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, while 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 filtering mollusks, penaeid 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,
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 nutritional 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, neoxanthine, zeaxanthine, violaxanthine, cryptoxanthine (Charón and Gonzalez, 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) (Hemaisswarya 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 (Hemaisswarya 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 galvania, 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; Kostopoulos 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; Kostopoulos et al., 2009; Conceição et al., 2010; Kostopoulos et al., 2012). Rotifiers, used as food during the first days after the larvae open 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 (Kostopoulos et al., 2009). The B. plicatilis complex is considered as a cosmopolitan species (Yin and Zhao, 2008; Kostopoulos et al., 2012). However, it includes several strains such as: (a) B. plicatilis sensu stricto, and Brachionus manjavacas (L-type), (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 on the size of the rotifer strain used (Kostopoulos 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 × 10⁶ cell.mL⁻¹) was analyzed by diluting the samples preparing a stock solution with similar 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 Mohheimani 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 (Sayedegh 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 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 λ = 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 λ = 490 nm. Samples were calibrated against D-Glucose anhydrous (Labessa) curve. Chlorphorm/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 λ = 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 λ = 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 ovigorous 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 × 10⁶ cell.mL⁻¹ at 25 ± 2 °C of one of each of the three microalgal 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 microalgal concentration, and eggs and offspring were counted, registered, and removed. Plates were monitored until the last original parent died. Live 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 (λ) (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 lowest 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 × 10⁶ 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,
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*).

Microalga 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. 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 10⁶ 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).
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 et al. 2008).

**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 × 10⁶ 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.
Table 1. Life table of the rotifer Brachionus sp. “Alvarado” fed with three types of microalgae.

<table>
<thead>
<tr>
<th>Algae</th>
<th>X</th>
<th>ex</th>
<th>Tc</th>
<th>r</th>
<th>Ro</th>
<th>λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. salina</td>
<td>300</td>
<td>8.94</td>
<td>44.33</td>
<td>0.1908</td>
<td>17.69</td>
<td>1.21</td>
</tr>
<tr>
<td>Isochrysis sp.</td>
<td>204</td>
<td>10.44</td>
<td>97.67</td>
<td>0.1822</td>
<td>19.19</td>
<td>1.19</td>
</tr>
<tr>
<td>N. oculata</td>
<td>252</td>
<td>10.32</td>
<td>126.31</td>
<td>0.1898</td>
<td>13.23</td>
<td>1.20</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average lifespan</td>
<td>4.291</td>
<td>2</td>
<td>2.146</td>
<td>0.2529</td>
<td>0.777294*</td>
</tr>
<tr>
<td>Microalgae</td>
<td>534.406</td>
<td>63</td>
<td>8.483</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Error</td>
<td>9.1652</td>
<td>2</td>
<td>4.5826</td>
<td>2.1332</td>
<td>0.126935*</td>
</tr>
<tr>
<td>Rate of natural increase</td>
<td>135.3384</td>
<td>63</td>
<td>2.1482</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Microalgae</td>
<td>2.36367</td>
<td>2</td>
<td>1.18184</td>
<td>1.18166</td>
<td>0.313475*</td>
</tr>
<tr>
<td>Error</td>
<td>63.00947</td>
<td>63</td>
<td>1.00015</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 (Moholeón 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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