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Morphometric study of *Brachionus angularis* in natural and culture populations.

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ABSTRACT

The present study was done to characterize morfometrically the rotifer *Brachionus angularis* present in the Canal Nacional, Xochimilco. The samples were obtained in february and september of 2010 in 5 sampling sites. The environmental parameters of each sample were determined and registered. The morfometric characterization was done by digital analysis using the Image Pro Plus of Media Cibernetics, determining for each individual; the distance between the median frontal spines (Feb = 6.35 ± 1.67 µm, Sep = 7.47 ± 2.47 µm, Culture Feb = 6.88 ± 2.91, Culture Sep = 8.26 ± 3.04), the frontal width (Feb = 63.04 ± 6.29 µm, Sep = 70.91 ± 5.34 µm, Culture Feb = 73.76 ± 3.92, Culture Sep = 74.92 ± 6.16), the maximum width (Feb = 86.34 ± 6.4 µm, Sep = 100.50 ± 6.17 µm, Culture Feb = 101.12 ± 7.89, Culture Sep = 103.16 ± 8.54), and the lorica total length (Feb = 103.21 ± 4.88µm, Sep = 110.08 ± 6.22 µm, Culture Feb = 109.84 ± 6.47, Culture Sep = 111.49 ± 9.76). Significative differences ($p < 0.001$) were found in the size of the individuals from the 4 different populations. Mainly between the natural population obtained in february and the other populations.

KEY WORDS: morfometric variation, rotifer, digital image analysis, Xochimilco lake.

INTRODUCTION

The rotifers are aquatic cosmopolitan organisms and some species are important live feed in aquaculture. Some of their characteristics, as nutritional quality, size and behavior, make these organisms an appropriate first food for fish larvae (Snell & Carrillo, 1984). *B. angularis* is one of the smallest species of rotifers (84 ± 4.9 to 127.8 ± 5.9), (Leutbecher, 2000; Yin & Niu, 2008; Ogata *et al*., 2011). On the other hand, this rotifer has been reported in 77 water bodies studied in 23 States of Mexico (Garza-Mouriño *et al*., 2006). In this manner, because of its size and distribution *B. angularis* is a good candidate to be used as live food in aquaculture. However, the massive stable culture of this species has not been developed yet (Ogata *et al*., 2010 & 2011). On the other hand, the morphometric studies allow the evaluation of the range of taxonomical and morphological variability exhibited by the rotifers in the water bodies in response to the biotic and abiotic factors (Green, 1981; Hillbricht-Ilkowska, 1983; Garza-Mouriño *et al*., 2005). The aim of this work was to make the morphometric description of *Brachionus angularis* in order to evaluate the size variation in the natural population and when it is cultivated under controlled conditions, giving important information to be used in the production of this rotifer as the first live food for fish larvae, which need a very small prey.

MATERIAL AND METHODS

Study area

The Canal Nacional, belongs to Xochimilco´s lacustrine system, the sampling sites are located at 2,274 meters on the sea level in the quadrant formed by 19º16'24" to 19º17'13" N

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latitude and 99º06'10" to 99º06'45" W longitude. This ecosystem receives an important contribution of water from the plant of treatment Cerro de la Estrella.

Samples

The samples were obtained in February and September of 2010. Two sampling methods were followed; a) 10 liters of water from the surface were taken in every sampling site, then the water was filtered trough a mesh of 30µm, the material was preserved with 4% formalin in a sterilized sampling bottle, and b) a zooplankton net of 50 µm of mesh size was used and the trawling was done at a velocity of 1 $km.h^{-1}$ along Canal Nacional. In order to obtain the ovigerous females to start the clonal cultures, the first method described above was followed but instead the formalin solution, water of the sampling place was filtered before and used to keep alive the organisms.

Ten egg-carrying amictic *B. angularis* females were isolated from the crude sample and were placed individually into wells of a STARSTED multiwell plate containing 2 ml of EPA medium (pH 8) with *Chlorella vulgaris* at a density of 1.6 x 10^6 cels/ml. The cultures were maintained under constant temperature of 25±1°C and photoperiod of 12L:12D, using an environmental chamber (Precision Scientific). When the population growth curve reached the maximum, the rotifers were fixed in 4% formalin.

Morphometric characteristics.

Semi-permanent preparations were made with the fixed samples of *B. angularis*. Later, the slides were examined using a BX50 Olympus microscope and each ovigerous female was photographed in high resolution digital images (1024 x 1024 pixels) with a Magna Fire Olympus camera of 5 megapixels. The photographs were analyzed using an Image Pro Plus V. 4.5 of Media Cybernetics analyzer. Figure 1 show the measurement protocol, where the DEFM segment corresponds to the distance between median frontal spines, AFL to the lorica frontal width, AMAX to the maximum width and LMAX to the body maximum length.

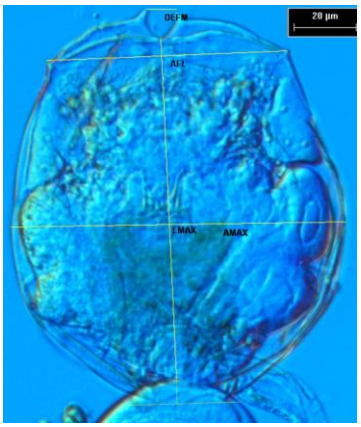

Figure 1. Morphometric characteristics protocol of *B. angularis*. The DEFM segment corresponds to the distance between median frontal spines, AFL to the lorica frontal width, AMAX to the maximum width and LMAX to the body maximum length.

Statistical analysis.

For the morphometric characterization, descriptive statistics was used in the global data presentation. Different statistical tests of significance were applied to verify if the data fitted the parametric assumptions (Zar, 1996). The one-way ANOVA was done to determine significant differences in the lorica characters; thereafter the Tukey test was applied (Montgomery, 1984), to determine the variables that show differences between the populations.

RESULTS

Table 1 show the values of environmental factors of the sampling sites at the time when the samples were collected. The analysis done show that *B. angularis* present morphometric changes depending of the environmental conditions of that they were obtained. The lorica size in the cultured populations was bigger than in the natural populations, showing a proportional growth. The natural population obtained in February had the smallest size (Figure 2), with significant differences

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in all the parameters studied when comparing with the others populations (Table 2). The cultured populations do not show significant differences between them. However, during the 20 days of culture, the cultured populations developed a bigger size compared with the size of the individuals of the natural populations.

Table 1. Sampling months mean values (± S.D.) of some ecological parameters and rotifer abundance.

Parameters	February		September	
	Mean value	(± S.D.)	Mean value	(± S.D.)
Transparency (cm)	35	15.80	35.4	15.3
pH	8.9	0.90	9.2	1.0
Temperature (ºC)	22.4	0.70	24.6	1.4
Water depth (cm)	70.4	15.00	73.4	13.8
Dissolved oxygen ($mg.L^{-1}$)	7.51	0.21	7.67	0.14
Clorophile *a* ($mg.m^{-3}$)	201.9	8.40	275.1	6.4
Clorophile *b* ($mg.m^{-3}$)	27.0	3.60	33.8	0.5
Clorophiles c_1 y c_2 ($mg.m^{-3}$)	26.3	3.30	39.6	3.1
Abundance of *B. angularis* (org L^{-1})	6.12	2.80	253.8	7.3

Table 2. Variance analysis and Tukey test values of studied parameters of *Brachionus angularis*.

Variance analysis							Tukey test				
Variation source	d.f.	Square sum	Mean square	F	P	Alpha = 0.05	Groups	Quantity	Differences between groups	d.f..	Critical value
DEFM							NAT FEB	47	CULT SEPT	199	3.63
A: Population	3	98.88	32.96	5.08	0.002058*	0.9162	CULT FEB	28			
S(A)	199	1289.85	6.48				NAT SEPT	75			
Total (adjusted)	202	1388.72					CULT SEPT	53	NAT FEB		
Total	203										
AFL							NAT FEB	47	NAT SEPT, CULT FEB, CULT SEPT	199	3.63
A: Population	3	3960.76	1320.25	41.61	0.000000*	1	NAT SEPT	75	NAT FEB, CULT SEPT		
S(A)	199	6314.82	31.73				CULT FEB	28	NAT FEB		
Total (adjusted)	202	10275.58					CULT SEPT	53	NAT FEB, NAT SEPT		
Total	203										
AMAX							NAT FEB	47	NAT SEPT, CULT FEB, CULT SEPT	199	3.63
A: Population	3	8542.84	2847.62	55.66	0.000000*	1	NAT SEPT	75	NAT FEB		
S(A)	199	10180.35	51.16				CULT FEB	28	NAT FEB		
Total (adjusted)	202	18723.19					CULT SEPT	53	NAT FEB		
Total	203										
LMAX							NAT FEB	47	NAT SEPT, CULT FEB, CULT SEPT	199	3.63
A: Population	3	2005.72	668.57	13.25	0.000000*	0.9998	CULT FEB	28	NAT FEB		
S(A)	199	10042.96	50.47				NAT SEPT	75	NAT FEB		
Total (adjusted)	202	12048.68					CULT SEPT	53	NAT FEB		
Total	203										

*alpha = 0.05.

DISCUSION

The lorica size of *B. angularis* Xochimilco been reported. Until now the smallest is the Laos strain (Ogata *et al*., 2011), with a size of 86 ± 4.9

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µm of lorica length and 75.6 ± 5.7 µm of width, followed by the Xochimilco strain with a maximum length of 111.49 ± 9.76 µm, and maximum width of 103.16 ± 8.54 µm.

The small and rounded lorica, as well as his incipient spines places to *B. angularis* inside the rotifers with high potential for the fresh water larviculture, as it has been the *plicatilis* complex for marine larviculture.

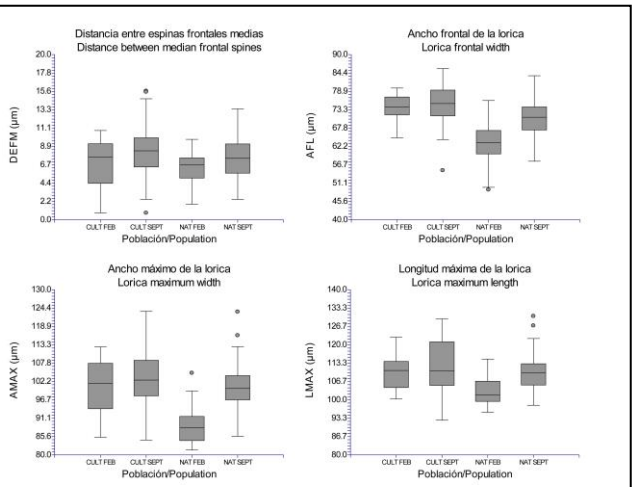

Figura 2. Caracterización morfométrica de *B. angularis* de dos poblaciones de ambiente natural (NAT FEB, NAT SEPT), y dos poblaciones cultivadas en laboratorio (CULT FEB, CULT SEPT).

The size variation in rotifers of different populations geographic or temporally separated can be of genetic origin (Serra & Miracle, 1987; Walsh & Zhang, 1992). In this study, the natural populations of *B. angularis* obtained in February and September had differences between them. However, these differences disappeared when the organisms of these populations were cultured under the same controlled conditions. Therefore, we can infer that the differences are due to the influence of the environment and not to the genetic origin.

On the other hand, Serra and Miracle (1987), found a strong interaction between the effects of salinity and temperature on the morphometric variation besides the genetic control. However, the evaluation of the zooplankton morphological changes under natural environmental conditions is very difficult due to the effects of several factors and the results can be mislead. Therefore, the present study was done with field collected organisms and cultures ones, in order to evaluate the morphometric variation between them.

CONCLUSIONS

The results of this work show that the individuals of *Brachionus angularis* obtained in two different climatic seasons present significative differences in their morphometry, which are minimal when the organisms were cultivated under the same controlled conditions in the laboratory. These results are usable in aquaculture when this species will be the first food for larval stages of freshwater fishes.

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