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Research Article

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-1)/*Chaetoceros* sp. (LPU-2), *Chaetoceros* sp. (LPU-2), *Chaetoceros* sp. (LPU-3), *Chaetoceros* 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 (Støttrup & 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).

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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 $\mu = (\ln N_1 - \ln N_0)/t_1 - t_0$, 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 $FI = (N_o - N_1) / N_t$, 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 Theilacker's equation (1971): $G = (\ln N_t - \ln N_0) / t$, 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 (C6H12). 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: 4.19×10^6 cells mL^{-1} for *Chaetoceros* sp. (LPU-3), 2.86×10^6 cells mL^{-1} for *Chaetoceros* sp. (LPU-2), and $.041\times10^6$ 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 265.19 ± 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

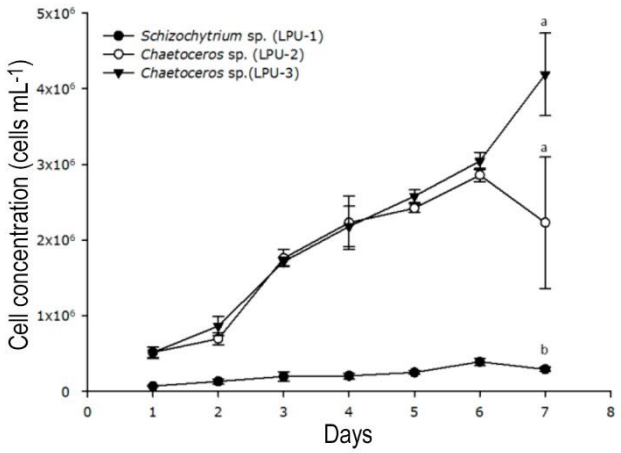

Figure 1. The mean and standard error of the cellular concentration of three species of microalgae: *Schizochytrium* sp. (LPU-1), *Chaetoceros* sp. (LPU-2), and *Chaetoceros* sp. (LPU-3). The strains were maintained in batch cultures at a temperature of 32 ± 2°C. The letters a, b, and c, indicate statistical differences ($P < 0.05$).

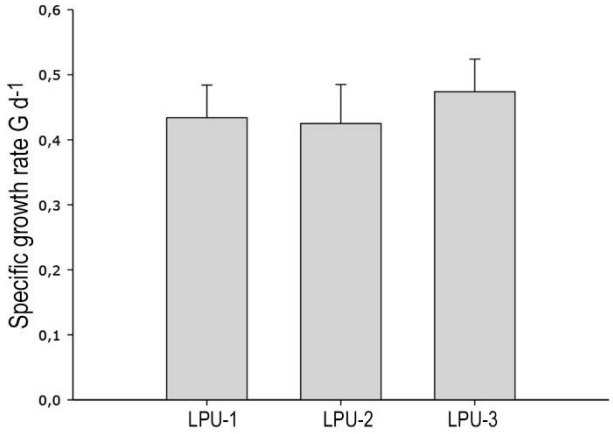

Figure 2. The mean and standard error of the specific growth rate (G d^{-1}) of three species of microalgae: *Schizochytrium* sp. (LPU-1), *Chaetoceros* sp. (LPU-2), and *Chaetoceros* sp. (LPU-3). The strains were maintained in batch cultures at a temperature of 32 ± 2°C.

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

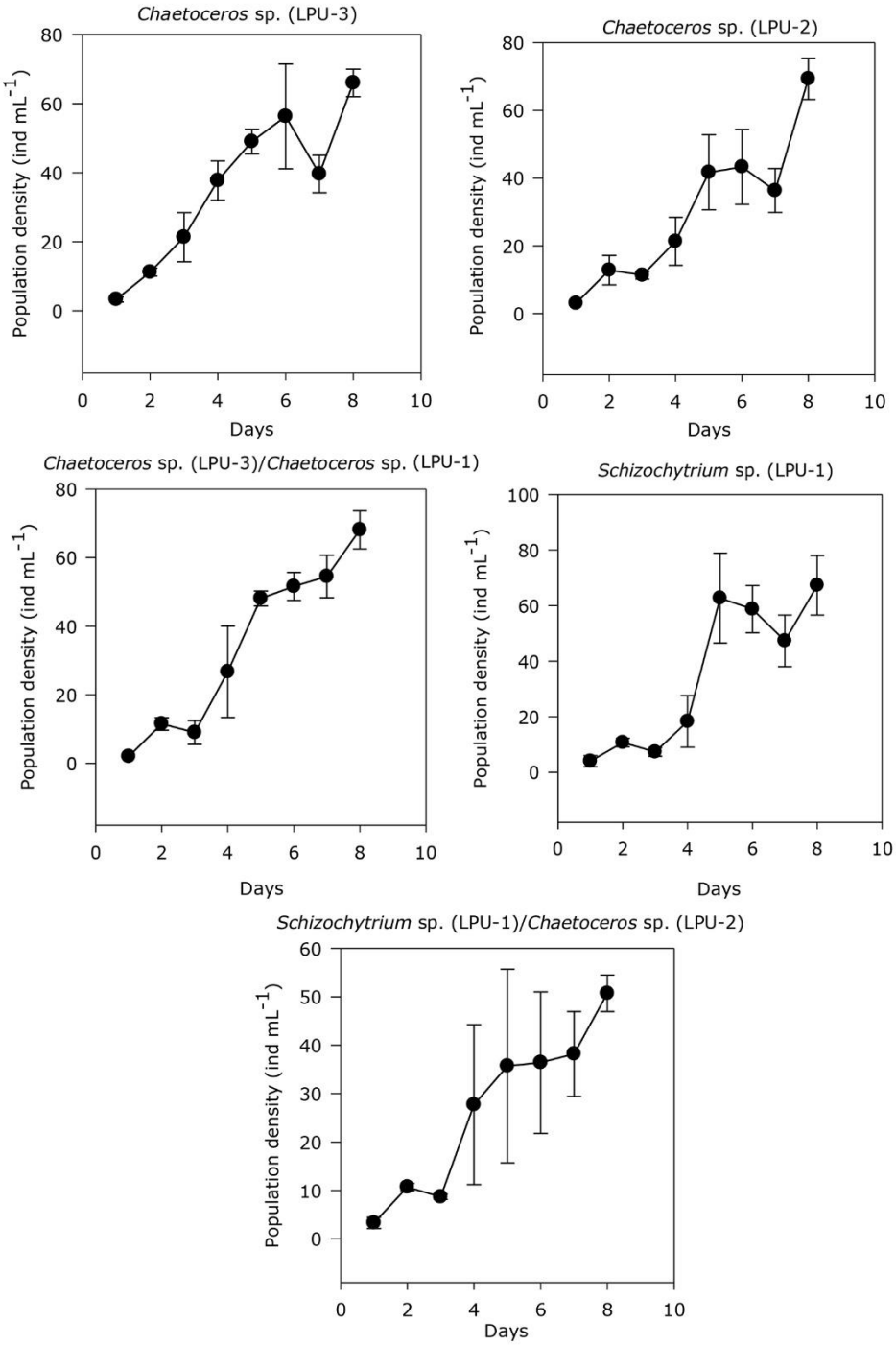

Figure 3. Mean and standard error of the population density (ind mL^{-1}) of *B. plicatilis* fed with five novel microalgae: T1: *Chaetoceros* sp. (LPU-3), *Chaetoceros* sp. (LPU-2), *Chaetoceros* sp. (LPU-3) / *Chaetoceros* sp. (LPU-2), *Schizochytrium* sp. (LPU-1), and *Schizochytrium* sp. (LPU-1)/*Chaetoceros* sp. (LPU-2).

cells mL^{-1} and also by Martínez-Córdoba *et al*. (2012) who reported a maximum density of *C. muelleri* 3.75×10^6 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.

Table 1. Daily food intake and culture growth rate G (d^{-1}) of *B. plicatilis* fed different microalgal strain.

Treatments	Food concentration (cells mL^{-1})	Daily food intake (cells) per rotifer	Culture growth rate G (d^{-1})
Chaetoceros sp. (LPU-3)	10,4895.83 ± 10,6105.46	97,292 ± 66,814	0.87 ± 0.37
Chaetoceros sp. (LPU-2)	84,166.67 ± 83,532.34	63,333 ± 67,884.7	0.74 ± 0.35
Chaetoceros sp. (LPU-3) / *Chaetoceros* sp. (LPU-2) (1:1)	421,145.83 ± 29,879.01	68,438 ± 15,194.2	0.87 ± 0.40
Schizochytrium sp.(LPU-1*)*	53,562.50 ± 44,034.06	50,472 ± 625.5	0.88 ± 0.43
Schizochytrium sp. (LPU-1) / *Chaetoceros* sp. (LPU-2) (1:5)	47,187.50 ± 34,031.59	59,514 ± 3,556.2	0.59 ± 0.24

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 ($P < 0.05$).

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

(LPU-1) (0.041×10^{6} 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 mangrovei*, 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.

Commonly, the microalgae cultures tolerate temperatures 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 number 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. Pacheco-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 3. Bromatological and fatty acids analysis (%) of *Brachionus plicatilis* rotifers fed with five different microalgae treatments: T1: *Chaetoceros* (LPU-3), T2: *Chaetoceros* (LPU-2), T3: *Chaetoceros* sp. (LPU-3)/*Chaetoceros* sp. (LPU-2), T4: *Schizochytrium* sp. (LPU-1), and T5: *Schizochytrium* sp. (LPU-1)/*Chaetoceros* sp. (LPU-2). 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 ($P < 0.05$).

Composition: bromatological & fatty acids	Rotifer's composition/treatment				
	T1	T2	T3	T4	T5
CHO	33.29 ± 1.11	50.34 ± 0.95	38.14 ± 2.96	3 0.66 ± 2.20	32.95 ± 2.80
PT	300.05 ± 30.03	287.20 ± 3.88	270.39 ± 4.25	238.10 ± 7.01	331.03 ± 2.01
LT	133.51 ± 6.06[b]	98.69 ± 2.65[c]	171.52 ± 3.83[a]	54.77 ± 0.80[d]	163.10 ± 5.80[a]
∑ Saturated	36.16 ± 1.34	36.70 ± 1.25	34.75 ± 0.42	38.05 ± 2.16	33.12 ± 2.96
∑ Monounsaturated	35.06 ± 0.79	36.58 ± 1.68	37.54 ± 5.22	38.37 ± 4.17	36.28 ± 2.02
18:3 (n-6)	3.12 ± 0.06	2.75 ± 0.14	3.34 ± 2.07	4.26 ± 1.15	4.63 ± 1.02
18:3 (n-3) ALA	0.96 ± 0.00	0.88 ± 0.02	0.96 ± 0.39	1.25 ± 0.19	0.94 ± 0.16
20:4 (n-6) ARA	6.09 ± 0.29	5.4 1 ± 0.63	7.08 ± 2.07	5.96 ± 1.78	1.09 ± 0.21
20:5 (n-3) EPA	7.38 ± 0.43	7.43 ± 0.87	9.77 ± 4.24	7.28 ± 2.79	0.91 ± 0.20
22:6 (n-3) DHA	4.94 ± 0.42	4.73 ± 0.61	6.42 ± 2.60	4.36 ± 1.52	0
∑ Polyunsaturated	28.79 ± 060	26.72 ± 2.20	27.72 ± 5.63	23.58 ± 5.22	30.60 ± 4.98
∑ n-3	13.25 ± 0.85	13.02 ± 1.50	11.42 ± 6.45	8.73 ± 4.12	1.91 ± 0.36
∑ n-6	9.22 ± 0.35	8.37 ± 0.73	10.42 ± 0.00	9.35 ± 1.35	5.91 ± 1.23

(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 microalgae 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 microalgae 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 microalgae rate of growth until it is null. It is important to consider the use of the combination of local microalgae species in tropical and subtropical areas to feed rotifers to ensure the least effort and cost possible.

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