Nutritional value and population growth of *Brachionus plicatilis* fed with endemic microalgae from North Pacific

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**ABSTRACT.** In the present study, the potential of three isolated microalgae strains from Bahía de La Paz, Baja California Sur, Mexico and identified as *Schizochytrium* sp. (Inner key: LPU-1), *Chaetoceros* sp. (LPU-2), and *Chaetoceros* sp. (LPU-3) for the cultivation of the rotifer *Brachionus plicatilis* was tested. The effects of isolated strains on the population growth and nutritional content (proteins, lipids, carbohydrates, and fatty acid composition) of *B. plicatilis* were evaluated. The feeding essay of *B. plicatilis* was carried out at 32 ± 2°C. Treatments were established using a monoalgal and dialgal diet: *Schizochytrium* sp. (LPU-1), *Schizochytrium* sp. (LPU-2), *Chaetoceros* sp. (LPU-3), *Chaetoceros* sp. (LPU-1)/*Chaetoceros* sp. (LPU-2), *Chaetoceros* sp. (LPU-1) and *Schizochytrium* sp. (LPU-2)/*Chaetoceros* sp. (LPU-3). The results show that the rotifers population growth ratio was higher with *Schizochytrium* sp. (LPU-1) 0.88 ± 0.43, *Chaetoceros* sp. (LPU-3), 0.87 ± 0.37, and from both 0.87 ± 0.40. The results show that the native microalgae of a North Pacific area mixed are an excellent source of nutrients for the growth and enhancement of the nutritional value of the rotifers, which can be used in the future to feed the larvae of marine fish more nutritionally and economically.

**Keywords:** *Brachionus plicatilis*; rotifer; microalgae; fatty acids; aquaculture

**INTRODUCTION**

Since the beginning of the '90s, marine fish larvae micro diets were tested by several research groups (Person Le Ruyet *et al.*, 1993; Cahu & Zambonino-Infante, 1994; Fernández-Díaz & Yúfera, 1997; Takeuchi *et al.*, 1998; Cahu *et al.*, 2003); however, live preys continues to be the main nutritional source for the early life stages of marine and freshwater fish in aquaculture (Støtttrup & McEvoy, 2003). Hence, the utilization of rotifers (*Brachionus plicatilis*) as starter feed for the rearing of marine fish larvae and crustaceans are still essential for commercial marine hatchery procedures (Bengtson, 2003).

A balanced nutritional diet is crucial for embryo development and further larvae metamorphosis (Watanabe & Kiron, 1994). Among all the nutritional requirements, lipids play an important role in larval growth and survival, eicosapentaenoic acid 20:5 (n-3) (EPA) and docosahexaenoic acid 22:6 (n-3) (DHA) are considered vital and essential acids due to their presence in the plasma membrane is highly abundant and marine fish larvae cannot synthesize them from the linoleic acid 18:3 (n-3). More specifically, DHA is present in higher concentrations in the neural and visual tissues. Therefore, a lack of this essential acid affects negatively several physiological and behavioral events (Estévez *et al.*, 1999; Sargent *et al.*, 1999a,b). All the above justifies that marine fish larvae must be acquired polyunsaturated fatty acids (HUFAs) through their diet eating zooplankton (*i.e.*, rotifers, crustaceans, etc.), which are enriched with these nutrients. Increasing the HUFA content of zooplankton before feeding larval fish and shrimp is a regular practice in the aquaculture industry (reviewed by Apt & Behrens, 1999).
The exit of massive rotifer culture brings many diets such as microalgae, yeast and commercial mixtures with the purpose of increasing the nutritional value and biomass. The use of microalgae and commercial mixtures are the best alternatives. Nonetheless, phytoplankton production in research centers that are located in tropical and subtropical ecosystems fails to be profitable because of the operational expenses in the hatcheries, mainly caused by the need of refrigeration systems for the growth and maintenance of the strains. Even more, it also fails to be profitable when the commercial mixtures contaminate the water used for larvae culture, decreasing their survival rate.

In the particular case of microalgae, they have been used for mass production and enrichment of rotifers due to the content of essential nutrients such as polyunsaturated fatty acids, vitamins, amino acids and pigments that can be transferred to superior trophic levels. The following species are commonly used for the above-mentioned purpose, *Nannochloropsis* sp., *Isochrysis galbana*, *Pavlova lutheri*, and *Chaetoceros muelleri* (Brown et al., 1997). However, they all require a controlled temperature of 19°C in tropical climates, which is incompatible with the range 28-35°C established for optimal growth for *B. plicatilis* (Dhert, 1996); this difference between temperatures trigger the inhibition of microalgae growth by heat stress (Pacheco-Vega et al., 2015). Then, we can hypothesize that endemic microalgae cultures can be obtained at room temperature and these can be used in the cultivation of rotifers *Brachionus plicatilis*, and will give them good nutrient content. Thus, this study aimed to evaluate three potential microalgae strains, one *Schizochytrium* sp. from the Thraustochytriaceae family and two strains from the *Chaetoceros* genus, as a diet for the rotifer *B. plicatilis* to diversify the microalgae species in aquaculture and improve their nutritional profile.

**MATERIALS AND METHODS**

Experiments were conducted at Unidad Pichilingue of the Universidad Autónoma de Baja California Sur. Microalgae strains: *Schizochytrium* sp. (LPU-1), *Chaetoceros* sp. (LPU-2), and *Chaetoceros* sp. (LPU-3), growth in natural seawater enriched with F/2 (Guillard, 1973) and silicate minerals for both *Chaetoceros* genus strains at 22 ± 1°C and constant artificial light of 2,500 lux.

For microalgae and rotifer culture we filtered sea water (5.0 and 1.0 µm), which then was deposited in a 3,000 L reservoir equipped with an ultraviolet light disinfection system. The water was recirculated for 24 h in this system and, depending on the use, transferred to a 400 L reservoir, where 1 mL of commercial sodium hypochlorite solution was added to 1 L of sea water and left at rest for 24 h. The posterior neutralization of sodium hypochlorite was performed with sodium thiosulfate at a rate of 0.05 g mL⁻¹, which was corroborated with the colorimetric orthotolidine test.

**Culture of microalgae**

All cultures were initiated in sterile 125 mL Erlenmeyer flask containing 90 mL of culture sea water with F/2 medium and 10 mL of microalgae inoculum. The culture was then transferred to a 1 L Erlenmeyer flask containing 900 mL of culture sea water with F/2 medium and 100 mL of microalgae inoculum. The culture sea water with F/2 was autoclaved under 1.02 kg cm⁻² of pressure for 20 min. After five days, the culture was inoculated into a 19 L polyethylene carboy, previously sanitized and filled with sea water disinfected with sodium hypochlorite. Cell counting was done with a Neubauer chamber for the following seven days for each microalgae in triplicate. The growth rate was calculated by the following equation

$$\mu = \left(\frac{\ln N_t - \ln N_0}{t_1 - t_0}\right)$$

where $N_1$ y $N_0$ are the cell densities at the beginning and end of the exponential stage, respectively, measured at times $t_1$ y $t_0$. For the bromatological analysis and further fatty acids profile determination from the microalgae biomass, 15 L from each microalgae culture was centrifuged in the exponential phase (day 5), frozen to -80°C and lyophilized.

**Rotifer culture**

The experimental units were 19 L polyethylene carboys. Fluorescent lamps were adjusted to 1,000 lux in the external edge of each culture unit. The physical and chemical parameters of the rotifer culture were inside the acceptable ranges for their optimal cultivation: 32 ± 2°C of temperature, 4.72 ± 0.34 mg mL⁻¹ of dissolved oxygen, 36.69 ± 0.23 of salinity. The air was filtered (5 and 1µm) before entering the experimental system. The treatments were applied as *Schizochytrium* sp. (LPU-1), *Schizochytrium* sp. (LPU-1)/*Chaetoceros* sp. (LPU-2), *Chaetoceros* sp. (LPU-2), *Chaetoceros* sp. (LPU-3), *Chaetoceros* sp. (LPU-2)/*Chaetoceros* sp. (LPU-3). Each treatment was done three times as an independent experiment for eight days. The feeding was carried out with cultures in the exponential stage, at the beginning of the culture of rotifers the food density was 80 cells mL⁻¹, and during the culture, 2 L daily of microalgae was supplied. The feed consumption of rotifers was estimated daily using the following equation

$$FI = \left(\frac{N_0 - N_1}{N_0}\right)$$

where $N_0$ corresponds to the total number of cells at the beginning of the culture of rotifers, $N_1$ is the total number of cells after 24 h in the culture of rotifers.
and N_t is the number of rotifers in the culture. The initial rotifer density surged from 2 rotifer mL^{-1}. A daily triplicate quantification was done with 1 mL sample of the zooplankton in a Sedgwick-Rafter counting chamber. We calculated the population growth rate according to Thielacker’s equation (1971): 
\[ G = \frac{\ln N_t - \ln N_0}{t} \]
where N_0 corresponds to the initial rotifer population, N_t corresponds to the number of rotifers at time t, and t is the number of days of the culture. Finally, the biomass was concentrated and frozen to -22°C for further processing.

Biochemical analysis and fatty acids profile determination from microalgae and rotifers

All the experiments were done in a dry matter. Protein quantification was done using the bicinchoninic acid (BCA) method as described by (Brown et al., 1989). Total lipids determination was done as described by Barnes & Blackstock (1973). Carbohydrate composition test was done as described by Roe et al. (1949). The first step to determine the fatty acids profile was to extract all lipids as described by Folch et al. (1957) and Bligh & Dyer (1959). For the fatty acid esterification, we added 2.5 mL of methanolic hydrochloric acid HCl:CH_3OH (5%, v/v) for a 2.5 h derivatization at 85°C (Sato & Murata, 1988). The methyl esterified fatty acids (FAME) obtained from the derivatization were extracted with 1 mL of hexane (C_6H_{12}). The fatty acids profile was determined with an Agilent Technologies 7820A gas chromatograph, with a fused silica capillary column compound with 2-polyethylene glycol as a stationary phase. The column has 30 m in length, 0.25 mm of internal diameter, and 0.25 μm of film thickness (Supelco Omegamax™ 250). The fatty acids present in the samples were identified by comparing the obtained mass spectra with the mass spectral database WIST/NBS. Data analysis was performed using the equipment’s software and displayed as the percentage of the area according to the identification of the total fatty acids.

Statistical analysis

All the presented data were given a homogeneity of variance test and normality tests according to the techniques of Levine and Kolmogorov-Smirnov, respectively. In the case where data was not homoscedastic, we applied the Kruskal-Wallis ranks’ test with a 0.05 of significance. We used SigmaPlot version 11.0 software for data analysis and graphing.

RESULTS

Microalgae

The maximum registered cell density (cells mL^{-1}) for each species was: 4.19x10^6 cells mL^{-1} for Chaetoceros sp. (LPU-3), 2.86x10^6 cells mL^{-1} for Chaetoceros sp. (LPU-2), and 0.041x10^6 cells mL^{-1} for Schizochytrium sp. (LPU-1). Statistical analysis (P < 0.05) shows that there is a significant difference between Chaetoceros (LPU-2, LPU-3) and Schizochytrium sp. (LPU-1). Growth kinetics are presented in (Fig. 1). The average growth rate for each species was: 0.47 for Chaetoceros sp. (LPU-3), 0.42 for Chaetoceros sp. (LPU-2), and 0.43 for Schizochytrium sp. (LPU-1) (Fig. 2).

Rotifers

In all rotifer cultures there was an increment of the population, the highest growth rate data were obtained from two microalgae: Schizochytrium sp. (LPU-1), 0.88 ± 0.43 and Chaetoceros sp. (LPU-3), 0.87 ± 0.3; and from the mixture of both microalgae 0.87 ± 0.40. The lowest growth rate value was obtained from the mixture of Schizochytrium sp. (LPU-1)/Chaetoceros sp. (LPU-2), 0.59 ± 0.24; nevertheless, no statistical difference was found between all treatments (P < 0.05) (Fig. 3). We then analyzed the rotifers’ density from each culture that was fed with a monoalgal and dialgal diet (Fig. 3); in this case the treatment with the highest density was Chaetoceros sp. (LPU-2) with 118 rot mL^{-1} at day 8 of culture. The values of food intake and food concentration per treatment are shown in (Table 1).

Microalgae’s nutritional value

The summary of the proximal and fatty acid analysis is shown in Table 2.

We sought to identify the total content of carbohydrates, proteins, and lipids from each microalgae species. Chaetoceros sp. (LPU-2) presented a value of 18.97 ± 0.3 mg g^{-1} for total carbohydrates which are significantly (P < 0.05) higher than in those obtained in Schizochytrium sp. (LPU-1) and Chaetoceros sp. (LPU-3). About total proteins, the highest content (P < 0.05) was shown in Schizochytrium sp. (LPU-1) and Chaetoceros sp. (LPU-2) with 256.19 ± 13.41 and 252.41 ± 0.78 mg g^{-1} respectively, and the lowest value was found in Chaetoceros sp. (LPU-3). Relative to lipid content, Schizochytrium sp. (LPU-1) presented the highest amount (P < 0.05) with 256.21 ± 1.31 mg g^{-1}, while Chaetoceros sp. (LPU-2) and Chaetoceros sp. (LPU-3) were 100.28 ± 0.27 and 100.16 ± 0.08 mg g^{-1} respectively. The fatty acids analysis was grouped into three categories: saturated, monounsaturated and polyunsaturated. Chaetoceros sp. (LPU-3) presented the highest saturated fatty acids value (P < 0.05) whereas Chaetoceros sp. (LPU-2) showed the highest polyunsaturated fatty acids value (P < 0.05) (Table 3). The linolenic acid (ALA) content of Schizochytrium sp. (LPU-1) and Chaetoceros sp. (LPU-2 and LPU-3) were not significantly different (P < 0.05). On the other hand
Figure 1. The mean and standard error of the cellular concentration of three species of microalgae: Schizochytrium sp. (LPU-1), Chaetoceros sp. (LPU-2), and Chaetoceros sp. (LPU-3). The strains were maintained in batch cultures at a temperature of 32 ± 2°C. The letters a, b, and c, indicate statistical differences ($P < 0.05$).

only in Chaetoceros sp. (LPU-2 and LPU-3), we found arachidonic acid (ARA) and eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA) was not detected in any species (Table 2).

Rotifers’ nutritional value
The bromatological and fatty acids composition under the effects of different treatments are shown in Table 3. After the feeding trial with three microalgae strains; the total carbohydrate content represented the smallest quantity to the total amount of proteins and lipids. The rotifers fed in all treatments do not show significant differences in carbohydrate and proteins content (Table 3).

The total lipid of rotifers fed with the mixture of Chaetoceros sp. (LPU-2)/Chaetoceros sp. (LPU-3) (T3) and Schizochytrium sp. (LPU-1)/Chaetoceros sp. (LPU-2) (T5) 171.52 ± 3.83 and 163.10 ± 5.80 mg g$^{-1}$, respectively (Table 3), was significant higher ($P < 0.05$) to the other treatments.

Regarding fatty acids content, a notable degree of similarity in rotifer saturated, monounsaturated and polyunsaturated acids composition was observed among all treatments. In the mixture Schizochytrium sp. (LPU-1)/Chaetoceros sp. (LPU-2) (T5) DHA was not detected.

DISCUSSION
The live food plays an essential role in the production of fish, crustaceans, and mollusks. In the particular case of some marine fishes, rotifers of the genus Brachionus spp. are the first source of exogenous food during the larval development. So, if the nutritional value of the rotifer is suitable as well as their availability, we can guarantee adequate exogenous nutrition for the larval stages of crop development.

Rotifers are non-selective filter feeders organisms that feed on a wide variety of food sources (Hotos, 2002; Yin & Zhao, 2008), mainly of algae and diatoms, that are considered by various authors to offer better results in terms of growth and contribution of fatty acids to the rotifers used in culture (Brown et al., 1997; Benavente-Valdés et al., 2012; Barclay, 2013; Torzillo & Vonshak, 2013). The most widely used species in the culture of rotifers are green algae of the genera Nannochloropsis, Nannochloris, and Chlorella which have been used in mass cultures by providing a high nutritional quality to the rotifer (Hee-Bae & Bum-Hur, 2011) as well as the marine diatoms such as Chaetoceros calcitrans obtaining in the rotifers good rates of population growth (Ortega-Salas et al., 2013).

The interest in improving the feeding for the rotifers for use in aquaculture at a lower cost is increasing, as well as the use of algae and diatoms, which are endemic to the North Pacific region, is an area where there is a primary concern for promoting the cultivation of marine fish. This study tested two species of Chaetoceros, isolated in the Bahía de La Paz, on the Northern Pacific coast of Mexico. These species are evaluated on their specific growth rate, and it was found that the maximum cell density attained was after seven days of culture, with a maximum concentration for Chaetoceros sp. (LPU-3) of 4.19×10$^6$ cells mL$^{-1}$, and for Chaetoceros sp. (LPU-2), 2.23×103 cells mL$^{-1}$. These densities are close to those reported by Pacheco-Vega et al. (2015) for Chaetoceros muelleri 2.028±106
Figure 3. Mean and standard error of the population density (ind mL$^{-1}$) of B. plicatilis fed with five novel microalgae: T1: Chaetoceros sp. (LPU-3), Chaetoceros sp. (LPU-2), Chaetoceros sp. (LPU-3) / Chaetoceros sp. (LPU-2), Schizochytrium sp. (LPU-1), and Schizochytrium sp. (LPU-1)/Chaetoceros sp. (LPU-2).

cells mL$^{-1}$ and also by Martínez-Córdoba et al. (2012) who reported a maximum density of C. muelleri $3.75 \times 10^6$ cells mL$^{-1}$, indicating that these diatoms can provide the necessary amounts to increase the production of rotifers. In this sense, the composition of the microalgae is influenced by the culture medium (Wikfors et al., 1984), the temperature (James et al., 1989) as well as by the light intensity (Thompson et al., 1990) and harvest times (Brown et al., 1997). On the other hand the low concentration of Schizochytrium sp.
inum co-
e cells that
(2015), noted that the
flocculation complicates easy access to fr
on the walls of the experimental system. This natural
together forming visible clumps that sediment and stick
for the culture of rotifers
umber of species. In this study, it was observed that
higher than 35°C turns out to be lethal for a large
strains used.

Commonly, the microalgae cultures tolerate tempera-
atures between 16 and 27°C, where temperatures
below 16°C decreases the growth, while a temperature
higher than 35°C turns out to be lethal for a large
umber of species. In this study, it was observed that
for the culture of rotifers Schizochytrium sp. (LPU-1)
used in isolation is not recommended, since at a
temperature of 35°C the cells have the ability to join
together forming visible clumps that sediment and stick
on the walls of the experimental system. This natural
flocculation complicates easy access to free cells that
can be consumed by rotifers, which causes an increase
in the mortality of the culture. On the other hand, it was
noted that the mixture of Schizochytrium sp. (LPU-
1)/Chaetoceros sp. (LPU-2) obtained for the highest
value of total protein and the mixture of Chaetoceros
sp. (LPU-2)/Chaetoceros sp. (LPU-3) represented the
highest lipid content. This result leads us to understand
that the two microalgae mixed may have a higher
contribution of macronutrients to the rotifer than when
isolated. Pache- Vega et al. (2015), noted that the
Schizochytrium sp. has a greater amount of
monounsaturated and saturated fatty acids, and does not
contain DHA and EPA.

In the study the same similarity was observed in
terms of the deficiency of DHA and EPA, however
when analyzing the T4: Schizochytrium sp. (LPU-1),
there was a concentration of 7.28% of EPA and 4.36%
of DHA, which suggests that the rotifer has the capacity
to transform the linolenic acid to EPA and DHA. About
the effect of the Chaetoceros sp. (LPU-2) and
Chaetoceros sp. (LPU-3) composition, on the cultured
rotifers, we can say that the protein content is higher

Table 1. Daily food intake and culture growth rate G (d⁻¹) of B. plicatilis fed different microalgal strain.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Food concentration (cells mL⁻¹)</th>
<th>Daily food intake (cells) per rotifer</th>
<th>Culture growth rate G (d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetoceros sp. (LPU-3)</td>
<td>10,489.83 ± 10,610.54</td>
<td>97,292 ± 66,814</td>
<td>0.87 ± 0.37</td>
</tr>
<tr>
<td>Chaetoceros sp. (LPU-2)</td>
<td>84,166.67 ± 83,532.34</td>
<td>63,333 ± 67,884.7</td>
<td>0.74 ± 0.35</td>
</tr>
<tr>
<td>Chaetoceros sp. (LPU-3) /</td>
<td>421,145.83 ± 29,879.01</td>
<td>68,438 ± 15,194.2</td>
<td>0.87 ± 0.40</td>
</tr>
<tr>
<td>Chaetoceros sp. (LPU-2) (1:1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizochytrium sp.(LPU-1)</td>
<td>53,562.50 ± 44,034.06</td>
<td>50,472 ± 625.5</td>
<td>0.88 ± 0.43</td>
</tr>
<tr>
<td>Schizochytrium sp. (LPU-1) /</td>
<td>47,187.50 ± 34,031.59</td>
<td>59,514 ± 3,556.2</td>
<td>0.59 ± 0.24</td>
</tr>
<tr>
<td>Chaetoceros sp. (LPU-2) (1:5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Biochemical analysis of fatty acids (%) of Schizochytrium sp. (LPU-1), Chaetoceros sp. (LPU-2), and Chaetoceros sp. (LPU-3). Data are shown as the mean ± and standard error. The values of total carbohydrates (CHO), total proteins (PT), and total Lipids (LT) are expressed in mg g⁻¹. The letters a, b, and c, indicate statistical differences (P < 0.05).

<table>
<thead>
<tr>
<th>Composition: chromatological &amp; fatty acids</th>
<th>Schizochytrium sp. (LPU-1)</th>
<th>Chaetoceros sp. (LPU-2)</th>
<th>Chaetoceros sp. (LPU-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>20.01 ± 0.33b</td>
<td>24.10 ± 0.30a</td>
<td>18.97 ± 0.30b</td>
</tr>
<tr>
<td>PT</td>
<td>265.19 ± 13.41±</td>
<td>252.41 ± 0.78b</td>
<td>149.52 ± 0.62b</td>
</tr>
<tr>
<td>LT</td>
<td>256.21 ± 1.31</td>
<td>100.28 ± 0.27b</td>
<td>100.16 ± 0.08b</td>
</tr>
<tr>
<td>∑ Saturated</td>
<td>37.14 ± 2.90</td>
<td>55.13 ± 2.11b</td>
<td>82.08 ± 2.09a</td>
</tr>
<tr>
<td>∑ Monounsaturated</td>
<td>48.81 ± 3.16</td>
<td>29.79 ± 0.61b</td>
<td>11.93 ± 0.30b</td>
</tr>
<tr>
<td>18:3 (n-6)</td>
<td>3.28 ± 0.07b</td>
<td>9.72 ± 1.47a</td>
<td>0.51 ± 0.07b</td>
</tr>
<tr>
<td>18:3 (n-3) ALA</td>
<td>0.66 ± 0.109</td>
<td>0.28 ± 0.081</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>20:4 (n-6) ARA</td>
<td>0b</td>
<td>0.87 ± 0.091a</td>
<td>0.67 ± 0.01a</td>
</tr>
<tr>
<td>20:5 (n-3) EPA</td>
<td>0</td>
<td>3.54 ± 0.06</td>
<td>3.33 ± 2.07</td>
</tr>
<tr>
<td>22:6 (n-3) DHA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>∑ Polyunsaturated</td>
<td>14.05 ± 0.26b</td>
<td>15.08 ± 1.51a</td>
<td>5.99 ± 1.79c</td>
</tr>
<tr>
<td>∑ n-3</td>
<td>0.66 ± 0.11</td>
<td>3.82 ± 0.024</td>
<td>0.66 ± 0.11</td>
</tr>
<tr>
<td>∑ n-6</td>
<td>3.28 ± 0.07b</td>
<td>10.71 ± 1.56a</td>
<td>3.28 ± 0.70b</td>
</tr>
</tbody>
</table>

(LPU-1) (0.041×10⁶ cells mL⁻¹) that was obtained, may
have been due to the high temperatures during the test,
that was 32°C; some authors such as Chatdumrong et
al. (2007) and Estudillo del Castillo et al. (2009), report
that to 25°C is the optimal temperature for
Schizochytrium limacinum and Schizochytrium mangrovi,
respectively. Mehlitz (2009) reports that the
optimum temperature for the culture of microalgae
is usually between 20 and 24°C, however, these may
vary depending on the culture medium, the species and
strain used.
(300.2 and 287.20 mg g\(^{-1}\)) to what Yin et al. (2013) reported for *Nannochloropsis* sp. 63.24 mg g\(^{-1}\); this indicates that it is possible to obtain a greater amount of protein by mixing *Chaetoceros* sp., than using only a single microalga as in the case of the *Schizochytrium* sp. (LPU-1).

In all polyunsaturated fatty acids (PUFA) treatments, significant amounts of arachidonic acid ARA (Table 3) were quantified, which plays a very important role in the production of eicosanoids (Sargent et al., 1999b). Some studies have concluded that the survival and growth of marine fish larvae are favored when the content of ARA in the live food (e.g., *B. plicatilis* and *Artemia* nauplii) is high (Koven et al., 2001; Park et al., 2006; García et al., 2008). About EPA, high concentrations were found in the rotifers; it is important to mention that in treatments involving *Schizochytrium* sp. (LPU-1) EPA also was detected. So, we can say that *B. plicatilis*, through the elongation and desaturation of PUFA C18 to C20 and C22 PUFA, is capable of producing EPA from linolenic acid (ALA), consistent with the hypothesis of Yin et al. (2013); they fed rotifers with the algae *Ulva pertusa* that is not rich in EPA but which gave high amounts of ALA and this, in turn, led to high levels of EPA.

The results of the treatments T1-T4 were unusually high for the DHA content as the microalgae proved to be deficient in this fatty acid, it is known that some species follow the metabolic pathway LNA→20:3n-3→20:4n-3→EPA→DPA, and 24:5n-3→24:6n-3 to 22:6n-3 (DHA) (Sprecher et al., 1995; Bergé & Barnathan, 2005) and that this may be the reason high content of DHA in the rotifers was detected.

In this study, we can show that the use of microalgal species from the North Pacific, such as *Chaetoceros* sp. (LPU-2) and *Chaetoceros* sp. (LPU-3) combined, have more nutrients and better performance for the culture of rotifers. The use of indigenous or regional microalgae is advisable to lower costs in the production of imported live food, mainly to feed rotifers, because depending on the region environmental conditions change. The light intensity is one of the main parameters to consider in a culture, as photosynthesis increases with the increase of the light intensity until reaching the maximum specific growth rate for each species at the saturation point for light (Park et al., 2011). Passing this point, photoinhibition starts, with detrimental results to the same cell, generally microalgae tend to exhibit photoinhibition during main hours of the day, due to the high light intensity (Martínez, 2008); if the microalgae are native it will support changes in the region’s light intensity. In this study, the microalgae were maintained at 2,500 lux, being the light intensity for the North Pacific in the Southern Baja California region of 1,000 to 5,500 lux (Vázquez-Pérez et al., 2016).

Concerning the temperature, the rotifer culture was maintained at an average temperature of 29°C, which turned out to be optimal for *Chaetoceros* sp. (LPU-2) and *Chaetoceros* sp. (LPU-3), thus maintaining the population growth. The algal production increases
proportionally with the temperature until it achieves the optimum temperature for each species; above this, the breathing and the photorespiration increases reducing the microalgal rate of growth until it is null. It is important to consider the use of the combination of local microalgal species in tropical and subtropical areas to feed rotifers to ensure the least effort and cost possible.

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