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Full Length Research Paper

Chemical composition of five marine microalgae that occur on the Brazilian coast

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Abstract

Five native species of marine microalgae were batch cultured and their chemical composition was measured in different growth phases. The highest cell yield was recorded in *Chlorella* sp. *Chlorella* sp. and *Bellerochea* sp. showed a reduction of the mean cell volume throughout growth. All microalgae showed protein as the main chemical component, with peaks in the exponential growth phase. The same was found for chlorophyll. Carbohydrates (*Chlorella* sp.), ash (*Bellerochea* sp. and *Chlorella* sp.), and lipids (*Rhodomonas* sp. and *Thalassiosira* sp.) were relatively high in some of the species, at least in one of the growth phases. The carbohydrate content increased throughout growth, but no clear relationship among growth, total lipid and ash content was identified. Total saturated fatty acids were higher in the exponential growth phase of all species, decreasing throughout growth. *Rhodomonas* sp. showed high levels of polyunsaturated fatty acid (PUFA), but low percentages of some essential fatty acids. *Bellerochea* sp., *Chaetoceros* sp. and *Chlorella* sp. showed low percentages of total PUFA, but all essential fatty acids were present, except for the green alga. Current results may contribute to selecting strains that possess suitable chemical composition and fast growth, useful characteristics for the sustainable use of native species in aquaculture.

Key words: Microalgae, chemical composition, native species, growth, mariculture.

INTRODUCTION

Capture fisheries and aquaculture supplied the world with about 142 million tons of food fish in 2008. Of this total, aquaculture accounted for 46% (FAO, 2010). Production of bivalves in hatcheries is undoubtedly related to the quality and the quantity of the supplied microalgae (Rico-Villa et al., 2006). With the development of the mariculture in the last two decades, microalgal cultivation has increasing its importance due the fundamental use of microalgae for feeding many animal species in aquaculture (Spolaore et al., 2006). There is particular reference to mollusk and crustacean rearing, and production of live food for fish larvae (Ponis et al., 2008), larvae of red shrimp *Farfantepenaeus brasiliensis* (Gaxiola et al., 2010), or specifically juvenile *Artemia* as prey for *Octopus vulgaris* paralarvae (Seixas et al., 2008). The growth of bivalves is dependent on the algae species and their nutritional value (Reitan, 2011).

Microalgae contain essential nutrients which determine the quality, survival, growth and resistance to disease of cultured animal species (Gouveia et al., 2008). Microalgae are some of the most important feed sources in aquaculture due to their nutritional value and their ability to synthesize and accumulate great amounts of ω3-polyunsaturated fatty acid (PUFA) (Patil et al., 2007), such as 22:6n-3 (DHA) and 20:5n-3 (EPA), two of the most important essential fatty acids required for gametogenesis (Ehteshami et al., 2011). Currently, 30% of the world microalgal production is sold for animal feed applications (Spolaore et al., 2006). The knowledge of the chemical composition of food-species has a key role for mariculture (Thompson et al., 1996; Laing and

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Psimopoulos, 1998; Southgate et al., 1998; Leonardos and Lucas, 2000; Rivero-Rodríguez et al., 2007), especially in larviculture (Pettersen et al., 2010). Many studies on the use of both monoalgal and combination of different species have been carried out in the last decades (Ben-Amotz et al., 1987; Laing et al., 1990; Carić et al., 1993; Thompson et al., 1996; Southgate et al., 1998; Tang et al., 2006). In this context, a variety of microalgal species has been studied, since many species are not suitable for use in commercial aquaculture systems (Enright et al., 1986a).

The microalgal culture system in hatcheries could be inadequate in qualitative or quantitative terms, and the quality of biomass produced could be subject to a nutritional drift (Ponis et al., 2006). In particular, the number of good quality microalgae currently available in hatcheries is limited, and several species which were previously used in commercial hatcheries have now been discarded due to their poor nutritional value (Ponis et al., 2006). Moreover, the availability of good quality seeds could be limited, partly due to the scarce knowledge of the requirements of juvenile stages, as occur with scallop *Nodipecten subnodosus* in Mexico (Cerón-Ortiz et al., 2009). In addition, some species may be edible and digestible but poorly assimilated by prey, like *Nitzschia* for the pearl oyster *Pinctada fucata* (Hashimoto et al., 2008). The nutritional value of the dietary algae is not only dependent on the chemical composition but also on factors such as the capability of bivalves to ingest and digest the algae and to assimilate their nutrients (Lora-Vilchis and Maeda-Martinez, 1997).

In Brazil, strains from foreign countries are largely used because they are world-wide recognized and because they are also chemically known. However, this procedure has been stimulating the introduction of many non-indigenous species (Assad and Bursztyn, 2000), and this option is tremendously contested in many cases (Renaud et al., 1999). There is a concern about the need of screening studies with native species, which could reduce the use of non-indigenous microalgae in com-mercial aquaculture (Brown et al., 1998; McCausland et al., 1999; Gouda et al., 2006; Ponis et al., 2006). Ideally, the chemical composition of potentially useful native species should be evaluated to assess the nutritional properties of them (Gouda et al., 2006). Despite this issue is obviously important with many positive conse-quences, experimental data in this field are needed in Brazil to make it feasible. Native species could generate a less environmental impact in the location where the system is operating, due more ecological compatibility and high level of fitness to local and/or regional characteristics.

The chemical content of microalgae can vary with culture age and with changes in environmental conditions (Fernández-Reiriz et al., 1989), and culture conditions (Araújo and Garcia, 2005). The effect of variation of these parameters on many algae species has been studied in order to better understand their physiology, as well as to answer specific and relevant questions for mass culture and nutrition of bivalves and others herbivores (Uriarte et al., 1993). Data on the chemical composition of micro-algae may also vary widely due to differences of the methods of measurement used (Barbarino and Lourenço, 2005), the physiological state of the microalgae (Fernández-Reiriz et al., 1989), as well as to the experi-mental conditions applied (Lourenço et al., 2002), like temperature (Durmaz et al., 2009), light intensity (Lourenço et al., 2008), medium cultivation (Huerlimann et al., 2010) or in outdoors conditions (Banerjee et al., 2011). In addition, due to the interaction of the organisms with the culture medium, a batch culture is under a continuous chemical change. These variations reflect on the cell metabolism and consequently on their chemical composition (Lourenço et al., 2002). Thus, the chemical composition of a given species may vary widely under different growth conditions, and such changes may be related to the growth phase of the culture (Fernández-Reiriz et al., 1989; Lourenço et al., 1997). However, studies focusing sampling in different growth phases are relatively scarce; most of the papers report the chemical profile of given species in a fixed momentum of the growth phase, ignoring the continuing process of interaction between microalgae and the medium.

The present study was designed to evaluate the chemical composition of five native microalgae in different growth phases, characterizing their chemical profile under standardized conditions. In addition, quantification of ash content and cell biovolume were also performed to increase information for a better interpretation of the dynamics of the microalgae in cultures.

MATERIALS AND METHODS

Microalgae

Five species were studied; all of them were obtained from the Elizabeth Aidar Microalgae Culture Collection, Department of Marine Biology, Fluminense Federal University, Brazil. The following strains were used: *Bellerochea* sp. Van Heurck 1885 (Heterokontphyta– Biddulphiales; strain SP1), *Chaetoceros* sp. Ehrenberg 1844 (Heterokontphyta–Bacillariales; strain SP1), *Chlorella* sp. M. Beijerinck 1890 (Chlorophyta–Chlorococcales; strain SP1), *Rhodomonas* sp. Karsten 1898 (Cryptophyta–Cryptomonadales; strain PB1) e *Thalassiosira* sp. Cleve 1873 (Heterokontphyta– Bacillariales; strain UB7). All strains were isolated from Brazilian coastal waters and were selected because of their fast growth responses and taxonomical diversity. The strains tested here do not form chains in cultures, always showing isolated individual cells.

Culture conditions

The species were grown in batch cultures, with three replicates (n = 3), in 3.0 L borosilicate round flasks, filled with 2.0 L of Conway culture medium (Walne, 1966). The seawater was diluted with distilled water to a salinity of 31 psu. Seawater (2.0 L) was autoclaved at 121ºC for 30 min at 1 atmospheric pressure, and enriched with nutrients, which had been a utoclaved separately. Nutrients were

added to the seawater before inoculation. Each cultures were exposed to a saturating irradiance (180 µmol photons m^{-2} s^{-1}, measured with a Biospherical Instruments quantum meter, model QLS100) provided from below by fluorescent lamps (Sylvania daylight tubes), on a 12:12 h light:dark cycle. Mean temperatures were 21±2°C. Cultures were continuously bubbled with filtered air at a rate of 2.0 L min^{-1} $flask^{-1}$, provided by aquarium air pumps. Daily sampling for cell counts was carried out four hours after the start of the light period. Cells were counted in Fuchs-Rosenthal or Neubauer chambers, depending on the cell size. Cell counts were also carried at the preparation of the experiments ("day 0") and the growth rates were calculated daily. Starter cultures of 50 to 100 ml in mid-exponential growth phase were inoculated into the medium. The initial cell densities of cultures were 3.0 x 10^4 cell ml^{-1} for *Bellerochea* sp., *Rhodomonas* sp. and *Chaetoceros* sp.; 1.0 x 10^4 cell ml^{-1} for *Thalassiosira* sp. and 8.0 x 10^4 cell ml^{-1} for *Chlorella* sp. In the experiments, differences in initial cell densities are due the remarkable differences in microalgae cell volumes (Lourenço et al., 2002).

Preparation of samples for chemical analyses

Sampling for chemical analysis was done three times: in the exponential growth phase, in the transitional phase (period between the end of exponential and early stationary growth phases) and in the stationary growth phase for all species, as indicated in Figure 1. Measured volumes (800 ml) were sampled from the cultures and centrifuged at 7000*g* (centrifuge Sigma, model σ-15), at room temperature, for 10 min, once or several times, to obtain highly concentrated material. All supernatants obtained from each centrifuged sample were collected, and any cell losses were quantified by means of cell counts of the supernatant. The centrifuged cell samples were frozen at -20°C and then freeze-dried, weighed, and stored in vacuum desiccators, sheltered from light for later analyses of protein, carbohydrate, and lipid/fatty acids. For chlorophyll *a*, total carotenoid analyses and ash content samples were obtained by filtering 10 to 30 ml culture onto Whatman GF/F glass fibre filters. The filters were kept at -20°C in flasks containing silica gel until analysis, one to ten days after sampling.

Measurements of biovolume

The cell volumes were measured using the equations provided by Hillebrand et al. (1999), assuming a suitable geometrical shape for each species. Mean volumes were based on measurements of 30-70 cells for each species in each culture flask, under a Hund (Wilovert S model) inverted microscope, giving three mean values used for statistics (n=3).

Chemical analyses

Protein extraction followed the method proposed by Barbarino and Lourenço (2005). The Lowry et al. (1951) method was used to evaluate protein in the samples, with bovine serum albumin as a protein standard. Spectrophotometric determinations were done at 750 nm, 35 min after the start of the chemical reaction.

Carbohydrates were extracted with 80% H_2SO_4 according to Myklestad and Haug (1972). Total carbohydrate concentration was determined spectrophotometrically by the phenol-sulfuric acid method (DuBois et al., 1956), using glucose as a standard. Total lipids were extracted according to Folch et al. (1957), and determined gravimetrically after total solvent evaporation.

Aliquots of the final lipid extracts in chloroform were used to prepare the fatty acid methyl esters (FAME) according to Metcalfe and Schmitz (1961). Samples were saponified with 50% KOH and the methyl- esters of fatty acids were prepared by esterification with 7% B_3F_3 in methanol. The separation of the FAME was carried out in a gas chromatograph Shimadzu, model GC-15 A, equipped with a flame and ionization detector, and a capillary fused silica column (Omegawax 320 x 30 m x 0.32 mm ID). The flow rate of hydrogen (carrier gas) was 40 ml min^{-1}. A Shimadzu, model C-R4, recorder and integrator was used to obtain the chromatograms and quantify the FAME. Standard mixtures of fatty acid methyl esters (Supelco, Bellefont, USA) were used to obtain relative retention times and to identify the fatty acids in the samples. Samples for fatty acid analysis were kept for no more than two weeks in N_2 atmosphere at -20°C until analysis in order to avoid possible oxidation.

Pigment extraction was performed in 90% acetone at 4°C for 20 h. Spectrophotometric determinations of chlorophyll *a* and total carotenoids were carried out as described by Lorenzen (1967) and by Parsons et al. (1984), respectively.

The ash content of the samples was measured gravimetrically, using previously weighed glass fibre filters, according to AOAC (1990). Samples in the filters were burned in a muffle furnace (Quimis, model Q-318M) at a 450°C for 24 h.

Statistical analysis

The results for chemical composition (except FA) and cell volumes were analyzed by one-way analysis of variance (ANOVA), comparing the three measurements (in different growth phases) along time for each species, using a significance level $\alpha = 0.05$ (Zar, 1996). Tukey's multiple comparison test followed the ANOVA, where applicable.

RESULTS

Microalgal growth and final yield

The growth curves of all microalgae are demonstrated in Figure 1. The exponential growth phase lasted at least for four days for all species. The stationary growth phase typically lasted four to five days, and the transitional phase occurred for two to three days, depending on the species. *Chlorella* sp. showed the higher final yield, with ca. 10.5x10^6 cell ml^{-1}. *Thalassiosira* sp. and *Rhodomonas* sp. showed the lowest values, ca. 6.4 x 10^5 and 1.3x10^6 cell ml^{-1}, respectively. For the diatoms *Bellerochea* sp. and *Chaetoceros* sp. the final yields were similar, varying around 6.2 x 10^6 cell ml^{-1}.

Cell volume

Significant differences in cell volume were detected throughout growth for all species (Table 1). *Thalassiosira* sp. and *Rhodomonas* sp. showed the largest cell volumes and *Bellerochea* sp. and *Chaetoceros* sp. the lowest. *Chlorella* sp., *Chaetoceros* sp. and *Bellerochea* sp. showed a decrease cell volume throughout growth ($0.0005 \leq P \leq 0.049$). Conversely, *Thalassiosira* sp. and *Rhodomonas* sp. increased their cell volume from exponential to stationary growth phase ($0.0053 \leq P \leq 0.044$).

Gross chemical composition

Significant differences in the protein content were

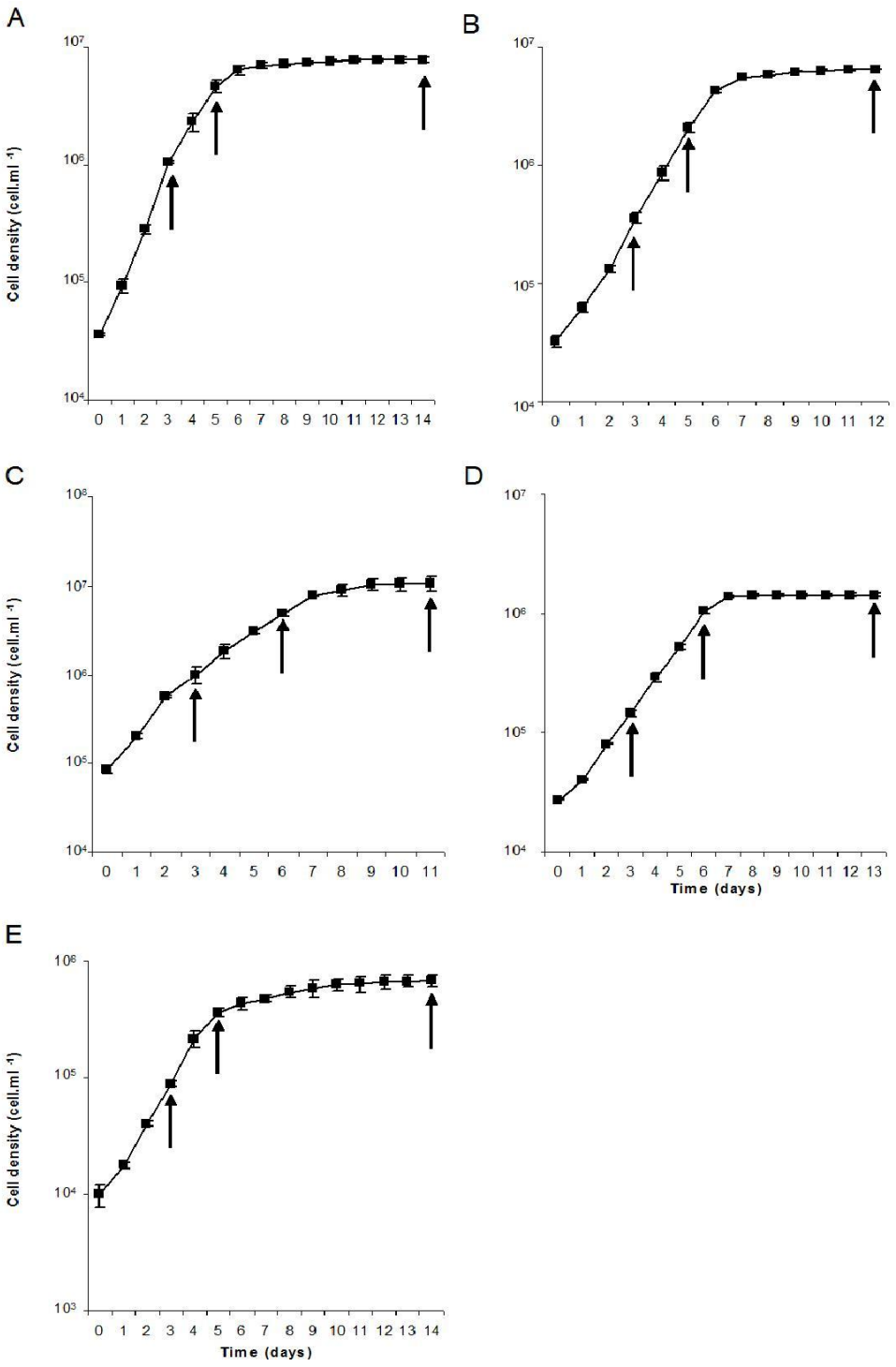

Figure 1. Growth curves *Bellerochea* sp. (A), *Chaetoceros* sp. (B), *Chlorella* sp. (C), *Rhodomonas* sp. (D), and *Thalassiosira* sp. (E) cultured with Conway culture medium. Each curve represents the mean of three replicates ± SD (n = 3). Arrows indicate the times of sampling for chemical analyses and cell measurements.

detected for all species along time (Table 2). All species achieved maximum levels of protein in the exponential growth phase, decreasing to the stationary growth phase ($0.001 \leq P \leq 0.04$), except for *Thalassiosira* sp., which showed maximum values in the transitional growth phase.

Changes in carbohydrate concentrations were detected in all experiments, with increasing values with time, achieving the highest concentrations in stationary growth

Table 1. Cell volumes of five marine microalgae in different growth phases. Values are expressed in μm^3 and represent the mean of three replicates ± SD (n = 3) (each culture flask is considered as a replicate)*. The determination of mean values for each culture flask included measurements in at least 30 cells, randomly chosen.

Growth phases	*Bellerochea* sp.	*Chaetoceros* sp.	*Chlorella* sp.	*Rhodomonas* sp.	*Thalassiosira* sp.
	*	*	*	*	*
Exponential	46.2 ± 4.41^a	68.1 ± 16.2^a	83.7 ± 7.12^a	226 ± 34.9^a	1475 ± 76.1^a
Transitional	43.9 ± 2.36^a	45.1 ± 2.21^a	51.4 ± 2.85^b	179 ± 6.89^b	1579 ± 164^a
Stationary	32.0 ± 3.35^b	30.3 ± 3.89^b	53.7 ± 4.90^b	235 ± 14.9^a	1707 ± 56.2^b

*Mean values significantly different: *P<0.05, a>b; ** P<0.05, a>b>c. Identical superscript letters (a,a; b,b) or absence of letters indicate that mean values are not significantly different.

Table 2. Concentrations of hydrosoluble protein, total carbohydrate, total lipid and total ash of five microalgae in different growth phases. The values are expressed in pg . $cell^{-1}$ and represent the mean of three replicates ± SD (n = 3)*.

Growth phase/species	*Bellerochea* sp.	*Chaetoceros* sp.	*Chlorella* sp.	*Rhodomonas* sp.	*Thalassiosira* sp.
			Protein		
	*	*	*	*	**
Exponential	21.9 ± 2.68^a	18.0 ± 0.28^a	25.1 ± 2.00^a	69.6 ± 4.43^a	115 ± 1.56^b
Transitional	21.9 ± 2.38^a	12.9 ± 0.48^b	9.93 ± 1.84^b	52.6 ± 6.39^b	163 ± 23.6^a
Stationary	14.2 ± 1.84^b	10.5 ± 1.52^b	6.07 ± 1.14^b	44.9 ± 6.52^b	73.1 ± 2.60^c
			Carbohydrate		
	*	*	**	**	**
Exponential	1.49 ± 0.02^b	1.61 ± 0.15^a	2.46 ± 0.19^c	3.95 ± 0.23^c	15.0 ± 0.87^c
Transitional	1.82 ± 0.17^b	0.93 ± 0.09^b	5.85 ± 1.13^b	5.06 ± 0.29^b	26.4 ± 2.63^b
Stationary	3.01 ± 0.39^a	1.50 ± 0.09^a	7.09 ± 0.84^a	8.60 ± 0.48^a	51.4 ± 5.46^a
			Lipid		
	**	**	*	*	
Exponential	6.96 ± 0.14^c	17.5 ± 4.01^a	12.6 ± 1.66^a	17.6 ± 1.81^b	26.8 ± 3.52
Transitional	13.9 ± 1.65^a	6.49 ± 0.99^b	1.82 ± 0.45^b	23.2 ± 7.14^b	29.0 ± 5.95
Stationary	9.87 ± 0.79^b	3.73 ± 0.54^c	1.82 ± 0.21^b	39.2 ± 3.85^a	39.6 ± 7.18
			Ash		
		**	*	**	*
Exponential	2.32 ± 0.25	3.05 ± 0.75^a	4.53 ± 0.91^b	6.75 ± 0.96 ^{a}	8.10 ± 1.53 ^{b}
Transitional	2.32 ± 0.52	1.14 ± 0.08^c	4.64 ± 0.46^b	3.45 ± 0.63 ^{c}	11.9 ± 1.53 ^{a}
Stationary	2.12 ± 0.31	2.32 ± 0.18^b	7.54 ± 1.21^a	5.38 ± 0.35 ^{b}	10.4 ± 0.80 ^{a}

*Mean values significantly different: *p < 0.05, a > b; **p < 0.05, a > b > c. Identical superscript letters (a,a; b,b) or absence of letters indicate that mean values are not significantly different.

phase (Table 2) (0.001≤P< 0.05), except for *Chaetoceros* sp. (maximum values in transitional growth phase).

Variations of total lipid showed different trends for the species throughout growth (Table 2). *Chaetoceros* sp. and *Chlorella* sp. showed a decrease in lipid concentrations from exponential to stationary growth phase (0.0004≤P≤0.03), but a significant increase of lipid was detected in *Rhodomonas* sp. during growth (with maximum values in stationary growth phase). For *Bellerochea* sp. maximum lipid concentrations were measured in transitional growth phase (P=0.018), while *Thalassiosira* sp. did not show differences in mean values of lipids during the experiment (P=0.63). Ash content of *Bellerochea* sp. did not vary during growth (Table 2), but in *Chlorella* sp. and *Thalassiosira* sp.a significant increase was detected from exponential to stationary growth phase. On the other hand, *Rhodomonas* *Rhodomonas* sp. and *Chaetoceros* sp. showed maximum

Table 3. Concentrations of chlorophyll *a*, chlorophyll *b* / chlorophyll *c* and total carotenoid of five microalgae in different growth phases. The values are expressed in pg . cell^{-1} and represent the mean of three replicates ± SD (n = 3)*.

Species	Growth phases	Chlorophyll *A*	Chlorophyll *B*	Chlorophyll *C*	Total carotenoid
		**		*	**
	Exponential	0.56 ± 0.05^{b}	_	0.26 ± 0.02^{a}	0.23 ± 0.01^{b}
Bellerochea sp.	Transitional	0.93 ± 0.38^{a}	_	0.39 ± 0.16^{a}	0.42 ± 0.18^{a}
	Stationary	0.25 ± 0.03^{c}	_	0.11 ± 0.02^{b}	0.12 ± 0.02^{c}
		*		*	*
	Exponential	0.35 ± 0.04^{a}	_	0.09 ± 0.01^{a}	0.13 ± 0.02^{b}
Chaetoceros sp.	Transitional	0.40 ± 0.04^{a}	_	0.09 ± 0.01^{a}	0.19 ± 0.02^{a}
	Stationary	0.20 ± 0.01^{b}	_	0.04 ± 0.01^{b}	0.11 ± 0.01^{b}
		*	**		**
	Exponential	0.64 ± 0.11^{a}	0.41 ± 0.09^{a}	_	0.37 ± 0.05^{a}
Chlorella sp.	Transitional	0.12 ± 0.03^{b}	0.08 ± 0.02^{b}	_	0.10 ± 0.01^{b}
	Stationary	0.04 ± 0.01^{b}	0.02 ± 0.00^{c}	_	0.04 ± 0.02^{c}
		*		*	
	Exponential	1.38 ± 0.12^{a}	_	0.59 ± 0.08^{a}	0.99 ± 0.13
Rhodomonas sp.	Transitional	1.12 ± 0.19^{a}	_	0.47 ± 0.08^{a}	0.92 ± 0.11
	Stationary	0.49 ± 0.10^{b}	_	0.31 ± 0.06^{b}	0.94 ± 0.09
		**		**	*
	Exponential	4.18 ± 0.18^{b}	_	1.47 ± 0.06^{b}	1.70 ± 0.07^{b}
Thalassiosira sp.	Transitional	7.16 ± 0.74^{a}	_	2.57 ± 0.26^{a}	2.52 ± 0.26^{a}
	Stationary	2.06 ± 0.14^{c}	_	0.85 ± 0.07^{c}	1.54 ± 0.12^{b}

*Mean values significantly different: *P<0.05, a>b; ** P<0.05, a>b>c. Identical superscript letters (a,a; b,b) or absence of letters indicate that mean values are not significantly different.

ash content in exponential growth phase, with lower values in the other phases (P≤0.0004). *Chaetoceros* sp., *Rhodomonas* sp., *Thalassiosira* sp. and *Chlorella* sp. showed a decrease in chlorophyll *a* throughout growth (0.0001≤P≤0.027) (Table 3). For *Bellerochea* sp. maximum values for chlorophyll *a* were detected in transitional growth phase. Similar trends (as described for chlorophyll *a*) were found for chlorophyll *b* and total carotenoid (Table 3), except for total carotenoid in *Rhodomonas* sp., with no difference in values along time.

Fatty acids

All species showed high percentages of saturated fatty acids (SFA) (Table 4). Most species tended to show maximum SFA in exponential growth phase, except *Rhodomonas* sp., with similar values in the three growth phases. The FA 12:0 was the most abundant SFA (0.001≤P≤0.013), except for *Bellerochea* sp., with higher concentrations of 14:0 (P<0.01). The FA 16:0 has been observed in high concentrations in *Chlorella* sp. and *Chaetoceros* sp.

The microalgae tended to show concentrations of monounsaturated fatty acids (MFA) progressively higher throughout growth (Table 4), with maximum values in stationary growth phase, except for *Chlorella* sp. (maximum values in transitional growth phase). The three diatoms and *Rhodomonas* sp. showed remarkable increase of MFA from transitional to stationary growth phase. Sixteen-carbon MFA were the most abundant FAs in all species (p < 0.01), especially the isomer 16:1n-7 (palmitoleic acid). High concentrations of 18:1n-9 was also detected in *Chlorella* sp. and in some samples of *Chaetoceros* sp.

Variations in polyunsaturated fatty acids (PUFA) were detected in the microalgae (Table 4). Lower percentages of PUFA were found in the diatoms *Bellerochea* sp. and *Chaetoceros* sp. (P<0.05), with no variations during growth for *Bellerochea* sp. (P>0.21). Arachidonic acid – AA (20:4n-6) has not been detected in *Thalassiosira* sp., and it occurred in low concentrations in *Bellerochea* sp., *Chaetoceros* sp. and *Chlorella* sp., but an increase of AA Concentrations occurred during growth of *Rhodomonas* sp. All species showed the eicosapentaenoic acid – EPA (20:5n-3), but in *Chlorella* sp. this FA was detected

Table 4. Fatty acid composition of five marine microalgae in different growth phases. Data are given as percentage of total fatty acids and refer to the actual res (n = 3), without corrections for 100% recovery. SFA = saturated fatty acids. MFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. n.d. = n sp. in the exponential growth phase were lost.

Fatty acid	*Bellerochea* sp.			*Chaetoceros* sp.			*Chlorella* sp.			*Rhodomonas* sp.	
	Transitional	Stationary	Exponential	Transitional	Stationary	Exponential	Transitional	Stationary	Exponential	Transitional	Stationary
12:0	2.50 ± 0.14	1.22 ± 0.31	51.0 ± 0.01	29.2 ± 1.05	21.5 ± 6.49	42.7 ± 5.94	28.9 ± 13.0	22.5 ± 0.12	45.4 ± 5.33	28.8 ± 5.09	26.1 ± 0.07
14:0	14.7 ± 1.82	15.9 ± 2.36	10.6 ± 0.04	6.71 ± 0.06	9.79 ± 0.01	2.14 ± 1.46	1.72 ± 0.26	1.77 ± 0.12	3.05 ± 0.79	6.80 ± 0.86	11.7 ± 0.13
15:0	2.31 ± 0.04	1.07 ± 0.21	2.72 ± 0.02	1.83 ± 0.05	2.12 ± 0.26	N.D.	1.72 ± 0.84	1.59 ± 0.08	1.07 ± 0.05	1.39 ± 0.81	1.24 ± 0.02
16:0	2.43 ± 0.25	6.77 ± 5.39	11.9 ± 0.01	9.85 ± 0.16	5.41 ± 1.12	3.16 ± 0.84	5.59 ± 2.04	10.7 ± 0.01	2.95 ± 1.23	5.33 ± 2.41	5.46 ± 0.13
17:0	5.83 ± 1.15	3.50 ± 1.50	0.46 ± 0.01	N.D.	0.54 ± 0.11	N.D.	0.77 ± 0.00	4.54 ± 1.16	0.84 ± 0.35	0.74 ± 0.18	6.73 ± 0.01
18:0	2.31 ± 0.78	1.43 ± 0.36	0.71 ± 0.01	0.41 ± 0.01	1.54 ± 0.50	1.19 ± 0.34	4.90 ± 1.58	6.23 ± 0.32	0.62 ± 0.14	1.07 ± 0.19	0.74 ± 0.01
20:0	0.32 ± 0.55	0.22 ± 0.21	N.D.	N.D.	N.D.	N.D.	N.D.	0.14 ± 0.01	N.D.	N.D.	N.D.
22:0	N.D.	0.25 ± 0.12	N.D.	0.34 ± 0.01	N.D.	N.D.	N.D.	0.44 ± 0.02	N.D.	N.D.	0.13 ± 0.00
∑ SFA	30.4 ± 4.73	30.4 ± 10.5	77.4 ± 0.10	48.4 ± 1.34	40.9 ± 8.49	49.2 ± 8.58	43.6 ± 17.7	47.8 ± 1.81	53.9 ± 7.89	44.1 ± 9.54	52.0 ± 0.37
16:1N-7	8.26 ± 0.07	23.2 ± 2.87	0.47 ± 0.03	3.12 ± 0.01	17.2 ± 3.29	N.D.	1.54 ± 0.38	1.53 ± 0.02	1.33 ± 0.19	1.90 ± 0.25	15.1 ± 0.16
16:1N-9	0.90 ± 0.06	0.90 ± 0.25	0.43 ± 0.00	1.24 ± 0.03	0.77 ± 0.40	N.D.	1.57 ± 0.47	0.34 ± 0.01	0.73 ± 0.42	0.63 ± 0.02	1.73 ± 0.01
18:1N-9	0.39 ± 0.02	0.29 ± 0.05	1.06 ± 0.03	4.50 ± 0.08	1.07 ± 0.65	5.19 ± 0.86	15.4 ± 4.74	1.10 ± 0.01	1.08 ± 0.04	1.66 ± 0.81	0.53 ± 0.06
18:1N-7	0.17 ± 0.18	0.27 ± 0.16	0.45 ± 0.01	0.35 ± 0.00	0.39 ± 0.08	N.D.	0.93 ± 0.00	0.85 ± 0.01	N.D.	N.D.	0.32 ± 0.01
20:1N7+9	0.09 ± 0.15	0.30 ± 0.06	0.40 ± 0.00	N.D.	N.D.	N.D.	N.D.	0.13 ± 0.00	0.95 ± 0.28	0.62 ± 0.00	0.24 ± 0.01
22:1	N.D.	0.17 ± 0.08	N.D.	N.D.	N.D.	N.D.	N.D.	0.13 ± 0.02	N.D.	N.D.	0.12 ± 0.00
∑ MFA	9.81 ± 0.48	24.8 ± 3.47	2.81 ± 0.07	9.21 ± 0.12	19.4 ± 4.42	5.19 ± 0.86	19.5 ± 5.59	4.09 ± 0.07	4.09 ± 0.93	4.81 ± 1.08	18.0 ± 0.25
18:2N-6	0.65 ± 0.36	0.28 ± 0.04	0.66 ± 0.01	0.42 ± 0.01	0.41 ± 0.07	N.D.	2.25 ± 0.75	7.48 ± 0.35	0.90 ± 0.38	3.48 ± 1.28	0.46 ± 0.08
18:3N-6	N.D.	N.D.	N.D.	0.26 ± 0.01	0.71 ± 0.07	N.D.	0.70 ± 0.00	0.84 ± 0.00	N.D.	N.D.	N.D.
18:3N-3	5.40 ± 0.18	5.23 ± 1.57	1.15 ± 0.01	1.06 ± 0.01	4.81 ± 0.16	3.05 ± 1.25	7.45 ± 2.90	8.92 ± 0.06	6.98 ± 0.84	7.07 ± 3.84	0.86 ± 0.01
18:4N-3	1.03 ± 0.60	0.23 ± 0.13	0.50 ± 0.01	1.14 ± 0.02	0.63 ± 0.22	N.D.	7.60 ± 2.95	5.56 ± 0.01	4.20 ± 0.58	13.6 ± 3.43	4.72 ± 0.01
20:2N-6	6.18 ± 0.82	3.33 ± 0.42	N.D.	3.55 ± 0.03	4.19 ± 0.77	8.70 ± 1.95	5.93 ± 1.53	3.47 ± 0.01	4.92 ± 0.56	2.75 ± 0.73	1.24 ± 0.00
20:4N-6	0.48 ± 0.09	0.74 ± 0.08	4.87 ± 0.04	0.68 ± 0.03	N.D.	N.D.	N.D.	0.14 ± 0.01	N.D.	N.D.	0.33 ± 0.00
20:3N-3	0.31 ± 0.02	0.11 ± 0.05	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.29 ± 0.10	0.54 ± 0.15	0.37 ± 0.03
20:4N-3	0.45 ± 0.09	0.25 ± 0.14	0.41 ± 0.01	0.36 ± 0.01	0.58 ± 0.18	N.D.	N.D.	0.19 ± 0.00	0.69 ± 0.16	1.83 ± 0.67	2.36 ± 0.01
20:5N-3	1.55 ± 0.29	1.90 ± 0.51	1.53 ± 0.02	4.01 ± 0.02	1.95 ± 0.43	N.D.	N.D.	0.66 ± 0.01	0.93 ± 0.06	3.78 ± 1.5	1.54 ± 0.00
22:4N-9	1.37 ± 0.06	1.10 ± 0.12	1.49 ± 0.02	1.22 ± 0.01	1.08 ± 0.21	3.36 ± 2.20	2.30 ± 1.00	1.36 ± 0.03	1.28 ± 0.06	1.25 ± 0.52	1.33 ± 0.01
22:4N-6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.16 ± 0.03	N.D.	N.D.	0.91 ± 0.00
22:5N-6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.71 ± 0.00	0.35 ± 0.04	N.D.	N.D.	0.34 ± 0.00
22:5N-3	0.10 ± 0.18	0.53 ± 0.38	N.D.	0.49 ± 0.08	N.D.	N.D.	N.D.	0.17 ± 0.00	N.D.	N.D.	0.18 ± 0.00
22:6N-3	1.15 ± 0.15	1.75 ± 0.60	1.09 ± 0.01	0.49 ± 0.03	2.46 ± 0.42	N.D.	N.D.	0.44 ± 0.11	2.06 ± 0.97	2.61 ± 1.46	1.78 ± 0.00
∑ PUFA	18.7 ± 2.84	15.5 ± 4.04	10.6 ± 0.12	13.2 ± 0.23	16.8 ± 2.53	15.1 ± 5.40	26.9 ± 9.13	29.7 ± 0.66	22.3 ± 3.71	36.9 ± 13.6	16.4 ± 0.15

in low percentage in stationary growth phase only. EPA tended to occur in low percentages in all species, typically lower than 2%, except for some samples of *Chaetoceros* sp., *Rhodomonas* sp. and *Thalassiosira* sp. The FA docoeicosapentaenoic acid – DHA (22:6n-3) was found in all species in low percentages. Only *Rhodomonas*sp., *Chaetoceros* sp. and *Thalassiosira* sp. sho some observations. centages relatively hi 18:3n-3 (*Chlorella* s

sp.) and 22:4n-9 (*Chlorella* sp.). The FA 20:2n-6 presented high measurements for at least one observation for all species.

DISCUSSION

Growth, final yield and cell volume

Small-sized species (*Chlorella* sp., *Chaetoceros* sp. and *Bellerochea* sp.) showed higher growth rates in exponential growth phase (data not shown), whereas greater species (*Rhodomonas* sp. and *Thalassiosira* sp.) exhibited lower rates. These trends are in accordance with many studies (Lourenço et al., 2002; Lavín and Lourenço, 2005; Borges-Campos et al., 2010), and show the critical importance of surface : volume relationship for phytoplankton. In this study, *Rhodomonas* sp. and *Thalassiosira* sp., species of larger biovolume, showed an increasing biovolume throughout growth. These characteristics are supported by findings of Hellebust (1976), who points that the increase in mean cell volume during growth reflects the accumulation of reserve products (especially long-chain polysaccharides) in the stationary growth phase, when growth conditions are unfavorable. Silva et al. (2009) observed the same trend for the cryptomonad *Rhodomonas* sp. in a study of nitrogen reduction in the culture media. However, for *Bellerochea* sp., *Chaetoceros* sp. and *Chlorella* sp. a decrease of cell volume occurred throughout the experiments. The same trend was reported for *Tetraselmis gracilis* by Lourenço et al. (1997), and it was interpreted as a result of a progressive reduction of resources (nutrients) available to the cells, since each individual could acquire a smaller amount of nutrients, allowing the biosynthesis of a small cellular mass. Such trend could also be related to the changes in the chemical profile of the species.

Considering final cell yield, species of smaller cell volumes (*Chlorella* sp., *Chaetoceros* sp. and *Bellerochea* sp.) showed higher values, while the larger cells (*Rhodomonas* sp. and *Thalassiosira* sp.) exhibited lower final yields, corroborating the studies of Borges-Campos et al. (2010) with *Synechococcus subsalsus* and *Chlorella minutissima*. These results were expected, especially taking into account the previous discussion for growth rates. However, among the three smaller species the behavior of *Chlorella* sp. compared to *Bellerochea* sp. and *Chaetoceros* sp. was remarkable. Even showing the larger cell volume among the three smaller species, *Chlorella* sp. achieved a final yield significantly higher than the other two species, suggesting a high efficiency of nutrient conversion into organic matter, according to Kaplan et al. (1986). Moreover, a possible cause for difference in final cell yield might be the demand for silicon. N and Si concentrations in Conway medium are 1,178 e 377 µM, respectively, with an atomic ratio N:Si of ca. 3.12. Some need an atomic ratio N:Si = 2.0, or even lower to get an optimum growth and cell yield (Brzezinski, 1985). As this hypothesis could not have been tested in this study, its consideration is speculative, but plausible. If *Bellerochea* sp. and *Chaetoceros* sp. had a high demand for Si, it could affect negatively their growth in the experiments, with smaller production of cells and biomass.

Chemical composition of microalgae

The evaluation of protein per cell revealed maximum protein content in the exponential growth phase, decreasing throughout growth. Fernández-Reiriz et al. (1989) found the same trend for the prasinophycean *Tetraselmis suecica*, but the diatoms *Phaeodactylum tricornutum* and *Chaetoceros calcitrans* did not change their protein content over growth, such as other diatoms tested by Knuckey et al. (2002). Fernández-Reiriz et al. (1989) also reported a peak in protein content of the cryptomonad *Rhodomonas* in transitional growth phase as well as Silva et al. (2009). Aidar et al. (1994) also reported higher protein concentrations per cell in exponential growth phase of the prasinophycean *T. gracilis* and the diatom *Cyclotella caspia*, decreasing protein per cell in the subsequent measurements over growth. Seixas et al. (2008, 2009) found a considerable high protein percentage (62 and 55%, d.w.), respectively, in *Rhodomonas lens* obtained in semi-continuous culture. All species showed a decreasing concentration of chlorophyll. Lourenço et al. (2004) reported this same trend for the most of the species cultivated with Conway media. Chlorophyll concentration is strongly affected by nitrogen availability, with variations similar to those described for protein (Fábregas et al., 1987).

For carbohydrates different trends were found, compared to protein. For all species, except *Chaetoceros* sp., increasing concentrations of carbohydrates were found throughout growth. This finding is in accordance with many other studies (Fernández-Reiriz et al., 1989; Lourenço et al., 1997; Knuckey et al., 2002). According to Enright et al. (1986a), when the rate of cell division in a phytoplankton culture is limited by nutrients, cells alter their metabolism and convert energy to produce reserve substances. Typically, at the end of a batch culture, with a frequent nutrient limitation, the synthesis of both protein and chlorophyll decreases, and the concentration of lipid and/or carbohydrate increases. There is a coupling between protein and carbohydrates in algal cells, which also reflects the budget of carbon and nitrogen available to the cells (Geider et al., 1993; Turpin, 1991).

The microalgae exhibited changes in ash content throughout growth, except *Bellerochea* sp. (with no change over growth). Knuckey et al. (2002) found the same behavior with the diatom *Nitszchia paleacea*, which did not change its chemical profile during different growth

phases. On the other hand, *Chlorella* sp. and *Thalassiosira* sp. exhibited an increase in ash content during growth. Fernández-Reiriz et al. (1989) found the same trend for the prasinophycean *T. suecica*, but for the diatoms *P. tricornutum* and *C. calcitrans* and the cryptomonad *Rhodomonas* sp. lower ash content was reported in the stationary growth phase. In the current study, a lower ash content for the cryptomonad *Rhodomonas* sp. was reported in transitional growth phase.

Changes in lipid concentration of *Chlorella* sp. and *Rhodomonas* sp. are similar to those reported by Fernández-Reiriz et al. (1989) with the prasinophycen *T. suecica* and with the cryptomonad *Rhodomonas* sp. Conversely, for those species a decreasing concentration of lipid per cell has been reported during growth. For the diatoms *C. calcitrans* and *P. tricornutum*, Fernández-Reiriz et al. (1989) found increasing concentrations of lipid throughout growth. The same was reported by Knuckey et al. (2002), who found an increase in lipid concentration in eight out of ten diatoms over growth. Knuckey et al. (2002) also reported that only the diatoms *Entomoneis punctulata* and *Papiliocellulus simplex* showed decreasing concentrations of lipid per cell over growth.

PUFA concentrations of *Bellerochea* sp., *Chaetoceros* sp., *Rhodomonas* sp. and *Thalassiosira* sp. showed lower values when compared to the diatoms and cryptomonads studied by Renaud et al. (1999) and to the cryptomonads studied by Seixas et al. (2009). *Chaetoceros* sp. showed higher values of PUFA com-pared to the results of Chen (2012) obtained with *Chaetoceros muelleri*. Knuckey et al. (2002) reported the same trends for MFA and PUFA described here for another *Thalassiosira* species, but changes in SFA content were opposite to those described in the present study. Knuckey et al. (2002) also found in *E. punctulata* exactly the same variations for SFA, MFA and PUFA reported here for *Bellerochea* sp. Chen (2012) cultivated 12 species of marine diatoms in three different envi-ronments and concluded that these conditions altered the fatty acid composition of the diatom species, emphasizing that production and storage of lipids is species-specific.

Fatty acids play a key role in aquaculture, since they are very important for the growth of marine organisms. Because of this, some microalgal species may be potentially indicated as useful for feeding marine animals, while others show to be unsuitable as food-species, if the essential PUFA lack in them. The microalgae may have superior lipid stability compared with traditional PUFAs, because they are naturally rich in antioxidant carotenoids and vitamins and because lipids are bioencapsulated by the algal cell wall (Patil et al., 2007). Regarding FA content, *Bellerochea* sp. and *Chaetoceros* sp., for instance, could be considered promising species, since they possess the three essential FA (AA, EPA e DHA) in all growth phases. *Chlorella* sp. should not be indicated for use in marine aquaculture as a monoalgal diet, since it is a poor source of PUFA, showing the three essential FA in low concentrations in stationary growth phase only. Concerns for the use of *Rhodomonas* sp. are essentially the same described for *Chlorella* sp. The cryptomonad exhibits the three essential FA in low concentrations.

Differences among the current study and others in the literature are evident. A large part of the data on chemical composition of marine microalgae is obtained using f/2 culture medium (Guillard, 1975), such as Enright et al. (1986a, b), Brown et al. (1998), McCausland et al. (1999), Renaud et al. (1999), Knuckey et al. (2002), Lafarga-De la Cruz et al. (2006), among others. However, in the current study, the microalgae were cultured with Conway culture medium (Walne, 1966), a richer culture medium. The f/2 medium contains 880 µM $NaNO_3$ and 36 µM NaH_2PO_4, while Conway medium contains 1,176 µM $NaNO_3$ and 128 µM NaH_2PO_4 (Walne, 1966). Despite changes in the chemical composition of microalgae reflect genetic differences, such variations are also influenced by culture conditions. Chemical composition of microalgae may change largely when the species are cultured under contrasting conditions, as well as in different growth phases (Brown et al., 1997). Thus, the detection of high concentration of protein and low concentration of carbohydrate in the experiments (even with the chlorophycean *Chlorella* sp.) may be an effect of high nitrogen concentration and other components of the Conway culture medium.

Conclusion

Data on the chemical composition of microalgal strains useful in tropical and subtropical mariculture are scarce in the literature (McCausland et al., 1999; Martínez-Fernández et al., 2006; Silva et al., 2009). This study provides basic information on the chemical composition of five native Brazilian microalgae. Possible uses of this knowledge are wide and could stimulate other studies. Besides the information on the chemical profile of the species (a tremendous gap in Brazil), the results may be useful for the selection of suitable native species in mariculture, to diminish the current dependence of Brazilian producers for exotic microalgal strains.

All strains tested in this study seem to be potentially useful as food-species in mariculture, and further studies on them in larger systems are required, as well as studies focusing the feeding of animals, such as oyster larvae and post-larvae. Since microalgae may be limited by one or more essential nutrients, a mixed algal cultures supplies a better equilibrium of nutrient properties, contri-buting for a better success in mariculture (Brown et al., 1997; Rico-Villa et al., 2006) probably because their combined nutrient is more likely to meet to nutritional requirements of the target species (Cerón-Ortiz et al., 2009). This is what could be interpreted for *Chlorella* sp.,

which presented a low FA content, but high percentages of other substances, such as carbohydrates. Thus, data on biovolume, cell density and chemical composition of microalgae (used all together) may provide important subsidies for choosing suitable diets for larvae of marine organisms, contributing to decrease their mortality.

We have been performing further studies with the same five native microalgae to evaluate their use in mariculture. In one of them, the conservation of them as live concentrated pastes was tested. In a second study, the micro algae were tested in trials as food-species for mangrove oyster larvae (*Crassotrea rhizophora*). These promising new results will be published soon.

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