Chemical composition of selected marine microalgae, with emphasis on lipid and carbohydrate production for potential use as feed resources

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Received: 4 May 2021 / Revised and accepted: 2 August 2021 / Published online: 14 September 2021
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Abstract

Marine microalgae are a promising sustainable source of lipids, omega-3 fatty acids, and carbohydrates. Selected microalgae species belonging to the Bacillariophyceae, Haptophyceae, Eustigmatophyceae, and Prasinophyceae were characterised for cellular content of carbon and nitrogen, and for production yields of lipids, fatty acids, total carbohydrates, and β -glucans. Carbon and nitrogen content showed a hyperbolic decrease with increasing cell numbers for *Chaetoceros calcitrans*, *C. muelleri*, *Skeletonema costatum*, *Tetraselmis* sp., and *Nannochloropsis oculata*. Cultures of *Pavlova lutheri* and *Tisochrysis lutea* showed an increase in carbon content per cell, but a decrease in nitrogen content. The total lipid content of *C. muelleri*, *C. calcitrans*, *N. oculata*, and *T. lutea* increased with decreasing relative growth rate; however, the highest productivity of lipids was found in *T. lutea* grown at 40% of the maximum specific growth rate. The highest content of eicosapentaenoic acid was found in *C. muelleri*, *C. calcitrans*, and *N. oculata*, and the highest content of docosahexaenoic acid was found in *T. lutea*. The β -glucan fraction of the carbohydrates was highest in *C. muelleri* and *C. calcitrans* and was very low in *N. oculata*. Out of the species investigated, *C. muelleri* had the highest production yield of β -glucans, obtained when cultivated at a 40% relative growth rate.

Keywords Marine microalgae · Lipids · Fatty acids · Carbohydrates · β -glucans · Cultivation

Introduction

Microalgae are a natural source of n-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are accumulated in the marine food chain (Reitan et al. 1994, 1997; Brown et al. 1997; Shah et al. 2018). The chemical composition of microalgae varies with species and classes, and the lipid content typically ranges from 10 to 60% of dry matter (Reitan et al. 1994; Brown et al. 1997; Chiu et al. 2009; Rodolfi et al. 2009; Doan et al. 2011; Wang et al. 2019). In addition, growth conditions such

as nutrient limitation and light intensity have been observed to influence significantly the growth rate and chemical composition of various microalgae species (Reitan et al. 1994; Tzovenis et al. 2003; Guedes et al. 2010; Vu et al. 2016). Temperature was found to have a pronounced effect on the growth rate, lipid content, and fatty acid profiles of microalgae (Roleda et al. 2013; Chaisutyakorn et al. 2018). The maximum specific growth rate increases with increasing temperatures to the optimal growth temperature, whereas it decreases for increasing temperatures above this point (Converti et al. 2009). Microalgae also differ in carbohydrate composition, again depending on both species and cultivation conditions (Pernet et al. 2003; Størseth et al. 2005). Microalgae species have been shown to accumulate carbohydrates as well as lipids when cultivated under nitrogen limitation (Harrison et al. 1990; Yang et al. 2013). The carbohydrates in microalgae may contain a high fraction of β -1,3-glucan (Størseth et al. 2004), and several studies suggest that β -1,3-glucan may act as an immunostimulant in fish and shellfish (Dahlmo et al. 1996; Chang et al. 2000; Vetricka et al. 2013).

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Because of their balanced composition of proteins and lipids (Brown et al. 1997), microalgae have been suggested as an alternative future feed resource, especially for use in aquafeed (Chauton et al. 2014). De novo synthesis of n-3 fatty acids can only take place in plant cells, and long-chain polyunsaturated fatty acids, such as EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid), are frequently found in high concentrations in marine microalgae (Brown et al. 1997; Patil et al. 2006; Shah et al. 2018). This makes marine microalgae a promising source for fish feed (Tacon 1996; Carter et al. 2003; Burr et al. 2011; Skrede et al. 2011; Sørensen et al. 2016). A further increase in the aquaculture production would require a supply of alternatives to fish meal and fish oil (Chauton et al. 2014).

The aim of this study was to provide comprehensive information on variation in cellular contents of carbon, nitrogen and phosphorous during the growth cycle of seven microalgae species in batch cultures. Furthermore, four species with potential for use as a feed resource were selected and cultivated at three different growth rates in semi-continuous culture in order to characterise the content of total lipids, fatty acids, carbohydrates, and β -glucans. The productivity of the different compounds in the selected four microalgae species was also evaluated. The chemical composition of the microalgae was reviewed for potential use as a feed resource.

Materials and methods

Culture method for microalgae

The following microalgae were used: the Bacillariophyceae *Chaetoceros muelleri* Lemmermann (CCAP, strain 1010/3), *Chaetoceros calcitrans* (Paulsen) H.Takano (CCMP strain 1315), and *Skeletonema costatum* (Greville) Cleve 1873, clone Skel-5, isolated from the Trondheimsfjord (Mykkestad 1974); the Haptophyceae *Pavlova lutheri* (Droop) Green (CCMP strain 1325) and *Tisochrysis lutea* El M.Bendif & I.Probert (Tahitian) (CCAP strain 927/14); the Eustigmatophyceae *Nannochloropsis oculata* (Droop) D.J. Hibberd (CCAP strain 849/1); and the Prasinophyceae *Tetraselmis* sp. (own isolate). They were grown in 1-L glass cylinders. The cultures were grown at five different temperatures (16, 20, 24, 27, and 32 °C) to determine the effect of temperature on the growth rate. The microalgae were cultivated in batch cultures and continuously illuminated (Phillips TLD 36 W/33 and Phillips TL 40 W/55) at an irradiance of 70–90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the culture surface. The cultures were aerated with filtered air with added 0.1% CO_2 using a purge Rotameter model 10A 6100. The F/2 growth medium (Guillard and Ryther 1962) was prepared using sand filtered and particle filtered (pore size of 1 μm) seawater taken from a depth of 70 m and autoclaved before use.

The increase in biomass of the microalgae cultures was monitored by sampling of 5 mL volumes for regular optical density measurements using a spectrophotometer at a wavelength of 750 nm. The specific growth rate (μ , day^{-1}) of the batch cultures was calculated as

$$\mu = \ln(N_t/N_0)/t$$

where N_0 is the biomass at time t_0 , and N_t is the biomass at time t . The maximum specific growth rate (μ_{max} , day^{-1}) was calculated in the initial exponential growth phase before the growth started to decline, when $\ln(N)$ versus time was linear.

The cell densities of the cultures were estimated using a Bürkner chamber and counted in microscope at 100 $\times$ magnification.

Cultures of *C. muelleri*, *C. calcitrans*, *N. oculata*, and *T. lutea* were then grown semi-continuously at 20 °C in 200-L Plexiglas cylinders at the same irradiance as for batch cultivation for determination of content and productivity of total lipids, fatty acids, carbohydrates, and β -glucans. The individual cultures were grown until steady state and each culture continued in steady state for 4–5 days (Kilham 1978) at dilution rates that corresponded to 4, 40, and 60% relative growth rate (% μ of μ_{max}). The cultures were sampled when the biomass of the cultures was in a steady state, meaning that the biomass did not vary by more than 5% between days.

Chemical analysis

The carbon, nitrogen, and phosphorous content of algae cells was analysed in batch cultures (20 °C) for all species. Precise volumes of the microalgae cultures were filtered using Whatman GF/F filters in six replicates for each culture at each sampling time during the growth phase of individual cultures: Three filters were used to measure carbon and nitrogen content. The filters were dried at 60 °C for 24 h and analysed with a Carlo Erba element analyser, model 1106, with acetanilide as standard. Phosphorus was analysed as described by Koroleff (1976) in the three remaining filter replicates.

Biomass for analysing the total lipids, fatty acids, carbohydrates, and β -glucans was harvested when the semi-continuous cultures had reached the steady state. Volumes of 2 L from each culture were harvested at dilution time during steady state and centrifuged at 3000–5000 rpm for 15 min (Wifug 400 E, and Heraeus Cryophuge 8000). The biomasses were frozen at –80 °C under a N_2 atmosphere before further analysis. The total lipid content was analysed gravimetrically ($n=2-3$) after extraction by a modified Bligh and Dyer (1959) method as described by Rainuzzo et al. (1994). The fatty acid methyl esters were prepared and analysed as described by Rainuzzo et al. (1994) using a Carlo Erba HRGC 5160 gas chromatograph equipped with a SP-2330 glass capillary column with an on-column

injection and flame ionization detector. The fatty acids were identified and quantified by comparison with known standards (NU-Chek Prep, USA) and use of response factors to internal standard (21:0). The total carbohydrate content was analysed using the phenol–sulphuric acid method (Dubois et al. 1956), and the β -glucan content was analysed using a method described by Myklestad and Haug (1972). The method is based on extraction with 0.1 N sulphuric acid, which omits the polysaccharides.

The productivity of the total lipid, fatty acid, carbohydrate, and β -glucan content of the biomass was calculated as

$$P = \text{Content} * V$$

where *Content* is the weight of the different chemical compounds per litre, and *V* is the daily harvested volume of the culture.

Statistical analysis

Means $\pm$ standard errors of the means are given throughout the text, tables, and graphs. Means were tested statistically using one-way ANOVA, followed by a Student–Newman–Keuls test using SigmaPlot for Windows version 13.0 (Systat Software Inc., USA). The 5% confidence level was used throughout the experiment.

Results

Growth parameters

The maximum specific growth rates of the seven microalgae investigated varied with the temperature (Fig. 1). The optimum growth temperature is the temperature that gives the highest maximum growth rate, and highest maximum specific growth rate of *C. calcitrans* and *N. oculata* was observed at 16 °C, for *S. costatum* at 20 °C, while *Tetraselmis* sp., *C. muelleri*, *P. lutheri*, and *T. lutea* obtained highest maximum specific growth rates at 24 °C. All species investigated showed decreased growth rates at temperatures above 24 °C and no growth at 32 °C.

The content of carbon and nitrogen per cell of each of the seven microalgae species grown in batch cultures is shown in Fig. 2. Both the carbon and nitrogen content per cell showed a hyperbolic decrease with increasing cell numbers for *C. calcitrans*, *C. muelleri*, *S. costatum*, *Tetraselmis* sp., and *N. oculata*. For *P. lutheri* and *T. lutea*, the carbon content per cell hyperbolically increased with increasing cell numbers, while the nitrogen content per cell decreased with increasing cell numbers.

The nitrogen to carbon ratio (N/C ratio) decreased in all algae species, except in *N. oculata*, where the ratio was roughly similar at all cell densities (Figs. 2). The reduced

Fig. 1 The maximum specific growth rate ($\mu_{\max}$) of the seven microalgae species grown at different cultivation temperatures

N/C ratio with increasing cell density shows that the nitrogen content was reduced more than the carbon content when the cultures grew and the cell density increased. In the stationary phase, at high cell densities, the N/C weight ratio varied between 0.051 (for *T. lutea*) and 0.126 (for *C. calcitrans*; Fig. 3), being significantly higher for the Bacillariophyceae than for the other algae groups. The P/C ratio at the stationary growth phase varied between 0.004 (*P. lutheri*, *T. lutea*, and *N. oculata*) and 0.011 (*C. calcitrans*; Fig. 3). The N/P ratios at the stationary phase ranged from 10.7 to 18.0.

Lipids and fatty acids

The total lipid content for all four microalgae species increased with reduced relative growth rate in semi-continuous cultures, meaning that the lipid content was highest in cultures with the strongest growth limitation for all species investigated (Fig. 4). The highest lipid content was found in *N. oculata*, at 335 mg g⁻¹ DW at 4% relative growth rate. Somewhat lower lipid content was observed in *C. muelleri* and *T. lutea*, at 313 and 290 mg g⁻¹ DW, respectively. The fatty acids accounted for 40% of the total lipids on average, without any systematic variation between species or growth conditions. The variation in total lipid content of the microalgae suggests that the species investigated stored lipids while the growth was limited.

Fatty acid composition

The diatoms *C. calcitrans* and *C. muelleri* had relatively similar fatty acid profiles, with high content of 14:0, 16:0, 16:1, and 20:5 n-3, and somewhat lower content of 16:3 (Table 1). *Chaetoceros muelleri* also had a significantly

Fig. 2 Content of carbon (C, pg cell⁻¹) and nitrogen (N, pg cell⁻¹) per algae cells and the N/C weight ratio at increasing cell density of the culture of **A** *C. calcitrans*, **B** *C. muelleri*, **C** *S. costatum*, **D** *Tetraselmis* sp., **E** *T. lutea*, **F** *P. lutheri*, and **G** *N. oculata*. Means with SE bars ($n=3$)

Fig. 3 The average N/C, P/C, and N/P weight ratios of the seven microalgae species sampled at the stationary phase. Means with SE bars ($n=3$). Different letters indicate significant differences ($p<0.05$)

Fig. 4 Content of fatty acids and total lipids of the microalgae cultivated by semi-continuous culture at different relative growth rates (4%, 40%, and 60% of μ_{max}). Means with SE bars ($n=2-3$)

higher content of 20:4 n-6 compared to *C. calcitrans*. The fatty acid composition of *T. lutea* was dominated by 14:0, 16:0, 16:1, 18:1 n-9, 18:3 n-3, 18:4 n-3, and 22:6

n-3. *Nannochloropsis oculata* had high content of 14:0, 16:0, 16:1, 20:4 n-6, and 20:5 n-3.

The reduction in specific growth rate from 60 to 4% in *C. muelleri* resulted in higher relative content of 16:0 and

Table 1 Fatty acid content (mg g⁻¹ DW) in microalgae grown at different relative growth rate (4%, 40%, and 60% of $\mu_{\max}$). Mean $\pm$ SE of two or three replicates (*C. muelleri* at 60% only $n = 1$). Open is not detected or < 0.1 . Asterisks indicate significant difference within species

Fatty acid	Bacillariophyceae					Prymnesiophyceae			Eustigmatophyceae		
	<i>C. calcitrans</i>		<i>C. muelleri</i>			<i>T. lutea</i>			<i>N. oculata</i>		
	40%	60%	4%	40%	60%	4%	40%	60%	4%	40%	60%
14:00	7.1 $\pm$ 0.4	4.4 $\pm$ 0.1*	11.8 $\pm$ 1.3	4.8 $\pm$ 0.6*	3.2	16.0 $\pm$ 3.2	12.2 $\pm$ 0.2	9.7 $\pm$ 1.2	10.4 $\pm$ 0.9	5.5 $\pm$ 0.1*	5.6 $\pm$ 0.2*
16:00	7.9 $\pm$ 0.1	6.8 $\pm$ 0.1*	35.2 $\pm$ 4.5	11.3 $\pm$ 2.0*	8.9	1.4 $\pm$ 0.1	4.6 $\pm$ 4.9	7.5 $\pm$ 0.1	35.6 $\pm$ 8.6	18.9 $\pm$ 0.6*	17.2 $\pm$ 2.8*
18:00	0.2 $\pm$ —	0.2 $\pm$ 0.1	2.6 $\pm$ 0.1	1.0 $\pm$ 0.1*	0.6	0.2 $\pm$ 0.1	4.0 $\pm$ 5.4	0.3 $\pm$ 0.1	0.9 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1
20:00	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1		0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1		
22:00		0.1 $\pm$ 0.1				0.1 $\pm$ 0.1					
16:01	13.5 $\pm$ 1.5	15.1 $\pm$ 1.0	42.6 $\pm$ 4.5	15.1 $\pm$ 2.3*	15	14.2 $\pm$ 0.2	7.4 $\pm$ 1.1*	3.2 $\pm$ 0.4*	36.3 $\pm$ 6.9	26.6 $\pm$ 2.2	23.9 $\pm$ 4.3
18:1 n-9	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	1.2 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3	12.1 $\pm$ 0.1	4.7 $\pm$ 4.5	8.3 $\pm$ 0.1	9.5 $\pm$ 0.3	1.5 $\pm$ 0.1*	2.3 $\pm$ 0.4*
18:1 n-7	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	2.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.3	2.1 $\pm$ 0.1	1.0 $\pm$ 1.2	1.2 $\pm$ 0.1	1.4 $\pm$ 0.3	0.8 $\pm$ 0.1	0.7 $\pm$ 0.2
22:01		0.1 $\pm$ 0.1	0.5 $\pm$ 0.1	0.1 $\pm$ 0.1							
16:2 n-4	2.9 $\pm$ 0.1	1.9 $\pm$ 0.1	4.6 $\pm$ 1.7	2.1 $\pm$ 1.6	0.8	0.6 $\pm$ 0.1	1.0 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	1.2 $\pm$ 0.1	0.9 $\pm$ 0.9
16:3 n-4	9.3 $\pm$ 0.3	5.4 $\pm$ 0.1*	0.3 $\pm$ 0.4	1.7 $\pm$ 2.2	2.6		0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.3 $\pm$ 0.1
16:04	1.4 $\pm$ 0.1	1.4 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1	0.9 $\pm$ 0.1	0.6 $\pm$ 0.8	0.7 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
18:2 n-6	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	1.2 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4	15.6 $\pm$ 0.6	3.5 $\pm$ 0.1*	2.3 $\pm$ 0.1*	4.8 $\pm$ 0.3	2.1 $\pm$ 0.1*	3.1 $\pm$ 0.1*
18:3 n-6	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	2.9 $\pm$ 0.1	1.3 $\pm$ 0.1*	0.9	0.9 $\pm$ 0.1	0.3 $\pm$ 0.1	0.1 $\pm$ 0.1	0.9 $\pm$ 0.1	0.7 $\pm$ 0.1	0.5 $\pm$ 0.1
20:3 n-6			0.5 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1				2.7 $\pm$ 0.2	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1
20:4 n-6	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	15.0 $\pm$ 0.3	2.3 $\pm$ 0.1*	2.6	1.4 $\pm$ 0.1	1.9 $\pm$ 0.1	2.8 $\pm$ 0.1*	11.3 $\pm$ 1.1	8.5 $\pm$ 0.1*	6.1 $\pm$ 0.2*
18:3 n-3	0.4 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1		8.4 $\pm$ 0.1	7.3 $\pm$ 0.1	4.0 $\pm$ 0.1*	0.9 $\pm$ 0.1	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1
18:4 n-3	1.1 $\pm$ 0.1	0.9 $\pm$ 0.1	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	0.4	10.2 $\pm$ 0.2	16.6 $\pm$ 0.1*	12.0 $\pm$ 0.2	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2
20:4 n-3		0.2 $\pm$ 0.2		0.1 $\pm$ 0.1		0.1 $\pm$ 0.1	0.1 $\pm$ 0.1		0.1 $\pm$ 0.1		
20:5 n-3	20.5 $\pm$ 0.4	12.5 $\pm$ 0.3*	16.5 $\pm$ 0.1	9.1 $\pm$ 0.1*	7.4	0.5 $\pm$ 0.1	0.7 $\pm$ 0.1	0.4 $\pm$ 0.3	49.6 $\pm$ 1.4	48.2 $\pm$ 1.1	38.4 $\pm$ 4.9
22:5 n-3	0.7 $\pm$ 0.9										
22:6 n-3	1.4 $\pm$ 0.1	0.9 $\pm$ 0.1	2.2 $\pm$ 0.1	1.3 $\pm$ 0.1	0.9	10.8 $\pm$ 1.2	13.8 $\pm$ 0.3	15.4 $\pm$ 0.3*			
EPA + DHA	21.8 $\pm$ 0.4	13.4 $\pm$ 0.1*	18.7 $\pm$ 0.1	10.3 $\pm$ 0.1*	8.3	11.2 $\pm$ 3.2	14.5 $\pm$ 0.3	15.8 $\pm$ 1.1	49.6 $\pm$ 1.4	48.2 $\pm$ 1.1	38.4 $\pm$ 4.9

20:4 n-6 and lower percentage of 20:5 n-3, whereas the rest of the fatty acids remained relatively constant with reduced growth rates (Table 2). *Tisochrysis lutea*'s relative content of 16:1 and 18:2 n-6 increased, while its content of 16:0 (not significant) and 22:6 n-3 decreased, when the growth rate was reduced. Reduced growth rate of *N. oculata* yielded increased content of 16:0 and 18:1, whereas the relative content of 20:5 n-3 was reduced.

These findings show that 14:0 and 16:0 were the main saturated fatty acids, and 16:1 was the main unsaturated fatty acid. The content of polyunsaturated fatty acids varied between the species investigated, with high content of EPA (20:5 n-3) in *C. muelleri*, *C. calcitrans*, and *N. oculata*, and high content of DHA (22:6 n-3) in *T. lutea*. Both *C. muelleri* and *N. oculata* had high content of 20:4 n-6 (AA), at 4.4–10.7% and 6.0–7.3%, respectively.

For all species investigated, reduced growth rates led to a general lower relative content of the polyunsaturated fatty acids 20:5 n-3 and 22:6 n-3 (Table 2). However, the relative content of 20:4 n-6 was not influenced in the same

manner, being constant in *N. oculata* and increasing in *C. muelleri* with decreasing growth rates.

Content of carbohydrates and β -glucans

The total carbohydrates in the microalgae varied from 54 mg g⁻¹ DW (for *T. lutea* at 40% relative specific growth rate) to 235 mg g⁻¹ DW (for *C. muelleri* at 4% relative specific growth rate) (Fig. 5). The total carbohydrate content varied with the change in the relative growth rate to a different extent in the different microalgae species. *C. muelleri* was the only species that yielded significantly increased content with reduced growth rate. The carbohydrate content of *C. calcitrans* and *N. oculata* did not vary significantly with the growth rate, and *T. lutea* had highest carbohydrate content at the highest growth rate ($p < 0.05$; Fig. 5).

The β -glucan content varied greatly between species, being highest in *C. calcitrans* and *C. muelleri*, and somewhat lower in *T. lutea* (Fig. 5). *Nannochloropsis oculata* had very low β -glucan content compared to the other microalgae

Table 2 Fatty acid content (% of sum) in microalgae grown at different relative growth rate (4%, 40%, and 60% of $\mu_{\max}$). Mean $\pm$ SE of two or three replicates (*C. muelleri* at 60% only $n = 1$). Open is not detected or < 0.1 . Asterisks indicate significant difference within species

Fatty acid	Bacillariophyceae					Prymnesiophyceae			Eustigmatophyceae		
	<i>C. calcitrans</i>		<i>C. muelleri</i>			<i>T. lutea</i>			<i>N. oculata</i>		
	40%	60%	4%	40%	60%	4%	40%	60%	4%	40%	60%
14:00	10.7 $\pm$ 2.9	8.5 $\pm$ 0.1	8.4 $\pm$ 0.4	9.1 $\pm$ 0.5	7.2	16.7 $\pm$ 3.2	15.3 $\pm$ 1.2	14.1 $\pm$ 1.8	6.3 $\pm$ 0.1	4.8 $\pm$ 0.3	5.6 $\pm$ 0.5
16:00	11.9 $\pm$ 2.7	13.2 $\pm$ 0.3	24.9 $\pm$ 1.7	21.5 $\pm$ 2.1	20	1.4 $\pm$ 0.1	5.6 $\pm$ 5.8	10.9 $\pm$ 0.1	21.3 $\pm$ 3.2	16.3 $\pm$ 0.1*	17.1 $\pm$ 0.8*
18:00	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	1.8 $\pm$ 0.1	1.9 $\pm$ 0.1	1.3	0.2 $\pm$ 0.1	5.2 $\pm$ 7.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
20:00	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1		0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1			
22:00		0.1 $\pm$ 0.1				0.1 $\pm$ 0.1					
16:01	17.8 $\pm$ 1.8	29.4 $\pm$ 2.0*	30.2 $\pm$ 1.4	28.7 $\pm$ 2.3	33.7	14.9 $\pm$ 0.1	9.3 $\pm$ 1.9*	4.7 $\pm$ 0.6*	21.8 $\pm$ 2.2	22.9 $\pm$ 1.1	23.7 $\pm$ 1.4
18:1 n-9	0.5 $\pm$ 0.2	0.7 $\pm$ 0.1	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	0.7	12.7 $\pm$ 0.3	5.7 $\pm$ 5.3	12.1 $\pm$ 0.1	5.8 $\pm$ 0.7	1.3 $\pm$ 0.1*	2.2 $\pm$ 0.1*
18:1 n-7	1.4 $\pm$ 0.3	1.7 $\pm$ 0.0	1.8 $\pm$ 0.1	1.0 $\pm$ 0.1	0.7	2.2 $\pm$ 0.1	1.2 $\pm$ 1.4	1.7 $\pm$ 0.1	0.9 $\pm$ 0.3	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1
22:01		0.1 $\pm$ 0.1	0.4 $\pm$ 0.0	0.1 $\pm$ 0.1							
16:2 n-4	4.4 $\pm$ 1.2	3.7 $\pm$ 0.1	3.3 $\pm$ 1.4	3.9 $\pm$ 2.7	1.8	0.6 $\pm$ 0.1	1.3 $\pm$ 0.1	0.9 $\pm$ 0.1	0.4 $\pm$ 0.1	1.0 $\pm$ 0.1	0.9 $\pm$ 1.0
16:3 n-4	14.1 $\pm$ 3.5	10.4 $\pm$ 0.1	0.2 $\pm$ 0.3	3.3 $\pm$ 4.4	5.8		0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1
16:04	2.1 $\pm$ 0.6	2.6 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2	0.9 $\pm$ 0.1	0.7 $\pm$ 0.9	0.9 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
18:2 n-6	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.9 $\pm$ 0.1	0.8 $\pm$ 0.1	0.9	16.4 $\pm$ 0.8	4.4 $\pm$ 0.3*	3.4 $\pm$ 0.1*	2.9 $\pm$ 0.4	1.8 $\pm$ 0.1	3.1 $\pm$ 0.2
18:3 n-6	0.2 $\pm$ 0.0	0.1 $\pm$ 0.1	2.1 $\pm$ 0.1	2.5 $\pm$ 0.2	2	0.9 $\pm$ 0.1	0.4 $\pm$ 0.1	0.1 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1
20:3 n-6			0.4 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2			0.1 $\pm$ 0.1	1.6 $\pm$ 0.3	0.4 $\pm$ 0.0	0.3 $\pm$ 0.1
20:4 n-6	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	10.7 $\pm$ 0.4	4.4 $\pm$ 0.6*	5.8	1.5 $\pm$ 0.2	2.4 $\pm$ 0.1	4.0 $\pm$ 0.1	6.9 $\pm$ 1.3	7.3 $\pm$ 0.2	6.1 $\pm$ 0.5
18:3 n-3	0.6 $\pm$ 0.1	0.4 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.1		8.8 $\pm$ 0.2	9.2 $\pm$ 0.4	5.8 $\pm$ 0.1	0.5 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1
18:4 n-3	1.7 $\pm$ 0.4	1.7 $\pm$ 0.0	0.5 $\pm$ 0.1	1.5 $\pm$ 0.1	0.9	10.7 $\pm$ 0.3	20.9 $\pm$ 1.4*	17.4 $\pm$ 0.1*		0.1 $\pm$ 0.1	0.2 $\pm$ 0.2
20:4 n-3		0.3 $\pm$ 0.4		0.2 $\pm$ 0.1		0.1 $\pm$ 0.1	0.1 $\pm$ 0.1		0.1 $\pm$ 0.1		
20:5 n-3	31.0 $\pm$ 7.3	24.3 $\pm$ 0.5	11.7 $\pm$ 0.7	17.3 $\pm$ 1.5*	16.6	0.5 $\pm$ 0.1	0.9 $\pm$ 0.1	0.6 $\pm$ 0.4	30.1 $\pm$ 1.9	41.6 $\pm$ 0.5*	38.2 $\pm$ 0.3*
22:5 n-3	0.8 $\pm$ 1.2							0.1 $\pm$ 0.1			
22:6 n-3	2.1 $\pm$ 0.6	1.7 $\pm$ 0.3	1.5 $\pm$ 0.1	2.4 $\pm$ 0.1	2	11.3 $\pm$ 1.4	17.4 $\pm$ 1.4*	22.5 $\pm$ 0.4*			
EPA + DHA	33.1 $\pm$ 7.9	26.0 $\pm$ 0.8	13.2 $\pm$ 0.8	19.7 $\pm$ 1.6*	18.6	11.8 $\pm$ 1.5	18.3 $\pm$ 1.5*	23.1 $\pm$ 0.8*	30.1 $\pm$ 1.9	41.6 $\pm$ 0.5*	38.2 $\pm$ 0.3*

Fig. 5 Content of total carbohydrates and β -glucans of the microalgae cultivated by semi-continuous culture at different relative growth rates (4%, 40%, and 60% of $\mu_{\max}$). Means with SE bars ($n = 3$). Asterisk indicate significant differences ($p < 0.05$)

species investigated. As for total carbohydrates, only in *C. muelleri*, the β -glucan content increased with the reduced relative growth rate.

Productivity of lipids, EPA, DHA, carbohydrates, and β -glucans

The productivity of total lipids in all species investigated varied with the relative growth rate and was highest when the algae were cultivated at 40% of the maximum specific growth rate (Fig. 6). Highest production yield of lipids was found in *T. lutea* cultivated at 40% relative growth rate, with 14 mg total lipid L⁻¹ culture volume per day.

Similarly to the total lipids, highest production yield of both EPA and DHA was obtained when the algae were cultivated at 40% relative growth rate (Fig. 6). The highest production yield of EPA was found in *N. oculata* at 40% relative growth rate, with 1.7 mg L⁻¹ culture volume per day. The highest production yield of DHA was found in *T. lutea* at 40% relative growth rate, with 0.8 mg L⁻¹ culture volume per day.

The production yield of total carbohydrates and β -glucans varied with the species and the growth rate

(Fig. 7). The two diatoms, *C. calcitrans* and *C. muelleri*, showed different patterns of production yields of total carbohydrates and β -glucans with different relative growth rates. *Chaetoceros calcitrans* obtained similar total carbohydrate content at 40 and 60% relative growth rates, while *C. muelleri* got significant highest production yield of both total carbohydrates and β -glucans at medium (40%) relative growth rate, and lower yields at 4% and 60%. A similar pattern was observed for total carbohydrates in *N. oculata* as well, with the highest yield at 40% relative growth rate. *T. lutea* had an increased yield of both total carbohydrates and β -glucans with increasing relative growth rates.

Discussion

The growth rates of microalgae are species-specific and also to a large extent depend on the cultivation conditions. Besides nutrients and light, temperature has been found to have a pronounced effect on the growth of microalgae, as shown in the present study. Most of the seven marine microalgae species grew between 16 and 24 °C, except for *C. calcitrans*. This is consistent with previous studies, which found that most microalgae species can tolerate a wide range of temperature between 15 and 30 °C (Ras et al. 2013; Chaisutyakorn et al. 2018). The maximum specific growth rate varied with the species and cultivation temperature. Out of the seven species investigated, *S. costatum* showed the highest specific growth, followed by *Tetraselmis* sp., indicating the great potential of these two species for commercial production. The optimal temperatures for the two diatoms (*C. calcitrans* and *C. muelleri*) were found to be 16 and 24 °C, respectively, with specific growth rates at 1.6 and 2.1 day⁻¹, respectively. These values are much higher than those found in a previous study for *Chaetoceros* sp. (0.54 day⁻¹) (Chaisutyakorn et al. 2018). *Tisochrysis lutea* was found to grow best at 24 °C, in agreement with Renaud et al. (1995), who found that the optimal temperature for *T. lutea* was 25 °C. In the present study, the highest specific growth rate was 1.73 day⁻¹, twice the value reported by Renaud et al. (1995). The optimal temperature for *N. oculata* was 16 °C, while other studies showed that *N. oculata* grew best at 20–25 °C (Converti et al. 2009; Wei et al. 2015; Chaisutyakorn et al. 2018). This indicates that the adaptability to temperature conditions may vary even within the same microalgae species. *N. oculata* showed a lower specific growth rate compared to other six species; however, this value is still much higher than those reported by Converti et al. (2009) and Chaisutyakorn et al. (2018) (0.13–0.33 day⁻¹). The growth rate of the species was also found to affect the productivity of the species (Converti et al. 2009); therefore, choosing the optimal cultivation

Fig. 6 Production yields (mg L⁻¹ culture volume day⁻¹) of total lipids (upper panel), and EPA and DHA (lower panel) of the microalgae cultivated at different relative growth rates (4%, 40%, and 60% of μ_{max}). Means with SE bars ($n=2-3$). Asterisks indicate significant differences ($p < 0.05$)

Fig. 7 Production yields of total carbohydrates and β -glucans (mg L^{-1} culture volume day^{-1}) of the microalgae cultivated at different relative growth rates (4%, 40%, and 60% of μ_{max}). Means with SE bars ($n=3$). Asterisks indicate significant differences ($p<0.05$)

temperature for the strain of species is important for the production yield of the culture.

The cellular content of carbon and nitrogen varied with the density of the culture for all species investigated in this study, in agreement with earlier studies (Pérez-Morales et al. 2015; Roopnarain et al. 2015). The N/C, P/C, and N/P ratios for the microalgae cells in the stationary phase in the present study were lower than what could be expected for nitrogen- and phosphorus-saturated cells (Sakshaug et al. 1983; Sakshaug and Olsen, 1986). The N/P ratio (by weight) of the growth medium used was 11 (F/2: Guillard and Ryther 1962), with corresponding values of the microalgae cells in the stationary phase (ranging 11–18) above the ratios for oceanic particulate matter, called the Redfield ratio (N/P = 7.1 by weight, Redfield et al. 1963). This indicates that the algae cultures in the present study were nutrient-limited and limited more by phosphorus than by nitrogen. The decreasing N/C ratio with increasing cell numbers was probably a result of constant lipid and carbohydrate synthesis, together with reduced protein synthesis due to exhaustion of nutrients in the growth media (Siron et al. 1989; Sukenik and Livne, 1991). The N/C ratio of *N. oculata* behaved differently from that of the other species, increasing up to a certain density, and then decreasing with further cellular density increases. Similar results were reported by Flynn et al. (1993), suggesting that this species can assimilate and store available nitrogen.

The total lipid content of the algae species investigated varied between 10 and 34% of dry matter, and all species obtained increased lipid content with reduced relative growth rates due to nutrient limitation. This is in agreement with earlier reports (Reitan et al. 1994; Brown et al. 1997;

Shokravi et al. 2020). The highest lipid content was found in *N. oculata* grown at 4% relative growth rate, followed by *C. muelleri* and *T. lutea*, both also at 4% relative growth rate. The lipid accumulation in the species investigated can be a result of reduced cell division and protein synthesis due to reduced availability of nutrients, as well as of increased neutral lipid synthesis when the algae became nutrient-limited (Sukenik and Livne 1991; Lombardi and Wangersky 1995; Wang et al. 2019). This indicates the great potential of using these three species for lipid production under nutrient limitation.

The content of n-3 PUFA in microalgae is of great interest when searching for aquafeed resources (Chauton et al. 2014). The fatty acid profiles of the species investigated showed relatively close taxonomic similarities, where the two diatom species (*C. calcitrans* and *C. muelleri*) obtained high content of 20:5 n-3, *T. lutea* had high content of 22:6 n-3; and *N. oculata* was rich in 20:5 n-3. Our results show that out of the species investigated, only *T. lutea* can be considered a source of DHA (22:6 n-3), with a quantitative DHA content of 11 to 15 mg g^{-1} DW. Our results show that *N. oculata* is a promising species for cultivation for EPA-rich biomass, with 38–50 mg g^{-1} DW, consistently with earlier reports (Delaunay et al. 1993; Reitan et al. 1994; Zhukova and Aizdaicher 1995).

The change in relative growth rate of the microalgae revealed different effects on the relative content of fatty acids. The per cent content of EPA in *N. oculata* and *C. muelleri* and content of DHA in *T. lutea* increased with increased relative growth rate.

Much attention has been paid to the content of total carbohydrates and β -glucans in diatoms (Granum and Mykkestad 2002; Chiovitti et al. 2004). In the present study, the highest

content of total carbohydrates was found in *C. muelleri*, and highest β -glucans was found in *C. calcitrans*. Among the species investigated, only *C. muelleri* accumulated both carbohydrates and β -glucans with decreasing relative growth rates. The other species did not accumulate carbohydrates with decreasing relative growth rates, as they had for total lipids, meaning that the accumulation of carbohydrates and that of lipids follow different metabolic pathways. The high content of β -glucans in *C. muelleri* was also reported by Størseth et al. (2004). β -glucans are increasingly used as immunostimulants in aquafeed because of their ability to be incorporated directly into aquafeeds and to enhance the immune system in finfish (Bruce and Brown 2017).

Microalgae are considered one of the most promising feedstocks for a sustainable supply of commodities and specialties for both food and non-food production (Singh and Gu 2010; Wijffels et al. 2010; Milledge 2011; Draaisma et al. 2013). Interestingly, all four microalgae species investigated obtained the highest production yield of lipids, calculated as the content of lipids in the culture volume harvested per day, when cultivated at a relative growth rate 40% of the maximum specific growth rate. High productivity is a key factor when selecting species and cultivation conditions for producing biomass and lipids for use in fish feed and other commodities (Chauton et al. 2014). Microalgae cultivation is increasingly recognised as a suitable technology for the production of the omega-3 PUFA, cultivated both under heterotrophic conditions (Oliver et al. 2020) and under traditional phototrophic conditions as well as photoheterotrophic and mixotrophic culture (Hamilton et al. 2014; Ryckebosch et al. 2014; Piasecka et al. 2020). The production yield of EPA and DHA also varied with the growth rate, and the highest production yield of EPA was found in *N. oculata* cultivated at 40% $\mu_{\max}$, whereas the highest production yield of DHA was found in *T. lutea* cultivated at 40% $\mu_{\max}$. The Marine Ingredients Organization, IFFO, considers microalgae the most promising and sustainable alternative source of EPA and DHA for fish feed (Chauton et al. 2014). However, the productivity of EPA and DHA from microalgae depends on the biomass production and the fatty acid content in the dry matter of the algae. Therefore, production of EPA and DHA from microalgae is a compromise between maximising the lipid content by modulating growth conditions without lowering biomass production, as discussed by Chauton et al. (2014).

The productivity of carbohydrates and β -glucans again showed higher values in *C. muelleri* and *N. oculata* when grown at 40% $\mu_{\max}$, while increasing with increased relative growth rate in *C. calcitrans* and *T. lutea*. Out of the four species, *C. calcitrans* showed the highest productivity of both carbohydrates and β -glucans when grown at 60% $\mu_{\max}$. Based on this result, *C. calcitrans* is a promising microalgae strains for producing β -glucans as immunostimulants for aquafeed.

Again, it is essential to maximise the carbohydrate content by manipulating the growth conditions without lowering the biomass production to achieve the maximum production of β -glucans.

Conclusion

Selected microalgae species belonging to the Bacillariophyceae, Haptophyceae, Eustigmatophyceae, and Prasinophyceae were characterised for cellular content of carbon and nitrogen, as well as production yields of lipids, fatty acids, total carbohydrates and β -glucans. Cellular content of carbon and nitrogen showed species-specific patterns with increasing cell numbers in the culture. The total lipid content of *C. muelleri*, *C. calcitrans*, *N. oculata*, and *T. lutea* increased with reduced relative growth rate in semi-continuous cultures and was highest in *N. oculata*. However, the highest productivity of lipids was found in *T. lutea* grown at a growth rate of 40% of the maximum specific growth rate. The content of polyunsaturated fatty acids varied between the species investigated, with high content of EPA in *C. muelleri*, *C. calcitrans*, and *N. oculata*, and high content of DHA in *T. lutea*. The β -glucan fraction of the carbohydrates was highest in *C. muelleri* and *C. calcitrans* and was very low in *N. oculata*. Among the species investigated, *C. muelleri* is a promising strain for β -glucan production due to its high yield of β -glucans for cells cultivated at a 40% relative growth rate.

Acknowledgements This research was carried out at NTNU Department of Biology in Trondheim, Norway.

Author contribution The authors Kjell Inge Reitan (KIR), Gunvor Øie (GO), Håvard Jørgensen (HJ), and Xinxin Wang (XW) have contributed to this paper. Conceptualisation: KIR, GO, and HJ; Methodology: KIR and HJ, Formal Analysis: HJ and GO; Investigation: KIR, HJ, GO, and XW; Resources: KIR; Data Curation: KIR, GO, and HJ; Writing—Original Draft Preparation: KIR and XW; Writing—Review and Editing: KIR and XW; Visualisation: KIR; Supervision: KIR and GO; Project Administration: KIR; Funding Acquisition: KIR.

Funding Open access funding provided by NTNU Norwegian University of Science and Technology (incl St. Olavs Hospital - Trondheim University Hospital). The work was supported by the Norwegian Research Council (project nr 127964/122).

Data Availability The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Declarations

Conflict of interest The authors declare no competing interests.

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