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Changes in fatty acid biosynthesis in marine microalgae as a response to medium nutrient availability

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ABSTRACT

To maximize and enhance the fatty acid (FA) profiles of microalgae, it is crucial to understand the metabolic pathways that lead to high levels of the desired product. *Nannochloropsis gaditana*, *Rhodomonas marina*, and *Isochrysis* sp. were cultivated in media with various nutrient concentrations, and the biomass was analyzed for protein and fatty acid contents. The long chain polyunsaturated fatty acid (LC-PUFA) and protein contents were maximized in media with high nutrient concentrations. Under these conditions, the reduction in the (SFA + MUFA)/PUFA ratio (by as much as 83%) indicated that *R. marina* and *N. gaditana* shifted preference towards PUFA synthesis. The $\sum\omega3/\sum\omega6$ ratio indicated that *R. marina* preferentially synthesized ω3 FA towards high nutrient concentrations (depicted by an eightfold increase) in contrast to *Isochrysis* sp. (which yielded a 35% reduction). In addition, the limitations of LC-PUFA biosynthesis at the level of its precursors (e.g. C18:2ω6, C18:3ω3, C18:4ω3) and further conversion to LC-PUFA as nutrients were increased, suggesting that external variations induced changes in the sets of enzymes that maintain the desaturation and elongation pathways of FA. The present study provides novel insights into the regulation of LC-PUFA biosynthesis and facilitates the modeling of microalgal FA patterns depending on the field of application.

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Contents

1. Introduction

Microalgae are fast growing organisms that produce a variety of compounds that have various commercial uses [19,25]. For instance, the synthesis of bioactive compounds like polyunsaturated fatty acids

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(PUFAs), sterols, pigments, and vitamins, makes them suitable for use in fish farming and human health [24]. In contrast, saturated and monounsaturated fatty acids, along with hydrocarbons produced by microalgal strains are suitable for biodiesel production [13,19].

Long chain PUFA (LC–PUFA) play key roles as components of biological membranes and precursors of a variety of signaling molecules (e.g. leukotrienes and eicosanoids) with multiple physiological and pathological responses [7,15]. For instance, the intake of PUFA, namely omega3 (ω3), by humans can prevent or improve cardiovascular diseases, hypertension, and arthritis [24]. Fish and their oils are considered dietary sources of ω3 LC–PUFA, particularly eicosapentaenoic (EPA – C20:5ω3) and docosahexaenoic (DHA – C22:6ω3) acids [1,12,23]. Nevertheless, as with humans, fish lack the ability to efficiently synthesize the ω3 or ω6 (omega 6) LC–PUFA required for normal growth and development, and must acquire these substances through their diet [15,19,28].

Microalgae are the primary sources of PUFA in the marine food chain. They are supplemented with zooplankton and other aquatic organisms and used as live feeds in marine hatcheries [19,34]. Consequently, fatty acid (FA) composition is an important parameter in the selection of microalgal strains for aquaculture [10]. Some of the most commonly used strains are *Nannochloropsis* sp. (EPA – producers) and *Isochrysis* sp. [*Isochrysis* aff. *Galbana*: DHA – producer; *Isochrysis galbana* (Parke): rich in both EPA and DHA] [10,15,22].

Although these photosynthetic organisms naturally produce high amounts of PUFA, their FA content and composition can be enhanced by subjecting them to changes in their growth conditions [15,22]. In this regard, manipulation of environmental parameters, such as the composition of the growth medium or the presence of critical media components could influence the nature, amount, and composition of the desired FA [16]. The aim of the present study was to assess the effects of growth media on the qualitative and quantitative characteristics of FA contents, and on the enhancement of EPA and DHA contents in three marine microalgae – *Nannochloropsis gaditana*, *Rhodomonas marina*, and *Isochrysis* sp. (used as feed in aquaculture), by applying a range of various nutrient concentrations.

2. Materials and methods

2.1. Algal growth and experiment design

The *Isochrysis* sp., *R. marina*, and *N. gaditana* were supplied by the Mariculture Center of Calheta (Madeira, Portugal). Each microalga strain was cultured in 500 mL of enriched seawater, with commercial culture medium Nutribloom Plus (Necton, Portugal). Five volumes: 250 (T1), 500 (T2), 1000 (T3), 2000 (T4), and 4000 (T5) μL of nutrient solution L^{-1} were used in the preparation of the growth media. The components and respective concentrations are presented in Table 1. Aerated cultures were maintained at 23 °C, with a photoperiod of 18:6 h light/dark cycles, at a light intensity of 52 μmol m^{-2} s^{-1}. The microalgae were harvested in the stationary phase. More detailed information on algal growth and the experimental design is outlined in Fernandes et al. [9].

2.2. Determination of specific growth rate and fatty acid productivity

After harvest in stationary phase, the productivity of fatty acids was estimated according to the methods of Hoffmann et al. [13], by the following equation:

$$P_x = r \times c_x \quad (1)$$

where P_x represents the productivity (mg g^{-1} d^{-1}) of a specific FA (x), r is the growth rate (d^{-1}), and c_x is the concentration of the fatty acid (mg g^{-1}). The results represented the mean values ± standard deviation (SD).

Table 1
Growth media composition (mg L^{-1}) from treatments T1 to T5.

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					
Tiamine	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

2.3. Fatty acid determination

The fatty acid composition of dried algal biomass was determined and expressed as FA methyl esters (FAME), as previously described by Lepage and Roy [20], and modified according to the methods of Cohen et al. [6]. Briefly, FAs were converted to FAMEs by adding a mixture of ethyl acetate-methanol (1:19 v/v) to dry biomass that was then left at 80 °C for 1 h, and then further extracted with heptane. FAMEs were analyzed by gas chromatography (Agilent HP 6890) equipped with a mass selective detector (Agilent 5973) and a capillary column DB-225J&W (30 m × 0.25 mm inner diameter, 0.15 μm film thickness) (Agilent). The chromatographic conditions were as follows: initial temperature of the oven was 35 °C for 0.5 min; was increased by 25 °C min^{-1} to 195 °C; followed by 3 °C min^{-1} to 205 °C; and 8 °C min^{-1} until reaching the final temperature of 230 °C for 3 min. The temperature of the injector was 250 °C, that of the transfer line, 280 °C; and the split ratio was 1:100. Helium was used as the carrier gas, with a flow rate of 2.6 mL min^{-1}. The identification of FAMEs was made by comparison of the retention times and mass spectra fragmentation to those of known standards (bacterial acid methyl esters CP mix and Supelco 37 component FAME mix). At least two replicates were performed for each gas chromatography analysis, and the results represented the mean values ± standard deviation (SD) of FAME expressed in mg g^{-1} of biomass dry weight (DW). The internal standard used was heneicosanoic acid (C21:0). The chemicals were all of analytical grade and used as received.

2.4. Protein determination

Total protein was assessed by multiplying the nitrogen content by 6.25, as described by Kim et al. [18]. Determination of the nitrogen content was performed with an elemental analyzer, TruSpec 630-200-200.

2.5. Statistical analysis

Statistical analysis of the data was carried out using the IBM SPSS Statistics 23 software. Differences in growth media nutrient concentrations between treatments were assessed by one-way analysis of variance (ANOVA), followed by a Scheffe's post hoc analysis; p-values of <0.05 were considered statistically significant.

3. Results and discussion

3.1. Protein and long chain polyunsaturated fatty acids (LC–PUFAs)

Microalgae are natural sources of protein and LC–PUFA, namely EPA and DHA, for organisms on higher levels of marine food webs [3]. EPA

and DHA are crucial for the maintenance of biomembranes and cellular functions (e.g. cell signaling), whereas proteins are involved in primary metabolism as biological catalysts of important reactions for cell growth [21,30,32]. Fig. 1 shows the effects of nutrient availability on protein and FA contents.

In *N. gaditana* treatments T1 to T4, EPA and protein contents presented similar increasing patterns with respect to nutrient concentration (Fig. 1a). In T5 however, the EPA content was reduced by half, in contrast to the protein content that remained constant. This might suggest that when the growth medium nutrient concentration is high, *N. gaditana* responds by accumulating nitrogen-related metabolites (proteins) and possibly by altering the membrane properties associated with EPA levels [4,29].

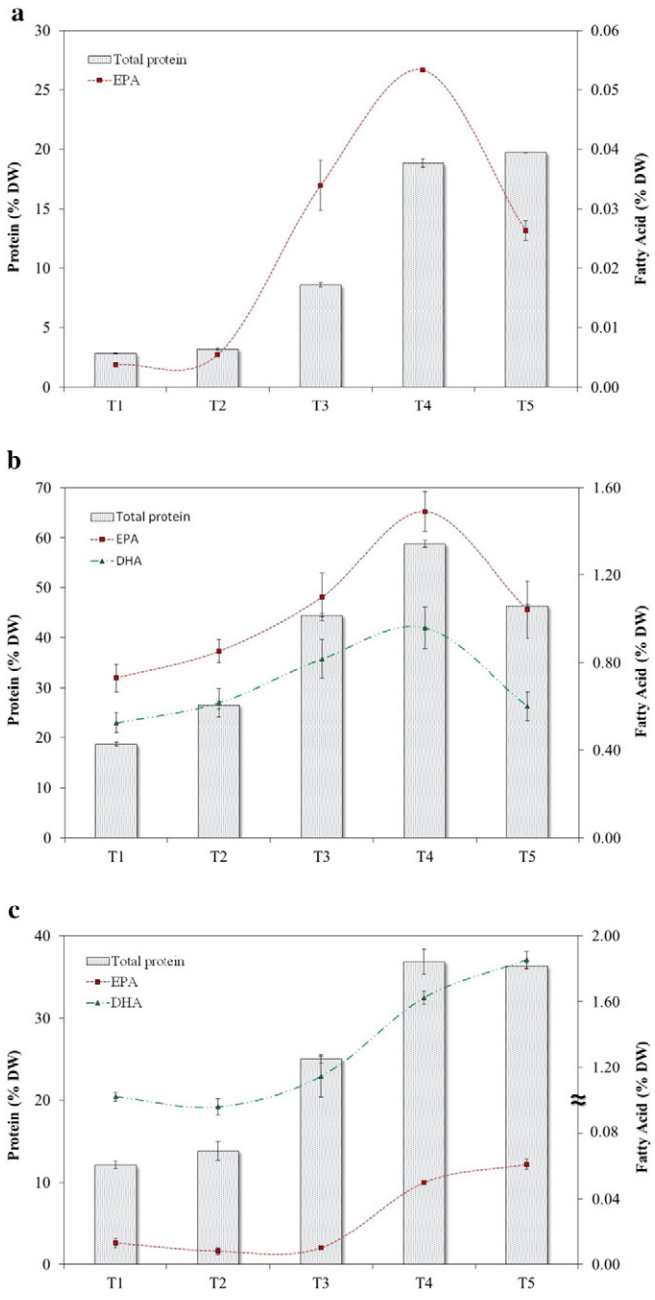

Fig. 1. The effect of growth media nutrient concentration on protein, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents on dry biomass weight (DW) of (a) *N. gaditana*, (b) *R. marina* and (c) *Isochrysis* sp.

R. marina exhibited an enhancement of EPA, DHA, and protein contents, with the increase in nutrient availability from T1 to T4 reaching a saturation point in the latter (Fig. 1b). Further nutrient input (T5) seemed to be detrimental to this microalga. In *Isochrysis* sp., biomolecules presented a similar trend with respect to nutrient concentrations, increasing with further nutrient input (Fig. 1c.).

Several factors are known to influence the activity of fatty acid elongases and desaturases – sets of enzymes that define the degree of unsaturation and chain length of FA – namely, the availability of vitamins and inorganic co-factors that can enhance or inhibit the activity of these enzymes [7]. For instance, nitrogen and sulfur are two essential components of proteins, vitamins, and coenzymes, whereas iron is a key element of the catalytic center of desaturases [2,11]. Thus, these observations suggest that higher nutrient availability comprising high levels of macronutrients and micronutrients, enhanced the quantity (evidenced by protein content) and/or activity of desaturases and elongases. Feng et al. [8] and Kaye et al. [17] studied the effects of nitrogen concentration on enzymes involved in the LC–PUFA pathway and concluded that unsaturated FA, particularly PUFA, increases with nitrogen supplementation and expression of the enzymes involved in LC–PUFA biosynthesis is down-regulated by nitrogen depletion.

The reduction observed in LC–PUFA levels in T5 for *N. gaditana* and *R. marina* might be related to increased salinity in the external environment. Therefore, the reduction in LC–PUFA content might indicate a reduction in membrane fluidity and permeability (related to the degree of unsaturation of the membrane) as a stress response to increased intracellular osmotic pressure, thereby preventing the diffusion of potentially harmful ions into the cell [33].

3.2. Fatty acid profile

According to Renaud et al. [26], LC–PUFAs are associated with high growth rates of aquaculture organisms. Knowing that the FA composition of microalgae varies with environmental factors such as nutrient availability, an attempt was made to improve the FA content by inducing changes in nutrient concentration and exploiting their physiological potential as high productivity strains [3,15].

Results from the experiments show that the FA composition of *N. gaditana* varied among the various nutrient availabilities (Table 2.). The four major FA in *N. gaditana* were linoleic acid (LA, C18:2ω6), alpha linolenic acid (ALA, C18:3ω3), palmitic acid (C16:0), and monounsaturated stearic acid (C18:1). Monounsaturated stearic acid was replaced by hexadecatrienoic acid (C16:3ω3) in T3, and by monounsaturated palmitic acid (C16:1) in T4 and T5. In *N. gaditana*, the major FA accounted for 75–89% of total FA.

In *R. marina*, nutrient availability in the growth medium induced a shift in the major FA detected (Table 3). In T1 and T2 for *R. marina*, the major FA detected were C16:0, LA, ALA, and stearidonic acid (SDA, C18:4ω3), representing 61–64% of total FA. Whereas in T3, T4, and T5, C16:0 and LA fatty acids were replaced by DHA and EPA, as predominant FA that together with ALA and SDA accounted for 73–79% of total FA.

Saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) are produced by *de novo* FA synthesis in the chloroplast, thereby providing the substrates needed for PUFA biosynthesis in the endoplasmic reticulum [4,15]. The experiments conducted on *N. gaditana* and *R. marina*, as evidenced by the (SFA + MUFA)/PUFA ratio, showed a shift in SFA and MUFA production towards PUFA synthesis as nutrient availability increased. Overall, the values observed for the (SFA + MUFA)/PUFA ratio in the marine microalgae studied were below 1, with the exception of *N. gaditana* in T1 and T2 with ratios of 2.81 and 1.30, respectively.

Previous studies have shown that CO_2 levels affect the desaturation of FA [27,31]. For instance, an increase in PUFA was reportedly associated with lower CO_2 availability, whereas increased CO_2 availability was

Table 2
Fatty acid profile of *N. gaditana* cultured under different nutrient concentrations.

Fatty acids (mg g^{-1} DW)	Treatment				
	T1	T2	T3	T4	T5
C14:0	0.07 ± 0.00^{ab}	0.06 ± 0.00^{a}	0.11 ± 0.02^{b}	0.19 ± 0.02^{c}	0.22 ± 0.02^{c}
C16:0	1.84 ± 0.02^{a}	1.25 ± 0.03^{b}	1.81 ± 0.02^{a}	2.79 ± 0.11^{c}	3.20 ± 0.11^{d}
C18:0	0.05 ± 0.01^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}	0.31 ± 0.04^{b}
Total – SFA[2]	1.98 ± 0.03^{a}	1.36 ± 0.04^{b}	2.00 ± 0.02^{a}	3.12 ± 0.13^{c}	3.84 ± 0.19d
C16:1[1]	0.18 ± 0.01^{a}	0.20 ± 0.03^{a}	0.76 ± 0.04^{b}	2.10 ± 0.12^{c}	1.94 ± 0.27^{c}
C18:1[1]	2.73 ± 0.04^{a}	1.01 ± 0.02^{b}	0.42 ± 0.03^{c}	0.50 ± 0.05^{c}	0.73 ± 0.12^{d}
Total – MUFA[2]	2.91 ± 0.04^{a}	1.21 ± 0.06^{b}	1.17 ± 0.05^{b}	2.60 ± 0.17^{a}	2.67 ± 0.39^{a}
C16:3ω3	0.26 ± 0.01^{a}	0.35 ± 0.01^{a}	1.11 ± 0.08^{b}	0.97 ± 0.18^{bc}	0.80 ± 0.02^{c}
C18:2ω6	0.66 ± 0.03^{a}	0.58 ± 0.03^{a}	1.63 ± 0.04^{b}	2.27 ± 0.18^{c}	2.66 ± 0.01^{d}
C18:3ω3	0.70 ± 0.02^{a}	0.89 ± 0.02^{a}	2.82 ± 0.17^{b}	3.00 ± 0.54^{b}	2.45 ± 0.18^{b}
C18:4ω3	n.d.	n.d.	0.04 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}
C20:5ω3 - EPA	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.34 ± 0.04^{b}	0.53 ± 0.00^{c}	0.26 ± 0.02^{d}
Total – PUFA[2]	1.74 ± 0.06^{a}	1.98 ± 0.07^{a}	6.46 ± 0.34^{b}	7.54 ± 1.17^{b}	7.19 ± 0.14^{b}
∑ω3	1.00 ± 0.03^{a}	1.29 ± 0.03^{a}	4.31 ± 0.30^{b}	4.39 ± 0.93^{b}	3.52 ± 0.22^{b}
∑ω6	0.66 ± 0.03^{a}	0.58 ± 0.03^{a}	1.63 ± 0.04^{b}	2.27 ± 0.18^{c}	2.66 ± 0.01^{d}
∑ω3/∑ω6	1.51 ± 0.04^{a}	2.22 ± 0.09^{b}	2.64 ± 0.12^{c}	1.92 ± 0.27^{b}	1.33 ± 0.08^{a}
ω3 LC–PUFA	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.34 ± 0.04^{b}	0.53 ± 0.00^{c}	0.26 ± 0.02^{d}
(SFA + MUFA)/PUFA	2.81 ± 0.09^{a}	1.30 ± 0.02^{b}	0.49 ± 0.03^{c}	0.77 ± 0.15^{d}	0.91 ± 0.10^{d}
Total[2]	6.62 ± 0.08^{a}	4.54 ± 0.15^{b}	9.63 ± 0.32^{c}	13.26 ± 1.13^{d}	13.70 ± 0.44^{d}

Values (means ± SD of at least two replications) in the same row, not sharing a common superscript are significantly different ($p < 0.05$). [1]Contains ω9 and ω7 isomers; [2]contains some minor components not shown; n.d. – non detected; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; LC–PUFA – long chain polyunsaturated fatty acid.

related to increased SFA over unsaturated FA [27,31]. As the inorganic carbon source (atmospheric CO_2) remained constant, the present findings suggest that the changes observed could be attributed to the carbon and mineral substrate ratio that was reduced as nutrient input was increased.

The FA profile of *Isochrysis* sp. varied across treatments (Table 4). For these microalgae, the major FA detected were myristic acid (C14:0), monounsaturated stearic acid (C18:1), SDA, and DHA, accounting for over 70% of the total FA among all treatments. *Isochrysis* sp. and *R. marina* yielded the highest LC–PUFA contents, with SDA as the major fatty acid. However, the FA profiles of *Isochrysis* sp. and *R. marina* presented qualitative and quantitative differences.

In microalgae, two pathways can lead to LC–PUFA production. LA is the common precursor that is converted to ALA or gamma linolenic acid (GLA, C18:3ω6), Fig. 2 [15]. In *Isochrysis* sp., both LA and ALA were present at concentrations below 10 mg g^{-1} of dry biomass, in contrast to the highly unsaturated final product, DHA. These results are consistent with those previously reported by Huerlimann et al. [15] for *Isochrysis* aff. *Galbana* (T. ISO). Moreover, the highest LA levels observed in T1 and T2 for *R. marina* could be attributed to the downregulation of desaturases and elongases, induced by low nutrient availability. This finding is supported by a reduction in ω3 LC–PUFA content and increase in intermediate fatty acid concentration. The present results suggest that for *N. gaditana*, the media used were not the most appropriate for LC–PUFA accumulation, since the highest levels of ω3 and ω6 FA obtained were LA and ALA. These FAs were previously reported as substrates for desaturation and elongation pathways that lead to LC–PUFA synthesis [15].

Table 3
Fatty acid composition of *R. marina* according to nutrient concentration.

Fatty acids (mg g^{-1} DW)	Treatment				
	T1	T2	T3	T4	T5
C14:0	9.43 ± 0.73^{a}	7.36 ± 1.08^{b}	5.32 ± 0.42^{c}	4.13 ± 0.45^{cd}	3.02 ± 0.27^{d}
C16:0	22.00 ± 1.26^{a}	13.00 ± 1.45^{b}	3.70 ± 0.21^{c}	3.15 ± 0.28^{c}	2.45 ± 0.16^{c}
C18:0	4.04 ± 0.26^{a}	1.86 ± 0.14^{b}	0.32 ± 0.02^{c}	0.34 ± 0.03^{c}	0.25 ± 0.06^{c}
Total – SFA[2]	42.40 ± 2.72^{a}	30.62 ± 4.01^{b}	15.44 ± 0.99^{c}	8.65 ± 0.79^{d}	6.26 ± 0.36^{d}
C16:1[1]	2.28 ± 0.20^{a}	1.52 ± 0.13^{bc}	1.28 ± 0.09^{b}	2.22 ± 0.26^{a}	1.87 ± 0.11^{ac}
C18:1[1]	17.66 ± 1.24^{a}	11.87 ± 1.05^{b}	5.66 ± 0.17^{c}	5.57 ± 0.25^{c}	4.48 ± 0.16^{c}
Total – MUFA[2]	19.98 ± 1.40^{a}	13.41 ± 1.17^{b}	6.94 ± 0.19^{c}	7.79 ± 0.51^{c}	6.36 ± 0.27^{c}
C18:2ω6	19.30 ± 1.33^{a}	12.21 ± 0.86^{b}	4.02 ± 0.09^{c}	4.83 ± 0.20^{c}	1.98 ± 0.22^{d}
C18:3ω3	32.75 ± 1.98^{a}	28.44 ± 1.98^{b}	26.88 ± 0.39^{bc}	23.82 ± 1.12^{c}	16.52 ± 0.15^{d}
C18:4ω3	22.08 ± 1.54^{a}	18.70 ± 1.12^{b}	26.86 ± 0.72^{c}	32.53 ± 0.66^{d}	23.50 ± 0.98^{a}
C20:4ω6 - AA	0.07 ± 0.02^{ab}	0.09 ± 0.02^{a}	0.01 ± 0.00^{c}	0.05 ± 0.02^{bc}	n.d.
C20:5ω3 - EPA	7.31 ± 0.62^{a}	8.52 ± 0.54^{ab}	10.99 ± 1.09^{c}	14.92 ± 0.90^{d}	10.41 ± 1.31^{bc}
C22:6ω3 - DHA	5.27 ± 0.47^{a}	6.18 ± 0.64^{a}	8.17 ± 0.88^{b}	9.59 ± 0.95^{b}	6.03 ± 0.65^{a}
Total – PUFA[2]	87.63 ± 5.69^{a}	74.61 ± 4.01^{b}	77.03 ± 2.55^{bc}	85.98 ± 1.82^{ac}	58.46 ± 3.28^{d}
∑ω3	67.41 ± 4.52^{ab}	61.84 ± 3.23^{ac}	72.89 ± 2.54^{bd}	80.87 ± 1.70^{d}	56.46 ± 3.06^{c}
∑ω6	20.22 ± 1.40^{a}	12.77 ± 0.87^{b}	4.13 ± 0.09^{c}	5.12 ± 0.17^{c}	2.00 ± 0.22^{d}
∑ω3/∑ω6	3.34 ± 0.15^{a}	4.85 ± 0.17^{a}	17.63 ± 0.69^{b}	15.81 ± 0.40^{b}	28.31 ± 1.54^{c}
ω3 LC–PUFA	12.58 ± 1.07^{a}	14.71 ± 1.18^{a}	19.16 ± 1.97^{b}	24.52 ± 1.82^{c}	16.44 ± 1.96^{ab}
DHA/EPA	0.72 ± 0.02^{a}	0.72 ± 0.03^{a}	0.74 ± 0.01^{a}	0.64 ± 0.03^{b}	0.58 ± 0.01^{c}
(SFA + MUFA)/PUFA	0.71 ± 0.03^{a}	0.59 ± 0.05^{b}	0.29 ± 0.02^{c}	0.19 ± 0.01^{d}	0.22 ± 0.01^{d}
Total[2]	150.01 ± 9.24^{a}	118.64 ± 8.72^{b}	99.40 ± 2.04^{c}	102.43 ± 2.34^{c}	71.08 ± 3.19^{d}

Values (means ± SD of at least two replications) in the same row, not sharing a common superscript are significantly different ($p < 0.05$). [1]Contains ω9 and ω7 isomers; [2]contains some minor components not shown; n.d. – non detected; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; LC–PUFA – long chain polyunsaturated fatty acid.

Table 4
Fatty acid profile of *Isochrysis* sp. grown under different nutrient concentration.

Fatty acids (mg g^{-1} DW)	Treatment				
	T1	T2	T3	T4	T5
C14:0	5.97 ± 0.18[a]	4.64 ± 0.09[b]	9.48 ± 0.69[c]	13.97 ± 0.51[d]	14.48 ± 0.43[d]
C16:0	3.91 ± 0.12[a]	2.98 ± 0.10[b]	5.34 ± 0.40[c]	6.77 ± 0.21[d]	6.78 ± 0.16[d]
C18:0	0.01 ± 0.00[a]	n.d.	n.d.	0.03 ± 0.00[b]	0.02 ± 0.01[b]
Total – SFA[2]	10.14 ± 0.29[a]	7.71 ± 0.14[b]	15.06 ± 1.10[c]	21.26 ± 0.65[d]	21.89 ± 0.61[d]
C16:1[1]	3.44 ± 0.16[a]	2.36 ± 0.20[b]	4.63 ± 0.51[c]	6.49 ± 0.16[d]	7.27 ± 0.43[d]
C18:1[1]	7.42 ± 0.27[a]	4.64 ± 0.32[b]	8.02 ± 0.84[a]	9.30 ± 0.32[c]	10.10 ± 0.23[c]
Total – MUFA[2]	10.89 ± 0.43[a]	7.03 ± 0.51[b]	12.68 ± 1.34[a]	16.12 ± 0.50[c]	17.72 ± 0.62[c]
C16:3ω3	0.07 ± 0.02[ab]	0.08 ± 0.02[ab]	0.01 ± 0.00[a]	0.15 ± 0.04[bc]	0.17 ± 0.05[c]
C18:2ω6	2.24 ± 0.15[ab]	1.81 ± 0.20[a]	2.91 ± 0.52[b]	6.30 ± 0.22[c]	7.33 ± 0.23[d]
C18:3ω3	3.10 ± 0.10[a]	2.88 ± 0.23[a]	5.47 ± 0.47[b]	7.64 ± 0.15[c]	8.49 ± 0.25[d]
C18:4ω3	14.11 ± 0.41[a]	14.27 ± 0.62[a]	25.24 ± 1.33[b]	35.43 ± 0.62[c]	38.90 ± 0.73[d]
C20:5ω3 - EPA	0.13 ± 0.03[a]	0.08 ± 0.02[a]	0.10 ± 0.01[a]	0.50 ± 0.01[b]	0.61 ± 0.03[c]
C22:6ω3 - DHA	10.20 ± 0.27[ab]	9.59 ± 0.49[a]	11.44 ± 1.26[b]	16.25 ± 0.39[c]	18.54 ± 0.51[d]
Total – PUFA[2]	30.10 ± 0.99[a]	28.86 ± 1.59[a]	45.44 ± 3.28[b]	67.80 ± 1.44[c]	76.09 ± 1.92[d]
∑ω3	27.61 ± 0.78[a]	26.90 ± 1.34[a]	42.25 ± 2.69[b]	59.91 ± 1.08[c]	66.66 ± 1.42[d]
∑ω6	2.31 ± 0.17[ab]	1.87 ± 0.23[a]	2.92 ± 0.52[b]	7.20 ± 0.30[c]	8.57 ± 0.38[d]
∑ω3/∑ω6	11.99 ± 0.56[a]	14.52 ± 1.10[b]	14.68 ± 1.91[b]	8.33 ± 0.21[c]	7.79 ± 0.19[c]
ω3 LC–PUFA	10.33 ± 0.30[ab]	9.67 ± 0.50[a]	11.53 ± 1.28[b]	16.69 ± 0.50[c]	19.10 ± 0.60[d]
DHA/EPA	83.85 ± 16.71[a]	119.35 ± 19.11[ab]	134.44 ± 30.34[b]	33.14 ± 0.59[c]	30.66 ± 1.32[c]
(SFA + MUFA)/PUFA	0.70 ± 0.01[a]	0.51 ± 0.01[b]	0.61 ± 0.02[c]	0.55 ± 0.01[d]	0.52 ± 0.01[bd]
Total[2]	51.13 ± 1.49[a]	43.60 ± 2.14[b]	73.18 ± 5.14[c]	105.18 ± 1.81[d]	115.70 ± 2.98[e]

Values (means ± SD of at least two replications) in the same row, not sharing a common superscript are significantly different ($p < 0.05$). [1]Contains ω9 and ω7 isomers; [2]contains some minor components not shown; n.d. – non detected; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; LC–PUFA – long chain polyunsaturated fatty a.

The three marine microalgae under investigation yielded ∑ω3/∑ω6 ratios >1, independently of the growth medium nutrient concentration. This suggests that the ω3 pathway is more active than that of ω6. For T5, *R. marina* achieved the highest ratio, comprising 28 times more ω3 FA than ω6 FA. According to Huerlimann et al. [15], this could be attributed to the substrate specificity of the enzyme set (namely desaturases) that link ω3 and ω6 pathways, and can be more active and preferentially diverted towards ω3 FA synthesis. Furthermore, the fatty acid profile of *R. marina* exhibited an increase in the ∑ω3/∑ω6 ratio with increasing nutrient input, whereas *Isochrysis* sp. exhibited the opposite trend. These findings support the assumption that the response to nutrient concentration is species-specific. They also suggest that nutrient availability affected the biosynthesis of PUFA, possibly by shifting enzyme specificity towards ω6 FA synthesis in *Isochrysis* sp., and ω3 FA synthesis in *R. marina*.

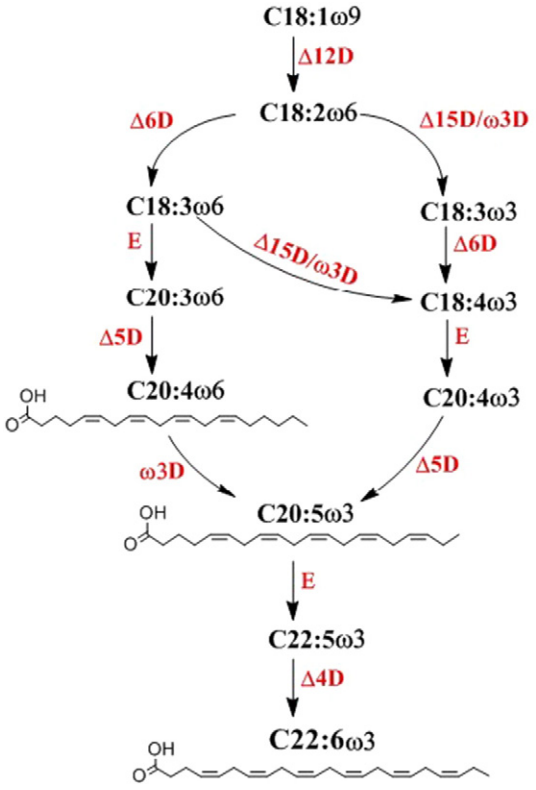

Fig. 2. Schematic representation of long chain – polyunsaturated fatty acid (LC–PUFA) biosynthesis in eukaryotic microalgae, based on Mühlroth et al. [22].

Since PUFAs are not synthesized by *de novo* FA synthesis and ω6 PUFA are not readily converted to ω3 PUFA in fish and humans, the levels of these biomolecules are largely determined by dietary intake [5]. Therefore, algal diets with low (SFA + MUFA)/PUFA ratios and ∑ω3/∑ω6 ratios higher than 2 are optimal for the feeding of larval and juvenile oysters [14]. For all treatments, *R. marina* and *Isochrysis* sp. exceeded the recommended minimal ratio, and their (SFA + MUFA)/PUFA ratios were <1 (Tables 2, 3, and 4). In contrast, the ∑ω3/∑ω6 ratio in *N. gaditana* was lower than 2, with the exception of those subjected to treatments T2 and T3, suggesting that these treatments are suitable for the production of this microalga for aquaculture.

3.3. EPA and DHA productivities

The implementation of strategies that induce the accumulation of specific compounds of high value may differentially influence microalgal growth [30]. In order to improve microalgal production for commercial applications, the productivity of the cultivation systems must be considered [3].

Regarding productivity, the microalgae with the highest EPA and DHA contents, along with the highest specific growth rates were those subjected to treatment T3 for *R. marina* and *N. gaditana*, and T4 for *Isochrysis* sp. (Fig. 3). DHA productivity was not calculated for *N. gaditana* microalgae since it was not present in their FA profile. Since *N. gaditana* demonstrated similar EPA productivities in T3 and T4, selection of the more favorable productivity had to take costs into account. *N. gaditana* productivities were low compared to those obtained for the other microalgal strains. This was mainly due to their low FA content.

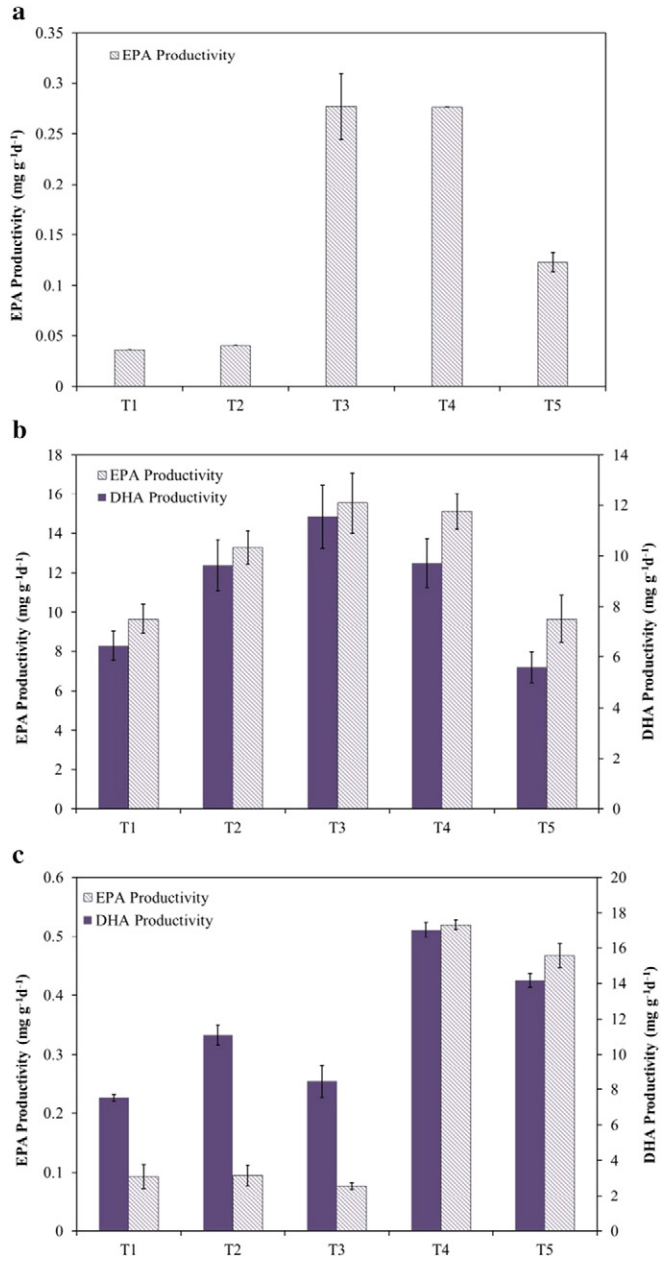

Fig. 3. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) productivities for (a) *N. gaditana*, (b) *R. marina* and (c) *Isochrysis* sp. according to the nutrients media composition.

4. Conclusion

The mobilization of FA groups with nutrient input constitutes an advantage for the modeling of the biochemical composition of microalgae, depending on the field of application. As such, at low nutrient concentrations, the possible interruption of desaturation and elongation pathways makes the FA patterns of microalgae more suitable for biodiesel production. In contrast, high nutrient inputs enhanced the role of the enzyme sets that lead to LC-PUFA accumulation, making microalgal composition more suitable for aquaculture and nutritional supplements. The present study revealed an effective strategy to assess the metabolic pathways that lead to desirable levels of FA accumulation in three biological systems.

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