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Research Article

Assessment of endemic microalgae as potential food for *Artemia franciscana* culture

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ABSTRACT. In this study, five microalgal strains were isolated from Bahía de La Paz, Baja California Sur, Mexico and identified as *Grammatophora* sp., *Navicula* sp., *Rhabdonema* sp., *Schizochytrium* sp., and *Nitzschia* sp., and their evaluation as potential food for *Artemia franciscana*. The isolated strains were cultured outdoors and harvested after four days. *Chaetoceros muelleri* was cultured under laboratory conditions and used as control. The protein, lipid, and carbohydrate composition and the fatty acid profiles of the strains were determined by gas chromatography. To assess the effect of microalgal strains on *A. franciscana*, decapsulated cysts were cultured at outdoor conditions in 15 L containers. The experiment was conducted for twelve days. Samples from the five different feeding treatments were taken at the beginning and end of the experiment to assess number, size, and weight of *Artemia* larvae. Treatment with *Rhabdonema* sp. showed larvae with a lower percentage of polyunsaturated fatty acids (PUFAs) while *Grammatophora* sp. showed those with the greatest PUFA proportion, even more than those fed *Chaetoceros muelleri* (control). Larvae consuming *Schizochytrium* sp. had no docosahexanoic (DHA) nor eicosapentaenoic (EPA) fatty acid content. Growth and survival of *A. franciscana* did not show significant differences among feed treatments, except when it was fed *Nitzschia* sp., showing lower survival and dry weight. Treatment based on *Schizochytrium* sp. and *Rhabdonema* sp. had a greater *A. franciscana* size but reduced dry weight; additional tests including two or more algal species for every treatment should be carried out to determine the best yield.

Keywords: brine shrimp, arid zones, nutrition, microalgae, fatty acids, aquaculture.

Evaluación del potencial de microalgas endémicas para el cultivo de *Artemia franciscana*

RESUMEN. Se aislaron cinco cepas de microalgas de la Bahía de La Paz, Baja California Sur, Mexico, identificadas como *Grammatophora* sp., *Navicula* sp., *Rhabdonema* sp., *Schizochytrium* sp., and *Nitzschia* sp., y su evaluación como alimento potencial para *Artemia franciscana*. Las cepas algales fueron aisladas, purificadas y cultivadas al exterior. *Chaetoceros muelleri* fue cultivada en condiciones de laboratorio y utilizada como control. El análisis bioquímico y el perfil de ácidos grasos correspondientes al cuarto día de cultivo de las microalgas, se efectuó mediante cromatografía de gases. El experimento con *Artemia franciscana* se realizó al exterior por doce días en tanques de 15 L. Se realizaron muestreos al inicio y al final del trabajo para determinar el incremento en talla, peso seco y sobrevivencia. El tratamiento con la microalga identificada como *Rhabdonema* sp. mostró larvas con menor porcentaje de ácidos grasos polinsaturados (PUFAs) mientras que aquellas alimentadas con *Grammatophora* sp. presentaron la mayor proporción, superando a las del control *Chaetoceros muelleri*. Con *Schizothyrium* sp. no presentaron los ácidos grasos docosahexanoico (DHA) y eicosapentanoico (EPA). La sobrevivencia obtenida al utilizarse las diferentes cepas no mostró diferencias significativas a excepción de la alimentación basada en *Nitzschia* sp. donde mostraron una sobrevivencia menor.

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Los tratamientos con base en *Schizothyrium* sp. y *Rhabdonema* sp. produjeron individuos de *A. franciscana* de mayor tamaño, pero se redujo el peso, por lo que se requieren mayores evaluaciones donde se incluyan dos o más especies de microalgas para determinar el mejor rendimiento.

Palabras clave: *Artemia*, zonas áridas, nutrición, microalgas, ácidos grasos, acuicultura.

INTRODUCTION

Feeding marine organisms under culture conditions is one of the most important efforts to improve performance and ensure production processes. Supplying live food is an irreplaceable activity at early culture stages, so efforts have been made to improve them. The microcrustacean, *Artemia* spp. is the most used species as live food for larval fish and crustacean species in aquaculture, from nauplius stage to adulthood, used by 85% of the marine species cultured (Godinez *et al*., 2004, Cisneros & Vinatea, 2009). The employment of *Artemia* in aquaculture has many advantages; however, the nauplius stages are a source of incomplete food since they have low content of eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) fatty acids, both important for the development of organisms in aquaculture (Sorgeloos *et al*., 1986; Mary-Leema *et al*., 2010), and which should be supplied in feed because of their limited ability to synthesize EPA and DHA. Fortunately, *Artemia* can be enriched by feeding it with microalgal species that have these fatty acids.

Various marine microalgae are used as food for *Artemia*, which must meet several nutritional and morphological features to enable their use. Tropical ecosystems have a diversity of these species that can be grown under different environments, and which may have different fatty acid composition (Luong-Van *et al*., 1999). Some microalgae have a high percentage of polyunsaturated fatty acids (PUFAs) that exceed the sources traditionally used because they include carotenoids and antioxidant vitamins naturally, which are bio-encapsulated in their cell wall (Patil *et al*., 2007).

In tropical climates microalgal species are usually grown in controlled environmental conditions at 19°C; however, the optimum temperature for cultivating *Artemia* is 24°C (Browne & Wanigasekera, 1999). This temperature difference causes growth inhibition and thermal stress to the microalgae. The use of microalgal strains isolated from tropical temperatures may enhance growth of cultured *Artemia* and avoids the need for temperature controlled environments. Mass production of marine microalgae under temperature-light controlled and hatchery-type environments has a high production cost for *Artemia* spp. The use of algal strains from local environments facilitates cultivation and reduces production costs in outdoor mass scale hatchery systems installed in tropical temperatures, which shows the importance of researching new species of marine microalgae.

Due to the increase of aquaculture activities and the need for alternative fish protein and lipid sources, it is necessary to isolate, characterize, and assess microalgal strains as live food. Our study reports five marine species of microalgae that were partially characterized by using conventional taxonomic tools and further evaluated as live food for *Artemia franciscana*.

MATERIALS AND METHODS

Isolation and characterization of microalgal strains

Sea water samples were collected from three coastal locations in Bahía de La Paz, Mexico. Isolation of microalgal cells was done by micropipetting, streaking on direct spray, and making serial dilutions in agar culture media. The selection of specific colonies and monospecific cultures was made taking into account some cell characteristics: size, shape, mobility, and pigments. Once monospecific microalgal cultures were obtained, they were processed with a mixture of streptomycin and dicloxacillin, each one at a concentration of 0.222 mg mL^{-1} for 24 h to obtain axenic strains. Strains were incubated at 19 ± 1°C under continuous illumination with daylight lamps and cultured in 1 µm filtered sea water, UV irradiated in F/2 enriched medium (Guillard, 1975).

For strain identification, a morphological observation of the cells was performed with a compound microscope (Cupp, 1943; Ruggiero *et al*., 2009; Spaulding *et al*., 2010). For individual dimensions of the cells forming the strains obtained, 30 different measurements were taken using Nikon camera equipment Sight Ds-L1 equipped with software previously calibrated and mounted to a Nikon compound microscope Optiphot-2.

For outdoor cultures of *Navicula* sp., *Nitzschia* sp., *Grammatophora* sp., and *Rhabdonema* sp. F/2 medium was used; they were cultured in 2.8 L flasks as above but incubated at outdoor conditions and then transferred to 18 L carboys, with filtered seawater (1 µm) sterilized with 0.1% sodium hypochlorite for 24 h, and any

residual chlorine was neutralized with a sodium thiosulfate solution before use.

Agitation of the microalgal culture was established by means of constant aeration with filtered air through a 0.2 µm membrane. The number of cells was counted daily using a Neubauer hemocytometer under a conventional compound light microscope. After the fifth day of culture, the microalgae were maintained in a semi-continuous system at the rate of 25% dilution per day. The control treatment (*C. muelleri*) was maintained at 20°C with constant aeration and illumination.

Biochemical composition of microalgae

Microalgal composition was determined in cells harvested from outdoor cultures. Samples were collected in triplicate on the fourth day of culture, freeze-dried, and then stored at -40°C until further analysis. Total weight was calculated according to Sorokin (1973), protein content according to Lowry *et al*. (1951), and procedure as modified by Malara & Charra (1972). Carbohydrate content was determined according to White (1987) and Dubois *et al*. (1959). Lipids were determined colorimetrically according to Bligh & Dyer (1959) and procedure as modified by Chiaverina (1972).

To determine the fatty acid profile of the strains, a lipid extraction from the cells was done with hexane by stirring at 22ºC for 24 h. Suspensions of the microalgae were then filtered, and supernatants were concentrated under reduced pressure. The methylated fatty acid esters were prepared according to AOAC International (AOAC, 1990), using 14% w/v boron trifluoride-methanol. The fatty acid profile was determined by gas chromatography coupled to mass spectrum (GC-MS) Saturn 2200 (Varian Inc.) ion trap in a SP-2380 capillary column 0.25 mm ID x 0.25 100 mx film (Supelco) using helium gas as carrier fluid. Signals were identified according to their mass spectrum. For fatty acid quantification, areas under the curve of the corresponding signal and the sum of the fatty acids were considered. Each sample was analyzed in triplicate.

***Artemia franciscana* cultures**

Cultivation of *A. franciscana* was performed on a series of fiberglass conical-bottom vessels with 15 L of sea water and covered with polyethylene sheets. Seawater was filtered through cotton yarn cartridges 20, 10, 5, and 1 µm of porosity and sterilized by means of an UV system and sodium hypochlorite. Sodium thiosulfate was used for further neutralization. *A. franciscana* cysts (Biogrow, Premium) were decapsulated and incubated following Sorgeloos *et al*. (1986). After hatching, counting was conducted to adjust a density of nauplii mL^{-1} in the experimental units. Each experimental diet was used to feed groups in triplicate. Feeding started after 10 h-post hatching to ensure food availability in the tanks. Food was provided twice daily throughout the test, adjusting to a concentration of 70,000 cells mL^{-1} for each treatment. Daily temperature, dissolved oxygen, salinity, and pH were recorded. Seven treatments were decided to run the experiment for twelve days. For the first five, a single culture of native species: *Schizothyrium* sp., *Nitzschia* sp., *Rhabdonema* sp. and *Navicula* sp., was retained to feed *A. franciscana*. For the sixth one, a mix of *Navicula* sp. and *Schizothyrium* sp. was chosen given previous results, and for the seventh treatment *Chaetoceros muelleri* was retained as control, given the wide utilization of this species as food for crustaceans. Twenty *Artemia* were sampled from each experimental unit at the beginning and end of the experiment and fixed in 5% formaldehyde to estimate their growth by measuring total length and dry weight (45°C for 36 h).

Statistical analysis

To determine cell size, 30 samples per microalgal species were measured, and their average size was calculated. In this experiment, the treatments were analyzed and compared in triplicate; before developing the statistical analysis, experimental data were tested for variance normality and homogeneity. Differences in the content of proteins, lipids, carbohydrates, and fatty acids were determined by one-way variance analysis (ANOVA). Similarly, the effect of the different microalgal species on survival, length, and weight gain in *A. franciscana* was evaluated by one-way (ANOVA) variance analysis for each case. Percentage data obtained were arcsine-square-root transformed prior to analysis. In cases where significant differences were observed, a Tukey HSD *post hoc* analysis was applied. Statistic 8.0 for Windows (StatSoft, USA) and $\alpha = 0.05$ was used for the statistical analysis.

RESULTS

The results of isolating and purifying the strains are shown in Table 1. Five species of microalgae were isolated showing various cell sizes with transeptical axial lengths from 7.26 to 44.65 µm in apical length for diatoms while *Schizochytrium* sp. showed zoospores of 9.06 µm (Table 1). Regarding major cellular constituents, the largest proportion of ashes was found in *Navicula* sp. The highest protein content was shown in *Rhabdonema* sp. and *Chaetoceros muelleri*. Regarding lipid content, *Grammatophora* sp. showed a greater percentage and lower proportion in *C. muelleri*. The

Table 1. Some characteristics of microalgae isolated from Bahía de La Paz, B.C.S., México. Number indicate median values ± SD (n = 30).

Microalgae (*genus*)	Family	Apical length (µm)	Axial length transeptic (µm)	Seta length (µm)
Schizochytrium sp.	Thraustochytriaceae	9.06 ± 1.41	-	-
Nitzschia sp.	Nitzschiaceae	44.65 ± 3.93	6.91 ± 1.00	44.94 (0.91)
Rhabdonema sp.	Fragilarioidea	7.26 ± 1.01	-	-
Navicula sp.	Naviculaceae	11.45 ± 1.47	7.87 ± 0.82	-
Grammatophora sp.	Fragilarioidea	11.58 ± 2.59	3.94 ± 0.69	-

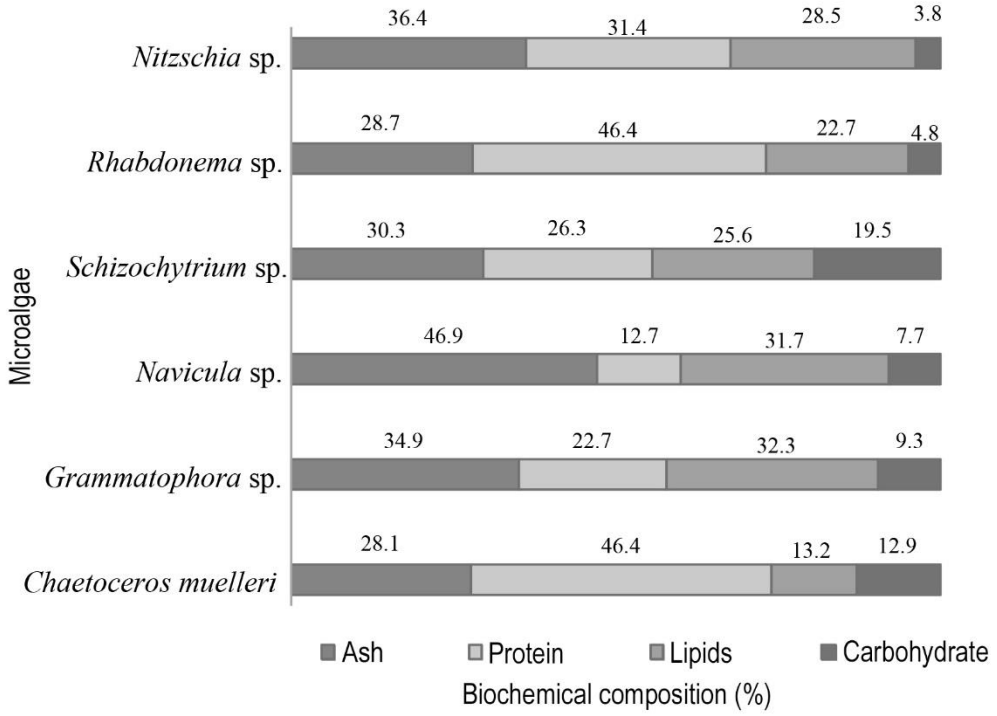

Figure 1. Biochemical composition (%) per species of microalgae isolated. Values represent the mean.

highest carbohydrate content was recorded in *Rhabdonema* sp. and in *Schizochytrium* sp., the lowest content was found in *Navicula* sp. (Fig. 1). Three types of lipids showed a variation of fatty acid composition of the microalgae; *Grammatophora* sp. showed a lower content of saturated fatty acids while the highest content was found in *Nitzschia* sp. In the case of monounsaturated fatty acids, *Rhabdonema* sp. showed a higher content while the smallest proportion was recorded *in Nitzschia* sp. The PUFA profile showed a variation in the microalgal species isolated where the percentage of docosahexaenoic acid (DHA) was higher in *Grammatophora* sp. and *C. muelleri*, but it was not found in *Nitzschia* sp. and *Schizochytrium* sp. whereas eicosapentaenoic acid (EPA) was higher in *Grammatophora* sp., and it was not found in *Schizochytrium* sp. (Table 2).

Growth kinetics of outdoor-cultured microalgae

The trends of the microalgae's growth kinetics until the fifth day of culture showed the biggest cell density for *Rhabdonema* sp. ($2{,}134.3 \times 10^3$ cell mL^{-1}), *Grammatophora* sp. ($2{,}128.7 \times 10^3$ cell mL^{-1}), and control culture of *Chaetoceros muelleri* ($2{,}028.5 \times 10^3$ cell mL^{-1}). While the least cell density was obtained in *Nitzschia* sp. (595.5×10^3 cell mL^{-1}) (Fig. 2). This results show significant differences ($P < 0.05$, ANOVA-One way) among microalgae. Temperature for the different outdoor cultures ranged from 17˚ to 29˚C and pH was 8.2 to 9.0.

Table 2. Fatty acid composition (%) from different microalgae. Values represent the mean. Different superscript letters (a, b, c, d, e) indicate on each column indicate significant differences ($P < 0.05$) between species. DHA: docosahexaenoic, EPA: eicosapentaenoic acids.

Fatty acid	*Chaetoceros muelleri* (control)	*Grammatophora* sp.	*Rhabdonema* sp.	*Schizochytrium* sp.	*Nitzschia* sp.	*Navicula* sp.
Saturated	42.83^{b}	34.97^{c}	57.62^{a}	37.51^{b}	60.41^{a}	41.54^{b}
Monounsaturated	31.66^{b}	36.67^{b}	35.85^{b}	51.02^{a}	22.62^{c}	33.86^{b}
Polyunsaturated						
C18:2 cis,trans	2.20^{a}	1.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
C 18:2 n-6	5.39^{b}	5.08^{b}	0.00^{c}	8.24^{a}	3.51^{b}	5.68^{b}
C 18:3 n-6	0.00^{c}	1.12^{b}	0.00^{c}	2.19^{a}	0.00^{c}	0.00^{c}
C20:2 n-6	0.00^{b}	0.84^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
C20:3 n-6	0.00^{c}	0.89^{b}	0.00^{c}	1.04^{b}	0.00^{c}	5.10^{a}
C22:4 n-6	4.02^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
C22:6 n-3 (DHA)	2.36^{a}	2.83^{a}	1.68^{b}	0.00^{c}	0.00^{c}	1.41^{b}
C20:5 n-3 (EPA)	11.55^{b}	16.61^{a}	4.85^{c}	0.00^{d}	13.46^{b}	12.41^{b}
Sum polyunsaturated	25.51^{b}	28.36^{a}	6.53^{e}	11.47^{d}	16.97^{c}	24.60^{b}

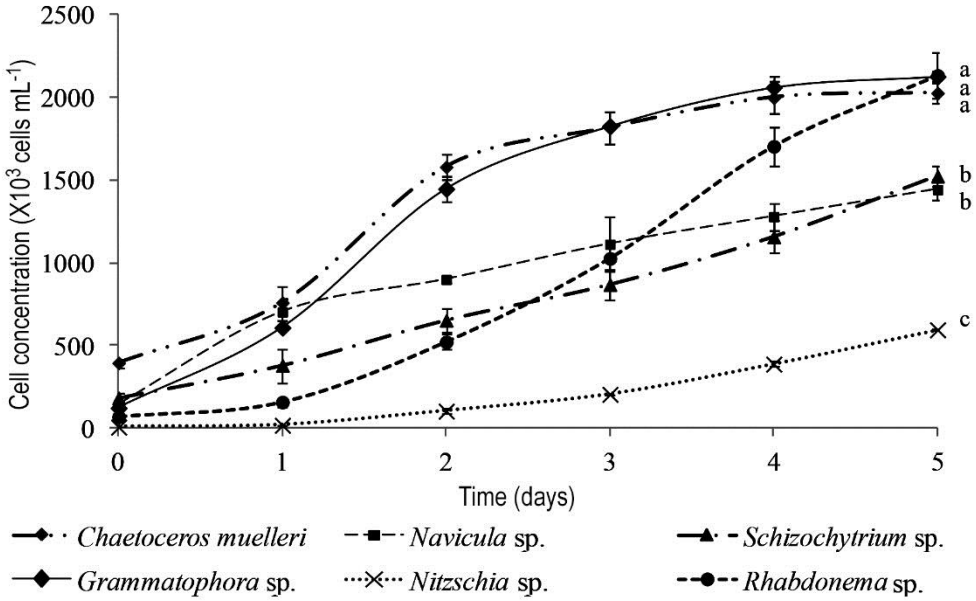

Figure 2. Mean values and standard deviations (SDs, n = 3) of cell concentrations (10^6 cells mL^{-1}) of five strains of microalgae cultures outdoors and control (*Chaetoceros muelleri*). Superscripts (a, b, c) express statistical significant differences among microalgae.

Feeding *Artemia franciscana* with microalgal strains

The results on cultured *A. franciscana* survival after 12-day feeding trials showed significant differences ($P > 0.05$, ANOVA-One way) by using different strains (Fig. 3). Tukey HSD test showed that *A. franciscana* fed with *Nitzschia* sp. was significantly smaller than the survival rates of the other treatments. The results on size increments, starting from nauplius to adult stage, showed significant differences ($P > 0.05$, ANOVA-One way) (Fig. 4).

After a 12-day culture, instead of the microalga used as food, dry weight of *A. franciscana* showed significant differences ($P > 0.05$, ANOVA-One way) from the beginning to the end of the experiment (Fig. 5); the mixed diet of *Navicula* sp. and *Schizothyrium* sp. yielded the highest weight while feeding trials with

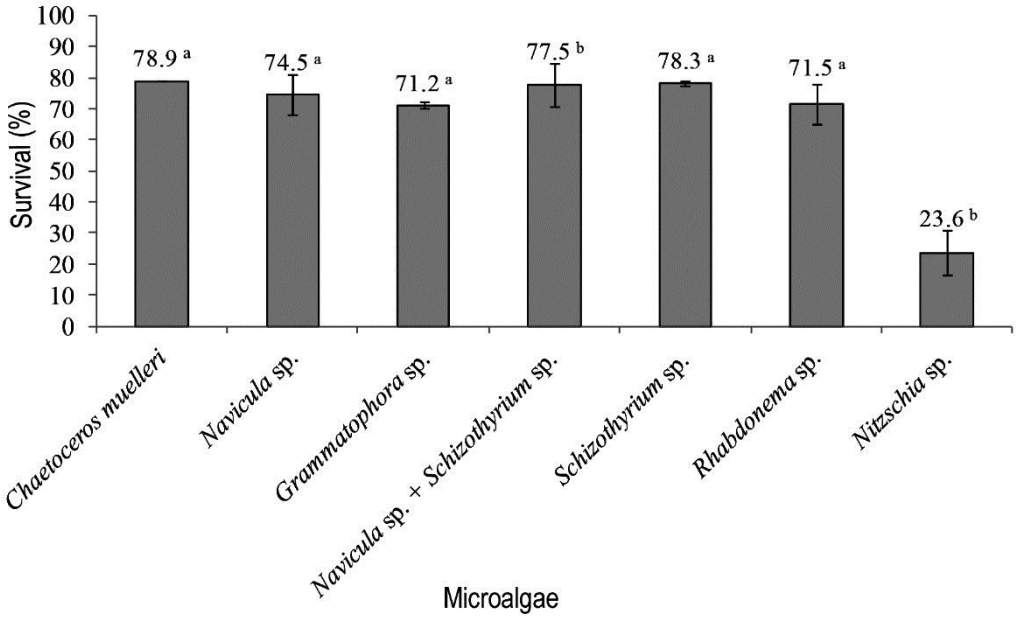

Figure 3. Survival of *Artemia franciscana* after 12 days, fed with different species of marine microalgae. Vertical bars indicate median values ± SDs Horizontal bars (n = 3). Superscripts (a, b) express significant differences between treatments.

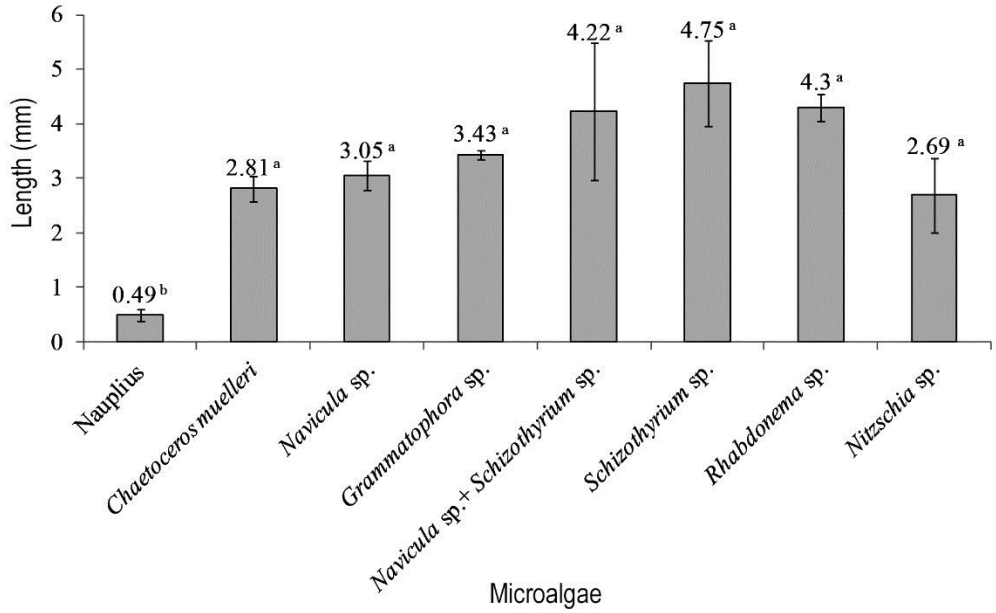

Figure 4. Length (mm) of *Artemia franciscana* fed with different species of marine microalgae after 12 days. Vertical bars indicate median values ± SDs Horizontal bars (n = 3). Superscripts (a, b) express significant differences between treatments.

Rhabdonema sp. and *Nitzschia* sp. resulted in less weight compared to the control diet with *C. muelleri.*

Water physical and chemical culture parameters are shown in Table 3; all of them were within normal and acceptable ranges for cultivating *A. franciscana*.

DISCUSSION

Microalgal cells with a wide range of forms, sizes, shapes, composition, and habits are found in nature, which may have a valuable application for aquaculture.

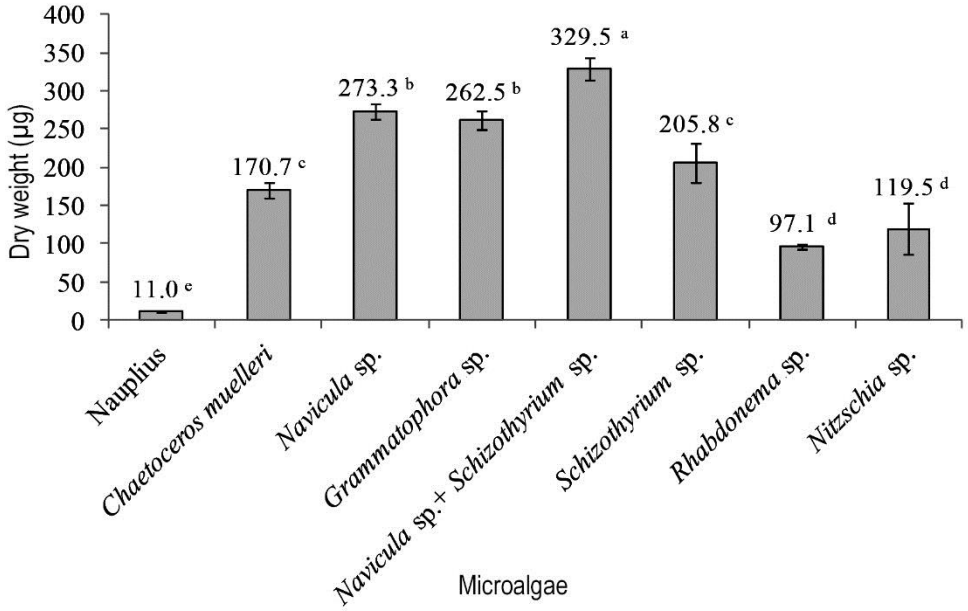

Figure 5. Dry weight (µg) of *Artemia franciscana* fed with different species of marine microalgae after 12 days. Vertical bars indicate median values ± SDs Horizontal bars (n = 3). Superscripts (a, b, c, d, e) express significant differences between treatments.

Table 3. Water quality parameters in cultures of *Artemia franciscana* fed with different microalgae, lower and maximum values.

Microalgae	Oxygen (mg mL^{-1})	Salinity	Temperature (˚C)	pH
Chaetoceros muelleri	4.3-6.6	36.6-46.0	19.9- 30.8	7.4-7.8
Navicula sp.	5.1-6.3	39.8-43.7	18.7-31.9	7.7-8.0
Grammatophora sp.	4.3-7.7	40.1-45.5	20.9-30.8	7.5-8.4
Schizothyrium sp.	4.1-5.1	40.8-46.6	19.3-30.8	7.6-7.9
Navicula sp. + *Schizothyrium* sp.	4.5-8.3	40.3-43.5	19.3-30.12	7.7-8.0
Rhabdonema sp.	4.3- 5.3	40.3-46.9	18.6-30.4	7.6-8.2
Nitzschia sp.	3.8-4.7	41.7-45.1	18.6- 30.2	7.6-8.8

In our study, five species of microalgae with variability in shape and size due to differences in taxon were isolated. One of the main differences that usually occur between microalgae belonging to different genera is their biochemical composition. According to the classification, those identified as *Navicula* sp., *Nitzschia* sp., *Grammatophora* sp., and *Rhabdonema* sp. belong to the class *Bacillariophyceae* (diatoms) from which a wide range of cell size is found, such as the genera isolated while the size of *Schizothyrium* sp. may range from 2.5 to 18 µm depending on its life cycle. Among the various microalgae assessed in our work, *Nitzschia* sp. was the strain with the largest size (44.9 µm), but the survival of *A. franciscana* fed by this microalga was affected. The size of the microalgae could determine the ingestion rate by the metanauplius *A. franciscana* since it has shown preference for microalgal cells of less than 16 µm (Díaz *et al*., 2006).

Significant differences ($P > 0.05$) were observed among ash, protein, lipid, and carbohydrate contents in the five strains. However, these variations are attributable to the different genera to which they belong, as their composition can vary by gender and culture conditions (Brown *et al*., 1997). Among the various microalgal constituents, the total carbohydrate content in *Schizotyhrium* sp. was the highest compared to the other four strains because they do not belong to *Bacillariophyceae*, which generally have lower carbohydrate content. However, this carbohydrate content makes them attractive for feeding oysters and scallops (Enright *et al*., 1986; Whyte *et al*., 1989). The feed control *C. muelleri* showed a low lipid percentage

due to the stable culture conditions; outdoor cultivated microalgae had higher lipid percentages because under stress conditions the metabolism is oriented to over produce lipids (Courchesne *et al*., 2009). Values less than 8% in lipids are recommended to feed crustaceans; in our work higher values did not affect growth for *A. franciscana*. The reported data on growth of *Artemia* sp. is difficult to compare due to differences in rearing parameters that also affect the composition of microalgae (Seixas *et al*., 2009). In addition, slight changes in factors as digestibility, palatability and composition will give variations in utilizing nutrients (Glencross *et al*., 2007).

Water quality parameters in *Artemia* cultures showed significant variations. Among these variables, oxygen, salinity, and temperature are the factors that can impact its growth and morphological features (Ben-Naceur *et al*., 2011). *Artemia* cultures can withstand and tolerate a wide range of variations in physical and chemical parameters and dissolved oxygen concentrations ranging from 1.0 mg L^{-1} to oxygen saturation. In the experimental units we registered concentrations above 3.8 mg L^{-1}. Salinity levels were within the required values for *Artemia* (Van Hoa *et al.*, 2011). Temperature was the parameter in which major variations were registered, and it was attributed to environmental variations throughout the day but within the tolerance range for *Artemia* (Sorgeloos *et al.*, 1986) cultivation. The pH was maintained in cultures without major changes around pH 8, as recommended by Treece (2000).

Our results on *A. franciscana* survival fed with the various species of microalgae showed similar values to the control group, except when *Nitzschia* sp. was used as food. These values are higher than those found by Atashbar *et al*. (2010), and similar to those obtained by Evjemo & Olsen (1999) with microalgae cultured under controlled ambient conditions. In our work, having no control over temperature, temperature variations were observed from 18.6 to 31.9ºC. *Artemia parthenogenetica* cultures have been successfully maintained in rustic culture tanks at 35ºC (Van Hoa *et al*., 2011) although Van Hoa (2002) mentions that growth and survival of *A. franciscana* can be influenced by temperature and salinity. Despite there was no control of environmental variables (outdoors) in our study, our results are comparable to those obtained under controlled culture conditions.

The results of protein, lipid, and carbohydrate contents of the various strains could not be correlated with survival and growth; feed conversion rate values could confirm the best algal feed to *Artemia* sp. (Maldonado-Montiel & Rodríguez-Canché, 2005).

The low dry weight in *Artemia* fed wth *Nitzschia* sp. is attributed to the low consumption of algae and consequently a low chitin synthesis which is the main constituent of the exoskeleton. However, our results on feeding *A. franciscana* with *Rhabdonema* sp. did not follow a pattern, requiring further evaluation to determine the cause of its low dry weight. Other authors suggest that energy content of these species of microalgae can affect the growth of *Artemia* (García-Ulloa *et al*., 1999; Godinez *et al*., 2004).

Among the different strains tested to feed *A. franciscana*, the ones fed with *Grammatophora* sp., *Schizothyrium* sp. and *Rhabdonema* sp. had a greater size than those fed with the control species (*C. muelleri*), reaching larger sizes as reported by Atashbar *et al*. (2010). Utilization of endemic microalgal species from tropical weather, suitable for cultivating *Artemia*, may facilitate the feeding process since adaptation to these temperatures allows microalgal cultures to be kept in better physiological condition and be utilized as food integrally. The results obtained from cultures of *Thalassiosira weissflogii* revealed that the diatoms changed their biochemical composition and decreased their growth rate when undergoing salt stress conditions at salinities greater than 25 (García *et al*., 2012). The same phenomenon may be related to the final composition of *C. muelleri* when it is ingested at high salt conditions as shown in our work. Additionally, the culture temperature (20˚C) of *C. muelleri* is below to the *Artemia* culture temperatures, the temperature difference can affect the growth and cell composition of microalgae, and thus cause a decrease in size. An evaluation of the nutritional quality of cold water species of microalgae showed that an increase in temperature decreased the amount of essential fatty acids and their growth rate, but the work with the microalgal strains isolated from temperate climates showed no variations were found in their cellular constituents due to their plasticity (Ming-Li *et al*., 2013). The various microalgal strains evaluated in our study can be exempted of this kind of event because of their origin.

Previous works in species of microalgae have shown the variation in their fatty acid profile (*i.e.*, Luong-Van *et al*., 1999; Patil *et al*., 2007), and our results are in acordance with these authors. Regarding the total content of saturated fatty acids, the lowest proportion was registered in *Grammatophora* sp. and the highest content was measured in *Nitzschia* sp. The content of monounsaturated fatty acids among the various species was in the range from 22.6% in *Nitzschia* sp. to 51.0% in *Schizothyrium* sp.

Regarding PUFAs, docosaexanoic (DHA) acid was not found in our cultures of *Nitzschia* sp. and *Schizothyrium* sp. However, Chatdumrong *et al*. (2006) has reported that this fatty acid is considered as a major constituent in *Schizothyrium* sp. and in a lesser amount in *Nitzschia* sp., and that its content in both species is increased under heterotrophic culture conditions. Because in our study both species were cultured under autotrophic conditions, it may be the reason for the absence of these fatty acids.

The temperate marine species of microalgae as those isolated in our study may have a high lipid content, the presence of polyunsaturated fatty acids, and docosahexanoic eicosapentanoic acids. The use of endemic species for aquaculture purposes may favor their cultivation under semi-controlled conditions, reducing their production costs. In addition, they can be used as live food. *Navicula* sp., *Grammatophora* sp., *Rhabdonema* sp., and *Schizothyrium* sp. are better than *C. muelleri*. According to the results in our study, the use of *Nitzschia* sp. for feeding and development of *A. franciscana* is not recommended.

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