

## Fatty acids profile and nutritional composition of two tropical diatoms from the Costa Rican Pacific Coast

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**SUMMARY:** Microalgae represent an important nutritional source for diverse organisms, therefore, their nutritional value, and more specifically, total lipid and fatty acid contents, must be considered. This study evaluated the nutritional contents and potential growth under controlled conditions of *Nitzschia* sp. and *Chaetoceros* sp. Tropical microalgae, isolated from the Gulf of Nicoya, Costa Rica. In both strains, the nutritional composition and the fatty acid profile were evaluated in exponential and stationary phases. With regards to fatty acids, *Nitzschia* sp. had more Eicosapentaenoic Acid (EPA) in both the exponential (32.80%) and stationary (27.20%) phases. The results in growth rate, production and biochemical composition indicated two tropical microalgae strains suitable for cultivation under controlled conditions. The studies of the phytoplankton in this geographical area is highly relevant because of its importance in the primary production of nutrients and the importance of finding sources of fatty acids such as the EPA.

**KEYWORDS:** *Bacillariophyceae*; *Chemical composition*; *Costa Rica*; *Fatty acids*; *Microalgae cultures*

**RESUMEN:** *Perfil de ácidos grasos y composición nutricional de diatomeas tropicales aisladas de la costa del Pacífico de Costa Rica.* Las microalgas representan una fuente alimenticia importante para diversos organismos, por lo que su valor nutricional debe ser considerado, especialmente su contenido de lípidos totales y el perfil de ácidos grasos. Este estudio tuvo como objetivo evaluar el crecimiento y determinar la composición bioquímica de dos cepas de microalgas tropicales aisladas de la costa del Pacífico Central de Costa Rica, *Nitzschia* sp. y *Chaetoceros* sp. En ambas se evaluó la composición nutricional y perfil de ácidos grasos en fase exponencial y estacionaria. *Nitzschia* sp. tuvo mayor cantidad de ácido eicosapentanoico (EPA) en ambas fases (32.80% y 27.20%, respectivamente). Los resultados en tasa de crecimiento, producción y composición bioquímica, presentan dos cepas de microalgas tropicales aptas para su cultivo en condiciones controladas. Estudiar el fitoplancton de esta zona es muy relevante debido a su importancia en la producción primaria y en la búsqueda de fuentes de ácidos grasos como el EPA.

**PALABRAS CLAVE:** *Ácidos grasos*; *Bacillariophyceae*; *Composición química*; *Costa Rica*; *Cultivos microalgales*

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## 1. INTRODUCTION

Microalgae are an important source of nutrients and food for diverse organisms (Conceição *et al.*, 2010). This importance arises due to a number of key characteristics of microalgae, including cell size, ease of production, protein and lipid contents and quality (Renaud *et al.*, 1994; Huerlimann *et al.*, 2010). Under appropriate conditions, these microorganisms can duplicate in biomass during the logarithmic growth phase in less than 24 h, although this rate varies among species (Abou-Shanab *et al.*, 2011). After the establishment of an algal culture, their nutritional value must be determined; particularly, the protein and lipid contents are the most important organic components to consider (Huerlimann *et al.*, 2010).

The biochemical composition of microalgae varies among species, although culture conditions are generally similar. However, each microalgae culture differentially responds to nutrient availability and proportions (Prieto *et al.*, 2005; Huerlimann *et al.*, 2010). Nutrient contents also fluctuate based on the developmental phase, where, for example, a typical culture under standard conditions contains 30-40% protein, 10-20% lipids, and 5-15% carbohydrates at the end of the logarithmic phase. In the stationary phase, when nitrates are limited, storage products such as carbohydrates and lipids can double (Roncarati *et al.*, 2004).

When selecting a microalgae species as a source of nutrients, the lipid profile should be analyzed. It is also necessary to determine the fatty acid (FA) profile (Renaud, 1994; Huerlimann *et al.*, 2010; Tocher, 2015). Since in the last years special interest in the nutritional balance contributed by the FA has arisen (Tocher, 2015) it is important to know the lipid profile. The FA composition is species-specific and is a relevant criterion in nutrition (Roncarati *et al.*, 2004). Particularly, it is important for diets containing marine organisms to contain highly unsaturated fatty acids (HUFAs), especially EPA (C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) (Delaporte *et al.*, 2005).

Currently, microalgae constitute a primary source of HUFAs (Hinzpeter *et al.*, 2006), with EPA being one of the most studied due to its well-known effects on the cardiovascular system of organisms, where, in humans, EPA effectively acts against cancer, coronary cardiomyopathy, schizophrenia, and rheumatoid arthritis (Ward and Singh, 2005; Hinzpeter *et al.*, 2006; Conceição *et al.*, 2010; Tocher, 2015). EPA may decrease muscle waste, can reduce inflammation and has the potential to modulate nutritional status/body composition. Marine fatty acids such as EPA and DHA inhibit leukotriene-mediated inflammation which leads to atherosclerosis in subpopulations with increased risk (Simopoulos, 2006).

The marine microalgae culture, like diatoms, could offer the ideal long-term, sustainable solution for EPA demand (Tocher, 2015). A variety of strategies has been devised to enhance the production of EPA and many authors have investigated processes for the increased production of EPA by diatom algae. The intensive production of FA would require algae to simultaneously demonstrate high growth and high proportions of EPA and DHA. These can be almost exclusive traits as lipid deposition is often associated with conditions when growth is limited (e.g. Nitrogen limitation, in stationary phase) (Tocher, 2015). *Nitzschia alba* and *N. laevis* have been studied because of the large EPA levels produced by these microorganisms (Ben-Amotz *et al.*, 1985; Ward and Singh, 2005).

Considering the importance of microalgae nutritional contents, the aim of the present study was to characterize the growth and nutritional composition of two Bacillariophyceae diatoms obtained from the Pacific coast of Costa Rica. These diatoms were *Nitzschia* sp. and *Chaetoceros* sp., which are often considered candidates for obtaining essential marine HUFAs.

## 2. MATERIALS AND METHODS

### 2.1. Algae cultures and growth rates

Two marine microalgae were selected from the strain database managed by the Center of Marine Biology (EBM) of the Universidad Nacional de Costa Rica. These strains were originally isolated off the Gulf of Nicoya, Costa Rica, and classified as belonging to the *Nitzschia* sp. and *Chaetoceros* sp. (Rodríguez-Núñez *et al.*, 2016).

For culture assays, the *f/2* culture medium was used (Guillard, 1975). This medium was prepared with seawater filtered at 1  $\mu\text{m}$  and sterilized with UV light (Model QL-240, eight lamps). For both strains, three culture volumes (150, 1500, and 8000 mL) were assessed in 250 and 2000 mL flasks and 10000 mL glass bottles, respectively. Each volume was cultured in triplicate. Luminous intensities were 14-16  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (150 mL), 30-35  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (1500 mL), and 50-55  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (8000 mL), while the remaining variables were constant for all assays, including salinity (33 psu), pH ( $8.3 \pm 0.2$ ), temperature ( $22 \pm 1$  °C), and photoperiod (12:12 h light:dark).

The doubling time was determined by the three repetitions used for each volume, with 1 mL samples taken every 12 h and fixed with 1% Lugol's iodine solution. A haemocytometer was used to perform at least four cell counts per sample. Once the doubling time for the 150 mL culture was obtained, the end-point of logarithmic growth was determined to inoculate the 1500 mL culture and, likewise, the 8000 mL culture.

## 2.2. Characterization of cultures and statistics

From the microalgae cultured, growth rate (K), culture productivity (P), and the accumulated media (in terms of cells per mL<sup>-1</sup>) were determined. For all volumes used in this study K and P (in logarithmic phase) were used to characterize the culture using the following formulas:  $K = (\ln C_f - \ln C_i) \cdot (t_f - t_i)^{-1}$  and  $P = (C_f - C_i) \cdot (t_f - t_i)^{-1}$ , where C<sub>i</sub>: initial concentration, C<sub>f</sub>: final concentration of the exponential phase, and t<sub>f</sub> - t<sub>i</sub>: duration of the exponential phase in days (Levasseur *et al.*, 1993).

Normal data distribution was confirmed using the Kolmogorov-Smirnov and Lilliefors Normality Tests, following the methodology proposed by Bhujel (2009). To determine the end-point for each culture phase (growth curve) the P values were used. A one-way analysis of variance was applied, using time as a variable. For the statistical analyses, the STATISTICA v7.0.61.0 software (Statsoft, Inc.) and Excel 2010 (Microsoft Corporation) were used.

## 2.3. Determination of chemical composition

Both strains were cultured in 10000 mL bottles filled with 8000 mL of f/2 culture medium. The nutritional composition was evaluated during two phases of the growth curve, with 12 repetitions during the logarithmic phase and 8 during the stationary phase. It was necessary to harvest 12 and 8 replicas for strain for biochemical analyzes of logarithmic and stationary phase. The harvesting points were previously defined in assays determining doubling time. Three samples of 1 mL were taken every 24 h and when harvesting the entire biomass. For observing the system conditions, culture samples were taken.

The harvested biomass was centrifuged for 10 min at 4000 rpm (Model U-320R, Boeco, Germany). The obtained sample was maintained at -20 °C. The total harvest was mixed and lyophilized (Ilshin FD-8505, Korea); except 5 g of biomass, which were used to determine moisture (%). To obtain the biomass of each strain the moisture value was used. The following lyophilized biomass characteristics were determined in triplicate: moisture, ash, crude protein, total lipids and fiber contents; and fatty acid profile.

Moisture was determined by drying the samples in an Oven (Model FD 110, Binder, USA) at 95 °C until reaching a constant weight (Method 930.04; AOAC, 2000). Ash content was determined by calcining the samples at 500 °C for 8 h in a muffle oven (Model Furnace 6000, Barnstead/Thermolyne, United Kingdom) (Method 930.05; AOAC, 2000). To determine crude protein content, Kjeldahl Nitrogen determinations were performed (DK 20, Velp Scientifica, Italy) (Method 978.04/955.04;

AOAC, 2000). Total lipids were assessed using the Soxhlet method (solvents: chloroform/metanol, 2:1, v/v; Ser 148, Velp Scientifica, Italy) (Method 920.39, AOAC, 2000). In turn, fiber quantity was assessed using the modified Weende Method (Fiwe, Velp Scientifica, Italy) (modified according to Method 962.09, AOAC, 2000.) on a supernatant sample taken from the previously performed total lipid assessments.

To establish the Fatty Acid Methyl Ester (FAME) profile (Supelco TM 37 Component FAME Mix, Sigma-Aldrich, 10 mg·mL<sup>-1</sup> in methylene chloride), which included saturated Fatty Acids (SFAs), Monounsaturated Fatty Acids (MUFAs), and Polyunsaturated Fatty Acids (PUFAs), the lipids were extracted when cold using the modified Folch Method (Folch *et al.*, 1957, Pierce Chemical Company Manual), fatty acid derivatization, and chromatographic analysis (Gas Chromatograph with Flame Ionization Detector GC-FID, CLARUS 600, Perkin Elmer). The conditions of chromatography were as follows: column, 30 m x 0.32 mm x 0.25 μm (Omega Wax320); injector, 250 °C; FID detector, 260 °C; Carrier-gas, He 1 mL·min<sup>-1</sup> (22 cm·s<sup>-1</sup>, approximately); injection volume, 1 μL; a split ratio, 100:1. The oven was held at 140 °C for 5 min before being increased to 240 °C at a rate of 2 °C·min<sup>-1</sup> over 60 min. All analyses were performed at the Algal Research and Tech Development Center of the Universidad Católica del Norte (CIDTA). FAMES were analyzed in duplicate.

The logarithmic and stationary phases of both strains were compared. The data obtained for total lipid, crude protein, biomass moisture, EPA and DHA levels were compared using the Student's *t*-test for the two samples (Bhujel, 2009).

## 3. RESULTS

### 3.1. Microalgae growth rates and characterizations

The culture acclimation time was 1-2.5 d for all volumes of *Nitzschia* sp. used (Figure 1) and 12 h and 2 d for all volumes of *Chaetoceros* sp. used (Figure 2). The end of the exponential phase in 8000 mL volume was 3.5 d for both microalgae species. The highest value of P was obtained in 150 mL volumes for both species (Table 1).

### 3.2. Microalgae nutritional compositions

*Nitzschia* sp. presented approximately 10% more biomass than *Chaetoceros* sp. (Table 2). Significant differences in moisture were found between the logarithmic and stationary phases for both *Nitzschia* sp. ( $P = 0.009$ ) and *Chaetoceros* sp. ( $P = 0.004$ ). Likewise, significant differences were found when comparing the logarithmic ( $P < 0.001$ ) and stationary ( $P < 0.001$ ) phases between strains.

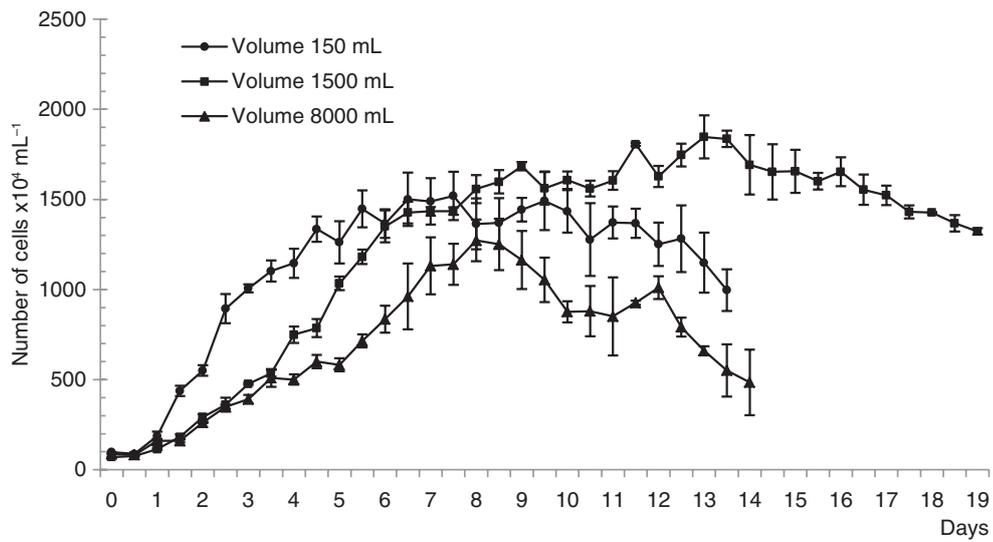


FIGURE 1. *Nitzschia* sp. growth kinetics when cultured with three volumes of the *f/2* medium.

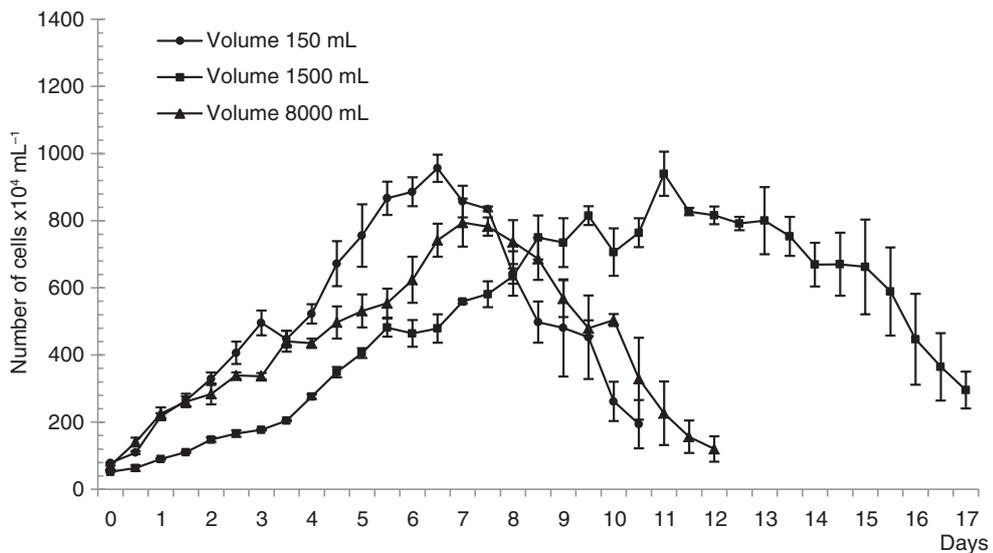


FIGURE 2. *Chaetoceros* sp. growth kinetics when cultured with three volumes of the *f/2* medium.

The main biochemical component of *Nitzschia* sp. was protein in the logarithmic and stationary phases. *Chaetoceros* sp. evidenced elevated protein levels in the logarithmic phase and the main biochemical component of the stationary phase was ash content (Table 2). For both strains, ash contents were higher in the logarithmic phase. Significant differences were also recorded between the logarithmic and stationary phases ( $P < 0.001$ ) of *Chaetoceros* sp. For both strains, fiber values were not detected in the two culture phases.

For both strains, crude protein contents were higher than total lipid contents in the logarithmic phase. Comparing the logarithmic and stationary phases, significant differences were found for *Nitzschia* sp. ( $P < 0.001$ ) and *Chaetoceros* sp. ( $P < 0.001$ ). Significant differences were also recorded between strains when comparing the logarithmic ( $P < 0.001$ ) and stationary phases ( $P < 0.001$ ). Regarding total lipid contents, the logarithmic and stationary phase comparisons showed significant differences for *Nitzschia* sp. ( $P < 0.001$ ) and

TABLE 1. *Nitzschia* sp. and *Chaetoceros* sp. density, growth rate, and productivity in three culture volumes

Strain	V	F	D	K	P	P
<i>Nitzschia</i> sp.	150	4.5	13 353 611 ± 703 520	0.58	2.75 x 10 <sup>6</sup>	P > 0.1
	1500	5	10 344 444 ± 370 928	0.54	1.93 x 10 <sup>6</sup>	P > 0.1
	8000	3.5	5 097 777 ± 493 305	0.51	1.21 x 10 <sup>6</sup>	P > 0.1
<i>Chaetoceros</i> sp.	150	4.5	6 386 666 ± 670 575	0.48	1.31 x 10 <sup>6</sup>	P > 0.1
	1500	5	4 061 944 ± 145 640	0.41	7.08 x 10 <sup>5</sup>	P = 0.0016
	8000	3.5	4 412 222 ± 309 663	0.52	1.06 x 10 <sup>6</sup>	P > 0.1

V: culture volume (mL), F: end of exponential phase (days), D: final density of exponential phase (cells mL<sup>-1</sup>) with standard deviation, K: rate growth, P: productivity (cells mL<sup>-1</sup>), P: significance between P values.

TABLE 2. Chemical composition and moisture content and of *Nitzschia* sp. and *Chaetoceros* sp., biochemical compounds expressed as percentages of dry weight, during the logarithmic and stationary phases (mean ± standard deviation, n = 3)

Strain	Phase	% Moisture		% Biochemical compound				
		Biomass**	Lyophilized biomass	Total lipids**	Crude protein**	Carbohydrates	Ash**	Fiber
<i>Nitzschia</i> sp.	L	81.13 ± 0.04	3.25 ± 0.22	18.36 ± 0.28	43.16 ± 0.13	18.88	19.60 ± 0.06	nd
	S	80.31 ± 0.38	3.25 ± 0.26	28.31 ± 0.32	39.62 ± 0.17	13.80	18.27 ± 0.09	nd
<i>Chaetoceros</i> sp.	L	88.60 ± 0.03	5.60 ± 0.18	19.28 ± 0.20	34.99 ± 0.10	10.78	34.95 ± 0.12	nd
	S	90.14 ± 0.36	5.37 ± 0.22	23.78 ± 0.17	27.84 ± 0.06	17.19	31.20 ± 0.11	nd

L: logarithmic phase, S: stationary phase, nd: undetected, \*\* Indicates significant differences between values of different phases between strain and the same phase between different strains using Student's *t*-test.

*Chaetoceros* sp. ( $P < 0.001$ ). The strain comparisons evidenced differences between the logarithmic ( $P = 0.009$ ) and the stationary phases ( $P < 0.001$ ).

According to the obtained FA profiles, both strains contained C14:0, C16:0, C16:1, and EPA as the principle FAs (Table 3). In the logarithmic phase of *Nitzschia* sp., PUFAs represented the majority of the lipid contents, while SFAs were the least represented. In the stationary phase of *Nitzschia* sp., MUFAs were the principal component, followed by PUFAs. In contrast, both phases of *Chaetoceros* sp. evidenced SFAs as the principal component, with PUFAs as the least represented. Significant differences were obtained when comparing the EPA levels between the logarithmic and stationary phases of *Nitzschia* sp. ( $P < 0.001$ ) and *Chaetoceros* sp. ( $P = 0.03$ ). In turn, no significant differences were found between *Chaetoceros* sp. culture phases for DHA levels ( $P = 0.49$ ).

#### 4. DISCUSSION

Biomass and lipid productivity are critical parameters in selecting micro-algal species for scale-up production and nutritional applications. The lipid content refers to quantitative and qualitative lipid composition (Conceição *et al.*, 2010; Huerlimann *et al.*, 2010). *Nitzschia* sp. and *Chaetoceros* sp. evidenced short acclimation

periods during scaling compared to other diatoms (Prieto *et al.*, 2005). Due to this short period, the desired biomass can be reached in less time, in addition to reducing the risks of improper management. Furthermore, both microalgae species of this study were sufficiently supported in the culture conditions. In terms of productivity, this result is particularly important. Notably, the significantly different growth rates of the three evaluated volumes for both strains are data which could be used to optimize the resource volumes used for scaling. Therefore, the tolerance and rate of adaptation to environmental variation are factors that should be considered when selecting microalgae species for culturing and biomass production (Conceição *et al.*, 2010).

Another characteristic used to evaluate microalgae potential is growth rate in terms of cell count or biomass. Ideally, microalgae should have a short lifecycle which is reproducible under controlled conditions (Araújo and García, 2005; Conceição *et al.*, 2010). Comparisons were made between traditionally cultured microalgae and the currently evaluated strains. Knuckey *et al.* (2002, p. 260) evaluated *Nitzschia cf. paleacea* and the *Isochrysis* aff. *galbana* clone T-ISO in a volume of 1600 mL, and obtained growth rates of 0.55 and 0.56, respectively. These values are very similar to those obtained for the *Nitzschia* sp. of this study.

TABLE 3. Fatty acid composition, expressed as percentages of total fatty acids, of *Nitzschia* sp. and *Chaetoceros* sp. during the logarithmic and stationary phases (mean  $\pm$  standard deviation,  $n = 2$ )

Fatty acids	% FAMES			
	<i>Nitzschia</i> sp.(L)	<i>Nitzschia</i> sp.(S)	<i>Chaetoceros</i> sp. (L)	<i>Chaetoceros</i> sp. (S)
SFAs				
C14:0	9.64 $\pm$ 1.06	8.68 $\pm$ 0.04	30.40 $\pm$ 0.40	31.70 $\pm$ 0.40
C15:0	0.53 $\pm$ 0.20	0.29 $\pm$ 0.01	0.75 $\pm$ 0.09	0.78 $\pm$ 0.03
C16:0	16.10 $\pm$ 0.40	15.30 $\pm$ 0.09	17.40 $\pm$ 0.10	17.30 $\pm$ 0.10
C17:0	0.50 $\pm$ 0.17	0.04 $\pm$ 0.01	nd	nd
C18:0	0.79 $\pm$ 0.24	0.41 $\pm$ 0.01	nd	nd
C21:0	nd	0.16 $\pm$ 0.01	nd	nd
C24:0	nd	nd	0.09 $\pm$ 0.01	nd
MUFAs				
C16:1	32.20 $\pm$ 0.40	39.70 $\pm$ 0.20	37.90 $\pm$ 0.90	38.20 $\pm$ 0.20
C18:1 <i>n</i> 9c/C18:1 <i>n</i> 9t	1.42 $\pm$ 0.47	1.99 $\pm$ 0.02	nd	nd
C22:1 <i>n</i> 9	nd	nd	0.40 $\pm$ 0.17	nd
C24:1 <i>n</i> 9	0.72 $\pm$ 0.20	0.58 $\pm$ 0.01	0.13 $\pm$ 0.03	nd
PUFAs				
C18:2 <i>n</i> 6t	4.41 $\pm$ 0.36	3.71 $\pm$ 0.01	nd	nd
C18:2 <i>n</i> 6c	nd	nd	2.00 $\pm$ 0.14	1.53 $\pm$ 0.13
C18:3 <i>n</i> 3	nd	0.60 $\pm$ 0.01	nd	nd
C18:3 <i>n</i> 6	0.89 $\pm$ 0.45	1.30 $\pm$ 0.01	1.08 $\pm$ 0.16	0.93 $\pm$ 0.04
C20:3 <i>n</i> 3	nd	nd	2.75 $\pm$ 0.25	3.48 $\pm$ 0.31
C20:5 <i>n</i> 3	32.80 $\pm$ 2.20	27.20 $\pm$ 0.09	6.44 $\pm$ 0.06	5.58 $\pm$ 0.16
C22:6 <i>n</i> 3	nd	nd	0.53 $\pm$ 0.22	0.52 $\pm$ 0.14
<i>n</i> -6/ <i>n</i> -3	0.16	0.18	0.32	0.26
$\Sigma$ SFAs	27.60	24.90	48.80	49.80
$\Sigma$ MUFAs	34.30	42.30	38.40	38.20
$\Sigma$ PUFAs	38.10	32.80	12.80	12.00

L: logarithmic phase, S: stationary phase, FAMES: fatty acid methyl esters, SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids, nd: undetected.

In contrast, the growth rates obtained by Renaud *et al.* (2002, p. 197) for *Chaetoceros* sp. ( $K = 0.74$ ) were superior to those obtained in this research. Knuckey *et al.* (2002, p. 260) also obtained higher values of  $K$  for other diatoms. Although the *Chaetoceros* sp. of this study presents a short culture acclimation time, its growth rate was inferior to other diatoms traditionally cultivated. That may be due the fact that this rate varies among species (Abou-Shanab *et al.*, 2011).

The produced biomass should have appropriate nutritional contents. These contents can vary depending on strain and the phase in which the culture is harvested. In algal cultures, crude protein is the primary organic constituent during the logarithmic growth phase, with contents decreasing during the stationary phase as a consequence of decreased nitrogenous compounds in the medium. This situation occurs in batch culture, like the one used in this research. In continuous or semi-continuous culture this effect is partially counteracted by adding

nutrients (Medina-Reyna and Cordero-Esquivel, 1998; Araújo and García, 2005; Tocher, 2015). This was observed in the present results for biochemical analyses. Renaud *et al.* (1994, p. 342) isolated tropical species from northern Australia, including marine diatoms that were cultivated under controlled conditions similar to those used in the current study. Comparisons between the protein contents of the *Nitzschia frustulum* in *Nitzschia* sp. of this study showed that it has higher protein levels than either of the previously assessed diatoms. Rivero-Rodríguez *et al.* (2007) assessed the biochemical compositions of five marine microalgae, including *Chaetoceros calcitrans*. The crude protein content of *Chaetoceros* sp. in this study was 6% less than *C. calcitrans*.

Ash represents a large portion of the biochemical composition in diatoms, as occurs in the microalgae species of this study. This is mainly found in the *Chaetoceros* genus, due to the formation and constitution of the frustula. The strains showed a

significant difference when comparing the percentage of ash in exponential and stationary phases. A higher value in the logarithmic stage may be due to a thicker frustula in the early stages of the culture (Medina-Reyna and Cordero-Ésquivel, 1998; Pacheco-Vega and Sánchez-Saavedra, 2009).

Many microalgae species present changes in lipid content during the different phases of growth (Huerlimann *et al.*, 2010; Su *et al.*, 2013). Lipid content generally increases during the stationary phase as photosynthetic energy of the culture is diverted from cell division to lipid production, which is often triggered by limitations of nitrogen (Ben-Amotz *et al.*, 1985; Huerlimann *et al.*, 2010; Su *et al.*, 2013). This behavior was found for the two presently studied strains, where *Nitzschia* sp. presented an increased total lipid content of 9.95% and *Chaetoceros* sp. of 4.5%. The *Nitzschia* genus is known for high lipid contents that can surpass even 40% (Ben-Amotz *et al.*, 1985). In comparing the total lipid contents of the *Nitzschia* sp. of this study, with the values obtained by Renaud *et al.* (1994, p. 341), the levels of *Nitzschia* sp. were 2.41% greater than previously reported. Likewise, the lipid contents of *Chaetoceros* sp. in the logarithmic phase were 7.9% higher than values reported by Rivero-Rodríguez *et al.* (2007), in addition to presenting values superior to those in other studies (Renaud *et al.*, 2002; Delaporte *et al.*, 2005; Pacheco-Vega and Sánchez-Saavedra, 2009).

As with lipids contents, the FA profile varies considerably between the different species or strain-specific (Hu *et al.*, 2008; Huerlimann *et al.*, 2010). However, in general terms for marine microalgae, PUFAs constitute most of the FA profile (Ben-Amotz *et al.*, 1985; Hu *et al.*, 2008), as evidenced by the *Nitzschia* sp. of this research. Moreover, the dominant FAs in both strains coincided with tendencies reported in the literature, specifically the C14:0, C16:0, C16:1, and EPA (Renaud *et al.*, 1994; Su *et al.*, 2013), values found in diatoms (Hu *et al.*, 2008).

Roncarati *et al.* (2004, p. 406) assessed four algal strains and confirmed that the FA profiles presented differences in the same strain depending on the culturing phase. The results for *Nitzschia* sp. and *Chaetoceros* sp. of this work coincided with prior findings by Su *et al.* (2013), in a study in which C16:1 FA content increased during logarithmic growth while EPA levels decreased. In the *Nitzschia* sp. of this study, C18:1 FA levels increased during the stationary stage, which could coincide with triacylglycerols acting as storage fats in algae, due to which, levels are generally low during the logarithmic phase (Ben-Amotz *et al.*, 1985; Su *et al.*, 2013).

Just as with diatoms previously studied, *Nitzschia* sp. and *Chaetoceros* sp. present high levels of EPA and low DHA levels (Delaporte

*et al.*, 2005; Hinzpeter *et al.*, 2006; Rivero-Rodríguez *et al.*, 2007; Conceição *et al.*, 2010). EPA plays an important role in the prevention of arrhythmia, atherosclerosis, cardiovascular disease and cancer (Simopoulos, 2006). It is important to know that EPA and/or DHA are necessary for the animal and human population (Roncarati *et al.*, 2004), and it is fundamental to study new sources of EPA and evaluate their potential, such as marine diatoms (Tocher, 2015), like the *Nitzschia* genus. Results of studies in *Nitzschia* reported high EPA levels (Conceição *et al.*, 2010). When compared to values obtained in previous studies, the *Nitzschia* sp. of this research presented up to 16.8% more EPA (Renaud *et al.*, 1994; Su *et al.*, 2013). High EPA levels are very important and are associated with the production of PUFAs.

The lipid contents of *Chaetoceros* sp. of this study were similar or greater than other studies on the genus *Chaetoceros* (Renaud *et al.*, 1994; Pacheco-Vega and Sánchez-Saavedra, 2009). The obtained EPA and DHA values were lower in both phases when compared to the values obtained for *C. muelleri* (13.44% EPA and 6.86% DHA, Pacheco-Vega and Sánchez-Saavedra, 2009) and *C. calcitrans* reported in other investigations (17.80% EPA and 1.30% DHA, Delaporte *et al.*, 2005). According to Ryckebosch *et al.*, (2012, p. 130) for the genus *Chaetoceros*, EPA levels should be between 8 and 22%, while DHA levels should be between 0.03 and 5%. This may be due to the fact that lipid and FA levels are strain-specific, and these concentrations may present differences between the two species of the same genus (Roncarati *et al.*, 2004; Huerlimann *et al.*, 2010).

The performed assessments support the conclusion that microalgae behavior and biochemical composition are specific to each strain. Although the evaluated strains were cultured under identical conditions, significant differences were found in growth, productivity, biochemical composition, lipid levels and FA profile between strains and between developmental phases of the same strain.

## 5. CONCLUSIONS

In conclusion, the culture characteristics and nutritional compositions of the studied strains support further consideration of these diatoms for nutritional applications in the Central American region. *Nitzschia* sp. presented approximately 10% more biomass than *Chaetoceros* sp., in addition to greater growth, higher productivity, and higher levels of protein, lipid, and EPA contents. Due to this, *Nitzschia* sp. is suggested as an option as a source of essential FA and EPA for animal and human nutrition. This study provides an initial foundation for further research, providing key information for the development of regional microalgae cultures.

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