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Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes

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ABSTRACT: The fatty acid (FA) composition of algae is an important determinant of their food quality for consumers, and FAs can also be used as biomarkers for biochemical and energetic pathways in food webs. FA analyses of 7 freshwater algal classes and 37 strains showed clear similarity within classes and strong differences amongst classes. Class was a dominant factor (66.4%) explaining variation in FA signatures of microalgae. The 7 algal classes comprised 4 separate groups according to their FA profiles: (1) Chlorophyceae and Trebouxiophyceae, (2) Bacillariophyceae, (3) Cryptophyceae, Chrysophyceae, and Raphidophyceae, and (4) Euglenophyceae. Each group had a characteristic FA composition, although the proportional abundance of individual FAs also differed between species and with environmental conditions. FAs found to be particularly representative for each group (i.e. diagnostic biomarkers) were as follows: 16:4ω3 and 16:3ω3 for Chlorophyceae and Trebouxiophyceae; 16:2ω7, 16:2ω4, 16:3ω4, 16:4ω1, and 18:4ω4 for Bacillariophyceae; 22:5ω6 and 18:4ω3 for Cryptophyceae and Chrysophyceae (Synurales), 16:3ω1 for Chrysophyceae (Ochromonadales), 16:2ω4, 16:3ω4, 16:3ω1, and 20:3ω3 for Raphidophyceae; and 15:4ω2, 20:4ω3, 20:2ω6, 20:3ω6, and 22:4ω6 for Euglenophyceae. FAs thus offer a powerful tool to track different consumer diets in a lacustrine food web. Based on the 20:5ω3 (eicosapentaenoic acid) and 22:6ω3 (docosahexaenoic acid) content among the investigated freshwater algal classes, Chlorophyceae, Trebouxiophyceae, and Chrysophyceae are of intermediate food quality for zooplankton, and Cryptophyceae, Bacillariophyceae, Euglenophyceae, and Raphidophyceae should be excellent resources for zooplankton.

KEY WORDS: Lipids · Diet quality · Omega-3 fatty acids · Lacustrine food web · Green algae · Diatoms · Cryptomonads

INTRODUCTION

In aquatic food webs, most fatty acids (FAs) are synthesized by phytoplankton and bacteria before being transferred via herbivorous invertebrates to fish and ultimately humans (Arts et al. 2001). Phytoplankton generate polyunsaturated FAs (PUFAs) from de novo synthesis of palmitic acid and further enzymatic elongase and desaturation reactions (Harwood & Guschina 2009, Cagilari et al. 2011). PUFAs

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can be divided into omega-3 (ω-3) and omega-6 (ω-6) families according to the location of the first double bond of the FA molecule, counted from the terminal methyl group. Because animals (e.g. crustaceans and fish as well as humans) cannot synthesize ω-3 and ω-6 FAs de novo, they need to obtain these molecules from their diet, and therefore some PUFAs are considered to be essential FAs (EFAs, see Table 1) or 'essential nutrients' (Parrish 2009) for animals. When adequate levels of ω-3 and ω-6 FAs are available from the diet, some mammals and freshwater fish can synthesize other forms of EFAs, whereas marine fish and freshwater zooplankton have very limited ability for bioconversion (Parish 2009, Taipale et al. 2011). While the role of EFAs varies among different organisms, they are generally required for optimal health and are not interconvertible in most animals (Parish 2009). For zooplankton, these EFAs are needed to achieve optimal somatic growth and reproduction, whereas fish also require these molecules for disease resistance, neural tissue and eye development, pigmentation, and reproduction (Sargent et al. 1999). The most critical EFAs for zooplankton and fish are eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA, 22:6ω3), and arachidonic acid (ARA, 20:4ω6) (Arts et al. 2001). The importance of marine phytoplankton (e.g. Bacillariophyceae and dinoflagellates) as an EFA source in ocean food webs is well documented (Kattner & Hagen 2009), but fewer studies have investigated freshwater algae (Ahlgren et al. 1992).

In addition to FAs, the growth and reproduction of zooplankton requires essential elements such as carbon, nitrogen, and phosphorus, as well as sterols (Martin-Creuzburg et al. 2009) and amino acids (Wilson 2003). Previous studies have shown that EPA might be the most important EFA supporting somatic growth and reproduction of *Daphnia* (Ravet & Brett 2006), whereas DHA appears to be the most important FA for copepods and many fish (Watanabe 1993, Sargent et al. 1999). Generally, phytoplankton with high proportions of EPA or DHA, such as Cryptophyceae and Bacillariophyceae, are excellent quality food resources for zooplankton. Furthermore, phytoplankton (e.g. Chlorophyceae) with high levels of α-linolenic acid (ALA), and an absence of EPA, are intermediate quality diets for zooplankton, and phytoplankton with a low concentration of PUFAs (e.g. cyanobacteria) are of very poor food quality for zooplankton (Brett et al. 2006, Burns et al. 2011). Bulk food quality is especially important for worldwide common daphnids, which do not feed selectively (DeMott 1986). Therefore, phytoplankton community composition in freshwater systems can define the biochemical composition of the pelagic community and subsequently influence the upper trophic level productivity of the pelagic food webs. Thus, it is important to know the FA profiles of a wide range of different freshwater phytoplankton to have a clear perspective on the nutritional quality of disparate producers to freshwater planktonic food webs.

Table 1. Essential fatty acids of zooplankton, fish, and humans. All of the ω-3 and ω-6 fatty acids can be synthesized by microalgae

Polyunsaturated fatty acid	Common name	Abbreviation
ω-3 family		
18:3ω3	α-linolenic acid	ALA
18:4ω3	Stearidonic acid	SDA
20:5ω3	Eicosapentaenoic acid	EPA
22:5ω3	Docosapentaenoic acid	DPA
22:6ω3	Docosahexaenoic acid	DHA
ω-6 family		
18:2ω6	α-linoleic acid	LIN
18:2ω6	γ-linolenic acid	GLA
20:4ω6	Arachidonic acid	ARA

In addition to the food quality, lipids or FAs have been used as trophic markers (Dalsgaard et al. 2003) to provide insight into consumer diets (Stott et al. 1997). The use of lipids in the study of food chain relationships was pioneered by Lee et al. (1971), and is now used extensively in marine ecosystem (e.g. reviewed by Iverson 2009) and freshwater food web studies (Kainz et al. 2004, Brett et al. 2006, Taipale et al. 2009). Pelagic food web studies often have difficulties in separating phytoplankton from bacteria or detritus when using carbon and nitrogen stable isotope analysis. Among freshwater systems, an ideal biomarker is specific for a particular basal resource, thus providing irrefutable evidence of the presence of each freshwater phytoplankton or bacterial taxon in the diet. Although not without problems, FAs are among the most promising tools to separate a phytoplankton signal from bacteria or detrital FA profiles, because bacteria do not contain PUFAs, and they mainly synthesize saturated FAs (SAFAs), monounsaturated FAs (MUFAs), and odd-chained branched FAs (Ratledge & Wilkinson 1988).

FAs and especially phospholipid FAs (PLFAs) have been successfully used as 'fingerprints' for different microbes and phytoplankton in a wide range of ecosystems (White et al. 1979, Bott & Kaplan 1985, Canuel et al. 1995, Wakeham 1995, Smoot & Findlay

2001, Boschker et al. 2005, Dijkman & Kromkamp 2006). Additionally, PLFAs are suitable for detecting rapid changes in the microbial community, due to their rapid decomposition after cell death (White et al. 1979). The FA profiles and compositions of phytoplankton are quite well recorded among marine phytoplankton (Dunstan et al. 1992, Viso & Marty 1993) and recently macrophyte-dominated benthic food webs (Galloway et al. 2012, Kelly & Scheibling 2012) as well, but analyses of the lipid profiles and associated phylogenetic relationships in freshwater microalgae have only recently been explored (Lang et al. 2011). Even though the FA profiles of some freshwater Cyanophyceae, Chlorophyceae, and Cryptophyceae classes were characterized by Ahlgren et al. (1992) over 20 yr ago, there is still poor knowledge and no studies of FA profiles of freshwater Chrysophyceae and Raphidophyceae, which are common microalgae in many boreal lakes. Because the FA composition of zooplankton in freshwater systems closely reflects seston FA composition (Taipale et al. 2009, Gladyshev et al. 2010, Ravet et al. 2010), FAs would be more useful in freshwater food web studies if the FA composition of a diversity of freshwater phytoplankton was better defined.

FAs that are common in microalgae or bacteria can be called characteristic FAs, but can be called diagnostic FAs only if they are not found in other groups. An ideal food web biomarker would be specific to 1 diet, but its signal should also be large enough to be detected in subsequent trophic levels. The most promising FA biomarkers are unusual short- or long-chain PUFAs. In marine Bacillariophyceae and Chlorophyceae, certain diagnostic C_{16} PUFAs have been identified (Dunstan et al. 1992, Viso & Marty 1993, Dijkman & Kromkamp 2006), but these molecules were not originally documented in the freshwater microalgal studies of Ahlgren et al. (1992). Because of the high sensitivity of new gas chromatography — mass spectrometry (GC-MS) instruments, it is now possible to detect trace levels of FAs and identify novel FA biomarkers for different phytoplankton taxa.

Here we studied the FA profiles of major freshwater microalgae groups, including 7 phytoplankton classes (Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cryptophyceae, Euglenoidea, Raphidophyceae, and Trebouxiphyceae), 22 genera, and 37 strains (Table 2). We describe diagnostic FA biomarkers that best differentiate each group. In addition, we used multivariate analyses to describe similarities and differences in the FA composition of these freshwater phytoplankton groups.

MATERIALS AND METHODS

Phytoplankton culturing

The phytoplankton strains were originally isolated from freshwater systems, and maintained at several universities prior to this project. Most of the phytoplankton strains were cultured at the University of Washington, USA, or at the University of Helsinki, Finland (Table 2). Additionally, some strains were cultured at the University of Otago, New Zealand (Burns et al. 2011). The strains in Washington and Otago were grown at 18°C under a 14h:10h light:dark cycle and in growth medium specific to the strains (Table 2). At the University of Helsinki, phytoplankton were obtained either from culture collections or isolated from boreal lakes of the Evo forest area in Finland. These strains were cultured at 20°C with a 16L:8D light:dark cycle and in growth medium specific to the strains (Table 2). We used plastic or glass bottles (volume >200 ml). Depending on the cell density, 0.5 to 3 ml of the phytoplankton stock was inoculated per 100 ml of fresh culture medium every 2 wk. The samples for phytoplankton analyses were harvested in the late phase of exponential growth, i.e. 2 to 3 wk after the inoculation.

Phytoplankton nomenclature

The algal classification followed mainly the taxonomy and common names of Algaebase (www.algaebase.org). However, *Mallomonas* and *Synura* were included in the class of Chrysophyceae (golden algae) together with *Dinobryon*, even though some studies separate them to Synurophyceae (Jordan & Iwataki 2012). Additionally, the 3 species of Trebouxiophyceae studied are referred to as eukaryotic picoplankton due to their small size.

FA analyses

Lipids were extracted with chloroform:methanol: water (4:2:1) from freeze-dried, homogenized phytoplankton (1–4 mg) samples. Sonication (10 min) was used to enhance lipid extraction, and samples were centrifuged to facilitate phase separation, after which the chloroform phase was transferred to a new tube. Chloroform was evaporated under an N_2 gas stream, and the remaining lipids were dissolved in toluene. Methanolic H_2SO_4 (1% v/v) was added to produce FA methyl esters (FAMEs), and samples were transmethylated in a water bath at 50°C overnight.

FAMEs were extracted twice with *n*-hexane, and excess *n*-hexane was evaporated under N_2 and stored at −20°C until analysis.

All samples excluding diatoms were analyzed using a gas chromatograph (Shimadzu Ultra) equipped with mass detector (GC-MS) at the University of Jyväskylä (Finland). Methyl esters of diatoms were analyzed with a gas chromatograph (Agilent® 6890N) connected with mass spectrometric detection (Agilent® 5973N) at the University of Eastern Fin-

Table 2. Freshwater algae strains used for this study were obtained from different culture collections and universities. Strain origin is according to the information received from culture collections or universities. Algae were cultured using optimal media for each strain. Cultures were maintained under either a 14:10 or 16:8 h light:dark cycle. Temperature of all cultures was 18–20°C. UWCC: Algal and Fungal University of Washington Culture Collection, at the University of Washington, Seattle, Washington, USA; Peltomaa: Lammi Biological Station, University of Helsinki, Finland; CCAP: Culture Collection of Algae and Protozoa, Ambleside, Cumbria, UK; Gilbert: Dartmouth College, New Hampshire, USA; NIVA: Norwegian Institute for Water Research, Oslo, Norway; UTEX: University of Texas Culture Collection, University of Texas at Austin, Texas, USA; CPCC: Canadian Phycological Culture Centre, University of Waterloo, Ontario, Canada; CCMP: National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences, Maine, USA; Carolina: Carolina Biological Supply Company, Burlington, North Carolina, USA

Class	Strain number	Species	Collection	Origin
Chlorophyceae (green algae)	1	*Ankistrodesmus* sp.	UWCC	Freshwater
	2	*Chlamydomonas reinhardtii*	UWCC	Freshwater
	3	*Chlamydomonas* sp.	Peltomaa, Finland	Musta-Kotinen, Finland
	4	*Pediastrum privum*	CCAP 261	Hokajärvi, Finland
	5	*Selenastrum* sp.	Peltomaa, Finland	Iso-Ruuhijärvi, Finland
	6	*Selenastrum capricornutum*	Culture collection USA[a]	Freshwater
	7	*Selenastrum capricornutum*	UWCC	Freshwater
	8	*Scenedesmus communis*	Peltomaa, Finland	Pääjärvi, Finland
	9	*Scenedesmus obliquus*	Max Planck Institute, Germany	Freshwater
	10	*Scenedesmus ecornis*	Peltomaa, Finland	Taka-Killo, Finland
	11	*Coenocystis* sp.	Peltomaa, Finland	Ormajärvi, Finland
Euglenophyceae (euglenoids)	12	*Euglena gracilis*	CCAP 1224/5Z	Freshwater
	13	*Euglena* sp. (small)	Peltomaa, Finland	Kyynärö, Finland
	14	*Euglena* sp. (big)	Peltomaa, Finland	Kyynärö, Finland
Chrysophyceae (golden algae)	15	*Dinobryon cylindricum*	UWCC FW 622	
	16	*Mallomonas caudata*	CCAP 929/8	Musta-Kotinen, Finland
	17	*Synura* sp.	Peltomaa, Finland	Kyynärö, Finland
Raphidophyceae (raphidophytes)	18	*Gonyostomum semen*	GSB 02[b]/04[c]	Lake Bökesjön, Sweden
Cryptophyceae (cryptomonads)	19	*Cryptomonas* sp.	Peltomaa, Finland	Kyynärö, Finland
	20	*Cryptomonas marssonii*	CCAP 979/70	Musta-Kotinen, Finland
	21	*Cryptomonas erosa*	Gilbert, USA[a]	
	22	*Cryptomonas pyrenoidifera*[a]	NIVA 2/81	Lake Gjersjøen, Norway
	23	*Cryptomonas obovoidea*[a]	CCAP 979/44	Freshwater
	24	*Cryptomonas ozolinii*	UTEX LB 2782	Crowdrey Lake, USA
	25	*Cryptomonas ovata*	CCAP 979/61	Hirschberg, Austria
	26	*Rhodomonas minuta*	CPCC 344	Freshwater
	27	*Rhodomonas lacustris*	NIVA 8/82	Nordbytjernet, Norway
Trebouxiophyceae (eukaryotic green picoplankton)	28	*Choricystis* sp.	CCMP 2201	North Deming Bond, USA
	29	*Choricystis coccoides*		Lake Tahoe, USA[a]
	30	*Stichococcus chodati*		Lake Tahoe, USA[a]
Bacillariophyceae (diatoms)	31	*Fragilaria crotonensis*	UTEX LB FD56	Wyoming, USA
	32	*Cyclotella meneghiniana*	PAE Lab, Belgium	Freshwater
	33	*Asterionella formosa*	PAE Lab, Belgium	Freshwater
	34	*Stephanodiscus hantzschii*	CCAP 1079/4	Esthwaite Water, England
	35	*Synedra* sp.	Carolina	Freshwater
	36	*Navicula pellicosa*	UTEX B664	Alaska, USA
	37	*Aulacoseira granulata* var. *angustissima*	CCAP 1002/2	Sydney, Australia

[a]For more information, see Burns et al. (2011)
[b]For more information, see Rengefors et al. (2008)
[c]Unpublished, isolated from Lake Bökesjön 2004

land. Both instruments were equipped with an Agilent® DB-23 column (30 m × 0.25 mm × 0.15 µm), under the following temperature program: 60°C for 1.5 min, then the temperature was increased at 10°C min^{-1} to 100°C, followed by 2°C min^{-1} to 140°C, and 1°C min^{-1} to 180°C and finally heated at 2°C min^{-1} to 210°C and held for 6 min. Helium gas was used as a carrier gas with an average velocity of 34 cm s^{-1}. FA concentrations were calculated using calibration curves based on known standard solutions of a FAME standard mixture. The Pearson correlation coefficient was >0.99 for each individual FA calibration curve.

FA identification

Identification of FAs was consistent among both laboratories and was based on authentic standard mixes (Supelco 37-component FAME mix, Supelco FAME mix, and reference standard GLC-68D from Nu Chek-Prep) and mass spectra. Identification of FAME mass spectra was based on the spectrum database maintained by the AOCS Lipid Library (http://lipidlibrary.aocs.org/ms/arch_me/index.htm). Identification of SAFAs and *iso*- and *anteiso*-branched FAs of bacterial origin were based on standards and mass

Table 2 (continued)

Place cultured	Media	Light cycle	Temperature (°C)
University of Washington	L16 (Lindström 1983)	14:10	18
University of Washington	L16 (Lindström 1983)	14:10	18
University of Helsinki	DY-V by CCMP	16:8	20
University of Helsinki	DY-V by CCMP	16:8	20
University of Helsinki	WC (Guillard & Lorenzen 1972, Guillard 1975)	16:8	20
University of Otago	MBL medium (Stemberger 1981)	14:10	18
University of Washington	L16 (Lindström 1983)	14:10	18
University of Helsinki	DY-V by CCMP	16:8	20
University of Washington	L16 (Lindström 1983)	14:10	18
University of Helsinki	WC (Guillard & Lorenzen 1972, Guillard 1975)	16:8	20
University of Helsinki	DY-V by CCMP	16:8	20
University of Helsinki		16:8	20
University of Helsinki	AF6 (Watanabe et al. 2000)	16:8	20
University of Helsinki	AF6 (Watanabe et al. 2000)	16:8	20
	Volvox	14:10	18
University of Helsinki	WC (Guillard & Lorenzen 1972, Guillard 1975)	16:8	20
University of Helsinki	WC (Guillard & Lorenzen 1972, Guillard 1975)	16:8	20
University of Washington	L16 (Lindström 1983)	14:10	18
University of Helsinki	AF6 (Watanabe et al. 2000)	16:8	20
University of Helsinki	DY-V by CCMP	16:8	20
University of Otago		16:8	20
University of Washington	L16 (Lindström 1983)	14:10	18
University of Washington	L16 (Lindström 1983)	14:10	18
University of Washington	L16 (Lindström 1983)	14:10	18
University of Washington	L16 (Lindström 1983)	14:10	18
University of Washington	L16 (Lindström 1983)	14:10	18
University of Washington	L16 (Lindström 1983)	14:10	18
University of Helsinki	DY-V by CCMP	16:8	20
University of Otago	WC (Guillard & Lorenzen 1972, Guillard 1975)	14:10	18
University of Otago	WC (Guillard & Lorenzen 1972, Guillard 1975)	14:10	18
University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18

spectra. The location of the double bond of MUFAs was verified with dimethyl disulfide (DMDS) adducts (Nichols et al. 1986). Diunsaturated FAs were identified by mass spectrum and relative retention times. Accurate identification of the double bond positions in dienoic FAs (2 double bonds) from mass spectra is in most cases impossible, but with the extended temperature program it was possible to chromatographically separate, for example, 16:2ω6 and 16:2ω7 from each other. Similar to dienoic FAs, the mass spectra alone provide limited information on the positions of double bonds in polyenoic FAs, but in most cases the relative retention data and the mass spectra together provide enough information to identify methylene-interrupted PUFAs (≥3 double bonds). The molecular weight of an FA is usually obtained from the mass spectra, and specific ions (the alpha ion and omega ion) can be used with caution to identify highly unsaturated FAs (http://lipidlibrary.aocs.org/ms/arch_me/index.htm). Briefly, the omega ion indicates the position of the first double bond from the terminal group, for example a peak at m/z = 150 commonly seen in ω-6 PUFAs and a peak at m/z = 108 in ω-3 PUFAs. All double bonds in the represented PUFAs were in a *cis*-configuration.

Data analyses

We used permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to test for differences in multivariate FA content between algal groups (e.g. Galloway et al. 2012). Because of the assumed relationships (*a priori* categorization based upon established phylogeny) among the classes, factors were treated as fixed in this analysis, and all analyses used Type III sums of squares. Because within groups sample sizes were limited for certain groups, Monte Carlo p-values were used to assess significance of the PERMANOVA test statistic by random sampling of the asymptotic permutation distribution (Anderson et al. 2006). PERMANOVA does not require multivariate normality, but may be sensitive to differences in dispersion. We confirmed that the results of the PERMANOVA test were not sensitive to an arcsine-square root transformation ($x' = \sin^{-1}\sqrt{x}$) and therefore present results for this test using the untransformed data. We calculated the percent variance explained by the factor 'algal class' in the PERMANOVA analysis (following Hanson et al. 2010 and Galloway et al. 2012). We did not evaluate the effects of culture conditions and media on phytoplankton FAs in this study because that would have required significant within-taxon replication across culture levels, which was beyond the scope of this research. However, we used a 2-way analysis of similarity (ANOSIM, 9999 permutations), where media was nested in algal class, to test whether samples within algal classes grouped by media type. We use the percent variance explained by the factor algal class to evaluate the relative contribution of phylogeny in describing algal FA composition. We used similarity percentage analysis (SIMPER; Clarke & Gorley 2006) on the untransformed data to identify and report the mean proportion and percent contribution of the top 5 FAs for taxonomic within-group similarity (e.g. see Kelly & Scheibling 2012). Finally, we used non-metric multidimensional scaling (NMDS) and principal component analysis (PCA) ordinations of arcsine-square root transformed percent FA composition data for multivariate pattern visualization (Euclidean distance). The results of a cluster analysis were overlaid on the NMDS to show separate groups with 75% similarity. An additional PCA was performed for visualization of the ANOSIM results evaluating whether culture media had within-class effects on interpretation of multivariate FA signature ordinations. All statistical routines were performed using PRIMER v.6.0 and PERMANOVA+ add on (Clarke & Gorley 2006, Anderson et al. 2008).

RESULTS

FA profiles of freshwater phytoplankton classes

We detected 54 different FAs from our freshwater algal strains. The FA profiles of 7 freshwater phytoplankton classes differed significantly from each other (PERMANOVA, p = 0.0001; Table 3). The factor 'class' accounted for 66.4% of the total variation in FA signatures (Table 3). Post hoc pairwise tests

Table 3. PERMANOVA results of the overall test of class level differences. Analysis assumes that the factor 'class' is fixed and uses Type III sums of squares. Significance determined with permutation and Monte Carlo (MC) p-values (see 'Materials and methods'). Percent variance (% Var) is the variance component estimated for the factor 'class,' and the residual is divided by the sum of all variance components to quantify the relative magnitude of effects

Source	df	MS	Pseudo-F	p(MC)	%Var
Class	6	2803.9	8.844	0.0001	66.4
Residual	30	317.04			33.6
Total	36				

Table 4. PERMANOVA results of the post hoc pairwise tests, showing the *t*-statistic, number of unique permutations (perms) in the procedure, and significance determined from Monte Carlo (MC) permutation (*p < 0.05, **p < 0.001; see 'Materials and methods')

Groups	*t*	Unique perms	p(MC)
Chlorophyceae, Euglenophyceae	2.1088	364	0.0117*
Chlorophyceae, Chrysophyceae	2.2452	364	0.0049*
Chlorophyceae, Raphidophyceae	1.4632	12	0.1148
Chlorophyceae, Cryptophyceae	2.4841	9662	0.0008**
Chlorophyceae, Trebouxiophyceae	0.72416	364	0.6544
Chlorophyceae, Bacillariophyceae	5.4079	8564	0.0001**
Euglenophyceae, Chrysophyceae	1.8634	10	0.0548
Euglenophyceae, Raphidophyceae	1.7276	4	0.1464
Euglenophyceae, Cryptophyceae	2.2607	220	0.0065*
Euglenophyceae, Trebouxiophyceae	2.6746	10	0.0096*
Euglenophyceae, Bacillariophyceae	4.6023	120	0.0002**
Chrysophyceae, Raphidophyceae	1.2566	4	0.2954
Chrysophyceae, Cryptophyceae	1.838	220	0.0268*
Chrysophyceae, Trebouxiophyceae	2.5056	10	0.0131*
Chrysophyceae, Bacillariophyceae	3.8773	120	0.0002**
Raphidophyceae, Cryptophyceae	1.0716	10	0.3229
Raphidophyceae, Trebouxiophyceae	2.3125	4	0.0778
Raphidophyceae, Bacillariophyceae	2.7886	8	0.0058*
Cryptophyceae, Trebouxiophyceae	2.134	220	0.0167*
Cryptophyceae, Bacillariophyceae	5.3347	6686	0.0001**
Trebouxiophyceae, Bacillariophyceae	5.7314	120	0.0001**

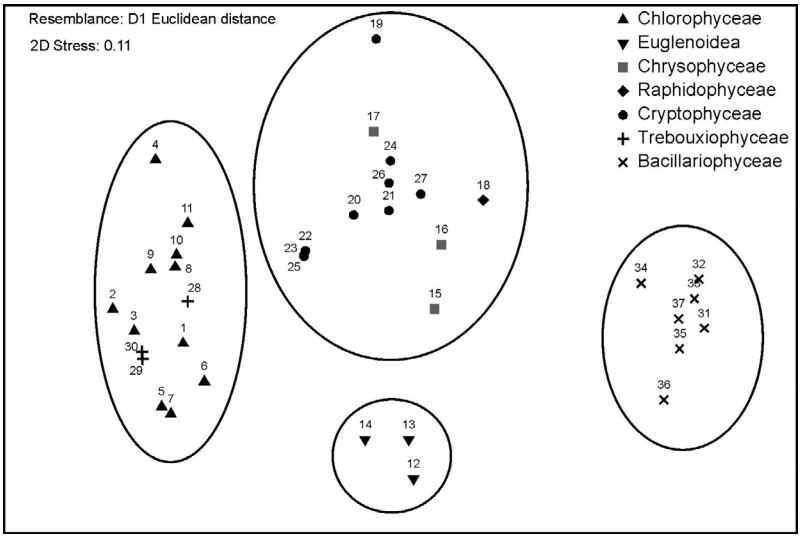

Fig. 1. Results of non-metric multidimensional scaling analysis (NMDS). The plot has a stress of 0.11, indicating a reasonable ordination of the data in 2 dimensions. The patterns evaluated here were tested using PERMANOVA. Axis 1 correlated positively with the diatom fatty acids (FAs). Axis 2 correlated positively with characteristic FAs for Cryptophyceae and Synurales and negatively with characteristic FAs of Euglenophyceae. The results of a cluster analysis, defined as the 'Distance' polygon, were overlaid on the NMDS plot to show the separate groups with 75% similarity. Numbers refer to different phytoplankton strains used in this study (see Table 2)

showed that most across-class comparisons were significantly different (Table 4) except for all comparisons involving Raphidophyceae (excluding Raphidophyceae versus Bacillariophyceae, p = 0.0058), Chlorophyceae versus Trebouxiophyceae (p = 0.654), and Euglenophyceae versus Chrysophyceae (p = 0.055).

According to multivariate ordination (Fig. 1), the 7 freshwater phytoplankton classes differed in multivariate space and formed 4 major groups (Fig. 1). The 2-dimensional stress of the NMDS was 0.11. A PCA (not shown) explained a total of 67.6% of the variation with the first 3 PC axes (PC1 = 40.1%, PC2 = 18.1%, PC3 = 9.3%). NMDS axis 1 (the *x*-axis) clearly separated Bacillariophyceae and Chlorophyceae from each other. All Bacillariophyceae clustered on the right side of axis 1, whereas Chlorophyceae together with Trebouxiophyceae clustered on the far left side of axis 1. Euglenophyceae together with *Dinobryon* formed a third group, and Cryptophyceae, Chrysophyceae, and the 1 Raphidopyceae taxon formed a fourth group (Fig. 1). NMDS axis 2 separated Euglenophyceae from the other algae. There were 3 Cryptophyceae strains (numbers 22, 23, and 25) which clustered separately from other Cryptophyceae (lower left section of the Chlorophyceae–Chrysophyceae polygon in Fig. 1). Additionally, *Dinobryon* (number 15) separated from other Chrysophyceae on NMDS axis 2, but clustered together with other Chrysophyceae on NMDS axis 1.

NMDS axis 1 (*x*-axis) was positively correlated most strongly (r = 0.69 to 0.84, p = 0.01) with the typical FAs of Bacillariophyceae (20:5ω3, 14:0, 16:1ω7, 16:3ω4, 16:2ω4, 16:2ω7) and negatively (r = −0.76 to −0.89, p = 0.01) with the typical FAs of Chlorophyceae (16:4ω3, 16:3ω3, 18:3ω3). NMDS axis 2 (*y*-axis) was generally positively correlated (r = 0.11, 0.34, 0.80) with 18:4ω3, 22:5ω6, and 22:6ω3, respectively, which are characteristic FAs for Crypto-

Table 5. Results of similarity percentage (SIMPER) analysis of freshwater microalgae fatty acid (FA) signatures in 6 classes. Analysis was run on the untransformed FA data. No results are reported for Raphidophyceae because only 1 strain was sampled within this group. The table shows mean proportions (Mean) of the 6 FAs that contributed the most (and % contribution of each FA) to within-group similarity. FA abbreviations shown in Table 1

Algal class (within-group n)	FA	Mean	Contribution to similarity (%)
Chlorophyceae (11)	18:1ω9c	13.8	40.2
	ALA	27.6	32.9
	16:0	20	5.8
	LIN	6.9	4.7
	16:4ω3	8.9	3.2
	18:4ω3	5	2.8
Euglenophyceae (3)	LIN	6.2	28.8
	ARA	5.1	13.8
	16:4ω3	6.3	10.3
	DHA	8.7	7.3
	DPA	1.6	6.7
	EPA	10.3	5.5
Chrysophyceae (3)	16:1ω7	6.9	19.4
	18:4ω3	13.3	14.9
	14:0	11	11.8
	18:1ω7c	3.3	8.4
	DHA	5.6	7.2
	22:5ω6	9.1	6.2
Cryptophyceae (9)	18:4ω3	17.3	31.8
	16:0	20.9	30.5
	ALA	23.7	12.4
	14:0	3.7	7.7
	EPA	9.8	5
	LIN	4.2	4.1
Trebouxiophyceae (3)	LIN	12.8	38.4
	18:1ω9c	9.9	17.5
	16:3ω3	6.6	11.3
	18:4ω3	2.2	9.1
	22:0	1.9	6.5
	16:4ω3	8.4	5
Bacillariophyceae (7)	16:1ω7	33.3	23.2
	EPA	13.2	22.4
	18:0	5	22.3
	14:0	9.2	8.5
	ARA	1.8	6.2
	16:0	16.8	5.1

phyceae and Chrysophyceae. NMDS axis 2 was generally negatively (r = −0.28 to −0.52) correlated (p = 0.01) with the long-chain PUFAs (22:4ω6, 20:2ω6, 20:4ω6, 20:3ω3) and the unusual C_{17} carbon chain PUFAs of 17:3ω2. Axis 2 separated the Euglenophyceae from other groups and also correlated negatively with 15:0, 15:4ω6, 15:4ω3, 20:2ω6, and 20:3ω3. The 2-way ANOSIM test (where culture media was nested in algal class) confirmed the significance of the algal class (Global R = 0.867, p = 0.0001), but phytoplankton FAs did not differ among culture media tested (Global R = −0.018, p = 0.531). In addition, there was no evident effect of culture media on groupings of samples within class in multivariate space (not shown).

Similarity and major FAs of freshwater phytoplankton

Six of the individual FAs that contributed the most to within-group (algal class) similarity and the mean proportion of that FA for the group are reported in the SIMPER analysis (Table 5). The 'Contributions' are the percentages that the FA contributed to dissimilarities among the taxa within that class. The FAs most responsible for within-group similarities also play an important role in separating the phytoplankton groups in the NMDS ordination.

The most abundant FAs (Table 6), viz. ω-3 and ω-6 (Fig. 2), varied among classes. The major FAs of Chlorophyceae and Trebouxiophyceae were oleic acid (18:1ω9), ALA, and palmitic acid (16:0). Oleic acid was the most abundant FA in *Selenastrum*, and ALA was the dominant FA amongst the genera *Chlamydomonas*, *Scenedesmus*, *Ankistrodesmus*, *Pediastrum*, *Choricystis*, and *Stichococcus*. Additionally, linoleic acid (18:2ω6) was 1 of the 3 dominant FAs of *Ankistrodesmus*, *Choricystis*, and *Stichococcus*. Four FAs (18:1ω9, 18:3ω3, 16:0, 18:2ω6) accounted for most

Table 6. Major fatty acids (FAs) and potential biomarkers for each algal class. FA abbreviations shown in Table 1. Asterisks indicate FAs that were only found in their respective phytoplankton group

Phytoplankton group	Major FA	FA biomarker
Chlorophyceae	ALA, 16:0, 18:1ω9, LIN	16:4ω3, 16:3ω3, 16:2ω6
Trebouxiophyceae	ALA, 16:0, 18:1ω9, LIN	16:4ω3, 16:3ω3, 16:2ω6
Cryptophyceae	ALA, 16:0, SDA	22:5ω6, 18:4ω3
Synuraphyceae	SDA, 14:0, ALA, 16:0	22:5ω6, 18:4ω3
Ocromonadales	16:1ω7c, 16:0, LIN, 18:1ω7	16:3ω1, 18:4ω3, 22:5ω6
Raphidophyceae	16:0, EPA, SDA, ALA	16:2ω4, 16:3ω4*, 16:3ω1, 20:3ω3
Bacillariophyceae	16:1ω7c, EPA, 16:0, 14:0	16:2ω7*, 16:2ω4, 16:3ω4, 16:4ω1*, 18:4ω4*
Euglenophyceae	16:0, ALA, EPA, DHA	15:3ω3*, 15:3ω1, 15:4ω3, 17:3ω2*, 17:2ω7/5*, 20:4ω3, 20:2ω6, 20:3ω6, 22:4ω6

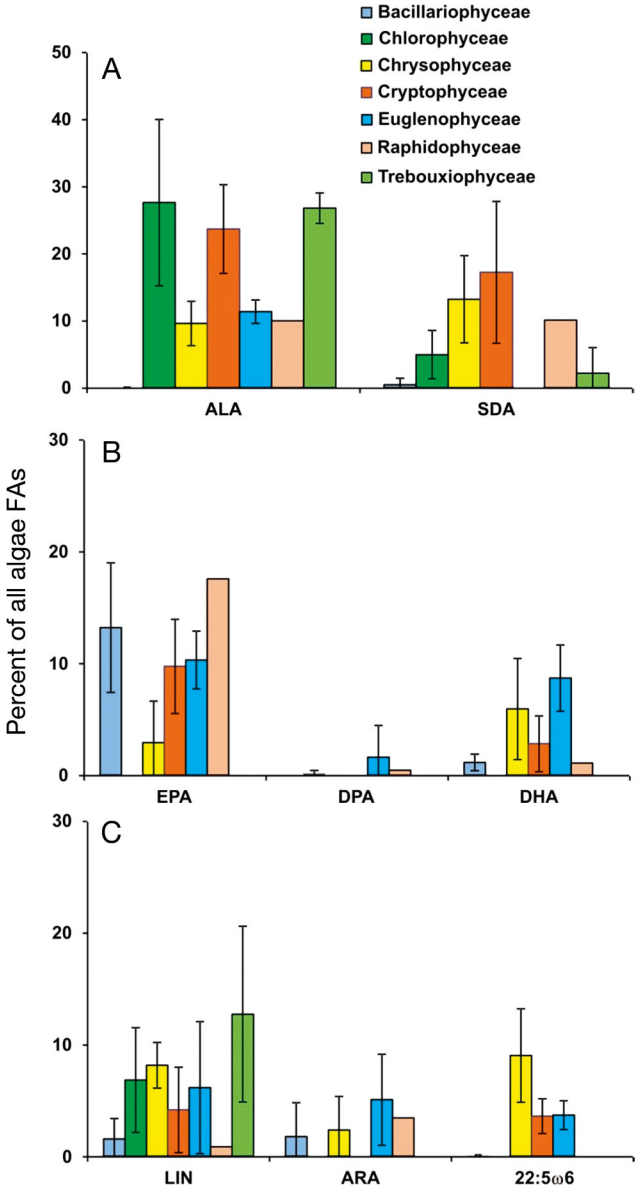

Fig. 2. Contribution (%, mean ± SD) of (A) ALA and SDA; (B) EPA, DPA, and DHA; and (C) LIN, ARA, and 22:5ω6 of all fatty acids (FAs) among 7 freshwater algal classes. FA abbreviations shown in Table 1

of the similarity among Chlorophyceae and were only slightly different compared to Trebouxiophyceae (18:2ω6, 18:1ω9, 16:3ω3, 18:4ω3).

Euglenophyceae contained a larger number of unique FAs than any other algal class (total of 22 FA). The most abundant FAs in this group were palmitic acid, ALA, and EPA, which each accounted for ≈10% of Euglenophyceae FAs. Linoleic acid, ARA, 16:4ω3, DHA, docosapentaenoic acid (DPA), and EPA contributed the most to the within-group similarity amongst the Euglenophyceae.

The most abundant individual FA and multivariate FA profiles of Chrysophyceae varied among the strains. The 3 most prevalent FAs in *Synura* and *Mallomonas* were stearidonic acid (SDA), 14:0, and ALA, which contributed only 30% of all FAs of *Mallomonas*, but ~50% of all FAs of *Synura*. In contrast to *Synura* and *Mallomonas*, 16:1ω7, 16:0, and 18:2ω6 were the most abundant FAs in *Dinobryon*. However, despite these different contributions, all 3 Chrysophyceae strains had similar FA profiles, excluding some minor differences among C_{20} PUFAs. Among the C_{20} PUFAs, 20:3ω3 was found only in *Dinobryon*, EPA and ARA were found only in *Mallomonas* and *Synura*, and 20:3ω6 was only found in *Synura*. Additionally, *Dinobryon* had more MUFAs than *Mallomonas* or *Synura*. According to the SIMPER analyses, 16:1ω7, 18:4ω3, 14:0, 18:1ω7, and DHA contributed most to within-group similarity amongst the chrysophytes.

All of the analyzed Cryptophyceae had the same FAs, but the contributions varied within this group. The 3 dominant FAs that contributed the most to within-group similarity in the Cryptophyceae were palmitic acid (16:0), ALA, and SDA. SDA was the most common FA in *Cryptomonas* sp. (strain 19), *C. erosa*, (21), *C. ozolinii* (24), and *Rhodomonas minuta* (826), whereas ALA was the most important FA in *C. marsonii* (20) and *R. lacustris* (27), and palmitic acid was the most important in strains *C. pyrenoidifera* (22), *C. obovoidea* (23), and *C. ovata* (25). These last 3 strains, which were separated previously by the NMDS analysis, were different from the other Cryptophyceae, and had more linoleic acid (18:2ω6), oleic acid, palmitic acid, and 17:0, and less SDA (only 6–7% of all FAs) than the other strains.

In the Bacillariophyceae, the major FAs were 16:1ω7, EPA, 16:0, and 14:0, which together accounted for more than 70% of all FAs. In addition to 16:1ω7 and EPA, stearic acid (18:0) also contributed the most to within-group similarity. Stearic acid was a major FA of *Navicula pellicosa* (~16%), but was not abundant in any other diatom. *Navicula* had also more ARA (8% cf. 1–2%) than any other diatom.

The FA profile of Raphidophyceae, i.e. *Gonyostomum semen*, was most similar to that of Bacillariophyceae, Cryptophyceae, and Chrysophyceae. The 5 major FAs for this group were palmitic acid, EPA, ALA, SDA, and myristic acid (14:0), which accounted for about 65% of all FAs. *G. semen* also had the highest contribution (~8%) of 16:2ω4 among the 37 phytoplankton strains analyzed.

The ω-3:ω-6 ratio of different freshwater microalgae strains varied between 0.5 and 45. The ω-3:ω-6

ratio was relatively low among Euglenophyceae (2 ± 0.5, mean ± SD), Chrysophyceae (2 ± 0.1), and Trebouxiophyceae (4 ± 3). This ratio was high in Bacillariophyceae (11 ± 14), Chlorophyceae (10 ± 12), Raphidophyceae (9), and Cryptophyceae (7 ± 3) but also varied considerably among Bacillariophyceae and Chlorophyceae.

DISCUSSION

The factor 'class' accounted for 66.4% of the total variation in the FA signatures (Table 3). Additionally, the 37 strains from 7 algal classes created 4 separate groups based on their FA composition: (1) Chlorophyceae and Trebouxiophyceae, (2) Bacillariophyceae, (3) Cryptophyceae, Chrysophyceae, and Raphidophyceae, and (4) Euglenophyceae (Fig. 1, Table 4). The FA composition of each taxonomic group was similar within each group even though the contribution of individual FAs differed, especially amongst the Cryptophyceae and Chrysophyceae. Only a few FAs have been reported as unique for specific algal groups. We found C_{16}, C_{15}, and C_{17} PUFAs, as well as 22:5ω6, to be the most useful FA biomarkers for freshwater phytoplankton. Among all classes, Euglenophyceae have the most unique FA profile, including C_{15}, C_{17}, and C_{20} PUFAs, which were not detected in any other class.

Effects of environment on algal FAs

Growth conditions, e.g. light intensity, temperature, salinity, or nutrients, can affect the phytoplankton lipid and FA composition (Guschina & Harwood 2009). Therefore, changes in the environment can influence the quality of microalgae or abundance of individual FAs in microalgae. Colder temperatures generally increase the unsaturation of microalgae membrane FAs, and thus temperature lowering can increase the relative amount of EPA or DHA which have melting points of −45 to −50°C (Tatsuzawa & Takizawa 1995, Ravet et al. 2010). This negative correlation between temperature and EPA was found for the seston of a eutrophic Siberian reservoir (Gladyshev et al. 2010) and could have an impact on zooplankton production. Our study shows that algal phylogenetic relationships (class level differences) are the dominant source of FA variation (66%) in our dataset, which included algal strains cultivated in taxon-specific optimal growth conditions. We did not have sufficient within-taxon replication at different culture levels to specify the proportion of variation attributable to culture conditions. Nevertheless, within any given class, the location of a sample plotted in multivariate space (e.g. as coded by media type) does not appear to be driven by the culture media used (not shown). This can be easily seen from Chlorophyceae, which clustered tightly together despite different media. These observations are consistent with the lack of media effects found in the ANOSIM. It should be noted that environmental conditions can affect the abundance of individual FAs, but do not stimulate microalgae to synthesize totally new FAs or change FA composition over taxonomic class. For example, EPA or DHA are not reported to be abundant among Chlorophyceae under any circumstances, but are prevalent in Cryptophyceae and Bacillariophyceae. Furthermore, field monitoring of Chlorophyceae in a small boreal lake revealed a strong correlation between the concentration of ALA in the seston and Chlorophyceae biomass throughout the open-water season. The ALA concentration also tracked Chlorophyceae biomass under different temperature and light conditions in a small boreal lake (Taipale et al. 2009).

FA biomarkers in freshwater food webs

Seston in freshwater systems and the diets of herbivorous zooplankton consists of different types of phytoplankton, bacteria, and terrestrial organic matter. Carbon and nitrogen isotope signatures do not naturally differ among these possible zooplankton diet sources with the exception of methane-oxidizing bacteria (MOB) which have very depleted $\delta^{13}C$ values. These very depleted $\delta^{13}C$ values have been found in zooplankton as well (Kankaala et al. 2006). Type I and II MOB have unique C_{16} and C_{18} MUFAs (Bowman et al. 1991) that are incorporated into zooplankton unmodified (Taipale et al. 2012), and thus are good biomarkers for MOB. Our phytoplankton cultures contained only trace amounts (<1%) of *iso*- and *anteiso*-branched FAs, which are dominant FAs in bacteria (Kaneda 1991). In freshwater systems, these FAs usually indicate gram-positive heterotrophic bacteria and have been shown to transfer quantitatively from bacterial diets to zooplankton that consume them (Ederington et al. 1995, Taipale et al. 2012). 16:1ω7 and/or 18:1ω7 are abundant FAs amongst heterotrophic Gram-negative bacteria (Ratledge & Wilkinson 1988), of which 16:1ω7 is also abundant (27–43% of all FAs in our study) in diatoms and has been classified as a diatom biomarker in

marine systems (Viso & Marty 1993). We also found considerable 16:1ω7 (15% of all FAs) in *Dinobryon*, and 8–9% 18:1ω7 in *Dinobryon* and *Chlamydomonas*, whereas 18:1ω7 contributed <6% to other phytoplankton strains considered. Therefore, the high abundance of 18:1ω7 in seston or zooplankton would most likely indicate assimilation of Gram-negative bacteria. However, 16:1ω7 most likely indicates diatom in freshwater systems, but 16:1ω7 of bacterial origin is also plausible. A low (<0.2) ω-3:ω-6 ratio has been used as an indicator of terrestrial organic particulate carbon FA in previous laboratory studies (Brett et al. 2009a, Taipale et al. in press). The ω-3:ω-6 ratio of different freshwater microalgae strains varied from 0.5 to 45 without any clear patterns, thus indicating that a low ω-3:ω-6 ratio does not necessarily refer to terrestrial origin and the ω-3:ω-6 ratio should be used with caution in food web studies.

PUFAs are most useful for separating different microalgal taxa from each other in freshwater systems because they are not generally prevalent FAs in bacteria or terrestrial particulate organic matter. Our study revealed that there were only a few FAs that belonged only to 1 or 2 algal classes (Table 5) and can therefore be used as specific FA biomarkers. The most specific FAs were found amongst the C_{15} to C_{18} PUFAs in 2 or 3 algal classes. Amongst all strains analyzed, only Cryptophyceae and Chrysophyceae (excluding *Dinobryon*) did not contain short-carbon-chain PUFAs. Euglenophyceae contained the unusual C_{15} and C_{17} PUFAs (15:3ω1, 15:4ω3, 17:2ω7/5, and 17:3ω2) (Korn 1964) and the C_{20} and C_{22} PUFAs (20:2ω6, 20:3ω6, 22:4ω6), which were not found in any other class and thus can be used as diagnostic FA biomarkers for Euglenophyceae. The C_{16} PUFAs 16:3ω3, 16:4ω3, and 16:2ω6 were detected from Chlorophyceae, Trebouxiophyceae (although 16:2ω6 was not found in *Scenedesmus ecornis* or *Coenocystis* sp.), and Euglenophyceae. The C_{16} PUFA 16:2ω7 was found only in the Bacillariophyceae, and 16:4ω1 and 18:4ω4 were only identified from *Cyclotella*, *Asterionella*, *Stephanodiscus*, and *Synedra*. The C_{16} PUFAs 16:2ω4 and 16:3ω4 were found in Bacillariophyceae as well as in *Gonyostomum semen*. The C_{16} PUFA16:3ω1 was abundant in *Dinobryon*, and was also detected in *G. semen.* Furthermore, division of FAs within the Chrysophyceae aligned with Synurophyceae (containing e.g. *Synura* and *Mallomonas*) and Chrysophyceae (e.g. *Dinobryon*), as already suggested by Jordan & Iwataki (2012). The PUFA 22:5ω6 was characteristic for Cryptophyceae and Chrysophyceae, and was also found in Euglenophyceae. It is also worth noting that the FA profiles of Cryptophyceae varied considerably, and therefore more biochemical studies should be undertaken to classify this group.

Biochemical quality of algal groups

Herbivorous zooplankton (e.g. cladocerans) are a crucial link between phytoplankton and fish production in many lakes; thus, the biochemical quality of the phytoplankton has a direct impact on the somatic growth and reproduction of e.g. *Daphnia*. *Daphnia* have limited capacity to bioconvert ALA to EPA de novo (von Elert 2002, Taipale et al. 2011), and thus phytoplankton species with high EPA concentration are very high-quality resources for *Daphnia* (Brett et al. 2006). Diets with high total concentrations of essential FAs without EPA are of intermediate quality for *Daphnia* (Brett et al. 2006), whereas diets with low concentrations of ω-3 FAs and sterols (see Brett et al. 2009a, Martin-Creuzburg et al. 2009) are biochemically inadequate resources for zooplankton. Field studies have demonstrated, for example, that the highest zooplankton biomass follows phytoplankton FA quality rather than phytoplankton quantity (Gladyshev et al. 2010).

We found that the Bacillariophyceae, Cryptophyceae, Euglenophyceae, Raphidophyceae, and Synuraphyceae all contain EPA and DHA, and thus they are potentially excellent food resources for zooplankton provided they can be ingested. The greatest contribution of EPA was found in Bacillariophyceae, with *Cyclotella* and *Asterionella* being particularly rich in EPA. A high proportion of EPA was found in *Gonyostomum semen*, but due to their large size (50–100 µm), this taxon is not easily consumed by daphnids. Euglenophyceae and Synuraphyceae, especially *Mallomonas*, were also rich in DHA. In addition to EPA and DHA, Euglenophyceae and Raphidophyceae have DPA (22:5ω3). Chlorophyceae, Trebouxiophyceae, and Ochromonadales (*Dinobryon*) are intermediate-quality food resources, because they almost entirely lack EPA and DHA. Even though Chlorophyceae and Trebouxiophyceae do not contain EPA or DHA, they had high levels of ALA and some SDA (see Fig. 2), which makes them much better diets than cyanobacteria for cladocerans (Brett et al. 2006, 2009b, Burns et al. 2011). Previous zooplankton studies have concluded that Cryptophyceae and Bacillariophyceae are excellent-quality diets for cladocerans (Ravet & Brett 2006, Brett et al. 2009a,b), but there are no studies

on Chrysophyceae, Raphidophyceae, or Euglenophyceae. It is possible that physical protection mechanisms of algae, e.g. silica spines (*Mallomonas* and *Synura*) or trichocysts (*G. semen*) or simply large size (e.g. *G. semen* or *Synura* colonies) might limit zooplankton grazing on these algae. There is very limited information on the food quality of freshwater algae for copepods (Burns et al. 2011), and more studies of zooplankton responses to different freshwater algal diets are needed.

Difference in FA profiles between marine and freshwater strains

Chlorophyceae are among the most studied classes of freshwater and marine microalgae, and the FA composition of this group is therefore generally well known. Both marine and freshwater Chlorophyceae have considerable ALA, and some genera also have substantial amounts of 18:1ω9. Freshwater Chlorophyceae do not contain any EPA or DHA, whereas marine species have trace amounts of these FAs (Ratledge & Wilkinson 1988). Marine Chlorophyceae are therefore a theoretically slightly better-quality diet than freshwater strains. The Chlorophyceae biomarker C_{16} PUFAs 16:3ω3 and 16:4ω3 have also been found more universally in marine and estuarine members of this class (Ratledge & Wilkinson 1988, Dunstan et al. 1992, Viso & Marty 1993, Dijkman & Kromkamp 2006), but are not routinely reported from freshwater taxa.

Bacillariophyceae are another well studied algal group (Ackman et al. 1968, Kattner et al. 1983), especially in marine systems. Their major FAs are 16:1ω7, EPA, 16:0, and 14:0 in both marine and freshwater strains. In our freshwater cultures, 16:1ω7 was the dominant FA, whereas in some marine diatoms, EPA is the dominant FA (Dunstan et al. 1993). The contribution of EPA from marine Bacillariophyceae varies between 12 and 30% (Dunstan et al. 1993), which is slightly more than what we found in our freshwater strains (EPA = 7–23% of all FAs). Thus marine diatoms are also of slightly higher food quality than freshwater strains. We detected very little 16:4ω1 in our freshwater Bacillariophyceae, whereas marine Bacillariophyceae have been reported to contain up to 19% of this FA (Dunstan et al. 1993). The presence of 16:4ω1 in marine Bacillariophyceae is not related to the morphology of Bacillariophyceae, because 16:4ω1 was found from both centric and pennate Bacillariophyceae. However, it seems that 16:4ω1 may only be a relevant FA biomarker in marine systems.

Raphidophyceae are more studied in marine environments, where *Heterosigma* and *Chattonella* are common. In freshwater systems, *Gonyostomum semen* is the most common representative of this class. Our analysis revealed that *G. semen* has the same primary FAs as *Heterosigma* and *Chattonella*, i.e. 16:0, SDA, EPA, and 14:0 (Nichols et al. 1987, Marshall et al. 2002), but *G. semen* has much more ALA than marine raphidophytes.

Because of very heterogeneous FA profiles amongst the Cryptophyceae, we were not able to determine any differences between marine and freshwater species. FA profiles of Cryptophyceae in our study varied considerably even under the same culture conditions. However, both marine (Dunstan et al. 2005) and freshwater (Ahlgren et al. 1992) Cryptophyceae contained 5 to 20% EPA, and thus a food quality difference was not found between marine or phytoplankton cultures. The best biomarker FA for Cryptophyceae, 22:5ω6, has been detected in marine as well as freshwater Cryptophyceae (Ahlgren et al. 1992, Dunstan et al. 2005). We were not able to compare cultures of Chrysophyceae and Synuraphyceae from both marine and freshwater systems due to limited research on the FA profiles of these groups (Cranwell et al. 1988).

CONCLUSIONS

Multivariate FA signatures can be used as 'fingerprints' for phytoplankton, bacteria, and terrestrial organic matter in food web studies. Our FA analysis of 37 microalgae strains revealed that algal class explained most of the total variation in FA signatures, and thus FAs can distinguish microalgae at the class level. Therefore, FAs can be used for the taxonomic primary production measurements in different freshwater systems. Moreover, FAs offer a powerful tool for lacustrine food web studies to track different diets in the food web. Zooplankton studies with a wide range of microalgae classes should be carried out to establish quantitative FA signature analysis or FA mixing models for zooplankton. Such FA-based models could give us more details regarding freshwater food webs, which cannot be gained by using stable isotope based mixing model analyses alone.

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