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Marine microalgae growth and carbon partitioning as a function of nutrient availability

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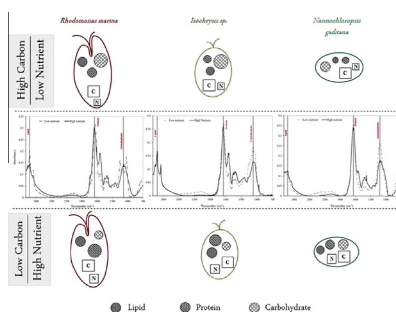
HIGHLIGHTS

- Different growth patterns were attained by microalgae throughout the experiment.
- The intracellular stoichiometry was affected by nutrients availability.
- In *N. gaditana*, the experiment triggered changes in lipid fractions proportions.
- Carbon allocation was channeled towards protein at lowest carbon availabilities.
- Carbohydrate was the main storage pool at high carbon availabilities.

GRAPHICAL ABSTRACT



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ABSTRACT

To understand in which way the structural differences of three marine microalgae (*Nannochloropsis gaditana*, *Rhodomonas marina* and *Isochrysis* sp.) affect their carbon partitioning, growth and applicability; a stoichiometric imbalance was imposed by steady carbon and other nutrients variation. Towards high nutrients concentrations/low carbon availability a decrease of 12–51% in C/N microalgae ratio was observed and maximum cell densities were achieved. Moreover, linear correlation between the nutrient input and microalgae protein content were observed. The macromolecular ratios pointed that carbohydrate was the main contributor for the C/N decrement. Although lipid content in *R. marina* remained constant throughout the experiment, a rise of 37–107% in *N. gaditana* and *Isochrysis* sp. was verified. Lipid fractions revealed high percentages of glycolipids in all microalgae (57–73% of total lipids). The present study shows an easy way to understand and modulate microalgae carbon partitioning relying on the field of application.

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1. Introduction

In aquaculture several species of microalgae are used for zooplankton enrichment in bioactive compounds (like proteins and neutral lipids) for feeding fish (Zmora et al., 2013). The selection of microalgae strains for aquaculture is based on parameters such as size, digestibility (e.g. cell wall), non-toxicity and nutritional value (Camacho-Rodríguez et al., 2013; Zmora et al., 2013). Meeting these criteria *Nannochloropsis* sp., *Rhodomonas* sp. and *Isochrysis* sp. are cultured as feed for farmed organisms such as bivalves, crab larvae and zooplankton that is then fed for crustacean and fish larvae (Brown, 2002; Camacho-Rodríguez et al., 2013; Zmora et al., 2013).

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Microalgae palatability is related with their gross biochemical composition (lipid, protein and carbohydrate) as with their aggregate energetic value, which, in turn, is dependent on factors such as microalgae phylogeny and variations in their environment conditions which may affect carbon allocation (Palmucci et al., 2011; Pernet et al., 2003; Ratha et al., 2013). Furthermore, the quantity of essential molecules, like polyunsaturated fatty acids, vitamins and sterols, as well as microalgae intracellular stoichiometry may also affect microalgae palatability and biomass applicability (Fidalgo et al., 1998; Palmucci et al., 2011). In order to modulate the latest factors, several strategies have been taken for enhancing the quality of these primary producers to feed aquatic organisms and/or for energy production (Fidalgo et al., 1998; Kim et al., 2014). The manipulation of biotic and abiotic factors related with algae culture conditions, for example temperature, nutrient availability and salinity, can induce changes in growth and biochemical composition of microalgae (Ratha et al., 2013; Xin et al., 2010). The response to changes at different environmental conditions is species-specific (Pernet et al., 2003).

Through photosynthesis microalgae can convert atmospheric CO_2 along with water and light into organic matter being carbohydrates the major products (Chen et al., 2013). The excess of fixed carbon is commonly allocated into carbohydrates and lipids, the latter more energy-demanding, and in stressful conditions, like nutritional imbalance, these molecular pools may be used as alternative energy sources for the production of raw materials required by cells (Chen et al., 2013; Chia et al., 2015; Palmucci et al., 2011). In addition, protein can function as nitrogen storage, in some organisms, and changes in this biochemical parameter can reflect the metabolic rate of actively growing/dividing cells (Andersen, 2013; Chen and Vaidyanathan, 2013). The biochemical assessment of microalgae, submitted to environment oscillations, may provide some insights about the structural modifications or molecular mechanisms that can lead to a successful adaptation (Chen and Vaidyanathan, 2013; Chia et al., 2015).

The configuration of core metabolic networks is highly varied across distinct algae classes (Hildebrand et al., 2013). This diversity along with organizational differences in the photosynthetic apparatus might affect processes such as nutrient acquisition and assimilation, carbon allocation and hence the response of microalgae to changes in their environment (Hildebrand et al., 2013). In order to contribute to the knowledge of the effect of carbon availability, the present study evaluated the impact of nutrients stoichiometry on the growth and carbon partitioning in three marine microalgae (*Nannochloropsis gaditana*, *Rhodomonas marina* and *Isochrysis* sp.).

2. Materials and methods

2.1. Chemicals

All the reagents used for analytical procedures had analytical grade.

2.2. Microorganisms and culture conditions

Microalgae strains of *Isochrysis* sp., *R. marina* and *N. gaditana* were supplied by Mariculture Center of Calheta (Madeira Island, Portugal). The cultivation of each microalga was performed by inoculating starter cultures into 500 mL of enriched seawater. The inoculation cell number was maintained at 2.6×10^6 cells mL^{-1}, for *Isochrysis* sp. and *N. gaditana*, and 1.4×10^5 cells mL^{-1}, for *R. marina*. The natural seawater used, for media preparation, was previously adjusted to a salinity of 25 g L^{-1} and sterilized in an autoclave (Uniclav 88) at 121 °C for 15 min. Five different volumes of nutrient solution Nutribloom Plus (Necton, Portugal), 250 (T1), 500 (T2), 1000 (T3), 2000 (T4) and 4000 (T5) µL L^{-1}, were used for the preparation of the growth medium. The nutrient concentrations in the final growth medium are presented in Table 1.

Table 1
Components of the growth medium culture and respective concentrations used in the final growth media (mg L^{-1}).

Component	Concentration in final growth medium (mg L^{-1})				
	T1	T2	T3	T4	T5
$NaNO_3$	43	85	170	340	680
KH_2PO_4	3	7	14	27	54
EDTA	2	4	8	15	31
$FeCl_3 \cdot 6H_2O$	1	3	5	11	22
$ZnCl_2$	0.03	0.07	0.14	0.27	0.54
$ZnSO_4$	0.07	0.14	0.29	0.57	1.15
$MnCl_2 \cdot 2H_2O$	0.04	0.08	0.16	0.32	0.65
$Na_2MoO_4 \cdot 2H_2O$	0.01	0.01	0.02	0.05	0.10
$CoCl_2 \cdot 6H2O$	0.01	0.01	0.02	0.05	0.10
$CuSO_4 \cdot 5H_2O$	0.01	0.01	0.03	0.05	0.10
$MgSO_4 \cdot 7H_2O$	0.12	0.25	0.49	0.98	1.97
Vitamins					
Thiamine	0.01	0.02	0.04	0.07	0.14
Biotin	0.001	0.003	0.005	0.010	0.020
B_{12}	0.001	0.002	0.003	0.006	0.012

Experiments were conducted at a temperature of 23 ± 2 °C, with a photoperiod of 18:6 h light/dark cycles, pH 8 ± 1, at a light intensity of 52 µmol m^{-2} s^{-1} and compressed air (125 mL min^{-1}) was used for aeration of cell cultures and as the only carbon source. The microalgae were harvested at stationary phase by centrifugation (centrifuge Labofuge 200 – Heraeus) for 5 min at 4500 rpm and washed with distilled water.

2.3. Cell concentration and specific growth rate determination

Microalgae growth was monitored daily by counting cells with a 0.1 mm deep improved Neubauer haemocytometer (Marienfeld – Superior) and a light microscope (Olympus BX41) using a 40x magnification. For cell counting, cells were fixed with lugol. A logistic model was used to describe algal growth, as previously presented by Xin et al. (2010), Eq. (1):

$$N = \frac{K}{1 + e^{a - rt}} \quad (1)$$

where K (cells mL^{-1}) is the carrying capacity, N (cells mL^{-1}) is the cell concentration in time t (days), a is a constant that refers to the position of the origin and r (d^{-1}) is the specific growth rate. The specific growth rate was calculated by the linearization of the logistic model.

2.4. Analytical procedures

For the determination of CHNS content in all experiments an elemental analyser Truspec 630 – 200 – 200 was used. Total protein was assessed by multiplying the nitrogen content for 6.25 as described by Kim et al. (2014). Fourier transform infrared spectroscopy (FT-IR) was used in order to perform the carbohydrate: lipid and carbohydrate: protein ratio analysis as previously reported by Montechiaro et al. (2006). FT-IR spectra were collected on a Perkin–Elmer Spectrum Two instrument and band assignments were made according to Dean et al. (2010), Giordano et al. (2001) and Montechiaro et al. (2006).

Extraction of total lipids was performed according to modified Bligh and Dyer (1959). Briefly, to dried algal biomass was added 3 mL of a methanol: chloroform mixture (2:1 v/v) followed by

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