Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in 7 classes

by

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ABSTRACT

Algal fatty acid (FA) composition is an important determinant of their food quality for consumers. FA can also be used as biomarkers for biochemical and energetic pathways in food webs. FA analyses of seven freshwater algal classes and 37 strains showed clear similarity within classes and strong differences amongst classes. The algal class was dominant factor (66.4%) explaining variation in FA signatures of microalgae. Seven algal classes created four separate groups according to their FA profiles: 1) Chlorophyceae and Trebouxiophyceae 2) Bacillariophyceae, 3) Cryptophyceae, Chrysophyceae, and Raphidophyceae, and 4) Euglenophyceae. Each group has a characteristic FA composition although the proportional abundance of individual FA also differs between species and with environmental conditions. The FA which were found to be particularly representative for each group (i.e., diagnostic biomarkers) are: 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. FA thus offer a powerful tool for lacustrine food web studies to track different consumer diets in a 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 intermediate food quality for zooplankton and Cryptophyceae, Bacillariophyceae, Euglenophyceae and Raphidophyceae should be excellent resources for zooplankton.

Key words: fatty acids, freshwater microalgae, biomarker

INTRODUCTION

In aquatic food webs, most fatty acids (FA) are synthesized by phytoplankton and bacteria before being transferred via herbivorous invertebrates to fish and ultimately humans (Arts et al. 2001). Phytoplankton generate polyunsaturated FA (PUFA) from *de novo* synthesis of palmitic acid and further enzymatic elongase and desaturation reactions (Cagilari et al. 2011, Harwood & Guschina 2009). PUFA 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 synthetize ω-3 and ω-6 FA de novo, they need to obtain these molecules from their diet, and therefore some PUFA are considered to be essential FA (EFA, see table 1) or 'essential nutrients' (Parrish 2009) for animals. When adequate levels of ω-3 and ω-6 FA are available from the diet, some mammals and freshwater fish can synthesize other forms of EFA, whereas marine fish and freshwater zooplankton have very limited ability for bioconversion (Parish 2009, Taipale et al. 2011). While the role of EFA varies among different organisms, they are generally required for optimal health and are not interconvertible in most animals (Parish 2009). For zooplankton these EFA are needed to obtain 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 EFA 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 there are fewer studies on freshwater algae (Ahlgren et al. 1992).

In addition to fatty acids, the growth and reproduction of zooplankton requires essential elements such as carbon, nitrogen and phosphorus, and sterols (Martin-Creuzburg et al. 2009) as well as amino acids (Wilson 2003). Previous studies have shown that EPA might be the most important EFA supporting somatic growth and reproduction of *Daphnia* (Ravet et al. 2006), whereas DHA appears to be the most important FA for copepods and many fish (Sargent et al. 1999, Watanabe 2007). 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 ALA, and an absence of EPA, are intermediate quality diets for zooplankton, and phytoplankton with a low concentration of PUFA (e.g., cyanobacteria) are 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 clear perspective on the nutritional quality of disparate producers to freshwater planktonic food webs.

In addition to the food quality, lipids or FA have also been used as trophic markers (FATM, 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 in 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 isotopes analyses (SIA). 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 bacteria taxa in the diet. Although not without problems, FA are one of the most promising tools to separate a phytoplankton signal from bacteria or detrital FA profiles, because bacteria do not contain PUFA, and mainly synthesize saturated FA (SAFA), monounsaturated FA (MUFA) and odd chained branched FA (Ratledge & Wilkinson 1989).

Fatty acids and especially phospholipid FA (PLFA) have been successfully used as "fingerprints" for different microbes and phytoplankton in wide range of ecosystems (White et al. 1979, Bott & Kaplan 1985, Canuel et al. 1995, Wakeham 1995, Smoot & Findlay 2001, Boschker et al. 2005, Dikjman & Kromkamp 2006). Additionally, PLFA 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 (Kelly & Scheibling 2012; Galloway et al. 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 twenty years ago, there is still a poor knowledge or no studies of FA profiles of freshwater Chrysophyceae or 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 2010, Ravet et al. 2010), FA would be more useful in freshwater food web studies if the FA composition of a diversity of freshwater phytoplankton was better defined.

FA that are common in microalgae or bacteria can be called characteristic FA, but can be called diagnostic FA only if they are not found in other groups. An ideal food web biomarker would be specific to one diet, but its signal showed to also be large enough to be detected in subsequent trophic levels. The most promising FA biomarkers are unusual short or long chain PUFA. In marine Bacillariophyceae and Chlorophyceae, certain diagnostic C_{16} PUFA have been identified (Dunstan et al. 1992, Viso & Marty 1993, Dikjman & 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 GC-MS instruments, it is now possible to detect trace levels of FA and identify novel FA biomarkers for different phytoplankton taxa.

Here we have studied the FA profiles of major freshwater microalgae groups, including seven phytoplankton classes (Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cryptophyceae, Euglenoidea, Raphidophyceae and Trebouxiphyceae), 22 genera and 37 strains (Table 1). 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 or at the University of Helsinki (Table 1). 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). In 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, 0.5–3 ml of the phytoplankton stock was inoculated per 100 ml of fresh culture medium every two weeks. The samples for phytoplankton analyses were harvested in the late phase of exponential growth, i.e., 2–3 weeks after the inoculation.

Phytoplankton nomenclature. The algal classification followed mainly the taxonomy and common names of Algaebase (2013). 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 three species of Trebouxiophyceae studied are referred to as eukaryotic picoplankton due to their small size.

Fatty acid 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 new tube. Chloroform was evaporated under a N_2 gas stream and the remaining lipids were dissolved in toluene. Methanolic H_2SO_4 (1% v/v) was added to produce fatty acid methyl esters, and samples were transmethylated in a water bath at 50 °C over night. FA methyl esters 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 Finland (Finland). Both instruments were equipped with an Agilent® DB-23 column (30 m x 0.25 mm x 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 sec^{-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.

Fatty acid identification. Identification of FA 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 data base maintained by the AOCS Lipid Library

(http://lipidlibrary.aocs.org/ms/arch_me/index.htm). Identification of SAFA and bacterial originated *iso*- and *anteiso* -branched FA were based on standards and mass spectra. The location of the double bond of MUFA was verified with dimethyl disulphide (DMDS) adducts (Nichols et al. 1986). Diunsaturated FA were identified by mass spectrum and relative retention times. Accurate identification of the double bond positions in dienoic fatty acids (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 FA, the mass spectra alone provide limited information on the positions of double bonds in polyenoic FA, but in most cases the relative retention data and the mass spectra together provide enough information to identify methylene-interrupted polyunsaturated fatty acids (≥ 3 double bonds). The molecular weight of the 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 FA (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 PUFA and a peak at *m/z*=108 in ω-3 PUFA. All double bonds in the represented PUFA 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 design the research to evaluate the effects of culture conditions and media on phytoplankton FA 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 two-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 five FA 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 affects on interpretation of multivariate FA signature ordinations.

All statistical routines were performed using PRIMER v.6.0 and PERMANOVA+ add on (Anderson et al. 2008, Clarke & Gorley 2006).

RESULTS

FA profiles of freshwater phytoplankton classes. We detected 54 different FA from our freshwater algal strains. The FA profiles of seven freshwater phytoplankton classes differed significantly from each other (PERMANOVA, P=0.0001; Table 3). The factor algal Class accounted for 66.4% of the total variation in FA signatures (Table 3). Post-hoc pairwise tests showed that most across-class comparisons were significantly different (Table 4) except for all comparisons involving Raphidophyceae (excluding Raphidophyceae vs. Bacillariophyceae, P=0.0058), Chlorophyceae vs. Trebouxiophyceae (P=0.654), and Euglenophyceae vs. Chrysophyceae (P=0.055).

According to multivariate ordination (Fig. 1) the seven freshwater phytoplankton classes were found to be different in multivariate space and formed four major groups (Fig. 1). The two-dimensional stress of the NMDS was 0.11. A principal component analysis (not pictured) explained at 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 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 one Raphidopyceae taxon formed a fourth group (Fig. 1). NMDS axis 2 separated Euglenophyceae from the other algae. There were three 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 FA 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 FA of Chlorophyceae (16:4ω3, 16:3ω3, and 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 FA for Cryptophyceae and Chrysophyceae. NMDS axis 2 was generally negatively (r=-0.28 to -0.52) correlated (p=0.01) with the long chain PUFA (22:4ω6, 20:2ω6, 20:4ω6, 20:3ω3) and the unusual C_{17} carbon chain PUFA 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 two-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 FA 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 pictured).

Similarity and major FA of freshwater phytoplankton. Six of the individual FA 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 FA most responsible for within group similarities also play an important role in separating the phytoplankton groups in the NMDS ordination.

The most abundant FA (Table 6), ω-3, and ω-6 FA (Fig. 2) varied among algal classes. The major FA 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 *Chlamydomonas*, *Scenedesmus*, *Ankistrodesmus*, *Pediastrum*, *Choricystis* and *Stichococcus* genera. Additionally, linoleic acid (18:2ω6) was one of the three dominant FA of *Ankistrodesmus*, *Choricystis*, and *Stichococcus*. Four FA (18:1ω9, 18:3ω3, 16:0 and 18:2ω6) accounted for most 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 FA than any other algal class (total of 22 FA). The most abundant FA in this group were palmitic acid, ALA and EPA, which each accounted for ≈ 10% of Euglenophyceae FA. Linoleic acid, ARA, 16:4ω3, DHA, 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 three most prevalent FA in *Synura* and *Mallomonas* were SDA, 14:0 and ALA, which contributed only 30% of all FA of *Mallomonas*, but ~50% of all FA of *Synura*. In contrast to *Synura* and *Mallomonas*, 16:1ω7, 16:0 and 18:2ω6 were the most abundant FA in *Dinobryon*. However, in spite of these different contributions all three Chrysophyceae strains had the similar FA profiles, excluding some minor differences among C_{20} PUFA. Amongst the C_{20} PUFA, 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 MUFA 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 FA, but the contributions varied within this group. The three dominant FA 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), *Cryptomonas erosa*, (21), *Cryptomonas ozolinii* (24) and *Rhodomonas minuta* 826), whereas ALA was the most important FA in *Cryptomonas marsonii* (20) and *Rhodomonas lacustris* (27), and palmitic acid was the most important in strains *Cryptomonas pyrenoidifera* (22), *Cryptomonas obovoidea* (23) and *Cryptomonas ovata* (25). These last three 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 FA) than the other strains.

In Bacillariophyceae the major FA were 16:1ω7, EPA, 16:0 and 14:0, which together accounted for more than 70% of all FA. 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. *G. semen*, was most similar to that of Bacillariophyceae, Cryptophyceae and Chrysophyceae. The five major FA for this group were palmitic acid, EPA, ALA, SDA and myristic acid (14:0), which accounted for about 65% of all FA. *G. semen* had also 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), Chrysophyceae (2±0.1) and in Trebouxiophyceae (4±3). This ratio was high in Bacillariophyceae (11±14), Chlorophyceae (10±12), Raphidophyceae (9) and Cryptophyceae (7±3) but varied also considerably among Bacillariophyceae and Chlorophyceae.

DISCUSSION

The factor algal Class accounted for 66.4% of the total variation in the FA signatures (Table 3). Additionally, the 37 strains from seven algal classes created four 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 taxonomical group was similar within each group even though the contribution of individual FA differed especially amongst the Cryptophyceae and Chrysophyceae. There are only a few FA that are reported as unique for specific algal groups. We found C_{16}, C_{15} and C_{17} PUFA, as well 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} PUFA, which were not detected in any other class.

Effects of the environment on algae FA. It is known that the growth conditions, e.g. light intensity, temperature, salinity or nutrients, can affect the phytoplankton lipid and FA composition (Guschina & Harwood 2009). Therefore changes in environment can possible influence to the quality of microalgae or abundance of individual FA in microalgae. Colder temperatures generally increase the unsaturation of microalgae membrane FA, thus temperature lowering can increase the relative amount of EPA or DHA which have melting points of -45 to -50 °C (Tatsuzawa and 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. 2009) 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 pictured). This can be easily seen from Chlorophyceae, which clustered tightly together in-spite of different media. These observations are consistent with the the lack of media effects found in the ANOSIM analysis. It should be noted that environmental conditions can affect the abundance of individual FA, but do not stimulate microalgae to synthesize totally new FA or change FA composition over taxonomical class. For example EPA or DHA is not reported to be abundant among Chlorophyceae under any circumstances, but is 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 open water season. The ALA concentration also tracked Chlorophyceae biomass under different temperature and light conditions in a small boreal lake (Taipale et al. 2008).

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 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} MUFA (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 FA, which are dominant FA in bacteria (Kaneda 1991). In freshwater

systems these FA 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). The FA of 16:1ω7 or/and 18:1ω7 are abundant FA amongst heterotrophic gram negative bacteria (Ratledge & Wilkinson 1988), of which 16:1ω7 is also abundant (27-43% of all FA 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 FA) in *Dinobryon*. We also found 8-9% 18:1ω7 in *Dinobryon* and *Chlamydomonas,* whereas 18:1ω7 contributed less than 6% to other phytoplankton strains considered. Therefore, the high abundance of 18:1ω7 in seston or zooplankton would most likely indicate 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 be used as indicator of terrestrial organic particulate carbon FA in previous laboratory studies (Brett et al. 2009a, Taipale et al. 2013). 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 ω-3:ω-6 ratio should be used with caution in the food web studies.

PUFA are most useful for separating different microalgae taxa from each other in freshwater systems because they are not generally prevalent FA in bacteria or t-POM. Our study revealed that there were only a few FA that belonged only to one or two algal classes (Table 5) and can be therefore used as specific FA biomarkers. The most specific FA were found amongst the C_{15} to C_{18} PUFA in two or three algal classes. Amongst all the strains analyzed, only Cryptophyceae and Chrysophyceae (excluding *Dinobryon*) did not contain short carbon chain PUFA. Euglenophyceae contained the unusual C_{15} and C_{17} PUFA (15:3ω1, 15:4ω3, 17:2ω7/5 and 17:3ω2) (Korn 1964), and

the C_{20} and C_{22} PUFA (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} PUFA 16:3ω3, 16:4ω3 and 16:2ω6 were detected from Chlorophyceae, Trebouxiophyceae (16:2ω6 not from *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} PUFA 16:2ω4 and 16:3ω4 were found in Bacillariophyceae as well as *G. semen*. The C_{16} PUFA16:3ω1 was abundant in *Dinobryon*, and was also detected in *G. semen.* Furthermore, division of FA 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, therefore more biochemical studies should be used to classify this group.

The 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 FA without EPA are intermediate quality for *Daphnia* (Brett et al. 2006), whereas diets with low concentrations of ω-3 FA and sterols (see Brett et al. 2009a, Martin-Creuzburg et al. 2009) are biochemically iandequate resources for zooplankton. Field studies have

demonstrated, for example, that the highest zooplankton biomass follows phytoplankton FA quality rather than phytoplankton quantity (Gladyshev 2009).

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 *G. semen*, but due to their large size (50-100 mm), 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 et al. 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 one of the most studied classes among 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 FA (Ratledge & Wilkinson 1989). Marine Chlorophyceae are therefore a theoretically slightly better quality diet than freshwater strains. The Chlorophyceae biomarker C_{16} PUFA 16:3ω3 and 16:4ω3 have also been found more universally in marine and estuarine members of the class (Ratledge & Wilkinson 1989, Dunstan et al 1992, Viso & Marty 1993, Dijkman & Kromkamp 2006), but are not routinely reported from freshwater taxa as well.

Bacillariophyceae are another well studied algal group (Ackman et al. 1968, Katner et al. 1983), especially in marine systems. Their major FA 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. 1994). The contribution of EPA from marine Bacillariophyceae varies between 12-30% (Dunstan et al. 1994), which is slightly more than what was found in our freshwater strains (EPA 7-23% of all FA). Thus marine diatoms are also a 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. 1994). 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 *G. semen* is the most common representative of this class. Our analysis revealed that *G. semen* has the same primary FA 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 heterogenous 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 5-20% 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 (Dunstan et al. 2005, Ahlgren et al. 1992). We were not able to compare cultures of Chrysophyceae and Synuraphyceae from both marine and freshwater systems due to limeted research on the FA profiles of these groups (Cranwell et al. 1988).

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 FA can distinguish microalgae at the class level. Therefore, FA can be used for the taxonomic primary production measurements in different freshwater systems. Moreover, FA offer a powerful tool for lacustrine food web studies to track different diets in the food web. Zooplankton studies with 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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Table 1. The 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	Linoleic acid	LIN
18:3ω6	γ-linolenic acid	GLA
20:4ω6	Arachidonic acid	ARA

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Table 2.The freshwater algae strains used for this study were obtained from different culture collections and universities. Strain origin is represented here is according to the information received from culture collections or universities. Algae were cultured using optimal media for each strain. Cultures were kept either 14:10 or 16:8 hour light:dark cycle. Temperature of all cultures was 18-20 °C.

Strain number	Class	Common name	Species	Collection	Origin	Place Cultured	Media	Light cycle	Temperature
1	Chlorophyceae	Green Algae	*Ankistrodesmus* sp.	UWCC[1]	Freshwater	University of Washington	L16 (Lindström 1983)	14:10	18
2	Chlorophyceae	Green Algae	*Chlamydomonas reinhardtii*	UWCC[1]	Freshwater	University of Washington	L16 (Lindström 1983)	14:10	18
3	Chlorophyceae	Green Algae	*Chlamydomonas* sp.	Peltomaa[2], Finland	Musta-Kotinen, Finland	University of Helsinki	DY-V by CCMP[8]	16:8	20
4	Chlorophyceae	Green Algae	*Pediastrum privum*	CCAP[3] 261	Hokajärvi, Finland	University of Helsinki	DY-V by CCMP[8]	16:8	20
5	Chlorophyceae	Green Algae	*Selenastrum* sp.	Peltomaa[2], Finland	Iso-Ruuhijärvi, Finland	University of Helsinki	WC (Guillard and Lorenzen 1972, Guillard 1975)	16:8	20
6	Chlorophyceae	Green Algae	*Selenastrum capricornutum*	Culture collection U.S.A.*	Freshwater	University of Ottago	MBL Medium (Stemberger 1981)	14:10	18
7	Chlorophyceae	Green Algae	*Selenastrum capricornutum*	UWCC[1]	Freshwater	University of Washington	L16 (Lindström 1983)	14:10	18
8	Chlorophyceae	Green Algae	*Scenedesmus communis*	Peltomaa[2], Finland	Pääjärvi, Finland	University of Helsinki	DY-V by CCMP[8]	16:8	20
9	Chlorophyceae	Green Algae	*Scenedesmus obliquus*	Max Planck Institute, Germany	Freshwater	University of Washington	L16 (Lindström 1983)	14:10	18
10	Chlorophyceae	Green Algae	*Scenedesmus ecornis*	Peltomaa[2], Finland	Taka-Killo, Finland	University of Helsinki	WC (Guillard and Lorenzen 1972, Guillard 1975)	16:8	20
11	Chlorophyceae	Green Algae	*Coenocystis* sp.	Peltomaa[2], Finland	Ormajärvi, Finland	University of Helsinki	DY-V by CCMP[8]	16:8	20
12	Euglenophyceae	Euglenoids	*Euglena gracilis*	CCAP[3] 1224/5Z	Freshwater	University of Helsinki	Euglena Gracilis Medium by CCAP[3]	16:8	20
13	Euglenophyceae	Euglenoids	*Euglena* sp. (small)	Peltomaa[2], Finland	Kyynärö, Finland	University of Helsinki	AF6 (Watanabe et al. 2000)	16:8	20
14	Euglenophyceae	Euglenoids	*Euglena* sp. (big)	Peltomaa[2], Finland	Kyynärö, Finland	University of Helsinki	AF6 (Watanabe et al. 2000)	16:8	20
15	Chrysophyceae	Golden Algae	*Dinobryon cylindricum*	UWCC[1] FW 622		University of Washington	Volvox	14:10	18
16	Chrysophyceae	Golden Algae	*Mallomonas caudata*	CCAP[3] 929/8	Musta-Kotinen, Finland	University of Helsinki	WC (Guillard and Lorenzen 1972, Guillard 1975)	16:8	20
17	Chrysophyceae	Golden Algae	*Synura* sp.	Peltomaa[2], Finland	Kyynärö, Finland	University of Helsinki	WC (Guillard and Lorenzen 1972, Guillard 1975)	16:8	20
18	Raphidophyceae	Raphidphyte Algae	*Gonyostomum semen*	GSB 02**/04***	Lake Bökesjön, Sweden	University of Washington	L16 (Lindström 1983)	14:10	18
19	Cryptophyceae	Cryptomonads	*Cryptomonas* sp.	Peltomaa[2], Finland	Kyynärö, Finland	University of Helsinki	AF6 (Watanabe et al. 2000)	16:8	20
20	Cryptophyceae	Cryptomonads	*Cryptomonas marssonii*	CCAP[3] 979/70	Musta-Kotinen, Finland	University of Helsinki	DY-V by CCMP[8]	16:8	20
21	Cryptophyceae	Cryptomonads	*Cryptomonas erosa*	Gilbert[4], U.S.A.*		University of Ottago	MBL Medium (Stemberger 1981)	16:8	20
22	Cryptophyceae	Cryptomonads	*Cryptomonas pyrenoidifera**	NIVA[5] 2/81	Lake Gjersjøen, Norway	University of Washington	L16 (Lindström 1983)	14:10	18
23	Cryptophyceae	Cryptomonads	*Cryptomonas obovoidea**	CCAP[3] 979/44	Freshwater	University of Washington	L16 (Lindström 1983)	14:10	18
24	Cryptophyceae	Cryptomonads	*Crytomonas ozolinii*	UTEX[6] LB 2782	Crowdrey Lake, U. S. A.	University of Washington	L16 (Lindström 1983)	14:10	18
25	Cryptophyceae	Cryptomonads	*Cryptomonas ovata*	CCAP[3] 979/61	Hirschberg, Austria	University of Washington	L16 (Lindström 1983)	14:10	18
26	Cryptophyceae	Cryptomonads	*Rhodomonas minuta*	CPCC[7] 344	Freshwater	University of Washington	L16 (Lindström 1983)	14:10	18
27	Cryptophyceae	Cryptomonads	*Rhodomonas lacustris*	NIVA[5] 8/82	Nordbytjernet, Norway	University of Washington	L16 (Lindström 1983)	14:10	18
28	Trebouxiophyceae	Eucaryotic green picoplankton	*Choricystis* sp.	CCMP[8] 2201	North Deming Bond, U.S.A.	University of Helsinki	DY-V by CCMP[8]	16:8	20
29	Trebouxiophyceae	Eucaryotic green picoplankton	*Choricystis coccoids*		Lake Tahoe, U.S.A.*	University of Ottago	WC (Guillard and Lorenzen 1972, Guillard 1975)	14:10	18
30	Trebouxiophyceae	Eucaryotic green picoplankton	*Stichococcus chodati*		Lake Tahoe, U.S.A.*	University of Ottago	WC (Guillard and Lorenzen 1972, Guillard 1975)	14:10	18
31	Bacillariophyceae	Diatoms	*Fragilaria crotonensis*	UTEX[6] LB FD56	Wyoming, U.S.A.	University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
32	Bacillariophyceae	Diatoms	*Cyclotella meneghiniana*	PAE Lab, Belgium	Freshwater	University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
33	Bacillariophyceae	Diatoms	*Asterionella formosa*	PAE Lab, Belgium	Freshwater	University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
34	Bacillariophyceae	Diatoms	*Stephanodiscus hantzschii*	CCAP[3] 1079/4	Esthwaite Water, England	University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
35	Bacillariophyceae	Diatoms	*Synedra* sp.	Carolina[9]	Freshwater	University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
36	Bacillariophyceae	Diatoms	*Navicula pellicosa*	UTEX[6] B664	Alaska, U.S.A.	University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18
37	Bacillariophyceae	Diatoms	*Aulacoseira granulata* var. *angustissima*	CCAP[3] 1002/2	Sydney, Australia	University of Washington	Diatom medium (Beakes et al. 1986)	14:10	18

[1] UWCC = Algal and Fungal University of Washington Culture Collection, at the University of Washington, Seattle, Washington. [2]Peltomaa= Lammi Biological Station, University of Helsinki, Finland. [3]CCAP = Culture Collection of Algae and Protozoa, Ambleside, Cumbria, U.K. [4]Gilbert, Dartmouth College, NH, U.S.A. [5]NIVA = Norwegian Institute for Water Research, Oslo, Norway. [6]UTEX = University of Texas Culture Collection, University of Texas at Austin, U.S.A. [7]CPCC = Canadian Phycological Culture Centre, University of Waterloo. [8]CCMP = National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences, Maine, U.S.A. [9]Carolina = Carolina Biological Supply Company, Burlington, U.S.A.

*see more information from Burns et al. (2011), **see more information from Rengefors et al. (2008),*** Unpublished, isolated from Lake Bökesjön 2004.

Table 3. PERMANOVA results of the overall test of class level differences. Analysis assumes the factor class is fixed and uses III sums of squares. Significance determined with permutation and Monte Carlo (P(MC)) *P*-values (see 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 in the procedure (Unique perms) and significance determined from Monte Carlo permutation (P(MC); *<0.05, **<0.001) (see 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

730 Table 5. Results of similarity percentage analysis (SIMPER) of freshwater microalgae FA signatures in six classes. Analysis is run on the untransformed FA data. There are no results reported for Raphidophyceae because only one strain was sampled within this group. The table shows mean proportions (Mean) of the six FA that contributed the most (and % contribution of each FA) to within-group similarity.

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

735

Table 6. The major FA and potential biomarkers for each algal class.

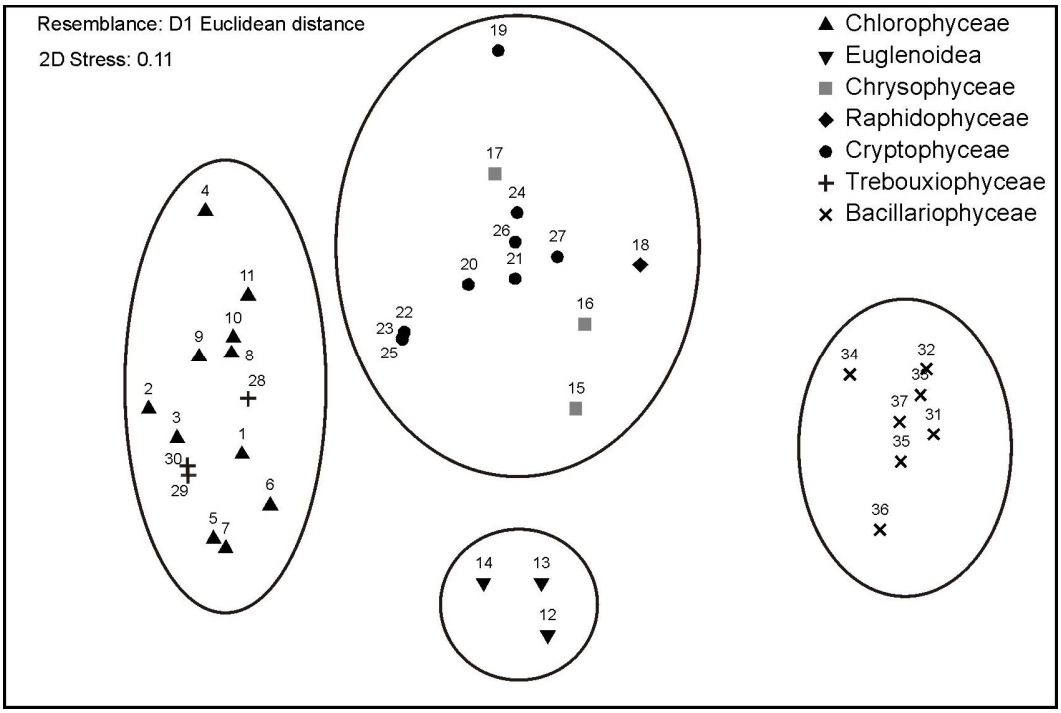
Phytoplankton group	Major FA	Fatty acid biomarker
Chlorophyceae	ALA, 16:0, 18:1ω9 and LIN	16:4ω3, 16:3ω3, 16:2ω6
Trebouxiophyceae	ALA, 16:0, 18:1ω9 and LIN	16:4ω3, 16:3ω3, 16:2ω6
Cryptophyceae	ALA, 16:0, and SDA	22:5ω6, 18:4ω3
Synuraphyceae	SDA, 14:0, ALA, and 16:0	22:5ω6, 18:4ω3
Ocromonadales	16:1ω7c,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

*These fatty acids were only found from this phytoplankton group

Figure legends

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 are tested using PERMANOVA. Axis 1 correlated positively with the diatom fatty acids. Axis 2 correlates positively with characteristic fatty acids for Cryptophyceae and Synurales and negatively with characteristic fatty acids 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 (Table 1).

Fig. 2 The contribution (%, mean±SD) of (A) ALA and SDA; (B) EPA, DPA and DHA; and (C) LIN, ARA and 22:5ω6 of all FA among seven freshwater algal classes.


Resemblance: D1 Euclidean distance
2D Stress: 0.11
Chlorophyceae
Euglenoidea
Chrysophyceae
Raphidophyceae
Cryptophyceae
Trebouxiophyceae
Bacillariophyceae
1
2
3
4
5
6
7
8
9
10
11
12
13
14
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17
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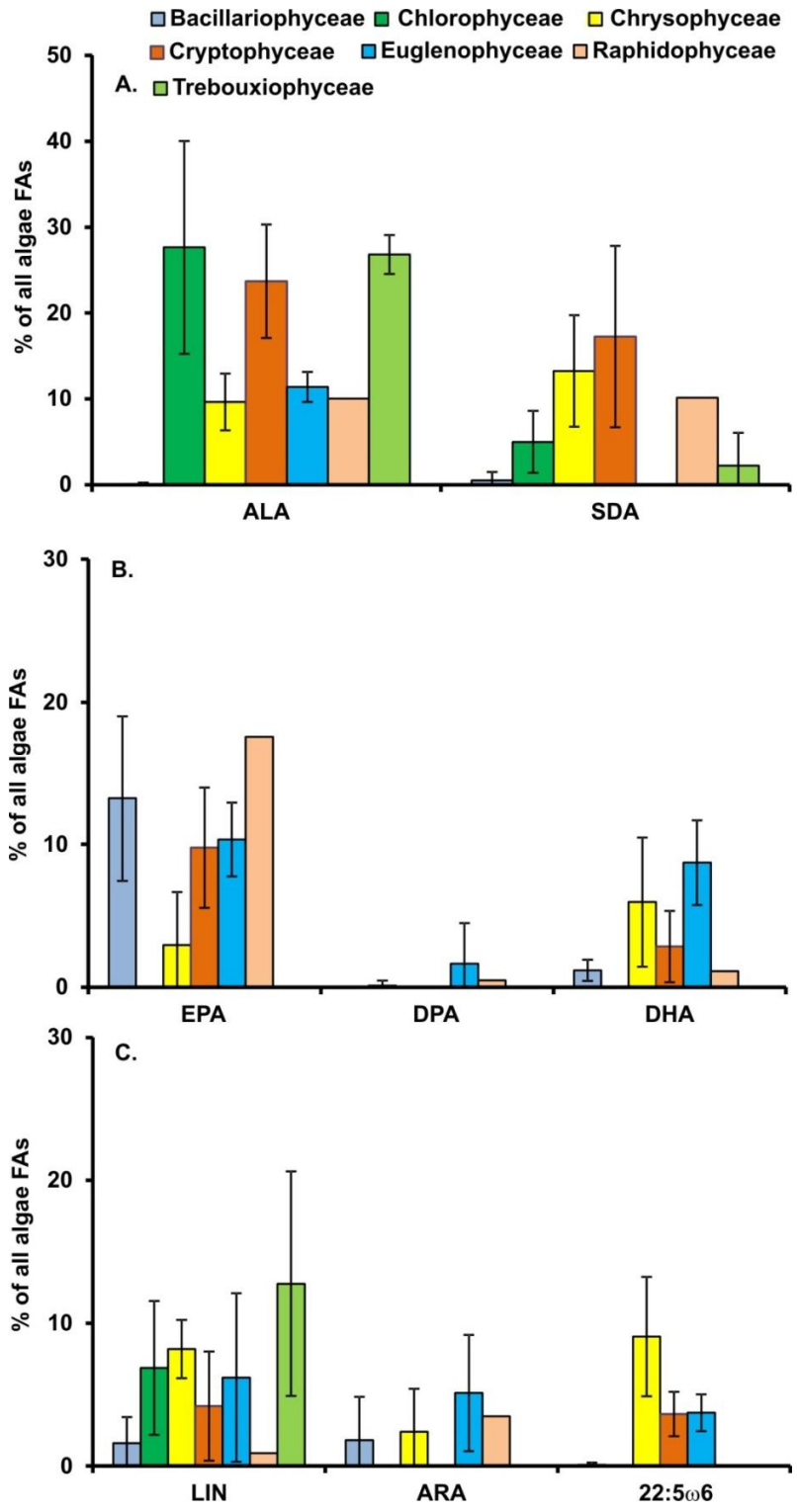
Fig. 1.


Bacillariophyceae
Chlorophyceae
Chrysophyceae
Cryptophyceae
Euglenophyceae
Raphidophyceae
Trebouxiophyceae
A.
% of all algae FAs
50
40
30
20
10
0
ALA
SDA
B.
% of all algae FAs
30
20
10
0
EPA
DPA
DHA
C.
% of all algae FAs
30
20
10
0
LIN
ARA
22:5ω6

755

Fig. 2.