Short communication

Temperature dependent growth rate, lipid content and fatty acid composition of the marine cold-water diatom *Porosira glacialis.*

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Abstract: In this study, the northern cold-water marine diatom *Porosira glacialis* was cultivated in a pilot-scale mass cultivation system at 5 different temperatures (-2 to 12 °C), in order to evaluate temperature-dependent growth rate (*in vitro* Chl *a*), lipid content (Folch's method) and fatty acid (FA) composition (GC-MS) in the exponential growth phase. We found that *P. glacialis* has a wide temperature range, with maximum growth at 12 °C and positive growth even at sub-zero water temperatures. The lipid content was inversely correlated with temperature, peaking at 33.4 $\pm$ 4.0% at 2 °C, and was highly desaturated independently of temperature; PUFA content varied from 71.50 $\pm$ 0.88% at 12 °C to 82 $\pm$ 0.64% at -2 °C. EPA was the main FA at all temperatures (31.0 $\pm$ 0.7 – 40.4 $\pm$ 1.2% of total FAs).

Keywords: Diatom; lipid; desaturation; psychrophilic.

1. Introduction

Microalgae are the main primary producers of the world's aquatic environments. Present in all habitats containing water, they display high physiological, chemical and morphological diversity, and are the fastest growing photoautotrophic organisms on the planet. Diatoms are the largest group of microalgae with an estimated 100,000 species worldwide [1]. They are the dominant primary producers in temperate & cold areas [2]. While their structural lipochemistry is similar to that of green algae and higher plants, they are distinguished by their ability to synthesize highly unsaturated fatty acids of more than 18 carbons [3], such as EPA and DHA to serve as structural components in membranes or as precursors for metabolites, which regulate biological functions [4]. Generally referred to as long-chained polyunsaturated fatty acids (LC-PUFAs), these fatty acids are in high-demand by aquatic and terrestrial animals and are preserved as they pass through the

food chain [5, 6]. In humans, studies have demonstrated the positive health-effects of LC-PUFA intake [7-9], and different agencies recommend an intake of approximately 500 mg EPA+DHA day^{-1} [10].

Many studies have investigated diatom lipid allocation as a function of temperature. As a general rule, there is an inverse relationship between temperature and degree of desaturation [11-18], as the main functions of fatty acids is to regulate membrane fluidity in response to changing temperatures. However, as species are inherently different in their environmental adaptations, environmental effects on fatty acid allocation must be explored for each species independently. In later years, much of the research on diatom lipids has moved from an ecological focus to an industrial one, seeking to uncover oleaginous species with potential for production of biodiesels and/or valuable PUFAs [19-22]. These studies regularly focus on batch cultivated warm-water strains of small Chlorophyta and Cyanophyta species with low iodine values. There is very little research on psychrophilic diatoms cultivated in very cold environments, or the technology required to perform large scale industrial cultivations in areas with pronounced seasons. Nevertheless, studies from polar regions have revealed highly unsaturated fatty acid compositions in diatom-dominated microalgal communities [23-26], making them excellent candidates for LC-PUFA production.

Today, our main sources of EPA and DHA are marine fish, of which approximately 1 million tonnes of fish oil are produced annually. Of these, the aquaculture sector uses about 75% [27]. The annual capture of wild fish has, however, stagnated between 80 and 100 million tonnes in the last 30 years, while the aquaculture production has grown from near negligibility to contributing more than 40% of the world's total fish production in 2014. This has had obvious implications for the composition of aquaculture feed; In 2013, Norwegian salmon producers were substituting up to 82% of the fish meal and 89% of the fish oil normally used in feed with terrestrial products [28]. Although this has no apparent negative effect for the growth of the salmon, it reduces the nutritional value for consumers by lowering the amount of n-3 LC PUFAs in the fillet [29, 30], and requires large areas of arable land that otherwise could be utilized for human food. Diatoms have an immense potential as feed for the salmon industry [31, 32], and could be the substitute for conventional sources if competitive large-scale production of LC-PUFA rich species can be achieved. Naturally, a potential salmon feed from diatoms should resemble (or improve upon) the nutrient content of the fish which it is meant to replace, and we believe the simplest way to achieve this is to harvest the diatom in the exponential growth phase. In this

study, a large cold-water centric diatom, *Porosira glacialis*, was cultivated in a nutrient replete environment at 5 different temperatures (-2, 2, 4, 8 and 12 °C), in order to investigate the effect of temperature on the growth rate, total lipid and fatty acid composition during the exponential growth phase.

2. Materials and Methods

2.1 Chemicals

All chemicals were obtained from Sigma Aldrich (Sigma Aldrich, St. Louis, Mo, USA) unless otherwise stated. Guillard's F2 Marine water enrichment solution (50x) was used for cell cultivation. Ethanol (96%) and 10% hydrochloric acid (Merck KgaA, 64271 Darmstadt, Germany) were used in Chlorophyll *a* (Chl *a*) extractions and quantification. Dichloromethane (99.9%), methanol (99.8%), sodium chloride (Merck KgaA) and sulfuric acid (95-97%) were used in lipid extractions and fatty acid derivation prior to GC-MS analysis.

Hexane (99%) pro analysis was used to dissolve the fatty acid methyl esters (FAMEs) before GC-MS analysis. Standards of the fatty acids 10:0, 12:0, 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-12, 18:2n-6, 18:3n-3, 20:0, 20:1n-9, 20:3n-6, 20:4n-6, 20:5n-3, 22:1n-9, 22:6n-3 and 24:1n-9 with purity >99% were purchased from Sigma Aldrich, while standards of the fatty acids 16:2n-4, 16:3n-3, 18:4n-3 with purity >98% were purchased from LGC Standards (Teddington, UK). The internal standards used for quantification were 14-methylhexadecanoic acid and 19-methylarachidic acid (>99%, Sigma Aldrich).

2.2 Species

The monoculture of *Porosira glacialis* used in this experiment was originally isolated from a sediment sample collected in the Barents Sea (N 76° 27.54', E 033° 03.54') during a 2014 cruise and identified using light microscopy and the diatom key from Tomas [33].

2.3 Cultivation & Harvesting

All cultures were cultivated in filtered seawater (32 PSU) added 4 mL L^{-1} Guillard's F2 Marine water enrichment solution (50x) and 12.32 µM sodium metasilicate nonahydrate (≥98%). To ensure sufficient CO_2 supply, all cultures were mixed by aeration (2-3 L min^{-1}) for the entirety of the experiment. To obtain samples cultivated at 2, 4, 8 and 12 °C, monocultures of *P. glacialis* were cultivated in 100 L clear plexiglass columns, placed in a light- and temperature-controlled room set to each temperature consecutively, beginning with 12 °C, then 8, 4

and 2 °C, respectively. The cultures received 14 hours of daylight per day using three North Light LED-strips (12 V) placed at regular intervals around the column, at a scalar irradiance of ca. 66 µmol m^{-2} s^{-1}. To obtain a sample cultivated at -2 °C, a 600 L plexiglass column was placed outside during February of 2016, using two LED work lights to achieve comparable irradiance conditions (measured each day). A larger volume was necessary to prevent sudden changes in the culture temperature as a result of the large variations in ambient air temperature during this time of year. The temperature was maintained by leading warm water (60 °C) through a silicone tube wrapped around the base of the column whenever necessary. All cultures' growth was monitored daily, both by cell-counts in 2 mL Nunc-chambers (Nunc A/S, Roskilde, Denmark) and by *in vitro* Chl *a* - extraction and quantification using the method described by Holm-Hansen and Riemann [34], using ethanol instead of methanol as the extractant. In vitro Chl *a* was used as a proxy for biomass. It is well known that Chl *a* may vary with cultivation conditions, especially irradiance, and the culture's growth phase. In the present experiment this error is assumed to be of minor importance, as we applied the same species throughout the experiment, and the cultures were never allowed to exit the exponential growth phase. Experiments prior to the present one also examined correlations between Chl *a* and cell concentrations, and this yielded the following results: Pearson r=0.67 at $p<0.05$, Min=39 µg Chl *a* L^{-1} and Max 886 µg Chl *a* L^{-1}, n=594. The *in vitro* Chl *a* measurements were used to calculate growth rates, visually represented both as the daily change of Chl *a*, and as doublings day^{-1} (µ) calculated from the increase in Chl *a* from the formula:

$$\mu = (\mathrm{Log2}(t_x) - \mathrm{Log2}(t_0))/x, \quad (1)$$

Where t equals the Chl *a* content and x equals the total number of days for each cultivation.

At each designated cultivation temperature, the photobioreactor was initiated with 20 L of stock culture and diluted every 1-2 days (determined by the cell counts; the culture density was never diluted below 1 million cells L^{-1} and never allowed to surpass 4 million cells L^{-1}) and harvested after 3-4 days of exponential growth at 100 L volume. The harvesting was performed by passing 80 L of culture through a plankton net (Sefar Nytal®), and collecting the biomass with a rubber spatula in 50 mL Falcon® tubes, which were subsequently placed in the freezer at -80 °C. The remaining 20 L of culture in the plexiglass-column was used as the new stock culture

for the next cultivation temperature. With this set-up, the culture was given a minimum of 72 hours to adapt to each temperature.

2.4 Lipid extraction & derivatization

The extraction procedure was adapted from Folch, Lees [35], using dichlormethane:methanol as the extractant [36]. Briefly, samples were freeze-dried and divided into triplicates of approximately 100 mg in 15 mL centrifuge tubes (Falcon). Each tube was added 2 mL dichloromethane:methanol (2:1 v/v) and 2 mL 5% (w/v) NaCl in MiliQ water. The tubes were gently shaken for 30 seconds by hand and then centrifuged for 4 minutes at 2000 G (Heraus Multifuge 1S-R, Germany). Following centrifugation, the organic phase was transferred to a pre-weighed and marked dram glass. The extraction procedure was repeated twice for each sample in order to increase the yield. Following extraction, the organic phase was evaporated under nitrogen and the total lipid was determined gravimetrically, as percentage of ash-free dry weight (AFDW). Due to lack of material, AFDW-calculations were based on samples from the same species harvested in the exponential growth phase, determined by combustion in a muffle furnace (AFDW = 46.04 $\pm$ 0.33% of dry weight, n=3). Finally, the extracted lipids were dissolved in dichloromethane:methanol (2:1 v/v) to a concentration of 10 mg mL^{-1} and esterified using a method adapted from Stoffel, Chu [37] using sulfuric acid as the catalyst:
Triplicate dissolved extracts from each cultivation temperature (3 x 100 µL) was transferred to a test tube (Duran®) and added 100 µL internal standard (0.1 mg mL^{-1}) and 800 µL dichloromethane. The samples were then added 2 mL 10% H_2SO_4 (v/v) in methanol and placed at 100 °C for 1 hour, before 3 mL hexane and 3 mL 5% (w/v) NaCl in MiliQ-water was added and the mixture shaken thoroughly. The resulting organic phase containing the fatty acid methyl esters (FAMEs) was transferred to and evaporated in GC-MS tubes (Waters TruView ™ LCMS Certified Vials), before being redissolved in 500 µL of hexane.

2.5 GC-MS

The method used here was originally described in Artamonova et al. [38]. The GC-MS analyses were performed on a Waters Quattro Premier GC (Waters, Milford, MA, USA) equipped with a 30-meter-long fused silica Restek FameWax 0.25 mm column with 0.25 µm film thickness. The injector temperature was set to 250 °C, the injection was in splitless mode and He 6.0 (Aga, Oslo, Norway) was used as carrier gas with a 1.0 mL min^{-1}

constant flow. One μl of the sample was injected, and the initial temperature on the column was 50 °C. The initial temperature was maintained for 3 minutes, and then increased by 2 °C per minute until the final temperature of 250 °C was reached. The final temperature was maintained for 10 minutes and the total runtime was 113 minutes. The GC-MS interface was kept at 250 °C, and the mass spectrometer was equipped with an EI ionization source operated at 70 eV. The MS source temperature was 210 °C and the trap current was 200 μA. The MS was run in full scan mode scanning m/z 150-400 with a scan time of 0.5 seconds. Each replicate was injected thrice, so that the final results are averages of 9 individual measurements for each cultivation temperature (triplicates measured three times each).

The quantification was based on relative peak area between the different analytes and the two internal standards. The choice of internal standard was based on retention time, and the FAs 10:0, 12:0, 14:0, 16:0, 16:1, 16:2, 16:3, 16:4, 18:0 and 18:1 were quantified with 14-methylhexadecanoic acid as internal standard, while the remaining longer chained FAs were quantified with 19-methylarichidic acid as internal standard. Standard curves were set up in the concentration range 10 - 1000 ng mL^{-1}. The quantification of 16:4 was based on the standard curve for 16:3 and gives an approximate value (while the relative amount between different samples is correct), as it was not possible to find a commercial supplier of 16:4 during the project period. The method does not distinguish between the position of the double bonds in mono-, di-, tri- and tetraenes where there is more than one possible configuration, e.g. 18:1n-9 will not be separated from 18:1n-12. All standards for the standard curve and the algae samples went through the same derivatization method to obtain FAMEs before analysis.

2.6 Statistical analyses

All data was presented as means and their standard deviations, either in tables or as figures with error bars representing one standard deviation. All data groups were subjected to normality tests (Shapiro Wilk). Normally distributed data was analysed with ANOVA, while data not normally distributed was analysed with a Mann-Whitney test, and post hoc Tukey's or Game-Howell tests, according to the error variance determined by a Levene's test,. Groups were determined homogenous at a significance level of >0.05. All analyses were performed using IBM SPSS v24 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1 Growth rate & total lipid

The algal culture displayed positive growth at all temperatures, and temperature had a significant effect on the total lipid content, see Table 1 for growth rates and lipid content, and Figure 1 for the daily increase in Chl *a*. A Levene's test revealed high error variance between all groups of Chl *a*-measurements (p=0.013). The highest average growth rate was measured at 12 °C (0.41 μ), but this measurement was not statistically different from the growth rates at 2, 4 and 8 °C (p=0.46, 0.652, 0.221, respectively). The lowest growth rate was measured at -2 °C (0.17 μ). The biomass measurements at -2 °C was influenced by water freezing; ice formation in the bioreactor trapped cells, resulting in an apparent reduction of biomass on day 1 and 2 (see Figure 1). On day 3, however, the culture was thoroughly mixed and the sample collected for Chl *a* measurement left to thaw before being filtered, thereby revealing the true average growth from day 0-3. The highest lipid content was measured in the algae cultivated at 2 °C (33.4%), but this measurement was not statistically different from the total lipid in algae cultivated at -2 °C (28.4%). The lowest lipid content was measured in the algae cultivated at 12 °C (19.5%), but this measurement was not significantly different from 8 °C (22.0%) or 4 °C (22.8%).

Table 1: Growth rates and total lipid content (arithmetic mean ± SD, n=3) for Porosira glacialis (P.g.) at each cultivation temperature. The growth rate was calculated from the increase in chlorophyll a (Chl a) from the start of cultivation to the point of harvest, while total lipids were measured from samples taken at the time of harvest.

Temperature (°C)	-2	2	4	8	12
Growth rate (μ)	0.17 ± 0.01	0.36 ± 0.07	0.34 ± 0.01	0.33 ± 0.04	0.41 ± 0.07
Lipid content (% of AFDW)	28.4 ± 1.3	33.4 ± 4.0	22.8 ± 1.8	22.0 ± 1.1	19.5 ± 1.5

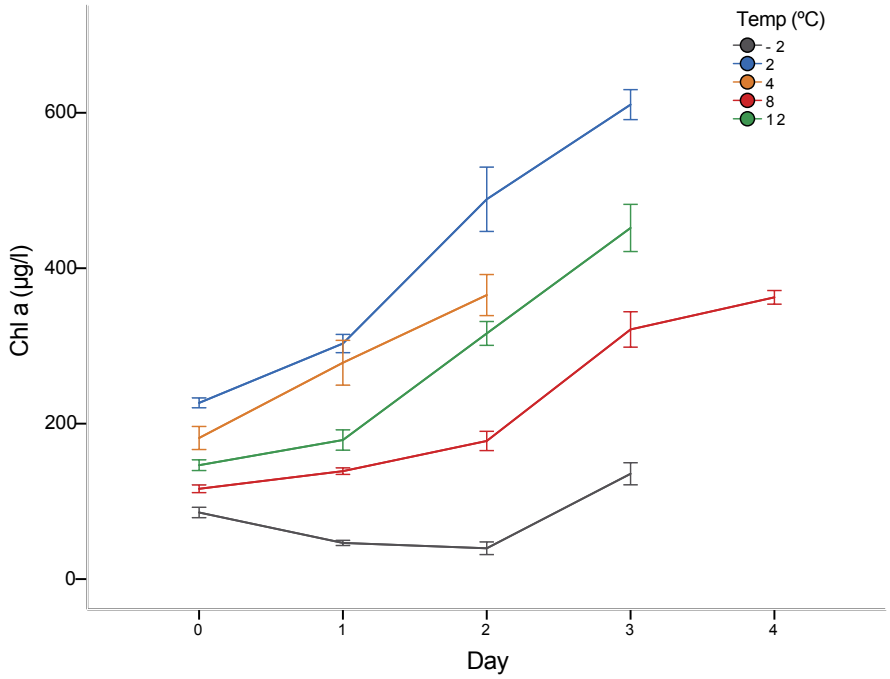

Figure 1: In vitro chlorophyll a (Chl a) daily increase at each cultivation temperature. Data shown is the mean ± SD, n=3. SDs are represented by T-bars.

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3.2 Desaturation

Temperature had an effect on the degree of fatty acid desaturation. Table 2 lists the relative amounts and standard deviation of all fatty acids measured. A total of 14 fatty acids were detected in the cultured diatom at all temperatures.

Table 2: Relative fatty acid content of Porosira glacialis (P.g.) at all treatment temperatures. Data shown are averages of replicates ± SD, n=3. TR=Trace values.

Fatty acid						
	14:0	3.7 ± 0.2	4.4 ± 0.2	5.7 ± 0.3	7.3 ± 0.2	7.8 ± 0.4
	16:0	5.3 ± 0.1	7.3 ± 0.3	8.0 ± 0.2	7.2 ± 0.3	7.4 ± 0.2
	16:1	8.7 ± 0.4	12.8 ± 0.4	10.3 ± 0.3	11.6 ± 0.2	11.6 ± 0.3
	16:2	2.09 ± 0.07	2.7 ± 0.1	2.68 ± 0.08	3.97 ± 0.05	4.7 ± 0.2
	16:3	8.6 ± 0.2	8.3 ± 0.3	16.5 ± 0.4	19.8 ± 0.2	16.3 ± 0.4
	16:4	14.4 ± 0.4	14.8 ± 0.5	10.5 ± 0.3	4.9 ± 0.2	4.8 ± 0.1
	18:0	0.23 ± 0.02	0.69 ± 0.09	0.90 ± 0.07	0.7 ± 0.1	0.95 ± 0.07
	18:1	0.18 ± 0.01	0.25 ± 0.02	0.44 ± 0.09	0.40 ± 0.03	0.39 ± 0.02
	18:2	0.25 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	0.26 ± 0.01	0.31 ± 0.01
	18:3	1.18 ± 0.05	1.8 ± 0.1	1.83 ± 0.05	1.65 ± 0.04	1.75 ± 0.06
	18:4	10.1 ± 0.4	9.6 ± 0.4	6.5 ± 0.3	5.3 ± 0.2	3.9 ± 0.2
	20:5	40.4 ± 1.2	32.6 ± 1.2	31.4 ±0.9	31.0 ± 0.7	33.8 ± 0.9
	22:6	4.9 ± 0.3	4.5 ± 0.3	4.9 ± 0.2	5.5 ± 0.3	6.0 ± 0.4
	24:0	*TR*	0.24 ± 0.05	0.31 ± 0.04	0.33 ± 0.09	0.4 ± 0.1
	Temperature (°C)	-2	2	4	8	12

Trends among individual fatty acids were observed; The amount of 14:0, 16:2 and 16:3 approximately halved from 12 to -2 °C. 14:0 decreased in increments as the temperature decreased (all changes were statistically significant). 16:2 decreased gradually, but more strongly at the transition from 8 to 4 °C, while for 16:3 the decrease was sudden during the transition from 4 to 2 °C. DHA (22:6) was also more abundant at 8 and 12 °C compared to the lower temperatures ($p<0.05$).

From 12 to 2 °C, 16:4 and 18:4 increased by 208% and 148%, respectively. At -2 °C, results were similar to those found at 2 °C and did not display the same increasing trend. For 16:4, the increase occurred between 8 and 2 °C, forming three significantly different subgroups; -2 and 2 °C (14.4-14.8%), 4 °C (10.5%), and 8 and 12 °C (4.9-4.8%). For 18:4, the decrease occurred in increments with increasing temperature (all measurements were statistically significant). The relative EPA content was highest in the algae cultivated at -2 °C (40.4%),

while all other measurements fluctuated between 31.0-33.8%. The relative contents of EPA at 2 and 12 °C were not statistically different (p>0.05).

3.3 SFA, MUFA & PUFA

Total values of, and trends among saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids are displayed in figure 2. The SFA content (Figure 2a) was lowest in the algae cultivated at -2 °C (10.43% of total FAs), and highest at 12 °C (16.48%). The SFA contents were statistically significant at all temperatures with the exception of 4 and 8 °C (p=0.061).

The MUFA content (Figure 2b), dominated by 16:1, was lowest in the algae cultivated at -2 °C (8.84%) and highest at 2 °C (13.31%). All MUFA contents were statistically significant at all temperatures apart from 8 and 12 °C (p=0.995).

The PUFA content (Figure 2c) was lowest in the algae cultivated at 12 °C (71.50%), and highest at -2 °C (82.0%). From -2 to 2 °C, the PUFA content was reduced by almost 8 percentage points, caused almost exclusively by the change observed in EPA. Two statistically similar groups were observed; 12 & 8 °C (p=0.71) and 4 & 0 °C (p=1.0).

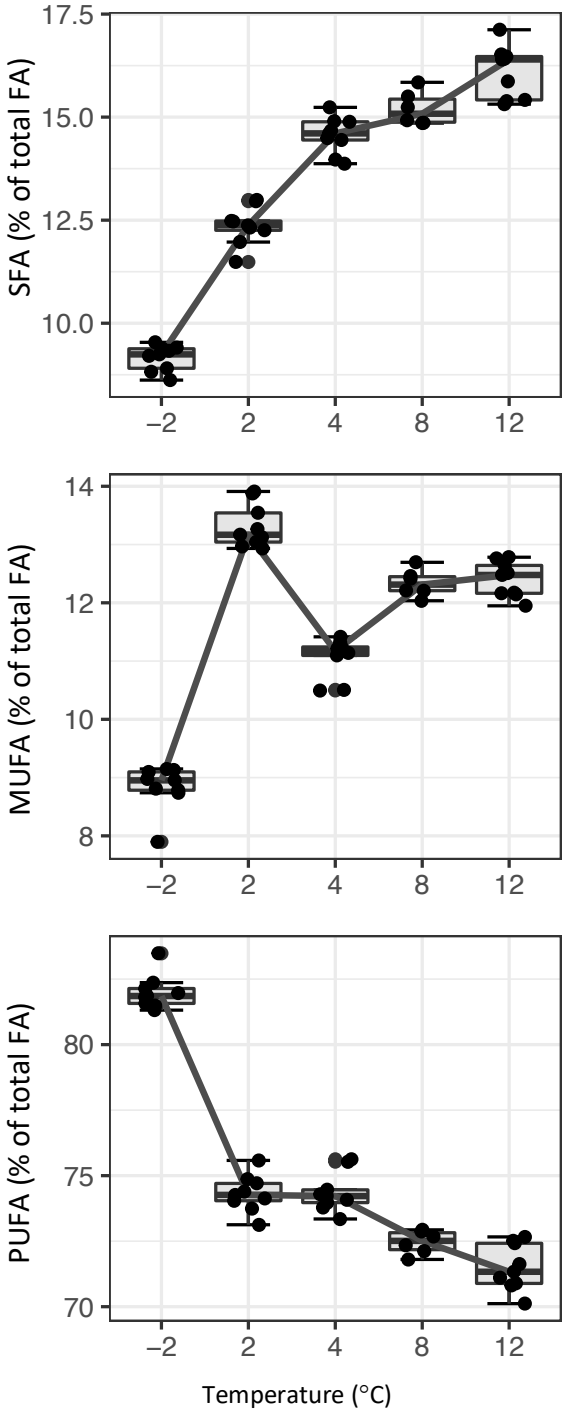

Figure 2: Trends among saturated (SFA, a), monounsaturated (MUFA, b) and polyunsaturated fatty acids (PUFA, c). Data shown is the mean ± SD, n=9. T-bars represent the SD for each sample.

4. Discussion

4.1 Temperature and growth

The algal culture displayed positive growth at all temperatures during the course of the experiment. The growth rates in table 1 showed highest and lowest growth at 12 and -2 °C, respectively, a result that follows the consensus of phytoplankton in general: Within a temperature range, diatom growth rates increase with temperature toward the species' optimum growth temperature [39-41]. A Tukey's test, however, revealed no statistical difference between growth rates at all temperatures other than -2 °C, indicating that from 2-12 °C the growth rate was independent of temperature. While this observation could be influenced by the large variation observed in the error variance of the measured growth rates, previous studies on cold-water diatom strains using comparable light regimes have also found that lowering temperatures within a species' natural temperature range do not necessarily slow growth: Teoh, Phang [12] found that the optimum cultivation temperature for an Antarctic strain of *Navicula* sp. was 4 °C ($\mu \approx 0.35$), with growth slowing drastically already at 6 °C. They did not try to cultivate at lower temperatures. Boelen, van Dijk [42] cultivated an Antarctic strain of *Chaetoceros brevis* and found higher growth rates at 3 °C compared to 7 °C ($\mu = 0.47$ and 0.41, respectively). In these studies, the differences in the growth rates were more pronounced than in our data, indicating that *P. glacialis* has a wider temperature range than both *Navicula* sp. and *C. brevis,* with a potential for yearlong cultivation in areas with pronounced seasons.

4.2 Temperature and lipids

The total lipid content was highest ($p<0.05$) in the samples harvested at the lowest temperatures. Other studies have found ambiguous effects of temperature on lipid content on diatoms [14, 15, 43], implying that responses to temperature are species specific and do not follow general trends. For the strain of *P. glacialis* studied here, the total lipid content was inversely correlated with cultivation temperature. The lipid content reported here is comparable to or higher than those found in other cultivated cold-water diatoms [12], but lower than those often reported for temperate and warm water cultivations [43, 44]. However, it is important to keep in mind that this strain of *P. glacialis* was harvested while still in the exponential growth phase, while lipid accumulation as observed in other studies is a result of the algae entering the stationary phase. The high lipid content often observed in such batch cultures comes at the expense of FA chain length and desaturation

[45] and protein content [46]. This has the unfortunate effect of reducing the versatility of the feed, as it would resemble those of terrestrial products such as soy- or rapeseed in FA composition, instead of offering a complete substitute for fish oil.

4.3 Desaturation

The degree of desaturation was also inversely correlated with temperature (see Figure 2), with SFAs increasing and PUFAs decreasing with temperature. Although the inverse correlation of PUFAs with temperature is strongly exaggerated by the large EPA content in the sample cultivated at -2 °C, the composition of the FA fraction did change notably with temperature: At both -2 and 2 °C, the five most abundant fatty acids were 20:5, 16:4, 18:4, 16:3 and 16:1. At 8 and 12 °C, the five most abundant fatty acids were 20:5, 16:3, 16:1, 16:0 and 14:0. At all temperatures, the five most abundant fatty acids constituted more than 75% of the total fatty acids. While this clearly shows that increasing the temperature increases saturation, it should be noted that the PUFA fraction dominated the fatty acids at all temperatures, constituting 71.49% of total FAs even at 12 °C. Furthermore, EPA was not observed to decrease with temperature from 2 to 12 °C, indicating that *P. glacialis* depends heavily on functional EPA during the growth phase, independently of temperature. These findings correlate well with a study by Gillan et al. [47], in which *Stauroneis amphioxys* was cultivated at 3 and 20 °C; while the ratio of the most desaturated fatty acids (16:4, 18:4, 20:5 and 22:6) to their equivalents with one less double bond was higher at 3 °C, there was no difference in the total amounts of PUFAs at the two growth temperatures.

Interestingly, the amount of 22:6 increased with temperature from -2 to 12 °C (4.9% at -2 °C to 6.0% at 12 °C, $p<0,05$), a result that is in direct opposition with other findings on diatoms [16, 48]. With a minimum content of 36.3% EPA+DHA at 4 °C, and a maximum content of 45.3% at -2 °C, this diatom contains far more LC-PUFA compared to most other autotrophic species of microalgae suggested for mass production [49]. Although the PUFA content was highest at -2 °C, the low growth rate observed and the increased difficulty associated with cultivation at this temperature reduces the potential for industrial production of PUFAs. However, by displaying growth at sub-zero temperatures, this strain represents an exciting potential for production of cold-adapted bioactive molecules for e.g. the pharmaceutical industry. In comparison, both 2 °C and 12 °C displayed the

highest growth rates, as well as high contents of EPA. Although there was some variation in the composition of the other PUFAs, the total PUFA content only varied by 2.92 percentage points from 2 to 12 °C. Based on these data, a high-quality feed especially rich in EPA can be produced at a large range of temperatures. Whether or not this production is economically feasible requires increased knowledge of the potential production and the associated costs in a large-scale production, which goes beyond the scope of this study. Therefore, future research should focus on optimizing growth or lipid content in an economically feasible manner, for example through increasing the relative concentration of CO_2 by addition of flue gas to the culture medium. While it would also have been interesting to investigate the growth and fatty acid composition of *P. glacialis* at higher temperatures, this specific strain has not been capable of maintaining growth at temperatures above 15 °C for a significant amount of time, also when temperatures have been gradually increased from lower temperatures.

4.4 Conclusion

To conclude, *P. glacialis* is a potential species for mass cultivation of diatoms. Its broad temperature range is well adapted for cultivation in cold areas with pronounced seasons and allows for yearlong cultivation at ambient sea-temperatures. The fatty acid composition was predominantly polyunsaturated, with EPA as the most abundant fatty acid at all temperatures. This makes *P. glacialis* an excellent source of marine fatty acids as a substitute for the conventional fish oil used in aquaculture feed, or as ingredients in other high-value products.

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Final approval of the version to be published: Jon Brage Svenning, Lars Dalheim, Hans Christian Eilertsen, Terje Vasskog.

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