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RESEARCH ARTICLE

# Effects of the biochemical composition of three microalgae on the life history of the rotifer *Brachionus plicatilis* (Alvarado strain): an assessment

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**Abstract –** The biochemical composition of microalgae used as food is essential for aquatic species in commercial production systems, such as rotifers and microcrustaceans. Life table bioassays with the rotifer *Brachionus sp.* "Alvarado" strain were performed using three microalgae (*Nannochloropsis oculata, Dunaliella salina* and *Isochrysis* sp.) as food. Microalgae growth rate, dry weight and biochemical composition (protein, lipid, carbohydrate) and pigments (chlorophyll and carotenoid) were determined. The microalgae showed significant differences in their biochemical composition. *N. oculata* showed the highest growth rate, while *D. salina* showed the slowest growth rate, but instead, it displayed a higher content of proteins, lipids, carbohydrates, chlorophyll, and carotenoids per cell. Rotifer life table analysis showed no significant differences among any of the microalgae as food bioassays. However, *Isochrysis* sp. had a higher effect on the net reproductive rate of the rotifer *Brachionus sp.* "Alvarado" followed by *D. salina, w*hile *N. oculata* showed a higher effect on life expectancy and generation time. In conclusion, the three microalgae are found to be useful to support rotifer cultures; however, both, *D. salina* and *Isochrysis* sp., might improve the rotifer culture due to better growth and reproduction in short time. This information is useful to implement the culture of this tropical strain of *Brachionus plicatilis* complex in order to obtain high population densities, making rotifers available for several applications such as the establishment of larviculture in hatcheries, bioassays for ecological studies or to assess its sensitivity through toxicity tests.

**Keywords:** Fatty acids / life table / reproductive value / strain

## 1 Introduction

Live food in aquaculture is composed primarily by phyto- and zooplankton (Prieto *et al.,* 2006) which are attractive for their capture in contrast with inert food since they have features such as movement and color. Live food also helps to preserve water quality because it is consumed before it reaches the bottom of tanks (Castro *et al.,* 2003). Microalgae are the base of aquatic food chains and the principal producers of dissolved oxygen in aquatic environments. Microalgae uptake is fundamental for many aquatic species with commercial value such as filtrating mollusks, penaeids shrimps and fish larvae (Muller-Feuga, 2000) and determines their survival, development and biotic success in controlled cultures (Pacheco *et al.,* 2010; Guedes and Malcata, 2012). *Chaetoceros*, *Thalassiosira*, *Tetraselmis*, *Isochrysis*, *Nannochloropsis*, *Pavlova* and *Skeletonema* microalgae genera are the most extensively used as live food (Guedes and Malcata, 2012). Their biochemical composition is variable depending on the species, type and the amount of light, temperature, salinity and growth phase (Gatenby *et al.,* 2003). The most important elements in algal biomass are proteins, which may constitute more than 50% of dry weight and together with lipids and carbohydrates they constitute up to 90% of it, while minerals, nucleic acids, pigments and other components constitute the remaining 10% (Arredondo *et al.*, 2007). Several studies indicate that dry weight microalgae in the logarithmic phase contains from 12 to 40% of protein, 7.2 to 23% of lipids and 4.6 to 23% of carbohydrates (Conceição *et al.*, 2010; Guedes and Malcata, 2012). Thus, the protein content defines their nutritional value,

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which depends on the culture media used (Conceição *et al.*, 2010).

Microalgae are widely used in aquaculture, mainly for larvae cultures. Their biochemical components have important roles; that is proteins have a major role in tissue regeneration, growth or the formation of new structures and as a source of energy. They also provide energy for the development of cultured organisms (Vásquez *et al.,* 2007). *Dunaliella salina, Isochrysis sp.* and *Nannochloropsis oculata* have been widely studied as live feed. *D. salina* has been recommended as direct feed or nutrimental complement in diets (Vásquez *et al.*, 2007), due to its ability to produce an excess of β-carotene and glycerol as a strategy to preserve its osmotic equilibrium (Blas-Valdivia *et al.,* 2012), and also because of its high concentration of other carotenes such as lutein, neoxanthyne, zeaxanthyne, violaxanthyne, cryptoxanthyne (Chacón and González, 2010), that is, it is used to increase vitamin levels in shrimp farms and also to provide an optimal coloration in fish. *Isochrysis* sp. has the potential to produce polyunsaturated fatty acids, especially docosahexaenoic acid (DHA) (Hemaiswarya *et al.,* 2010). It is used in mariculture to feed larvae (Liu and Lin, 2001) and enriches zooplankton such as *Artemia* and is used to feed shrimp, copepods, oysters and scallops (Hemaiswarya *et al.*, 2010). *N. oculata* is used in mariculture as a source of omega-3, eicosapentaenoic acid (EPA), arachidonic acid (ARA), DHA (Sánchez *et al.,* 2008; Zou *et al.,* 2010). *Nannochloropsis sp., Nannochloris sp., Chaetoceros sp., Dunaliella sp., Pyramimonas, Isochrysis sp., Isochrysis galvana, Pavlova lutheri,* and *Tetraselmis* sp. are among the most used marine microalgae in rotifer cultures (Hoff and Snell, 2008; Conceição *et al.*, 2010; Rico-Martínez *et al.,* 2016), sometimes mixed to improve the rotifer culture.

Rotifers are important in aquatic environments due to their high reproduction rate. They are dominant in planktonic communities and link the microbial community with higher trophic levels (Rico-Martínez *et al.*, 2016). The most cultivated rotifers of the *Brachionus* genus are *Brachionus plicatilis, Brachionus rotundiformis*, and *Brachionus calyciflorus* (Yúfera, 2001; Kostopoulou *et al.,* 2012; Rico-Martínez *et al.*, 2016). *B. plicatilis* is widely used in larviculture of marine fish and crustaceans (Hagiwara *et al.,* 2001; Yin and Zhao, 2008; Kostopoulou *et al.,* 2009; Conceição *et al.*, 2010; Kostopoulou *et al.*, 2012). Rotifers, used as food during the first days after the larvae opens the mouth, contribute to diminish the high mortalities that occur at this early phase. Rotifer availability helps to overcome the economic bottleneck that high mortalities represent for aquaculturists during larvae development (Kostopoulou *et al.,* 2009). The *B. plicalitis* complex is considered as a cosmopolitan species (Yin and Zhao, 2008; Kostopoulou *et al.*, 2012). However, it includes several strains such as: (a) *B. plicatilis sensu stricto*, and *Brachionus manjavacas* (L-type), (b) *B. rotundiformis* (S-type), and (c) *Brachionus ibericus* and *Brachionus* "Almenara" (SM-type), mainly defined by size. The size of the rotifer strain determines its suitability to feed a certain larvae species based on a relation with the larvae mouth opening. Thus, the success of the larvae culture depends of the size of the rotifer strain used (Kostopoulou *et al.,* 2009). According to Moha-León *et al.* (2015), *Brachionus* sp. "Alvarado" belongs to SM clade, and it shows worthy features for larviculture such as size, fast growth rate, and ease of culture. Therefore, the goal of this study was to assess the effect of the biochemical composition of three microalgae species *D. salina*, *N. oculata* e *Isochrysis sp*., and their effect on *Brachionus* sp. 'Alvarado' population dynamic, in order to contribute to improve the knowledge on the culture conditions for this tropical strain for further studies such as larviculture, ecological, or toxicological research.

## 2 Materials and methods

The microalgae *D. salina* (DUS1), *N. oculata* (NNO1), and *Isochrysis sp*. (ISX1), were obtained from the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE) microalgae collection; located in Ensenada, B.C. Mexico. Microalgae were cultured in Guillard "f/2" media (Stein, 1979) at 18 ± 2 °C with constant illumination (without dark period) using cold white light lamps (59.91 μE.m$^{-2}$ sec$^{-1}$), providing continuous aeration and adding a new f/2 media to refresh the culture. *N. oculata* was cultured at 20‰ and both *D. salina* and *Isochrysis sp*. at 35‰.

Rotifer culture of *Brachionus sp*. 'Alvarado' strain has been maintained continuously for more than 6 years in 25 ± 2 °C in synthetic marine media with 15‰, 8.2–8.4 pH, and using *N. oculata* as feed, using the illumination of 3 000 luxes, according to Pérez-Legaspi and Rico-Martínez (1998) with slight modifications performed by Moha-León *et al.* (2015). This rotifer strain was collected from the tropical Alvarado lagoon system (18° 46'–18° 42' N and 95° 34'–95° 58' W), Veracruz, Mexico (Moha-León *et al.*, 2015); and identified as belonging to the *B. plicatilis complex* (Rico-Martínez *et al.,* 2013). The morphometric measures performed in a previous study allowed to include this strain within the medium morphotype (150–220 μm) with a total length of 177.5–204.3 μm and lorica amplitude of 145.75–153.9 μm (Moha-León *et al.*, 2015). This strain has not been fully evaluated neither with a DNA sequence, nor by reproductive isolation.

### 2.1 Microalgae analysis

Duplicate 400 mL samples of each microalgae culture in logarithmic phase were obtained to perform further analysis; also equal cell density ($1 \times 10^6$ cell.mL$^{-1}$) was analyzed by diluting the samples preparing a stock solution with similar cell density for each microalgae strain. Growth rate was measured using cell density. Every 24 h cell counts were performed by triplicate using a Neubauer chamber (Loptik Labor) and optical compound microscope (Carl Zeiss) at 40×, considering all the cells in both sets of the grid from the chamber and using a hand counter, following the protocol of Hoff and Snell (2008), and Moheimani *et al.* (2013). Dry weight biomass was determined using the gravimetric method. All samples were centrifuged (3 000 revs for 30 min at 10 °C) (Cence H1650R), washed with 0.5 M ammonium format solution (Sayegh *et al.,* 2007), filtered using filter paper (55 mm Whatman GF/C Microfiber) and algae were collected in a membrane and stored at − 20 °C. Biomass wet weight in the filter paper was registered using an analytical balance (210 g × 0.1 mg) Mod. TP-214 (Denver Instrument, Co.) and then transferred to petri dishes and dried in a culture oven (ECOSHEL 9162) for 24 h at 70 °C. Dry weight was determined using an analytical balance

and desiccator to assess the weight of the filters plus algal sample in triplicate until reading a constant weight, and biomass determined by the difference in weight.

The Bradford method (Kruger, 2002) was used to determine protein, after hydrolysis with 0.1 N NaOH for *Isochrysis sp*. and *D. salina* at 100 °C during 10 m and 1 h, respectively; for *N. oculata* 1 N NaOH during 1 h at 100 °C as described in Arredondo *et al.* (2007). Absorbance was recorded at $\lambda = 595$ nm using a uv/vis spectrophotometer (Thermo Scientific Genesys 10S UV-Vis). Samples were calibrated against egg albumin (Golden Bell) curve. Carbohydrates were determined using the phenol-sulphuric acid colorimetric method (Dubois *et al.,* 1956). Absorbance was recorded at $\lambda = 490$ nm. Samples were calibrated against D-Glucose anhydrous (Labessa) curve. Chlorophorm/methanol (1:2 v/v) Soxhlet extraction was used to obtain lipids, according to Halim *et al.* (2012). Total lipids were determined using the gravimetric method Del Ángel *et al.* (2007). Pigment analysis was determined using acetone extraction (Arredondo and Voltolina, 2007). Both, chlorophyll extraction (*a* and *b*) and total carotenoid concentration were calculated from 20-mL algal sample in exponential phase. Samples were precipitated using a centrifuge, supernatant was extracted and 100% acetone was added, agitating sample with a vortex (Science Med MX-S) and sonicating the sample for 5 min in a sonicator (Branson). Samples were stored for 16 h at 4 °C and sonicated again. Samples were then centrifuged (3 000 rev for 10 min at 4 °C), and supernatant recuperated and transferred to a quartz cell. Absorbance was read at three wavelengths $\lambda = 630$, 647, 664 nm for chlorophyll "a" and "b". Jeffrey and Humphrey (1975) equations were used to determine the concentration. Carotenoid absorbance was read at $\lambda = 480$ nm for carotenoids using Strickland and Parsons (1972) equations to determine concentration.

### 2.2 Rotifer bioassays

Full cohort life table bioassays of *Brachionus* sp. 'Alvarado' fed with *D. salina, N. oculata* and *Isochrysis* sp were performed according to Moha-León *et al.* (2015). The neonates were obtained from parthenogenetic eggs by shaking ovigerous females in order to induce egg delivering collecting all of them. Bioassays used 5 parthenogenetic neonates (<2 h old) per well in synthetic marine media (15‰), 2-mL volume containing $1 \times 10^6$ cell.mL$^{-1}$ at 25 ± 2 °C of one of each of the three microalgae species. Five replicates of each bioassay were performed and monitored every 12 h ($x$). Original females (parents) were transferred to a fresh medium with the same microalgae concentration, and eggs and offspring were counted, registered, and removed. Plates were monitored until the last original parent died. Life table analysis for each bioassay included hatching rates using 12 h intervals ($x$), mean generation time (Tc), net reproductive potential (Ro), intrinsic growth rate ($r$), life expectancy (ex), reproductive value (Vx), and finite index of increase ($\lambda$) (Begon *et al.,* 1996). One-way analysis of variance (ANOVA) was performed using Statistica 7.0 (Statsoft, Inc. 2004) to determine the effect between the biochemical composition of microalgae and the net reproductive potential of *Brachionus* sp. 'Alvarado'. One-way ANOVA and Tukey test ($p < 0.05$) post hoc comparisons were performed to assess the differences in the biochemical composition for the microalgae.

## 3 Results

The comparison of cell densities between the three microalgae species showed that both *Isochrysis* sp. and *N. oculata* have similar growth rates but different yield rates. *N. oculata* had the highest yield rate reaching higher cell density faster than the other two species, while *D. salina* has the slowest growth rate (Fig. 1). The Biochemical composition varied significantly among the three assessed species: *D. salina, N. oculata,* and *Isochrysis sp*. On the biochemical composition, when matching the same density ($1 \times 10^6$ cell. mL$^{-1}$), *D. salina* showed significant differences ($p < 0.05$) in the content of proteins and carbohydrates; and higher lipid content (not significant ($p < 0.05$)) probably associated to its biggest size and biomass. The analysis in a 400 mL sample resulted in *D. salina* showing only significant ($p < 0.05$) content of carbohydrates and proteins. However, *N. oculata* showed the highest content of lipids although are not significant ($p < 0.05$), followed by *Isochrysis* sp. and *D. salina* probably associated to the difference in growth and yield rates (Figs. 1 and 2). The pigment analysis shows that *D. salina* had higher chlorophyll "a" and "b" concentration and *Isochrysis* sp. had higher carotenoids concentration (μg.mL$^{-1}$) followed by *D. salina* (Fig. 3). Rotifer *Brachionus* sp. 'Alvarado' strain reproduces better, after 48 h, when fed with *D. salina* and *Isochrysis* sp. according to their reproductive value (Vx) (Fig. 4) and net reproductive rate (Ro) (Tab. 1). Rotifers fed with *Isochrysis* sp. had the highest reproductive value, followed by those fed with *D. salina*. However, the highest life expectancy (ex) and generation time (Tc) were observed when rotifers were fed with *N. oculata*, followed by those fed with *Isochrysis* sp. Rotifers fed with *D. salina* showed greater longevity (Tab. 1) and highest fertility rate (87.13%) in contrast with those fed with *Isochrysis sp.* (69.64%) and *N. oculata* (62.03%). Statistical analysis (ANOVA) showed a non significant effect between the algae used as food on the reproductive value (Vx), average life span (ex) or the rate of natural increase ($r$) (Tab. 2). No further analysis was performed.

## 4 Discussion

Our results show that it is possible to use any of the three microalgae as food to cultivate the rotifer *Brachionus* sp. strain 'Alvarado' as they display similar values in the intrinsic rate of natural increase ($r$). However, the highest values in the net reproductive rate (Ro) and reproductive value (Vx) were observed when supplied *Isochrysis* sp. and *D. salina,* although differences are not statistically significant ($p < 0.05$) (Fig. 4, Tab. 1). Therefore, these two microalgae contribute favorably to the reproduction of this rotifer strain, despite the density any of the three algae reached (Fig. 4). These results are comparable to those obtained in similar studies, where the higher net reproductive rate for *B. plicatilis* occurs when fed with different strains of *I. galvana* in contrast with those fed with *Nannochloropsis sp.* (Sayegh *et al.*, 2007). In addition,

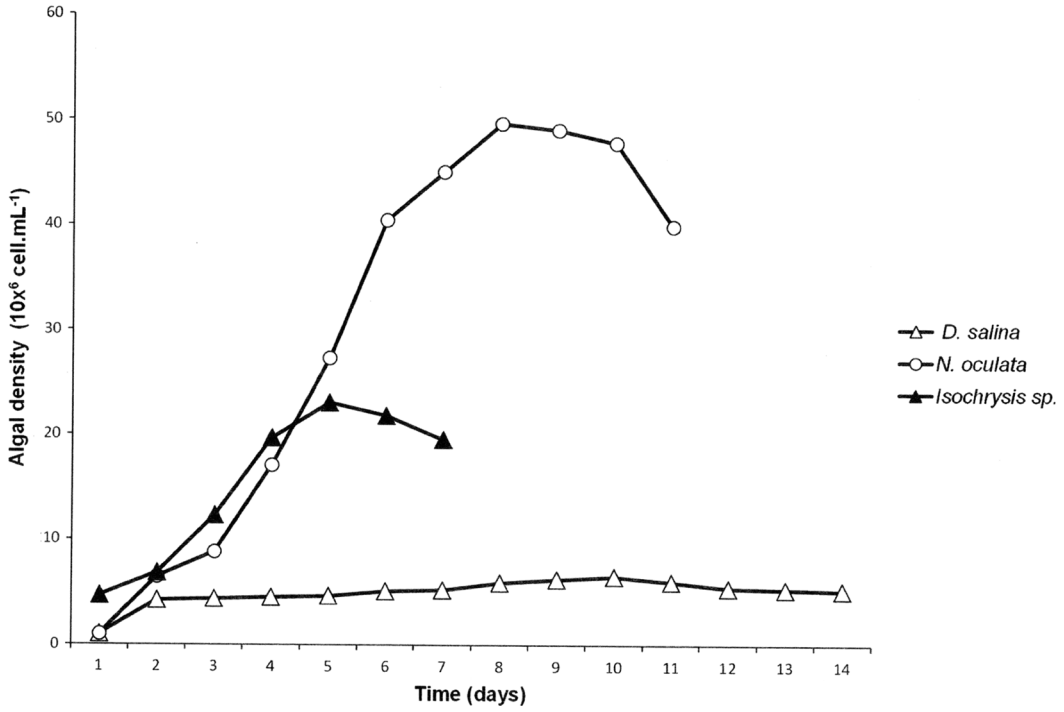


**Fig. 1.** Growth phase of the three algae used as food for the rotifer *Brachionus* sp. "Alvarado" strain.

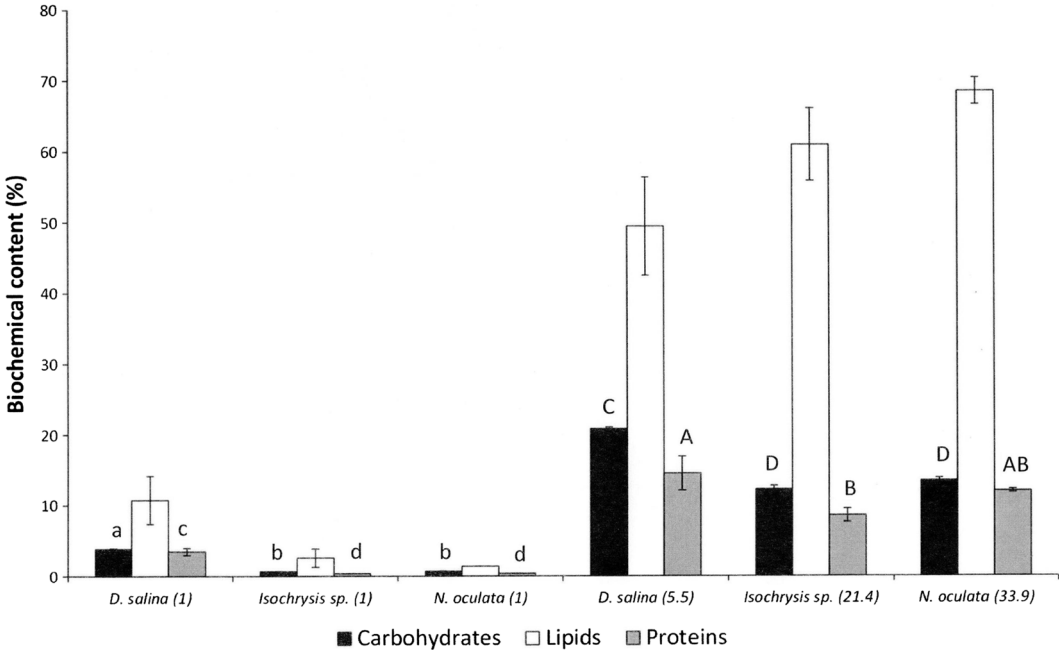


**Fig. 2.** Biochemical analysis of the algae used in this study. Numbers in parenthesis refer to the cell density at $10^6$ cell.mL$^{-1}$. The bars correspond to the mean ± one standard deviation. Different letters represent statistically significant differences in nutrient content among species ($p < 0.05$).

*B. plicatilis* has a higher reproductive rate when fed with *I. galvana* than with *Tetraselmis sp*. and *Nannochloris atomus* (Korstad *et al.,* 1989). On the other hand, Yin and Zhao (2008) suggest that *D. salina* is a better food source for *B. plicatilis* s.s. They obtained a higher population growth rate than with other microalgae (*Synechococcus sp., Chlorella pyrenoidosa, Isochrysis zhanjiangensis,* and *Tetraselmis cordiformis*).

Microalgae species possess features that make them adequate for its use as food for rotifer species, such as appropriate size and shape for ingestion, easy digestion, fast growth rates and stability to fluctuations in temperature, as well as light and nutrient profile (Brown, 2002). Under the culture conditions for the three microalgae used for this study, we found *N. oculata* had the highest yield rate. This was also

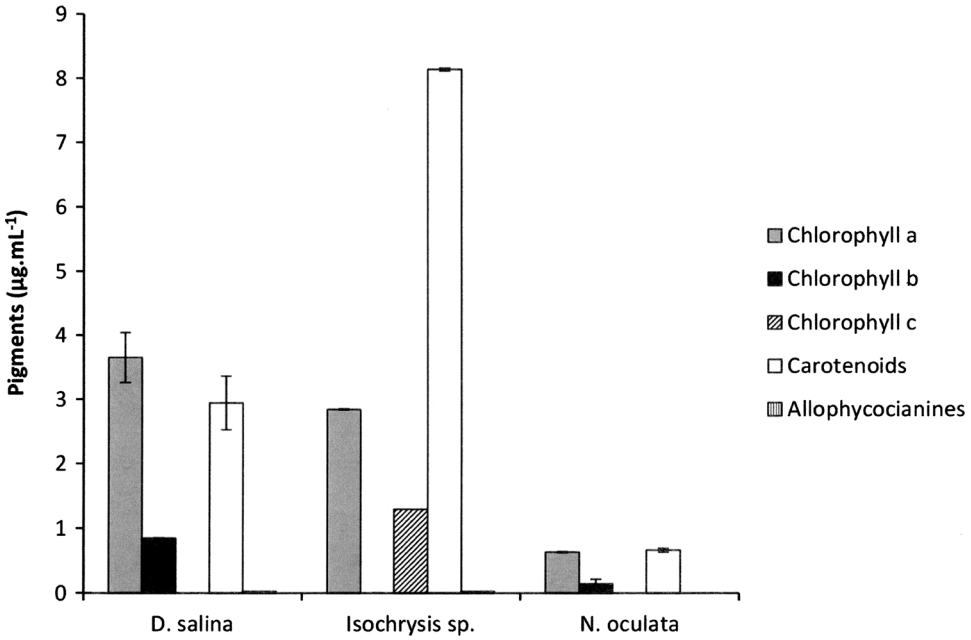


**Fig. 3.** Pigment analysis of the three algae in log phase. The cell densities from each algae correspond to 5.6, 16.2, and 32.6 × $10^6$ cell.mL$^{-1}$ for *D. salina*, *Isochrysis* sp., and *N. oculata*; respectively. The bars correspond to the mean ± one standard deviation. All the pigments content among the three species were statistically different ($p < 0.05$).

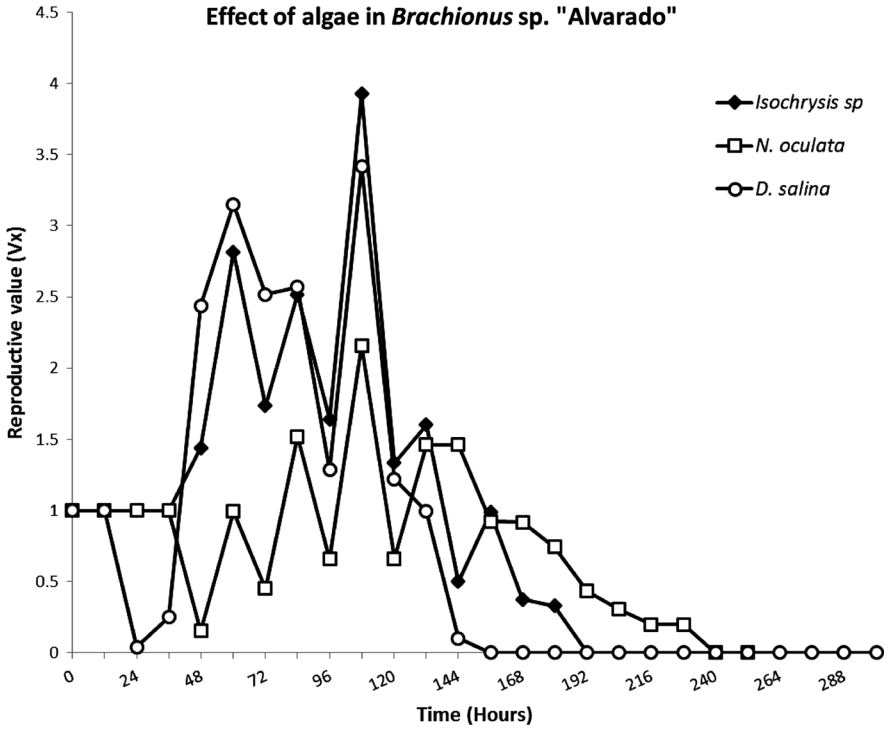


**Fig. 4.** Reproductive value (Vx) of *Brachionus* sp. "Alvarado" strain, fed with three different algae.

reported by Kobayashi *et al.* (2008). *D. salina* showed the slowest growth rate (Fig. 1), but in turn, it showed an adequate biochemical composition for its use as feed for rotifer mass cultures. Sayegh *et al.* (2007) recorded significant differences in growth rate, cell volume, and dry weight between different strains of *I. galbana* and *Nannochloropsis* sp.

In our experiment *N. oculata* showed less carbohydrates, lipids and proteins content per cell than *I. galbana*. *N. oculata* is widely used for rotifer cultures (Hoff and Snell, 2008; Conceição *et al.*, 2010; Rico-Martínez *et al.*, 2016), to obtain higher growth rates associated to its high amounts of fatty acids, such as EPA, omega-3, ARA and DHA (Kobayashi

**Table 1.** Life table of the rotifer *Brachionus* sp. "Alvarado" fed with three types of microalgae.

| Algae | *X* | ex | Tc | *r* | Ro | λ |
|---|---|---|---|---|---|---|
| *D. salina* | 300 | 8.94 | 44.33 | 0.1908 | 17.69 | 1.21 |
| *Isochrysis sp.* | 204 | 10.44 | 97.67 | 0.1822 | 19.19 | 1.19 |
| *N. oculata* | 252 | 10.32 | 126.31 | 0.1898 | 13.23 | 1.20 |

*Abbreviations*: *X* (Hours), ex (Life expectancy); Tc (Mean generation time); *r* (Intrinsic rate of natural increase); Ro (Net reproductive rate), ⅄(Infinite index of increase).

**Table 2.** Analysis of variance (one-way ANOVA) performed for life table parameters, and the microalgae used as food for the rotifer *Brachionus* sp. "Alvarado" strain.

| Effect | SS | DF | MS | *F* | *p* |
|---|---|---|---|---|---|
| Average lifespan | | | | | |
| Microalgae | 4.291 | 2 | 2.146 | 0.2529 | $0.777294^{ns}$ |
| Error | 534.406 | 63 | 8.483 | – | – |
| Rate of natural increase | | | | | |
| Microalgae | 9.1652 | 2 | 4.5826 | 2.1332 | $0.126935^{ns}$ |
| Error | 135.3384 | 63 | 2.1482 | – | – |
| Reproductive value | | | | | |
| Microalgae | 2.36367 | 2 | 1.18184 | 1.18166 | $0.313475^{ns}$ |
| Error | 63.00947 | 63 | 1.00015 | | |

*Abbreviations*: SS (Sum of Squares), DF (Degrees of Freedom), MS (Mean Squares), *F* (*F*-ratio), *p* (*p* value). ns (non-significant $p > 0.05$), $N = 25$.

*et al.*, 2008; Sánchez *et al.*, 2008; Ferreira *et al.*, 2009; Zou *et al.*, 2010). *D. salina* and *Isochrysis* sp. had a higher content of carbohydrates, lipids, proteins, and pigments than *N. oculata*; thus, favoring an increase in the reproductive rate of the assessed rotifer, *Brachionus* sp. 'Alvarado' (Figs. 2 and 3). Korstad *et al.* (1989) registered a highest survival and reproduction in *B. plicatilis* when fed with *I. galvana* than whith *Tetraselmis* sp. and *N. atomus*. Yin and Zhao (2008) suggested that flagellated algae such as *Dunaliella* and *Isochrysis* do not adhere to surfaces distributed in the water column, favoring food availability for ingestion and thus, increasing grazing efficiency. There is support for selective feeding in *B. plicatilis* (Pagano *et al.*, 1999; Hotos, 2002). Grazing selectivity for *Dunaliella sp*. has been reported by Corcoran and Boeing (2012). They concluded that its particle size would be closer to the optimal particle size for *B. plicatilis* than the other five microalgae species assessed. Our findings showed that any of the three species could be used as feed for *B. plicatilis* "Alvarado" strain. We highlight that *D. salina* is also an appropriate algae species to feed *Brachionus* sp. "Alvarado" and other rotifer species belonging to the SM clade from *B. plicatilis* complex due to its biochemical composition (high carbohydrate, protein, and lipid content) and also, because its size and mobility. Even when, *D. salina* showed the lowest growth rate within the three species assessed, it is also appropriate for mass cultures and shows worthy features for larviculture such as size, fast growth rate, ease of culture and the absence of cysts generation (Moha-León *et al.*, 2015). The aim of intensive rotifer culture systems for feeding fish or crustacean larvae is to achieve efficient mass culture to obtain sufficient biomass (Hagiwara *et al.*, 2001) and the biochemical composition of feed is one of the key factors to succeed (Brown, 2002). The advantage of the local strain *Brachionus* sp. "Alvarado" is that it is already adapted to tropical aquatic systems. Thus, becoming an alternative of live food for larvae cultures in the tropical zones, or for develop toxicity tests to assess the health of tropical coastal ecosystems.

## 5 Conclusions

Our results provide information on the biochemical composition of three microalgae species used as food for the rotifer *Brachionus* sp. Alvarado. *D. salina and Isochrysis sp*. have a higher content of carbohydrates, lipids, and proteins than *N. oculata* had. There were no significant differences among the three types of feed. However, our results support the recommendation of using both *D. salina* and *Isochrysis* sp. as feed for the rotifer, because they have an appropriate biochemical composition, size, and availability and may increase the rate of reproduction, in order to improve rotifer culture for different aquaculture and environmental research applications.

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