Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*

J.P Fidalgo[a], A Cid[a], E Torres[a], A Sukenik[b], C Herrero[a1],

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Abstract

The marine microalga *Isochrysis galbana* was cultured in nitrate, nitrite or urea media to examine changes in the gross biochemical composition, with special emphasis on the growth phase associated changes in the compositions of the lipid classes and fatty acids. The gross biochemical composition was affected more by the growth phase than by the nitrogen source. Protein was higher during exponential growth (about 37–45% AFDW) but the pattern changed as the cultures aged, and lipids were the main algal constituents on all nitrogen sources in the late stationary phase. In all cultures, the relative abundance of neutral lipid increased in the late stationary phase concomitant with a proportional reduction in phospholipids, whereas galactolipids only slightly changed during the growth of the cultures. Total fatty acid content was affected by nitrogen source and growth phase. Maximal PUFA values were obtained at the early stationary phase and decreased throughout the stationary phase. The highest fatty acid contents in the early stationary phase were produced in urea cultures; these cultures also had higher PUFA content, with EPA and DHA contents of 27.66% and 14.13% of total fatty acids, respectively.

Keywords

Isochrysis galbana; Nitrogen sources; Lipids; Fatty acids

1. Introduction

Microalgae play an important role in mariculture as food for larval and juvenile molluscs, crustacean and fish species. The nutritional value of microalgae is related to their biochemical composition, especially the lipid and fatty acid compositions (Sukenik and Wahnon, 1991;

[1] a Laboratorio de Microbiología, Facultad de Ciencias, Universidade da Coruña, Campus da Zapateira s/n, 15071 A Coruña, Spain
b National Institute of Oceanography Israel Oceanographic and Limnological Research Tel Shikmona, P.O.B. 8030, Haifa 31080, Israel

Dunstan et al., 1992 and Dunstan et al., 1993;Sukenik et al., 1993). The quality and quantity of microalgal lipid are important to the nutrition of marine animals (Enright et al., 1986; Gallager et al., 1986; Koven et al., 1989; Sargent et al., 1989). Dietary lipids are sources of both metabolic energy and specific metabolites that are essential for animal growth. The most important aspect of lipid in animal nutrition is the content and proportions of certain fatty acids. In particular, it has been shown that some polyunsaturated fatty acids (PUFAs) synthesized by algae, are essential for the growth and development of marine fish larvae (Koven et al., 1989), shrimp and molluscs (De Pauw and Persoone, 1988). The presence of long-chain *n*−3 highly unsaturated fatty acids is important in selecting unicellular marine algae for aquacultural purposes, in particular, eicosapentaenoic acid (EPA), 20:5*n*−3, and docosahexaenoic acid (DHA), 22:6*n*−3.

The golden-brown flagellate *Isochrysis galbana* has been utilized successfully as a food source for a variety of bivalve molluscs (Enright et al., 1986) and is one of the most commonly used marine unicellular algae in many mariculture systems (Sukenik and Wahnon, 1991). *I. galbana* is rich in polyunsaturated fatty acids (PUFAs), and grows well in mass cultures, either indoors or outdoors (Kaplan et al., 1986).

The manipulation of the algal growth environment can alter the growth characteristics and chemical composition of cells cultured under the conditions applied (Brown et al., 1989). Among the different components of the culture medium, the source and concentration of nitrogen can provoke important changes in the growth and biochemical composition of microalgal species (Kaplan et al., 1986; Levasseur et al., 1993;Fidalgo et al., 1995). The chemical composition of microalgal cells is also known to vary during their growth phase, particularly with respect to their lipid component (Sukenik and Carmeli, 1990; Hodgson et al., 1991).

In order to optimize the lipid and fatty acid production, *I. galbana* was cultured with different nitrogen sources. The gross biochemical composition (protein, carbohydrate and lipid) was examined, with special emphasis on the growth phase associated changes in the composition of the lipid classes and fatty acids.

2. Materials and methods

2.1. Organism

I. galbana Parke clon ISO (Haptophyceae) was obtained from the Culture Center of Algae and Protozoa, Cambridge, England.

2.2. Growth conditions

Cultures were carried out in sterilized and enriched seawater (salinity 36 g l^{-1}) (Fidalgo et al., 1995). Nitrogen sources used were: nitrate, as $NaNO_3$, nitrite, as $NaNO_2$, and urea ($(NH_2)_2CO$), all at a concentration of 4 mg atom N l^{-1}.

Cultures were grown in a controlled environmental incubator at 18±1°C and illuminated with Mazda Fluor C7 TF40 fluorescent lamps, at an irradiance level of 115 μmol m^{-2} s^{-1}. A 12:12

light–dark cycle was maintained in order to obtain synchronous cultures. Cultures were continuously bubbled with 0.22 μm filtered air at a rate of 10 l min^{-1}. Axenic stock cultures were grown in nitrate media. Inocula were taken from the same stock culture at late exponential growth phase, and initial densities were adjusted to 2.5×10^6 cells ml^{-1}. Algal cells were not preadapted to the new N source. Experiments were carried out in triplicate.

2.3. Growth measurement

Cell numbers were measured daily using an electronic particle counter (Coulter Electronics), pH was recorded, and instantaneous growth rates (μ) were calculated.

2.4. Analytical methods

Cells were harvested at three different points in the growth curve: during the exponential growth phase, in an early stationary phase, and during the late stationary phase (Fernandez-Reiriz et al., 1989). Samples were always collected at the same moment in the photoperiod. Dry weight was determined according to Vonshak (1986)and ash content as A.O.A.C. (1980). C/H/N was determined with an elemental analyser (Carlo-Herba). Protein content was derived from nitrogen content using the factor proposed by Gnaiger and Bitterlich (1984). Carbohydrates were measured by the phenol sulphuric acid method (Kochert, 1978). Pigments were extracted in 90% acetone and concentrations were calculated as previously described (Sukenik and Wahnon, 1991).

2.5. Extraction and quantification of lipids

Total lipids were extracted with methanol:chloroform:water (2:1:0.8 v/v/v). Total lipids were quantified by the charring method (Marsh and Weinstein, 1966). Total lipid extracts were fractionated to the major lipid groups on heat activated silicic acid columns (Sep-Pak silica cartridges, Waters) as previously described (Sukenik et al., 1989).

2.6. Preparation and determination of fatty acids

Fatty acid methyl esters were analyzed from freeze-dried biomass following the procedure described by Miller (1984)using a Perkin-Elmer 8310 gas–liquid chromatograph with FID detector equipped with a 30-m fused silica capillary column SP-2330 (Supelco). Identification of fatty acid methyl esters was accomplished by comparing the retention times of experimental samples to those of known standards. Identification was verified by GC–MS using a Hewlett-Packard gas–liquid chromatograph connected to an HP5972 mass selective detector using both SP-2330 and Omegawax 250 (Supelco) analytical columns. Margaric acid (C17:0) was used as internal standard.

2.7. Statistical analysis

Data were analyzed using ANOVA and the Duncan multiple range test (α=0.05).

3. Results

3.1. Algal growth

I. galbana grew, in the three different nitrogen sources at a similar rate, with no significant differences among them ($P>0.05$) (Fig. 1). Maxima instantaneous growth rates (μ) were 0.78 $^{-1}$ for nitrate, 0.76 for nitrite and 0.73 for urea media. There were no significant differences among the cellular densities reached at the stationary phase ($P>0.1$).

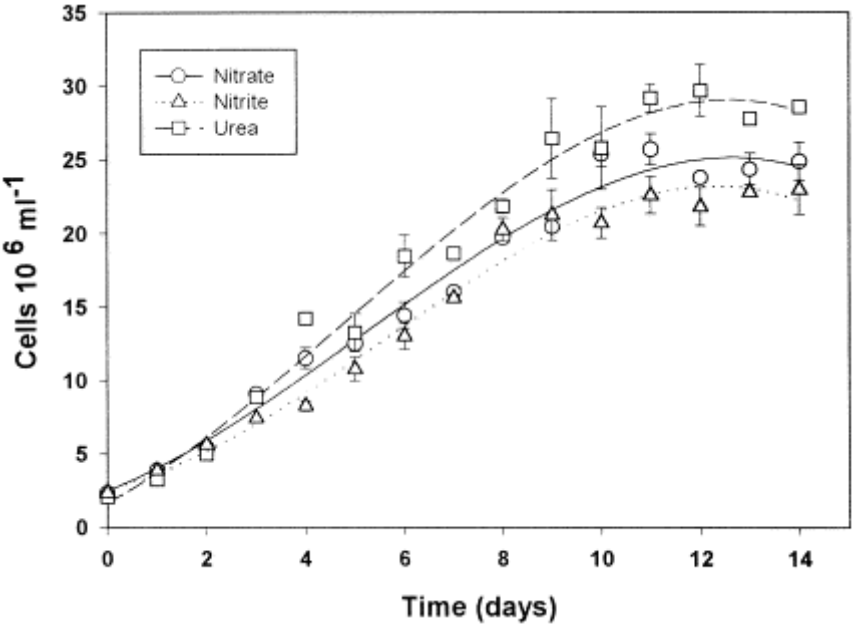

Fig. 1.

Growth curves of *I. galbana* cultured with different nitrogen sources.

Dry weights, ash percentages and organic matter (AFDW, ash free dry weight) of cells were affected by the growth phase and by the N-source (Table 1). In all the N-sources assayed, cellular dry weight tended to increase when the culture aged, from values of about 41–53 pg cell $^{-1}$ at the logarithmic phase to values of about 65–83 pg cell^{-1} at the late stationary phase. Similar results were obtained for the organic cell weights, increasing from 30–40 pg cell^{-1} to 55–68 pg cell^{-1}. There were no significant differences in cell dry weights and organic dry weights between the logarithmic and early stationary phases in nitrate and urea cultures, but significant differences occurred between early and late stationary phases in all cultures. In late stationary phase, cells grown in nitrate and urea had significantly higher dry weights and organic weights than cells grown in nitrite (Table 1). Consequently, dry weight yields and organic matter (AFDW) yields at late stationary phase were significantly higher in nitrate and urea cultures than in nitrite cultures. Ash content was significantly higher in the logarithmic phase than in the stationary phase in all cultures (Table 1).

Table 1.

Dry weight (DW), ash content, and ash-free dry weight (AFDW) of *I. galbana* grown in different N sources

Parameter	Phase	Nitrate	Nitrite	Urea

Parameter	Phase	Nitrate	Nitrite	Urea
DW (pg cell^{-1})	L	47.50±1.65 (a,b)	53.38±0.95 (b)	41.05±0.16 (a)
	ES	49.00±1.47 (a,b)	44.68±0.45 (a)	42.68±1.29 (a)
	LS	76.46±10.78 (d)	65.40±6.48 (c)	83.44±2.19 (d)
Ash (%DW)	L	27.82±1.29 (b)	24.82±1.21 (b)	25.83±0.10 (b)
	ES	15.73±0.20 (a)	16.25±0.75 (a)	17.25±0.24 (a)
	LS	16.21±0.65 (a)	16.24±0.72 (a)	17.81±0.50 (a)
AFDW (pg cell^{-1})	L	34.20±1.28 (a,b)	40.03±2.51 (b)	30.38±2.76 (a)
	ES	41.16±1.25 (a,b)	37.53±0.47 (a)	35.42±1.12 (a)
	LS	64.23±9.07 (d)	54.94±5.46 (c)	68.42±2.00 (d)

The values presented are means of three replicates and standard deviations. Means were compared using the multiple range test of Duncan (α=0.05); differences were not significant for groups with the same letter.

L: Logarithmic phase; ES: early stationary phase; LS: late stationary phase.

3.2. Proximate cellular composition

The cellular contents of both N and C increased with the age of the culture, especially between early and late stationary phase (Table 2). The increase was higher in C, consequently the ratio C/N increased throughout the culture, with maxima at the late stationary phase. The C/N ratio ranged from 8.24–9.41 for cells in the logarithmic phase to 10.46–13.29 for cells in late stationary phase. Regarding the N source, in the logarithmic phase, the C/N ratio is significantly lower in urea cultures; no significant differences appear in C/N ratio among the three N sources at early stationary phase, but at late stationary phase, this ratio is significantly lower in nitrite cultures (Table 2).

Table 2.

Cellular quotas (Q) of C and N of *I. galbana* grown in different N sources and in different phases of the growth cycle

Parameter	Phase	Nitrate	Nitrite	Urea
QN (pg cell^{-1})	L	2.38±0.02 (a,b,c)	2.59±0.04 (c)	2.36±0.01 (a,b,c)
	ES	2.46±0.10 (b,c)	2.24±0.02 (a,b)	2.20±0.07 (a)
	LS	3.10±0.11 (d)	3.17±0.09 (d,e)	3.38±0.10 (e)
QC (pg cell^{-1})	L	19.20±0.09 (a,b)	20.75±0.06 (b,c)	16.71±0.08 (a)
	ES	22.39±0.07 (c)	19.64±0.05 (b)	19.40±0.11 (a,b)
	LS	32.70±0.09 (e)	28.37±0.11 (d)	38.43±0.15 (f)
C/N (mol/mol)	L	9.41±0.13 (b)	9.35±0.15 (b)	8.24±0.05 (a)
	ES	10.60±0.16 (c)	10.23±0.06 (c)	10.27±0.36 (c)
	LS	12.33±0.75 (d)	10.46±0.38 (c)	13.29±0.38 (e)

The values presented are means of three replicates and standard deviations. Means were compared using the multiple range test of Duncan (α=0.05); differences were not significant for groups with the same letter.

L: Logarithmic phase; ES: early stationary phase; LS: late stationary phase.

The biochemical profile was more affected by the growth phase than by the nitrogen source. In the logarithmic phase, protein was the main constituent in all cultures, accounting for 37–45%

AFDW (Table 3). Lipids were second, accounting for 22–32%. Carbohydrates showed less variation during the exponential growth with values between 7.65% and 9.84% (Table 3). This pattern changed as the cultures aged and in the late stationary phase, lipids were the main constituents in cells grown in all nitrogen sources. Urea cultures showed significantly higher values of lipids than nitrate and nitrite cultures in the logarithmic and early stationary phases. The values of chlorophyll *a* and carotenes as percentages of AFDW, decreased when populations entered the stationary phase, the decrease in chlorophyll *a* content being greater; therefore, the carotenoids/chlorophyll *a* ratio increased.

Table 3.

Biochemical composition, as %AFDW, of *I. galbana* cells cultured with different N sources and in different phases of the growth cycle

Parameter	Phase	Nitrate	Nitrite	Urea
Protein	L	40.19±0.79 (b)	37.36±1.23 (b)	44.96±0.83 (c)
	ES	34.50±0.94 (b)	34.58±0.96 (b)	35.98±1.49 (b)
	LS	28.01±1.31 (a)	33.46±1.41 (b)	28.48±1.51 (a)
Lipids	L	21.87±0.47 (a)	27.50±3.11 (a)	32.33±0.58 (b)
	ES	34.03±0.58 (b)	33.58±0.58 (b)	42.05±0.51 (c)
	LS	38.49±0.51 (c)	41.61±0.51 (c)	31.36±0.98 (b)
Carbohydrates	L	7.65±0.43 (a)	8.19±0.50 (a)	9.84±0.44 (b)
	ES	10.64±0.48 (c)	9.04±0.45 (b)	10.18±0.49 (b,c)
	LS	11.95±0.47 (d)	13.58±0.50 (d)	10.85±0.47 (c)
Chlorophyll *a*	L	0.58±0.01 (b)	0.53±0.01 (b)	0.78±0.02 (c)
	ES	0.35±0.02 (a)	0.36±0.04 (a)	0.38±0.00 (a)
	LS	0.26±0.05 (a)	0.28±0.08 (a)	0.31±0.02 (a)
Chlorophyll *c*	L	0.05±0.00 (a,b)	0.03±0.00 (a)	0.11±0.01 (c)
	ES	0.08±0.01 (c)	0.10±0.02 (c)	0.13±0.01 (d)
	LS	0.05±0.01 (a,b)	0.06±0.01 (b)	0.08±0.00 (c)
Carotenes	L	0.44±0.02 (b)	0.40±0.01 (b)	0.59±0.02 (c)
	ES	0.31±0.01 (a)	0.35±0.02 (a)	0.34±0.01 (a)
	LS	0.25±0.01 (a)	0.31±0.02 (a)	0.25±0.02 (a)
Carotenoids/chlorophyll *a*	L	0.75±0.04 (a)	0.76±0.01 (a)	0.77±0.01 (a)
	ES	0.92±0.02 (b)	0.92±0.03 (b)	0.91±0.02 (b)
	LS	0.94±0.15 (b)	1.06±0.10 (c)	1.33±0.11 (c)

The values presented are means of three replicates and standard deviations. Means were compared using the multiple range test of Duncan (α=0.05); differences were not significant for groups with the same letter.

L: Logarithmic phase; ES: early stationary phase; LS: late stationary phase.

3.3. Lipid composition

Lipid composition of *I. galbana* grown with different nitrogen sources in the different growth phases is shown in Fig. 2. Little differences occurred in lipid fractionation, expressed as percentage of total lipid, among the different nitrogen sources. Lipid composition was more affected by the growth phase. In all cultures, neutral lipid increased with the growth phase, from

27–30% of total lipid in logarithmic phase to 54–61% in late stationary phase, whereas phospholipid decreased from 31–39% in logarithmic phase to 10–12% of total lipid in late stationary phase. The percentage of galactolipid was not affected by the growth phase, with values between 34% and 28% in nitrate cultures, 37% and 28% in nitrite cultures, and 40% and 35% in urea cultures.

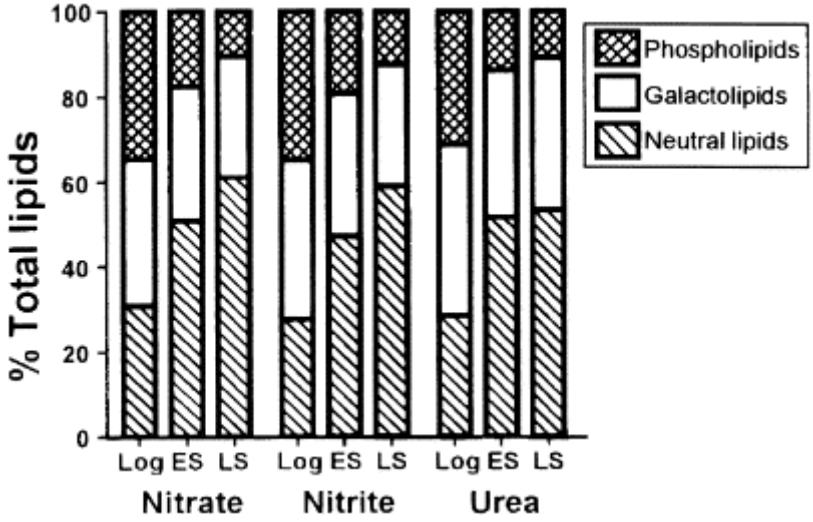

Fig. 2.

Relative proportions of the major lipid fractions in *I. galbana* cells cultured with different nitrogen sources and in different phases of the growth cycle.

3.4. Fatty acid composition

Total fatty acid cell content increased with culture time, from 103–125 mg g^{-1} DW of exponential cells to 146–177 mg g^{-1} DW of late stationary cells, although maxima contents were obtained at early stationary phase in cells grown in nitrite and urea (Table 4). In this haptophyte, the main fatty acids, as percent of total fatty acids, were: 14:0 (9.8–19.9%), 16:0 (12.2–18.8%), 16:1*n*−7 (12.4–25.8%), 18:4*n*−3 (7.1–15.4%), 20:5*n*−3 (12.8–27.7%) and 22:6*n*−3 (5.1–14.1). Reliable identification of 20:0 and 18:3*n*−4 fatty acids was not possible under these analytical conditions due to their low occurrence.

Table 4.

Fatty acid composition (as % of Total fatty acids) of *I. galbana* grown with different N sources and in different phases of the growth cycle

FA	Nitrite			Nitrite			Urea		
	L	ES	LS	L	ES	LS	L	ES	LS
14:0	19.23	14.44	10.27	18.51	12.70	12.50	19.93	10.25	9.80
15:0	0.26	0.27	0.32	0.21	0.22	0.33	0.29	0.24	0.34
16:0	14.94	12.58	18.62	13.86	12.58	18.81	17.74	12.17	18.00
16:1*n*−7	20.50	19.43	24.59	20.55	19.09	25.84	19.92	12.43	21.58
16:2*n*−7	0.41	0.33	0.22	0.40	0.34	0.20	0.32	0.41	0.28
16:2*n*−4	0.41	0.16	0.14	0.42	0.17	0.14	0.45	0.11	0.09
16:3*n*−6	tr[a]	0.16	0.10	–	–	0.40	tr[a]	0.11	0.14

FA	Nitrite			Nitrite			Urea		
	L	ES	LS	L	ES	LS	L	ES	LS
18:0	0.52	0.42	0.67	0.79	0.20	0.54	tr	0.15	0.52
16:3*n*−4	tr	tr	tr	tr	0.13	tr	tr	0.07	tr
18:1*n*−9	1.78	1.41	2.37	1.48	1.29	2.02	1.53	1.17	2.07
18:1*n*−7	1.76	1.68	3.88	1.99	2.11	4.08	1.79	1.17	2.66
18:2*n*−6	2.00	0.68	1.23	1.95	0.91	1.10	2.01	0.43	0.96
18:3*n*−6	0.54	0.33	0.41	0.96	0.26	1.18	0.61	0.20	0.35
20:0+18:3*n*−4	0.29	tr	0.22	–	–	0.73	0.37	–	–
18:3*n*−3	1.37	1.23	0.68	1.04	1.03	0.73	1.19	1.88	0.67
20:1*n*−9	tr	tr	0.25	1.24	0.13	0.27	tr	tr	0.15
18:4*n*−3	12.03	14.28	8.30	11.71	13.99	7.06	12.13	15.37	9.78
18:4*n*−1	0.51	0.89	0.38	0.43	0.16	0.38	tr	–	tr
20:2*n*−6	0.41	tr	0.39	tr	0.14	0.35	tr	0.34	0.15
20:3*n*−6	[b]–	–	0.34	–	–	0.19	[b]–	–	0.45
20:4*n*−6	tr	tr	0.31	1.04	0.28	0.33	tr	0.34	0.43
20:4*n*−3	tr	tr	–	tr	tr	tr	tr	tr	tr
20:5*n*−3	14.64	19.09	16.14	14.94	22.74	13.10	12.76	27.66	19.69
21:5*n*−3	2.23	1.49	0.51	0.65	0.98	1.15	1.65	0.95	0.64
22:5*n*−3	–	1.64	0.48	–	–	0.51	–	–	0.56
22:6*n*−3	5.64	7.85	8.26	6.30	10.14	5.85	5.14	14.13	9.94
Total (mg g^{-1} DW)	119.42	146.92	177.45	125.16	182.65	146.24	102.55	200.82	151.30
%(SFA+MUFA)	59.30	51.33	61.54	59.54	48.52	65.86	62.54	37.73	55.54
%PUFA	40.70	48.67	38.46	40.46	51.48	34.14	37.46	62.27	44.46
(SFA+MUFA)/PUFA	1.46	1.05	1.60	1.47	0.94	1.93	1.67	0.61	1.25
n−6/*n*−3	0.09	0.03	0.09	0.13	0.04	0.13	0.09	0.03	0.07

The values presented are means of three replicates. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. L: Logarithmic phase; ES: early stationary phase; LS: late stationary phase.

[a]tr: trace; [b]–: no detected.

In the three media assayed, maxima PUFA values were obtained at the early stationary phase and decreased throughout this phase. Conversely, SFA and MUFA subtotals decreased at the beginning of the stationary phase, presenting minima values at early stationary phase (Table 4). *Isochrysis* cells grown in nitrate or nitrite media showed very similar fatty acid patterns in any phase of growth, but slightly different from urea grown cells. *Isochrysis* cultured in urea media had the highest contents of saturated and monounsaturated fatty acids (SFAs+MUFAs) in the log phase. At the beginning of the stationary phase, these cells showed a sharp decline in these fatty acids and a strong increase in polyunsaturated fatty acids (PUFAs) (Table 4). Therefore, PUFAs reached 62.27% of total fatty acids in the early stationary phase of cells grown in urea, as opposed to 48.63% and 51.48%, obtained in nitrate and nitrite media, respectively, in the same phase (Table 4). The highest content of eicosapentaenoic acid (EPA) (20:5n−3) and docosahexaenoic acid (DHA) (22:6*n*−3) was obtained in urea cultures at the early stationary phase: EPA, 55.55 mg g^{-1} DW (27.66% of total fatty acids) and DHA, 28.37 mg g^{-1} DW (14.13%

of total fatty acids). Ratio of SFAs+MUFAs to PUFAs and that of *n*–6 PUFAs and *n*–3 PUFAs (*n* −3/*n*–6) declined at the beginning of the stationary phase and increased in later stages (Table 4).

4. Discussion

Nitrate, nitrite and urea were suitable N sources for growth of *I. galbana*, but the nitrogen source affected the biochemical composition of cells. Changes in cellular composition have also been reported for *Dunaliella tertiolecta* (Fábregas et al., 1989) and *P. tricornutum* (Fidalgo et al., 1995) cultured with different N sources. Biochemical composition was also affected by the growth phase, as has been reported previously, particularly with respect to their lipid components (Sukenik and Carmeli, 1990; Hodgson et al., 1991). Not only the gross composition, but also lipid classes and fatty acids were affected.

The increase in cellular contents of N and C throughout the growth phase (Table 2) can be explained in function of the increase in cellular dry weight (Table 1), but lower variations occur if N and C are considered as %DW, with values between 4% and 5% DW for N and 40–45% for C. The increase in the ratio C/N throughout the growth time is in accordance with the consumption of the available nitrogen of the culture media in a batch growth (Brown et al., 1993; Uriarte et al., 1993). Variations in N and C quotas and in C/N ratio due to N source have also been reported in other marine microalgae (Levasseur et al., 1993).

The increase in the C/N ratio indicates that the consumption of nitrogenous compounds can be the main cause of the relative decrease in the synthesis of protein and the increase in the synthesis of storage products observed throughout the growth cycle (Table 3).

Low protein and high carbohydrate contents were found in nitrate, nitrite and urea cultures at the stationary phase in *P. tricornutum* (Fidalgo et al., 1995). However, in *I. galbana* lipid appears as the main reserve product in all the nitrogen sources assayed. It has also been suggested that carotene/chlorophyll a ratio is inversely related to N status (Davidson et al., 1991); in *I. galbana* carotenoids/chlorophyll *a* ratio increased throughout the culture in the three nitrogen sources assayed (Table 3).

Lipids are important sources of energy for growing bivalve larvae (Webb and Chu, 1983; Gallager et al., 1986;Whyte et al., 1987). The relative amounts of each lipid class in microalgal cells can change considerably with variations in culture conditions (Yongmanitchai and Ward, 1989; reviewed by Roessler, 1990). Triacylglycerols (neutral lipids) are considered to be storage lipids for algae (Sukenik and Carmeli, 1990); the more polar lipid fractions are likely to be associated with the photosynthetic membranes of the algae. Triacylglycerols increased between exponential and stationary phases (Fig. 2).

The proportions of the two polar lipid fractions of the total lipids were generally highest in the logarithmic phase. In the early stationary phase, the proportion of galactolipids slightly declined, whereas there was a strong decrease in the phospholipids fraction (from 31–34% of total lipids to 10–12%) for all the nitrogen sources assayed. Similar results were found in three species of

marine microalgae (Dunstan et al., 1993): stationary phase cultures of all three species showed increased proportions (%) of triacylglycerols with correspondingly decreased proportions of polar lipids relative to logarithmic phase cultures. However, no significant differences were found in lipid fractionation among the nitrogen sources assayed.

Generally, accumulation of triacylglycerols has been correlated with the cessation of cellular division at the onset of stationary phase in aged cultures (Hodgson et al., 1991). The phenomenon appears to be a complex matter of the dynamics of cellular division, nitrogen metabolism, and photosynthetic fixation of carbon (Suen et al., 1987; Sukenik and Carmeli, 1990).

Some of the variations in the fatty acid composition of *I. galbana* (Table 4) caused by the growth phase can be related to the relative distribution of the major lipid classes in this alga. The C18:1 and C16:0 fatty acid percentage increased as the relative content of neutral lipids increased, mostly under severe nitrogen-limiting conditions as occurred in the late stationary phase. Similar results were found by Sukenik and Wahnon (1991).Urea media yielded higher contents of total fatty acids as mg per g of dry weight, at the beginning of the stationary phase. Amounts of PUFAs, as mg g^{-1} DW followed a similar pattern, representing the 62.5% of total fatty acids. Because the *n*−3 family of fatty acids are essential in the diets of many commercially important marine fish and shellfish (Sargent et al., 1989) it is in the interest of the aquaculturist to maximize the production of the *n−3* family of fatty acids in dietary algae used in culture of larvae of many marine species (Hodgson et al., 1991). Urea grown cells showed at the beginning of stationary phase the highest relative abundances of the *n*−3 PUFAs linolenic acid (18:3*n*−3), eicosapentaenoic acid and docosohexaenoic acid (about 2%, 28% and 14%, respectively). Therefore, urea could be a good nitrogen source to culture *I. galbana* to maximize PUFA production, and harvesting the biomass at specific growth phases may enable better yields in lipid and PUFA composition. Lower ratios SFA+MUFAs to PUFAs and *n*−6 to *n*−3 PUFAs were also obtained in urea cultures, followed by nitrite cultures. These ratios are considered of nutritional value in bivalve nutrition. Diets with lower values of SFA+MUFAs to PUFAs and ratios *n*−6:*n*−3<0.5 were optimal for larvae and juvenile oysters (Webb and Chu, 1983; Enright et al., 1986). Urea is one of the lowest cost nitrogen source for microalgal culture. Similar results have been obtained in *P. tricornutum* (Yongmanitchai and Ward, 1991; Fidalgo et al., 1995). These findings indicate that the opportunity exists to maximize PUFA production by microalgae with the potential to improve animal growth and reduce production costs in mariculture operations and may be of use in the large scale culture and harvesting of microalgae for the biotechnology industry (Dunstan et al., 1993).

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Corresponding author. Tel.: +34-81-167000; Fax: +34-81-167065; E-mail: herreroc@udc.es