

Study of the effects of photoperiod and salinity in the Alvarado strain of the *Brachionus plicatilis* species complex (Rotifera: Monogononta)

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Abstract – The survivorship and distribution of planktonic rotifers are influenced by salinity and photoperiod. In this study we performed life table experiments with the estuarine rotifer *Brachionus* sp., “Alvarado” strain (collected in Veracruz, Mexico). Three salinities: 7.5, 15 and 30‰, and two photoperiods: constant light and 16:8 h (light:dark) photoperiod at one temperature ($25 \pm 2^\circ\text{C}$) were assessed. The microalgae *Nannochloropsis oculata* (1×10^6 cells.mL⁻¹) was supplied as food. We recorded the hatching percentages at different temperatures, and we included morphometric data of adult rotifers, neonates and parthenogenetic eggs. Results showed that the highest estimated values of net reproductive potential (Ro), intrinsic growth rate (*r*) and reproductive value (Vx) were obtained at 15‰ and 16:8 h (light:dark) photoperiod, followed by continuous light, respectively. The lowest reproductive rate was observed at 30‰ and 16:8 h (light:dark) photoperiod. Hatching reached 100% after 20 h at temperatures of 25 and 30 °C. The morphometric analysis of the lorica suggests that this *B.* “Alvarado” strain belongs to the SM clade of the *B. plicatilis* species complex. This strain of *Brachionus* is: (a) euryhaline, (b) high growth rate, (c) small size. All these characteristics suggest that this strain can be potentially used as live food for larval fish and for the development of toxicity tests.

Key words: Lifetable / growth potential / rotifer / clade / sympatry

Introduction

Rotifers are microscopic invertebrates present in estuarine, freshwater and marine environments (Pennak, 1989). There are three Classes currently recognized in the Phylum Rotifera: Seisonacea with three species, Bdelloidea with 461 species and Monogononta with 1570 species (Fontaneto *et al.*, 2008; Segers, 2008). However, more species should be considered as a result of cryptic speciation (Segers, 2008), their size ranges between 50 and 2000 µm in length (Wallace *et al.*, 2006; Segers, 2008). Their small size and integument permeability make them susceptible to chemical and physical changes in their environments, establishing the limits of the habitat of species (Nogrady *et al.*, 1993; Pérez-Legaspi and Rico-Martínez, 1998; Wallace *et al.*, 2006). *Brachionus plicatilis* is a

euryhaline rotifer which has been considered cosmopolitan due to its ample distribution (Yin and Zhao, 2008; Stelzer *et al.*, 2011; Kostopoulou *et al.*, 2012). However, its taxonomic status nowadays is not clear, because several molecular studies suggest that it is a complex of cryptic species (Gómez, 2005; Stelzer *et al.*, 2011; Alcántara-Rodríguez *et al.*, 2012; Kostopoulou *et al.*, 2012), with different osmoregulatory capabilities (Lowe *et al.*, 2005).

Salinity is an important factor that influences rotifer cultures (Lowe *et al.*, 2005), where *B. plicatilis* is widely used in fish and crustacean marine larviculture (Kostopoulou *et al.*, 2009, 2012; Conceição *et al.*, 2010). The *B.* “Cayman” morphotype has been recognized as the most representative for aquaculture (Kostopoulou and Vadstein, 2007). Life tables offer useful information on the effect of environmental factors in the population structure of rotifers (Pérez-Legaspi *et al.*, 2015), making it possible to estimate their population dynamics based on growth

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rates and mortality (Krebs, 1994; Wallace *et al.*, 2006; Wallace and Snell, 2010). Population parameters like “*r*” (intrinsic growth rate) depend on the type of clone of *B. plicatilis*, as well as on the interactions between temperature and salinity (Miracle and Serra, 1989). The highest growth rates have been recorded in salinities ranging from 5 to 20‰ for clones of *B. plicatilis sensu stricto* (Yin and Zhao, 2008); while in sister species the highest growth rates are recorded in salinities of 16–18‰ (Lowe *et al.*, 2007). On the other hand, Lubzens *et al.* (1985) suggest that salinity can be a modulator of growth rates in *B. plicatilis* influencing the genetic variation of the strain. Salinity causes local adaptation, genetic population divergence and speciation cryptic in the populations of the *B. plicatilis* species complex (Alcántara-Rodríguez *et al.*, 2012). In addition, rotifers are very prone to local and regional differentiation because its ability to disperse; consequently the sympatry are very common in the *B. plicatilis* species complex, coexisting among them, being possible to find additional species in the sampled lineages (Gómez, 2005). *Brachionus* sp. “Alvarado” represents a new lineage (and possibly a new species) of the *B. plicatilis* species complex which has not been completely genetically characterized. However, a preliminary analysis of the COI (cytochrome c oxidase subunit I) sequence shows a strong relation with *Brachionus ibericus* and *B.* “Cayman”, in a publication where the Alvarado strain was named *Brachionus* VER strain (Rico-Martínez *et al.*, 2013); although the Alvarado strain also shows close relation to the *B.* “Almenara” strain (Jorge Ciro-Pérez, personal communication). Recently, García-Morales and Elías-Gutiérrez (2013) reported the presence of *B.* “Almenara” through the analysis of the COI sequence at the Sontecomapan Lagoon (118.7 km away from Alvarado Lagoon), suggesting that this strain has great capability for dispersal and colonization. In the present study, we have decided to temporarily name our population as *Brachionus* sp. “Alvarado” strain base in the original collection site. Our strain shows a high reproductive rate and ease of culture. Therefore, it is important to assess its growth rate potential under different conditions of photoperiod and salinity, to determine its usefulness in aquaculture or for the development of further toxicity tests; in the meantime contributing to the knowledge of its reproductive biology, and comparing its characteristics with those of similar clades of the *B. plicatilis* species complex. The objective of this study was to assess the effect of the salinity and photoperiod in the life history of the rotifer *Brachionus* sp. “Alvarado” strain using: (a) life table experiments, (b) morphometric data and (c) hatching percentages.

Materials and methods

The rotifer *Brachionus* sp. “Alvarado” strain was collected in the Alvarado Lagoon, off the coast of southwest Veracruz, Mexico, in the Gulf of Mexico (18°46′–18°42′N and 95°34′–95°58′W). The region has an

annual temperature between 18 and 26 °C and an average of 7.5‰ (2.2–14.3). This lagoon system receives freshwater from the rivers Papaloapan, Blanco, El Limón and Acula; besides, it is connected to the sea (De la Lanza-Espino and Lozano, 1999). Rotifer culture was implemented according to the protocol of Pérez-Legaspi and Rico-Martínez (1998) with slight modifications, and maintained for over 5 years in the following conditions: synthetic seawater at 15‰, using distilled water and aquarium salts (Oceanic, GAL aquarium, USA), with a pH of 8.2–8.4, and a temperature of 25 ± 2 °C and constant light at 3000 luxes. Additionally, the green algae *Nannochloropsis oculata* was acquired from the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE, Strain Number NNO1), used as food, and cultured in Guillard “F2” medium at 15‰ (Stein, 1979).

To establish the temperature to be used during the experiments, we studied the hatching percentages at different temperatures (20, 25 and 30 ± 2 °C), in the following way: ten parthenogenetic eggs per well ($n=100$) were placed in a new sterile transparent polystyrene plate (Costar, Corning Inc.), with 2 mL of synthetic seawater at 15‰. The plates were placed under constant light at 3000 luxes inside a bioclimatic chamber (Felisa), exposing the eggs to each temperature and recording the different times needed to achieve 100% hatching.

The life table experiments included a week of previous acclimation for each rotifer culture at 7.5, 15 and 30‰, respectively. The definitive experiments started using neonates < 2 h old. These neonates were obtained from parthenogenetic eggs and then placing one individual per well ($N=24$) in a 24-well polystyrene plate containing a total volume of 2 mL of synthetic seawater at each of the tested salinities (7.5, 15 and 30‰), with an algal density of 1×10^6 cells.mL⁻¹ of *N. oculata* which was determined with a Neubauer chamber (Optik Labor, Germany). Consecutively, the plates were exposed to a temperature of 25 ± 2 °C, at 3000 luxes to assess: (a) continuous light, and (b) 16:8 h, light:dark photoperiod. Then, the adult organisms (parents) were observed every 12 h, and the neonates (descendants) were counted and removed from the well. The medium was replaced with the same corresponding salinity and food concentration. Life tables were maintained until the last original parent died in each treatment.

Besides, according to Kostopoulou *et al.* (2009); we measured the lorica of parthenogenetic females (adult and neonates) and eggs cultured at the same salinities tested (7.5, 15 and 30‰), adding the 2.5‰ only for the morphology. These measures were taken with a 10:100 reticulated micrometer (Carl Zeiss). We measure: total Lorica length (TL), maximum width (MW), the distance between the first and second external spine (DS1-2), and distance between spines (DS), and the external cover of the egg (E).

Data analysis included the estimation of the following population parameters: time interval of 12-h (x), mean generation time (T_c), net reproductive potential (R_0), intrinsic growth rate (r), life expectancy (ex), reproductive value (V_x), and finite index of increase (λ); according to Krebs (1994), and Begon *et al.* (1996). These parameters

Table 1. Life table of the rotifer *Brachionus* sp. “Alvarado” at different salinities and two types illumination regimes.

Conditions	Salinity (‰)	<i>X</i>	Ex	Tc	<i>r</i>	Ro	λ
Photoperiod 16:8 h (light:dark)	7.5	144	7.12	5.27	0.4817	12.69	1.6189 ± 0.028
	15	144	8.25	4.77	0.5336	18.09	1.7051 ± 0.043
	30	180	7.60	7.82	0.3100	11.31	1.3630 ± 0.026
Continuous light	7.5	144	7.41	5.27	0.5296	16.38	1.6980 ± 0.043
	15	216	9.18	6.72	0.4427	19.64	1.5570 ± 0.048
	30	216	9.18	6.91	0.4301	19.58	1.5375 ± 0.051

X, hours; ex, life expectancy; Tc, mean generation time; *r*, intrinsic rate of natural increase; Ro, net reproductive rate; λ , infinite index of increase.

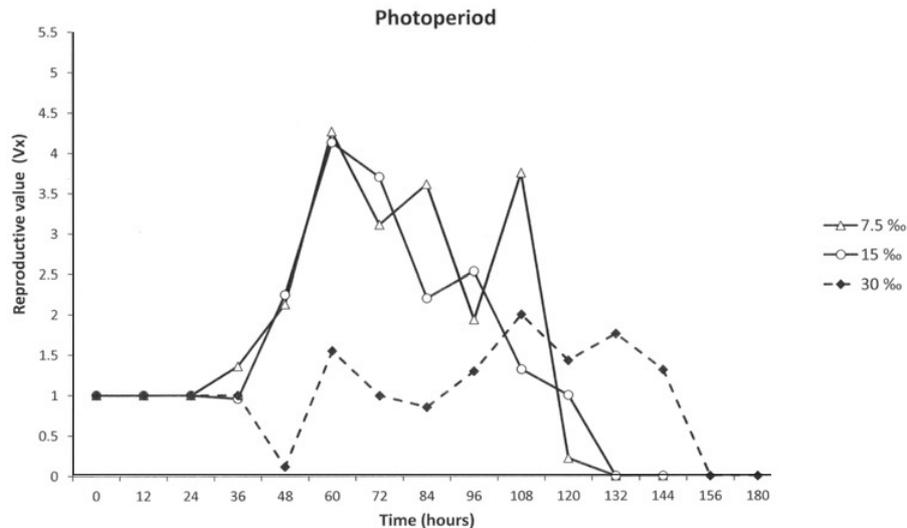


Fig. 1. Reproductive value (*V_x*) of *Brachionus* “Alvarado” cultured at different salinities at $25 \pm 2^\circ\text{C}$ under a 16:8 h light:dark photoperiod.

were calculated using the software PopTools 2.7.5 (2006) for Microsoft Excel, with confidence intervals of 95%, through 1000 Monte Carlo simulations (Alvarez-Buylla and Slatkin, 1993). We also performed single regression analysis between survivorship (*lx*) and life expectancy (*ex*) for each treatment. The morphometric data and the effects of photoperiod and salinities for all individuals in each treatment were analyzed by one way analysis of variance and *post hoc* comparisons by Tukey’s tests ($P < 0.05$). The analysis was done using the software Statistica 7.0 (Statsoft, Inc., 2004).

Results

The best conditions to culture *Brachionus* sp. “Alvarado” were recorded when the population grows at 15‰, regardless of photoperiod (Table 1). However, it is possible to obtain high population densities in this strain when exposed to continuous light and salinities of 7.5 and 15‰, as shown in our analysis of *V_x* (Figs. 1 and 2). Under continuous light rotifers produce neonates after 36 h for the three tested salinities, and the highest *V_x* was attained at 7.5‰, but with a moderate longevity (Fig. 1). The highest values of Ro were recorded at 15 and 30‰ when rotifers are exposed to continuous light. The lowest Ro value was obtained at 7.5‰ under continuous

light, but this value is higher than any of the Ro values obtained at (16:8, l:d) photoperiod. The highest Ro values with photoperiod were obtained at 15‰. Similarly, the *r* values were higher under continuous light that is, under photoperiod at salinities of 7.5 and 30‰. However, the highest *r* value was attained at 15‰ under photoperiod (Table 1). The statistical analysis shows that there is no significant effect among the continuous light and the salinities tested; while the photoperiod and salinities are significant ($P < 0.05$) (Table 2). In general, rotifers grow better at 15‰ in photoperiod and continuous light, respectively; followed by 30‰ under continuous light (Table 1). On the other hand, the correlation coefficient (adjusted R^2) between survivorship (*lx*) and life expectancy (*ex*), shows that there is good correlation for all treatments (Table 3).

The hatching percentage of parthenogenetic eggs was 100% at 20 h at 25 and 30°C, while only 80% was recorded at 20°C, respectively (Fig. 3). The morphometric data of the lorica of parthenogenetic females (adults and neonates) and eggs of *Brachionus* “Alvarado” is shown in Table 4. We found significant differences ($P < 0.05$) in TL and MW between adults and neonates, and adults grew larger at 30‰ (Fig. 4). Our morphometric data support the inclusion of *Brachionus* “Alvarado” (Fig. 5) in the SM clade of *B. plicatilis* species complex. Neither sexual females nor males were measured, because they have never

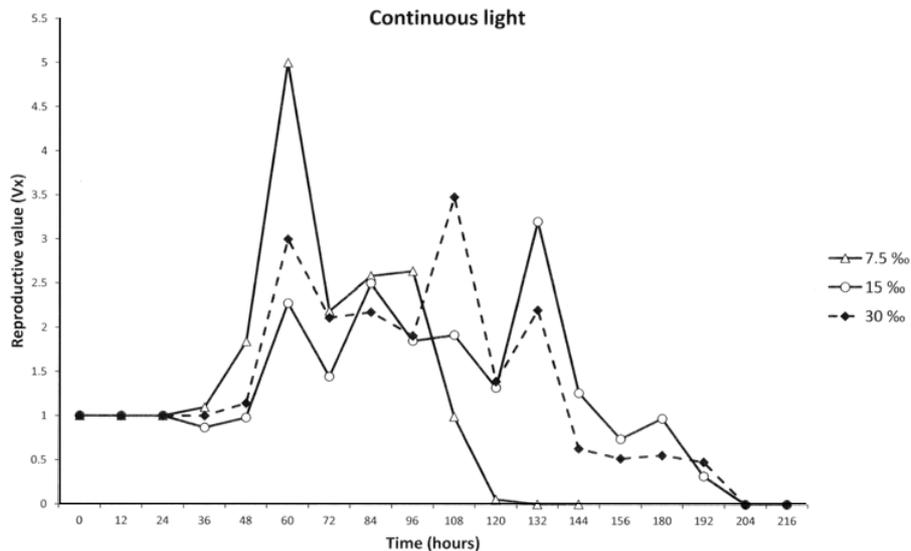


Fig. 2. Reproductive value (Vx) of *Brachionus* “Alvarado” cultured at different salinities at 25 ± 2 °C under continuous light.

Table 2. Analysis of variance (one-way ANOVA) performed for continuous light, photoperiod and the morphometry for the rotifer of *Brachionus* sp. “Alvarado” exposed to several salinities.

Effect	SS	d.f.	MS	F	P
Continuous light					
Intercept	52 710.14	1	52 710.14	100.5386	0.000000
Salinity	313.18	2	156.59	0.2987	0.743225 ^{ns}
Error	24 116.78	46	524.28		
Photoperiod					
Intercept	32 606.59	1	32 606.59	65.74141	0.000000
Salinity	8403.64	2	4201.82	8.47171	0.000907*
Error	18 847.33	38	495.98		
Adult (TL)					
Intercept	2852 390	1	2852 390	18 840.42	0.000000
Salinity	8249	3	2750	18.16	0.000000*
Error	11 506	76	151		
Neonate (TL)					
Intercept	1701 486	1	1701 486	14 255.57	0.000000
Salinity	1482	3	494	4.14	0.008995*
Error	9071	76	119		
Adult (MW)					
Intercept	1808 410	1	1808 410	15 025.11	0.000000
Salinity	737	3	246	2.04	0.115233 ^{ns}
Error	9147	76	120		
Neonate (MW)					
Intercept	909 084.8	1	909 084.8	5636.677	0.000000
Salinity	3981.9	3	1327.3	8.230	0.000082*
Error	12 257.3	76	161.3		

TL, total length of the lorica; MW, maximum width of the lorica; SS, sum of squares; d.f., degree of freedom; MS, mean squares; F, F-ratio; P, P value. *Significant (P < 0.05), ns, non-significant P > 0.05; N = 24.

been observed for this strain since the initial culture was established 5 years ago.

Discussion

The Ro, r and Vx values indicate that the best culture conditions for *Brachionus* sp. “Alvarado” are achieved at 15‰ salinity and under photoperiod or continuous light

(Figs. 1 and 2, Table 1), which falls under the range reported by Yin and Zhao (2008) for *B. plicatilis* s.s. However, it is also possible to obtain high densities of rotifers under continuous light at salinities of 7.5 and 30‰. It appears that continuous light positively influences reproduction of *Brachionus* “Alvarado”, increasing life expectancy (ex) and generation time (Tc) where the highest values were recorded (Table 1). However, there is no significant difference among the treatments in continuous

light, but still it is possible to distinguish that 15‰ favors reproduction in this rotifer strain regardless of illumination type or photoperiod (Fig. 3); although the photoperiod has a significant effect on the salinities tested ($P < 0.05$) (Table 2).

In our strain only parthenogenetic females are present, thus the life tables were performed with parthenogenetic females; they are considered as the step of life history exposed most of the time to natural selection (Stelzer, 2005). In a recent toxicological study where our strain was used,

Table 3. Coefficient of determination (R^2) between survivorship (lx) and the mean life expectancy (ex) for all treatments assessed with the rotifer *Brachionus* sp. “Alvarado”.

Condition/Salinity	7.5‰	15‰	30‰
Photoperiod	0.7962	0.8240	0.7723
Continuous light	0.8096	0.7120	0.7120

sexual females and males were never encountered in spite of the culture was maintained for 10 months (Rico-Martínez *et al.*, 2013). These authors suggest that perhaps this strain is unable to produce cysts under laboratory conditions. Some authors argued that salinity is one of the most important factors that influence cyst production in *B. plicatilis* (Hagiwara *et al.*, 1989). The lack of cyst production under laboratory conditions could be considered as an advantage for the use of *B.* “Alvarado” in aquaculture, because parthenogenesis eliminates the cost of producing males which allows for rapid parthenogenetic population growth (Kostopoulou *et al.*, 2012). However, further studies are needed to determine if sexuality can be induced in this strain with the appropriate stimulus.

Salinity may influence evolutionary and ecological processes of rotifers (Alcántara-Rodríguez *et al.*, 2012). The range of salinity tolerance shown by our strain is characteristic of the *B. plicatilis* species complex, with

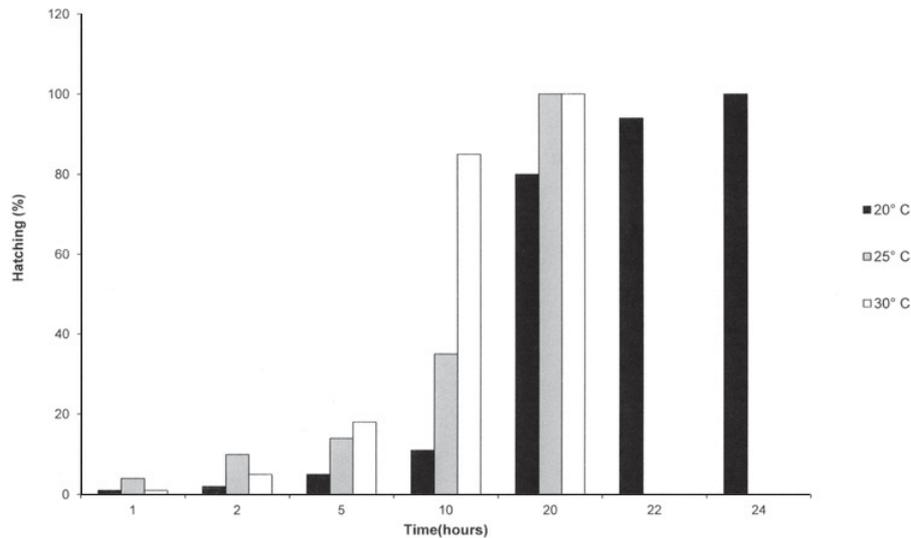


Fig. 3. Hatching percentage of the rotifer *Brachionus* sp. “Alvarado” exposed to different temperatures at 15‰ salinity.

Table 4. Morphological characterization of the rotifer *Brachionus* sp. “Alvarado”. Organisms were taken randomly from different cultures maintained at several salinities at $25 \pm 2^\circ\text{C}$; we measured adult females (A), neonates (N) (< 30 min old) and parthenogenetic eggs (E). The values are in micrometers and correspond to the mean \pm one standard deviation ($N = 20$).

Salinity (‰)	Stage	Lorica		Spines	
		TL	MW	DS1-2	DS
2.5	A	177.5 \pm 15.34	153.9 \pm 15.87	21.45 \pm 25.6	89 \pm 6.40
	N	151.2 \pm 7.23	114.25 \pm 8.15	11.85 \pm 2.97	67.75 \pm 11.9
	E	107.5 \pm 11.06	90.2 \pm 6.34		
7.5	A	182.5 \pm 8.66	149.75 \pm 8.34	22.1 \pm 2.46	91.5 \pm 8.12
	N	144.85 \pm 8.45	122.65 \pm 16.3	12.6 \pm 42.0	69.05 \pm 9.33
	E	119.75 \pm 6.38	87.95 \pm 4.68		
15	A	191 \pm 9.67	152 \pm 8.94	22.2 \pm 3.99	94.5 \pm 12.34
	N	139.5 \pm 15.03	97.5 \pm 12.92	11.4 \pm 2.34	64.5 \pm 9.98
	E	116.25 \pm 11.5	78 \pm 7.67		
30	A	204.3 \pm 14.19	145.75 \pm 8.92	22.35 \pm 2.68	91 \pm 7.18
	N	147.7 \pm 11.29	102 \pm 12.07	13.2 \pm 2.19	66.75 \pm 6.54
	E	113.5 \pm 10.01	81.55 \pm 9.9		

TL, total length; MW, maximum width; DS1-2, distance between first spine and second external spine; DS, distance between spines; E, external cover of egg.

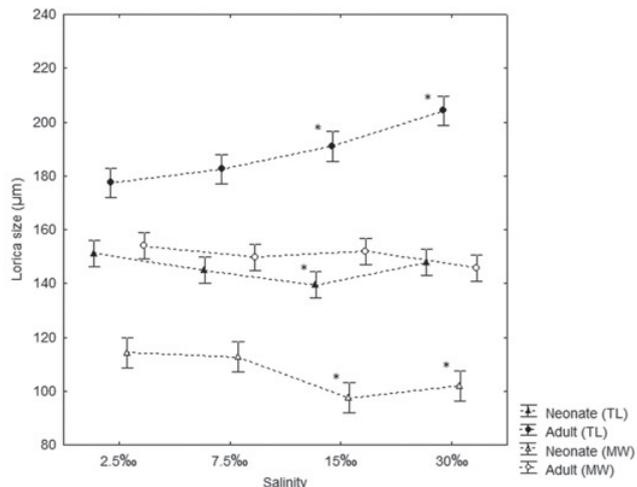


Fig. 4. Morphometric analysis of the lorica of *Brachionus* sp. “Alvarado” at different salinities. *Significant ($P < 0.05$).

rapid maximum growth occurring at moderate salinities at 10–20‰, increasing at high salinities for marine species, which means that the *B. plicatilis* species complex shows close relation with its habitat depending on the genotype (Miracle and Serra, 1989; Anitha and George, 2006); showing a higher “*r*” as a result of adaptation to an optimum salinity (Anitha and George, 2006). *B.* “Alvarado” shows a decrease in the growth rate when salinity increases at 30‰, perhaps because this strain is originated in a coastal lagoon with moderate salinity. The experiments performed in this study demonstrate that reproductive value decreases at 30‰ in comparison with other salinities under the same illumination conditions. This can be explained by a high consumption of ATP due to the intense activity of the ATPase Na^+/K^+ at high salinities, which drives a decrease in growth and reproduction (Lowe *et al.*, 2005).

The R^2 values show a strong relationship between survivorship and life expectancy in all treatments (Table 3). On the other hand, the 100% hatching of parthenogenetic eggs were completed at 20 h at 30 and 25 °C, while at 20 °C, at 20 h only 85% was recorded. The 100% hatching rate was achieved at 24 h. These experiments are allowed to consider 25 °C as an adequate temperature for all treatments and to obtain a better hatching percentage for this rotifer strain (Fig. 3). Fielder *et al.* (2000), mention that salinity has higher effect than temperature. However, it is convenient to develop further studies to assess the effect of temperature in the life history of *Brachionus* “Alvarado”.

Kostopoulou *et al.* (2009) suggest that the length of the lorica seems to be the most conserved character in spite of environmental variations. However it should be included in the genetic analysis to discriminate the lineages. Nevertheless, morphometry is useful to study the size range of species/lineages in the *B. plicatilis* species complex to detect phenotypical variation. The size and morphology of the lorica are not good indicators of the species boundaries (Suatoni *et al.*, 2006). The morphometric analysis of the lorica of *Brachionus* sp. “Alvarado”

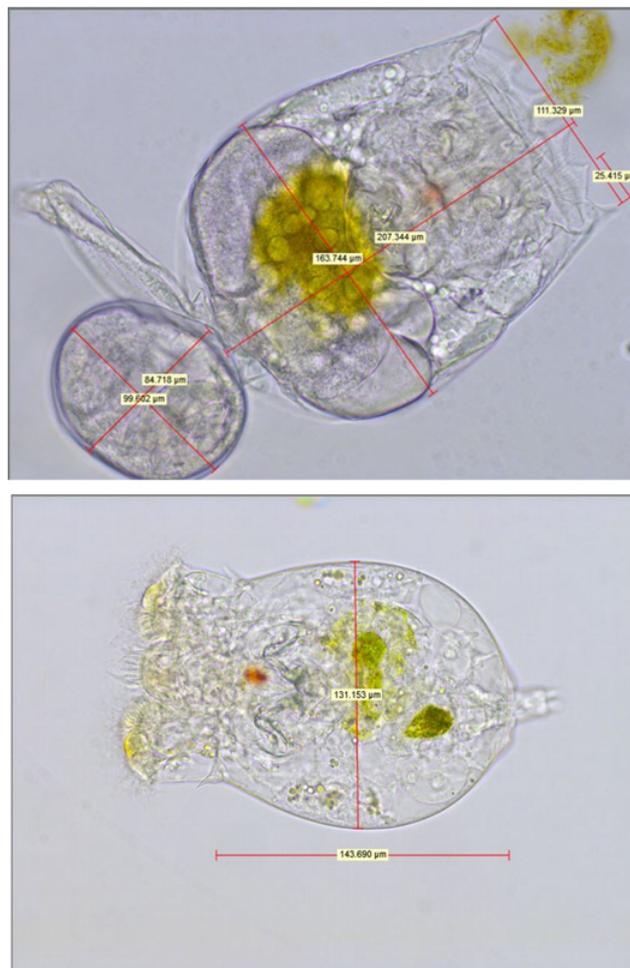


Fig. 5. Parthenogenetic female of *Brachionus* sp. “Alvarado” at 15‰ (40 ×). Adult with one parthenogenetic egg (upper) and neonate < 24 h old (lower).

shows that this strain acts as an osmoconformer organism (Table 4) (Lowe *et al.*, 2005), showing a more slender and elongated lorica when the salinity is increased. Anitha and George (2006) suggest that salinity influences in the osmoregulatory ability in rotifers which is in turn dependent of the genotype. Furthermore, the dimensions of *B.* “Alvarado” (177.5–204.3 µm) include this strain in the Medium Morphotype such as SM-clade (150–220 µm), in agreement with Conceição *et al.* (2010) and Rico-Martínez *et al.* (2013), and related to the dimensions reported for *B. ibericus* (193.5 µm) and *B.* “Almenara” (164–231 µm) (Kostopoulou *et al.*, 2009). Previous genetic analysis through an incomplete COI sequence and morphometric analysis suggest that our strain is closely related to *B.* Almenara (Jorge Ciro-Pérez, personal communication), and the strain *B.* Cayman and *B. ibericus* (Rico-Martínez *et al.*, 2013); *B.* Almenara was recorded for the Sontecomapan Lagoon (García-Morales and Elías-Gutiérrez, 2013) near of Alvarado Lagoon, both are connected to the Gulf of Mexico. This does not rule out the possibility that several strains coexist in the same aquatic system (Jorge Ciro-Pérez, personal

communication). Perhaps, one rotifer strain with higher growth rate such as *B. Cayman* would be the most representative (Kostopoulou and Vadstein, 2007). It is possible to find many species of the complex *B. plicatilis* that sometimes coexist in sympatry, subject to the seasonal successions, temporary partition of the niche, predation or parasitism (Gómez, 2005). Depending on the environmental variations such as salinity affects the differentiation of niches for each strain, as in the case of *B. plicatilis* and *Brachionus manjavacas*; which can coexist with different growth rates as adaptive differential response to salinity (Montero-Pau *et al.*, 2011). These ecological differences can occur when the clones belong to different lineages with optimal growth rates and patterns of mixis (Gómez, 2005). It is necessary to develop further studies that evaluate the limits of gene flow between sympatric species of the *B. plicatilis* complex, using specific mate recognition systems (Gómez *et al.*, 1997), as well as molecular methods (Gómez *et al.*, 1997; Gómez, 2005). This data provide preliminary information that contributes to the characterization of this new strain, although it is necessary to perform a thorough genetic analysis. The information generated in this study is useful to improve the intensive culture of the rotifer *Brachionus* “Alvarado” in the same way as those other strains such as *B. Cayman* are used in aquaculture, then *B.* “Alvarado” can be potentially used as live fed considering the fast growth, easy to culture, size and salinity tolerance; or to supply enough organisms to develop toxicity tests with a strain that is part of the *B. plicatilis* species complex from tropical aquatic ecosystems.

Conclusions

The best culture conditions for the rotifer *B.* “Alvarado” occurred at 15‰ salinity regardless of the illumination provided. This strain did not produce cysts in the laboratory conditions tested; this can be an advantage for aquaculture use because it allows producing parthenogenetic populations with rapid growth. The 100% hatching rate was achieved at 20 h at 25 and 30 °C. The morphometric data of *Brachionus* “Alvarado” showed that it is part of the SM clade in the *B. plicatilis* species complex. This study shows the feasibility of the use of this strain for aquaculture as live fed or to develop toxicity tests.

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