ELSEVIER

Aquaculture 216 (2003) 223–242

Aquaculture

www.elsevier.com/locate/aqua-online

Optimisation of T-ISO biomass production rich in essential fatty acids II. Effect of different light regimes on the production of fatty acids

Ioannis Tzovenis [a,*], Niels De Pauw [b], Patrick Sorgeloos [a]

[a] *Laboratory of Aquaculture and "Artemia Reference Centre", Ghent University, Rozier 44, B-9000 Ghent, Belgium*

[b] *Department of Applied Ecology and Environmental Biology, Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, J. Plateaustraat 22, B-9000 Ghent, Belgium*

Received 29 November 2001; received in revised form 26 July 2002; accepted 26 July 2002

Abstract

It is well documented that culture conditions affect the fatty acid content of microalgae. We report in this study the fatty acid profiles and $n-3$ HUFA productivity of T-ISO, a popular haptophyte in the aquaculture industry, cultured under three photoperiods (24:0, 16:08 and 12:12 h L:D) combined with three photon flux densities (PFD: 120, 220 and 460 μmol photon m^{-2} s^{-1}), at 25 °C. Sampling took place in both the exponential and post-exponential (light-limited) phase.

In general, fatty acid proportions were effected by a strong interaction of L:D × PFD resulting in metabolic changes difficult to be modelled. At the 12:12 and 24:0 h L:D the fatty acid pattern can be summarised as PUFA>SAFA>MUFA, while at 16:08 h L:D as SAFA>PUFA>MUFA reflecting a differential acclimation of the strain under light–dark cycles. At the 12:12 h L:D the PUFA content of biomass was significantly higher than at the other photocycles. PUFA content differences were located in the $n-3$ fraction with the $n-6$ content being rather constant. The $n-3/n-6$ and DHA/EPA ratios under 24:0 h or 12:12 h L:D were optimal according to the literature for fish and shellfish nutrition requirements. In contrast, the 16:08 h L:D regimes, especially at low PFD, produced inadequate ratios.

The production of $n-3$ HUFA in T-ISO is essentially influenced by the total photon flux available per day in a similar manner with growth. The capacity of the strain for storing lipid is limited under the conditions tested; consequently, the fatty acid content follows the biomass yield

* Corresponding author. Present address: Department of Ecology and Systematics, Faculty of Biology, University of Athens, Panepistimioupolis, Zografou 15784, Greece. Tel.: +30-1-7274042; fax: +30-1-7274885.

E-mail address: itzoveni@eexi.gr (I. Tzovenis).

PII: S0044-8486(02)00375-7

and productivity pattern. Hence, in the context of aquaculture a light regime of 12:12 h L:D and a PFD within the photolimitation–photoinhibition range offers advantages for the culture of T-ISO. If the high investment could be substantiated, continuous cultures under 24:0 h L:D at the same PFD range could serve as an optimisation basis using advanced photobioreactors.

Keywords: T-ISO; Photoperiod; Fatty acids; Photon flux density

1. Introduction

Opting for best product at minimum cost is the principle that drives industrial applications. In the case of T-ISO (*Isochrysis* aff. *galbana* Green, Haptophyceae), a widely acclaimed strain in aquaculture (De Pauw and Persoone, 1988; Benemann, 1992; Gladue and Maxey, 1994; Borowitzka, 1997), the best product would be considered a biomass harvest with a desired lipid profile, either for direct use in aquaculture or for lipid extraction for the chemical industry. Such a profile should include essential fatty acids (Brown and Jeffrey, 1992; Sargent et al., 1995) as well as fatty acids that provide energy and carbon skeletons for biosynthesis to the aquacultured animals (Sargent et al., 1997).

The general fatty acid profile of T-ISO (e.g. Napolitano et al., 1990) is suitable because, besides DHA, 18:2$n-6$ and 18:3$n-3$, the high content of 22:5$n-6$ has been suggested as a potential source of 20:4$n-6$ to marine fish larvae (Sargent et al., 1997), and 18:5$n-3$ is a putative source of 20:5$n-3$ (Napolitano et al., 1990). The high content of 18:4$n-3$ (OTA) of no obvious metabolic significance for bivalve larvae (Napolitano et al., 1990) has anti-inflammatory action (Coupland et al., 1996). Furthermore, Thompson et al. (1993, 1996) have concluded that the sum of 14:0 and 16:0 in T-ISO is of major importance for providing energy to oyster larvae and this sum in T-ISO is quite high and constant under the examined culture conditions.

Nevertheless, the fatty acid profiles of microalgae are susceptible to major changes according to the prevailing culture conditions (Harrison et al., 1990). The light regime imposed to the culture is a fundamental environmental factor since it determines the yield and productivity of the biomass (Kirk, 1983; Raven, 1984; Falkowski et al., 1985; Dubinsky, 1986; Fogg and Thake, 1987; Falkowski and Raven, 1997) and there is much evidence that influences the fatty acid composition as well (Nichols, 1965; Constantopoulos and Bloch, 1967; Sicko-Goad et al., 1988; Sukenik et al., 1989; Picaud et al., 1991; Sanchez Saavedra and Voltolina, 1994; Harwood, 1996). Studies so far published about the influence of light on the fatty acids of T-ISO have focused on comparisons between different PFD under a given photoperiod [12:12 h L:D indoors and outdoors, Renaud et al. (1991); 24:0h L:D, Thompson et al. (1990); 24:0h L:D in a turbidostat, Sukenik and Wahnon (1991); 12:12h L:D Brown et al. (1993)]. Therefore, a direct comparison of different photoperiods and PFD seems necessary in order to decide on the most efficient light regime for industrial purposes (chemicals or aquaculture alike).

Growth results under a range of photocycles combined with a range of PFD showed that specific growth rate of T-ISO maximised with the increase of the total photon flux supplied per day (Tzovenis et al., 2003), while growth was saturated at 170 μmol photon $m^{-2} s^{-1}$ for all photocycles under the culture set-up used. The objective of this study was

Table 1
Fatty acid profile of T-ISO (% of total) cultured under different light regimes. All fatty acids can be expressed as mg g^{-1} or fg $cell^{-1}$ by multiplying the corresponding TFA value given at the bottom of the table

Regime	A1		a1		A2		a2		A3		a3	
L:D	24:0 h											
PFD	120 μmol photon m^2 s^{-1}				220 μmol photon m^2 s^{-1}				460 μmol photon m^2 s^{-1}			
GP	exp		p-exp		exp		p-exp		exp		p-exp	
ΣPFD	10.368 mol photon m^2 day^{-1}				18.998 mol photon m^2 day^{-1}				39.744 mol photon m^2 day^{-1}			
%	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
14:0	18.78	1.18	15.63	0.79	19.19	1.45	17.81	0.32	19.78	0.91	14.68	0.84
16:0	10.19	0.99	9.65	0.16	10.85	0.67	12.38	0.14	12.16	0.91	12.72	0.53
16:1$n-7$	2.47	0.18	4.36	0.18	2.75	0.55	3.95	0.02	2.67	0.29	3.56	0.14
18:0	1.53	1.07	2.15	0.60	1.18	0.77	0.27	0.00	2.21	1.00	0.38	0.12
18:1$n-9$	9.17	0.67	10.29	0.50	11.21	0.65	15.92	0.06	11.89	0.67	15.51	0.38
18:1$n-7$	1.37	0.21	1.22	0.18	1.85	0.30	1.64	0.09	2.05	0.58	1.81	0.20
18:2$n-6$	3.41	0.50	4.74	0.37	3.05	0.27	3.35	0.04	3.31	0.25	2.62	0.11
18:3$n-6$	0.54	0.12	0.52	0.04	0.44	0.18	0.30	0.01	0.46	0.23	0.13	0.06
18:3$n-3$	6.26	0.49	6.77	0.40	5.22	0.67	6.13	0.10	4.71	0.52	5.82	0.23
18:4$n-3$	19.47	0.79	15.46	1.14	16.33	1.37	17.05	0.10	14.21	1.31	17.22	0.43
18:5$n-3$	3.28	0.72	1.70	0.37	3.39	1.77	1.35	0.03	4.18	0.51	1.83	0.07
20:5$n-3$	0.75	0.05	0.63	0.02	0.75	0.08	0.91	0.03	0.78	0.23	0.68	0.39
22:5$n-6$	1.48	0.43	1.08	0.10	1.52	0.11	1.40	0.02	1.48	0.14	1.22	0.30
22:6$n-3$	12.96	0.91	7.87	0.70	13.78	1.00	10.98	0.11	13.52	1.01	13.49	0.43
SAFA	32.46	2.02	34.35	1.51	33.10	1.46	33.09	0.43	35.61	1.18	31.10	1.41
MUFA	15.13	0.51	19.41	0.48	18.55	0.91	23.11	0.17	18.33	1.40	22.09	0.49
PUFA	52.41	2.08	46.24	1.65	48.35	1.27	43.80	0.42	46.06	2.03	46.81	1.51
$n-3$ HUFA	13.99	0.83	10.14	0.85	14.93	1.18	12.08	0.13	14.72	1.07	15.25	1.19
$n-3$	43.18	1.65	34.29	1.78	39.88	1.64	36.65	0.33	37.85	2.01	40.16	1.00
$n-6$	7.09	0.64	7.31	0.49	6.60	0.50	6.06	0.10	7.14	0.65	5.13	0.40
TFA (mg g^{-1})	131.16	8.54	189.01	9.85	129.57	9.69	128.48	1.08	144.78	23.43	91.42	2.79
TFA (fg $cell^{-1}$)	1853.8	138.6	2563.0	133.6	1987.7	117.4	2342.3	19.7	2759.5	464.5	1980.0	60.4

Regime	B1		b1		B2		b2		B3		b3	
L:D	16:08											
PFD	120 μmol photon m^2 s^{-1}				220 μmol photon m^2 s^{-1}				460 μmol photon m^2 s^{-1}			
Phase	exp		p-exp		exp		p-exp		exp		p-exp	
ΣPFD	6.912 mol photon m^2 day^{-1}				12.672 mol photon m^2 day^{-1}				26.496 mol photon m^2 day^{-1}			
%	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
14:0	18.69	0.70	19.44	0.56	23.06	0.82	25.10	1.76	20.26	0.27	22.86	1.31
16:0	16.30	0.46	11.98	0.16	15.70	0.54	16.67	0.79	15.38	1.05	18.63	0.94
16:1$n-7$	7.46	1.50	1.61	0.09	1.71	0.09	1.95	0.15	2.31	0.05	2.51	0.14
18:0	1.59	0.31	1.70	0.51	1.24	0.35	1.00	0.24	1.40	0.16	0.86	0.19

(continued on next page)

Table 1 (*continued*)

Regime	B1		b1		B2		b2		B3		b3	
L:D	16:08											
PFD	120 μmol photon m^2 s^{-1}				220 μmol photon m^2 s^{-1}				460 μmol photon m^2 s^{-1}			
Phase	exp		p-exp		exp		p-exp		exp		p-exp	
ΣPFD	6.912 mol photon m^2 day^{-1}				12.672 mol photon m^2 day^{-1}				26.496 mol photon m^2 day^{-1}			
%	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
18:1$n-9$	11.74	1.03	10.92	0.84	20.76	2.04	20.19	4.44	20.88	1.79	25.48	1.61
18:1$n-7$	2.11	0.25	1.73	0.09	1.55	1.31	0.61	1.43	2.00	0.07	0.00	0.00
18:2$n-6$	5.66	0.43	6.42	0.16	7.64	0.46	6.11	0.41	7.67	0.04	3.90	0.37
18:3$n-6$	0.38	0.01	0.38	0.03	0.11	0.06	0.16	0.03	0.17	0.01	0.09	0.02
18:3$n-3$	6.80	0.11	8.44	0.46	5.02	0.51	5.93	0.99	4.36	0.46	3.85	0.18
18:4$n-3$	5.62	0.29	8.05	0.34	4.98	0.42	5.83	0.52	4.80	0.37	5.52	0.25
18:5$n-3$	3.22	0.28	5.64	0.30	3.01	0.31	2.73	0.31	3.04	0.38	2.35	0.20
20:5$n-3$	1.72	0.58	0.69	0.06	0.47	0.36	0.38	0.24	0.67	0.07	0.19	0.11
22:5$n-6$	2.27	0.22	3.19	0.10	2.26	0.18	2.43	0.31	2.57	0.23	2.41	0.39
22:6$n-3$	6.93	0.69	9.43	0.44	8.15	0.44	7.03	0.66	9.10	0.97	6.77	0.44
SAFA	40.05	0.72	37.38	0.38	41.60	0.99	44.98	2.12	38.88	0.84	44.78	1.32
MUFA	22.90	0.84	16.33	0.91	25.43	1.85	23.37	4.43	26.25	1.85	28.90	1.37
PUFA	37.04	0.72	46.29	1.05	32.97	1.18	31.64	2.57	34.87	2.61	26.32	0.47
$n-3$ HUFA	8.66	0.47	10.42	0.46	8.64	0.50	7.44	0.71	9.82	0.91	7.01	0.46
$n-3$	25.31	0.87	32.61	1.01	21.65	1.20	21.93	2.47	22.03	2.11	18.74	0.50
$n-6$	8.98	0.40	11.23	0.31	10.37	0.47	8.92	0.43	11.83	0.51	6.75	0.38
TFA (mg g^{-1})	130.88	6.64	154.70	4.84	150.26	8.61	140.19	9.15	162.47	22.31	160.32	7.49
TFA (fg $cell^{-1}$)	3153.2	230.9	3102.0	65.1	3193.7	262.0	2192.7	157.4	3441.2	523.8	3011.8	128.5

Regime	C1		c1		C2		c2		C3		c3	
L:D	12:12											
PFD	120 μmol photon m^2 s^{-1}				220 μmol photon m^2 s^{-1}				460 μmol photon m^2 s^{-1}			
Phase	exp		p-exp		exp		p-exp		exp		p-exp	
ΣPFD	5.184 mol photon m^2 day^{-1}				9.504 mol photon m^2 day^{-1}				19.872 mol photon m^2 day^{-1}			
%	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
14:0	17.92	1.43	16.21	0.98	18.68	1.33	18.00	0.74	19.01	1.66	15.04	0.55
16:0	9.71	0.63	8.21	0.13	9.49	1.04	10.49	0.35	9.68	0.88	10.15	0.53
16:1$n-7$	3.09	0.43	4.98	0.10	2.78	0.17	3.42	0.03	2.73	0.14	3.61	0.10
18:0	1.68	0.44	1.70	0.57	1.04	0.81	0.27	0.10	0.94	0.49	1.55	1.03
18:1$n-9$	7.00	0.55	7.50	0.20	10.53	0.44	15.73	0.15	10.84	0.65	10.99	0.59
18:1$n-7$	1.62	0.72	1.15	0.13	1.02	0.20	1.41	0.04	1.07	0.37	1.09	0.59
18:2$n-6$	2.96	0.10	6.16	0.43	3.56	0.29	3.53	0.04	3.03	0.25	3.33	0.09
18:3$n-6$	0.20	0.05	0.56	0.03	0.42	0.27	0.34	0.01	0.37	0.18	0.18	0.04
18:3$n-3$	6.67	0.84	9.78	0.53	6.46	0.24	7.00	0.07	5.63	0.28	9.50	0.14
18:4$n-3$	17.54	1.95	15.65	0.96	15.32	4.26	18.22	0.18	16.01	1.60	18.71	0.38
18:5$n-3$	7.29	1.10	3.63	0.27	8.07	0.22	3.91	0.25	8.27	0.76	6.25	0.08

Table 1 (*continued*)

Regime	C1		c1		C2		c2		C3		c3	
L:D	12:12											
PFD	120 μmol photon m^2 s^{-1}				220 μmol photon m^2 s^{-1}				460 μmol photon m^2 s^{-1}			
Phase	exp		p-exp		exp		p-exp		exp		p-exp	
ΣPFD	5.184 mol photon m^2 day^{-1}				9.504 mol photon m^2 day^{-1}				19.872 mol photon m^2 day^{-1}			
%	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
20:5$n-3$	1.04	0.33	0.56	0.02	0.85	0.30	0.74	0.02	0.87	0.24	0.41	0.04
22:5$n-6$	1.17	0.29	0.89	0.06	1.28	0.28	1.27	0.04	1.42	0.08	1.04	0.15
22:6$n-3$	11.74	1.67	7.80	0.63	12.67	0.48	9.95	0.13	14.14	1.20	12.02	0.24
SAFA	32.42	1.72	32.97	0.85	31.14	1.60	31.25	0.28	30.68	1.88	29.92	0.80
MUFA	14.76	2.99	16.52	0.45	16.54	1.04	22.01	0.21	16.77	1.07	16.77	1.46
PUFA	52.82	4.65	50.51	1.23	52.32	2.13	46.74	0.20	52.55	2.69	53.32	0.84
$n-3$ HUFA	13.59	1.16	9.95	0.29	13.79	0.61	10.77	0.12	15.39	0.94	12.46	0.27
$n-3$	45.16	4.80	39.20	1.82	43.67	4.14	39.93	0.16	45.35	3.18	46.92	0.80
$n-6$	5.59	0.22	8.32	0.46	6.58	0.69	6.02	0.07	6.40	0.45	5.46	0.12
TFA (mg g^{-1})	151.56	32.40	175.68	20.41	148.34	15.18	150.79	8.23	155.25	11.55	147.40	3.42
TFA (fg $cell^{-1}$)	3437.1	806.1	2698.5	313.6	3056.8	215.4	2331.3	127.3	3136.0	64.9	2837.5	65.9

L:D, light:dark cycle; PFD: photon flux density; GP: growth phase; ΣPFD: total photon flux density available per day.

to establish light regimes that favour the production of essential fatty acids and in particular the $n-3$ highly unsaturated fatty acids (HUFA) in T-ISO.

2. Materials and methods

The Haptophyte *Isochrysis* aff. *galbana* Green, "ahitian strain" (T-ISO), was obtained from the Tinamenor hatchery in Spain, and stocked as a monospecific culture in the Artemia Reference Center (ARC) in Belgium. The experimental design was a 3^2 factorial of photon flux density (PFD), photoperiod (L:D) plus growth phase (GP) at two levels (exponential and post-exponential). As L:D, 24:0, 12:12 and 16:08 h L:D were used at three PFD levels, namely, 120, 220, and 460 μmol photon m^{-2} s^{-1}. Triplicate monospecific batch cultures were set-up in round flasks with Walne medium (Walne, 1966). Detailed description of culture set-up and conditions can be found in Tzovenis et al. (2003). Sampling took place at the end of the exponential phase of well-acclimated cultures, representing the nonlimited growth, and at the end of the post-exponential phase (~ 50–70% drop of the exponential specific growth rate μ, due to light limitation caused by increased cell density). The latter was used to simulate the harvesting point, typically employed in hatcheries and still avoid severe nutrient limitation. Adding nutrients at this point tested the nutrient limitation and if an increase of μ was found, the particular experiment was aborted. The following codes were used for the light regimes (see Table 1): 'A'=24:0 h L:D; 'B'=16:08 h L:D; 'C'=12:12 h L:D; '1'=120 μmol photon m^{-2} s^{-1}; '2'=220 μmol photon m^{-2} s^{-1};

'3'= 460 μmol photon m^{-2} s^{-1}. Caps denote exponential, and lower case letters the corresponding post-exponential phase (e.g. 'A1', 'a1').

Samples for lipid analysis were collected on pre-combusted GF/C filters (450 °C) and stored at −40 °C, under N_2 until processing. A modified method of Lepage and Roy (1986) was used for the analysis of the fatty acids. Details for analysis can be found in Tzovenis et al. (1997). Fatty acids normalised per total offer a fingerprint of taxonomic importance (Mourente et al., 1990), therefore fatty acids were expressed as percentage of the total identified ones (TFA). The nonidentified did not exceed 5% of the total. Biomass and cellular content can be calculated from TFA in mg g^{-1} and fg $cell^{-1}$, respectively. The $n-3$ μUFA yields and productivities were obtained via the corresponding culture kinetic data (Tzovenis et al., 2003).

Statistical analysis was carried out using multivariate analysis of variance (MANOVA) for all responses of fatty acids, in order to provide a means of discrimination among the 18

Table 2
Statistic analysis of the fatty acid responses of T-ISO grown under different light regimes

ANOVA for TFA
DESIGN: three-way ANOVA, fixed and random effects
DEPENDENT: one variable: TFA mg g^{-1}
BETWEEN: 1—L:D (3): 12:12, 16:08, 24:0 h; 2—PFD (3): 120, 220, 460 μmol photon m^{-2} s^{-1}; 3—GP (3): exponential, post-exponential phase
WITHIN: none
RANDOM: GP

Summary of all effects

Effect	*df* Effect	MS Effect	*df* Error	MS Error	*F*	*P*-level
L:D	2 *	4850.15 *	2 *	78.82 *	61.53769 *	0.015990 *
PFD	2	3282.21 *	2	11579.94	0.28344	0.779156
GP	1	570.55	191	243.97	2.33862	0.127856
L:D × PFD	4	4861.83 *	4	2907.46	1.67219	0.315317
L:D × GP	2	78.82	191	243.97	0.32306	0.724326
PFD × GP	2 *	11579.94 *	191 *	243.97 *	47.46512 *	0.000000 *
L:D × PFD × GP	4 *	2907.46 *	191 *	243.97 *	11.91742 *	0.000000 *

MANOVA for exponential fatty acid profiles
DESIGN: two-way MANOVA, fixed effects (exponential phase)
DEPENDENT: 11 variables: % 14:0, % 16:0, % 16:1$n-7$, % 18:0, % 18:1$n-9$, % 18:2$n-6$, % 18:3$n-3$, % 18:4$n-3$, % 18:5$n-3$, % 22:5$n-6$, % 22:6$n-3$
BETWEEN: 1—L:D (3): 12:12, 16:08, 24:0 h; 2—PFD (3): 120, 220, 460 μmol photon m^{-2} s^{-1}
WITHIN: none

Summary of all effects (type III SS)

Effect	Wilks' Lambda	Rao's *R*	*df* 1	*df* 2	*P*-level
L:D	0.000923 *	351.0940 *	22 *	242 *	0.00 *
PFD	0.042382 *	42.4322 *	22 *	242 *	0.00 *
L:D × PFD	0.027666 *	16.4205 *	44 *	464 *	0.00 *

L:D, photoperiod; PFD, photon flux density; GP, growth phase.
* Significant value.

different light regimes (9 exponential and 9 post-exponential). To isolate the effects for each response (univariate analysis), the model was reduced to three-way analysis of variance (ANOVA). For the homogeneity of variance assumption, when necessary, variances were transformed to their logarithms (square root for percentages), and tested for heteroscedasticity with Cochran's C-test. Tuckey's honest significance distance (HSD, modified for unequal number of replicates as the Spjotwol and Stoline test) was used to compare and rank the means of fatty acids resulting under each regime. For comparison of the fatty acid profiles resulting from each light regime discriminant analysis and subsequently cluster analysis were curried out. The latter based on the similarities (single linkage method on Euclidean distances) was used to plot tree diagrams. Fatty acids expressed as % to TFA were used for clustering. All statistics were performed with STATISTICA® software (StatSoft, 1994 and references therein).

3. Results

3.1. Acclimated response (exponential phase)

During exponential phase TFA averaged 144.3 ± 20.5 mg g^{-1} (Table 1) and were influenced strongly by L:D with lesser influence of PFD and their interaction L:D × PFD (Table 2). MANOVA for the fatty acid profile of T-ISO [% TFA (w/w); Table 1] showed the same pattern (Table 2). Discriminant analysis revealed that all exponential culture

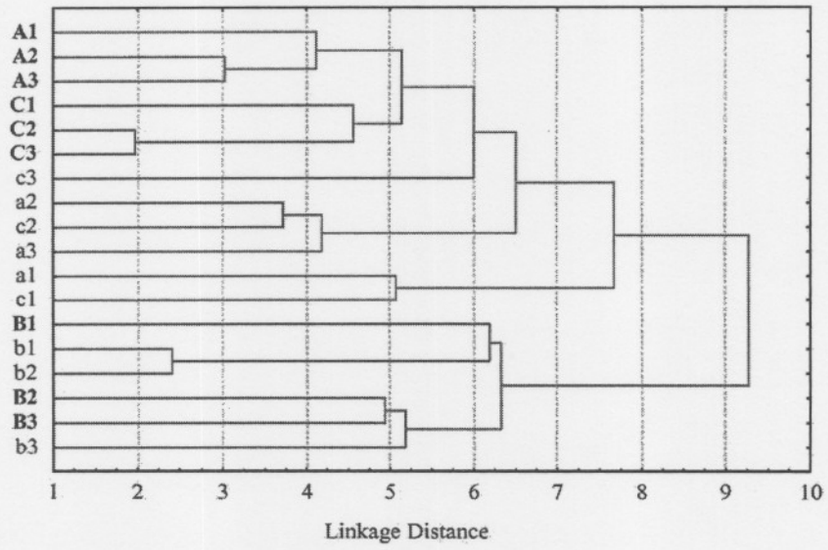

Fig. 1. Tree diagram for 18 light regimes, based on relative content of the following fatty acids: 14:0, 16:0, 16:1$n-7$, 18:0, 18:1$n-9$, 18:1$n-7$, 18:2$n-6$, 18:3$n-6$, 18:3$n-3$, 18:4$n-3$, 18:5$n-3$, 20:5$n-3$, 22:5$n-6$, 22:6$n-3$ (data from Table 1). Method: single linkage upon squared Euclidean distances. Codes as in Table 1.

regimes were different and demonstrated the strong contribution to this variability of all major fatty acids. In Fig. 1, a tree diagram from cluster analysis illustrates the differences and groups the culture regimes according to their similarities. Groups were discriminated according to L:D, and within each L:D by PFD. Apparently the 16:08 h L:D regimes were the most different ones.

To find a trend for the different fatty acid profiles the unsaturation degree [(PUFA + MUFA)/SAFA] was plotted for each regime (Fig. 2a). Although no common trend was found, it seems that the 'B' regimes were not optimal for high desaturation, while 'A' and

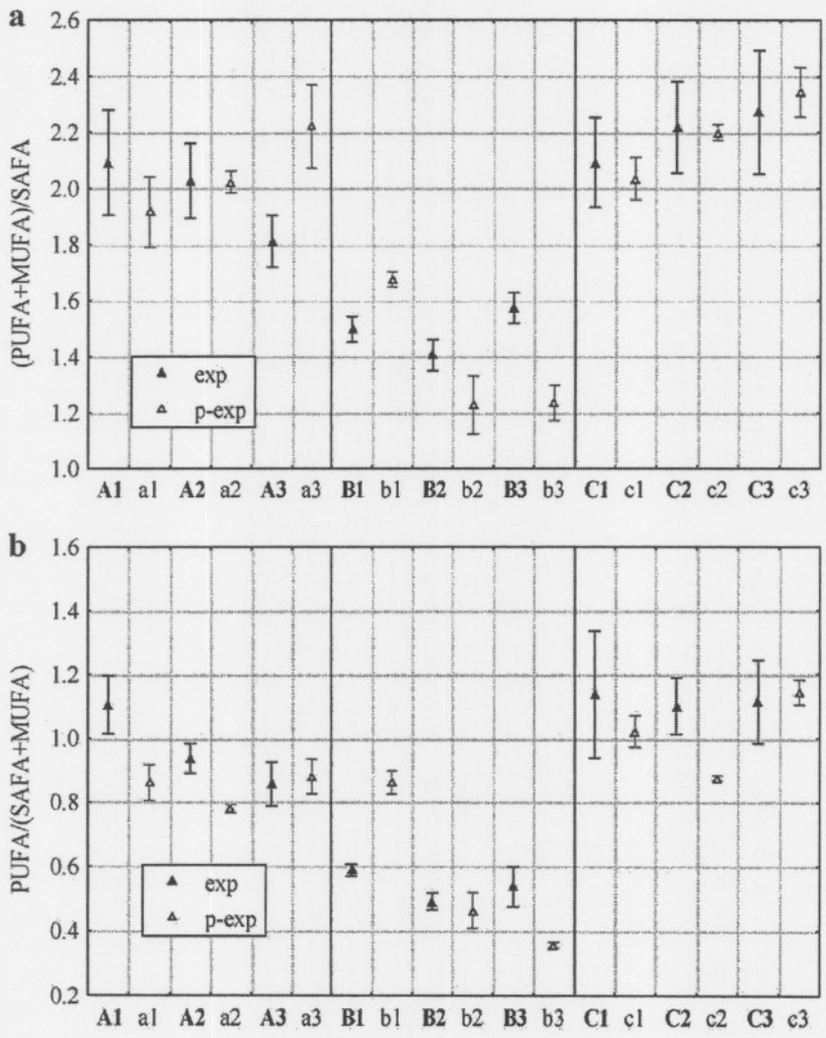

Fig. 2. Degree of unsaturation [(PUFA + MUFA)/SAFA] (a), and the index [PUFA/(SAFA + MUFA)] (b) of T-ISO cells cultured under different light regimes (mean ± standard deviation). Codes as in Table 1.

'C' regimes' difference was, in this sense, smaller, rendering both optimal for PUFA accumulation. This was confirmed by plotting the [PUFA/(SAFA + MUFA)] ratio, grossly representing in T-ISO the ratio of fatty acid elongation/desaturation to the de novo pathway. Detailed comparison of the two optimal regimes revealed some different trends imposed by L:D × PFD interactions. Increasing PFD resulted in a proportional increase of 16:0 with a concomitant decrease of 18:4$n-3$ and 18:3$n-3$ in 'A', whereas SAFA and 18:4$n-3$ were constant in 'C'. In 'A' DHA was PFD-independent whereas in 'C' DHA increased with increasing PFD reaching at 'C3' equivalent levels with the average in 'A'. In contrast, 18:3$n-3$ was decreasing with increasing PFD in 'C'. That trend for DHA and 18:3$n-3$ was inherent to the discontinuous light since it was evident in both 'B' and 'C'

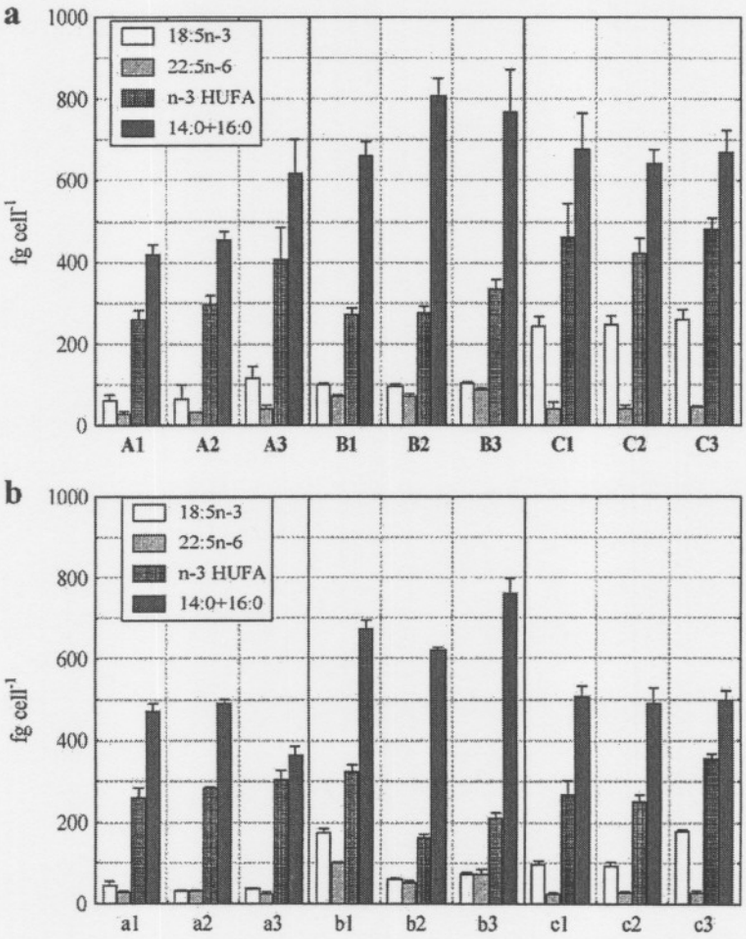

Fig. 3. Selective fatty acid profiles of T-ISO plotted vs. light regimes (mean ± standard deviation). (a): $n-3$ HUFA, 22:5$n-6$, 18:5$n-3$, and the sum 14:0 + 16:0 per cell (fg cell $^{-1}$) exponential phase; (b): post-exponential phase; (c): $n-3/n-6$ ratio; (d): DHA/EPA ratio. Codes as in Table 1.

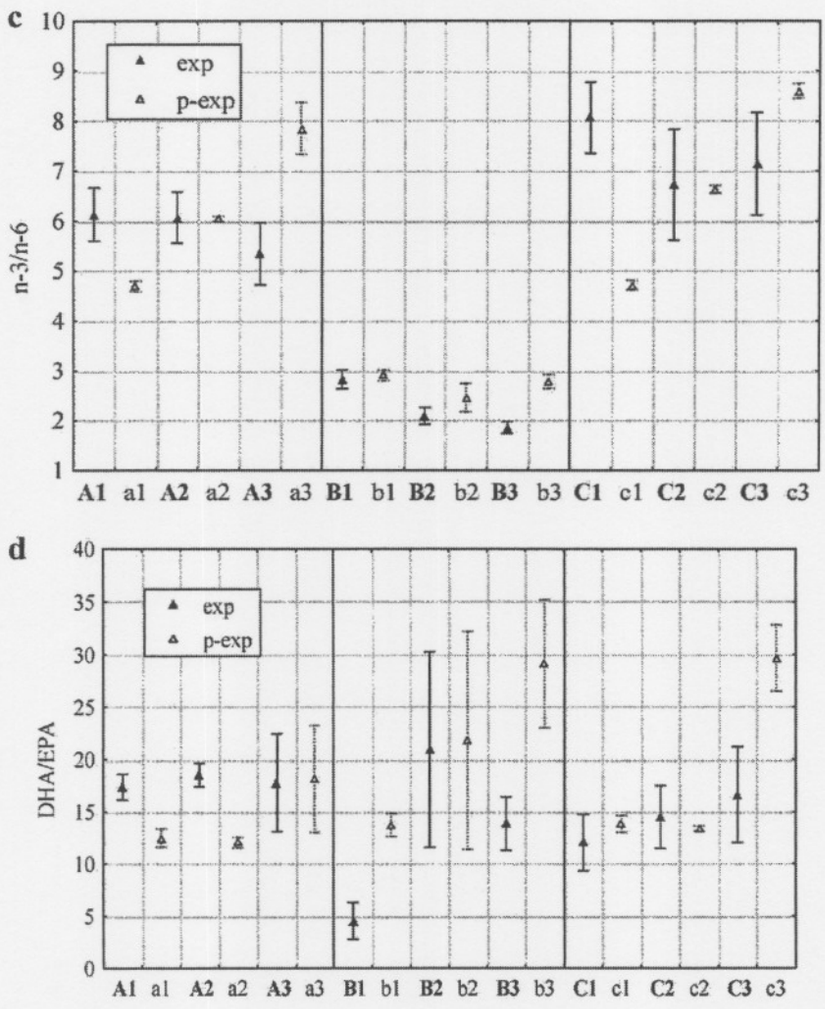

Fig. 3 (*continued*).

(DHA levels lower in 'B'). The levels of 18:5$n-3$ in 'C' (high and PFD independent) contrasted also with 'A'. On the other hand, the proportions of 18:2$n-6$, 18:1$n-9$, and 22:5$n-6$ were very high in 'B' with 18:4$n-3$ very low.

3.2. Transient response (light-limited post-exponential phase)

In the post-exponential phase under continuous light TFA exhibited a high negative correlation to PFD (Table 1). This outcome influenced the proportions, so that unsaturation (Fig. 2a) tended to increase with PFD although the PUFA/de novo index did not (Fig. 2b). Few trends were apparent with an overall pattern PUFA>SAFA>MUFA for most 'A', 'a',

'C', and 'c' regimes contrasting most 'B' and 'b' regimes' SAFA>PUFA>MUFA. Post-exponential proportions of MUFA in 'a' regimes increased at the slight expense of both SAFA and PUFA. In 'c' regimes only a MUFA increase at 'c2' at the expense of PUFA was noted. Under 16:08 h L:D the trends were far more complicated. During exponential phase there was no strong effect of PFD (MUFA increased slightly, though statistically significant, at the expense of PUFA in B2 and remain as such in B3), while during post-

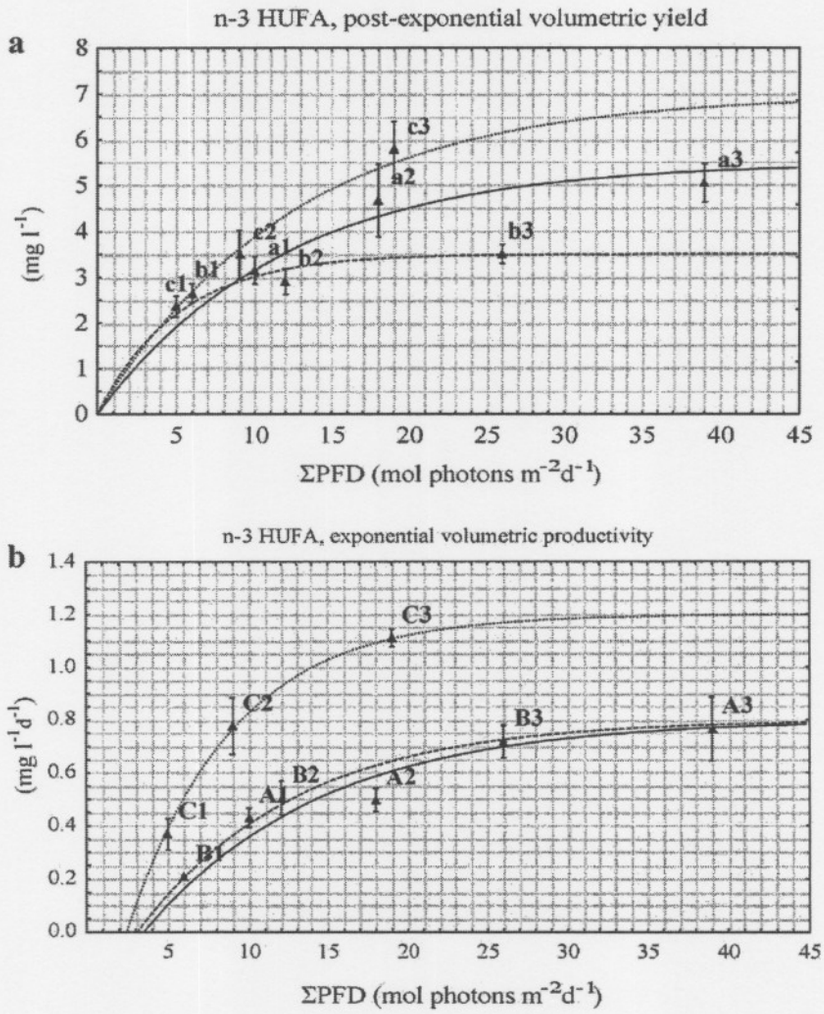

Fig. 4. $n-3$ HUFA volumetric yield and productivity of T-ISO cells cultured under different light regimes plotted vs. total photon flux density available per day (ΣPFD, mol photon m^{-2} day^{-1}). (a) $n-3$ HUFA exponential productivity (mg l^{-1} day^{-1}); (b) post-exponential yield $n-3$ HUFA per culture time (mg l^{-1} day^{-1}); (c) $n-3$ HUFA post-exponential yield (mg l^{-1}). Data fitted according to $y=y_{max}$ $\{1-\exp(-\alpha\Sigma PFD/y_{max})\}$ for each photoperiod. Points are mean ± standard deviation. Codes as in Table 1.

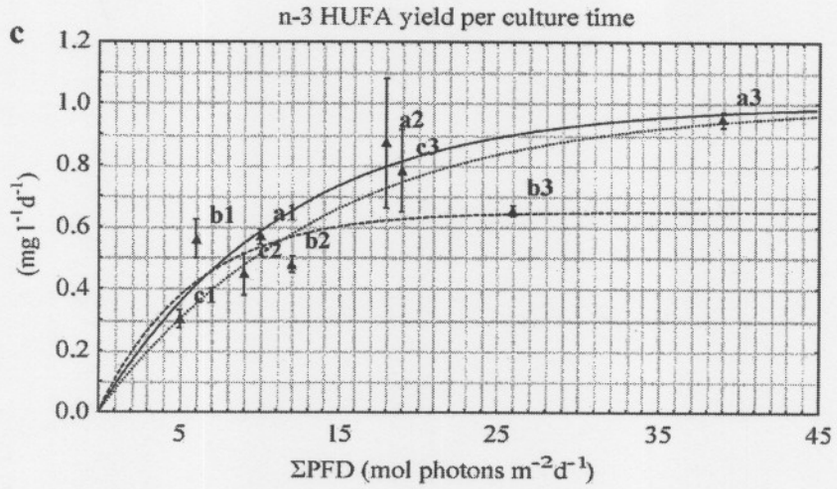

Fig. 4 (*continued*).

exponential phase the proportions of SAFA and MUFA increased with PFD at the expense of PUFA. However, comparing the fatty acid groups within each PFD, at 'b1' PUFA increased at the expense of both MUFA and SAFA, at 'b2' SAFA increased at the expense of MUFA and only at 'b3' PUFA dropped in favour of both SAFA and MUFA. Only 18:3$n-3$ (%) showed a common trend during the post-exponential phase increasing the exponential values. 14:0 and 18:5$n-3$ decreased in 'a' and 'c', while 16:1$n-7$ increased. The proportions of these fatty acids in 'b' were apparently unchanged. DHA compared to the exponential levels dropped in 'a' and 'c' with the remarkable exception of the 'a3' levels that remained unchanged, while in 'b' regimes DHA dictated their PUFA pattern described before. As a conclusion proportions were effected by a strong interaction of L:D × PFD resulting in metabolic changes difficult to be modelled.

3.3. Cell and biomass profiles

For cell rations in aquaculture it is important to know the specific profile per cell. Therefore, fatty acids important for industry were plotted for exponentially (Fig. 3a) and post-exponentially (Fig. 3b) harvested cells: 14:0+16:0 for high energy content, $n-3$ HUFA for DHA and EPA content, 18:5$n-3$ for EPA, and DPA for ARA. Evidently, 'C' cells had the most interesting profile since they contained the highest amounts of the different PUFA as well as a high content of high energy fatty acids. The $n-3$ content was significantly high in 'A' and 'C' (Fig. 3c), while DHA/EPA (Fig. 3d) did not differ significantly in any regime.

3.4. Productivity and yield of n−3 HUFA

The ultimate goal of this study was to define suitable regimes for production of T-ISO biomass rich $n-3$ HUFA. Evidently $n-3$ HUFA yield and productivity were a function

of ΣPFD per day (Fig. 4). Surprisingly, exponential volumetric productivities under 12:12 h L:D deviate to higher values with 'C3' almost double than the other regimes.

4. Discussion

4.1. Effect of light regimes on total fatty acids (TFA)

At conditions close to the ones adopted in this study there have been reported various values for TFA in T-ISO ranging from 31.5 to 148.2 mg g^{-1} (Brown et al., 1993; Burgess et al., 1993; Mourente, 1993; Albentosa et al., 1994; Reitan et al., 1993; Renaud et al., 1994a,b). Values reported on TFA per cell seem to be in better accord (Volkman et al., 1989; Brown et al., 1993; Renaud and Parry, 1994; Saoudi-Helis, 1994). In general, TFA in T-ISO seem to be about half of the lipid content (e.g. Brown et al., 1993; Dunstan et al., 1993; Albentosa et al., 1994) with the fraction of lipids per ash free CDW ranging from 20% to 30%. Consequently, the lipid variation in T-ISO is not high and the variation of TFA in mg g^{-1} can be explained by the variability of CDW, particularly of its nonorganic part (e.g. Brown et al., 1993).

During the exponential phase T-ISO showed a trend for more fatty acids at discontinuous light. Probably there is less demand for photosynthetic membranes under continuous light than under discontinuous light. The effect of PFD during the exponential phase was not significant. Fernandez-Sevilla et al. (1998), working with *I. galbana* under a range of high irradiance, concluded that the fatty acid content was not significantly affected by PFD but by the state of growth of the alga, depending on whether the light regime was photoinhibiting or photolimiting. Brown et al. (1993) demonstrated that in exponentially growing T-ISO there was a high level of polar lipids (ca. 75%), practically not changing with PFD in contrast to alkenones and an unidentified part. Hence, it seems that T-ISO at the exponential phase synthesise more or less a basal level of fatty acids needed for the membranes of the fast growing cell.

During the light-limited post-exponential phase under subsaturating PFD (120 μmol photon m^{-2} s^{-1}) fatty acids accumulated in biomass, whereas over this PFD evidently there were no significant changes (except for the TFA drop at 'a3'). Hence, deviations should be attributed to short-term changes within the fatty acid profile. At photolimiting conditions there might have also been fundamental metabolic changes towards esters and alkenones that accumulate at high PFD (Brown et al., 1993), thus decreasing TFA under 'a3'. These unsaturated compounds are nondegradable end products of fatty acid regulation under slow growth or stress conditions (Ben-Amotz et al., 1985; Suen et al., 1987) and have been detected in T-ISO as C_{37}–C_{39} unsaturated methyl and ethyl ketones (Volkman et al., 1989).

4.2. Fatty acid profiles

The PUFA>SAFA>MUFA pattern observed under 24:0 h or 12:12 h L:D seems typical for T-ISO growing under such regimes (Ben-Amotz et al., 1985; Volkman et al., 1989; Mourente et al., 1990; Napolitano et al., 1990; Thompson et al., 1990, 1993; Renaud et al.,

1991, 1994a,b, 1995; Brown et al., 1993; Delaunay et al., 1993; Dunstan et al., 1993; Reitan et al., 1993; Servel et al., 1994). Deviations from the typical pattern were found under extreme conditions of temperature [in Thompson et al. (1990, 1992) but not in Napolitano et al. (1990) or Renaud et al. (1995)] subsaturating PFD (Sukenik and Wahnon, 1991) and under nutrient- or photolimitation (Sukenik and Wahnon, 1991; Saoudi-Helis et al., 1994; Reitan et al., 1994). These effects could be attributed to an impediment of desaturation due to energy or nutrient-limitation resulting in SAFA and/or MUFA accumulation.

The SAFA>PUFA>MUFA pattern of fatty acids exhibited under 16:08 h L:D in the present study was similar only with the study of Albentosa et al. (1994), but the culture conditions were different (24:0 h L:D × ~ 180 μmol photons $m^{-2} s^{-1}$ × 18 °C). In fact, the PUFA content per cell under 16:08 h L:D was the same as with continuously illuminated cells but with a higher accumulation of SAFA and MUFA. Cells under 12:12 h L:D in comparison to 24:0 h L:D simply accumulated more PUFA. The major difference in the 16:08 h L:D fatty acid profile was the lower $n-3$ PUFA content while the $n-6$ fatty acids along with SAFA and $18{:}1n-9$ were much higher than in the other two photocycles. Since this regime was not suboptimal for growth (in terms of μ, post-exponential harvest and productivity) evidently high $n-3$ PUFA levels simply were not needed.

Algae respond to suboptimal growth conditions by optimising the photosynthetic process via thylakoid resizing and/or Calvin–Benson cycle up-regulation (Falkowski and Raven, 1997). Extra-plastid membranes involved in key metabolic pathways may get modified as well (Thompson, 1996). In both cases, the constant presence of certain fatty acids in the membranes facilitates an immediate acclimation (Harwood, 1996; Klyachko-Gurvich et al., 1999) and their constant storage in triacylglycerols (TAG) supports quick turnover and supply (Hodgson et al., 1991). The fatty acid profile of T-ISO conforms very well to this hypothesis with the presence of a high amount of PUFA, both in membranes and in storage lipids (Sukenik and Wahnon, 1991; Saoudi-Helis, 1994).

The thylakoid lipids in T-ISO contain mainly 14:0, 16:0, $18{:}1n-9$, $18{:}3n-3$, $18{:}4n-3$, $18{:}5n-3$ and $22{:}6n-3$, which are present also in high amounts in storage lipids (Sukenik and Wahnon, 1991; Saoudi-Helis, 1994). At subsaturating and light-limiting conditions these fatty acids accumulate indicating a thylakoid expansion, a response reversed at PFD equal to or higher than the saturating one. The only fatty acid that clearly correlates negatively to ΣPFD is $18{:}3n-3$, which accumulates under light limitation at subsaturating PFD along with $16{:}1n-7$. At oversaturating PFD $18{:}3n-3$ did not change while $18{:}3n-6$ and $18{:}5n-3$ decreased. Probably, their thylakoid contents were masked by a mobilisation of reserves from TAG to the elongation/desaturation process towards $22{:}5n-6$ and $22{:}6n-3$. In phospholipids $18{:}3n-6$ was not detected so its fluctuation can be attributed to changes in the photomembranes.

DHA, a fatty acid with an ambiguous role (see Kyle et al., 1992), increased with increasing PFD (constant in 'A') and decreased during the post-exponential phase. Considering its high content and omnipresent positioning (optimally ca. 15% in glycolipids, 35% in phospholipids and 50% in TAG; Saoudi-Helis, 1994) along with the fact that it represents the end product of the PUFA pathway, DHA should play a central role in the membrane physiology of T-ISO. Therefore, it must be kept at high levels in the

membranes, and its decrease in situations that limit its synthesis should be associated with mobilisation of its TAG reserves. Because it correlates positively with the available light energy it seems reasonable to assume that at energetically favourable regimes DHA accumulates in TAG.

If the 16:08 h L:D regime could not be considered suboptimal for growth, then an idea of the basal levels of $n-3$ PUFA could be obtained and changes be explored. All photosynthesis-associated $n-3$ fatty acids maximised at 12:12 h L:D indicating an expansion of the photomembranes associated with an increase in cell volume (Tzovenis et al., 2003) to relieve the packaging (Raven, 1986). Under 24:0 h L:D these fatty acids have intermediate contents. If one abides with the assumption that without dark intervals there is a higher degree of photoinhibition (Marshall et al., 2000), then probably a larger thylakoid surface would be required to host a reasonable number of functioning reaction centres. DHA on the other hand increased both under 12:12 h L:D and 24:0 h L:D, thus proving its critical role as the end product of the $n-3$ pathway under photosynthesis stressing conditions.

4.3. N–3 HUFA yield and productivity: significance for aquaculture and industry

Results showed that $n-3$ HUFA under any light regime complies well with the general requirements of the aquacultured animals. Diets for bivalves should, in general, contain about 1–20 fg/μm^3 $n-3$ HUFA (Brown et al., 1989), which in T-ISO ranged from 7 to 13 fg/μm^3 with a maximum at 12:12 h L:D. Crustaceans require at least 1% in the diet and should be satisfied as well. Fish requirements (e.g. 5.1 mg/g diet for sea bream larvae, Koven et al., 1990; 5.7 mg for stripped sea bass, Tuncer and Harrell, 1992) were within range in T-ISO; however, for most fish $n-3$ HUFA could be efficiently administered only through zooplankter intermediates, of which rotifers and *Artemia* exhibit problems in lipid retention (e.g. Whyte et al., 1994; Zhukova et al., 1998).

To make complete the profile of desired fatty acids the DHA/EPA and $n-3/n-6$ ratios should be considered as well. In many cases, EPA was proven either insufficient in comparison to DHA (e.g. Delaunay et al., 1993) or even suppressive (e.g. Thompson et al., 1996). Therefore, Sargent et al. (1997) suggest a ratio of DHA/EPA well over unity in the diets for marine fish larvae. Both continuous light and 12:12 h L:D grown T-ISO represent excellent sources of DHA with very high ratios of DHA/EPA. At low PFD and 16:08 h L:D the ratio is lowest and hence this regime should be avoided. The high fluctuation in some cases was due to the very low EPA content prone to errors in the final quantification. Webb and Chu (1983) suggested that a $n-3/n-6$ ratio lower than 2 is suboptimal. However, such a ratio without further information on the length of the carbon chains seems insufficient (Volkman, 1989). Napolitano et al. (1990) in a test with oyster and scallop larvae fed with T-ISO commented on the absence of C_{20} NMID (nonmethylene interrupted) fatty acids which serves as an index of poor PUFA metabolism in animals, thus rendering T-ISO an effective fatty acid source for aquaculture. Fernández-Reiriz et al. (1998) reported a positive correlation between $n-3/n-6$ and growth of *Ruditapes decussatus* with best results among others observed with a monospecific diet of T-ISO with a ratio of 3.4, less than half of the corresponding maxima obtained in the present study. The $n-3/n-6$ ratio in T-ISO should be more than sufficient under 24:0 or 12:12 h

L:D but might be inadequate under 16:08 h L:D, although the adequate $n-3$ HUFA contents should compensate for that.

In brief, cells harvested at the end of the exponential phase from cultures under discontinuous light of 12:12 h L:D and any PFD between photoinhibition and photolimitation are the most compliant to the desired profile since they have the highest $n-3$ HUFA together with the highest 18:5$n-3$ contents and have excellent DHA/EPA and $n-3/n-6$ ratios. Alternatively, if a high sum of 14:0+16:0 together with 22:5$n-6$ (targeting 22:4$n-6$) is desired, then 16:08 h L:D regimes should be ideal as they contain also an adequate amount of $n-3$ HUFA. These regimes can be applied outdoors in hatcheries located from the temperate zone to the tropics, rendering the production process more economic.

In volumetric production terms, the post-exponential harvests of T-ISO gave maxima of $n-3$ HUFA volumetric yields between 5 and 6 mg l^{-1} (max DHA yield 5.8 mg l^{-1} at 'c3') and were influenced by the total amount of energy given to the culture per day. Any light regime supplying more than *ca.* 15 mol photon m^{-2} day^{-1} (i.e. 'a2', 'c3', 'b3', 'a3') seems to be optimal for a maximal $n-3$ HUFA volumetric yield. These values are comparable to the 6.95 mg l^{-1} obtained by Chen et al. (1997) with *I. galbana* TK2 (a strain close to T-ISO) under 24:0 h L:D × ~ 200 μmol photon m^{-2} s^{-1} × 25 °C × urea medium. In contrast, Dunstan et al. (1993) reported for T-ISO a rather low value of 1.48 mg l^{-1} DHA in the early stationary phase of a culture under 12:12 h L:D × 100 μmol photon m^{-2} s^{-1} × 22 °C, probably due to the low cell concentration achieved in their system (7.25×10^6 cells ml^{-1}). The latter represents a major problem for microalgae production in aquaculture units because the 'Millford' method usually employed (De Pauw and Persoone, 1988; Benemann, 1992) rarely leads to high cell concentrations contrary to novel culture systems based on sophisticated photobioreactors capable of productivities over 1 g biomass l^{-1} day^{-1} for T-ISO (Borowitzka, 1997).

In this study, maximal volumetric productivities of $n-3$ HUFA for post-exponential harvests ranged between 0.8 and 1.0 mg l^{-1} day^{-1} comparable to the values of Dunstan et al. (1993) for semi-continuous culture (0.84 to 0.77). Burgess et al. (1993) reported a value of 4.3 mg l^{-1} day^{-1} obtained with an optical fiber photobioreactor which manifests the possibilities of such advanced technology systems. Nevertheless, such systems require high investment substantiated only for production of high added value products. The production of purified lipids or high-pressure lipid extracts falls within this view and there is an emerging interest in the development of such products from microalgae and other microorganisms for various applications, ranging from the baby food industry to cosmetics (Yongmanitchai and Ward, 1989; Kyle and Ratledge, 1992; Boswell et al., 1996; Burns et al., 1999).

Finally, the calculated volumetric productivity of $n-3$ HUFA at exponential harvests can be used for extrapolations of continuous culture because it gives an insight of instantaneous rates for a given regime, although, as steady-state biomass cannot be calculated by batch culture data alone, continuous culture experiments cannot be avoided. The exponential productivity pattern was similar to the post-exponential except for 12:12 h L:D where values were much higher (at 460 μmol photon m^{-2} s^{-1}: *ca.* 1.15 mg $n-3$ HUFA mg l^{-1} day^{-1}). If this observation is confirmed by further studies 12:12 h L:D regimes can then serve as a basis for a continuous culture optimisation. This regime

represents an economical solution considering the high cost of artificial illumination in the hatcheries. Extrapolating from the post-exponential productivity (yield over time), which can simulate continuous culture at low dilutions (achieving higher biomass), 12:12 h L:D × 460 μmol photon m^{-2} s^{-1} × p-exp regime seems the best choice for optimisation.

Acknowledgements

Ioannis Tzovenis is a scholar of the Empeirikion Foundation's fellowship program. This study has been supported by the Belgian National Science Foundation (FKFO project) and the Belgian Ministry of Science Policy (GOA project).

References

Albentosa, M., Labarta, U., Pérez-Camacho, A., Fernández-Reiriz, M.J., Beiras, R., 1994. Fatty acid composition of *Venerupis pullastra* spat fed on different microalgae diets. Comp. Biochem. Physiol. 108A, 639–648.

Ben-Amotz, A., Tornabene, T.G., Thomas, W.H., 1985. Chemical profile of selected species of microalgae with emphasis on lipids. J. Phycol. 21, 72–81.

Benemann, J., 1992. Microalgae aquaculture feeds. J. Appl. Phycol. 4, 233–245.

Borowitzka, M.A., 1997. Microalgae for aquaculture: opportunities and constraints. J. Appl. Phycol. 9, 393–401.

Boswell, K., Koskelo, E., Carl, L., Glaza, S., Hensen, D., Williams, K., Kyle, D., 1996. Preclinical evaluation of single-cell oils that are highly enriched with arachidonic acid and docosahexaenoic acid. Food Chem. Toxicol. 34, 585–593.

Brown, M.R., Jeffrey, S.W., 1992. The nutritional properties of microalgae used in mariculture: an overview. In: Allan, G.L., Dall, W. (Eds.), Proceedings of Aquaculture Nutrition Workshop, Salamander Bay, 15–17 April 1991. NSW Fisheries Brackish Water Fish Culture Station, Salamander Bay, Australia, pp. 174–179.

Brown, M.R., Jeffrey, S.W., Garland, C.D., 1989. Nutritional aspects of microalgae used in mariculture; a literature review. CSIRO Mar., Lab. Rep. no. 205, 44 pp.

Brown, M.R., Dunstan, G.A., Jeffrey, S.W., Volkman, J.K., Barret, S.M., LeRoi, J.-M., 1993. The influence of irradiance on the biochemical composition of the Prymnesiophyte *Isochrysis* sp. (clone T-ISO). J. Phycol. 29, 601–612.

Burgess, G.J., Iwamoto, K., Miura, Y., Takano, H., Matsunaga, T., 1993. An optical fibre photobioreactor for enhanced production of the marine unicellular alga *Isochrysis* aff. *galbana* T-ISO (UTEX LB 2307) rich in docosahexaenoic acid. Appl. Microbiol. Biotechnol. 39, 456–459.

Burns, R.A., Wibert, G.J., Diersen-Schade, D.A., Kelly, C.M., 1999. Evaluation of single-cell sources of docosahexaenoic acid and arachidonic acid, 3 month rat oral safety study with an utero phase. Food Chem. Toxicol. 37, 23–36.

Chen, J.S., Lii, C., Chen, H., 1997. Production of docosahexaenoic acid by marine microalga *Isochrysis galbana* TK2. J. Chin. Agricult. Chem. Soc. 35, 96–106.

Constantopoulos, G., Bloch, K., 1967. Effect of light intensity on the lipid composition of *Euglena gracilis*. J. Biol. Chem. 242, 3538–3542.

Coupland, K., Coupland, D., Packer, C., 1996. New sources of stearidonic acid and the moderation of inflamation by plant oils containing stearidonate. ISSFAL 1996.

Delaunay, F., Marty, Y., Moal, J., Samain, J.-F., 1993. The effect of mono-specific algal diets on growth and fatty acid composition of *Pecten maximus* (L.) larvae. J. Exp. Mar. Biol. Ecol. 173, 163–179.

De Pauw, N., Persoone, G., 1988. Micro-algae for aquaculture. In: Borowitzka, M.A., Borowitzka, L.J. (Eds.), Micro-algae Biotechnology. Cambridge Univ. Press, Cambridge, pp. 197–221.

Dubinsky, Z., 1986. Productivity of algae under natural conditions. In: Ricmont, A. (Ed.), CRC Handbook of Microalgal Mass Culture. CRC Press, Boca Raton, FL, pp. 101–115.

Dunstan, G.A., Volkman, J.K., Barret, S.M., Garland, C.D., 1993. Changes in the lipid composition and maximisation of the polyunsaturated fatty acid content of three microalgae grown in mass culture. J. Appl. Phycol. 5, 71–83.

Falkowski, P.G., Raven, J.A., 1997. Aquatic Photosynthesis. Blackwell Scientific, Oxford, 375 pp.

Falkowski, P.G., Dubinsky, Z., Wyman, K., 1985. Growth–irradiance relationships in phytoplankton. Limnol. Oceanogr. 30, 311–321.

Fernández-Reiriz, M.J., Labarta, U., Albentosa, M., Pérez-Camacho, A., 1998. Effect of microalgal diets and commercial wheat germ flours on the lipid profile of *Ruditapes decussatus* spat. Comp. Biochem. Physiol. 119A, 369–377.

Fernandez-Sevilla, J.M., Molina-Grima, E., Garcia-Camacho, F., Acien-Fernandez, F.G., Sanchez-Perez, J.A., 1998. Photolimitation and photoinhibition as factors determining optimal dilution rate to produce eicosapentaenoic acid from cultures of the microalga *Isochrysis galbana*. Appl. Microbiol. Biotechnol. 50, 199–205.

Fogg, G.E., Thake, B., 1987. Algal Cultures and Phytoplankton Ecology. The University of Wisconsin Press, Madison, 268 pp.

Gladue, R.M., Maxey, J.E., 1994. Microalgae feeds for aquaculture. J. Appl. Phycol. 6, 131–141.

Harrison, P.J., Thompson, P.A., Calderwood, G.S., 1990. Effects of nutrient and light limitation on the biochemical composition of phytoplankton. J. Appl. Phycol. 2, 45–56.

Harwood, J.L., 1996. Recent advances in the biosynthesis of plant fatty acids. Biochim. Biophys. Acta 1301, 7–56.

Hodgson, P.A., Henderson, R.J., Sargent, J.R., Leftley, J.W., 1991. Patterns of variation in the lipid class and fatty acid composition of *Nannochloropsis oculata* (Eustigmatophyceae) during batch culture: I. The growth cycle. J. Appl. Phycol. 3, 169–181.

Kirk, J.T.O., 1983. Light and Photosynthesis in Aquatic Ecosystems. Cambridge Univ. Press, Cambridge, 401 pp.

Klyachko-Gurvich, G.L., Tsoglin, L.N., Doucha, J., Kopetskii, J., Shebalina, (Ryabykh), I.B., Semenenko, V.E., 1999. Desaturation of fatty acids as an adaptive response to shifts in light intensity. Physiol. Plant. 107, 240–249.

Koven, W.M., Tandler, A., Kissil, G.W., Sklan, D., Friezlander, O., Harel, M., 1990. The effect of dietary ($n-3$) polyunsaturated fatty acids on growth, survival and swim bladder development in *Sparus aurata* larvae. Aquaculture 91, 131–141.

Kyle, D., Ratledge, C. (Eds.), 1992. Industrial Applications of Single Cell Oils. AOCS Press, Champaign, 400 pp.

Kyle, D.J., Sicotte, V.J., Singer, J.J., Reeb, S.E., 1992. Bioproduction of docosahexaenoic acid (DHA) by microalgae. In: Kyle, D., Ratledge, C. (Eds.), Industrial Applications of Single Cell Oils. AOCS Press, Champaign, pp. 287–300.

Lepage, G., Roy, C.C., 1986. Direct transesterification of all classes of lipids in a one-step reaction. J. Lipid Res. 27, 114–120.

Marshall, H.L., Geider, R.J., Flynn, K.J., 2000. A mechanistic model of photoinhibition. New Phytol. 145, 347–359.

Mourente, G., 1993. Effects of dietary docosahexaenoic acid (DHA; 22, $6n-3$) on lipid and fatty acid compositions and growth in gilthead sea bream (*Sparus aurata* L.) larvae during first feeding. Aquaculture 112, 79–98.

Mourente, G., Lubián, L.M., Odriozola, J.M., 1990. Total fatty acid composition as a taxonomic index of some marine microalgae used as food in marine aquaculture. Hydrobiologia 203, 147–154.

Napolitano, G.E., Ackman, R.G., Ratnayake, W.M.N., 1990. Fatty acid composition of three cultured algal species (*Isochrysis galbana*, *Chaetoceros gracilis* and *Chaetoceros calcitrans*) used as food for bivalve larvae. J. World Maric. Soc. 21, 122–130.

Nichols, B.W., 1965. Light induced changes in the lipids of *Chlorella vulgaris*. Biochim. Biophys. Acta 106, 274–279.

Picaud, A., Creach, A., Trémolières, A., 1991. Studies on the stimulation by light of fatty acid synthesis in *Chlamydomonas reinhardtii* whole cells. Plant Physiol. Biochem. 29, 441–448.

Raven, J.A., 1984. A cost-benefit analysis of photon absorption by photosynthetic unicells. New Phytol. 94, 593–625.

Raven, J.A., 1986. Physiological consequences of extremely small size for autotrophic organisms in the sea. In: Platt, T., Li, W.K.W. (Eds.), Photosynthetic Picoplankton. Can. Bull. Fish. Aquat. Sci., vol. 214, pp. 1–70.

Reitan, K.I., Rainuzzo, J.R., Øie, G., Olsen, Y., 1993. Nutritional effects of algal addition in first feeding of turbot (*Scophthalmus maximus* L.) larvae. Aquaculture 118, 257–275.

Reitan, K.I., Rainuzzo, J.R., Olsen, Y., 1994. Effect of nutrient limitation on fatty acid and lipid contents of marine microalgae. J. Phycol. 30, 972–979.

Renaud, S.M., Parry, D.L., 1994. Microalgae for use in tropical aquaculture: II. Effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. J. Appl. Phycol. 6, 347–356.

Renaud, S.M., Parry, D.L., Thinh, L.-V., Kuo, C., Padovan, A., Sammy, N., 1991. Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochrysis* sp. and *Nannochloropsis* oculata for use in tropical aquaculture. J. Appl. Phycol. 3, 43–53.

Renaud, S.M., Parry, D.L., Thinh, L.-V., 1994a. Microalgae for use in tropical aquaculture: I. Gross chemical and fatty acid composition of twelve species of marine microalgae. J. Appl. Phycol. 6, 337–345.

Renaud, S.M., Zhou, H.C., Parry, D.L., Thinh, L.-V., Woo, K.C., 1994b. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, and commercial species *Isochrysis* sp. (clone T. ISO). J. Appl. Phycol. 7, 595–602.

Renaud, S.M., Zhou, H.C., Parry, D.L., Thinh, L.-V., Woo, K.C., 1995. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, and commercial species *Isochrysis* sp. (clone T.ISO). J. Appl. Phycol. 7, 595–602.

Sanchez Saavedra, M.d.P., Voltolina, D., 1994. The chemical composition of *Chaetoceros* sp. (Bacillariophyceae) under different light conditions. Comp. Biochem. Physiol. 107B, 39–44.

Saoudi-Helis, L., 1994. Influence du taux de croissance et de stress thermiques sur la composition biochimique et lipidique de *Isochrysis* aff. *galbana* clone T. iso. Culture en conditions contrôlées. Thèse de Docteur de l' Universitè d' Aix-Marseille 2, 148 pp.

Saoudi-Helis, L., Dubacq, J.P., Marty, Y., Samain, J.F., Gudin, C., 1994. Influence of growth rate on pigment and lipid composition of the microalga *Isochrysis* aff. *galbana* clone T-ISO. J. Appl. Phycol. 6, 315–322.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Dietary origins and functions of long-chain ($n-3$) polyunsaturated fatty acids in marine fish. J. Mar. Biotechnol. 3, 26–28.

Sargent, J.R., McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture 155, 117–127.

Servel, M.O., Claire, C., Derrien, A., Coiffard, L., De Roeck-Holtzhauer, Y., 1994. Fatty acid composition of some marine microalgae. Phytochemistry 36, 691–693.

Sicko-Goad, L., Simmons, M.S., Lazinsky, D., Hall, J., 1988. Effect of light cycle on diatom fatty acid composition and quantitative morphology. J. Phycol. 24, 1–7.

StatSoft, 1994. Statistica for Windows version 4.3. Vol. I: Conventions and Statistics I, 659 pp.; Vol. II: Graphics, 777 pp.; Vol. III: Statistics II, 911 pp. StatSoft, Tulsa.

Suen, Y., Hubbard, J.S., Holzer, G., Tornabene, T.G., 1987. Total lipid production of the green alga *Nannochloropsis* sp. QII under different nitrogen regimes. J. Phycol. 23, 289–296.

Sukenik, A., Wahnon, R., 1991. Biochemical quality of marine unicellular algae with special emphasis on lipid composition: I. *Isochrysis galbana*. Aquaculture 97, 61–72.

Sukenik, A., Carmeli, Y., Berner, T., 1989. Regulation of fatty acid composition by irradiance level in the eustigmatophyte *Nannochloropsis* sp. J. Phycol. 25, 686–692.

Thompson Jr., G.A., 1996. Lipids and membrane function in green algae. Biochim. Biophys. Acta 1302, 17–45.

Thompson, P.A., Harrison, P.J., Whyte, J.N.C., 1990. Influence of irradiance on the fatty acid composition of phytoplankton. J. Phycol. 26, 278–288.

Thompson, P.A., Guo, M.X., Harrison, P.J., 1992. Effects of variation in temperature: II. On the fatty acid composition of eight species of marine phytoplankton. J. Phycol. 28, 488–497.

Thompson, P.A., Guo, M.X., Harrison, P.J., 1993. The influence of irradiance on the biochemical composition of three phytoplankton species and their nutritional value for larvae of the Pacific oyster (*Crassostrea gigas*). Mar. Biol. 117, 259–268.

Thompson, P.A., Guo, M.X., Harrison, P.J., 1996. Nutritional value of diets that vary in fatty acid composition for larval Pacific oysters (*Crassostrea gigas*). Aquaculture 143, 379–391.

Tuncer, H., Harrell, R.M., 1992. Essential fatty acid nutrition of larval striped bass (*Morone saxatilis*) and palmetto bass (*M. saxatilis* × *M. chrysops*). Aquaculture 101, 105–121.

Tzovenis, I., De Pauw, N., Sorgeloos, P., 1997. Effect of different light regimes on the docosahexaenoic (DHA) content of *Isochrysis* aff. *galbana* (clone T-ISO). Aquacult. Int. 5, 489–507.

Tzovenis, I., De Pauw, N., Sorgeloos, P., 2003. Optimisation of T-ISO biomass rich in essential fatty acids: I. Effects of different light regimes on growth and biomass production. Aquaculture 216, 203–222.

Volkman, J.K., 1989. Fatty acids of microalgae used as feedstocks in aquaculture. In: Cumbie, R.C. (Ed.), Fats for the Future. Ellis Horwood, Chichester, pp. 263–283.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128, 219–240.

Walne, P.R., 1966. Experiments in the large scale culture of the larvae of *Ostrea edulis* L. Fish. Invest., London, II 25 (53 pp.).

Webb, K.L., Chu, F.L.E., 1983. Phytoplankton as a food source for bivalve larvae. In: Pruder, G.D. (Ed.), Biochemical and Physiological Approaches to Shellfish Nutrition. Spec. Publ.-World Maric. Soc., vol. 2, pp. 272–291.

Webb, W.L., Newton, M., Starr, D., 1974. Carbon dioxide exchange of *Alnus rubra*: a mathematical model. Oecologia 17, 281–291.

Whyte, J.N.C., Clarke, W.C., Ginther, N.G., Jensen, J.O.T., Townsend, L.D., 1994. Influence of composition of *Brachionus plicatilis* and *Artemia* on growth of larval sablefish (*Anoploma fimbria*) Pallas. Aquaculture 119, 47–61.

Yongmanitchai, W., Ward, O.P., 1989. Omega-3 fatty acids: alternative sources of production. Process Biochem. 24, 117–125.

Zhukova, N., Imbs, A.B., Yi, L.F., 1998. Diet-induced changes in lipid and fatty acid composition of *Artemia salina*. Comp. Biochem. Physiol. 120B, 499–506.