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RISING WATER TEMPERATURES ALTER LIPID DYNAMICS AND REDUCE N-3 ESSENTIAL FATTY ACID CONCENTRATIONS IN *SCENEDESMUS OBLIQUUS* (CHLOROPHYTA)[1]

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The biosynthesis of nutritionally important polyunsaturated fatty acids (PUFAs) in phytoplankton is influenced by environmental temperature. We investigated the potential of climate warming to alter lipid dynamics of *Scenedesmus obliquus* (Turpin) Kütz. by comparing lipid and fatty acid (FA) profiles as well as FA metabolism (using [1-^{14}C] acetate) at 20°C and 28°C. We documented an overall decline (53%–37%) in the proportion of n-3 PUFA (in particular, of α-linolenic acid [ALA; 18:3n-3]), and a concomitant increase in saturated fatty acids (SAFAs) in total lipids (TLs) at 28°C, consistent with enhanced incorporation of radioactivity from [1-^{14}C] acetate into total 16:0, 18:1, and decreased incorporation into 18:2 and 18:3 FA (from 36% to 22% of the total) at 28°C. Glycerophospholipids were also affected by warming; ALA and stearidonic acids (SDAs; 18:4n-3) both decreased (by 13% and 15%, respectively) in phosphatidylcholine (PC) and (by 24% and 20%, respectively) in phosphatidylethanolamine (PE). The characteristic FA in phosphatidylglycerol (PG; 16:1n-13*t*) increased (by 22%) at 28°C. The activities of desaturases, which add double bonds to FA moieties, comprised the major suite of reactions affected by the temperature increase in TL and polar lipid (PL) classes. Climate modelers predict an increase in the number of extreme heat days in summer at temperate latitudes, with parallel projected increases in water temperatures of shallow water bodies. Our results suggest that the overall decrease in the essential n-3 FA ALA in *S. obliquus* at higher water temperatures may lower food quality for higher tropic levels, adding another climate-warming stress.

Key index words: **chlorophyte; climate change; essential fatty acids; polar lipids; radiolabel; *Scenedesmus*; temperature; unsaturation**

Abbreviations: **ALA, α-linolenic acid; ARA, arachidonic acid; BBM, Bold's basal medium; CL, cardiolipin (diphosphatidylglycerol); DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine; DHA, docosahexaenoic acid; EFA, essential fatty acid; EPA, eicosapentaenoic acid; FA, fatty acid; Fads, fatty acid desaturases; FAME, fatty acid methyl esters; FFA, free (nonesterified) fatty acid; GC, gas chromatography; GL, glycosylglycerides; HUFA, highly unsaturated fatty acid; LIN, linoleic acid; MGDG, monogalactosyldiacylglycerol; n-3, omega-3; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, polar lipid; PTV, programmed temperature vaporization; PUFA, polyunsaturated fatty acid; SAFA, saturated fatty acid; SDA, stearidonic acid; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol; TL, total lipid; TNL, total nonpolar lipid**

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Phytoplankton contribute approximately half of the planet's annual primary productivity and are thus crucial components of the biosphere (Beardall et al. 2009). They represent a highly diverse group of photosynthetic organisms that are distributed in every climatic region (Chi et al. 2008). Their ability to colonize environments with large temperature extremes means that they must have mechanisms to cope with temperature stress. One such adaptation is the ability to adjust the lipids and their molecular species in cell membranes (Thompson 1996, Guschina and Harwood 2006), a process referred to as ''homeoviscous adaptation'' (Sinensky 1974).

As temperature is lowered, membrane lipids increase the degree of unsaturation in their FAs through the action of fatty acid desaturases (Fads) (Nishida and Murata 1996, Cossins et al. 2002). These findings came partly from studying the response of plants to chilling (Nishida and Murata 1996), but fish and invertebrates exhibit the same increase in FA unsaturation with lowered temperatures (Farkas et al. 1984, Logue et al. 2000). However, we know much less about changes in algal lipid dynamics in relation to the elevated temperatures predicted from climate change models. Due to the pivotal role that phytoplankton play globally, it is critically important to more fully develop this knowledge.

PUFAs and highly unsaturated fatty acids (HUFAs; a subset of PUFAs with ≥20 carbons and three or more double bonds) are critical regulators of the survival, reproduction, and population growth in invertebrates and fish (Copeman et al. 2002, von Elert 2004). Phytoplankton are the primary source for the production of these compounds (Kainz et al. 2004). As they are highly retained in freshwater aquatic food webs, any changes in the quantity and quality of PUFAs in phytoplankton, as a consequence of thermal adaptation, could have direct impacts on growth rates, reproductive capacities, and general health (e.g., disease resistance) of aquatic invertebrates and fish.

Diet is extremely important in determining FA composition of primary consumers, such as herbivorous zooplankton (Brett et al. 2006). This is because herbivorous zooplankton and the fish that prey on them either cannot synthesize all the FAs that they require or cannot synthesize them in amounts required for optimal physiological performance (Arts and Wainman 1999, Arts et al. 2009). Thus, consumers must acquire at least some of the required essential fatty acids (EFAs) from dietary lipids ultimately supplied by phytoplankton FAs. Common examples include linoleic acid (LIN; 18:2n-6), ALA, eicosapentaenoic acid (EPA; 20:5n-3), arachidonic acid (ARA; 20:4n-6), and docosahexaenoic acid (DHA; 22:6n-3). Furthermore, different algal taxa exhibit different FA profiles (Harwood 1998, Napolitano 1999). For example, cryptophytes, diatoms, and dinoflagellates contain the most HUFAs, whereas green alga contain mostly shorter chain FAs including LIN and ALA (Ahlgren et al. 1990).

In this study, we examined the effects of two different culture temperatures on lipid profiles of *S. obliquus*, a freshwater chlorophyte. This species has been used extensively in controlled laboratory experiments as a food source for consumers, primarily due to its ease of culture and its adequacy as a herbivore food source. Chlorophytes contain EFAs such as LIN and ALA, the precursors for EPA and DHA, which zooplankton need for survival (Sundbom and Vrede 1997, Abrusan et al. 2007). Although the response of the FA and lipid composition of algae to chilling has received some attention (Thompson 1996, McLarnon-Riches et al. 1998), less is known about the effects of higher temperatures on FA and lipid composition especially in relation to assessing the nutritional quality of algae for herbivorous zooplankton.

Therefore, our objective was to characterize the ability of *S. obliquus* to adjust its FA and PL composition in response to a warm-water challenge (i.e., an 8°C warming from 20°C to 28°C) by investigating lipid and FA metabolism using [1-^{14}C]acetate. An increase in the number of extreme heat days is one of the predicted outcomes of global warming for northern temperate (e.g., Canadian, North European) ecosystems (IPCC 2007). Therefore, we were interested in determining if changes in EFA proportions in response to increasing temperature affected the nutritional quality of this alga. We hypothesized that, as temperature increased, *S. obliquus* would adjust its FA and lipid composition by decreasing the proportion of unsaturated FAs.

MATERIALS AND METHODS

Culturing of phytoplankton. *S. obliquus* CPCC 5 (Canadian Phycological Culture Centre, University of Waterloo, Waterloo, Canada) was used for all experiments. The algae were grown in 2 L batch cultures on a 16:8 h (L:D) cycle (PAR = 35.4 μmol · m^{-2} · s^{-1}) at two temperatures (20°C and 28°C) in Bold's basal medium on a table shaker (125 rpm) (BBM; Bold 1949, Bischoff and Bold 1963). Algal cells were counted daily with a Bright-Line Hemacytometer (Reichert, Buffalo, NY, USA) so as to determine when to harvest them in their exponential growth phase (usually after 2 weeks of incubation). After an ~2-week cultivation in 2 L flasks as described above, algal cells were harvested by centrifugation (2,500 rpm, 908*g*) (Hettich, Tuttlingen, Germany; model# Rotanta 460R). Eight 2 L flasks were used for each temperature treatment. Each 2 L flask gave an algal biomass yield of ~40 mg wet weight, which was used as a replicate for the metabolic experiments (four replicates were used in these experiments for each temperature treatment, see below). In addition, four replicates were used for the determination of dry biomass (samples at 70°C to constant weight) and lipid yields (calculated as FAs). These were repeated for growth at each temperature. All experiments were independently repeated at least once with similar results.

Radiolabeling experiments. Radiolabeling experiments were conducted in the following manner: Four 2 L flasks were used for each temperature treatment. After centrifugation (as described above), 37 mg algal pellets (fresh biomass) were

resuspended in fresh BBM and transferred to 100 mL flasks. Then 6 μCi of [1-^{14}C]acetate (Amersham Life Sciences Ltd., Bucks, UK; specific activity = 1.85–2.29 GBq · mmol^{-1}; where ''[1-^{14}C]'' indicates that the radioactivity in the acetate molecule is on the carboxyl carbon) was added to each flask, and the algae were incubated under the same conditions as described in ''Culturing of phytoplankton'' for 24 h.

Lipid extraction. Algal cells were harvested by centrifugation, the pellets were washed once with dechlorinated water, and total lipids were preextracted using hot isopropanol to inactivate endogenous lipases (see Kates 1986). The isopropanol extracts were then dried under nitrogen gas and redissolved in chloroform/methanol (2:1, by vol.). TLs were further separated using the method of Garbus et al. (1963), which was designed to give quantitative extraction even for highly polar membrane lipids, and solvents were evaporated under a stream of nitrogen. TL extracts were resuspended into known volumes of chloroform and stored under nitrogen at −20°C prior to further lipid analysis. The following analyses were performed using these total lipid extracts:

1. Analysis of total FAs (methods used: transmethylation followed by the capillary gas chromatography [GC] separation, see ''Analysis of fatty acids'' below).
2. Analysis of radio-incorporation from [1-^{14}C]acetate into the total FAs (methods used: transmethylation followed by the radio-GC separation, see ''Analysis of fatty acids'' below).
3. Analysis of radio-incorporation from [1-^{14}C]acetate into individual lipid classes (methods used: TLC followed by a scintillation counting, see below).
4. Analysis of radio-incorporation from [1-^{14}C]acetate into individual FAs of individual lipid classes (methods used: TLC, transmethylation followed by radio-GC separation, see below).
5. Analysis of FA profiles for individual lipids and determination of the individual lipid concentrations (methods used: TLC, transmethylation followed by capillary-GC separation, see below).

Separation of phospholipids, glycosylglycerides, and total nonpolar lipids (TNLs). Phospholipids and glycosylglycerides (GLs) were separated using two-dimensional TLC on silica gel G plates (Merck KGaA, Darmstadt, Germany) using chloroform/methanol/water (65:25:4, by vol.) in the first dimension and then chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5, by vol.) in the second (Benning et al. 1995). TNLs were separated using one-dimensional TLC on 10 × 10 cm silica gel G plates with double development using toluene:hexane:formic acid (140:60:1, by vol.) for the entire plate followed by hexane:diethyl ether:formic acid (60:40:1, by vol.) to half height (Hansen and Rossi 1990). After drying, the plates were sprayed with a 0.05% solution of 8-anilino-4-naphthosulphonic acid in methanol and viewed under UV light to reveal lipids. Identification was made routinely by reference to authentic standards and was also confirmed using specific color reagents (Kates 1986). The lipids were then scraped off the plates along with the silica gel. In the case of the FA analyses, transmethylation was performed (as described below in ''Analysis of fatty acids'') followed by either radio GC or capillary GC. To measure the incorporation of radioactivity from [1-^{14}C]acetate into individual lipid classes, we transferred the lipid and silica directly into a scintillation vial to which 10 mL of scintillant was added (OptiFluor, PerkinElmer Inc., Waltham, MA, USA). The samples were counted in a Perkin-Elmer Tri-Carb 2800 TR liquid scintillation counter.

Analysis of fatty acids. Aliquots of the total lipid extracts (for analysis of the total FAs) or individual lipid classes separated using TLC (for analysis of FA profiles in these lipids as well as to determine the concentrations of these lipids) were used for fatty acid methyl ester (FAME) preparation. FAMEs were prepared by transmethylation with 2.5% H_2SO_4 in dry methanol/toluene (2:1, by vol.) at 70°C for 2 h. A known amount of pentadecanoate (15:0) was added as an internal standard, so that subsequent quantification of peaks (and, consequently, lipids) could be performed. FAMEs were extracted with HPLC-grade hexane after addition of 5% NaCl.

Radiolabeled FAs were analyzed by radio GC using a Unicam Pro-GC connected via an effluent splitter to a LabLogic RAGA (LabLogic, Sheffield, UK) gas flow proportional counter. Glass columns (1.5 m × 4 mm i.d.) were packed with 10% SP-2330 on a 100/120 Supelcoport (Supelco, Bellefonte, PA, USA). The oven temperature was programmed as follows: 190°C for 10 min, programmed to 220°C at 4°C · min^{-1}, then 220°C for 30 min. The carrier gas was helium (flow rate = 30 mL · min^{-1}). Quantification of radioactive peaks used Laura software (LabLogic). Although four replicates were used for each temperature in all experiments, in some cases where individual lipid classes were poorly labeled, we had to combine samples.

TABLE 1. Effects of temperature on the endogenous fatty acid (FA) composition (% of total FAs) of individual glycerophospholipids in *Scenedesmus obliquus* grown at 20°C or 28°C.

	PC		PE		PI		PG	
FA	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C
16:0	6.2 ± 1.1	6.6 ± 2.5	2.3* ± 0.4	4.1* ± 0.7	40.2* ± 2.3	31.2* ± 3.8	6.6* ± 0.7	13.7* ± 1.3
16:1n-9	1.0 ± 0.1	0.6 ± 0.2	n.d.	n.d.	1.1 ± 0.4	0.4 ± 0.4	0.2 ± 0.0	0.1 ± 0.2
16:1n-13*t*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	21.6* ± 2.0	26.4* ± 1.6
16:1n-7	0.7* ± 0.1	1.0* ± 0.2	0.5* ± 0.2	1.8* ± 0.3	0.9 ± 0.2	1.0 ± 0.7	2.6* ± 0.4	0.4* ± 0.3
16:3n-3	0.2* ± 0.0	0.4* ± 0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16:4n-3	0.8* ± 0.1	0.6* ± 0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18:0	0.4 ± 0.3	0.5 ± 0.1	1.1 ± 0.9	4.6 ± 2.0	2.6 ± 3.0	4.6 ± 2.7	0.6* ± 0.3	1.2* ± 0.2
18:1n-9	14.2 ± 1.7	14.2 ± 0.7	13.6* ± 0.7	15.2* ± 0.4	38.5 ± 1.8	42.1 ± 2.1	9.2 ± 0.3	10.1 ± 0.7
18:1n-7	2.8* ± 1.3	3.4* ± 0.7	13.1 ± 1.9	11.4 ± 0.4	n.d.	1.0 ± 0.1	1.1* ± 0.1	3.7* ± 0.1
18:2n-6	17.4* ± 1.4	23.0* ± 1.1	23.5* ± 1.4	27.8* ± 0.4	11.1* ± 0.6	14.6* ± 0.7	7.5* ± 0.2	12.9* ± 1.0
18:3n-6	1.2* ± 0.1	2.1* ± 0.1	1.0 ± 0.1	1.0 ± 0.1	n.d.	n.d.	n.d.	n.d.
18:3n-3	44.9* ± 2.0	38.9* ± 1.6	39.0* ± 2.4	29.4* ± 1.0	5.6* ± 0.2	5.0* ± 0.4	35.2* ± 1.3	28.1* ± 2.2
18:4n-3	10.3* ± 0.7	8.7* ± 0.7	5.9* ± 0.6	4.7* ± 0.6	n.d.	0.1 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
22:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10.4* ± 1.0	2.7* ± 0.9
24:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.7* ± 0.4	0.4* ± 0.5

*Significantly different pairs ($P < 0.05$); n.d., not detected; mean ± SD, where $n = 4$ for each temperature.
PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol.

TABLE 2. Effects of temperature on the endogenous fatty acid (FA) composition (% of total FAs) of individual glycosylglycerides and betaine lipid in *Scenedesmus obliquus* grown at 20°C or 28°C.

	SQDG		MGDG		DGDG		DGTS	
FA	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C
16:0	42.6 ± 2.2	45.4 ± 4.2	0.3 ± 0.1	0.5 ± 0.1	8.6* ± 0.6	14.4* ± 0.8	22.0 ± 1.4	23.0 ± 4.8
16:1n-9	0.5 ± 0.1	0.5 ± 0.3	0.2 ± 0.0	0.3 ± 0.1	1.8 ± 0.2	2.2 ± 0.3	0.7 ± 0.2	0.5 ± 0.0
16:1n-7	0.2 ± 0.2	0.7 ± 0.2	0.3 ± 0.0	0.4 ± 0.1	0.9* ± 0.1	1.3* ± 0.1	0.9 ± 0.2	0.9 ± 0.4
16:2n-6	n.d.	n.d.	0.4 ± 0.0	0.4 ± 0.0	1.1 ± 0.1	1.2 ± 0.2	n.d.	n.d.
16:3n-3	n.d.	n.d.	0.9 ± 0.1	3.2 ± 1.7	3.5* ± 0.4	7.6* ± 1.0	0.6 ± 0.0	0.6 ± 0.2
16:4n-3	n.d.	n.d.	35.6 ± 3.2	32.1 ± 3.8	3.0* ± 0.5	1.5* ± 0.2	0.7 ± 0.1	1.2 ± 0.4
18:0	0.5 ± 0.2	0.7 ± 0.3	0.1 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	1.5 ± 0.7	1.6 ± 0.2	2.0 ± 0.5
18:1n-9	2.9 ± 0.1	3.8 ± 0.7	0.7 ± 0.2	1.1 ± 0.3	5.6* ± 0.2	7.9* ± 0.3	11.8 ± 0.4	10.0 ± 0.9
18:1n-7	16.0 ± 1.0	13.0 ± 1.0	0.3 ± 0.0	0.2 ± 0.0	1.3 ± 0.3	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.0
18:2n-6	1.7 ± 0.1	3.0 ± 0.9	2.6 ± 0.2	4.0 ± 1.1	11.8* ± 0.3	17.0* ± 0.4	5.1 ± 0.1	6.0 ± 1.0
18:3n-6	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	7.4 ± 0.2	8.8 ± 1.5
18:3n-3	33.6 ± 1.1	30.6 ± 2.5	55.7 ± 2.7	54.4 ± 0.6	55.7* ± 1.2	40.6* ± 1.2	14.4 ± 0.2	14.2 ± 2.3
18:4n-3	1.3 ± 0.1	1.5 ± 0.8	2.8 ± 0.2	2.9 ± 0.6	5.6* ± 0.1	3.0* ± 0.5	34.0 ± 1.4	31.8 ± 4.8

*Significantly different pairs ($P < 0.05$); n.d., not detected; mean ± SD, where $n = 4$ for each temperature; FAs present in proportions of <1% were not included.

SQDG, sulfoquinovosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryl-*N,N,N*-trimethylhomoserine.

TABLE 3. Fatty acid (FA) categories (% of total endogenous FAs) in different lipid classes of *Scenedesmus obliquus* grown at 20°C or 28°C.

	SAFA		MUFA		PUFA	
Lipid/FA category	20°C	28°C	20°C	28°C	20°C	28°C
Glycerophospholipids						
PC	6.6 ± 4.8	7.0 ± 2.6	18.6 ± 3.2	19.1 ± 1.8	74.8 ± 4.4	73.9 ± 4.0
PI	42.8 ± 5.2	36.0 ± 9.9	40.5 ± 2.4	44.5 ± 3.2	16.7* ± 0.8	19.5* ± 1.2
PG	22.3* ± 4.4	18.0 ± 2.8	44.4 ± 4.6	40.7 ± 2.8	43.0 ± 1.6	41.3 ± 3.2
PE	3.4* ± 0.8	8.6* ± 2.8	27.3 ± 2.6	28.5 ± 2.2	69.3* ± 3.8	62.9* ± 2.0
Glycosyldiacylglycerols						
SQDG	42.2 ± 2.4	46.0 ± 4.4	20.3 ± 1.6	18.2 ± 2.4	37.5 ± 1.4	35.8 ± 4.4
DGDG	8.9* ± 0.8	15.9* ± 1.9	9.6* ± 0.8	12.4* ± 0.8	81.5* ± 2.6	71.7* ± 3.6
MGDG	0.3 ± 0.2	0.7 ± 0.2	1.5 ± 0.4	2.0 ± 0.6	98.2 ± 6.8	97.3 ± 7.8
Betaine lipid						
DGTS	23.6 ± 1.0	25.0 ± 5.2	14.3 ± 2.0	12.4 ± 1.4	62.1 ± 2.0	62.6 ± 10.2
Total lipid	12.9* ± 0.2	22.1* ± 4.2	17.7* ± 0.2	19.6* ± 2.0	64.1 ± 0.6	54.8 ± 8.8
Total nonpolar lipid	10.8* ± 0.4	17.0* ± 2.8	42.7* ± 2.4	36.6* ± 5.6	26.9 ± 1.8	27.5 ± 2.8

*Significantly different pairs ($P < 0.05$); n.d., not detected; mean ± SD, where $n = 4$ for each temperature.

PC, phosphatidylcholine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGTS, diacylglyceryl-*N,N,N*-trimethylhomoserine; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SAFA, saturated fatty acid.

TABLE 4. Effects of temperature on labeling of fatty acids (FAs) (% of total labeled FAs) from [1-^{14}C]acetate in individual classes of the glycerophospholipids in *Scenedesmus obliquus* grown at 20°C or 28°C.

	PC		PE		PI		PG	
Fatty acid	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C
16:0	4.8 ± 0.8	3.0 ± 1.2	n.d.	n.d.	47.8 ± 8.2	33.9 ± 11.2	9.5 ± 1.0	6.9 ± 0.3
16:1	n.d.	2.2 ± 2.2	n.d.	n.d.	n.d.	n.d.	17.2* ± 2.4	38.0* ± 4.8
18:1	63.6 ± 1.7	66.2 ± 3.5	87.8 ± 14.2	100 ± 0.0	52.2 ± 8.2	66.1 ± 11.2	22.6 ± 2.8	22.8 ± 2.2
18:2	31.6 ± 0.8	28.6 ± 5.0	12.2 ± 14.2	n.d.	n.d.	n.d.	25.4 ± 2.1	21.2 ± 2.1
18:3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.1 ± 2.3	11.1 ± 5.6
22:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13.2 ± 0.9	n.d.

*Significantly different pairs ($P < 0.05$); n.d., not detected; mean ± SD, where $n = 2$ for PI (per temperature treatment), and $n = 2$ at 20°C and $n = 4$ at 28°C for PC and PG, and $n = 4$ for PE (per temperature treatment) (samples pooled, where necessary, for radio GC).

PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol.

TABLE 5. Effects of temperature on labeling of fatty acids (FAs) (% of total labeled FAs) from ^{14}C-labeled acetate in individual classes of the glycosylglycerides and betaine lipid in *Scenedesmus obliquus* grown at 20°C or 28°C.

	SQDG		MGDG		DGDG		DGTS	
Fatty acid	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C
16:0	32.5* ± 5.4	65.4* ± 0.3	n.d.	0.7 ± 0.5	12.9 ± 3.9	22.8 ± 6.2	53.4 ± 2.8	66.8 ± 7.1
16:1	n.d.	n.d.	n.d.	0.9 ± 0.5	8.4 ± 2.8	9.0 ± 2.9	n.d.	n.d.
16:2	n.d.	n.d.	n.d.	1.6 ± 0.7	n.d.	n.d.	n.d.	n.d.
16:4	n.d.	n.d.	11.0 ± 1.8	11.3 ± 3.1	n.d.	n.d.	n.d.	n.d.
18:1	36.2 ± 7.2	24.0 ± 0.5	25.2 ± 8.4	13.9 ± 2.6	38.0 ± 4.4	29.1 ± 1.0	46.6 ± 2.8	33.2 ± 7.1
18:2	18.3* ± 0.4	10.6* ± 0.3	39.1 ± 1.4	37.0 ± 2.1	28.7 ± 0.8	29.7 ± 4.3	n.d.	n.d.
18:3	13.0* ± 2.3	n.d.	24.7 ± 3.7	34.6 ± 6.2	12.0 ± 1.4	9.6 ± 3.6	n.d.	n.d.

*Significantly different pairs ($P < 0.05$); n.d., not detected; mean ± SD, where $n = 2$ at 20°C and $n = 3$ at 28°C for DGDG and DGTS, and $n = 2$ at 20°C and $n = 4$ at 28°C for SQDG and MGDG (samples pooled, where necessary, for radio GC).

SQDG, sulfoquinovosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryl-*N,N,N*-trimethylhomoserine.

Where this occurred (Tables 4 and 5), it is indicated in the table legend.

A Clarus 500 gas chromatograph with a flame ionizing detector (FID) (Perkin-Elmer 8500, Norwalk, CT, USA) and fitted with a 30 m × 0.25 mm i.d. capillary column (Elite 225, Perkin Elmer) was used for separation and analysis of FAs. The oven temperature was programmed as follows: 170°C for 3 min, programmed to 220°C at 4°C · min^{-1}, hold for 15 min (Guschina et al. 2003). FAMEs were identified routinely by comparing retention times of peaks with those of N-15-A and 1A FA standards (Nu-Chek Prep. Inc., Elysian, MN, USA). In addition, gas chromatography-mass spectrometry (GC-MS) of FAs as their 4,4-dimethyloxazoline (DMOX) derivatives was used (see below) for precise identification.

Identification of individual fatty acids using DMOX derivatives. DMOX derivatives were made for two reasons: (i) to unequivocally identify the position of the double bond in a number of isomers of mono-, di-, and polyunsaturated C16 FAs (commercial standards were not available for many of them) and (ii) to confirm the position of the double bonds in the other FAs that were identified in this study. Lipid samples were hydrolyzed with 0.1 M potassium hydroxide in 90% aqueous ethanol (0.25 mL per mg of sample) at 50°C for 3 h. After acidification with acetic acid and addition of water (2 mL), the free fatty acids were extracted twice with diethyl ether-isohexane (1:1, by vol; 6 mL then 3 mL). The combined organic layers were passed through a short (3 cm) column of anhydrous sodium sulfate prepared in a Pasteur pipette and were taken to dryness using a centrifugal evaporator (Jouan, Saint-Herblain, France; model RC 10–22). The free fatty acids were converted to DMOX derivatives by heating with 2-amino-2-methyl-1-propanol (0.25 mL) at 190°C for 16 h. On cooling, water (5 mL) was added, and the DMOX derivatives were extracted with diethyl ether-isohexane (1:1, by vol; 5 mL). The aqueous layer was reextracted with fresh solvent (2 mL), and the combined solvent extracts were washed with water (3 mL) and dried over anhydrous sodium sulfate. Finally, the solvent extract was passed through a short (3 cm) column of anhydrous sodium sulfate prepared in a Pasteur pipette. The column was washed with isohexane (2 mL), and the sample containing the DMOX derivatives was taken to dryness. The DMOX derivatives were dissolved in isohexane containing butylated hydroxytoluene (50 ppm) and kept at −20°C until they were analyzed. FAME and DMOX derivatives, made from a number of representative samples, were analyzed using GC-MS on a 7890A gas chromatograph (Agilent, Wilmington, DE, USA), fitted with a split/splitless (split ratio 50:1) injector and a Supelcowax^{TM} 10 (0.25 mm i.d. × 30 m in length, 0.25 μm film thickness) capillary column (Supelco, Bellefonte, PA, USA), connected to an Agilent 5975C inert XL MSD quadrupole mass spectrometer. The column temperature was held at 170°C for 3 min, temperature programmed to 220°C at 4°C min^{-1}, and finally held at 220°C for 30 min. Helium was the carrier gas at a constant flow rate of 1 mL · min^{-1}. The mass spectrometer was operated in electron impact mode at an ionization energy of 70 eV, and the mass range was 50–550 a.m.u. at a rate of 3 spectra · s^{-1}. Agilent ChemStation software was used for data acquisition.

Statistics. Comparison of the two temperature treatment means was performed using *t*-tests in SigmaStat (ver. 3.5). In cases where raw data were not normally distributed, the data were transformed by the square root arcsine transformation. The false discovery rate (FDR) was used for the multiple comparison test to avoid the increased error rates that result from multiple testing (Benjamini and Hochberg 1995). Significant effects were reported at $P < 0.05$.

RESULTS

Effects of temperature on the proportions of FAs in TLs and TNLs of S. obliquus. There was an overall decline in the proportion of n-3 PUFAs as growth temperature was increased from 20°C to 28°C, with a concomitant increase in SAFAs and LIN in the overall distribution of FAs in TLs (Fig. 1). This decrease in n-3 PUFAs was attributable to decreases in 16:4n-3 (~66%), 18:4n-3 (~48%), and ALA (~25%). Palmitic acid (16:0) increased by ~70% and was the only SAFA identified that was significantly affected by an increase in growth temperature. The dominant identified FAs at 20°C (i.e., those present in proportions >5%) were 18:3n-3, 16:4n-3, 16:0, 18:1n-9, and 18:2n-6 at 33.1, 13.6, 11.8, 9.6, and 5.8%, respectively. The dominant FAs at 28°C were 18:3n-3, 16:0, 18:2n-6, and 18:1n-9 at 24.6, 20.0, 9.3, and 9.0%, respectively (Fig. 1). In addition, it should be noted that the yield of lipid (as measured both gravimetrically or by total FAs · g^{-1} dry weight) was approximately halved for *S. obliquus* grown at 28°C compared with 20°C (data not shown).

The growth temperature increase resulted in the enhanced relative incorporation of radioactivity from [1-^{14}C] acetate into saturated 16:0 FA and

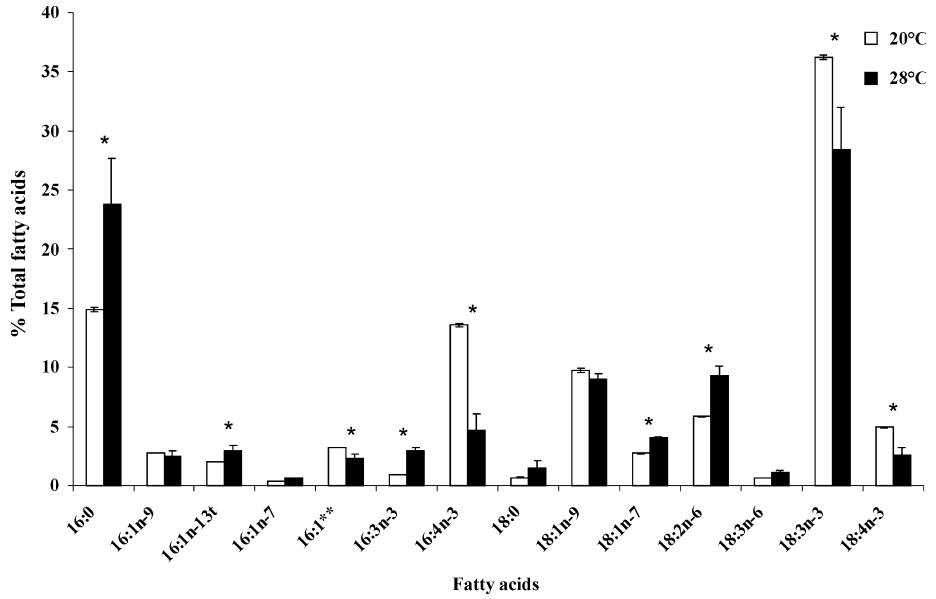

FIG. 1. Effects of temperature on the endogenous fatty acid (FA) composition (% of total FAs) of total lipids in *Scenedesmus obliquus* (means ± SD with $n = 4$ for each temperature). Pairs with an * were significantly different ($P < 0.05$) after applying the false discovery rate correction. All FAs present at >1% of total FAs are included. **The position of the double bond on the 16:1 isomer is unknown. FAs are abbreviated with the first number indicating the number of carbon atoms and the second figure showing the number of double bonds. The position of the first double bond from the methyl end of the chain is shown as n-x.

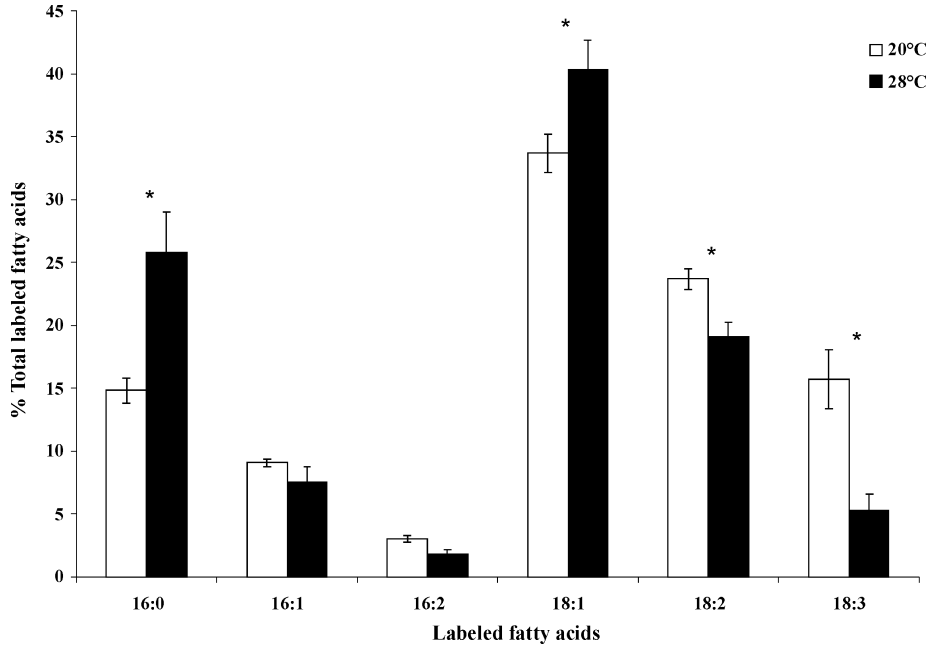

FIG. 2. Effects of temperature on the incorporation of radioactivity from ^{14}C-labeled acetate into total fatty acids (FAs) in *Scenedesmus obliquus* (% of total labeled FAs). Results are means ± SD, where $n = 3$ for 20°C and $n = 4$ for 28°C. Pairs with an * were significantly different ($P < 0.05$) after applying the false discovery rate correction.

monounsaturated 18:1 FA, and decreased radiolabel incorporation into unsaturated 18:2 and 18:3 FAs (Fig. 2) within the time-course of the labeling experiment. The percentage incorporation of radiolabel in 16:0 increased by ~90% at 28°C when compared with 20°C, whereas 18:1 increased by ~28% at 28°C. As growth temperature increased, the incorporation of [1-^{14}C] acetate into 18:3 decreased by ~62%, which was consistent with the decline in the proportion of ALA (Fig. 1).

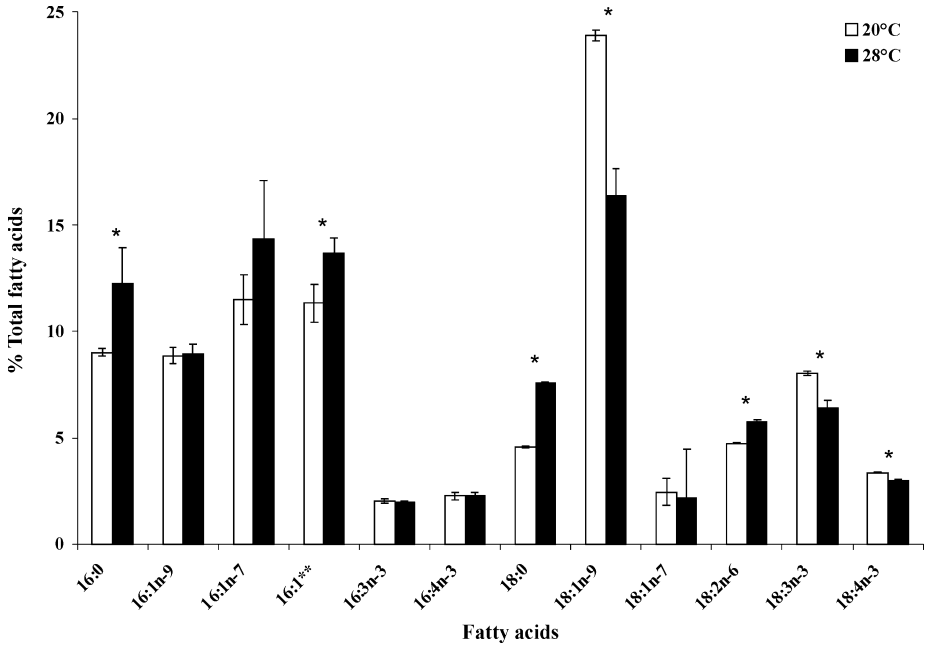

FIG. 3. Effects of temperature on the endogenous fatty acid (FA) composition (% of total identified FAs) in total nonpolar lipids of *Scenedesmus obliquus*. Results are means ± SD, where $n = 4$ for each temperature. Pairs with an * were significantly different ($P < 0.05$) after applying the false discovery rate correction. Only FAs present in proportions >1% were included. **The position of the double bond in the 16:1 isomer is unknown.

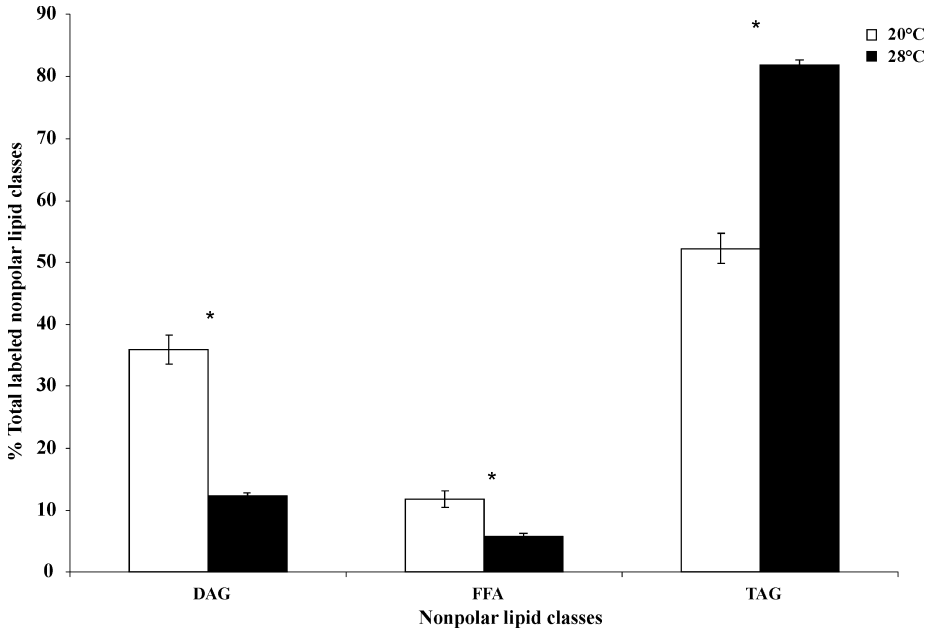

FIG. 4. Effects of temperature on the relative labeling of individual nonpolar lipid classes from ^{14}C-labeled acetate in *Scenedesmus obliquus* (% of labeled total nonpolar lipid classes). Results are means ± SD, where $n = 4$ for each temperature. Pairs with an * were significantly different ($P < 0.05$) after applying the false discovery rate correction. DAG, diacylglycerol; FFA, free (nonesterified) fatty acid; TAG, triacylglycerol.

FAs with 16-carbon chain lengths and 18:1n-9 dominated the TNL-FA profile at both temperatures (Fig. 3) in contrast with TLs. The relative amounts of both 16:0 and 16:1 (double bond position unknown) increased by ~37% and ~20%, respectively, whereas 18:1n-9 decreased by ~34% at the warmer temperature. The SAFA 18:0 almost doubled at 28°C in comparison to 20°C.

The TNLs of *S. obliquus* were composed of triacylglycerols (TAGs), diacylglycerols (DAGs), and free

(nonesterified) fatty acids (FFAs), and the majority of the radiolabel was incorporated in TAGs at both temperatures (Fig. 4). An increase in growth temperature resulted in a ~55% increase, a ~65% reduction, and a ~50% reduction in the relative incorporation of radiolabel from [1-^{14}C]acetate into TAGs, DAGs, and FFAs, respectively. The monoenoic FA 16:1 was the only labeled FA in the TNL fraction that changed (i.e., labeled at 20°C but not at 28°C) in response to an increase in temperature (data not shown).

Effects of temperature on proportions of FAs in total PL fractions of S. obliquus. At both temperatures, the PL composition of *S. obliquus* consisted of the following major glycerophospholipids: phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and cardiolipin (CL); and the following glycosyldiacylglycerols: sulfoquinovosyldiacylglycerol (SQDG), digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG); as well as the betaine lipid, diacylglyceryl-*N,N,N*-trimethylhomoserine (DGTS). The major radiolabeled PL classes at both temperatures were DGDG, MGDG, SQDG, PC, and PG (Fig. 5). The proportion of radiolabel in DGDG, PG, and DGTS decreased at 28°C when compared with 20°C with a simultaneous increase in the labeling of MGDG (Fig. 5).

The FAs of individual PL classes in *S. obliquus* resembled the distribution typical of green algae (Hitchcock and Nichols 1971, Harwood 1998); however, only the FA composition of the glycerophospholipids (PC, PE, PI, and PG) and the glycosylglyceride DGDG responded to the increase in temperature (Tables 1 and 2). Both PC and PE were enriched in PUFAs, especially with the two 18-carbon unsaturated EFAs, LIN and ALA. Both ALA and another n-3 PUFA, SDA (18:4n-3), decreased by ~15% at 28°C. In comparison to PC, overall PUFA accumulation in PE slightly decreased (Table 3). Increasing the temperature to 28°C resulted in a ~9% decrease in total PUFAs in PE, again, as a result of decreases in the proportions of ALA and SDA. The saturated and monounsaturated FAs 16:0 and 18:1n-9, respectively, comprised the dominant FAs of PI (Table 1). Unlike other SAFAs, which increased with increasing temperature, 16:0 in PI decreased by >20%. The phospholipid PG contained a relatively large amount of Δ^3-*trans*-hexadecenoic acid (16:1n-13*t*). As expected, this FA was found only in PG, and the shift to 28°C resulted in an increase of ~22%. The EFA ALA was the dominant FA in PG at both temperatures, but increasing the temperature to 28°C resulted in a reduction of ~20%. For all phospholipids, shift of growth temperature to 28°C caused an increase in the relative content of 18:2n-6, whereas 18:3n-3 was reduced.

DGDG contained a large amount of ALA with much lower levels of 16-carbon PUFAs. Increasing the temperature to 28°C decreased the proportion of ALA by ~28% and increased the proportion of 16:0 by ~66% in DGDG. The proportions of 18:1n-9 and LIN were higher in DGDG in comparison to its precursor lipid, MGDG (Table 2). Again, the decrease in ALA content in DGDG was accompanied by an increase in LIN.

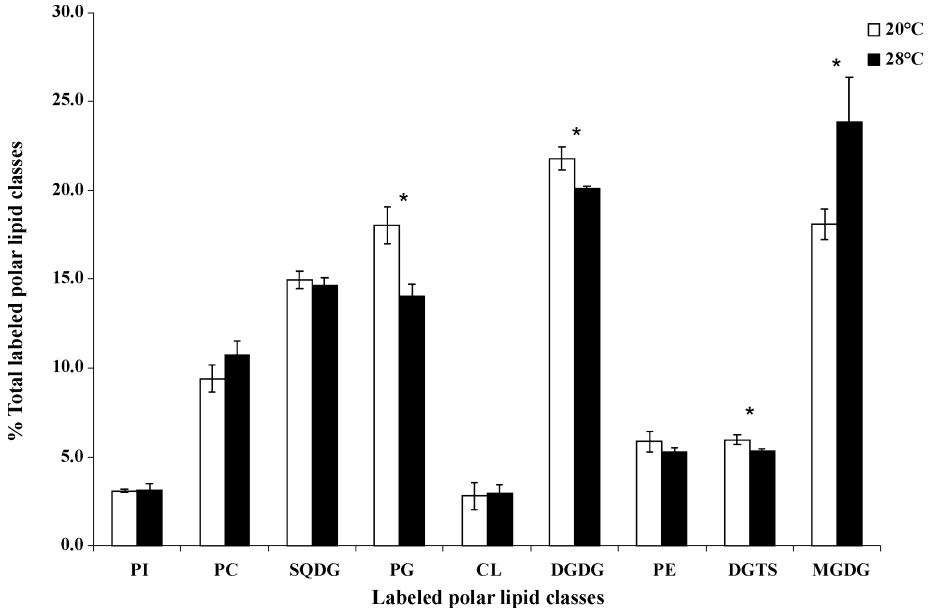

FIG. 5. Effects of temperature on the relative labeling of individual polar lipid (PL) classes from ^{14}C-labeled acetate in *Scenedesmus obliquus* (% of total labeled PL classes). Results are means ± SD, where $n = 4$ for each temperature. Pairs with an * were significantly different ($P < 0.05$) after applying the false discovery rate correction. CL, cardiolipin (diphosphatidylglycerol); DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryl-*N,N,N*-trimethylhomoserine; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol.

Newly synthesized 16:0 and 18:0 acyl chains were rapidly incorporated into the PLs and further desaturated and elongated during a 24 h incubation period at both temperatures (Tables 4 and 5). From these results, we conclude that PC could serve as a substrate for desaturation of 18:1 to produce 18:2; no further conversion of 18:2 into 18:3 was noted after 24 h incubation with [1-^{14}C]acetate, and temperature did not change these processes. In PE, the radiolabel was incorporated into 18:1 FA and further desaturated into 18:2, which was only detected at 20°C. Significant amounts of radiolabeled 16:0, 16:1, unsaturated 18C acids, and 22:0 were detected in PG at 20°C. An increase in temperature led to an accumulation of 16:1 and a lack of 22:0. Labeling of all 18-carbon unsaturated FAs suggested that the significant rates of desaturation reactions could be taking place using PG as substrate; however, these reactions were not affected by temperature (Table 4). In accordance with the FA distribution in MGDG, DGDG, and SQDG (Table 2), labeled FA patterns suggested that these lipids could also serve as substrates for 18:1 desaturation to produce LIN and ALA in *S. obliquus* (Table 5). Desaturation of 16:0 → 16:4 takes place exclusively on MGDG as substrate (Ohlrogge and Browse 1995) (see Tables 2 and 5), whereas only radiolabeled 16:1 was found in DGDG. However, no significant temperature effects were found on the labeling of FAs in MGDG, DGDG, or DGTS. Increasing the temperature decreased the labeling of 18:2 and 18:3 in SQDG by ~42% and 100%, respectively (Table 5).

DISCUSSION

We examined the effects of temperature on the lipid and FA composition and biosynthesis in *S. obliquus* under a temperature regime chosen to simulate what may well occur in pond waters of north-temperate ecosystems as a result of climate warming. The biochemical consequences of the ensuing temperature adaptations, especially with respect to changes in the nutritional quality of this alga, were of particular interest. Although nutritional quality of algae as food for herbivores has many features (e.g., amino acid and/or carbohydrate profiles, nitrogen and phosphorous concentrations, trace metals, sterols, vitamins, digestibility, toxicity, colonial vs. unicellular, etc.), herein, we consider a more narrowly defined, but important feature, the concentrations of EFAs.

As temperature increased from 20°C to 28°C, the FA composition of *S. obliquus* shifted from proportional domination by n-3 18-carbon PUFAs to a profile that was relatively depleted in 18-carbon n-3 PUFAs and enriched in SAFAs (particularly 16:0 and 18:0). This was determined for TLs, TNLs, and PLs. As both total lipid yield and the proportion of ALA were significantly lower at 28°C, we conclude that much less ALA was accumulated by *S. obliquus* in absolute terms (μg ALA · mg^{-1} dry weight) when this algae was grown at 28°C compared to 20°C.

The overall FA composition of *S. obliquus* was largely consistent with previous research. For example, chlorophytes are known to have an FA profile rich in 16:0, 16-carbon PUFAs, ALA, and LIN (Thompson 1996, Napolitano 1999). Of these FAs, the major component in lipids of most green algae is ALA, followed by 16:0. Minor amounts of SDA can be observed in some species of chlorophytes (Brett et al. 2009) and were also found in the strain of *S. obliquus* we used.

In accordance with previous studies that have examined lipid adaptations in response to temperature (Lynch and Thompson 1982, Badea and Basu 2009), the decrease in unsaturated FAs is associated with an acclimation to increasing temperature. Lynch and Thompson (1982) found that chilling the halophile chlorophyte *Dunaliella salina* from 30°C to 12°C resulted in an increase in overall FA unsaturation, particularly in ALA, although the underlying molecular mechanism by which this occurred was not explained. Other researchers have shown that the regulation of desaturase gene expression was involved in this process (De Palma et al. 2008, Badea and Basu 2009). Although the underlying mechanisms by which alterations in FAs occur were not the focus of this study, our data confirm that the activities of desaturases, which add an additional double bond to an existing 18:1 acid, comprise the major suite of reactions affected by the temperature increase in this particular alga. We suggest that n-3 desaturase activity on 18:2 and γ-18:3 acyl chain substrates significantly decreased at 28°C in the TL fraction together with a concomitant increase in accumulation of 18:1 monounsaturated fatty acid (MUFA) species, as well as the SAFAs 16:0 and 18:0, as its biosynthetic precursor molecules. This evidence suggests that, as environmental temperature increases, membrane FAs are modified to favor MUFA and SAFA accumulation. The results are consistent with Lynch and Thompson (1982), Nishida and Murata (1996), and Badea and Basu (2009), who reported that the level of FA unsaturation in membrane lipids increases as growth temperature is lowered. We demonstrated that as growth temperatures increase to levels that may routinely be experienced in shallow ponds under a climate-warming scenario in the summer, the level of FA unsaturation will decrease compared with what would be expected under more moderate water temperature scenarios typical of pre-climate-warming conditions. In our *S. obliquus* cultures, the proportion and absolute concentration of ALA, a product of direct desaturation of LIN (Hitchcock and Nichols 1971), decreased in TLs and decreased in proportion in all glycerophospholipids and DGDG when the growth temperature was increased to 28°C.

PUFAs tend to dominate the FA composition in the different glycerophospholipid classes because of their importance in the structure and functions of biological membranes (Thompson 1996). In algae, the major glycerophospholipids are PC, PE, and PG with minor amounts of phosphatidylserine, PI, and CL (Guschina and Harwood 2009). This finding is consistent with the preferential labeling of PC, PE, and PG in this alga. Of the glycerophospholipids, the largest accumulation of PUFAs was in PC and PE at both temperatures, followed by PG and PI (PI had the smallest amount of PUFAs). SAFAs, MUFAs, and PUFAs in PC did not differ between the two temperatures, suggesting that the overall level of FA unsaturation in PC is unaffected by an increase in temperature.

Substantial amounts of ALA were accumulated in PC and PE, and this accumulation was temperature sensitive. Incorporation of radioactivity from [1-^{14}C] acetate into ALA was not found in either lipid after 24 h incubation at either temperature. As labeling of this FA was significant in PG and GLs, especially MGDG, its absence in two major phospholipids (PC and PE) was not attributable to low instrument sensitivity. A more likely explanation is that the bulk of biosynthetic conversion of LIN into ALA takes place on chloroplast lipids such as MGDG, so that radiolabeling of such FAs in PC and PE requires more than the 24 h incubation period used in this study.

PG is found exclusively in the thylakoid membranes in algae and higher plants and is required for photosynthesis and growth (Harwood 1980). Unlike all other lipids, PG contains the unusual FA Δ^3-*trans*-hexadecenoic acid (16:1n-13*t*), in the *sn*-2 position of PG (Harwood 1998, Wada and Murata 2007). This FA is found in the PG of all eukaryotic photosynthetic organisms. The freezing tolerance in some higher plants is correlated with a decreased level of 16:1n-13*t* acid in PG (Xu and Siegenthaler 1997). Our data confirm this trend (i.e., the proportion of this FA slightly increased when algae were incubated at the higher temperature). The labeling of FAs from [1-^{14}C] acetate revealed a quick increase in biosynthetic rate of 16:1 in response to warming. Its labeling was much higher at 28°C when compared with 20°C suggesting that the desaturase responsible for its biosynthesis is more active at the warmer temperature.

GLs are found almost exclusively in photosynthetic membranes in algae and higher plants. They are characterized by a high content of PUFAs (Hitchcock and Nichols 1971). This was consistent with our findings where the proportion of PUFAs in MGDG and DGDG comprised >98% and 81%, respectively, at 20°C. The PUFAs contained mainly ALA (with >50% of the total % FA present in both lipid classes) as well as >30% of 16:4n-3 in MGDG at 20°C. The FA profiles of MGDG and the sulpholipid, SQDG, and the only betaine lipid, DGTS, were broadly consistent with those of other green algae (Thompson 1996) and were not altered by the change in temperature, suggesting that ALA and 16:4n-3 may be required in these membrane bilayers for maintaining proper cellular functions but may not be required to maintain average membrane lipid order (fluidity) in relation to this 8°C temperature difference.

As a result of greenhouse gas emissions, many regions across the globe will experience higher surface air temperatures (IPCC 2007). In conjunction with this climate, modelers have predicted that the number of extreme heat days will also rise significantly during the summer months (IPCC 2007). These extreme heat days will have a direct effect on large surface area-to-volume ratio aquatic ecosystems such as freshwater ponds and shallow lakes, as their water temperatures reflect air temperatures (McKee et al. 2002, Van Doorslaer et al. 2007). Water temperatures have already attained maximum summer highs of ~29°C in some lakes in Canada (Sharma et al. 2007). In addition, maximum summer temperatures ≥29°C have been documented in coastal bays connected to Lake Ontario (Wells and Sealock 2009). We must improve our understanding of the effects of such high water temperatures on the nutritional quality of phytoplankton as a food source for zooplankton and other herbivores because the EFAs are highly retained in aquatic foodwebs (Kainz et al. 2004) and are required for optimal animal growth and reproduction. In addition, EFAs such as ALA and its long chain homologs (EPA and DHA) are exported into adjacent terrestrial ecosystems (Gladyshev et al. 2009) where they are quickly assimilated by consumers.

The results arising from analysis of endogenous lipids provide an integrative, longer-term perspective on the FA profiles that developed in this alga under the two temperature regimes and were in broad agreement with the short-term ^{14}C-labeling experiments, which demonstrated that, even after 2 weeks habituation to the two temperatures, there were still significantly different effects on metabolism (as assessed by ^{14}C-uptake into the different FAs and lipid classes measured here). Increasing the water temperature to 28°C decreased total lipid yield, the proportion of the essential fatty acid ALA in TLs, TNLs, and PLs, and the proportional contribution of all PG and DGDG in PL. Thus, we conclude that the nutritional quality of *S. obliquus* was reduced by our experimental warming protocol.

We realize that extrapolating from controlled laboratory experiments to real-life situations is difficult because in natural systems variables such as temperature, nutrients, light, salinity, toxins, and so forth, act together (additively and synergistically) to produce the final outcomes on algal lipid yield and nutritional quality and therefore that more realistic field experiments are ultimately required to address the broad question, will climate warming have a net

negative effect on EFA production on aquatic primary producers in temperate and subpolar regions? Along these same lines, it is also becoming clear that rising water temperature favors the dominance of cyanobacteria (Paerl and Huisman 2008), a group of prokaryotes, many of which are known to be relatively depleted in ALA compared with Chlorophyta (Ahlgren et al. 1992). This is an issue of considerable importance because it is now well known that algal-derived EFAs have many positive effects on the health (e.g., growth rates, reproductive capacity) of aquatic organisms and their consumers (Tocher 2003, Brett et al. 2009, Lands 2009, Parrish 2009). Therefore, based on the results presented here, we suggest that the nutritional status of organisms at higher tropic levels may be compromised because of changes in the lipid composition of their algal food base, if water temperatures of ponds and small lakes increase in response to climate change.

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