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ORIGINAL PAPER

Effects of prolonged darkness and temperature on the lipid metabolism in the benthic diatom *Navicula perminuta* from the Arctic Adventfjorden, Svalbard

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Abstract The Arctic represents an extreme habitat for phototrophic algae due to long periods of darkness caused by the polar night (~4 months darkness). Benthic diatoms, which dominate microphytobenthic communities in shallow water regions, can survive this dark period, but the underlying physiological and biochemical mechanisms are not well understood. One of the potential mechanisms for long-term dark survival is the utilisation of stored energy products in combination with a reduced basic metabolism. In recent years, water temperatures in the Arctic increased due to an ongoing global warming. Higher temperatures could enhance the cellular energy requirements for the maintenance metabolism during darkness and, therefore, accelerate the consumption of lipid reserves. In this study, we investigated the macromolecular ratios and the lipid content and composition of *Navicula* cf. *perminuta* Grunow, an Arctic benthic diatom isolated from the microphytobenthos of Adventfjorden (Svalbard, Norway), over a dark period of 8 weeks at two different temperatures (0 and 7 °C). The results demonstrate that *N. perminuta* uses the stored lipid compound triacylglycerol (TAG) during prolonged dark periods, but also the pool of free fatty acids (FFA). Under the enhanced temperature of 7 °C, the lipid resources were used significantly faster than at 0 °C, which could consequently lead to a depletion of this energy reserves before the end of the polar night. On the other hand, the membrane building phospho- and glycolipids remained unchanged during the 8 weeks darkness, indicating still intact thylakoid membranes. These results explain the shorter survival times of polar diatoms with increasing water temperatures during prolonged dark periods.

Keywords Benthic diatoms · Arctic Ocean · Lipids · Storage products · Polar night · Dark survival · Global warming

Abbreviations

Cer	Ceramide
CH	Carbohydrates
Chol	Cholesterol
DAG	Diacylglycerole
DGCC	Diacylglycerylcarboxyhydroxymethyl-choline
DGDG	Digalactosyldiacylglycerols
DM	Dry mass
Eb	Ester bond
FA	Fatty acids
FAME	Fatty acid methyl esters
FFA	Free fatty acids
FTIR	Fourier transform infrared
GC–MS	Gas chromatography–mass spectrometry
HPLC	High-pressure liquid chromatography
IS	Internal standard
LPC	Lysophosphatidylcholine
MD	Mean deviation from the median
MGDG	Monogalactosyldiacylglycerols
MPB	Microphytobenthos
MS	Mass spectrometry
MUFA	Monounsaturated fatty acids
PC	Phosphatidylcholine

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PE	Phosphatidylethanolamine
PG	Phosphatidylglycerols
PI	Phosphatidylinositole
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
Si	Silicate
SQDG	Sulphoquinovosyldiacylglycerols
TAG	Triacylglycerols

Introduction

The Arctic ocean is for phototrophic organisms with respect to light availability an extreme habitat (Hop et al. 2002; Berge et al. 2015b). In Svalbard, the polar night lasts for about 4 months from the end of October to the mid of February. During this period, photoautotrophic production stops completely, while many heterotrophic organisms are surprisingly active (Berge et al. 2015a, b). Additionally, the long period of darkness can be further extended in the inner part of Svalbard's fjords due to sea ice formation during winter, although sea ice extent and break-up varies with season between April and July (Muckenhuber et al. 2016). Consequently, phototrophic algae can be exposed up to about 10 months to darkness or very low light conditions (Chapman and Lindley 1980; Dunton 1990). Diatom taxa from different polar habitats, such as sea ice, pelagial and microphytobenthos (MPB), are known to survive long periods of complete darkness (Bunt and Lee 1972; Palmisano and Sullivan 1983; Peters and Thomas 1996; Zhang et al. 1998; Schlie et al. 2011; Veuger and Van Oevelen 2011). The species-specific maximum survival periods range from 3 months to 3 years, whereby benthic diatoms showing the longest survival times (Antia 1976).

The water masses in the Svalbard fjords are in the winter months well mixed with water temperatures ranging between −1.2 and 1.5 °C (Iversen and Seuthe 2011; Pawłowska et al. 2011). Signals of a warming Arctic as reflected in raised surface air temperatures, a retreat and thinning sea ice cover, a longer melt season and increasing river discharges are observed since the 1980s (Serreze and Francis 2006; IPCC 2007; Bintanja and van der Linden 2013). Predictions of future surface air temperature increases prognosticate that particularly winter temperatures will increase much more markedly than summer temperatures. By the end of this century, winter air temperatures in the Arctic may increase by even up to 7 °C, while summer air temperatures might only increase by 2–4 °C (IPCC 2007; MacDonald 2010). Over the period 1971–2010, the ocean absorbed 90% of the climate change induced heat of the climate system, leading to averaged ocean warming of 0.11 °C per decade of the upper 75 m (IPCC 2014). This heat uptake by the surface ocean will continue and linked with the predicted surface air temperature increase (IPCC 2014). In the well mixed Svalbard fjords during winter times this could lead to temperature increases even at the sea floor.

Higher temperatures generally stimulate the metabolic activity of all organisms, which could reduce the dark survival potential of benthic diatoms. Cold water species exhibit significant longer dark survival times at low than under higher water temperatures (Smayda and Mitchell-Innes 1974; Antia 1976). Reeves et al. (2011) demonstrated for three Antarctic sea ice diatoms a reduced dark survival time at 10 °C compared to −2 °C, but no negative effect at 4 °C. The latter authors showed, for example, for *Fragilariopsis cylindrus* a maximum survival time of 60 days at both −2 and 4 °C, while a maximum value of only 7 days was observed at 10 °C.

The physiological state in which polar diatoms survive darkness and the underlying biochemical mechanisms are still badly understood. In diatoms different mechanisms have been described for long-term dark survival (McMinn and Martin 2013). Those include the utilization of stored energy products (Palmisano and Sullivan 1982), the reduction of metabolic activity (Palmisano and Sullivan 1982), formation of resting stages (Durbin 1978; McQuoid and Hobson 1996), and/or a facultative heterotrophic lifestyle (Lewin and Lewin 1960; Hellebust and Lewin 1977; Tuchman et al. 2006). The utilization of energy storage products, such as lipids (triacylglycerol) and carbohydrates (chrysolaminarin), could provide energy for the cellular maintenance metabolism during long periods of darkness. Experimental evidence concerning the usage of these storage products for long-term dark survival, however, is still rare.

Storage lipids consist of the neutral lipid triacylglycerol (TAG), a glycerine backbone esterified with 3 fatty acids (FA), which are deposited in densely packed lipid bodies located in the cytoplasm of the algal cell (Darley 1977; Hu et al. 2008). High proportions of such lipid droplets have often been detected in polar plankton and sea ice diatom taxa, particularly in late autumn prior onset of the polar night (Fryxell 1989; Fahl and Kattner 1993; Zhang et al. 1998). Other important lipid classes in diatoms are polar lipids (glyco- and phospholipids) and free fatty acids (FFA) (Dunstan et al. 1994). Polar lipids are common membrane constituents consisting of high proportions of polyunsaturated fatty acids (PUFA), whereas TAG have generally more saturated (SFA) and monounsaturated fatty acids (MUFA) (Sukenik and Wahnon 1991). Concentrations of FFA in diatoms seem to be highly species specific and can range from 0.4 to 26% of total lipids (Volkman et al. 1989; Parrish et al. 1991; Dunstan et al. 1994). The FA composition in diatoms

has been studied intensively (Kates and Volcani 1966; Ackman et al. 1968; Volkman et al. 1989; Dunstan et al. 1994), and reported as sensitive trophic and chemical marker for marine food webs (Kattner and Brockmann 1990; Parrish et al. 1991; Graeve et al. 1994) as well as an indicator for changes of environmental conditions (Kattner and Brockmann 1990).

In the present study, we examined the lipid content and composition in the benthic diatom *Navicula* cf. *perminuta* Grunow, isolated from an Arctic microphytobenthic (MPB) community from Adventfjorden (Svalbard), during a dark period of 8 weeks at two temperatures (0 and 7 °C). The temperatures are representative for average ambient winter sea temperatures in the fjord system and that of a predicted increase due to global warming. The following hypothesis were addressed: (1) the total lipid content in the diatom cells decreases with increasing period of dark exposure; (2) from all lipid classes in diatoms, TAG is preferentially used as storage product during darkness; and (3) under higher temperatures significantly more TAG is metabolized.

Material and method

For all experiments, we used a unialgal culture of the benthic diatom isolate *Navicula* cf. *perminuta* Grunow (strain ROS_AF06) from the Rostock stock collection of Arctic benthic diatoms. Shallow water sediment samples were taken in 2009 in Adventfjorden, Svalbard. *Navicula* cf. *perminuta* was isolated in 2010 from this microphytobenthic assemblage and established as unialgal culture. The species determination of the strain was morphologically and genetically (18S V4, *rbc*L) performed, but did not result in a clear taxon identification (Stachura-Suchoples et al. 2015). Algae were cultivated as batch cultures in 250 mL glass bottles in a climate chamber at 8.2 ± 1.4 °C with optimal light conditions of 25 µmol photons m^{-2} s^{-1} (Karsten et al. 2012) at a light–dark cycle of 16:8 h. We used Osram Daylight Lumilux Cool White lamps (L36W/840) (Osram, Germany) as light sources. Radiation measurements were carried out with a Li-Cor LI-190-SZ quantum sensor connected to a Li-Cor LI-250 Light meter (LI-COR Corp., USA). The growth medium was prepared from sterilized Baltic seawater (salinity of ~12) enriched with f/2 medium (after Guillard (1975), Sigma–Aldrich Chemie GmbH, Germany), salt (Sel marin hw professional, Wiegandt GmbH, Germany), and metasilicate (0.108 mmol l^{-1}, Sigma–Aldrich Chemie GmbH, Germany) resulting in seawater with a salinity of 34 and a pH of 8.6.

Experimental setup and sample preparation

The cultures were divided in two biological replicates for Fourier transform infrared (FTIR) spectroscopy, and in another four replicates for high-pressure liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) analysis. Every replicate was cultivated for several weeks to gain sufficient biomass, and subsequently aliquots in five subsamples for the corresponding sampling times. Afterwards, the resulting 30 culture flasks were maintained under light conditions (25 µmol photons m^{-2} s^{-1}) with fresh medium at 1.4 ± 0.8 °C and 8.2 ± 1.3 °C for a week. After one week, cells were, with growth rates of 0.32 d^{-1} at 1 °C and 0.5 d^{-1} at 8 °C (Schlie and Karsten 2016), in the 3rd and 4th generation, respectively, and should, therefore, be acclimated to the changed temperatures. Subsequently, culture flasks for the dark incubation experiment were transferred to total darkness for 1, 2, 4 and 8 weeks, respectively, at both temperature treatments of 0.4 ± 0.3 °C and 7.2 ± 0.8 °C. The control sample was taken from the light immediately prior transfer to the dark (=week 0). For FTIR spectroscopy, the algae were harvested by centrifugation at 4000*g* for 10 min. The algal pellets were washed with distilled water and shock frozen, lyophilized and stored at −70 °C until FTIR spectroscopy measurements. For HPLC and GC–MS the algal samples were suspended by gentle shaking and filtered on precombusted (450 °C for 6 h) and weighted glass fibre filters (GF6 glass fibre filters, <1.0 µm, Whatman GmbH, Germany). After lyophilisation, filters were weighted for dry mass (DM) determination and stored at −70 °C until chemical analysis.

FTIR: macromolecular composition

For FTIR spectroscopy (for details see Wagner et al. 2010), the lyophilized diatom cells were re-suspended with distilled water to concentrations of app. 7×10^5 cells μL^{-1}. A 2 µL aliquot per sample with 5 replicates were applied to a microtiterplate (HTS-XT module, Bruker, Germany) and dried for 10 min at 40 °C. FTIR transmission spectra were measured with a FTIR spectrometer (Vector22, Bruker, Germany) in the range between 4000 and 700 cm^{-1} (32 scans per sample, spectral resolution of 4 cm^{-1}) and analysed using OpusLab Software (Version 5.0, Bruker Optics, Germany). The measured cell spectra were corrected to the background spectra and baseline corrected by the rubber band method (64 data points). All cellular macromolecular changes are expressed as peak ratios (mean ± standard deviation) with respect to the silicate peak, assuming that shell size is not changing during the slowed metabolism in the dark.

Lipid extraction

For the separation of lipid classes and the FA analysis, total lipids of the benthic diatom were extracted three times with 4 mL dichloromethane:methanol (2:1, v/v) from lyophilized filters using an ultrasonic bath (Bandelin, Germany). 20 µL of the fatty acid methyl ester (FAME) 23:0 (1 µg/µL in cyclohexane) were added as an internal standard (IS). The lipid containing organic solvent was evaporated under nitrogen in a heat block (30 °C). The lipid extract residue was re-dissolved in 4 mL dichloromethane:methanol (2:1, v/v) and stored at −20 °C until further analysis.

HPLC: separation of lipid classes

For HPLC analysis of the major lipid classes, 3 mL aliquot of the respective sample was further prepared. The solvent dichloromethane:methanol was evaporated under nitrogen in a heat block (30 °C), the residue diluted in 50 µL cyclohexane and transferred to HPLC-vials. The lipid classes were separated with a HPLC system [monolithic silica column, 100 × 4.6 mm I.D., macropore size of 2 µm, mesopore size of 13 nm (130 Å), total porosity of >80%, drift tube temperature of 40 °C, 3.5 bar internal nitrogen pressure; Chromolith®Performance-Si, LaChrom Elite HPLC system, VWR, Germany] using a gradient programme in combination with three Eluents after Graeve and Janssen (2009). For detection, an evaporative light scattering detector (Sedex 75, Sedere, France) was utilised. Data acquisition was performed using LaChrom Elite software (version 3.1.7, VWR, Germany). Lipid classes were identified by comparison with known standards of animal and plant lipids [cerebroside, cholesterol, glycerol trioleate, oleyl alcohol, palmitic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, lysophosphatidylcholine (Larodan, USA); digalactosyldiglyceride, monogalactosyldiglyceride, phosphatidylglycerol, sulphoquinovosyldiglyceride (Lipid Products, England)]. We used for quantification calibration curves of the standard substances. Lipid classes in terms of DM [median ± mean deviation from the median (MD)] were additionally corrected with the IS 23:0 FAME.

GC–MS: separation of fatty acids

For GC–MS analysis of the FA, 1 mL aliquot of the extracted lipids was transferred to 12 mL tubes and evaporated under nitrogen to dryness. For transesterification 250 µL hexane and 1 mL of a 3% concentrated sulphuric acid in methanol were added to the dried extracts and heated for 4 h at 80 °C. Afterwards the FAMEs were extracted three times with 2 mL cyclohexane. The combined extracts were concentrated under nitrogen down to 80 µL volume and transferred to GC-Vials. FAMEs were subsequently analysed on a fused silica capillary column (WCOT; 60 m × 0.25 mm I.D.; film thickness 0.25 µm; liquid phase: DB-FFAP; J&W, Germany) with a HP 6890 gas–liquid chromatograph coupled to a 5970 Series mass selective detector (MSD; Hewlett–Packard GmbH, Germany). A temperature programme [60–160 °C (20 °C min^{-1}), 160–240 °C (3 °C min^{-1}) and 15 min hold] was used according to Kattner and Fricke (1986). The samples were injected at 60 °C in splitless mode, with helium as carrier gas. Quantification of FA was achieved by comparison of mass spectral responses of selected ions compared to those of the internal standard 23:0 FAME. FAMEs were identified by comparison of retention times with known standard mixtures, according to mass spectral data and NIST (National Institute of Standards and Technology) library. Peak areas were converted with the added IS to the absolute weight values (median ± MD), which were normalised to DM.

Statistical analysis

Statistical analyses were conducted using SPSS Statistics version 20 (IBM Inc.). If the data did not meet the assumption of normality and the homogeneity of variance, a Wilcoxon signed-rank test was used to replace the dependent *t*-test and a Friedman test to replace the repeated measures ANOVA. For all analysis significance level was set to $\alpha = 0.05$.

Results

FTIR: macromolecular composition

With the FTIR spectroscopy, the lipids are detected in three forms: through the vibration of the FA carbon chains (CH_2 and CH_3) (C–H ~2923 and ~2852 cm^{-1}) (Coates 2000) and the vibration of the ester bond (Eb) (C=O ~1742 cm^{-1}) (Giordano et al. 2001). In *N.* cf. *perminuta* all three lipid band absorption heights decreased remarkably during the course of the 8 weeks darkness at both temperature treatments (Figs. 1a, c, 2a, c). This was also reflected in the declining Eb, CH_2 and CH_3 to silicate (Si) ratios, whereby the drop of the Eb/Si ratio was equal between the temperature treatments, but higher at 7 °C for the CH_2/Si and CH_3/Si ratios (Table 1). The lipid bands showed the strongest decline after the first week in darkness at both temperatures and slowly continued to decrease until the eighth week of darkness (Figs. 1a, c, 2a, c). Additionally to the declining lipids, the protein band height (C=O of amide I ~1648 cm^{-1} and N–H of amide II ~1546 cm^{-1}) (Giordano et al. 2001) dropped

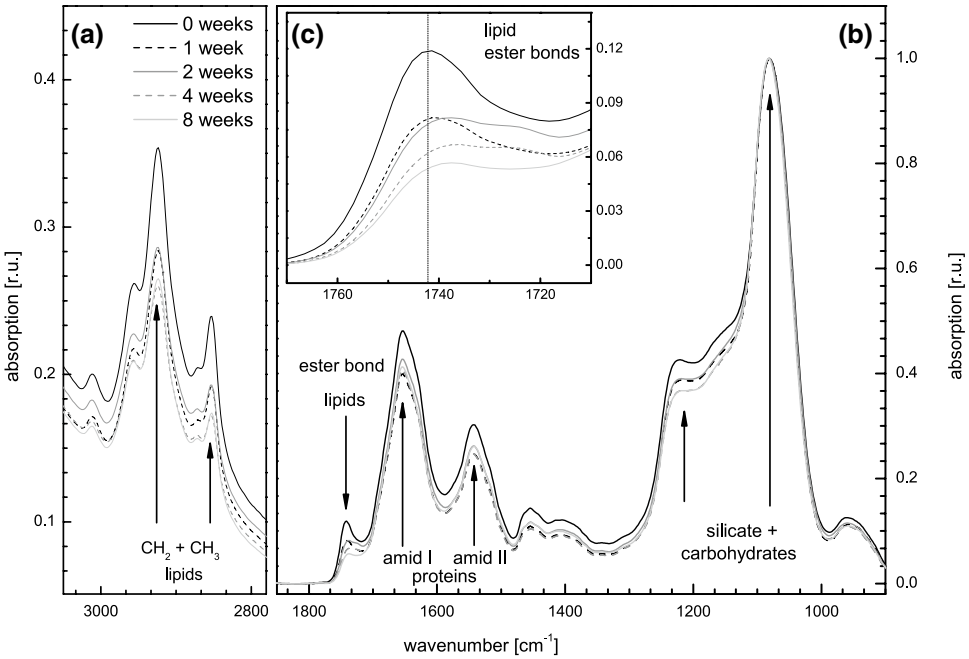

Fig. 1 Fourier transform infrared (FTIR) spectra of *Navicula* cf. *perminuta* after 0 (*light* conditions), 1, 2, 4 and 8 weeks of dark incubation at 0 °C (mean, $n = 2$, measuring replicates = 5). Cut out **c** shows the changes in lipid ester bonds in detail. For comparison the spectra are normalized to the silicate vibration band (~1068 cm^{-1}) and plotted as relative units (r.u.)

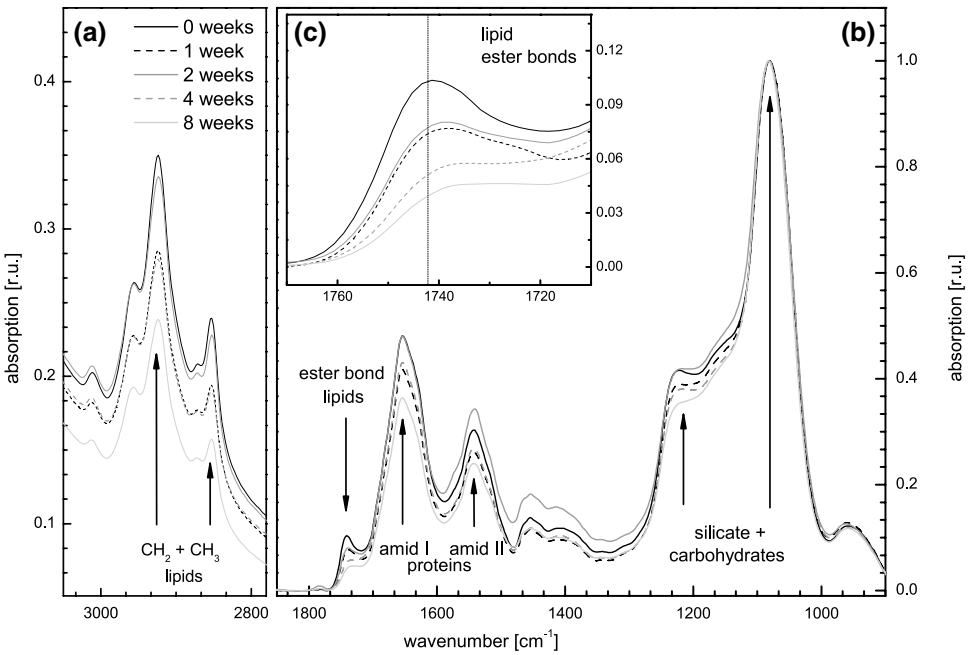

Fig. 2 Fourier transform infrared (FTIR) spectra of *Navicula* cf. *perminuta* after 0 (*light* conditions), 1, 2, 4 and 8 weeks of dark incubation at 7 °C (mean, $n = 2$, measuring replicates = 5). Cut out **c** shows the changes in lipid ester bonds in detail. For comparison the spectra are normalized to the silicate vibration band (~1068 cm^{-1}) and plotted as relative units (r.u.)

also at both temperature treatments (Figs. 1b, 2b). The strong band between 1300 and 1000 cm^{-1} is attributed to silicate which overlaps with bands for carbohydrates (Carb) (C–O–C between 1200 and 900 cm^{-1}) (Giordano et al. 2001). Nevertheless, the band at ~1159 cm^{-1}, which belongs to the carbohydrates, decreased in the dark at both temperatures (Figs. 1b, 2b). At 0 °C, the carbohydrate band declined distinctly more from the light to the first week in darkness, remained at this level and showed a second drop after the fourth week of darkness (Fig. 1b). In contrast, the protein bands decreased only after the first week in darkness and remained more or less unchanged at this level (Fig. 1b). At 7 °C, however, the carbohydrate and protein bands decreased after the first week of darkness, recovered after the second week in darkness to the original level and dropped again until the eighth week of darkness (Fig. 2b). Overall, the Protein/Si and Carb/Si ratios declined just slightly more at 7 °C than at 0 °C (Table 1).

Table 1 Changes of lipids [Ester bound (Eb) and carbon chains C–H (CH_2 and CH_3)], proteins and carbohydrates (Carb) to silicate (Si) ratios of *Navicula* cf. *perminuta* cells in response to 0 (light conditions) and 8 weeks of dark incubation at 0 and 7 °C (mean, $n = 2$, measuring replicates = 5)

Temperature	0 °C		7 °C	
Weeks of darkness	0	8	0	8
Eb/Si	0.12 (0.006)	0.06 (0.005)	0.11 (0.012)	0.05 (0.001)
CH_2/Si	0.35 (0.008)	0.27 (0.007)	0.35 (0.000)	0.24 (0.020)
CH_3/Si	0.24 (0.006)	0.17 (0.004)	0.24 (0.004)	0.16 (0.014)
Protein/Si	0.30 (0.001)	0.26 (0.012)	0.30 (0.004)	0.24 (0.047)
Carb/Si	0.47 (0.006)	0.42 (0.004)	0.46 (0.006)	0.42 (0.009)

The standard deviations are shown in parentheses. Ratios were extracted from FTIR spectra using the peak maxima (Carb ~1159 cm^{-1}, CH_2 ~2923 cm^{-1}, CH_3 ~2852 cm^{-1}, Eb ~1742 cm^{-1}, protein ~1546 cm^{-1}, Si ~1068 cm^{-1})

HPLC: lipid class composition

Neutral lipids and phospholipids from the diatom *N.* cf. *perminuta* could be effectively separated and quantified with known standards (Fig. 3). The glycolipids showed a more complex lipid class composition. The most common neutral lipids were TAG and FFA (Table 2). In minor quantities, cholesterol (Chol) and diacylglycerole (DAG) were found. The polar lipids comprised sulphoquinovosyldiacylglycerols (SQDG), digalactosyldiaacylglycerols (DGDG), phosphatidylglycerols (PG), ceramide (Cer), monogalactosyldiaacylglycerols (MGDG), phosphatidylinositole (PI), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) (Fig. 3; Table 2). The plant specific polar lipids emerged between 11 and 19 min of retention time (Fig. 3). These peaks could not be seperated as clear as the other lipid classes with the used HPLC method. Additionally, three minor and two major peaks remained unidentified with the used standard substances and are, therefore, unquantified (Fig. 3). The calculated total lipid content is, therefore, underestimated and will only be used for comparisons over time. At 0 °C the total lipid content decreased from 71 ± 22.2 µg mg^{-1} DM in the light to 51.6 ± 7.7 µg mg^{-1} DM after 8 weeks darkness and from 78.3 ± 37.7 µg mg^{-1} DM to 32.1 ± 8.8 µg mg^{-1} DM at 7 °C, respectively (Table 2; Fig. 4a, b). The total lipid decomposition rate over the whole period of 8 weeks was much higher at 7 °C (0.82 µg mg^{-1} DM d^{-1}) than at 0 °C (0.35 µg mg^{-1} DM d^{-1}). The neutral lipids were at both temperatures responsible for the general decrease of total lipids. At 0 °C the neutral lipids decreased by 47.3%, with the strongest decline from the first to the second week of darkness (Fig. 4). TAG decreased from 23.0 ± 14.1 µg mg^{-1} DM in the light to 15.6 ± 0.7 µg mg^{-1} DM after 8 weeks darkness, and the FFA content from 13.6 ± 4.3 µg mg^{-1} DM to 6.5 ± 1.7 µg mg^{-1} DM, respectively. The

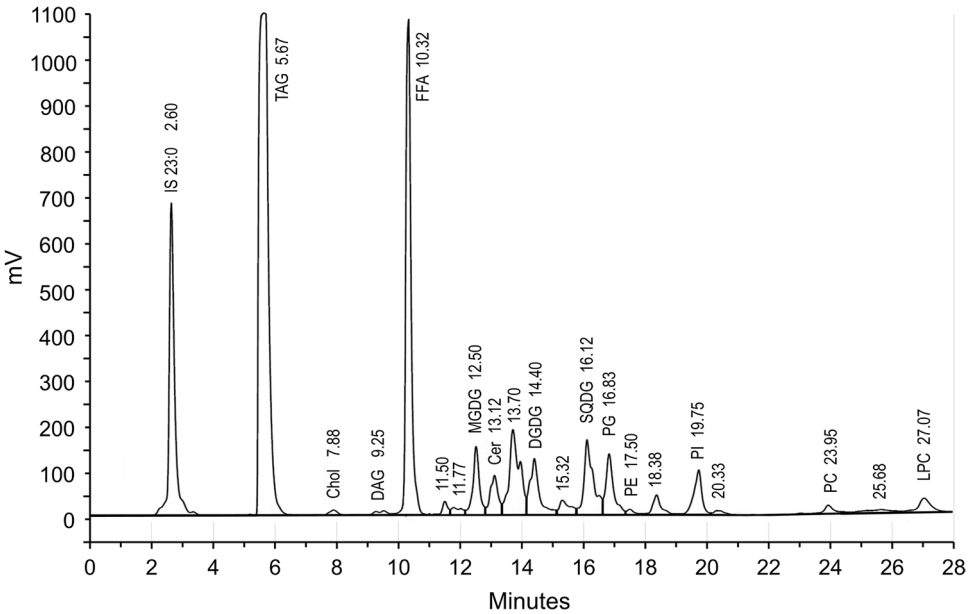

Fig. 3 Representative chromatogram of the lipid classes in *Navicula* cf. *perminuta* using HPLC (sample: 0 °C, light condition, replicate 4). From 0 to 11 min retention time the neutral lipids and from the 11 to 28 min retention time the polar lipids emerge. *Cer* ceramide, *Chol* cholesterol, *DAG* diacylglycerole, *DGDG* digalactosyldiaacylglycerols, *FFA* free fatty acids, *IS* internal standard, *LPC* lysophosphatidylcholine, *MGDG* monogalactosyldiaacylglycerols, *PC* phosphatidylcholine; *PE* phosphatidylethanolamine, *PG* phosphatidylglycerols, *PI* phosphatidylinositole, *PS* phosphatidylserine, *SQDG* sulphoquinovosyldiacylglycerols, *TAG* triacylglycerols

Table 2 Lipid class composition, total neutral, total polar and total lipid content (μg mg^{-1} DM) and the ratio of neutral/polar lipids of *Navicula* cf. *perminuta* after 0 (light conditions), 1, 2, 4 and 8 weeks of dark incubation at 0 and 7 °C (median, $n = 4$)

Temperature	0 °C					7 °C				
Weeks of darkness	0	1	2	4	8	0	1	2	4	8
TAG	23.0 (14.1)	21.6 (4.8)	16.9 (3.8)	16.1 (1.8)	15.6 (0.7)	26.8 (12.9)	11.1 (4.4)	9.8 (4.4)	10.2 (4.9)	1.9 (1.4)
Chol	0.9 (0.1)	1.1 (0.2)	0.6 (0.1)	0.8 (0.2)	0.6 (0.1)	0.8 (0.3)	0.5 (0.3)	0.5 (0.1)	0.8 (0.2)	0.7 (0.1)
DAG	0.3 (0.2)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.5 (0.3)	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
FFA	13.6 (4.3)	13.3 (5.8)	4.3 (3.5)	5.0 (3.1)	6.5 (1.7)	16.0 (10.0)	5.2 (4.1)	3.8 (0.8)	4.7 (1.7)	3.8 (0.6)
Total neutral lipids	42.1 (15.3)	37.9 (6.0)	23.4 (5.8)	22.3 (2.4)	22.2 (2.1)	47.0 (23.3)	16.8 (8.8)	15.3 (4.3)	15.7 (7.0)	6.5 (1.9)
MGDG	3.2 (1.1)	2.1 (0.6)	2.4 (0.7)	3.3 (0.4)	3.1 (0.4)	3.4 (1.8)	2.0 (0.3)	3.0 (0.3)	4.0 (1.5)	3.3 (0.7)
Cer	3.3 (1.0)	2.7 (1.0)	2.4 (0.7)	4.1 (1.0)	3.1 (0.5)	3.9 (2.0)	1.9 (0.2)	2.5 (0.3)	3.7 (1.5)	2.6 (0.5)
DGDG	5.8 (1.4)	4.5 (1.5)	4.0 (1.1)	5.3 (0.8)	4.9 (0.6)	5.5 (2.3)	3.2 (0.4)	4.2 (0.3)	5.1 (1.8)	4.0 (1.1)
SQDG	6.6 (1.9)	3.7 (1.7)	4.7 (1.8)	5.7 (1.1)	6.7 (1.2)	6.6 (3.9)	3.1 (0.5)	5.1 (0.6)	7.0 (4.1)	5.9 (2.3)
PG	2.7 (2.2)	3.4 (1.4)	4.7 (1.4)	4.4 (1.4)	4.5 (1.5)	5.8 (2.8)	3.3 (1.2)	4.8 (1.7)	5.1 (2.0)	2.0 (1.9)
PE	0.3 (0.4)	0.5 (0.2)	0.9 (0.3)	0.8 (0.3)	0.8 (0.2)	0.8 (0.6)	0.5 (0.1)	0.6 (0.3)	0.7 (0.3)	0.1 (0.2)
PI	3.1 (1.0)	4.0 (1.9)	3.6 (2.0)	3.0 (1.5)	2.8 (0.7)	2.1 (1.0)	2.3 (1.0)	4.5 (2.2)	3.3 (1.6)	3.7 (0.7)
PC	0.6 (0.2)	0.6 (0.3)	0.7 (0.2)	0.9 (0.1)	0.9 (0.2)	0.7 (0.4)	0.5 (0.1)	0.9 (0.1)	1.3 (0.6)	0.9 (0.4)
LPC	1.3 (0.6)	4.1 (2.3)	2.9 (2.0)	2.1 (1.3)	1.7 (1.0)	2.2 (0.5)	1.6 (0.8)	2.5 (1.0)	1.9 (0.6)	2.8 (1.0)
Total polar lipids	28.0 (7.0)	28.7 (4.6)	29.4 (7.5)	30.0 (2.4)	29.4 (6.1)	31.3 (14.4)	18.2 (2.5)	29.1 (5.5)	34.1 (12.6)	26.1 (6.9)
Total lipids	71.0 (22.2)	69.6 (7.7)	52.9 (13.3)	50.4 (2.9)	51.6 (7.7)	78.3 (37.7)	36.2 (11.3)	45.0 (9.8)	49.9 (19.5)	32.1 (8.8)
Ratio neutral/polar	1.5 (0.2)	1.2 (0.2)	0.9 (0.1)	0.8 (0.1)	0.9 (0.3)	1.3 (0.2)	1.0 (0.4)	0.6 (0.3)	0.5 (0.1)	0.3 (0.1)

The mean deviations from the median are shown in parentheses. *Cer* ceramide, *Chol* cholesterol, *DAG* diacylglycerole, *DGDG* digalactosyldiaacylglycerols, *FFA* free fatty acids, *LPC* lysophosphatidylcholine; *MGDG* monogalactosyldiaacylglycerols, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerols, *PI* phosphatidylinositole, *PS* phosphatidylserine, *SQDG* sulphoquinovosyldiacylglycerols, *TAG* triacylglycerols

decrease of the TAG and FFA content at 0 °C was, however, not significant. At 7 °C, the decline of the neutral lipid classes TAG and FFA showed the same pattern as at 0 °C with the difference, that the strongest decrease occurred during the change from the light to the first week in darkness. Furthermore, the decrease of the neutral lipids was with 86.1% much higher than at 0 °C. Nevertheless, the differences between 0 and 7 °C for the TAG and FFA fraction were only significant at the eighth week of darkness. TAG decreased ($p < 0.05$) from 26.8 ± 12.9 μg mg^{-1} DM in the light to 1.9 ± 1.4 μg mg^{-1} DM after 8 weeks darkness and the FFA ($p < 0.05$) from 16.0 ± 10.0 μg mg^{-1} DM to 3.8 ± 0.6 μg mg^{-1} DM, respectively. In contrast, the amount of (quantifiable) polar lipids remained more or less unchanged over the time in darkness at both temperatures. The glycolipids MGDG, DGDG, SQDG and Cer declined slightly but not significant after the first week of darkness and recovered afterwards at both temperatures (Table 2; Fig. 4a, b). Most of the phospholipids showed a slight but unsignificant increase over the time in darkness at both temperatures (Table 2). The large peak at 13.7 min of retention time could not be quantified (Fig. 3), however, the area ratio DGDG/peak at 13.7 min also remained constant over time in darkness at both temperatures (data not shown). Due to the relative constant polar lipid content over the 8 weeks in darkness, the relative amount of polar lipids increases at both temperatures in relation to the neutral lipid content, which results in the significant decrease of the neutral/polar lipids ratio at 0 °C from 1.5 ± 0.2 to 0.9 ± 0.3 and at 7 °C from 1.3 ± 0.2 to 0.3 ± 0.1 (Table 2). The MD were general very high in the light samples and decreased with the time of dark incubation.

GC–MS: fatty acid composition

The FA composition of *N.* cf. *perminuta* at 0 and 7 °C over 8 weeks of darkness is presented in Table 3. The diatom predominately produced 16:1(n-7), 16:0 and 20:5(n-3) FA. FA of minor quantities were 14:0, 16:4(n-1), 16:2(n-4), 22:6(n-3), 18:1(n-7), 16:3(n-4), 18:3(n-3) and 18:4(n-3). In the light, the FA composition between the temperature treatments differed, with slightly higher (but not significant) proportions of PUFA at 0 °C, which resulted in a lower SFA + MUFA/PUFA ratio of 2.6 ± 0.2 at 0 °C compared to 3.1 ± 0.5 at 7 °C (Table 3). During the dark incubation of 8 weeks, the FA content decreased significantly at 0 °C from 72.8 ± 7.7 μg mg^{-1} DM to 32.9 ± 4.3 μg mg^{-1} DM and to an even greater extend at 7 °C significantly from 77.7 ± 19.1 μg mg^{-1} DM to 18.1 ± 4.0 μg mg^{-1} DM (Table 3; Fig. 5a, b).

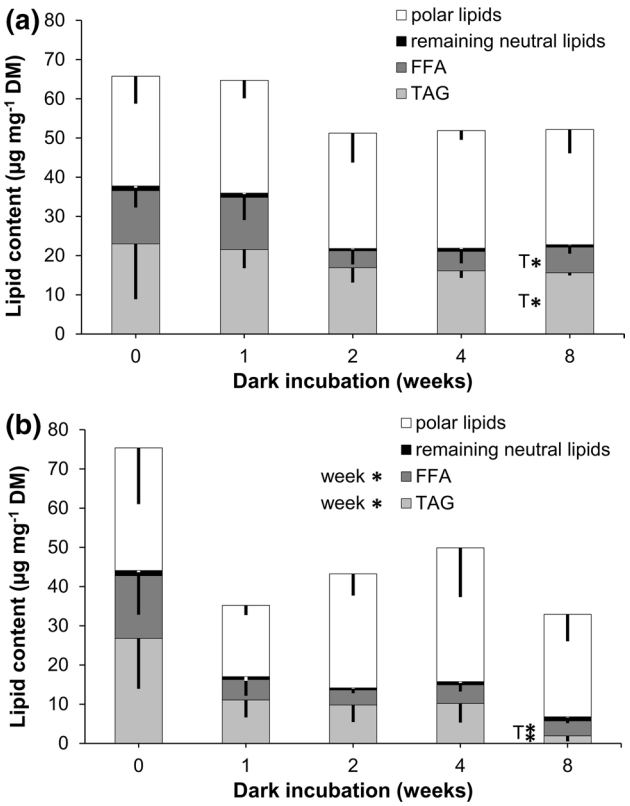

Fig. 4 Lipid content (µg mg^{-1} DM) in *Navicula* cf. *perminuta* after 0 (light conditions), 1, 2, 4 and 8 weeks of dark incubation at **a** 0 °C and **b** 7 °C. Total lipid content is separated into polar lipids, remaining neutral lipids, free fatty acids (FFA) and triacylglycerols (TAG) (median ± mean deviation from the median, $n = 4$). Significance of effects (Friedman or RMANOVA) of darkness (week) from the different lipid class fractions are indicated by $*p < 0.05$ next to the corresponding legend. The *asterisks* next to the columns segments indicate significant differences between temperature (*T*) treatments

The decrease of the FA content in darkness was faster at 7 °C, reaching low values of <30 µg mg^{-1} DM already after 1 week in darkness, in contrast to 2 weeks at 0 °C (Fig. 5a, b). After 8 weeks of darkness the FA content at 7 °C was significant lower as at 0 °C. All FAs contributed to the declining FA content (Table 3). But SFA and MUFA decline proportionally much more compared to PUFA which resulted in a shift of the relative FA composition to lower proportions of SFA and MUFA and higher proportions of PUFA. Thus, the SFA + MUFA/PUFA ratio decreased significantly at 0 °C from 2.6 ± 0.2 in the light to 1.8 ± 0.1 after 8 weeks darkness and from 3.1 ± 0.5 to 1.6 ± 0.2 at 7 °C, respectively. This shift from MUFA dominated to a more PUFA dominated composition was a bit more (but not significantly) distinct at 7 °C than at 0 °C. After 8 weeks of darkness, the amount of PUFA changed from 24.7% in the light to 39% as the highest portion at 7 °C, in contrast to MUFA being still the major constituent at 0 °C with 42%, followed by 35.5% of PUFA.

Discussion

Macromolecular composition

The FTIR spectra of *N*. cf. *perminuta* shows a FTIR spectra typical for a diatom (Stehfest et al. 2005; Jungandreas et al. 2012) with a large vibration band of the silicate frustules, which overlays the peaks of the carbohydrates and partly the band of the nuclear acids. The decreasing Eb/Si ratio reflects the catabolism of lipids and the declining CH_2/Si and CH_3/Si ratios the catabolism of fatty acids (Table 1). However, not only lipids seem to be used for energy requirements under prolonged darkness. Additionally, the declining Protein/Si and Carb/Si ratios indicate the catabolism of protein and carbohydrates in the diatom cells (Table 1). This is in agreement with the quantitative decline of the lipid, carbohydrate and protein spectral region on a per cell level in *Cyclotella meneghiniana* during 34 days darkness, measured also with FTIR spectroscopy (Stehfest et al. 2005). In this diatom, the lipids showed the fastest decline, followed by the carbohydrates and to a slower rate by the proteins. In contrast, *Skeletonema costatum* catabolized 58% of the proteins, 44% of the carbohydrates and only 27% of the lipid pool within 10 days of darkness (Handa 1969). From the peak ratios in *N*. cf. *perminuta* no assumptions can be made on the quantitative change between the declining macromolecules. However, comparisons between the temperature treatments are possible. The decrease of the Eb/Si ratio (Table 1) showed no differences between the temperature treatments, which contradicts the significantly higher decrease of TAG at 7 °C compared to 0 °C (Fig. 4a, b). The CH_2/Si and CH_3/Si ratios showed, in contrast, a higher decline over the time in darkness at 7 °C which is in accordance to the significantly higher catabolism of FFA and TAG at 7 °C. The decrease of the Carb/Si ratio over time in darkness was not different between temperature treatments, but the decline of the Protein/Si ratio was higher at 7 °C. In the green alga *Scenedesmus acuminatus* the protein and carbohydrate consumption during a dark period increased with temperature, while the lipid degradation was temperature independent (Dehning and Tilzer 1989). The change of the macromolecular composition during darkness and the impact of the temperature seems, therefore, species specific (Sackett et al. 2013).

Lipid class composition

The overall lipid class compositon in diatoms resembles closely that of green algae and higher plants with the main

Table 3 Fatty acid composition, total fatty acid content (µg mg^{-1} DM) and ratio of saturated plus monounsaturated fatty acids to polyunsaturated fatty acids (SFA + MUFA/PUFA) of *Navicula* cf. *perminuta* after 0 (light conditions), 1, 2, 4 and 8 weeks of dark incubation at 0 and 7 °C (median, $n = 4$)

Temperature	0 °C					7 °C				
Weeks of darkness	0	1	2	4	8	0	1	2	4	8
14:0	2.0 (0.3)	1.3 (0.2)	0.8 (0.4)	0.9 (0.3)	1.0 (0.2)	2.3 (1.0)	0.8 (0.1)	0.9 (0.3)	1.0 (0.2)	0.6 (0.2)
15:0	0.4 (0.1)	0.3 (0.1)	0.1 (0.0)	0.2 (0.1)	0.2 (0.1)	0.4 (0.1)	0.1 (0.0)	0.1 (0.1)	0.1 (0.0)	0.1 (0.1)
16:0	15.2 (3.0)	8.4 (1.9)	5.6 (1.6)	5.6 (0.6)	6.3 (0.8)	18.3 (8.3)	5.3 (0.9)	5.6 (1.6)	5.5 (1.7)	4.0 (1.0)
18:0	0.4 (0.2)	0.4 (0.1)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.5 (0.3)	0.2 (0.6)	0.2 (0.0)	0.3 (0.1)	0.3 (0.1)
16:1(n-7)	32.2 (5.1)	20.1 (3.8)	10.4 (4.5)	11.5 (2.4)	12.3 (3.1)	35.7 (17.6)	7.8 (2.1)	10.6 (3.8)	9.2 (3.0)	5.1 (2.1)
16:1(n-5)	0.2 (0.1)	0.1 (0.1)	0.0 (0.0)	0.0 (0.1)	0.1 (0.0)	0.3 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
18:1(n-9)	0.3 (0.0)	0.3 (0.1)	0.1 (0.0)	0.2 (0.0)	0.2 (0.1)	0.4 (0.2)	0.1 (0.3)	0.1 (0.1)	0.1 (0.1)	0.0 (0.1)
18:1(n-7)	1.1 (0.3)	1.3 (0.4)	1.0 (0.1)	1.1 (0.1)	1.2 (0.1)	1.1 (0.4)	0.9 (0.2)	0.9 (0.0)	1.1 (0.2)	0.9 (0.2)
16:2(n-4)	2.7 (0.5)	1.9 (0.6)	1.3 (0.4)	1.4 (0.4)	1.5 (0.2)	2.5 (1.1)	1.0 (0.1)	1.2 (0.2)	1.4 (0.3)	0.9 (0.3)
16:3(n-4)	1.2 (0.2)	0.8 (0.3)	0.6 (0.2)	0.7 (0.2)	0.7 (0.1)	1.1 (0.5)	0.5 (0.1)	0.5 (0.1)	0.7 (0.2)	0.4 (0.1)
16:4(n-1)	2.8 (0.5)	2.3 (0.5)	1.5 (0.4)	1.5 (0.4)	1.6 (0.2)	2.6 (1.2)	1.3 (0.1)	1.4 (0.2)	1.8 (0.4)	1.0 (0.3)
18:2(n-6)	0.3 (0.1)	0.3 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.5 (0.2)	0.1 (0.2)	0.1 (0.1)	0.0 (0.1)	0.0 (0.0)
18:3(n-3)	1.3 (0.4)	0.7 (0.2)	0.5 (0.2)	0.5 (0.1)	0.7 (0.2)	1.0 (0.6)	0.3 (0.1)	0.4 (0.2)	0.4 (0.1)	0.3 (0.1)
18:4(n-3)	0.9 (0.2)	0.4 (0.1)	0.3 (0.1)	0.3 (0.1)	0.3 (0.2)	0.6 (0.5)	0.1 (0.0)	0.2 (0.1)	0.2 (0.1)	0.1 (0.1)
20:5(n-3)	10.0 (2.1)	9.7 (2.4)	5.8 (2.2)	6.0 (1.4)	6.1 (1.5)	8.3 (4.9)	4.3 (0.6)	4.9 (1.4)	6.2 (2.0)	3.8 (1.7)
22:6(n-3)	1.4 (0.3)	1.5 (0.4)	0.8 (0.2)	0.8 (0.2)	0.7 (0.1)	1.2 (0.7)	0.6 (0.1)	0.6 (0.2)	0.8 (0.2)	0.5 (0.2)
Total FA	72.8 (12.8)	51.5 (10.0)	29.3 (10.3)	30.7 (6.1)	32.9 (6.8)	77.7 (38.0)	25.5 (4.2)	28.4 (8.3)	29.4 (8.6)	18.1 (6.4)
Ratio SFA + MUFA/PUFA	2.6 (0.2)	1.9 (0.1)	1.7 (0.2)	1.7 (0.1)	1.8 (0.1)	3.1 (0.5)	2.0 (0.3)	2.2 (0.5)	1.7 (0.3)	1.6 (0.2)

The mean deviations from the median are shown in parentheses. Fatty acid abbreviation: first number indicates chain length, second indicates the number of double bonds, third number indicates position of first double bond counted from the carboxyl group

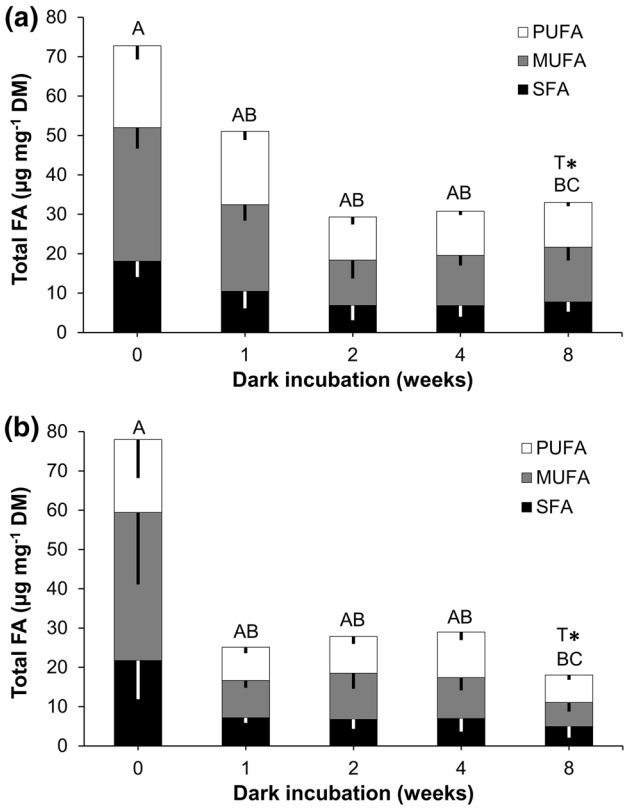

Fig. 5 Total fatty acid (FA) content (µg mg^{-1} DM) in *Navicula* cf. *perminuta* after 0 (light conditions), 1, 2, 4 and 8 weeks of dark incubation at **a** 0 °C and **b** 7 °C. Total FA content is separated into polyunsaturated (PUFA). monounsaturated (MUFA) and saturated (SFA) fatty acids (median ± mean deviation from the median, $n = 4$). Total FA content over time differed when they did not share an *upper case letter* and an *asterisk* ($*p < 0.05$) indicates significant differences between temperature (*T*) treatments

components TAG, FFA, MGDG, DGDG, SQDG, PG, PI, PC and PE (Kates and Volcani 1966; Opute 1974a; Darley 1977). Additionally, diatoms possess according to Kates and Volcani (1966) diphosphatidylglycerol and phosphatidic acid, two minor unidentified sulfur-containing lipids (less polar than SQDG) which are more produced in the dark (Kates and Volcani 1966; Opute 1974b) and an unidentified spingolipide, which could be cerebroside (Kates and Volcani 1966). In *Thalassiosira pseudonana* the betaine lipid diacylglycerylcarboxyhydroxymethyl-choline (DGCC) was found under phosphorus limitation conditions (Martin et al. 2011). In *N.* cf. *perminuta* we determined the lipid classes TAG, FFA, MGDG, DGDG, SQDG, PG, PI, PC, PE and additionaly Cer which belongs to the sphingolipids and LPC, low concentrations of Chol and trace amounts of DAG (Table 2; Fig. 3). Additionally, four minor and one major peaks were separated which could not be identified with the available standards (Fig. 3). Those peaks could potentially refer to diphosphatidylglycerol, phosphatic acid, the unidentified sulfur-containing lipids or DGCC (Kates and Volcani 1966; Opute 1974b; Martin et al. 2011).

Comparison with other studies indicate, that the relative composition of the individual lipid classes in diatoms depend on species, growth conditions, growth phase and also on extraction methods (Volkman et al. 1989; Parrish and Wangersky 1990; Dunstan et al. 1994; Berge et al. 1995; Brown et al. 1996; Lynn et al. 2000). Concentrations of FFA are highly species specific and can range from 0.4 to 26% of total lipids (Volkman et al. 1989; Parrish et al. 1991; Dunstan et al. 1994). Berge et al. (1995), however, asserts that high quantities of FFA in diatom cells are often artefacts, indicating lipolytic degradation and are, therefore, in unsuitable conditions for lipid extraction. Nevertheless, in *N.* cf. *perminuta* we found high concentrations of FFA, which decreased during dark incubation (Table 2). Would the FFA content be a source of artefacts, the relative concentration should be constant throughout the experiment. The TAG concentration in diatoms ranges from 0.3 to 47.7% of total lipids (Volkman et al. 1989; Dunstan et al. 1994) and depends on nutrient availability, irradiance, growth phase and medium, salinity, pH and temperature (Brown et al. 1996; Lynn et al. 2000; Hu et al. 2008; Obata et al. 2013). The thylakoid membrane of diatoms consists of the same lipid classes like in vascular plants (Goss and Wilhelm 2010) and green algae (Vieler et al. 2007) with the difference that diatoms have a much higher content of negatively charged lipids, such as SQDG and PG (Vieler et al. 2007; Goss et al. 2009). This supports our findings with SQDG being the most abundant polar lipid in *N.* cf. *perminuta* and having PG in high portion. Another difference to vascular plants is the significant concentration of the phospholipid PC in diatom thylakoid membranes (Lepetit et al. 2012). Lepetit et al. (2012) reported in *C. meneghiniana* and *Phaeodactylum tricornutum* thylakoids PC concentrations half of the SQDG content. In contrast, *N.* cf. *perminuta* showed low PC concentrations with 10–18% of the SQDG content.

Fatty acid compostition

The FA composition of *N.* cf. *perminuta* conforms with other studies, that 16:1(n-7), 16:0, 20:5(n-3) and 14:0 are the main FAs in diatoms (Darley 1977; Nichols et al. 1986; Volkman et al. 1989; Dunstan et al. 1994; Berge et al. 1995). Additional FAs are more species-specific (Dunstan et al. 1994). The FAs 16:1(n-7) and 20:5(n-3) are used as trophic markers for diatoms in algal blooms and associated food webs (Parrish et al. 1991). The FA composition of *N.* cf. *perminuta* closely resembles with *Navicula* sp. studied in Dunstan et al. (1993) and with the Antarctic sea ice diatom *Navicula glaciei* (Whitaker and Richardson 1980).

The relative proportions of individual FAs, however, differ as reflected in higher quantities of 20:5(n-3) and lower 16:1(n-7) content in both *Navicula* species compared to *N.* cf. *perminuta*. Such differences in the quantitative proportions can result from different growth conditions regarding nutrients, temperature and irradiance (Ackman et al. 1968; Mortensen et al. 1988; Parrish et al. 1991). Decreasing temperature is one of the main factors which can shift the FA composition of membrane lipids from MUFA dominated to a more PUFA dominated composition (Mortensen et al. 1988; Thompson et al. 1992; Jiang and Gao 2004) to maintain the membrane fluidity (Murata and Los 1997). In *N.* cf. *perminuta* we also detected a slightly higher relative amount of PUFA at 0 °C than at 7 °C during light conditions. The SFA + MUFA/PUFA ratio decreases, however, even more with increasing period of dark exposure, which is probably a consequence of the simultaneously decreasing TAG content. Since TAG consists dominantly of SFA and MUFA whereas membrane lipids dominate in PUFA (Sukenik and Wahnon 1991; Berge et al. 1995), this shift in the relative distribution of saturated and unsaturated FAs reflects the increased TAG consumption and the constant level of membrane lipids. To our best knowledge, this is the first study, which determined the FA composition in a diatom species over a prolonged dark period. Fisher and Schwarzenbach (1978) demonstrated in *T. pseudonana* a decrease of the FAs 16:0 and 16:1(n-7), but only for short-term darkness (24 h). This observation also indicates TAG consumption in the dark phase (Fisher and Schwarzenbach 1978; Chauton et al. 2013). In the green alga *Selenastrum capricornutum* the MUFA/PUFA ratio also decreased together with a decrease of the FA content by 50% after exposure to 7 days darkness (McLarnon-Riches et al. 1998).

The lipids metabolism under darkness

Diatoms possess, in contrast to other algae and higher plants, two β-oxidation pathways for the FA degradation. One is located in the peroxisomes like in plants, whose end product acetyl-CoA presumably goes via the glyoxylate cycle into the gluconeogenesis for carbohydrate production. A second pathway is located in the mitochondria, which serves acetyl-CoA into the TCA cycle and provides the cell with ATP, carbon skeletons and other metabolites (Armbrust et al. 2004; Chauton et al. 2013). Enzymes involved in the mitochondrial β-oxidation are upregulated at the end of a light phase and during a dark period (Chauton et al. 2013). In contrast, peroxisomal β-oxidation enzymes are down regulated at night and show enhanced activity during the light period (Chauton et al. 2013). Furthermore, the acetyl-CoA carboxylase which regulates the carbon flux to the fatty acid production shows low activity under dark conditions and a significant activity increase with irradiation (Hellyer et al. 1986; Harwood 1988). As a consequence, storage lipids in the form of TAG get synthesized under light, while degradation happens during darkness (Sicko-Good et al. 1988; Armbrust et al. 2004; Chauton et al. 2013) providing energy and carbon skeletons for respiration, cell maintenance and cell division (Armbrust et al. 2004).

Apart from the diurnal light–dark rhythm, diatoms store lipids in excess to better cope with unfavorable growth conditions like nutrient limitation (Roessler 1990; Fahl and Kattner 1993), low irradiance and low temperatures (Smith and Morris 1980; Palmisano and Sullivan 1982). Lipid droplets have been detected at high quantities in polar plankton and sea ice diatom taxa in summer and late autumn (Fryxell 1989; Fahl and Kattner 1993; Zhang et al. 1998). In the benthic diatom *Nitzschia* cf. *dubiiformis* from Svalbard growing at 7 °C and low light conditions (25 µmol photons m^{-2} s^{-1}), also large amounts of cytoplasmic lipid droplets were observed, which gradually disappeared with ongoing dark incubation (Karsten et al. 2016). Therefore, diatoms seem to alter their metabolic lipid pathways triggered by favoured or unfavourable growth conditions towards an enhanced incorporation or remobilisation of assimilated carbon into or out of the lipid fraction (Hu et al. 2008).

The chemical energy of lipid reserves can subsequently be used during long dark periods (McMinn and Martin 2013). Experimental evidence, however, about the utilization of lipid storage products for long term dark survival in diatoms, is still rare. Palmisano and Sullivan (1982) reported in three polar sea ice diatoms the biosynthesis and accumulation of lipid- and carbohydrate reserves during the middle of a simulated summer-winter transition, and the subsequent utilization of these carbon reserves during the onset of a simulated winter. *Navicula* cf. *perminuta* catabolized during the 8 weeks of darkness the lipid storage product TAG to 32% at 0 °C and even to 93% at 7 °C, as well as the pool of FFA to 52% at 0 °C and to 76% at 7 °C, whereas the polar lipids remained more or less unchanged (Fig. 4). The highest decrease of the neutral lipids occurred in the first 2 weeks of darkness. Differences between temperatures reveal a faster and stronger decrease of TAG and FFA at the higher temperature (Table 2; Fig. 4). Under light exposure, high deviations of the different lipid classes between the replicates indicate a more variable cell-specific lipid production, which could be caused by slightly different photon fluence rates, for example, due to self-shading effects. With increasing duration of dark exposure, the deviations between the replicates declined (Fig. 4), pointing to a selection process among individual cells, in which only cells with a similar quantitative lipid composition survived.

Under dark conditions some diatoms reduce their metabolic rate down to the maintenance metabolism, which serves to keep all cell functions ready for photosynthesis after a transfer back to the light (Geider and Osborne 1989). In *S. costatum* very low maintenance metabolic rates of <0.01 d^{-1} (g C g^{-1} cell C d^{-1}) were measured (Vårum et al. 1986; Geider and Osborne 1989). To meet such energy requirements, storage products, such as lipids and carbohydrates, have to be used (Geider and Osborne 1989). Planktonic diatoms from the Antarctic Ocean regulate their cellular chlorophyll *a*, carbon and nitrogen content at the beginning of a prolonged darkness period down to constant low levels, which suggests a strongly reduced maintenance metabolism (Peters and Thomas 1996). In *N.* cf. *perminuta*, only one-third of the stored TAG at 0 °C was used during the 8 weeks of darkness, which leads to the assumption, that the rate of the maintenance metabolism was relatively low at this temperature. In contrast, at 7 °C, although the TAG content was in the light about 14% higher than at 0 °C, the TAG consumption during the 8 weeks of darkness was very strong, leaving only 7% of the initial energy storage compound for further dark survival (Fig. 4). The total lipid decomposition rate over the whole period of 8 weeks was 2.4 times higher at 7 °C compared to 0 °C. Furthermore, the stronger decrease of the protein/Si ratio at 7 °C indicates a higher catabolism of proteins. All these results point to a much higher maintenance metabolism at 7 °C. Bunt et al. (1966) reported that respiratory oxygen consumption in the ice diatom *Fragilaria sublinearis* was substantially increased at 10 °C compared to 3 °C. The green alga *S. acuminatus* preferentially catabolised the protein (34% at 22 °C; 22% at 7 °C), but also to a minor extend the carbohydrate (20% at 22 °C; 15% at 7 °C) and lipid (15% at 22 °C; 13% at 7 °C) pool during 7 weeks of darkness (Dehning and Tilzer 1989). The lipid degradation was temperature independent, whereas the protein and carbohydrate consumption increased with temperature, especially at the beginning of the dark period (Dehning and Tilzer 1989). Consequently, a higher metabolic rate caused by an increase of temperature, as predicted in global warming scenarios, can lead to a more rapid catabolism of stored energy products and hence possibly to shorter dark survival times (McMinn and Martin 2013). This could be followed by the utilization of other potential energy sources, such as the degradation of organelles like chloroplasts (Baldisserotto et al. 2005; Karsten et al. 2012) or the switch to a heterotrophic lifestyle, i.e. uptake of bioavailable sugars and amino acids from the environment (Hellebust and Lewin 1977; Palmisano and Sullivan 1982; Tuchman et al. 2006).

Numerous species of diatoms, especially benthic taxa, are known to be facultative heterotrophic (Lewin and Lewin 1960; Hellebust and Lewin 1977; Rivkin and Putt 1987). Active transport mechanisms for the uptake of various organic substrates are identified, which seem to be substrate and light regulated (Hellebust and Lewin 1977; Armbrust et al. 2004). Studies on freshwater benthic diatoms showed that the uptake rates of organic substrates increased substantially with dark conditions (Tuchman et al. 2006). Since these transporters are energetically expensive, they are possibly only activated when essential for survival or when external substrates are present at high concentrations (Tuchman et al. 2006). In a simulated summer-winter transition experiment the heterotrophic potential (measured as glucose uptake) from three polar sea ice diatoms showed a 7–60-fold stimulation at the onset of winter conditions (Palmisano and Sullivan 1982). However, the assimilated glucose contributed to only <0.3% of the total carbon requirement, making it inadequate for growth, but may be sufficient for maintenance metabolism during darkness (Palmisano and Sullivan 1982).

The degradation of photosynthetic pigments can also provide nitrogen and carbon for the maintenance metabolism during darkness. Veuger and van Oevelen (2011) observed during the first weeks of a 12 months dark incubation period a degradation of chlorophyll *a* and *c*, fucoxanthin and other carotenoids in benthic diatoms, followed by much slower or even completely reduced degradation rates at the end of the dark period. Schlie et al. (2011) mentioned for the benthic diatom *Surirella* sp. from the Arctic a shrinkage of the ovoid chloroplasts already after 2 weeks in darkness, and also the benthic *Fragilaria striatula* from Kongsfjorden, Svalbard exhibited a 30–40% reduction in chloroplast length after 3 months in darkness (Karsten et al. 2012). As a consequence of the chloroplast degradation, diatoms showed long lag phases upon re-irradiation, which length increased with dark incubation time (Karsten et al. 2012), because the photosynthetic apparatus had to be rebuilt. Manoharan et al. (1999) reported in the dinoflagellate *Prorocentrum minimum* a decrease in the glycolipid content together with a TAG reduction during a dark incubation of 10 days, whereas the total phospholipids remained unchanged. *Navicula* cf. *perminuta* in this study, however, showed no reduction of the glycolipids during the course of the 8 weeks darkness at 0 °C and only a slight degradation of glycolipids after the first week of dark exposure with a subsequent recovery close to the light value at 7 °C (Fig. 4a, b). Since the thylakoid membrane compose to 70–85% of glycolipids (Goss et al. 2009), it is assumed that *N.* cf. *perminuta* did not start to degrade its chloroplasts for energy mobilization. Also Berge et al. (2015a) confirmed in natural phytoplankton communities from Svalbard at the end of the polar night a missing lag-phase with respect to initiation of photosynthesis upon re-illumination, which indicates that chloroplast were not degraded during the dark season. In which way temperature affects

the heterotrophic uptake potential or the potential degradation rate of chloroplasts under dark conditions in polar diatoms is not known. Nevertheless, energy requirements increase with rising temperature that can reduce the survival potential of polar benthic diatoms at prolonged dark periods, such as the polar night.

Summary

Navicula cf. *perminuta* used during the 8 weeks in darkness the pools of lipids, carbohydrates and proteins as energy source. From the lipid pool the long term storage product TAG and the FFA are catabolized, while the phospho- and glycolipids remained unchanged. This suggests that the photosynthetic membranes of chloroplasts remained untouched and, therefore, functional. While at 0 °C the lipid catabolism was relative low, at 7 °C the pool of TAG and FFA were almost depleted after the 8 weeks of darkness, assuming a drastic increase in the overall energy requirements. This clearly points to shorter survival times under higher temperatures, which might be a fundamental problem for Arctic benthic diatoms during the polar night when global warming is further increasing.

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References

Ackman RG, Tocher CS, McLachlan J (1968) Marine phytoplankter fatty acids. J Fish Res Board Can 25:1603–1620. doi:10.1139/f68-145

Antia NJ (1976) Effects of temperature on the darkness survival of marine microplanktonic algae. Microb Ecol 3:41–54. doi:10.1007/BF02011452

Armbrust EV, Berges JA, Bowler C et al (2004) The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. Science 306:79–86. doi:10.1126/science.1101156

Baldisserotto C, Ferroni L, Andreoli C, et al (2005) Dark-acclimation of the chloroplast in Koliella antarctica exposed to a simulated austral night condition. Arct Antarct Alp Res 37:146–156. doi:10.1657/1523-0430(2005)037[0146:.CIK]2.0.CO;2

Berge J, Gouygou J, Dubacqt J, Durand P (1995) Reassessment of lipid composition of the diatom, *Skeletonema costatum*. Phytochemistry 39:1017–1021

Berge J, Daase M, Renaud PE et al (2015a) Unexpected levels of biological activity during the polar night offer new perspectives on a warming Arctic. Curr Biol. doi:10.1016/j.cub.2015.08.024

Berge J, Renaud PE, Darnis G et al (2015b) In the dark: a review of ecosystem processes during the Arctic polar night. Prog Oceanogr. doi:10.1016/j.pocean.2015.08.005

Bintanja R, van der Linden EC (2013) The changing seasonal climate in the Arctic. Sci Rep 3:1556. doi:10.1038/srep01556

Brown MR, Dunstan GA, Norwood SJ, Miller KA (1996) Effects of harvest stage and light on the biochemical composition of the diatom *Thalassiosira pseudonana*. J Phycol 32:64–73. doi:10.1111/j.0022-3646.1996.00064.x

Bunt JS, Lee CC (1972) Data on the composition and dark survival of four sea-ice microalgae. Limnol Oceanogr 17:458–461

Bunt JS, H Owens O, Hoch G (1966) Exploratory studies on the physiology and ecology of a psychrophilic marine diatom. J Phycol 2:96–100. doi:10.1111/j.1529-8817.1966.tb04601.x

Chapman ARO, Lindley JE (1980) Seasonal growth of *Laminaria solidungula* in the Canadian High Arctic in relation to irradiance and dissolved nutrient concentrations. Mar Biol 57:1–5. doi:10.1007/BF00420961

Chauton MS, Winge P, Brembu T et al (2013) Gene regulation of carbon fixation, storage, and utilization in the diatom *Phaeodactylum tricornutum* acclimated to light/dark cycles. Plant Physiol 161:1034–1048. doi:10.1104/pp.112.206177

Coates J (2000) Interpretation of infrared spectra, a practical approach. In: Meyers RA (ed) Encyclopedia of analytical chemistry. Wiley, Chichester, pp 10815–10837

Darley WM (1977) Biochemical compostion. In: Werner D (ed) The biology of diatoms, vol. 13. University of California Press, California, pp 198–233

Dehning I, Tilzer MM (1989) Survival of *Scenedesmus acuminatus* (Chlorophyceae) in darkness. J Phycol 25:509–515

Dunstan GA, Volkman JK, Barrett SM, Garland CD (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

Dunstan GA, Volkman JK, Barrett SM et al (1994) Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35:155–161

Dunton KH (1990) Growth and production in *Laminaria solidungula*: relation to continuous underwater light levels in the Alaskan High Arctic. Mar Biol 106:297–304. doi:10.1007/BF01314813

Durbin EG (1978) Aspects of the biology of resting spores of *Thalassiosira nordenskioeldii* and *Detonula confervacea*. Mar Biol 45:31–37. doi:10.1007/BF00388975

Fahl K, Kattner G (1993) Lipid content and fatty acid composition of algal communities in sea-ice and water from the Weddell Sea (Antarctica). Polar Biol 13:405–409

Fisher NS, Schwarzenbach RP (1978) Fatty acid dynamics in *Thalassiosira pseudonana* (Bacillariophyceae): implications for physiological ecology. J Phycol 14:143–150. doi:10.1111/j.1529-8817.1978.tb02439.x

Fryxell GA (1989) Marine phytoplankton at the Weddell Sea ice edge: seasonal changes at the specific level. Polar Biol 10:1–18

Geider RJ, Osborne BA (1989) Respiration and microalgal growth: a review of the quantitative relationship between dark respiration and growth. New Phytol 112:327–341. doi:10.1111/j.1469-8137.1989.tb00321.x

Giordano M, Kansiz M, Heraud P et al (2001) Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalga *Chaetoceros muellerii* (Bacillariophyceae). J Phycol 37:271–279. doi:10.1046/j.1529-8817.2001.037002271.x

Goss R, Wilhelm C (2010) Lipids in algae, lichens and mosses. In: Wada H, Murata N (eds) Lipids in photosynthesis: essential regulatory functions, 30th edn. Springer, Dordrecht, pp 117–137

Goss R, Nerlich J, Lepetit B et al (2009) The lipid dependence of diadinoxanthin de-epoxidation presents new evidence for a macrodomain organization of the diatom thylakoid membrane. J Plant Physiol 166:1839–1854. doi:10.1016/j.jplph.2009.05.017

Graeve M, Janssen D (2009) Improved separation and quantification of neutral and polar lipid classes by HPLC–ELSD using a monolithic silica phase: application to exceptional marine lipids. J Chromatogr B 877:1815–1819. doi:10.1016/j.jchromb.2009.05.004

Graeve M, Kattner G, Hagen W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J Exp Mar Bio Ecol 182:97–110. doi:10.1016/0022-0981(94)90213-5

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Springer, Boston, pp 29–60

Handa N (1969) Carbohydrate metabolism in the marine diatom *Skeletonema costatum*. Mar Biol 4:208–214. doi:10.1007/BF00393894

Harwood JL (1988) Fatty acid metabolism. Annu Rev Plant Physiol Plant Mol Biol 39:101–138

Hellebust JA, Lewin J (1977) Heterotrophic nutrition. In: Werner D (ed) The biology of diatoms. University of California Press, California, pp 169–197

Hellyer A, Bambridge HE, Slabas AR (1986) Plant acetyl-CoA carboxylase. Biochem Soc Trans 14:565–568

Hop H, Pearson T, Hegseth EN et al (2002) The marine ecosystem of Kongsfjorden, Svalbard. Polar Res 21:167–208

Hu Q, Sommerfeld M, Jarvis E et al (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639. doi:10.1111/j.1365-313X.2008.03492.x

IPCC (2007) Change, Intergovernmental panel on climate. Climate change 2007: the physical science basis. Agenda 6.07:333

IPCC (2014) Climate change 2014: synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. Pachauri RK, Meyer LA (ed). IPCC, Geneva, p 151

Iversen KR, Seuthe L (2011) Seasonal microbial processes in a high-latitude fjord (Kongsfjorden, Svalbard): I. heterotrophic bacteria, picoplankton and nanoflagellates. Polar Biol 34:731–749. doi:10.1007/s00300-010-0929-2

Jiang H, Gao K (2004) Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornatum* (Bacillariophyceae). J Phycol 40:651–654. doi:10.1111/j.1529-8817.2004.03112.x

Jungandreas A, Wagner H, Wilhelm C (2012) Simultaneous measurement of the silicon content and physiological parameters by FTIR spectroscopy in diatoms with siliceous cell walls. Plant Cell Physiol 53:2153–2162. doi:10.1093/pcp/pcs144

Karsten U, Schlie C, Woelfel J, Becker B (2012) Benthic diatoms in Arctic Seas—ecological functions and adaptations. Polarforschung 81:77–84

Karsten U, Schaub I, Woelfel J et al (2016) Living on cold substrata—new insights and approaches to study microphytobenthos ecophysiology and ecology in Kongsfjorden. In: Hop H, Wienche C (eds) Advances in polar ecology. Springer, Berlin (**press**)

Kates M, Volcani BE (1966) Lipid components of diatoms. Biochim Biophys Acta 116:264–278

Kattner G, Brockmann UH (1990) Particulate and dissolved fatty acids in an enclosure containing a unialgal *Skeletonema costatum* (Greve.) Cleve culture. J Exp Mar Bio Ecol 141:1–13

Kattner G, Fricke HSG (1986) Simple gas–liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J Chromatogr 361:263–268. doi:10.1016/S0021-9673(01)86914-4

Lepetit B, Goss R, Jakob T, Wilhelm C (2012) Molecular dynamics of the diatom thylakoid membrane under different light conditions. Photosynth Res 111:245–257. doi:10.1007/s11120-011-9633-5

Lewin JC, Lewin RA (1960) Auxotrophy and heterotrophy in marine littoral diatoms. Can J Microbiol 6:127–134. doi:10.1139/m60-015

Lynn SG, Kilham SS, Kreeger DA, Interlandi SJ (2000) Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). J Phycol 36:510–522. doi:10.1046/j.1529-8817.2000.98251.x

MacDonald GM (2010) Global warming and the Arctic: a new world beyond the reach of the Grinnellian niche? J Exp Biol 213:855–861. doi:10.1242/jeb.039511

Manoharan K, Lee TK, Cha JM et al (1999) Acclimation of Prorocentrum minimum (Dinophyceae) to prolonged darkness by use of an alternative carbon source from triacylglycerides and galactolipids. J Phycol 35:287–292. doi:10.1046/j.1529-8817.1999.3520287.x

Martin P, Van Mooy BA, Heithoff A, Dyhrman ST (2011) Phosphorus supply drives rapid turnover of membrane phospholipids in the diatom *Thalassiosira pseudonana*. ISME J 5:1057–1060. doi:10.1038/ismej.2010.192

McLarnon-Riches CJ, Rolph CE, Greenway DLA, Robinson PK (1998) Effects of environmental factors and metals on Selenastrum capricornutum lipids. Phytochemistry 49:1241–1247. doi:10.1016/S0031-9422(98)00095-8

McMinn A, Martin A (2013) Dark survival in a warming world. P R Soc Lond B Biol 280:20122909. doi:10.1098/rspb.2012.2909

McQuoid MR, Hobson LA (1996) Diatom resting stages. J Phycol 32:889–902

Mortensen SH, Børsheim KY, Rainuzzo J, Knutsen G (1988) Fatty acid and elemental composition of the marine diatom *Chaetoceros gracilis* Schütt. Effects of silicate deprivation, temperature and light intensity. J Exp Mar Bio Ecol 122:173–185. doi:10.1016/0022-0981(88)90183-9

Muckenhuber S, Nilsen F, Korosov A, Sandven S (2016) Sea ice cover in Isfjorden and Hornsund, Svalbard (2000–2014) from remote sensing data. Cryosph 10:149–158. doi:10.5194/tc-10-149-2016

Murata N, Los DA (1997) Membrane fluidity and temperature perception. Plant Physiol 115:875–879

Nichols PD, Palmisano AC, Smith GA, White DC (1986) Lipids of the Antarctic sea ice diatom Nitzschia cylindrus. Phytochemistry 25:1649–1653

Obata T, Fernie AR, Nunes-Nesi A (2013) The central carbon and energy metabolism of marine diatoms. Metabolites 3:325–346. doi:10.3390/metabo3020325

Opute FI (1974a) Lipid and fatty-acid composition of diatoms. J Exp Bot 25:823–835

Opute FI (1974b) Physiological studies of the sulpholipids of diatoms. J Exp Bot 25:798–809. doi:10.1093/jxb/25.4.798

Palmisano AC, Sullivan CW (1982) Physiology of sea ice diatoms. I. Response of three polar diatoms to a simulated summer–winter transition. J Phycol 18:489–498

Palmisano AC, Sullivan CW (1983) Physiology of sea ice diatoms. II. Dark survival of three polar diatoms. Can J Microbiol 29:157–160. doi:10.1139/m83-026

Parrish CC, Wangersky PJ (1990) Growth and lipid class composition of the marine diatom, *Chaetoceros gracilis*, in laboratory and mass culture turbidostats. J Plankton Res 12:1011–1021

Parrish CC, DeFreitas ASW, Bodennec G, et al (1991) Lipid composition of the toxic marine diatom *Nitzschia pungens*. Phytochem 30:113–116.

Pawłowska J, Włodarska-Kowalczuk M, Zajączkowski M et al (2011) Seasonal variability of meio- and macrobenthic standing stocks and diversity in an Arctic fjord (Adventfjorden, Spitsbergen). Polar Biol 34:833–845. doi:10.1007/s00300-010-0940-7

Peters E, Thomas DN (1996) Prolonged darkness and diatom mortality I: Marine antarctic species. J Exp Mar Bio Ecol 207:25–41

Reeves S, McMinn A, Martin A (2011) The effect of prolonged darkness on the growth, recovery and survival of Antarctic sea ice diatoms. Polar Biol 34:1019–1032. doi:10.1007/s00300-011-0961-x

Rivkin RB, Putt M (1987) Heterotrophy and photoheterotrophy by Antarctic microalgae: light-dependent incorporation of amino acids and glucose. J Phycol 23:442–452. doi:10.1111/j.1529-8817.1987.tb02530.x

Roessler PG (1990) Environmental control of glycerolipid metabolism in microalgae: commercial implications and future research directions. J Phycol 26:393–399

Sackett O, Petrou K, Reedy B et al (2013) Phenotypic plasticity of southern ocean diatoms: key to success in the sea ice habitat? PLoS One 8:e81185. doi:10.1371/journal.pone.0081185

Schlie C, Karsten U (2016) Microphytobenthic diatoms isolated from sediments of the Adventfjorden (Svalbard): growth as function of temperature. Polar Biol. doi:10.1007/s00300-016-2030-y

Schlie C, Woelfel J, Rüdiger F, et al (2011) Ecophysiological performance of benthic diatoms from Arctic waters. In: Seckbach J, Kociolek JP (eds) The diatom world, 19th edn. Springer, Netherlands, pp 425–436

Serreze MC, Francis JA (2006) The Arctic on the fast track of change. Weather 61:65–69. doi:10.1256/wea.197.05

Sicko-Good L, Simmons MS, Lazinsky D, Hall J (1988) Effect of light cycle on diatom fatty acid composition and quantitative morphology. J Phycol 24:1–7. doi:10.1111/j.1529-8817.1988.tb04448.x

Smayda TJ, Mitchell-Innes B (1974) Dark survival of autotrophic, planktonic marine diatoms. Mar Biol 25:195–202

Smith AE, Morris I (1980) Synthesis of lipid during photosynthesis by phytoplankton of the Southern Ocean. Science 207:197–199. doi:10.1126/science.207.4427.197

Stachura-Suchoples K, Enke N, Schlie C et al (2015) Contribution towards a morphological and molecular taxonomic reference library of benthic marine diatoms from two Arctic fjords on Svalbard (Norway). Polar Biol. doi:10.1007/s00300-015-1683-2

Stehfest K, Toepel J, Wilhelm C (2005) The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. Plant Physiol Biochem 43:717–726. doi:10.1016/j.plaphy.2005.07.001

Sukenik A, Wahnon R (1991) Biochemical quality of marine unicellular algae with special emphasis on lipid-composition 1. *Isochrysis galbana*. Aquaculture 97:61–72. doi:10.1016/0044-8486(91)90279-g

Svendsen H, Beszczynska- Møller A, Hagen JO et al (2002) The physical environment of Kongsfjorden – Krossfjorden, an Arctic fjord system in Svalbard. Polar Res 21:133–166

Thompson PA, Guo M, Harrison PJ, Whyte JNC (1992) Effects of variation in temperature. II. on the fatty acid composition of eight species of marine phytoplankton. J Phycol 28:488–497. doi:10.1111/j.0022-3646.1992.00488.x

Tuchman NC, Schollett MA, Rier ST, Geddes P (2006) Differential heterotrophic utilization of organic compounds by diatoms and bacteria under light and dark conditions. Hydrobiologia 561:167–177. doi:10.1007/s10750-005-1612-4

Vårum KM, Østgaard K, Grimsrud K (1986) Diurnal rhythms in carbohydrate metabolism of the marine diatom *Skeletonema costatum* (Grev.) Cleve. J Exp Mar Bio Ecol 102:249–256. doi:10.1016/0022-0981(86)90180-2

Veuger B, Van Oevelen D (2011) Long-term pigment dynamics and diatom survival in dark sediment. Limnol Oceanogr 56:1065–1074

Vieler A, Wilhelm C, Goss R et al (2007) The lipid composition of the unicellular green alga *Chlamydomonas reinhardtii* and the diatom *Cyclotella meneghiniana* investigated by MALDI-TOF MS and TLC. Chem Phys Lipids 150:143–155. doi:10.1016/j.chemphyslip.2007.06.224

Volkman JK, Jeffrey SW, Nichols PD et al (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Bio Ecol 128:219–240

Wagner H, Liu Z, Langner U et al (2010) The use of FTIR spectroscopy to assess quantitative changes in the biochemical composition of microalgae. J Biophot 3:557–566

Whitaker TM, Richardson MG (1980) Morphology and chemical compositon of a natural population of an ice-associated Antarctic diatom *Navicula glaciei*. J Phycol 16:250–257. doi:10.1111/j.1529-8817.1980.tb03027.x

Zhang Q, Gradinger R, Spindler M (1998) Dark survival of marine microalgae in the high Arctic (Greenland Sea). Polarforschung 65:111–116