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SPECIES-SPECIFIC VARIATION IN FATTY ACID CONCENTRATIONS OF FOUR PHYTOPLANKTON SPECIES: DOES PHOSPHORUS SUPPLY INFLUENCE THE EFFECT OF LIGHT INTENSITY OR TEMPERATURE?[1]

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We tested, in the laboratory, the influence of light intensity, temperature, and phosphorus (P) supply on fatty acid (FA) concentrations of four freshwater algae: the green algae *Scenedesmus quadricauda* (Turpin) Bréb. and *Chlamydomonas globosa* J. Snow, the cryptophyte *Cryptomonas ovata* Ehrenb., and the diatom *Cyclotella meneghiniana* Kütz. We investigated the main and interactive effects of two variables on algal FA concentrations (i.e., light intensity and P supply or temperature and P supply). Interactive effects of light intensity and P supply were most pronounced in *C. meneghiniana*, but were also found in *S. quadricauda* and *C. ovata*. Changes in several saturated and unsaturated FA concentrations with light were more distinct in the low-P treatments than in the high-P treatments. Interactive effects of temperature and P supply on various FA concentrations were observed in all four species, but there was no consistent pattern. In lake ecosystems, P limitation often coincides with high light intensities and temperatures in summer. Therefore, it is important to examine how combinations of these environmental conditions affect FA concentrations of primary producers that are important sources of FAs for higher trophic levels.

Key index words: ***Chlamydomonas*; *Cryptomonas*; *Cyclotella*; fatty acids; light; lipids; phosphate; PUFA; *Scenedesmus*; temperature**

Abbreviations: **ALA, α-linolenic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FAME, fatty acid methyl ester; GLA, γ-linolenic acid; LIN, linoleic acid; MUFA, monounsaturated fatty acid; OD, optical density; POC, particulate organic carbon; PUFA, polyunsaturated fatty acid; SAFA, saturated fatty acid; TFA, total fatty acid**

The FA composition of algae is of interest in many fields of research, such as physiology, ecology, taxonomy, biotechnology, and pharmacology. The nutritional quality of phytoplankton for higher trophic levels depends on FA composition, especially on concentrations of polyunsaturated fatty acids (PUFAs) (Müller-Navarra et al. 2000, Wacker and Von Elert 2001). Therefore, algal FA profiles are important in ecological research because, through their effects on nutrition and condition of herbivores, they can directly influence energy transfer efficiency across the primary producer–herbivore interface. FA profiles also serve as chemotaxonomic markers to distinguish between species (Dalsgaard et al. 2003) or to identify sources of organic matter in sediments (e.g., Dijkman and Kromkamp 2006). In aquaculture, FAs are important because commercially important fish species require dietary C20 and C22-PUFA (Tocher 2003, Arts and Kohler 2009), the ultimate source of which are PUFA-rich algae. Furthermore, a high yield of total lipid and energy-yielding FAs is also of interest for the production of biofuels (Hu et al. 2008). FAs are indispensable components of membranes and storage lipids and are, among other things, involved in signal transduction (Sumida et al. 1993). Finally, in human and mammalian health and nutrition, essential long-chain PUFAs are known to have generally favorable effects on the cardiovascular system (Connor 2000), vision (Sapieha et al. 2011), and brain (Lim et al. 2005) including effects on psychiatric disorders (Hallahan and Garland 2005, Lafourcade et al. 2011).

FA profiles of phytoplankton are species specific, but there are similarities between taxonomically

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related species. Furthermore, environmental conditions are known to affect the FA composition of algae (Solovchenko et al. 2008, Guiheneuf et al. 2009, Guschina and Harwood 2009). Algae adapt to cold temperatures by increasing the activity of desaturases, which results in an increase in the degree of unsaturation in the FAs of their cell membranes. Double bonds in the FA tail of monounsaturated fatty acids (MUFAs) and PUFAs increase their cross-sectional area, causing higher degrees of disruption in the membrane and therefore enhancing fluidity (Morgan-Kiss et al. 2006).

The FAs are also known to be influenced by the function of the photosynthetic apparatus in autotrophic organisms (Klyachko-Gurvich et al. 1999). For example, increasing light intensities (below photoinhibitory levels) result in increased synthesis of carbon compounds, such as lipids and their components, the FAs, in autotrophic organisms. This is mainly due to the light regulated activity of the acetyl CoA carboxylase, which catalyzes the first step of FA synthesis (Wainman et al. 1999). Excess carbon, thus produced, is often stored in the form of neutral storage lipids (triacylglycerols), a lipid class that is dominated by saturated fatty acids (SAFAs) and MUFAs. Therefore, higher concentrations of SAFAs and MUFAs have been observed in high light compared to low light. Alternately, low light intensities stimulate the synthesis of galactolipids, which are important components of photosynthetic membranes (Sukenik et al. 1989, Khotimchenko and Yakovleva 2004). In contrast to triacylglycerols, galactolipids contain high amounts of PUFAs (Guschina and Harwood 2009). Thus, we might expect to see changes in concentrations and relative proportions of algal FAs between low and high light intensity growth conditions. Finally, nutrient limitation has been determined to increase the FA concentration of phytoplankton (Thompson 1996, Lynn et al. 2000, Guschina and Harwood 2009). This, however, seems not to be a direct effect of nutrient limitation on FA synthesis, but a consequence of a limited growth rate and the consequent storage of excess carbon in the form of lipids.

In spite of the above-mentioned general findings, there are large species-specific differences and contrasting results with respect to the concentrations of individual FAs in phytoplankton in response to changing environmental conditions. For example, reactions of PUFA concentrations to light limitation have been contradictory in several studies performed with various species: some increase their PUFA concentration under light limitation (Sukenik et al. 1989, Mock and Kroon 2002, Guiheneuf et al. 2009), while others increase PUFA concentrations in high light (Seto et al. 1984, Zhukova 2007, Solovchenko et al. 2008). It is very hard to compare results among such studies and form general conclusions since, in most cases, only one alga was analyzed and the culture conditions varied.

We conducted a comprehensive analysis of how FA concentrations of four freshwater phytoplankton species change with different environmental conditions. We chose the genera *Scenedesmus*, *Chlamydomonas*, *Cryptomonas*, and *Cyclotella* as representatives of common algal groups Chlorophyceae, Cryptophyceae, and Mediophyceae (diatoms), all of which are important components of phytoplankton communities in temperate lakes and therefore as food organisms for herbivorous zooplankton. Monocultures of these species were cultured under varying conditions of light intensity, temperature as well as P supply. The experiments were designed to not only reveal variations in FA concentrations with one environmental factor, but also to document interactive effects between P and light intensity or P and temperature on the FA concentrations of the four species. We hypothesized that many of the reported contradictory results describing the reactions of FAs to environmental conditions derive from interactive effects of nutrient supply and another factor (e.g., temperature and light intensity).

MATERIALS AND METHODS

Cultures. We performed two different experiments using the two Chlorophyceae *S. quadricauda* and *C. globosa*, the Cryptophyceae *C. ovata* (all three species from the culture collection of the Limnological Institute, University of Constance), and the Mediophyceae *C. meneghiniana* (collection of algal cultures, Göttingen, Germany, SAG 1020-1a). The species were cultivated in WC medium (Nichols 1973) in semicontinuous cultures (i.e., we diluted the cultures every day at the same time). The dilution rate differed between the two experiments (see below for details). To avoid daily fluctuations and short-term adaptations in the biochemical composition of the algal cells we applied continuous lighting. A circadian rhythm would have entailed synchronization of cell division rates and with it great daily fluctuations in biochemical composition dependent on the stage of the cell cycle. Continuous light conditions desynchronize algal cell division patterns (Roenneberg et al. 1989) and therefore lead to an average biochemical composition that is constant over time. Optical density (OD 800 nm, UV mini-1240; Shimadzu, Duisburg, Germany) was measured daily, and the growth rate was calculated ($\mu = \ln[OD_2] - \ln[OD_1]/t$; μ: growth rate, OD_1: OD [800 nm], OD_2: OD [800 nm] after time t). All experiments were carried out until growth rates of all cultures remained constant to ensure that all cells of a culture had the same cell division rate and to achieve acclimation to the respective experimental conditions. Carbon deficiency was avoided by aerating the media with sterile filtered air.

Light experiment. To achieve light intensities of 30, 60, 140, 230, and 490 μmol PAR photons $\cdot m^{-2} \cdot s^{-1}$ (LI-1400 datalogger; LI-COR Environmental GmbH, Bad Homburg, Germany, equipped with a 4π quantum sensor), a climate chamber (20°C) was divided into five compartments (climate chamber: VB 1514, Vötsch GmbH, Balingen-Frommern, Germany; fluorescent lamps: FLUORA L30W/77 and LUMILUX L30W/830, warm white, Osram AG, München, Germany). Each compartment was shaded differently with neutral density foil filters (Lee Filters, Hampshire, England). Although light intensities in lakes can reach much higher values than applied here, lipid synthesis in algae has been reported to be light saturated at light intensities ranging from 300 to 800 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (Wainman et al. 1999). Therefore, we chose a gradient covering the range from light-limited to light-saturated lipid synthesis.

The four species were cultivated in 1 L Erlenmeyer flasks each in 300 mL of one high-P (50 μM P, provided in the form of K_2HPO_4) and one low-P-medium per compartment. The low-P medium was 1 μM P for *Scenedesmus*, 5 μM P for *Cryptomonas* and *Chlamydomonas*, and 10 μM P for *Cyclotella* because of species-specific differences in their optimum P requirements (Rhee 1978, Klausmeier et al. 2004). We had two replicates per light compartment for each treatment. The dilution rate for all cultures was 0.2 per day. We chose constant dilution rates, and with it constant growth rates, for all cultures to reduce the effect of light intensity on the growth rates of the different species. At the end of the experiment light intensities within all cultures were determined separately. Slightly different growth of replicates of each species in the same light compartment resulted in differing cell densities, presumably due to slightly differing initial inocula concentrations, and therefore in slightly differing light conditions due to shading (difference between light intensities measured in the same light compartment was on average 11%). We therefore decided to deal with all cultures as individual levels in a continuous light gradient (i.e., regression approach) instead of referring to the cultures in the same light compartment as replicates (analysis of variance [ANOVA] approach).

Temperature experiment. The species were cultivated in 1 L Erlenmeyer flasks under two different temperature conditions (10°C and 25°C) each in 500 mL of one high-P (50 μM P, provided in the form of K_2HPO_4) and one low-P-medium per temperature at a light intensity of 200 μmol PAR photons $\cdot$ $m^{-2} \cdot s^{-1}$ (three replicates per treatment). We chose 10°C and 25°C as two extremes that might occur during the growth season in the euphotic zone of temperate lakes (Mitchell et al. 2004), depending on depth and latitude (Mitchell and Lampert 2000). The low-P medium was 1 μM P for *Scenedesmus*, *Chlamydomonas*, and *Cyclotella* and 5 μM P for *Cryptomonas*. Different growth of high-P and low-P treatments would have caused differences in cell densities and therefore different shading inside the cultures. To avoid such variances in lighting, the cultures were diluted every day to the same optical density (OD: 0.05, 800 nm, UV mini-1240; Shimadzu). However, this unavoidably led to varying dilution rates.

FA analyses. Samples for FA determination were obtained by filtering 0.5–1 mg algal carbon on 25 mm glass fiber filters (GF/F; Whatman, Dassel, Germany). Filters were stored at −25°C under nitrogen atmosphere in glass tubes with Teflon seal after adding 7 mL of dichlormethane-methanol (2:1 v/v). Extraction of lipids was done twice with dichlormethane-methanol (2:1 v/v). Before further analysis, a defined concentration of methyl nonadecanoate (19:0; Fluka 74208, purchased from Sigma-Aldrich GmbH, Steinheim, Germany) and methyl tricosanoate (23:0; Fluka 91478) was added as internal standard. After extraction of lipids, identification and quantitation of fatty acid methyl esters (FAMEs) was done using gas chromatography (6890N; Agilent Technologies, Böblingen, Germany) according to Wacker and Martin-Creuzburg (2007), but with the following configuration: 1 μL of sample was injected in split mode (5:1), vaporized in the injector at 250°C, and mixed with the carrier gas (helium). FAMEs were separated on a 50% cyanopropyl-phenyl methyl-polysiloxane column (Agilent Technologies J&W DB-225, 30 m × 0.25 mm × 0.25 μm) using the following temperature gradient: 60°C for 1 min, increasing 20°C $\cdot$ min^{-1} until 150°C, 10°C $\cdot$ min^{-1} until 220°C, for 13.75 min. FAMEs were detected using a flame ionization detector at 250°C. FAMEs were quantified using multipoint standard calibration curves determined for each FAME (using six different concentrations per FAME) from mixtures of known composition (Supelco® 37 Component FAME Mix; Sigma 47885-U, Sigma-Aldrich GmbH). Identification of FAME from the samples was done via known retention times of the Supelco® FAME Mix reference substances and the mass spectra, which were recorded using a gas chromatograph-mass spectrometer (Finnigan MAT GCQ, Bremen, Germany) equipped with a fused-silica capillary column (Agilent Technologies J&W DB-225 ms; see Martin-Creuzburg et al. 2009).

Particulate organic carbon (POC) determination. POC was measured by first filtering ~0.25 μg carbon of the algal suspension onto 25 mm, precombusted, glass fiber filters (GF/F; Whatman) and then quantifying algal C using an elemental analyzer (Euro EA 3000; HEKAtech GmbH, Wegberg, Germany).

Statistics. Algal FA concentrations were expressed on a per carbon basis. In the light experiment an analysis of covariance (ANCOVA) was done with light intensity as continuous variable and medium phosphate concentration as factor. To meet homogeneity of variance assumptions, light intensities were $\log_e$ transformed. FA concentrations arising from the temperature experiment were analyzed by a two-way ANOVA with temperature and medium phosphate concentration as factors. The statistical tests resulted in four possibilities to describe the reaction of FA concentrations to experimental conditions: (i) there is a significant reaction to light or temperature, but not to P concentrations (i.e., no difference between the high-P and low-P treatments); (ii) there is a significant difference between the high-P and low-P treatments, but no reaction to light or temperature; (iii) both light/temperature and P supply have a significant influence on the concentrations of FAs; (iv) the two factors interact (i.e., reaction to light or temperature differs depending on P supply). For the data of the temperature experiment, in addition to the ANOVA, we calculated means and standard deviations of FA concentrations to visualize significant differences between the two temperature levels and the high-P and low-P treatments. In the light experiment, we analyzed FA concentrations in a continuous light gradient (i.e., we compared regression lines of high-P and low-P treatments and did not estimate means). Instead, we calculated adjusted means, which are the predicted FA concentrations for high-P and low-P treatments at the mean value of the applied light intensities. Adjusted means make it easier to compare between low-P and high-P treatments than if we had used the intercept from the model. Furthermore, for the light experiment, we show confidence intervals (95%) instead of standard deviations (Table 1). Confidence intervals allow distinguishing easily between significant and nonsignificant slopes, in that the slope is significant if the interval does not include zero. All statistical calculations were carried out using the software package R (R Development Core Team, version 2.6.0, 2007, http://www.r-project.org). Results reported in the text are statistically significant at the $P < 0.05$ level.

RESULTS

Light experiment. We observed interactive effects of light intensity and P supply on FA concentrations especially in the diatom *C. meneghiniana*. In this species, the summary FA indices total fatty acid (TFA), SAFA, and MUFA increased with increasing light intensity in both the low-P treatment and the high-P treatment (Fig. 1). However, the slope was higher in the low-P treatment than in the high-P treatment. Increasing concentrations of TFA, SAFA, and MUFA with increasing light were also observed in the two green algae (*S. quadricauda* and *C. globosa*; Fig. 1), but there was no difference between the P levels in the summary FA indices. In the Cryptophyceae *C. ovata*, TFA, SAFA, MUFA, and PUFA concentrations did not change with light intensity (Fig. 1).

TABLE 1. Statistics of the light experiment.

	Scenedesmus quadricauda		*Chlamydomonas globosa*		*Cryptomonas ovata*		*Cyclotella meneghiniana*	
	Slope	Adj. mean	Slope	Adj. mean	Slope	Adj. mean	Slope	Adj. mean
C14:0	**L**		**L, P**		**L**		**L × P, L, P**	
P+	−0.68 (−1.4 to 0.1)	2.32 (1.59 to 3.06)	0.80 (0.5 to 1.1)	4.03 (3.67 to 4.39)	−0.69 (−1.0 to −0.4)	3.66 (3.33 to 3.98)	2.40 (1.8 to 3.0)	21.0 (20.4 to 21.6)
P−	−0.88 (−1.4 to −0.4)	2.52 (2.07 to 3.25)	0.47 (0.2 to 0.8)	3.12 (2.82 to 3.48)	−1.42 (−2.8 to −0.0)	4.59 (3.15 to 4.92)	4.91 (3.9 to 5.9)	24.6 (23.7 to 25.2)
C16:0	**L**		**L, P**		**P**		**L × P, L, P**	
P+	22.7 (19.7 to 25.6)	63.4 (60.6 to 66.3)	4.70 (2.1 to 7.3)	28.50 (25.65 to 31.35)	−1.49 (−3.4 to 0.4)	21.54 (19.66 to 23.42)	4.24 (−1.6 to 10.0)	96.9 (91.1 to 102.7)
P−	14.0 (0.4 to 27.5)	71.1 (58.2 to 73.9)	3.40 (−0.3 to 7.2)	24.11 (20.56 to 26.96)	1.01 (−1.1 to 3.2)	17.89 (15.67 to 19.77)	24.94 (17.9 to 32.0)	116.8 (110.6 to 122.6)
C18:0	**L**							
P+	3.78 (2.7 to 4.9)	6.74 (5.70 to 7.78)	0.57 (−0.2 to 1.3)	2.87 (2.06 to 3.69)	0.67 (−0.3 to 1.7)	5.06 (4.08 to 6.03)	1.32 (−0.7 to 3.4)	4.73 (2.70 to 6.76)
P−	3.78 (0.8 to 6.8)	9.31 (6.47 to 10.35)	−0.19 (−0.9 to 0.5)	3.00 (2.36 to 3.81)	−0.22 (−2.0 to 1.6)	4.70 (2.86 to 5.68)	−0.48 (−2.8 to 1.8)	5.81 (3.79 to 7.85)
C16:1*n*−7	**L, P**		**L**		**L, P**		**L × P, L, P**	
P+	0.05 (−0.2 to 0.3)	2.23 (2.03 to 2.43)	0.34 (−0.1 to 0.7)	2.42 (1.98 to 2.86)	−0.34 (−0.6 to −0.0)	3.75 (3.45 to 4.04)	13.6 (3.3 to 24.0)	181.8 (171.5 to 192.1)
P−	−0.12 (−0.2 to −0.0)	0.97 (0.88 to 1.17)	0.28 (0.0 to 0.5)	2.21 (1.98 to 2.65)	−0.61 (−1.5 to 0.2)	4.73 (3.83 to 5.02)	45.9 (31.5 to 60.2)	200.1 (187.4 to 210.4)
C16:1*n*−9	**L**		**L**		**L**		**P**	
P+	0.23 (−0.1 to 0.6)	2.32 (1.99 to 2.66)	2.57 (1.8 to 3.4)	5.99 (5.15 to 6.84)	−0.25 (−0.4 to −0.2)	1.30 (1.20 to 1.39)	0.01 (−0.5 to 0.6)	1.72 (1.16 to 2.28)
P−	0.42 (−0.1 to 0.9)	2.59 (2.13 to 2.92)	2.80 (0.7 to 4.9)	7.94 (5.98 to 8.78)	−0.15 (−0.3 to 0.0)	1.35 (1.18 to 1.45)	−0.57 (−2.0 to 0.8)	3.00 (1.75 to 3.56)
C18:1*n*−7	**L, P**		**L**		**L, P**		**L × P, P**	
P+	1.05 (0.2 to 1.9)	3.22 (2.36 to 4.07)	2.34 (1.0 to 3.6)	11.6 (10.2 to 13.0)	0.17 (−0.3 to 0.7)	6.03 (5.52 to 6.53)	0.12 (−0.1 to 0.4)	3.47 (3.24 to 3.70)
P−	0.52 (0.2 to 0.9)	2.06 (1.71 to 2.92)	1.18 (−0.5 to 2.8)	11.4 (9.82 to 12.8)	0.86 (0.1 to 1.7)	7.49 (6.66 to 8.00)	−0.05 (−1.0 to 0.0)	4.29 (3.82 to 4.52)
C18:1*n*−9	**L**		**L**		**P**		**P**	
P+	44.9 (29.3 to 60.4)	73.1 (58.2 to 88.0)	17.3 (12.0 to 22.6)	38.5 (32.8 to 44.2)	0.22 (−0.1 to 0.5)	2.83 (2.55 to 3.11)	0.18 (−0.6 to 0.9)	3.08 (2.35 to 3.81)
P−	30.2 (8.2 to 52.3)	75.8 (54.9 to 90.7)	9.99 (2.5 to 17.5)	35.5 (28.3 to 41.2)	−0.12 (−0.9 to 0.6)	4.62 (3.83 to 4.90)	−0.50 (−1.8 to 0.8)	4.40 (3.30 to 5.13)
C16:3*n*−3	**L, P**		**L**				**L**	
P+	1.64 (−0.6 to 3.9)	10.9 (8.8 to 13.1)	4.58 (2.4 to 6.8)	15.5 (13.1 to 17.9)	0.21 (−0.6 to 1.0)	2.60 (1.83 to 3.37)	−5.27 (−6.8 to −3.7)	34.8 (33.2 to 36.3)
P−	2.40 (−0.6 to 5.4)	14.3 (11.5 to 16.4)	3.09 (0.5 to 5.6)	15.3 (12.9 to 17.7)	−0.26 (−1.0 to 0.4)	2.25 (1.52 to 3.02)	−4.12 (−6.2 to −2.1)	34.8 (33.0 to 36.3)
C16:4*n*−3	**P**						**P**	
P+	0.47 (−2.9 to 3.8)	21.2 (18.0 to 24.4)	−5.12 (−10.3 to 0.1)	25.6 (20.0 to 31.3)	−0.16 (−0.4 to 0.1)	1.04 (0.81 to 1.27)	0.08 (−0.1 to 0.3)	3.47 (3.27 to 3.66)
P−	1.96 (−1.3 to 5.3)	15.9 (12.7 to 19.1)	0.73 (−4.0 to 5.5)	24.3 (19.8 to 29.9)	−0.02 (−0.2 to 0.2)	0.87 (0.68 to 1.09)	−0.87 (−2.7 to 0.9)	5.53 (3.94 to 5.73)
C18:2*n*−6					**L, P**		**L × P, L, P**	
P+	2.28 (−4.5 to 9.1)	36.2 (29.7 to 42.7)	0.91 (−0.3 to 2.1)	7.54 (6.26 to 8.82)	−1.47 (−2.3 to −0.6)	7.66 (6.78 to 8.53)	−0.08 (−0.2 to 0.1)	2.48 (2.32 to 2.64)
P−	−2.65 (−11.0 to 5.7)	40.5 (32.6 to 47.0)	−0.50 (−1.5 to 0.5)	6.92 (5.96 to 8.20)	−1.49 (−3.4 to 0.4)	10.24 (8.24 to 11.11)	−0.41 (−0.7 to −0.1)	3.06 (2.78 to 3.22)
C18:3*n*−6	**L × P, L**				**L**		**L, P**	
P+	−1.04 (−2.0 to −0.1)	3.99 (3.09 to 4.88)	n.d.		−0.23 (−0.4 to −0.1)	1.16 (1.02 to 1.30)	−0.16 (−0.3 to −0.0)	2.12 (1.98 to 2.26)
P−	−2.73 (−3.6 to −1.9)	4.66 (3.82 to 5.56)	n.d.		−0.04 (−0.2 to 0.1)	1.10 (0.94 to 1.24)	−0.36 (−0.6 to −0.1)	2.71 (2.47 to 2.85)
C18:3*n*−3	**L**						**L × P, L, P**	
P+	10.1 (−2.8 to 23.0)	55.9 (43.6 to 68.3)	−2.75 (−12.6 to 7.1)	51.5 (40.8 to 62.2)	−3.62 (−8.9 to 1.6)	36.6 (31.4 to 41.9)	−0.21 (−0.4 to 0.0)	2.71 (2.50 to 2.91)
P−	9.74 (−4.1 to 23.6)	58.6 (45.5 to 70.9)	5.96 (−4.7 to 16.7)	45.9 (35.7 to 56.6)	−0.92 (−7.8 to 6.0)	36.2 (29.1 to 41.5)	−0.66 (−1.0 to −0.3)	3.10 (2.78 to 3.31)
C18:4*n*−3	**L, P**				**P**		**L × P, L, P**	
P+	2.19 (0.3 to 4.1)	8.30 (6.48 to 10.1)	n.d.		1.58 (−2.8 to 6.0)	32.3 (27.9 to 36.7)	0.24 (0.1 to 0.4)	3.40 (3.23 to 3.57)
P−	0.44 (−2.1 to 3.0)	12.1 (9.68 to 13.9)	n.d.		1.74 (−1.3 to 4.8)	20.3 (17.1 to 24.7)	0.97 (0.6 to 1.4)	5.43 (5.08 to 5.60)
C20:5*n*−3					**L × P**		**L**	
P+	n.d.		n.d.		−2.85 (−5.2 to −0.5)	12.3 (9.94 to 14.6)	3.14 (1.1 to 5.1)	40.2 (38.2 to 42.2)
P−	n.d.		n.d.		0.30 (−1.8 to 2.4)	13.5 (11.4 to 15.8)	4.40 (1.6 to 7.2)	38.5 (36.0 to 40.5)
C22:6*n*−3							**L**	
P+	n.d.		n.d.		−1.13 (−2.4 to 0.1)	4.29 (2.99 to 5.59)	1.03 (0.4 to 1.7)	12.1 (11.4 to 12.7)
P−	n.d.		n.d.		0.07 (−1.4 to 1.6)	4.60 (3.07 to 5.86)	1.01 (−0.0 to 2.1)	12.4 (11.4 to 13.0)

Data shown are slopes and adjusted means of regression lines as well as confidence intervals (95%). Bold letters indicate statistically significant effects of light intensity (L), phosphorus (P) supply, or interactive effects of these two factors (L × P). To facilitate comparison between low-P and high-P treatments we included adjusted means instead of intercepts. n.d., not detected.

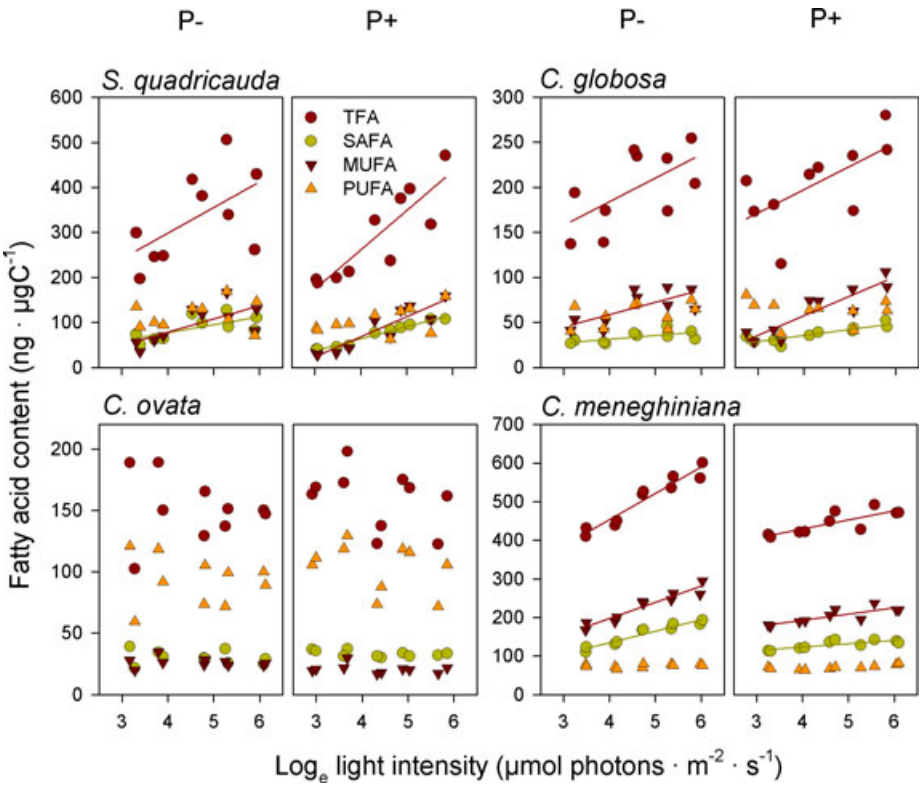

FIG. 1. Change with light intensity in total fatty acid (TFA, significant effects: *Scenedesmus quadricauda*: L; *Chlamydomonas globosa*: L; *Cyclotella meneghiniana*: L × P), saturated fatty acid (SAFA, *S. quadricauda*: L; *C. globosa*: L, P; *C. meneghiniana*: L × P), monounsaturated fatty acid (MUFA, *S. quadricauda*: L; *C. globosa*: L; *Cryptomonas ovata*: P; *C. meneghiniana*: L × P), and polyunsaturated fatty acid concentration (PUFA, *C. meneghiniana*: P) in the four analyzed species. Each data point represents a single culture. Regression lines were added only if effect of light intensity was significant.

We observed interactive effects on many of the individual FAs of *C. meneghiniana*. For example, 14:0, 16:0, 16:1*n*−7, and 18:4*n*−3 concentrations increased with increasing light, and the slope was in each case higher in the low-P treatment than in the high-P treatment (Table 1). In contrast, 18:1*n*−7, 18:2*n*−6 (linoleic acid, LIN), and 18:3*n*−3 (α-linolenic acid, ALA) decreased with increasing light intensity, but only in the low-P treatments. In *S. quadricauda*, only 18:3*n*−6 (γ-linolenic acid, GLA) differed in its reaction to light depending on P supply (i.e., its concentration decreased with increasing light, but the slope was more negative in the low-P treatment than in the high-P treatment, Table 1). In all cases described above, the change in FA concentration with increasing light intensity was more pronounced in the low-P cultures than in the high-P cultures. The only exception to this trend was seen for 20:5*n*−3 (eicosapentaenoic acid, EPA) in *C. ovata*; this FA decreased with increasing light in the high-P treatment, but did not change in the low-P treatment (Table 1).

Temperature experiment. We observed interactive effects of temperature and P supply on FA concentrations of the analyzed species. TFA, SAFA, and MUFA concentrations in *C. meneghiniana* were higher at 25°C than at 10°C, but only in the low-P cultures (Fig. 2). In *C. globosa*, SAFA concentration also was higher at 25°C than at 10°C, however, only in the high-P treatment in the summary FA indices (Fig. 2).

For the individual FAs, all possible kinds of interactions were detected. Some FA concentrations were higher at 25°C than at 10°C, either only in the low-P treatment (such as 18:0 in *S. quadricauda*, 18:0 and 18:4*n*−3 in *C. globosa*, and 18:1*n*−7 in *C. ovata*, Table 2) or only in the high-P treatment (such as 16:3*n*−3 in *C. globosa* and 18:1*n*−9, LIN, and GLA in *C. ovata*, Table 2). Others were higher at 10°C than at 25°C only in the low-P treatment (16:0 in *C. globosa* and 16:1*n*−7 in *C. meneghiniana*, Table 2) or only in the high-P treatment (16:1*n*−7 in *S. quadricauda*, Table 2). We observed also contrary results for the two P levels: the concentration of 16:1*n*−7 in *C. globosa* was higher at 25°C in the low-P treatment but higher at 10°C in the high-P treatment. Conversely, the concentration of 18:1*n*−9 in *C. globosa* was higher at 10°C in the low-P cultures, but higher at 25°C in the high-P cultures (Table 2).

DISCUSSION

FA concentrations of microalgae in nature are affected by different environmental conditions

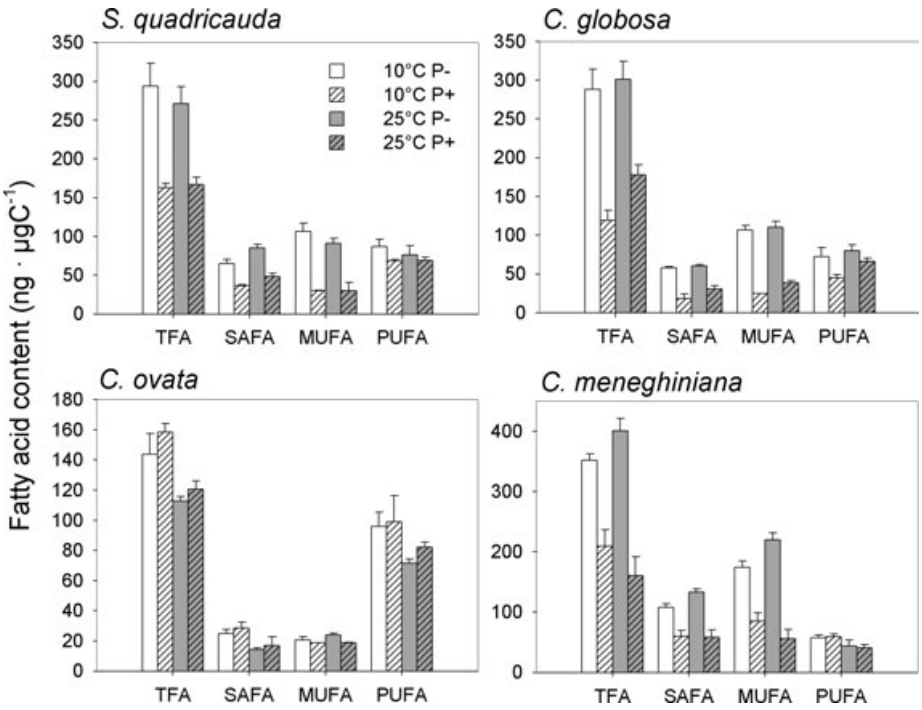

FIG. 2. Change with temperature in total fatty acid (TFA, significant effects: *Scenedesmus quadricauda*: P; *Chlamydomonas globosa*: T, P; *Cryptomonas ovata*: T, P; *Cyclotella meneghiniana*: T × P), saturated fatty acid (SAFA, *S. quadricauda*: T, P; *C. globosa*: T × P; *C. ovata*: T; *C. meneghiniana*: T × P), monounsaturated fatty acid (MUFA, *S. quadricauda*: P; *C. globosa*: T, P; *C. ovata*: T, P; *C. meneghiniana*: T × P), and polyunsaturated fatty acid concentration (PUFA, *S. quadricauda*: P; *C. globosa*: T, P; *C. ovata*: T; *C. meneghiniana*: T) in the four analyzed species. Data shown are means and standard deviations.

simultaneously. In our laboratory experiments, the influence of light intensity and temperature on FA concentrations depended to some extent on P supply. This finding is in agreement with a former study on phytoplankton sterol concentrations that also showed different reactions to light intensities depending on P supply (Piepho et al. 2010). In the light experiment, changes in several FA concentrations were more pronounced in the low-P treatment than in the high-P treatment. For example, TFA concentration of *C. meneghiniana* increased from 400 to 460 $\mu g \cdot mgC^{-1}$ in the high-P cultures, but from 400 to 570 $\mu g \cdot mgC^{-1}$ in the low-P treatment.

At high light intensities, excess carbon is often stored in the form of triacylglycerols; storage lipids that are rich in SAFAs and MUFAs (Guschina and Harwood 2009). The observed increase of TFA, SAFA, and MUFA concentrations in *C. meneghiniana*, *S. quadricauda*, and *C. globosa* in this study are in agreement with this general observation. P limitation has a similar effect because as cell division rates decrease less carbon is needed for the synthesis of new membrane components. This leads to an accumulation of carbon, which is again stored in the form of lipids (Guschina and Harwood 2009). Therefore, we observed a higher accumulation of TFA with increasing light in low-P cultures of *C. meneghiniana*.

Furthermore, the concentration of some FAs decreased with increasing light at low-P supply, but did not change at high-P supply. This was observed, for example, for LIN and ALA in *C. meneghiniana*, two PUFAs that usually do not accumulate in triacylglycerols of diatoms (Orcutt and Patterson 1974, Pahl et al. 2010). Thus, we might assume that the above-mentioned effect occurred predominantly in the membrane lipids of the diatom. Also as described above, increasing light intensities as well as increasing P limitation resulted in enhanced TFA concentrations in *C. meneghiniana*, and with it the general carbon concentration increased. Since we expressed FA concentration on a per carbon basis, increasing carbon concentrations (e.g., due to higher total lipid contents) might explain the apparent decrease of LIN and ALA per carbon in the low-P treatment of *C. meneghiniana*. A similar explanation might apply to GLA concentration in *S. quadricauda*, which decreased in both P treatments, but with a more negative slope in the low-P culture.

The EPA concentration in *C. ovata* increased with decreasing light intensity in the high-P treatment, but remained constant over the light gradient in the low-P treatment. We assume that the increased amount of EPA in *C. ovata* grown at low-light supply originates from its role in chloroplast lipids, which possess higher concentrations of unsaturated FAs

TABLE 2. Statistics of the temperature experiment.

	Scenedesmus quadricauda		*Chlamydomonas globosa*		*Cryptomonas ovata*		*Cyclotella meneghiniana*	
	10°C	25°C	10°C	25°C	10°C	25°C	10°C	25°C
C14:0	**T, P**		**T, P**		**T**		**T × P**	
P+	3.71 (±0.25)	5.07 (±0.19)	4.28 (±0.02)	5.53 (±0.45)	3.95 (±0.18)	5.46 (±0.33)	7.39 (±0.83)	18.4 (±2.94)
P−	2.83 (±0.12)	3.86 (±0.74)	7.05 (±0.44)	8.01 (±0.37)	4.01 (±0.58)	5.10 (±0.37)	8.24 (±0.57)	28.6 (±0.99)
C16:0	**T, P**		**T × P**		**T**		**P**	
P+	26.6 (±1.74)	34.3 (±3.49)	10.7 (±5.82)	20.0 (±1.51)	20.2 (±1.73)	8.36 (±6.29)	45.3 (±9.18)	40.6 (±3.85)
P−	48.5 (±4.71)	62.9 (±2.73)	43.4 (±2.00)	38.0 (±1.82)	17.4 (±2.13)	3.52 (±0.11)	88.2 (±5.70)	89.4 (±4.26)
C18:0	**T × P**		**T × P**				**T**	
P+	3.16 (±0.08)	4.25 (±1.24)	2.16 (±0.13)	2.31 (±0.15)	2.97 (±0.07)	2.93 (±0.03)	2.04 (±0.12)	2.99 (±0.56)
P−	7.97 (±0.45)	12.9 (±0.59)	3.16 (±0.19)	5.20 (±0.42)	3.16 (±0.43)	2.68 (±0.08)	2.90 (±0.02)	2.67 (±0.06)
C16:1*n*−7	**T × P**		**T × P**		**T, P**		**T × P**	
P+	4.39 (±0.30)	2.75 (±0.28)	4.17 (±0.17)	2.88 (±0.28)	5.04 (±0.08)	4.30 (±0.09)	78.2 (±13.8)	54.6 (±7.5)
P−	1.20 (±0.06)	1.57 (±0.26)	3.47 (±0.25)	9.25 (±0.75)	5.98 (±0.24)	5.06 (±0.49)	163.0 (±10.6)	203.3 (±10.1)
C16:1*n*−9	**T, P**		**T × P**		**P**		**T**	
P+	1.61 (±0.09)	2.01 (±0.15)	1.47 (±0.13)	2.81 (±0.25)	1.54 (±0.09)	1.66 (±0.03)	0.84 (±0.12)	1.41 (±0.14)
P−	2.21 (±0.14)	3.04 (±0.31)	9.45 (±0.45)	17.11 (±1.53)	1.45 (±0.19)	2.79 (±0.07)	1.09 (±0.12)	1.77 (±0.55)
C18:1*n*−7	**T**		**T × P**		**T × P**		**P**	
P+	1.47 (±0.02)	2.04 (±0.10)	3.22 (±0.25)	9.31 (±0.14)	5.33 (±0.26)	5.30 (±0.13)	2.42 (±0.13)	3.15 (±0.69)
P−	1.56 (±0.12)	2.49 (±0.50)	9.86 (±0.78)	23.25 (±2.62)	5.74 (±0.82)	7.15 (±0.13)	3.57 (±0.13)	4.44 (±0.70)
C18:1*n*−9	**P**		**T × P**		**T × P**		**P**	
P+	21.0 (±0.55)	21.3 (±10.8)	14.9 (±0.11)	21.0 (±1.14)	3.48 (±0.27)	2.87 (±0.16)	1.87 (±0.17)	2.37 (±0.42)
P−	98.1 (±10.1)	79.7 (±9.0)	82.0 (±5.60)	55.4 (±4.84)	4.02 (±0.64)	4.61 (±0.16)	2.82 (±0.24)	2.54 (±0.17)
C16:3*n*−3	**T × P**		**T × P**		**T, P**		**T, P**	
P+	6.30 (±0.45)	4.25 (±0.53)	6.91 (±0.64)	13.2 (±0.85)	1.22 (±0.12)	1.62 (±0.05)	1.03 (±0.06)	1.20 (±0.34)
P−	14.1 (±1.69)	8.86 (±0.89)	19.1 (±2.45)	19.7 (±2.61)	1.47 (±0.12)	1.83 (±0.11)	1.55 (±0.04)	1.42 (±0.05)
C16:4*n*−3	**P**						**P**	
P+	21.8 (±1.81)	15.5 (±2.15)	23.7 (±3.14)	28.7 (±2.29)	1.19 (±0.09)	1.30 (±0.10)	4.24 (±0.08)	4.90 (±1.32)
P−	22.1 (±2.60)	10.5 (±1.38)	32.3 (±4.70)	30.5 (±5.01)	0.77 (±0.68)	1.12 (±0.07)	3.73 (±0.10)	3.37 (±0.41)
C18:2*n*−6	**T, P**		**T, P**		**T × P**			
P+	12.3 (±0.30)	19.0 (±1.41)	2.89 (±0.28)	7.43 (±0.12)	4.81 (±0.69)	7.04 (±0.16)	1.41 (±0.02)	2.07 (±0.37)
P−	19.6 (±1.69)	28.3 (±3.59)	8.29 (±1.12)	11.1 (±0.94)	6.85 (±0.46)	6.14 (±0.72)	1.65 (±0.19)	1.65 (±0.10)
C18:3*n*−6	**T, P**				**T × P**			
P+	2.05 (±0.09)	3.91 (±0.54)	n.d.	n.d.	1.36 (±0.05)	1.72 (±0.04)	1.15 (±0.05)	1.49 (±0.23)
P−	1.20 (±0.03)	3.40 (±0.59)	n.d.	n.d.	1.39 (±0.16)	1.30 (±0.06)	1.22 (±0.10)	1.22 (±0.08)
C18:3*n*−3	**T**		**P**				**T**	
P+	47.8 (±2.05)	38.7 (±6.66)	41.0 (±3.97)	55.4 (±3.83)	20.9 (±2.88)	21.8 (±1.70)	n.d.	2.73 (±0.81)
P−	58.4 (±8.10)	33.4 (±6.04)	59.6 (±12.90)	61.9 (±9.27)	25.5 (±3.63)	22.3 (±2.36)	n.d.	1.99 (±0.09)
C18:4*n*−3	**T**		**T × P**		**T, P**		**T × P**	
P+	5.24 (±0.28)	7.07 (±1.19)	0.99 (±0.10)	0.37 (±0.65)	46.2 (±9.79)	34.2 (±1.31)	10.1 (±1.25)	n.d.
P−	5.05 (±0.34)	9.79 (±1.85)	0.88 (±0.07)	1.15 (±0.05)	31.5 (±3.68)	22.0 (±1.60)	18.3 (±1.92)	1.12 (±0.14)
C20:5*n*−3					**T, P**		**T, P**	
P+	n.d.	n.d.	n.d.	n.d.	16.3 (±4.11)	11.2 (±0.42)	31.8 (±2.01)	28.4 (±5.60)
P−	n.d.	n.d.	n.d.	n.d.	21.6 (±1.06)	12.8 (±0.90)	19.7 (±1.11)	18.0 (±3.72)
C22:6*n*−3					**T**		**P**	
P+	n.d.	n.d.	n.d.	n.d.	6.58 (±1.89)	4.00 (±0.14)	11.7 (±0.80)	9.64 (±2.14)
P−	n.d.	n.d.	n.d.	n.d.	6.88 (±0.65)	3.05 (±0.11)	12.3 (±1.14)	12.3 (±3.20)

Data shown are means and standard deviations. Bold letters indicate statistically significant effects of temperature (T), phosphorus (P) supply, or interactive effects of these two factors (T × P). n.d., not detected.

under light limitation (e.g., Klyachko-Gurvich et al. 1999). Simultaneously, P-limited *Cryptomonas* might not be able to synthesize more phospholipids under low light. Since EPA is quite abundant in the chloroplast phospholipid phosphatidylglycerol of *Cryptomonas* (Sato 1991), this could explain why EPA does not increase with decreasing light in the low-P treatment of *C. ovata*.

The temperature experiment revealed a variety of interactive effects. For example, TFA, SAFA, and MUFA concentrations of *C. meneghiniana* were higher at 25°C than at 10°C only in the low-P treatment. In the high-P treatment there was no difference between 10°C and 25°C, but the trend was for FA concentrations to be slightly higher at 10°C. Although we supplied constant P concentrations to the cultures in all treatments, we observed that the cellular P:C ratio was somewhat lower at 10°C than at 25°C in the high-P treatment (data not shown). Assuming that at lower P:C the FA concentration is slightly higher (as can be seen by the generally higher concentration of TFAs, SAFAs, and MUFAs in the low-P treatment), the temperature effect in the high-P treatment might be hidden by the described P effect. The latter explanation could, for example, also explain what happened to the concentration of 18:0 in *C. globosa*. However, for other interactive effects this explanation did not apply,

because the effect of P:C on the FA concentrations was not as pronounced.

Interactive effects of light and P as well as of temperature and P were species specific and did not generally affect trends in concentrations of individual FAs. In some cases, interactions might explain contradictory results of FA concentrations to environmental conditions, but in other cases they did not. Therefore, we conclude that additional factors play a role in explaining the inconsistent trends in concentrations of individual FAs in response to the environmental conditions that we manipulated in our experiments. It is striking that we observed interactive effects of light intensity and P as well as of temperature and P on the concentrations of TFAs, SAFAs, and MUFAs of the diatom *C. meneghiniana*. For the other three investigated species interactive effects on FAs seem to occur at random. This finding raises the question in what way the response of the diatom to the experimental conditions differs from the other species.

We suggest that to generalize responses of FA concentrations to environmental conditions it might be necessary to know the optimal growth conditions for each alga in terms of light, temperature, and P supply and then to analyze the changes of FA concentrations around these optima. This would result in apparently different experimental conditions for the different species, but physiologically the algae would be growing at a comparable reference point. For example, it has been described that diatoms, such as *Cyclotella* in this study, are adapted to grow best in relatively bright light (Eppley et al. 1969) but that cryptophytes, such as *Cryptomonas*, do better under low light (Gervais 1997). If we apply these facts to the here analyzed species of *Cyclotella* and *Cryptomonas*, one could argue that, since we used rather low light intensities, *Cryptomonas* might have grown near its optimal light condition, whereas *Cyclotella* might prefer still higher light intensities. In the light experiment, we observed only rare and moderate changes of FA concentrations with light in *Cryptomonas*. FA concentrations in *Cyclotella* changed to a much higher degree with increasing light. It would therefore be interesting to analyze if phytoplankton species are able to maintain homeostatic FA concentrations near their growth optima, while the biochemical concentration changes significantly as soon as the algae are stressed by environmental conditions that are farther away from their optima.

Also, diatoms grow most efficiently at high nutrient concentrations, but are poor competitors when nutrients are limiting (Egge and Aksnes 1992, Egge 1998). In this study, changes in FA concentrations of *Cyclotella* with light or temperature were more pronounced in the low-P treatments than in the high-P treatments. This would support the idea that near optimum growth conditions, in this case higher P concentrations, changes in biochemical composition of algae are less pronounced. Although all of the analyzed species can be found in temperate, mesotrophic lakes, and therefore are adapted to the same habitat, such differences might be behind the great variety of physiological adaptations to environmental conditions.

Furthermore, it is obvious that variations of FA concentrations are much less pronounced in *C. ovata* than in the other species, especially in the light experiment. Of the analyzed species *Cryptomonas* is the only one with aspects of a heterotrophic lifestyle (Tranvik et al. 1989). If changes in FA concentration are, to some extent, adaptations of the photosynthetic apparatus, it is likely that for this species light affects FA production in a different way.

We quantified all FAs on a per carbon basis. Thus, we avoided the potential problem of inferring changes in FA concentrations due to changes in FA proportions (e.g., assuming that decreasing PUFA proportions represent changes in absolute PUFA concentrations when this effect could be caused merely by an increase in absolute concentrations of SAFAs or MUFAs). However, when showing data on a per carbon basis, it has to be kept in mind that carbon concentrations of algae are not independent of environmental conditions and apart from lipids there are other carbon storage molecules, such as carbohydrates (e.g., Granum et al. 2002) that can change the way the data must be interpreted. Blanchemain and Grizeau (1996) compared the change of EPA concentration of the diatom *Skeletonema* with light intensity expressed as percent of total FAs or on a chl *a*, dry weight or per cell basis. The authors found increasing FA concentrations with light when they normalized EPA to chl *a*, a decreasing concentration per dry weight, and no change on a per cell basis or in percent of total FAs. Therefore, contradictory results of different studies on reactions of FA concentrations to environmental conditions might also be caused by the unit used to quantitate the data. Which unit is most appropriate depends on the aim of the study. For analyses that deal with aspects of cell physiology, reporting lipid quantities on a per cell basis might be ideal, whereas studies focused on detailed aspects of photosynthesis might benefit from the expression of lipid quantities on a chl *a* basis. However, reporting lipid quantities on a per cell basis encounters the problem of different biochemical composition of cells in different reproduction stages on the one hand and the problem of different cell sizes and carbon contents of cells of different species on the other hand. Comparing the FA concentrations of different species on a per cell basis would involve considering cell sizes and per cell carbon contents of each species. The option of normalizing FA concentrations to chl *a* must entail recognizing the known variation of chl *a* concentration with varying light intensities (Kohl and Nicklisch 1988). In our study, we focused on describing changes in FA concentrations of algae in relation to their quality as

food for herbivores. Thus, we suggest that reporting FAs on the basis of a well-accepted measure of food quantity (i.e., on a per carbon basis) is appropriate and desirable. Herbivorous zooplankton ingests a certain amount of food quantity per time, which cannot be described as the number of cells ingested, since it might ingest much more cells of smaller phytoplankton than of larger ones. Therefore, we conclude that reporting FAs on a per carbon basis is the most appropriate way to present our results. Furthermore, when tracking the transfer of nutrients through food webs carbon is an appropriate unit, since it still applies after phytoplankton cells have been digested by consumers.

The FAs are components of various lipid classes that differ in their function in phytoplankton cells. Although there are some general assumptions concerning the FA composition of the individual lipid classes, for instance, there are mainly SAFAs and MUFAs in triacylglycerols, but many PUFAs in glycolipids, FA composition of the lipid classes is still species specific. Adaptation to varying environmental conditions might occur at the level of lipid classes instead of the level of FAs. For example, high light intensities increase triacylglycerols (Guschina and Harwood 2009), and, depending on which FAs dominate in the triacylglycerols, changes in the composition of TFAs might vary between different species. This would mean that species-specific changes in FA concentration could be caused by the differing FA concentrations of the lipid classes. To analyze the adaptation of lipid classes to changing environmental conditions it would therefore be necessary to first separate the lipid classes of a plankton sample and then analyze the FA composition of each class separately. To find general reaction norms of FA concentrations to varying environmental conditions, we would suggest choosing this approach in the future.

Our study shows that interactive effects of two environmental conditions on the FA concentration of algae might explain species-specific contradictory results that have been found in earlier studies, which only considered one environmental factor at a time. Although we also discussed further possible reasons for the described inconsistencies, we suggest that when dealing with the influence of light or temperature on the FA concentration of phytoplankton, nutrient supply should also be considered. The effect of P can be of great importance in lakes that might be limited by this nutrient in summer. High light intensities and temperatures further play a role for the phytoplankton community during this time of the year.

Since algal primary producers are an important source of FAs for higher trophic levels, the influence of the analyzed environmental conditions on the FA concentrations of algae are of great interest for ecological food web studies. The anthropogenic influence on freshwater ecosystems, for example, eutrophication or climate change, makes it crucial for us to understand the possible impact it can have on aquatic food webs. This understanding is even more important when we consider that humans are part of this food web and are ultimately as dependent on the intake of essential FAs as are small zooplankton species.

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