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DRY WEIGHT, CARBON, C/N RATIO, HYDROGEN, AND CHLOROPHYLL VARIATION DURING EXPONENTIAL GROWTH OF SELECTED MICROALGAE SPECIES USED IN AQUACULTURE

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ABSTRACT. Microalgae are commonly used as food source in aquaculture, mainly for shellfish and larvae of crustacean and fish. All hatcheries need an excellent inoculum to produce high-quality microalgae when cultured outdoor in extensive systems, and this depends largely on the health of the microalgae cultured under laboratory conditions as a primary step. Therefore, the aim of this work was to assess variations of dry weight, carbon, C/N ratio, hydrogen and chlorophylls as physiological indicators of nutrients uptake and growth rate during exponential growth of *Isochrysis galbana*, *Chaetoceros calcitrans* and *Dunaliella tertiolecta*, using f/2 as culture medium. *Chaetoceros calcitrans* and *D. tertiolecta* had higher carbon content (~30 pg cell^{-1}). The C/N ratio varied widely, gradually decreasing on *I. galbana.* Chlorophyll *a* varied among the three microalgae tested, ranging from <0.05 to >0.25 pg cell^{-1}. Growth rate was higher in *I. galbana* (K' 0.83) followed by *D. tertiolecta* and *C. calcitrans*. Results showed that nutrient incorporation by cell change when cell density increases; this information provides new insights in the physiology of marine microalgae and confirms that nutrient uptake dynamics is different in each microalga species. Finally, this study indicates that using one culture medium is not equally efficient for all microalgae used in aquaculture since each species has specific nutritional requirements.

Keywords: Carbon, C/N ratio, Chlorophyll, Growth rate, Microalgae.

Variación de peso seco, carbono, relación C/N, hidrógeno y clorofilas durante el crecimiento exponencial de especies selectas de microalgas utilizadas en acuacultura

RESUMEN. Las microalgas son comúnmente utilizadas como fuente de alimento en acuacultura, principalmente para cultivo de moluscos y para las fases larvarias de crustáceos y peces. Los criaderos de larvas necesitan un excelente inóculo para producir microalgas de alta calidad cuando se cultivan al exterior en sistemas extensivos; esto depende principalmente de la salud de las microalgas cultivadas bajo condiciones de laboratorio como primer paso. Por lo tanto, el objetivo de este trabajo fue evaluar variaciones de peso seco, carbono, relación C/N, hidrógeno y clorofilas como indicadores fisiológicos de la asimilación de nutrientes y tasa de crecimiento durante el crecimiento exponencial de *Isochrysis galbana*, *Chaetoceros calcitrans* y *Dunaliella tertiolecta*, usando f/2 como medio de cultivo. *Chaetoceros calcitrans* y *D. tertiolecta* presentaron el mayor contenido de carbono (~30 pg cél^{-1}). La relación C/N varió ampliamente, decreciendo gradualmente en *I. galbana*. La clorofila *a* fue la que más varió entre las tres microalgas evaluadas, en el intervalo de <0.05 a >0.25 pg cél^{-1}. La tasa de crecimiento fue mayor en *I. galbana* (K' 0.83) seguido por *D. tertiolecta* y *C. calcitrans*. Los resultados mostraron que la incorporación de nutrientes por célula cambia cuando la densidad celular se incrementa; esta información provee nuevo conocimiento sobre la fisiología de microalgas marinas y confirma que la dinámica de incorporación de nutrientes es diferente en cada especie de microalga. Por último, este estudio indicó que el uso de un solo medio de cultivo no es igualmente eficiente para todas las microalgas usadas en acuacultura, debido a que necesitan requerimientos nutricionales específicos.

Palabras clave: Carbono, Clorofila, Microalgas, Relación C/N, Tasa de crecimiento.

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INTRODUCTION

Microalgae play a key role in aquaculture development, they are the main food source for many farm-raised marine fish, crustaceans, and shellfish, as well as for hatchery-raised zooplankton (rotifers, copepods, cladocerans, and brine shrimp) that serve as food for the larvae of farm-raised marine animals (Brown *et al*., 1999; Muller-Feuga, 2000; Pérez-Morales, 2006).

All hatcheries need an excellent inoculum to produce high-quality microalgae when cultured outdoor in extensive systems, and this depends largely on the health of the microalgae cultured under laboratory conditions as a primary step (Abu-Rezq *et al*., 1999; Lourenço *et al*., 2002; Banerjee *et al*., 2011). Several factors, such as temperature, salinity, pH, light intensity, photoperiod and aeration can alter the health and the quality of a microalgae culture, but the most important factor depends largely on the appropriate media culture with an adequate amount of nutrients and minerals for each microalgae species (Gopinathan, 1986; Keller *et al*., 1987; Geider & La Roche, 2002; Lananan *et al*., 2013).

The most popular media used for the culture of

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marine microalgae are Artificial Sea Water, Conway, ESM, Erd-Schreiber, f/2, K, L1, Miquel's, and Walne's medium (Miquel, 1890; Guillard & Ryther, 1962; Okaichi *et al*., 1982; Keller & Guillard, 1985; Kaplan *et al*., 1986; Keller *et al*., 1987; Guillard & Hargraves, 1993; Tompkins *et al*., 1995). These media show significant variations in mineral composition such as nitrogen or phosphorus compounds, incorporation of trace metals, vitamins, and several inorganic and organic salts; it is worth noting that in hatcheries, culture medium is used routinely unspecified, most of the time using the same for several microalgae species.

In aquaculture, microalgae cultures should involve a complete knowledge of their particular nutritional requirements; hence each microalgae species will be assessed through the elemental composition such as carbon, hydrogen, nitrogen and phosphorous content, C/N ratio as well as the chlorophyll content, because these indicators can be quantified, and related to healthy microalgae cultures (Ríos *et al*., 1998; Geider & La Roche, 2002; Roopnarain *et al.*, 2015).

In marine hatcheries, diatoms, flagellates and chlorophytes are important groups of phytoplankton commonly used as food sources, most often, *Isochrysis galbana* (Prymnesiophyceae), *Chaetoceros calcitrans* (Bacillariophyceae), and *Dunaliella tertiolecta* (Chlorophyceae).

Therefore, the aim of this work was to experimentally assess daily variations of dry weight, carbon, C/N ratio, hydrogen, and chlorophyll (*a*, *b*, and *c*) as physiological indicators of nutrient uptake and growth rate during exponential growth of three microalgae (*Isochrysis galbana*, *Chaetoceros calcitrans,* and *Dunaliella tertiolecta*), using f/2 as medium culture.

MATERIAL AND METHODS

Algal culture

Microalgae strains of *Isochrysis galbana* (3-5 µm), *Chaetoceros calcitrans* (8-10 µm), and *Dunaliella tertiolecta* (10-12 µm) were cultured under laboratory conditions at a temperature of 23 ± 1 °C, salinity of 36 ± 1 ups, pH of 8.0 ± 0.1, photoperiod of 12:12 h light:dark cycle, and artificial illumination of 150 µmol m^{-2} s^{-1}. Light was provided by fluorescent lamps (cool white) placed behind the culture bottles. The microalgae cultures were continuously aerated with gentle influx of air.

Seawater was collected from the Gulf of California and filtered under a low vacuum on Whatman GF/F filters, autoclaved and enriched with f/2 medium containing (in µg L^{-1}): $NaNO_3$ (883), NaH_2PO_4•H_2O (36.3), Na_2SiO_3•$9H_2O$ (54), EDTA•Fe (11.7), $FeCl_3$•$6H_2O$ (11.7), $CuSO_4$•$5H_2O$ (0.04), $ZnSO_4$•$7H_2O$ (0.08), $CoCl_2$•$6H_2O$ (0.05), $MnCl_2$•$4H_2O$ (0.9), Na_2MoO_4•$2H_2O$ (0.03), thiamine•HCl (B_1) (0.1), biotin (B_7) (0.5), and cobalamin (B_{12}) (0.5), following Guillard & Ryther (1962).

Triplicate cultures of *I. galbana*, *C. calcitrans* and *D. tertiolecta* were initiated with a cell density of 2 x 10^5 cell mL^{-1} in bottles of 19 L. Measurement and characterization of microalgae strains were done every 24 h during five days of culture, which correspond to the late exponential growth phase, according to previous work done by Pérez-Morales (2006).

Dry weight

Dry weight was determined daily, filtering 30 mL of each microalgae culture through pre-weighed fiberglass filters (Ø 25 mm and nominal pore size of 0.7 µm; Whatman GF/F, Whatman International, Kent, UK). Samples were dried in a convection oven at 60 °C for 24 h, and then combusted at 400 °C in the muffle furnace for 2 h. Samples were weighed three times in succession at room temperature with an analytical balance (marca) (±0.00001 g).

Carbon, hydrogen and nitrogen

After determining dry weights of each microalgae species, the content of carbon, hydrogen, and nitrogen were determined by combustion with an elemental analyzer (440HA model, Exeter Analytical, Coventry, UK) using a pneumatic auto-sampler. The filters were placed in aluminum capsules, and incinerated at high-temperature (1,000 °C). The helium (99.9 %) carrier gas pressure was set to 1.2 bars with a flow rate of ~100 mL min^{-1}. For combustion, oxygen (99.9 %) was set at 1.9 bars, and air (no water or oil present) at 2.4 bars. Three empty tin capsules were folded, and sealed in the same manner; these were used as blanks to calibrate the standard, and samples. Combustion products (CO_2, N_2, and H_2O) were separated by selective chemical traps, and measured with three pairs of detectors of high-precision thermal conductivity, and simultaneously determining C, H, and N as weight percentages.

Chlorophylls

To quantify chlorophylls, microalgae samples of known volume were filtered under low vacuum on Whatman GF/F filters, as described earlier. The filters were frozen, and analyzed following the method given by Parsons *et al.* (1984). Chlorophyll *a*, *b*, and *c* concentration were estimated using equations proposed by Jeffrey & Humprey (1975).

Algal growth

Cell densities were estimated during the exponential growth phase by direct counting, using a Neubauer haemocytometer. The growth rate (K') was estimated following Fogg & Thake (1987), where $K' = Ln (N_2 / N_1) / (t_2 - t_1)$, and N_1 and N_2 are biomass at time 1 (t_1), and time 2 (t_2). Once K' is

known, was calculated division per day, where Div day^{-1} = K' / Ln2 and generation time = 1 / (Div day^{-1}).

Statistical analysis

Data were tested with standard process for homoscedasticity (Levene's test), and normality (Shapiro-Wilk test). All data were then statistically tested to quantify the differences among the treatments using one-way statistical analyses of variance (ANOVA, $p<0.01$) with Tukey post-hoc tests, also the Pearson Correlation Coefficient was measured between cell density, and carbon content for each microalgae culture (Sokal & Rohlf, 1981).

RESULTS

Dry weight

The greatest change in dry weight of cells occurred in *C. calcitrans* (Fig. 1), reaching at day 5 about twice (~90 pg $cell^{-1}$) that observed on day 3. *Isochrysis galbana* slightly decrease on days 3 and 4 (~10 pg $cell^{-1}$). *Dunaliella tertiolecta* cells at day 1 were heavier (~80 pg $cell^{-1}$), and decreased to ~60 pg $cell^{-1}$. Dry weight by cell of each microalgae exhibited significant differences over time (Table 1).

Carbon

Chaetoceros calcitrans showed the greatest change in cellular carbon content, reaching ~30 pg $cell^{-1}$ on day 5 (Fig. 2); this was about twice carbon content that was found in days 2 and 3. *Isochrysis galbana* had similar carbon content at the beginning and end of the trial (~8 pg $cell^{-1}$); the maximum content occurred on day 2, and it steadily decreased until day 4. *Dunaliella tertiolecta* carbon content was similar over days 1 to 5, with a slight decrease from day 1 to day 2. The carbon content in microalgae showed significant differences during the bioassay period (Table 1). Correlation between cell density and carbon content was higher in *D. tertiolecta* (r= 0.98) compared to *I. galbana* or *C. calcitrans* (r= 0.9, and 0.8, respectively).

C/N ratio

Highest values of C/N ratios were observed in *C. calcitrans* and *D. tertiolecta* at day 1 (35 and 26, respectively). *Isochrysis galbana* showed steadily decline from day 1 until day 5 (Fig. 3). The C/N ratio in *C. calcitrans* and *D. tertiolecta* declined after day 1, remaining almost constant on days 2 until 4. Major differences were observed with *I. galbana*, where statistical analysis demonstrated significant differences in C/N ratio (Table 1).

Hydrogen

The highest concentration of hydrogen (>7 pg $cell^{-1}$) was present at day 1 in *D. tertiolecta*, falling to ~4.5 pg $cell^{-1}$, and remained constant in the following days without significant differences (Table 2). The most remarkable changes in hydrogen content were observed in *C. calcitrans* (Fig. 4),

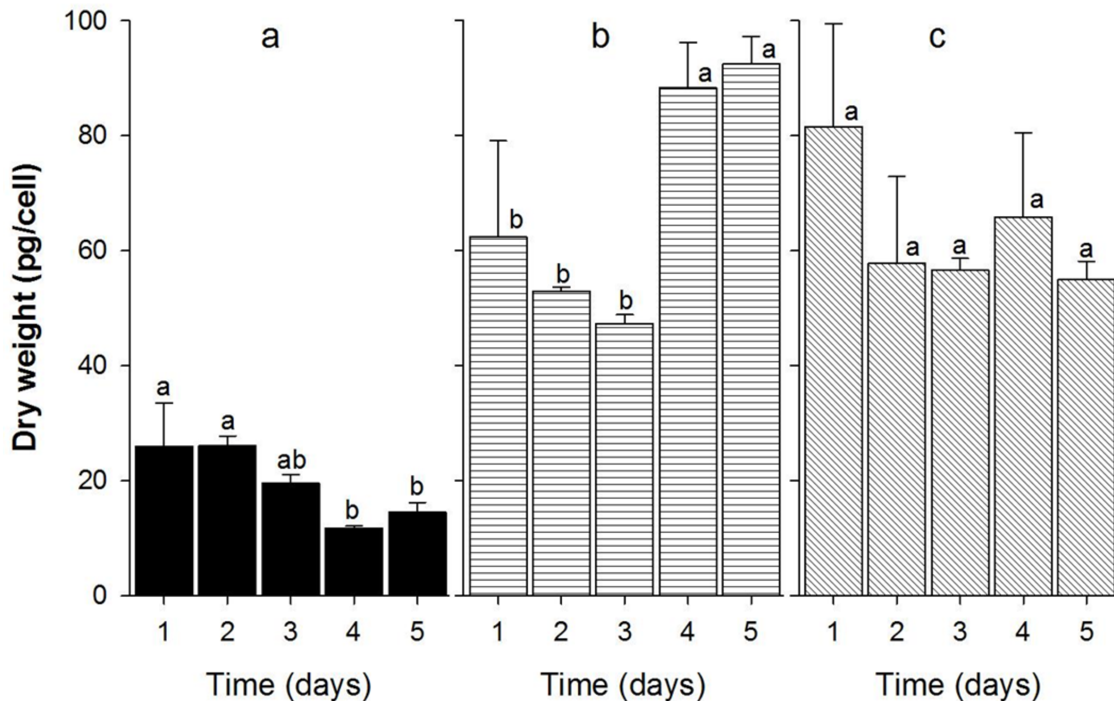

Figure 1. Dry weight by cell (pg/cell ± SE) of a) *Isochrysis galbana*, b) *Chaetoceros calcitrans*, and c) *Dunaliella tertiolecta* cultured on f/2 medium (n=3). For each variable, data carrying similar alphabet are not statistically significant (p>0.01, Tukey test).

Table 1. Results of one-way analysis of variance performed on dry weight, carbon content, and C/N ratio by cell of *Isochrysis galbana, Chaetoceros calcitrans*, and *Dunaliella tertiolecta* subjected to time (5 days).

Source of Variation	DF	SS	MS	F	p
Dry weight					
Isochrysis galbana					
Between groups	4	505.67	126.42	9.7	0.002
Residual	10	130.54	13.05		
Chaetoceros calcitrans					
Between groups	4	5,078.82	1,269.70	17.2	<0.001
Residual	10	739.62	73.96		
Dunaliella tertiolecta					
Between groups	4	1,443.95	360.99	2.3	0.129
Residual	10	1,561.75	156.17		
Carbon					
Isochrysis galbana					
Between groups	4	79.89	19.97	147.8	<0.001
Residual	10	1.35	0.135		
Chaetoceros calcitrans					
Between groups	4	566.61	141.65	27.3	<0.001
Residual	10	51.92	5.19		
Dunaliella tertiolecta					
Between groups	4	95.91	23.98	4.6	0.023
Residual	10	52.49	5.25		
C/N ratio					
Isochrysis galbana					
Between groups	4	152.45	38.11	1429.6	<0.001
Residual	10	0.27	0.03		
Chaetoceros calcitrans					
Between groups	4	1,801.85	450.46	107.1	<0.001
Residual	10	42.06	4.21		
Dunaliella tertiolecta					
Between groups	4	985.38	246.35	95.7	<0.001
Residual	10	25.75	2.58		

DF: degrees of freedom; SS: sum of squares; MS: mean square; F: *F*-ratio

starting with >6 pg cell^{-1} on day 1, dropping to half this level on days 2 and 3, and returning to the initial level on days 4 and 5. *Isochrysis galbana* reached a maximum (>2 pg cell^{-1}) on day 2, and decreasing until day 4; this change was significant (Table 2).

Chlorophylls

For all microalgae tested, the main changes in chlorophyll content occurred in chlorophyll *a* (Fig. 5). *Isochrysis galbana* had the lowest content (<0.05 pg cell^{-1}), *C. calcitrans* exhibited a significant increase from day 2 to day 4 (0.07 to 0.14 pg cell^{-1}), whilst in *D. tertiolecta* the most notable increase occurred from day 2 to day 3 (0.18 to 0.28 pg cell^{-1}). Chlorophyll *c* was present in low amounts in *I. galbana*, and *D. tertiolecta*, whereas with *C. calcitrans* was slightly higher remained constant from days 1 until day 5. *Dunaliella tertiolecta* had lower content (<0.05 pg cell^{-1}) of chlorophyll *b* (pigment restricted to Chlorophyta). Statistical analyses indicated significant differences in chlorophyll *a* and *c* for all microalgae evaluated (Table 2).

Algal growth

Regarding cell densities, *Isochrysis galbana* increased in density until day 4 (3.9 x 10^6 cell mL^{-1}), decreasing on day 5 (Fig. 6), with a high growth rate and div day^{-1} (K′ of 0.58, and 0.83, respectively). Cell density of *C. calcitrans* increased until day 3 (1.22 x 10^6 cell mL^{-1}). From the beginning of the bioassay, *D. tertiolecta* slightly increased in cell density until day 5 (1.36 x 10^6 cell mL^{-1}). *Chaetoceros calcitrans* and *D. tertiolecta* had similar K′, div day^{-1}, and generation time (Table 3).

DISCUSSION

Microalgae represent the main source of food in the initial ontogeny of many marine animals in the open sea, for this reason the microalgae culture is very important for hatchery production of the larval forms of farmed crustaceans, finfish and shellfish. The microalgae are also prey for zooplankton, which later become prey for larger larval fish stages. Thus, healthy microalgae cultures are essential for aquaculture production (Abu-Rezq *et al*., 1999; Brown *et al*., 1999; Muller-Feuga, 2000).

Physiological indicators of dry weight, carbon, C/N ratio, hydrogen, and chlorophyll content were consistent with healthy cultures reported by other authors (Estep & Hoering, 1981; Kaplan *et al*., 1986; Timmermans *et al*., 2001; Sebastien & Klein, 2006; Raghavan *et al*., 2008; Lananan *et al*., 2013; Roopnarain *et al*., 2015). In this study, dry weight by cell varied between species (because *I. galbana* cells are smaller, dry weight was less than *C. calcitrans* and *D. tertiolecta* cells), and growth phases. Regarding to variations in growth phases, some authors have reported that the increase in cell bio-

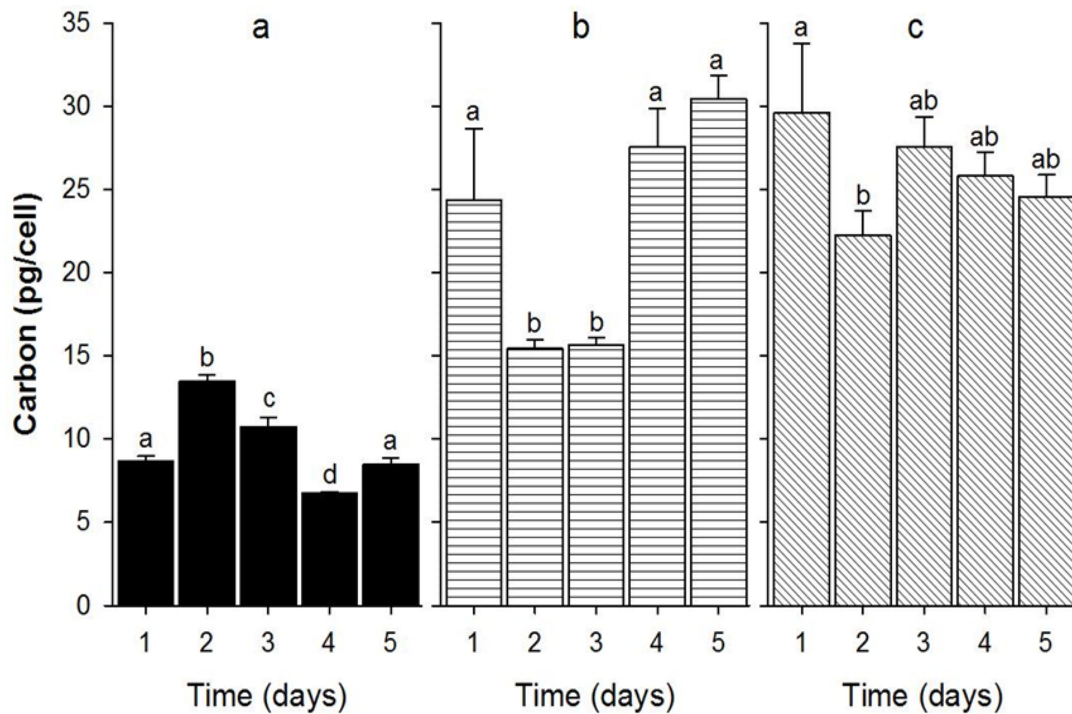

Figure 2. Carbon content by cell (pg/cell ± SE) of a) *Isochrysis galbana*, b) *Chaetoceros calcitrans*, and c) *Dunaliella tertiolecta* cultured on f/2 medium (n=3). For each variable, data carrying similar alphabet are not statistically significant (p>0.01, Tukey test).

mass is influenced by availability of nutrients; most phytoplankton store nutrients and increase their cell volume during central growth phase (Banerjee *et al*., 2011; Roleda *et al*., 2013; Roopnarain *et al*., 2015). Variations in dry weight observed in this work, suggest that *I. galbana*, *C. calcitrans* and *D. tertiolecta* process accumulated nutrients into the cells at different times, assimilating these later when needed for growth. Hence microalgae cells could change their cell volume and weight during this process, which is a typical daily cycle when binary fission occurs; once finished the cells start again to accumulate and store nutrients changing with this their sizes over the time (Tantanasarit *et al*., 2013).

Carbon content and C/N ratios of the three microalgae were consistent with values reported by others authors (Bienfang & Harrison, 1984; Cuhel *et al*., 1984; Brutemark *et al*., 2009). Variations in carbon content in different phases of cell growth in nutrient-enriched batch cultures are affected by available carbon source, because cell growth is based on different inorganic carbon sources (CO_2, H_2CO_3, HCO_3^-, CO_3^{2-}), and are transformed into complex molecules. Moreover, it has been reported that these variations differ between phytoplankton species, and depend of their exponential and stationary growth phase as well as of the nutrients sources (Brutemark *et al*., 2009; Roleda *et al*., 2013). In this study, the best correlation (*r*= 0.98) between cell density and carbon content was shown in *D. tertiolecta*, indicating the best nutrients incorporation into the cell to increase the population.

The decline of C/N ratio during the culture time was consistent in all the three species, suggesting that stored carbon in the initial days was gradually converted into biochemical components such as carbohydrates (mainly β-glucose for cell wall formation), lipids, proteins, and nucleic acids which are primary products of photosynthesis (Bienfang & Harrison, 1984; Cuhel *et al*., 1984). In this work *I. galbana* showed a marked gradual decrease in C/N ratio (Fig. 3a), which has been related to a high growth rate and to the increase in the proportion of proteins into the cell (Ríos *et al.,* 1998). Otherwise, *C. calcitrans* and *D. tertiolecta* showed values of C/N ratio slightly greater than the Redfield ratio of 6.6 from day 2 to 5, but this is commonly observed in nutrient repleted phytoplankton cultures (Geider & La Roche, 2002). Further, some authors have reported an inverse relationship between C/N ratio and nutrient concentration, showing that higher C/N ratios (11 to 7) are present when nitrogen is low (0.5 to 2 mmol N/L), but when nitrogen is high (4 to 16 mmol N/L) the C/N ratio stabilizes around 6 (Fábregas *et al*., 1995).

Roleda *et al*. (2003) found that cell growth and carbon assimilation can be affected by pH variations and determined that C/N ratio is directly related to cell growth, showing a positive correlation between lipid increase and C/N ratio decrease. Besides, it has

Table 2. Results of one-way analysis of variance performed on hydrogen, and chlorophyll (Chl *a*, and *c*) content by cell of *Isochrysis galbana, Chaetoceros calcitrans*, and *Dunaliella tertiolecta* subjected to time (5 days).

Source of Variation	DF	SS	MS	F	p
Hydrogen					
Isochrysis galbana					
Between groups	4	2.46	0.62	78.5	<0.001
Residual	10	0.078	0.0078		
Chaetoceros calcitrans					
Between groups	4	27.06	6.77	22.8	<0.001
Residual	10	2.96	0.30		
Dunaliella tertiolecta					
Between groups	4	18.44	4.61	20.7	<0.001
Residual	10	2.22	2.22		
Chl *a*					
Isochrysis galbana					
Between groups	4	0.00046	0.00011	45.7	<0.001
Residual	10	0.000025	0.0000025		
Chaetoceros calcitrans					
Between groups	4	0.0085	0.0021	28.9	<0.001
Residual	10	0.00073	0.000073		
Dunaliella tertiolecta					
Between groups	4	0.028	0.0069	19.3	<0.001
Residual	10	0.0036	0.00036		
Chl *c*					
Isochrysis galbana					
Between groups	4	0.000069	0.000017	21.3	<0.001
Residual	10	0.000008	0.0000008		
Chaetoceros calcitrans					
Between groups	4	0.000387	0.000097	16.4	<0.001
Residual	10	0.000059	0.0000059		
Dunaliella tertiolecta					
Between groups	4	0.00014	0.000034	9.1	0.002
Residual	10	0.000038	0.0000038		

DF: degrees of freedom; SS: sum of squares; MS: mean square; F: *F*-ratio

been documented that variations in carbon content and C/N ratio by cell are functions of cell nutrient quota of each microalgae species and are directly related to the increase in cell density (Cuhel *et al*., 1984; Cloern *et al*., 1995).

Hydrogen cell content was observed in high quantities in both *C. calcitrans* and *D. tertiolecta* cultures at first day, whilst *I. galbana* cultures had slight variations. These values showed a direct relation between hydrogen content, and chlorophyll *a* content for all microalgae evaluated. Variations in hydrogen content have been associated with the formation of chlorophyll, and essential molecules (carbohydrates, lipids, and proteins). Higher values of hydrogen content by cell are directly related with both photosynthesis process, and high respiration rates, which indicate good nutrient uptake (Estep & Hoering, 1981).

Concentration of chlorophylls observed in this study were consistent with their corresponding phytoplankton group (Fogg & Thake, 1987; Lourenço *et al*., 2002). Chlorophyll production over time is related to use and availability of nutrients in batch cultures, which is an indicator of growth efficiency, this due to that the instantaneous photosynthetic rate is limited by either, light energy or a nutrient (Cuhel *et al*., 1984; Cloern *et al*., 1995).

Table 3. Growth rate (K’), divisions per day (Div/day), and generation time (Gen. t) of *Isochrysis galbana, Chaetoceros* calcitrans, and *Dunaliella tertiolecta* cultured on f/2 medium (n=3).

Species	K’	Div/day	Gen. t
Isochrysis galbana	0.58	0.83	28 h 44 min
Chaetoceros calcitrans	0.35	0.51	47 h 38 min
Dunaliella tertiolecta	0.38	0.55	43 h 23 min

Cell densities and growth rates of microalgae evaluated in this study differ from those reported by other authors. In this work, *I. galbana* showed higher cell density (3.9 x 10^6 cell mL^{-1}) than occurs with Erd-Schreiber medium (3.2 x 10^6 cell mL^{-1}) or Walne’s medium (~1 x 10^6 cell mL^{-1}) (Gopinathan, 1986; Sebastien & Klein, 2006). Roleda *et al*. (2013) reported that *I. galbana* cultured with f/2 medium have a low growth rate of K’ = 0.45, whilst this study was of K’ = 0.58. Kaplan *et al*. (1986), state that temperature influences performance of microalgae cultures because the optimum temperature for achieving highest algal yield in *I. galbana* was found at 27 °C.

Several reports claim that f/2 medium is a good promoter for rapid growth in *I. galbana*, principally because the microalga requires thiamine (B_1), and cobalamin (B_{12}), as well as Fe^{3+}, one of the main trace metals that enhance the growth rate under laboratory conditions (Kaplan *et al*., 1986; Brown *et al*., 1999). Results in this work showed lower values than ob-

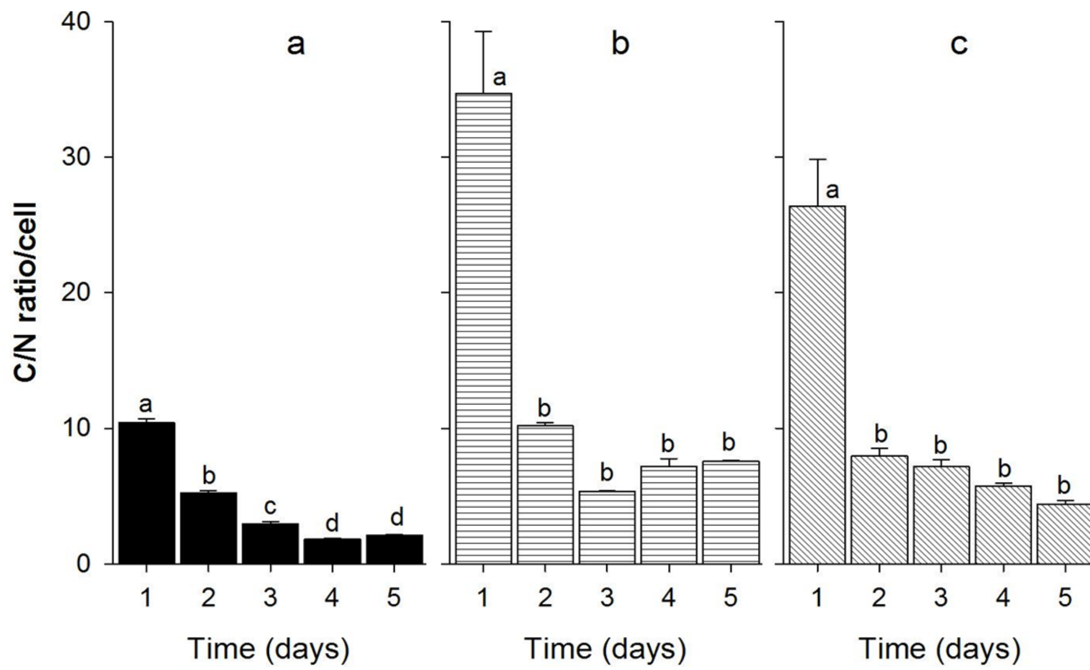

Figure 3. Carbon/nitrogen (C/N) ratio by cell of a) *Isochrysis galbana*, b) *Chaetoceros calcitrans*, and c) *Dunaliella tertiolecta* cultured on f/2 medium (n=3). For each variable, data carrying similar alphabet are not statistically significant (p>0.01, Tukey test).

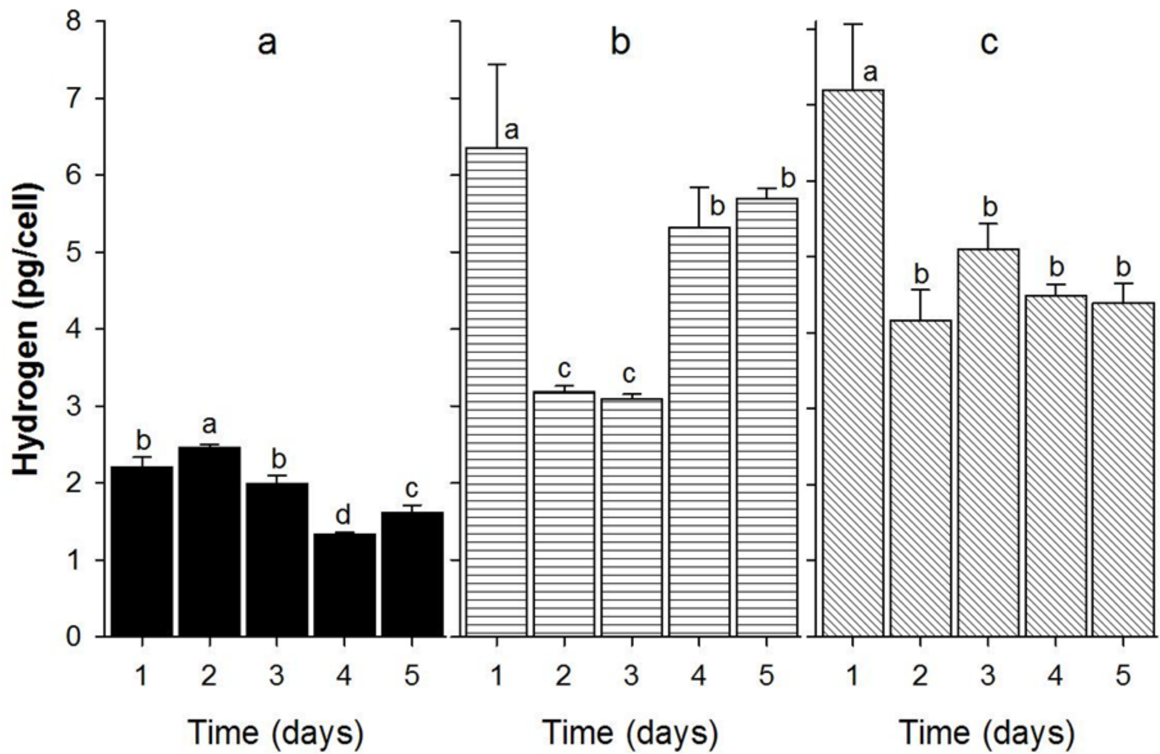

Figure 4. Hydrogen content by cell (pg/cell ± SE) of a) *Isochrysis galbana*, b) *Chaetoceros calcitrans*, and c) *Dunaliella tertiolecta* cultured on f/2 medium (n=3). For each variable, data carrying similar alphabet are not statistically significant (p>0.01, Tukey test).

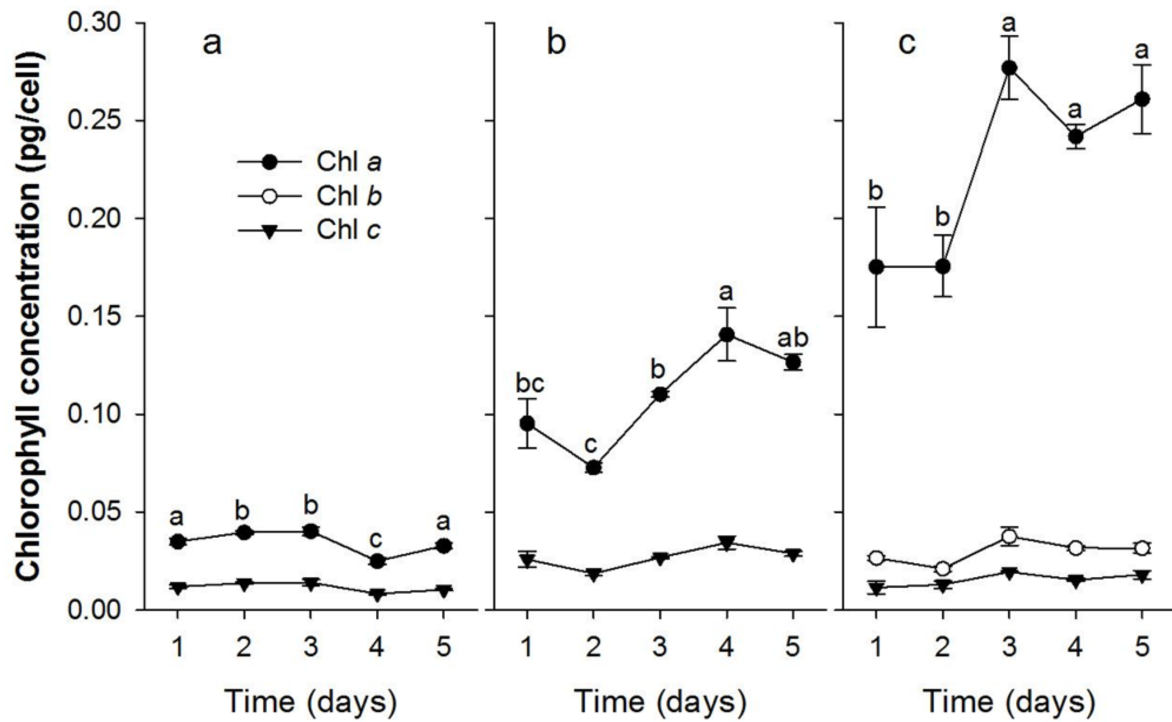

Figure 5. Chlorophyll content (Chl *a, b, c*) by cell (pg/cell ± SE) of a) *Isochrysis galbana*, b) *Chaetoceros calcitrans*, and c) *Dunaliella tertiolecta* cultured on f/2 medium (n=3). For each variable, data carrying similar alphabet are not statistically significant (p>0.01, Tukey test).

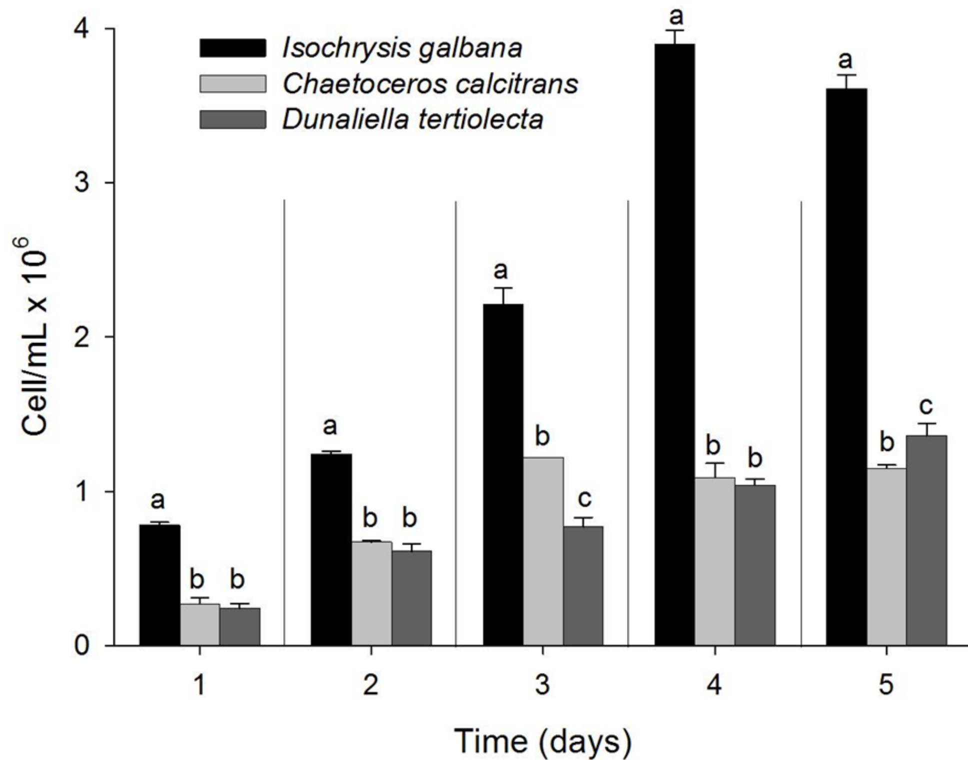

Figure 6. Cell density (cell/mL ± SE) of *Isochrysis galbana, Chaetoceros calcitrans*, and *Dunaliella tertiolecta* cultured on f/2 medium (n=3). For each variable, data carrying similar alphabet are not statistically significant (p>0.01, Tukey test).

served with Artificial Sea Water medium (ASW) or Conway medium (5 x 10^6 cell mL^{-1}) during the same time of culture (Kaplan *et al*., 1986; Lananan *et al*., 2013). The main differences observed in this study with f/2 medium could be related to the level of sodium bicarbonate ($NaHCO_3$), potassium nitrate (KNO_3) or some trace metal present in both medium cultures, *e.g*. boric acid (H_3BO_3), as well as different forms of copper ($CuCl_2$), and zinc ($ZnCl_2$) which could be more suitable for *I. galbana* cells compared with copper ($CuSO_4$) and zinc ($ZnSO_4$) forms in f/2 medium.

Cell densities of *C. calcitrans* in Conway medium (3.5 x 10^6 cell mL^{-1}) and Walne's medium (2.5 x 10^6 cell mL^{-1}) were higher compared to the results quantified in this work (1.2 x 10^6 cell mL^{-1}) (Raghavan *et al*., 2008; Banerjee *et al*., 2011; Lananan *et al*., 2013). This may indicate that *C. calcitrans* required certain nutrients in quantities different to those offered by f/2 media to increase growth. Moreover, some authors have established that low iron, low light, and addition of carbon dioxide are more adequate conditions for enhancing growth of *C. calcitrans* to ensure a higher content of important macromolecules, such as carbohydrates, lipids, and proteins (Timmermans *et al*., 2001; Raghavan *et al*., 2008).

Lananan *et al*. (2013) state that f/2 is a better media for culturing chlorophytes, leading to high cell densities, such as 4 x 10^6 cell mL^{-1} in *Dunaliella* sp., compared with Conway medium that yields 3 x 10^6 cell mL^{-1}. Roleda *et al*. (2013) also find higher growth rate (K′ = 0.74) in *D. tertiolecta* cultured with f/2; in this study, were observed lower densities (~1.4 x 10^6 cell mL^{-1}) and growth rate (K′ = 0.38), which are consistent with those recently reported by Barakoni *et al*. (2015). However, differences in cell densities between cultures using the same culture medium may be due to light intensity and photoperiod. Cuhel *et al*. (1984) and Roleda *et al*. (2013) reported that light intensity influence the growth of *Dunaliella tertiolecta*, since batch cultures improves their growth with lower light intensity (100 µmol m^{-2} s^{-1}) and longer photoperiod (16:8 light to dark); both conditions may improve *D. tertiolecta* cultures assessed here.

Differences in cell densities and growth rates for each microalga evaluated in this work were mainly dependent of nutritional conditions, although some of these variations have been related to intrinsic characteristics for each strain (Fogg & Thake, 1987; Abu-Rezq *et al*., 1999). Whether other culture media, addition of trace elements and/or vitamins may improve the growth of microalgae strains evaluated here remains to be tested.

Studies of nutrients uptake in microalgae for aquaculture purposes are catered rarely, this because in aquaculture systems a routine management is performed using the same culture medium for several microalgae species in order to reduce costs, which may result in a lower nutritional quality of cultures.

In summary, the most important aspects observed in this work were daily variations in nutrient uptake during exponential growth, which was reflected in the elemental composition (dry weight, C/N ratio, carbon, hydrogen and chlorophyll *a* content) of microalgae cells. This information provides new insights about physiology of marine microalgae and confirms that nutrient uptake dynamics is different in each microalga species. These results further demonstrate that to study nutrient uptake in microalgae, daily assessments are more efficient than experiments of few hours, this due to variations over time. Lastly, this study indicates that using one culture medium is not equally efficient to all microalgae used in aquaculture since each species has specific nutritional requirements.

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