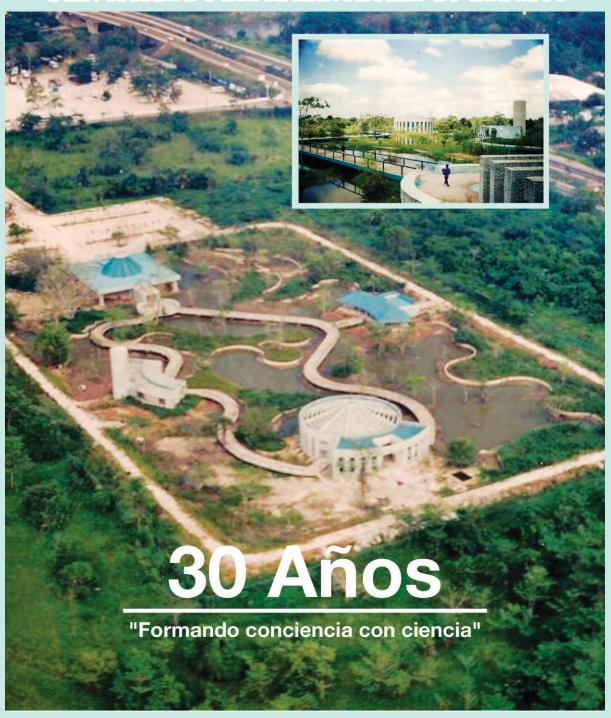




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# **D**REVISTA DE **LOS PREVISTA DE LOS PORTOS DE**

### División Académica de Ciencias Biológicas Universidad Juárez Autónoma de Tabasco

Kuxulkab´ Voz chontal - tierra viva, naturaleza

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#### Nuestra Portada

Retrospéctivo del Centro de Investigación para la Conservación de Especies Amenazadas (CICEA-DACBiol)

Diseño de:

Lilianna López Gama

Fotografías:

Francisco Maldonado Mares Profesor-Investigador de la DACBiol



#### **Estimados lectores:**

sumir el compromiso de la edición de una revista, es realmente un reto que exige una búsqueda de mejora continua, es una responsabilidad que requiere un equipo de apoyo. Nuestra revista ha pasado por diferentes etapas de evolución, gracias al interés y la colaboración de muchos de nuestros profesores desde su inicio. Este año, bajo la dirección de la Maestra Rosa Martha Patrón López y con su decidido apoyo, se han redoblado los esfuerzos para evaluar el sistema de manejo de la revista, hacerlo más eficiente y congruente con las necesidades y facilidades actuales.

Nuestra Universidad inició también un plan de rescate y refuerzos a las revistas universitarias, promoviendo diferentes apoyos y capacitaciones a través de una serie de autoevaluaciones. Hoy la División Académica de Ciencias Biológicas es pionera en la Universidad por contar con un Área Editorial, la cual dará apoyo a todas aquellas actividades de la División que lo requieran; ésta se encuentra a cargo del Biól. Fernando Rodríguez Quevedo. El Biólogo además de ser el editor de apoyo de la revista, con una comprometida diligencia, ha implementado un programa de reorganización del sistema de manejo de Kuxulkab', que dentro de poco, nos permitirá en tiempo real dar respuesta y visualización a todo el proceso editorial, esto como parte de la estrategia del plan de mejoras de nuestra revista. Además en este año que se festeja el 30 aniversario de la enseñanza de las ciencias ambientales en la UJAT, varios eventos se están llevando a cabo y nosotros queremos unirnos a los festejos buscando una nueva cara para Kuxulkab', como la revista que representa nuestra División Académica; como parte de estos nuevos cambios, destaca mencionar que a partir de éste número el volumen de nuestra revista pasa a ser renombrada cada inicio de año y no a mediados como se venía realizando, como una de las recomendaciones que nos señalaron para facilitar su identidad.

Este número cuenta con una interesante recopilación de doce artículos, todos ellos seleccionados de las diferentes áreas en las que trabajan profesores, investigadores y estudiantes de Tabasco, siendo la UJAT muestra de la diversidad y el desarrollo de investigaciones con el paso del tiempo. Como siempre agradecemos tanto a nuestros contribuidores como a los revisores que amablemente se han tomado el tiempo de colaborar con nosotros, y los invitamos a seguir considerando usar esta opción de publicación como una ventana para compartir investigación, así como desarrollo de temas de interés, tanto a nuestros colegas, alumnos y compañeros en la División como en la región.

Lilia Gama Editor en Jefe Rosa Martha Padrón López
Directora

División Académica de Ciencias Biológicas Universidad Juárez Autónoma de Tabasco

### Freshwater rotifer: (part I) importance, larvi food, and culture

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#### **Abstract**

n finfish larval rearing often depends on the production of phytoplankton as well as zooplankton. Phytoplankton serve as feed for the zooplankton which, in turn, nourish the fish larvae. Different size and types of zooplankton may be raised for different larval stages. Feed production at all stages must be consistent in quality and quantity for the duration of the hatchery cycle if larval rearing is to be successful. We investigated native freshwater rorifer Brachionus angularis, B. quadridentatus brevispinus, and. B. durgae where the last one is reported as a rare an uncommon species in the world which have been collected in Tabasco. The report of freshwater rotifer from Tabasco is divided in two parts, part 1 describes freshwater rotifer from Tabasco, Mexico, as live food, and how to culture them. The second part reports on two species which have been succesfully cultivated in laboratory.

#### Introduction

# 1. Importance phytoplankton as live food in aquatic communities

The initial source of food for many larval organisms is phytoplankton. This is probably associated with the size of the larvae at hatching. After a certain period of time the larvae of most species can be fed exclusively on zooplankton or a combination of plant and animal matter i.e. plankton. The term 'plankton' can be defined chiefly as microscopic drifting or floating organisms in the sea and fresh waters and may be having feasible floating devices. The plant components of all the plankton are the phytoplankton and are the primary producers for the

entire aquatic body, whereas the animal components of the plankton are the zooplankton and are the primary consumers (Bold and Wynne, 1985).

Microalgae are unicellular microscopic algae called phytoplankton ('phyto'= plant; 'planktos'= made to wander). These small plants range in size form 1/1000 of a mm to 2 mm floating in the upper 200 m of the ocean where sunlight is available for photosynthesis. Phytoplankton species range from primitive blue-green algae (cyanobacteria) to diatoms, dinoflagellates and green flagellates". They are a rich source of micronutrients, protein, carbohydrates, and a special specific fatty acid. especially specific essential fatty acid. They also provide essential pigments such as astaxanthin, zeaxanthin, chlorophyl, and phycocyanin, which enhance coloration and health in fish and invertebrates, they are also extremely important for primary production within intertidal habitats and constitute a major food source for higher trophic levels, and are capable of rapid population growth and play a major role in nutrient recycling. In addition, they help balance the pH of aquatic systems by removing excess carbon dioxide and adding oxygen, and most importantly they have been cultured to produce oils, chemicals, pharmaceuticals, and polysaccharides, as well as for eutrophication control Round (1981), Bold and Wynne (1985) and Hoff and Snell (1999).

Finfish larval rearing, often depends on the production of phytoplankton as well as zooplankton. Phytoplankton serve as feed for the zooplankton which, in turn, nourish the fish larvae. Different size and types of zooplankton may be raised for diffeent larval stages. Feed production at all stages must be consistent in quality and quantity for the duration of

the hatchery cycle if larval rearing is to be successful (Sato, 1993).

# 2. Importance zooplankton as live food in aquatic communities

Zooplankton is an important component of aquatic communities linking phytoplankton to icthyoplankton (Lampert and Sommer, 1997). Freshwater systems zooplankton has been recognized as an important energy resource for fish of small body size that, in turn, provide energy to piscivorous fish consumers higher up the food web, and are also used to evaluate the role of the biotic and abiotic factors at the population level because of their short life span, phenotypic plasticity, and easy their easy to culture in small volume (Hoff and Snell, 1999; Lampert and Sommer, 1997; and Bold and Wyne, 2000; Medeiros and Arthington, 2008).

To feed the delicate larvae, juveniles and even adults fish, prawn and shrimp in nurseries and growout ponds protein-rich and naturally occurring zooplanktonic organisms are essential. Different types of zooplanktons like larval forms of brine shrimp (Artemia salina), rotifers (Brachionus sp.), cladocerans (Moina sp.) Euchlanis sp., Daphnia sp., Ceriodaphnia sp.; copepods (calanoid, cycloid and harpacticoid copepods) and larval forms of different aquatic organisms etc., are considered as natural food for the prawn/shrimp/fish. Rotifers rapidly occupy all available niches in ponds and lakes and thus help in transferring energy with high efficiency from primary producers (alga and bacteria) to secondary consumers (such as larval insects and fish) (Wallace et al., 2006).

In general, fry must have zooplankton to survive, or at least to be healthy and grow rapidly. Most fry are not particular about the types of zooplankton they eat, but the organisms must be small enough to fit into their mouths. To maximize survival, stock any fry just as populations of zooplankton small enough for the fry to eat are rapidly increasing and before invading predators become numerous.

Most fish fry eat three main types of zooplankton-rotifers (the smallest of the main group), copepods and cladocerans. For the tiniest fish fry, such as the newly hatched fry of sunshine bass or white bass, small rotifers may be the only zooplankton small enough to eat. For larger fry, the

smallest rotifers may not provide enough nutrients to make chasing and ingesting them worth the effort. Copepod nauplii, which are just-hatched copepods, are important first foods for larval fish, too. Protozoans may also be eaten, but little is known about their contribution to fry diets (Ludwig 1999).

#### 3. Importance of rotifer as live food

There are over 1000 rotifer species of rotifers, 90 % which inhabit in freshwater (Hoff and Snell, 1999). Rotifers and cladocers are important components of most freshwater aquatic communities where rotifer is the most dominant zooplankton in all the freshwater aquatic ecosystems and considered as ideal food for fish larvae and they are valuable live food for the culture of larvae of most fish species (MacIsaac and Gilbert, 1991a).

Several characteristics of rotifers including their very small size, and relatively slow smimmig velocity make them a suitable prey for fish larvae that have just resorbed their yolk sac but cannot yet ingest the larger food (Lubzens *et al.*, 1989). However, the greatest potential for rotifer culture resides in the possibility of rearing these animals at very high density where densities of 2000 individuals/ml-1 have been reported. Even at high densities, animals reproduce rapidly and can thus contribute to the build up of large quantities of live food in a very short preiod of time (Hirata, 1979). Furthermore, rotifers can be easily enriched with fatty acids, antibiotics, etc. and used to transfer these substances to 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 temperature of between 15 and 31 °C. The optimal pH 6-8 at 25 °C (Ludwig, 1993). Earlier reported (Fujita, 1979) indicated that importance of long chain -3 poluynsaturated fatty acid in rotifers as food, and later Gatesoupe (1982) stated that a rotifer can be seen as a living food capsule, which transmite adequate supplies of macro-and micronutrientss, vitamins, or even antibiotics to the fish larvae. Last, but no least, the filter-feeding nature of rotifers facilitates the inclusion into their body tissues of specific nutrients essential for the larval predators.

Freshwater rotifers *Brachionus angularis, B.* patulus, B. calyciflorus, B. rubens, B. quadridentatus are used in freshwater aquaculture and laboratory

experiments. A single rotifer can become thousands of rotifers in a few days. Its primary mode of reproduction is called parthenogenesis, which is a form of asexual reproduction. Usually when environment conditions are suitable, females rotifers produce up to 7 eggs simultneously, without any genetic input from a male rotifer. These eggs are genetically identical, and hatch to form new "daughter" rotifer within 12 h. By 18 h post hatching, the daughter rotifers begin to reproduce themsleves, and egg production is maintained for up to a week or more (Schluter and Groeneweg, 1981; Walz, 1983; Lubzens, 1987; Dahril, 1997; Arimoro, 2006; Sarma et al., 2009, Alva-Martinez et al., 2009, Alanis et al., 2009, Park et al., 2001, Kabir et al., 2010; Ajah, 2010).

In finfish aquaculture, rotifers are offered to finfish larvae for seven to 30 days after exogenous feeding has begun (Lubzens *et al.*, 1989). Anywhere, from 40,000 to 173,000 rotifers are needed to feed one fish larvae from hatching until it can be feed on another type of food,(Okauchi *et al.*, 1980; Kafuku and Ikenoue, 1983), although the exact number depends on the species of fish culture and *et al.*, so on the size of the rotifers.

#### 4. Freshwater native rotifer culture

#### 4.1 Culture vessels and water

They are quite a number of culture vessels for rotifer production. The volume of the vesel 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 amonia. For large scale feeding programmes, concrete tanks are recommended normally measuring 5 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 in height can be used for rotifer (Arimoro, 2006). Tank should not be filled to the capacity so as it ensure adequate light penentration and ease of manual stirring if mechanical aeration are not available. It is advisable to use dechlorinated tap water for rotifer culture, rain water or screened pond water to avoid contamination.

#### 4.2 Nutrient source

Research is currently exploring alternative and cheap food source for rotifers. Algae are plants that require large amount of plant 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 Scenedesmus obliquus, S. quadricauda, S. pectinatus, Chlorella vulgaris, Chorella sp, Tetraselmis suecica, Eudorina elegans (Dahril, 1997; Kennari et al., 2008; Park et al., 2001; Indy et al, 2008; Lürling, 2006; Ajah, 2010; Arimoro, 2006) are important useful algae for the culture of freshwater rotifers 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 (Arimoro 2006).

#### 4.3 Isolation methods

For starting and initiating a rotifer culture, mixed population of zooplankton comprising of rotifers, copepods and cladocers are collected from wild. To collect rotifers one usually needs a net constructed of fine, soft, and sheer fabric of plain weave. These nets work best in open waters but in areas where the vegetation abounds masses of submerged plants can be removed and washed out in pans or buckets or clear pond water before using the net. The size of plankton net can be varied from 35-65 m. Larger animals can be removed with coarse screen also.

To achieve pure culture of rotifer, Arimoro and Ofojekwu (2004) recommended to use "Basudine" an organopshosporic acid ester applied at the rate of 1.5 mgl-1. This concentration was achieved through series of toxicity experiments to determine the safe concentration for the rotifer (Angbon *et al.*, 2002; Arimoro, 2006; Kabir *et al.*, 2010). At this concentration, custacean including mosquito larval fail to survive thereby alowing 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; Kabir, *et al.*, 2006; Arimoro, 2006; Opuszynski *et al.*, 1984; Thomson, 1989; Ajah, 1995).

Stock cultures should be kept in closed vials in an isolated room to prevent contamination with bacteria and ciliates. The culture should be aerated and exposed to fluorescent lights and generally maintained on algal concentrate. Care should be taken not to over-heat the cultures.

#### 4.4 Culture methods

Starter culture: The stock culture was up scaled to starter culture. The rotifers were carried out in static system consisting of erlenmeyers of 500 ml placed 2 cm from fluorescent light tubes (5000 Lux). The temperature readings in the erlenmeyers were 26 -28 °C. The rotifers were stocked at a density of 10 individuals ml-1 and fed 400 ml freshly-harvested algae, approximately 50 ml of algae being added everyday to supply enough food. After 2-3 days they were rinsed on a submerged filter. The concentrated rotifers were then distributed in several 15 L bottle filled with 2 L water at a density of 10-20 individuals. ml-1 and a wild tube aeration provided. Freshwater algae were supplied daily. Everyday the cultures were cleaned (double-screen filtration) and restocked at densities of 200 rotifers ml-1. After one week the 15 L bottles were completely filled and the culture was ready to be used for inoculation of mass culture (Indy et al., 2008; Arimoro, 2006).

**Batch culture:** Optimal temperature of culture is usually 20-30 C with a pH of 8.0. Phytoplankton or any of the food substitute metioned above are added to the container. Cultures can be started by adding a minimum of 10-20 rotifer/ml to minimize the possibility to crash (Hoff and Snell, 1999; Arimoro, 2006; Indy *et al.*, 2008).

**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 the 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 (Arimoro, 2006).

Mass production of freshwater rotifer, is possible by the use of the appropriate alga band supplemented with baker yeast. The amount of baker yeast fed on daily basis is about 1 g million-1 of

rotifers. Although beaker yeast has a small particle size (5-7 m) 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.* (1998) opined that, rotifer raised on yeast alone lack the essential fatty acids and vitamin to sustain the larval requirements of the predator organisms. Furthermore, the first trials to replace the complete natural rotifer diet by baker 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 requieres the presence of bacteria for digestion.

#### Harvesting of Rotifers

Harvest by the use of a rotating drum filter may be an efficient method for the concentration of the 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). When rotifers reach their peak in the plastic vessels, ponds, or small-scale culture; its is advisable to harvest them to avoid sudden crash. A hand net of mesh size (50 m) 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 (Arimoro, 2006).

# 4.5 Maintenance and management of rotifers culture

It is very important that the alga tanks/ponds should be renewed weekly. 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. 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 bacth 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 amonia and reducing the dissolved oxygen level in the water.

#### 4.6 Native freshwater rotifers from Tabasco

We investigated several native freshwater rotifers from Tabasco fed marine algae Nannochloropsis oculata adapted from marine N. oculata in different low salt concentration and identified as Brachionus angularis (Fig.1), B. quadridentatus brevispinus (Fig. 2), and other rotifer B. calyciflorus (Fig. 3) and B. durgae (Fig. 4) fed with freshwater algae Tetraselmis suecica and Chlorella vulgaris in laboratory and have been reported elsewhwere (Indy et al., 2008, 2009) and as a preliminary work on investigations of native freshwater rotifers from Tabasco. B. calvciflorus, B. angularis, and B. guadridentatus brevispinus are the most common rotifers suitable found in large numbers in ponds and in wild populations in Tabasco, however, B. durgae is rare and uncommon species according to Segers (pers. Comm.). None the less were found were found in specific occasions during November-December or sometimes during floods. However, there are many more rotifers species needed to be studied.

#### 5. Conclussion

Larviculture, more particularly the start feeding of early larval stages, appears to be te major bottleneck for the industrial upscaling of the culture of fish and shelfish. Evolutionay, larvae of most fish and crustaceans are fixed on the scheme of motile prey organisms and encounter problems to accept inert/dry diets. Even if they accept the diets, their poor enzymatics activity and not functional stomach will not allow them to digest the existing formulated diets (Pederson et al., 1987; Pederson and Hjelmeland, 1988). Improving the acceptance of dry diets for fish larvae and formulate digestible and less polluting diets remains thus still a central task for freshwater aquaculturists. Before this is achieved, live food (phyto - and zooplankton) will remain an important food source for the start of early larval stages.



Figure. 1. Brachionus angularis



Figure 2. Brachionus quadridentatus brevispinus

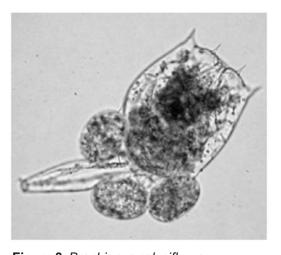


Figure 3. Brachionus calyciflorus

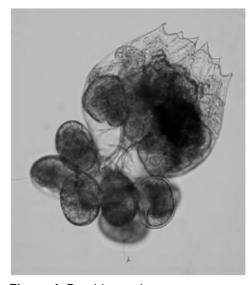


Figure 4. Brachionus durgae

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