *B. calyciflorus* can be maintained continuously in a 'feed back' culture system. Many articles have been written on the usefulness of rotifers for the raising of fish fry and how one can expect to raise a high percentage of the spawned fishes and end up with quality specimens from feeding rotifers. Rotifers are also used as a conditioning food to induce adult fish to spawn.

Freshwater larviculturist will avail themselves with the findings reported in this review in order to improve larval performance, increase yield and facilitate breeding of new fish species. This will ensure an overall satisfactory performance in hatchery operations.

REFERENCES

Agbon AO, Ofojekwu PC, Ezenwaka IC, Alebeleye WO (2002). Acute toxicity of diazinon on rotifers, Cyclops, mosquito larvae and fish. J. Appl. Sci. Environ.Managt. 6(1): 18-21.

Aragao C, Conceicao LEC, Dinis MT, Fyhn HJ (2004). Amino Acid pools of rotifers, and *Artemia* under different conditions. Nutritional Implication for fish larvae Aquaculture 234: 429 – 445.

Arimoro FO, Ofojekwu PC (2003/2004). Incidence of feeding, growth, and survival of the toothed carp, *Aphyosemion gairdneri* larvae reared on the freshwater rotifer*, B. calyciflorus*. Trop.Freshwater Biol. (12,13): 35-43.

Arimoro FO, Ofojekwu PC (2004). Some aspects of the culture, population dynamics and reproductive rates of the freshwater rotifer, *B. calyciflorus* fed selected diets. J. Aquatic Sci. 19(2): 95-98.

Awaiss, Kestemont (1998). Feeding sequences (rotifer and dry chemical composition of African catfish, (*Clarias gariepinus*) Burchell (Pisces: Clariidae) Larvae. Aquaculture Res. 29(10): 731.

Burtle G, Morrison J (1987). Dimilin for the control of *Lernaea* in golden shiner ponds.*Proc.* Arkansans Acad. Sci. 41: 17-19.

Castell J, Blair T, Neil S, Howes K, Mercer S, Reid Young-Lai J, Gullison B, Dhert P, Sorgeloos P (2003). The effect of different HUFA enrichment emulsions on the nutritional value of rotifers (*B. plicatilis*) fed to larvae haddock (Melanogrammus aeglefinus) Aquacult. Int.11(1–2): 109 – 117.

Craig SR, Connie R, Holt JG (1994). The effects of enriching live foods with unsaturated fatty acids on the growth and fatty acids on the growth and fatty acid composition of larval red drum *Sciannops ocellatus*. J. World Aquacult.Soc. 25(3): 424-434.

Cho SH, Hur SB, Jo JY (2001). Effect of enriched live feeds on the survival and growth rates in the larval Korean rockfish, *Sebates schlegeli* Hilgendorf. Aquacult. Res. 32(3): 199.

Cruz- Hernandez C.M, Salhi M., Bessonart M, Izquiero M.S, Gonzalez M.M, Fernandez-Palacious H (1999). Rearing techniques for the red porgy (*Pagrus pagrus)* during larval development. Aquaculture 179(1-4): 489-497.

Fengqi L (1996). Production and application of rotifers in aquaculture. Aquacult. Mag. 22: 16 – 22.

Fukusho K (1989). Biology and mass production of rotifer, *Brachionus plicatilis*. Int. J. Int. J. Aqric. Fish Technol. 1:232-240.

Fulks W, Main KL (1991). Rotifer and Microalgae culture systems. The oceanic inst., Honolulu, USA. p. 364.

Hagiwara A (1989). Recent studies on the rotifer, *Brachionus plicatilis* as a live food for the larval rearing of marine fish. La Mer 27: 116-121.

Hirata H, Yamasaki S (1983). Steady state zooplankton community in a feed back culture system. Microcosms Ecol. Rec. Doe. Symp. Ser 52: 402-407.

Hirata H, Yamasaki S, Kanaguchi T, Ogawa M (1983). Contionuous culture of the rotifer, *Brachionus plicatilis*fed recycled algal diets. Hydrobiol. 104: 71-75.

Hirayama K, Mariyama I, Maeda T (1989). Nutritional effect of freshwater *chlorella* on the growth of the rotifer, *Brachionus plicatilis*. Hydrobiologia 186/187: 39-42.

Kafuku T, Ikenoue H (1983). Modern methods of aquaculture in Japan, Developments in Aquaculture and Fisheries sciences. 11 Kodansha Ltd, Tokyo and Elsevier, Amsterdam. p. 216.

Lim LC, Wong CC (1997). Use of the rotifer, *Brachionus calyciflorus* Pallas, in freshwater ornamental fish larviculture Hydrobiologia 358 (1/3): 273 (5).

Lubzens E, Tandler A, Minloff G (1989). Rotifers as food in Aquaculture. Hydrobiologia 186/187, 387– 400.

Lubzens E, Gibson O, Zmora O, Sukenik A (1995): Potential advantages of frozen algae (Nannochloropsis sp.) for rotifer (*Brachionus plicatilis*) Culture. Aquaculture 133: 295–309.

Lubzens E, Zmora O, Barr Y (2001). Biotechnology and aquaculture of rotifers. *Hydrobiologia* 446/447: 37–353.

Ludwig GM (1993). Effects of Trichlorfon, Fenthion, and Diflubenzuron on the zooplankton community and on the production of the reciprocal-cross hybrid stripped bass fry in culture ponds. Aquaculture 110: 301-319.

Ludwig GM (1994). Tank culture of sunshine bass *Morone chrysops X M. saxatilis* fry with freshwater rotifer *B. calyciflorus* and salmon starter meal as first food sources J . world Aquacult. Soc. 25(2): 337-345.

Ludwig GM, Lochmann S (2000). Culture of the sunshine bass, *Morone chrysops X M. saxatilis* fry in tanks with zooplankton cropped from ponds with a drum filter. J. Appl. Aquacult. 10(2): 11-26.

Mercier L, Audet C, Joel de la N, Parent B, Parish CC, Ross NW (2004). First feeding of winter flounder (*Pseudopleronectus americanus*) larvae: use of *B. plicatilis* acclimated at low temperature as

live prey Aquaculture 229(229 (1-4): 273-278.
Moore BR, Mitchell AJ, Griffin BR, Hooffman GL (1984). Parasites and diseases of pond fishes In; Dupree HK, Huner JV (Editors), 3rd report to the fish farmers. U.S Fish and Wild Life Service, Washington, D.C pp. 177-205.
Opuszynski K, Shireman JV, Aldridge FJ , Rottmann RW (1984). Environmental manipulation to stimulate rotifers in fish rearing ponds. Aquaculture, 42: 343- 348.
Ovie SI (1997). *Brachionus* species of some inland waters in Nigeria with a note on a new record and zoogeography.Trop. Freshwater Biol. 6: 27-39.
Ottera H (1993). Feeding, growth and survival of Atlantic cod (*Gadus morhua*) Larval reared in replicate plastic enclosure. Can J. Fish Aquat. Sci. 50(5): 913 – 924.
Reitan L, Kjell T, Jose R, Gunvor O, Olsen Y (1993). Nutritional effects of algal addition in the first feeding of turbot *Scophthalmus maximus* larvae. Aquaculture 188(3): 257-275.
Rimmer MA, Reed AW, Levith MS, Lisle AT (1994). Effect of Nutritional enhancement of live food organisms on growth and survival of Barramundi *(Lates calcarifer*) Larvae. Aquaculture 14: 247 – 260.
Shiri HA, Charlery D, Auwerx J, Vught J, Slycken J, Dhert P, Sorgeloos P (2003). Larval rearing of burbot (*Loto lota)* using *Brachionus calyciflorus* rotifer as starter food. J. Appl. Ichthyol. 19(2): 84-87.
Thompson WT (1989). Agricultural Chemicals, Book 1, Insecticides, Acaricades and Ovicides. Thomson Publications,, Fresno, CA.
Villegas CT, Millamena O, Escritor F (1990): Food value of *B plicatilis* fed three selected algal species as live food for milk fish *Chanos chanos* Forsskal fry production. Aquacult. Fish. Mgt. 21: 213 – 219.
Watanabe T, Kitajina C, Fujita S (1983). Nutritional value of live organisms used in Japan for the mass production of fish (a review). Aquaculture 34: 115-143.