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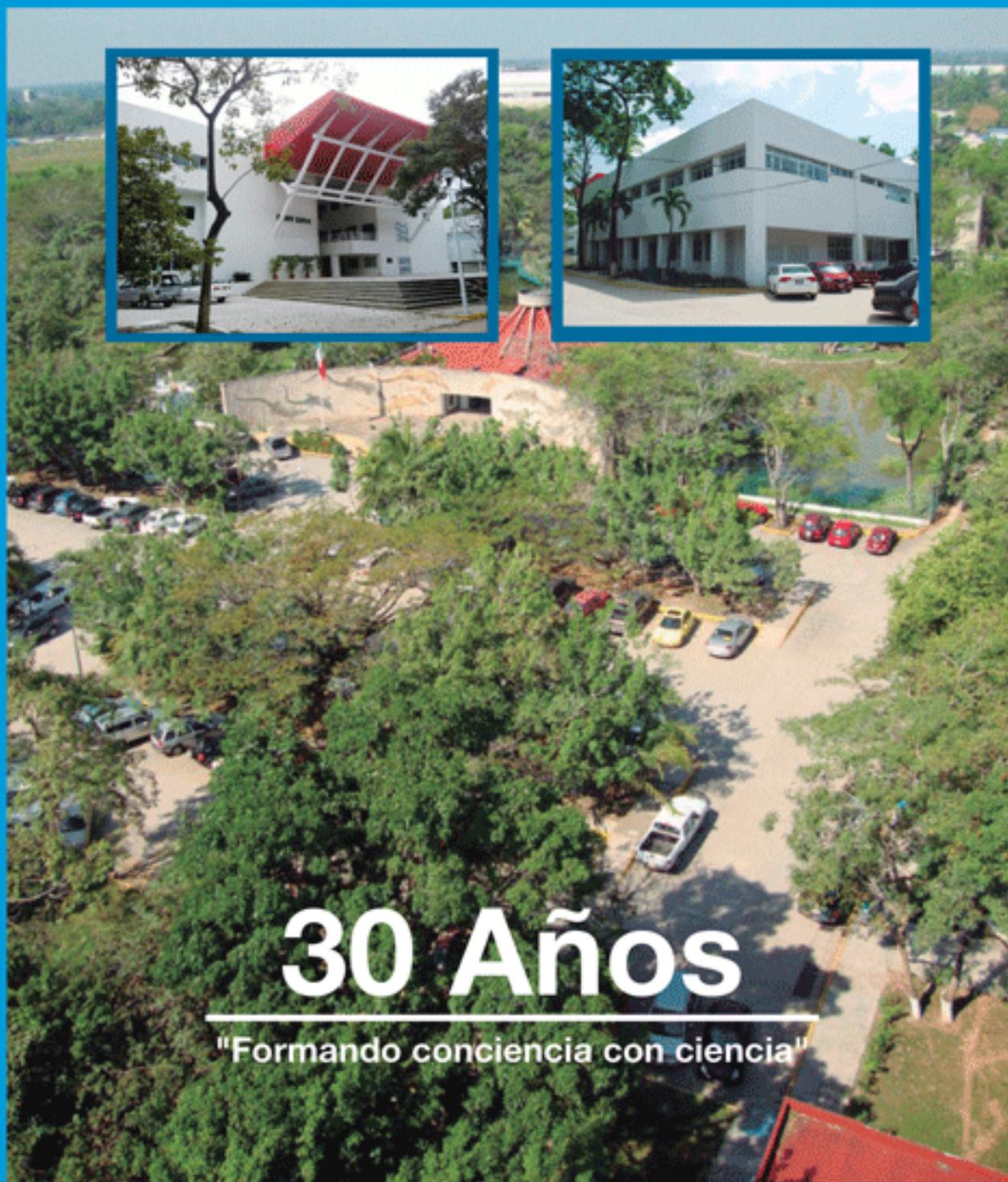
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## 30 Años

"Formando conciencia con ciencia"

## REVISTA DE DIVULGACIÓN

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*Kuxulkab' Voz chontal - tierra viva, naturaleza*

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Edificios emblemáticos de la DACBiol-UJAT; el Centro de Investigación para la Conservación de Especies Amenazadas (CICEA), el Centro de Investigación para la Conservación y Aprovechamiento de Recursos Tropicales (CICART) y el Herbario UJAT.

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**E**ste año se llevó a cabo un importante número de eventos para festejar el 30 aniversario de la enseñanza de las ciencias ambientales en la UJAT, tuvimos la oportunidad de conocer a investigadores que enriquecieron con sus participaciones los conocimientos de todos los que formamos la comunidad de la División Académica de Ciencias Biológicas.

La Universidad se encuentra en un proceso, que sin duda alguna, fortalecerá todos los medios de comunicación que forman parte de la misma, como lo es nuestra revista. El Área Editorial se encuentra ya funcionando como fortaleza no solo de Kuxulkab' sino de otros aspectos de divulgación y editoriales de la DACBIol. El programa de reorganización del sistema de manejo de Kuxulkab', permite hoy en día, brindar una respuesta mucho más rápida a todos aquellos artículos sometidos para publicar; igualmente nos encontramos participando en la implementación de un nuevo sistema propuesto por el Departamento de Publicaciones Periódicas de la Universidad, para la administración de manuscritos que permita agilizar el vínculo con la impresión como parte de la estrategia del plan de mejoras de dichas revistas.

Este número cuenta con un conjunto de cinco artículos y seis notas de temas de actualidad relacionados a las áreas de investigación que se llevan a cabo en la DACBIol y desarrollados por investigadores, estudiantes y colegas en la región. Como siempre agradecemos a todos los autores que nos enriquecen con sus contribuciones, así como a los revisores que amablemente se han tomado el tiempo de colaborar con nosotros y que cada día forman un grupo más nutrido, lo que nos fortalece en la revisión de una mayor diversidad de temas. Los invitamos a seguir considerando y usar esta opción de publicación como una ventana para compartir sus investigaciones, así como el desarrollo de temas de interés, tanto para nuestros colegas, alumnos y compañeros de la DACBIol y de la región.

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# Freshwater rotifer: (part II) a laboratory study of native freshwater rotifers *Brachionus angularis* and *B. quadridentatus brevispinus* from Tabasco

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## Abstract

This study is a first report of rotifer from the South of Mexico. The rotifers were collected from the fishponds around Biological Division, UJAT and we examined their morphometric characters, cultivated and identified them. We conducted experiment at different salt concentrations tested at 0g L<sup>-1</sup> water (no add NaCL), 2g L<sup>-1</sup>, 4g L<sup>-1</sup>, 6g L<sup>-1</sup>, 8g L<sup>-1</sup>, 10g L<sup>-1</sup> water. The species were identified as *Brachionus angularis* Gosse (1851) characterized by having two anterior spines, which are almost invisible while *B. quadridentatus brevispinus* Ehrenberg (1832) with six anterior spines, which can be seen clearly. Lorica size of *B. angularis* and *B. quadridentatus brevispinus* are about 12.14 and 12.8 μm, respectively and the size is much smaller than *B. plicatilis* (Mazatlan strain). Generally, the measurement of the two native freshwater rotifers showed much smaller than marine *B. plicatilis* strain from Mazatlan. The size of lorica length in both native rotifers was almost similar but quite smaller than marine rotifer *B. plicatilis*. We tested the tolerance growth of freshwater rotifer in different salt concentrations. *B. angularis* and *B. quadridentatus brevispinus* growth in 0 ppt lasted for one week and it reached 5342 individuals in 500 ml. Their growth in 2 ppt and 4 ppt was almost similar, however it decreased when the concentration of salt increased was increased to 6 ppt, 8 ppt, and 10 ppt, and between two species, *B. quadridentatus brevispinus* had higher growth rate than *B. angularis*. In general conclusion, both freshwater rotifers grew and reproduced in 2 ppt, 4 ppt, 6 ppt, 8 ppt, and 10 ppt. The tolerance of both native freshwater rotifers and their ability to survive in low salt concentration should be considered as one step towards rotifer

culture for finfish larval rearing worldwide of super small rotifer until now, large scale culture of marine fishes and shrimps are depend on marine rotifers *B. plicatilis* and *B. rotundiformis*. However, it is still necessary to maintain culture to accomplish their growth in large scale. This step remains for future studies.

## Introduction

There are over 1000 species of rotifers, 90% which inhabit in freshwater. Rotifers and cladocers are important components of most freshwater aquatic communities where rotifer is the most dominant zooplankton in all the freshwater aquatic ecosystem and considered as ideal food for most fish larvae (Hoff and Snell, 1999). Several characteristics of rotifers including their very small size and relatively slow swimming 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<sup>-1</sup> have been reported. Even at high densities, the animals reproduce rapidly and can thus contribute to the build up of large quantities of live food in a very short period of time (Hirata, 1979). Furthermore, rotifers can easily be enriched with fatty acids, antibiotics, 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 temperatures 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 polyunsaturated fatty acid in rotifers as food, and later Gatesoupe (1982) stated that a rotifer can be looked upon as a living food capsule, which transmits adequate supplies of macro- and micronutrients, vitamins, or even antibiotics to the fish larvae. Last, but not the least, the filter-feeding nature of rotifers facilitates the inclusion into their body tissues of specific nutrients essential for the larval predators. However, the level of polyunsaturated -3 fatty acid in rotifer is affected both survival and growth rate of fish larvae. In this sense, the most common rotifer used as food for finfish larvae rearing world-wide, is the SS monotype of rotifer called *B. rotundiformis* that has been used in the research line for many projects, while in freshwater fish and shrimp aquaculture, *B. calyciflorus* and *B. angularis* are the commonly cultured freshwater rotifer. However, for larvae with very small mouths, it is intended to have greater proportion of super small rotifer strain that also cover the nutritional requirement.

The freshwater rotifer *Brachionus angularis*, *B. patulus*, *B. calyciflorus*, *B. rubens* and *B. quadridentatus* are used in freshwater aquaculture and laboratory experiments. A single rotifer can become thousands of rotifer in 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 simultaneously, 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 themselves, 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 utilize another type of food (Okauchi *et al.*, 1980; Kafuku & Ikenoue, 1983), although the exact

number depends in the species of fish cultured and also on the size of the rotifers.

In the investigation reported here, we firstly examined the morphometric characteristics in two native freshwater rotifers, and experimentally cultured them in laboratory fed with marine algae *Nannochloropsis oculata*, and then evaluated the low salinity tolerance fed with *N. oculata*. Since there is no information yet on the salt stress tolerance of freshwater wild rotifers up to date, we tested the lowest salinity tolerance of wild freshwater rotifers from our collections. This method is proposed to brackish water larvae fish or shrimp culture to provide new information of native freshwater rotifers collected from Tabasco, Mexico.

## Materials and Methods

**Collecting site.** The freshwater rotifers used were collected from several fish ponds located in Biological Sciences Division, Universidad Juárez Autónoma de Tabasco, Mexico. The sampling sites were man-made fishponds with maximum depth of 1.5 m. The salinity, water temperature, and pH of the pond were recorded. Wild rotifers *B. quadridentatus brevispinus* were observed to be abundant when the water temperature gradually decreased to 24-26 °C on November, while *Brachionus angularis* were found during January-February when the water temperature dropped to 20-22 °C.

**Isolation and Inoculation.** Rotifer were collected by plankton net of 50 m mesh size, then concentrated zooplankton were then shifted to a one liter conical flask and poured with filtered water. The rotifers were then separated by a modified fine pipette then immediately transferred to a 100 ml Erlenmeyer flask contained 50 ml of microalgae *Nannochloropsis oculata* (A). Initial culture of rotifer was carried out by collected 50 female bearing eggs rotifers from flask A and reared in 250 ml flask Erlenmeyer contained of microalgae *N. oculata*, prior to individual culture experiments at different salt concentrations. Observation of rotifer growth was conducted every day during three days, and then the culture was scaled up to stock culture. Temperatures reading in Erlenmeyer flask were 26-27 °C.

**Stock culture and starter culture.** The female rotifers bearing eggs were stocked at a density of 50 individual.ml<sup>-1</sup> in Erlenmeyer flask of 500 ml and fed with fresh microalgae *Nannochloropsis oculata*. Fluorescent continuous lighting and aeration for 24 hours at 25 °C for better growth of rotifers were maintained constant in laboratory. About 50 ml of algae were added every day to supply enough food. After 3-4 days they were rinsed on a submerged filter. The concentration of rotifers was then distributed in several 10 L bottles filled with 2 L at a density of 50 individual.ml<sup>-1</sup> in a mild tube with aeration provided. Fresh algae *N. oculata* were supplied daily. Every day the culture was cleaned (double-screen filtered) and restocked at densities of 200 individual.ml<sup>-1</sup>. After one week the culture was completely filled and the culture was ready to be used for different salt concentrations.

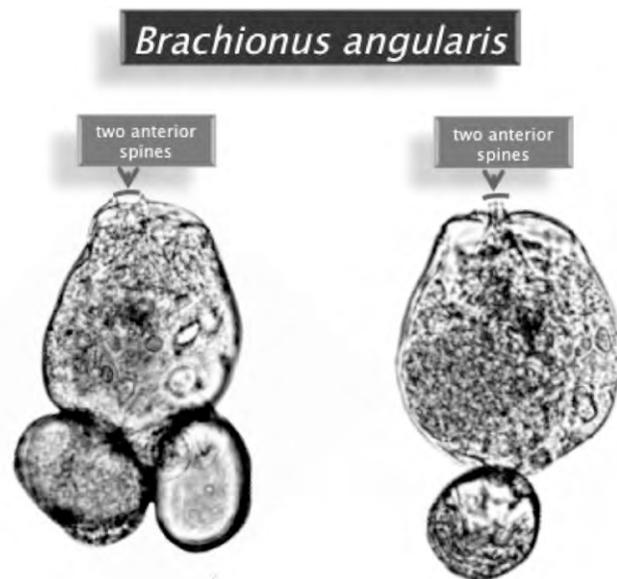
**Rotifer cultivation in different low salt concentrations.** We used marine microalgae *Nannochloropsis oculata* strain from Centro Investigación y Alimento Desarrollo (CIAD) Mazatlan, Mexico and have been adapted to grow in different salt concentrations during a year in the Laboratory of Tropical Biology, Universidad Juarez Autonoma de Tabasco (UJAT), Tabasco. To know their ability to growth and survive in different salt concentrations, we tested their growth and observed them in low salt concentration. Saline concentrations for the experiments were prepared using dried Ocean natural salt. We used different concentrations to be tested at 0g L<sup>-1</sup> water (no add NaCL), 2g L<sup>-1</sup>, 4g L<sup>-1</sup>, 6g L<sup>-1</sup>, 8g L<sup>-1</sup>, 10g L<sup>-1</sup> water and tested 50 individual.ml<sup>-1</sup> of each species of rotifer *Brachionus angularis* and *B. quadridentatus brevispinus* in each concentration. The experiments lasted one week.

**Species identification and morphometric characterization.** For a clarification of identification rotifer, 10 rotifers of each species were fixed with 10% of formalin and Lugol solution. Photographs were taken by Axio microscope Zeiss Scope A1 (@Zeiss, Germany) with compound digital camera Canon Power shot A640 10 megapixel (@Canon, Japan) with a magnification of 40X. The images were sent to Laboratory of Aquaculture and Artemia Reference Centre, Ghent University, Belgium. The morphometric characters in terms of lorica length and width, width and height of anterior spines were

measured based on Fu *et al.* (1991) and Hagiwara *et al.* (1995).

## Results

**Species identification and morphometric characteristic.** The species were identified as *Brachionus angularis* Gosse 1851 characterized by having two anterior spines which are almost invisible (Fig. 1) while *B. quadridentatus brevispinus* Ehrenberg, 1832 with six spines which can be seen clearly (Fig. 2). Generally, the measurement of the two native freshwater rotifers showed much smaller than marine *B. plicatilis* strain from Mazatlan (Table 1) The size of lorica length in both native rotifers was almost similar but quite smaller than marine rotifer *B. plicatilis*.



**Figure 1.** Figure 1. Native freshwater rotifer *Brachionus angularis* Gosse 1851

**Environmental conditions of freshwater rotifers.** Water temperature and pH conditions from the collecting site varied from 27-28 °C, while lower in laboratory from 22-25 °C (Table 2).

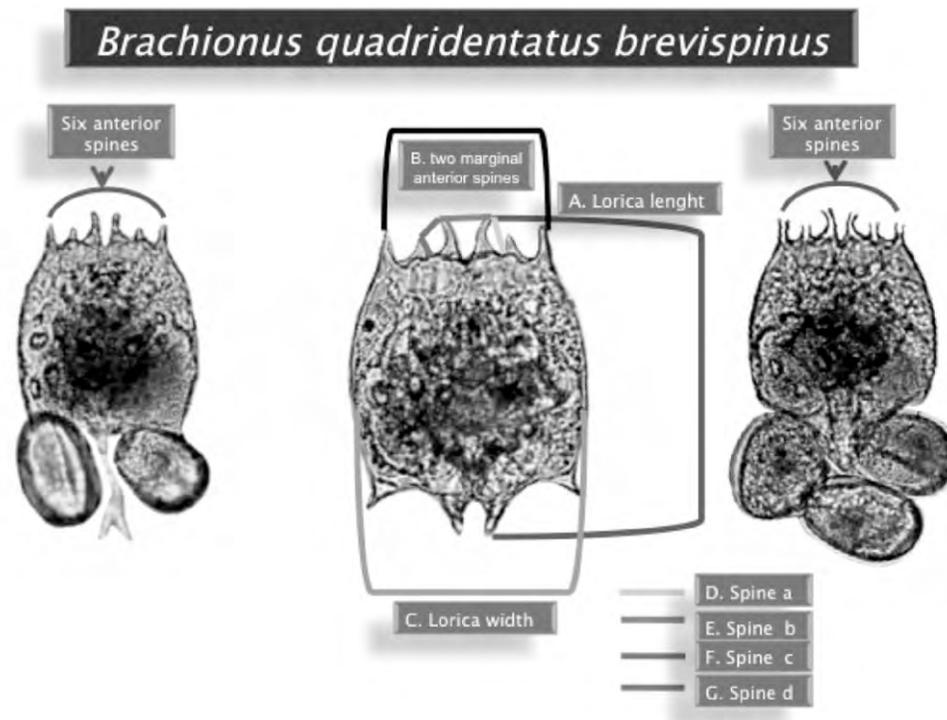


Figure 2. Native freshwater rotifer *B. quadridentatus brevispinus* Ehrenberg, 1832

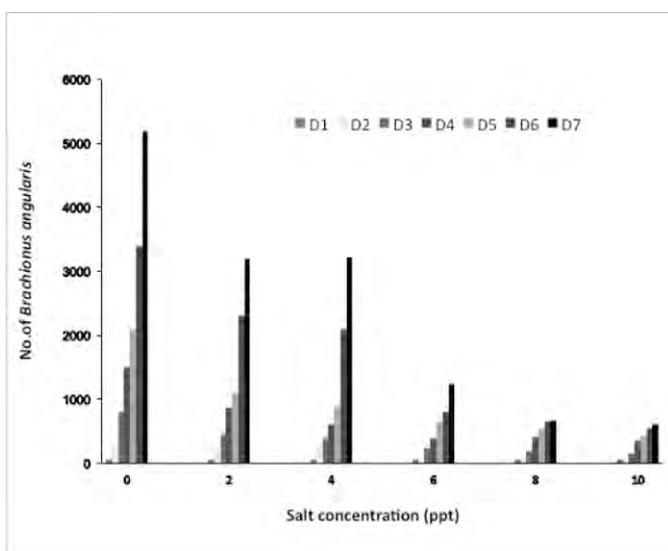
Variable ( $\mu\text{m}$ )	<i>B. angularis</i>	<i>B. quadridentatus brevispinus</i>	<i>B. plicatilis</i> (Mazatlan strain)
A			
B	12.14	12.8	174.87
C	10.10	9.20	141.63
D	4.70	6.42	80.11
E	0.42	1.01	12.07
E	0.42	1.42	10.08
F	0.57	0.71	15.02
	0.52	0.71	10.02

Table 1. Mean morphometric variables of two native freshwater *Brachionus* rotifer (Tabasco strain) compared with laboratory culture of *B. plicatilis* (Mazatlan strain).

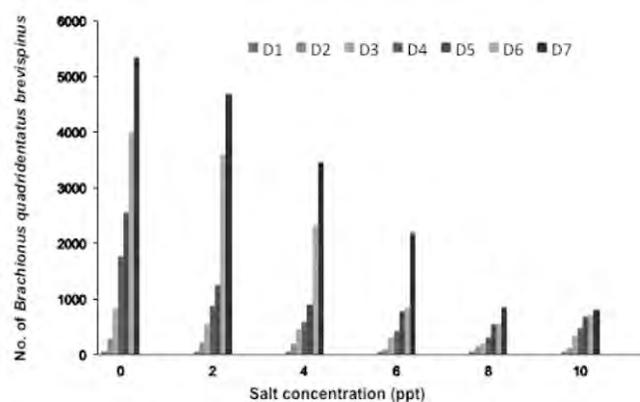
**Table 2.** Environmental conditions of native freshwater rotifer strains from Tabasco

Variable	Sampling site condition	Laboratory condition
Water temperature (°C)	27-28	22-25
Salinity (ppt)	0	0, 2, 4, 6, 8,10
pH	5-6	6-7

**Growth of *Brachionus angularis* and *B. quadridentatus brevispinus* in different low salt concentrations.** The tolerance of freshwater rotifer growth, which was tested in different salt concentrations, is shown in Figure 3 and 4. Rotifer *B. angularis* and *B. quadridentatus brevispinus* growth in 0 ppt lasted for one week, it reached 5342 individual in 500 ml. Their growth in 2 ppt and 4 ppt were almost similar, however it decreased when the salt concentration was increased to 6 ppt, 8 ppt, and 10 ppt, and between two different species of rotifer, *B. quadridentatus brevispinus* reproduced higher than *B. angularis*. However, both freshwater rotifers showed that were growth and reproduced in 2 ppt, 4 ppt, 6 ppt, 8 ppt, and 10 ppt (Fig. 3 and Fig. 4 respectively).



**Figure 3.** Growth of Rotifer *Brachionus angularis* in different low salt concentrations in laboratory



**Figure 4.** Growth of Rotifer *Brachionus quadridentatus brevispinus* in different low salt concentrations in laboratory.

## Discussion

This study is a first report of rotifer cultivation from the South of Mexico. The tolerance of both native freshwater rotifers and their ability to survive in low salt concentration are to be considered as one step towards rotifer culture for finfish larval rearing worldwide of super small rotifer until now, for large scale culture of marine fishes and shrimps are depending on marine rotifers *B. plicatilis* and *B. rotundiformis*. However, it is still necessary to maintain the culture to achieve their growth in large scale. This step remains for future studies.

The use of both rotifers has some relevance to the management of freshwater bodies and aquaculture ponds. First, it permits the limits of salt tolerance to be established so that remedial measures can be taken to protect freshwater water bodies from salinization (Peredo-Alvarez *et al.*, 2003). Second, if a certain freshwater zooplankton

species can tolerate high salinity, its mass culture can be attempted to use as live food for both fish and shrimp cultures. It has been documented that several fish belonging to the Aetherinidae family prefer mildly saline conditions (up to 10 g l<sup>-1</sup>), particularly during their early life history stages (Martinez-Palacios *et al.*, 2004). Third, it has been reported that with gradual acclimatization, some euryhaline zooplankton may eventually tolerate very high salinity levels (>50 g l<sup>-1</sup>) (Mustahal *et al.*, 1991). If attempts to culture freshwater zooplankton at various salinity levels succeed, it may reduce the dependence on the costly, imported and often mixed *Artemia* cysts for aquaculture (Campos-Ramos *et al.*, 2003).

Only a few species of rotifer among the approximately 1,800 species described are able to tolerate to tolerate brackish and saline water (Miracle and Serra, 1989; Onwudinjo and Egborge, 1994; Fontaneto *et al.*, 2006; Zakaria *et al.*, 2007). Therefore, compared to saline and brackish environments, freshwater environments generally sustain considerably larger quantities of rotifer species (Remane, 1971; Fontaneto *et al.*, 2006). Due to the prominent physiological constraints of saltwater on most rotifers, this group is of particular interest to test for ecological responses with regards to salinity variations on both spatial and seasonal scales on estuarine systems. The relationship between zooplankton species and salinity have been evaluating and as a central goals for many community ecologists worldwide (Miracle and Serra, 1989; Hammer, 1993; Lansac-Tôha & Lima, 1993; Keller & Conlin, 1994; Onwudinjo & Egborge, 1994; Williams, 1998; Epifanio & Garvine, 2001; Herbst, 2001; Ara, 2002; Derry *et al.*, 2003; Toumi *et al.*, 2005; Fontaneto *et al.*, 2006; Zakaria *et al.*, 2007). However, despite these previously accomplished studies, new questions frequently arise and contrasting results suggest that the influence of environmental factors on species compositions is site-dependent. Thus, rotifer distribution may be different than generally expected when environmental particularities are taken into account.

On the other hand, body size is one of the rotifer characteristics that is considered as a critical feature and determines their adequacy as food for a young larva. The present results on morphometric

variables revealed that the local strain classified as *B. angularis* and *B. quadridentatus brevispinus* as according to the category of Segers (pers. communication) have a smaller size than *B. plicatilis*. For larvae with smaller mouths, the body size of rotifer *B. angularis* and *B. quadridentatus* could be considered in using it as living food for fish larvae.

On the use marine algae *Nannochloropsis oculata*, our results showed that the rotifers in 0 ppt fed in green water microalgae *N. oculata*, could reproduce well (see Fig. 3 and 4) - although there is no comparison yet with other freshwater rotifer culture - seems that the environmental factors were favorable for their growth. Shiri *et al.* (2003) observed the survival rate of 69.2% in the rotifer fed on green water containing algae 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.

## Conclusion

The two native freshwater rotifers were identified as *Brachionus angularis* and *B. quadridentatus brevispinus* used in this study have present several characteristics: smaller size than *Brachionus plicatilis* (strain from Mazatlan), slow mobility, could survive and reproduced in low salt concentration at 2, 4, 6, 8 and 10 ppt fed with marine *Nannochloropsis oculata*. This performance could be considered as a potential candidate rotifer to use as prey in the culture of freshwater larvae, brackish or marine finfish.

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