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Daphnia fatty acid composition reflects that of their diet

Michael T. Brett[1]
Department of Civil and Environmental Engineering, Box 352700, University of Washington, Seattle, Washington 98195

Dörthe C. Müller-Navarra[2]
Department of Environmental Science and Policy, University of California, 1 Shields Ave., Davis, California 95616

Ashley P. Ballantyne[3] *and Joseph L. Ravet*
Department of Civil and Environmental Engineering, Box 352700, University of Washington, Seattle, Washington 98195

Charles R. Goldman
Department of Environmental Science and Policy, University of California, 1 Shields Ave., Davis, California 95616

Abstract

We conducted a series of experiments feeding *Daphnia pulex* nine different phytoplankton monocultures with widely varying fatty acid composition and nutritional values to test the extent to which *Daphnia* fatty acid composition was affected by diet. In general, *Daphnia* fatty acid composition matched that of their diet much more closely than it did the fatty acid composition of *Daphnia* consuming other diets. However, *Daphnia* had consistently less saturated fatty acids and more arachidonic acid than did their diet, and *Daphnia* consuming cyanobacteria had substantially less saturated fatty acids and more monounsaturated fatty acids than their diets. *Daphnia* that consumed cryptophytes, which are rich in ω3 polyunsaturated fatty acids (PUFAs), had on average 47% ± 8% (±1 SD) ω3 PUFAs within their fatty acid pool, whereas *Daphnia* that consumed ω3 PUFA–poor cyanophytes only had 6% ± 3% ω3 PUFAs. The ratio of ω3 to ω6 fatty acids in *Daphnia* was also strongly dependent on diet, and averaged ≈ 10 : 1, 2 : 1, and 1 : 1 for *Daphnia* that consumed cryptophytes, chlorophytes, and cyanophytes, respectively. Furthermore, the sum of C_{20} and C_{22} ω3 and ω6 fatty acids in *Daphnia* was highly correlated with that of their diet ($r^2 = 0.94$). These results suggest analyses of *Daphnia* fatty acid composition may be a powerful means of inferring diet in the field. These results also suggest the nutritional benefits of consuming ω3-rich phytoplankton will transfer up the food web, making zooplankton both more efficient at converting phytoplankton biomass to their own biomass as well as much more nutritious for the zooplanktivorous fish that consume them.

There is tremendous interest in developing approaches for inferring diet in organisms based on their elemental and biochemical composition. The classic approach is to use analyses of stable isotopes to infer dietary source (carbon and sulfur) and trophic position (nitrogen), thereby providing three variables with which to characterize an organism's trophic niche (Peterson and Fry 1987). However, this approach can only be used to infer dietary sources if the sources of interest have distinct stable isotope ratios, which is not always the case, especially for generalist herbivores. A number of authors have explored the utility of using fatty acids as trophic markers of dietary composition, i.e., the FATM concept (Dalsgaard et al. 2003). This concept has been most commonly applied to marine systems (Dalsgaard et al. 2003), but has also been applied to studies of freshwater streams (Desvilettes et al. 1997; Napolitano 1999; Heintz et al. 2004). Fatty acids (FAs) are critical constituents of all biota, where they comprise the main component of neutral and polar lipids. Polar lipids provide the basic cellular membrane matrix into which other membrane constituents such as cholesterol and proteins are embedded (Vance 1996). The FATM concept is based on the fact that the major primary producer taxa have distinctive fatty acid profiles that may be, to varying degrees, transferred conservatively to consumers. FAs can be incorporated into the neutral lipids of primary consumers virtually unaltered, especially when catabolic activity is low, such as when accumulating lipid reserves. However, because consumers selectively metabolize FAs and can convert some forms to others, FAs can only be used as semiquantitative food web tracers (Dalsgaard et al. 2003). Because the fatty acid composition within phytoplankton is largely dependent on systematic affiliation, primary producers may "lay-down" the basic fatty acid pattern in aquatic food webs (Jefferies 1970; Ahlgren et al. 1996). The main phytoplankton groups, such

[1] Corresponding author (mtbrett@u.washington.edu).
[2] Present address: Center for Marine and Climate Research, Institute of Hydrobiology and Fisheries Research, University of Hamburg, D-22767 Hamburg, Germany.
[3] Present address: Nicholas School of the Environment, Division of Earth and Ocean Sciences, Duke University, Box 90229, Durham, North Carolina 27708-0229.

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Table 1. The nomenclature and structure of the FAs considered in this study.

Type	Structure	Abbreviation
Saturated fatty acids	14:0i, 14:0, 15:0i, 15:0, 16:0i, 16:0, 17:0i, 17:0a, 17:0, 18:0, 20:0	SAFA
Monounsaturated fatty acids	16:1, 18:1ω6/ω9, 18:1ω7, 20:1ω7, 20:1ω9	MUFA
α-Linolenic acid	18:3ω3	ALA
Stearidonic acid	18:4ω3	SDA
Eicosapentaenoic acid	20:5ω3	EPA
Docosahexaenoic acid	22:6ω3	DHA
Linoleic acid	18:2ω6	LIN
γ-Linolenic acid	18:3ω6	GLA
Arachidonic acid	20:4ω6	ARA
Polyunsaturated fatty acids	ALA, SDA, LIN, GLA, EPA, DHA & ARA	PUFA
Highly unsaturated fatty acids	EPA, DHA, ARA	HUFA
C_{18} ω3 PUFAs	e.g., primarily GLA and SDA	
C_{18} ω6 PUFAs	e.g., primarily LIN and GLA	
ω3 PUFAs	ALA, SDA, EPA and DHA	
ω6 PUFAs	LIN, GLA and ARA	
Bacterial fatty acids	14:0i, 15:0i, 15:0, 17:0i 17:0a, 17:0	Bact FAs

as diatoms, cryptophytes, dinoflagellates, chlorophytes, and cyanobacteria, can be distinguished based on the presence and especially the ratios of particular FAs (Pohl and Zurheide 1979; Ahlgren et al. 1992; Dalsgaard et al. 2003). Prokaryote (i.e., bacteria) FAs are quite different from those of eukaryotes (Perry et al. 1979). Odd-chain (C_{13} to C_{19}) and branched FAs, generally of iso-antiso structure, often comprise a high proportion of bacterial FAs. In addition, certain monoenoic FAs are characteristic of bacteria (Gillan et al. 1981). Finally, cyanobacteria usually lack C_{20} or C_{22} polyunsaturated fatty acids (PUFAs), and only some taxa contain C_{18} PUFAs (Parker et al. 1967; Murata et al. 1992).

Most of what is known about dietary impacts on zooplankton fatty acid composition is from the marine copepod literature (Dalsgaard et al. 2003). Classic studies by Gatten et al. (1983) and Fraser et al. (1989) showed the diet of larval herring could be inferred by comparing their fatty acid profile to that of phytoplankton (which they consumed during the first month post-hatch) and zooplankton (which they consumed in the second and third months post-hatch). In general, omnivorous or carnivorous copepods accumulate triacylglycerols instead of wax esters. However, when omnivorous or carnivorous copepods do accumulate wax esters they primarily contain fatty alcohols dominated by 14:0 and 16:0 (Sargent and Henderson 1986; Graeve et al. 1994) and not 20:1ω9 and 22:1ω11, as is generally the case for herbivorous marine copepods (Sargent and Henderson 1986; Kattner and Hagen 1995). The fatty acid 18:1ω9 and the ratio between 18:1ω7/18:1ω9 can also be used to distinguish between herbivory and carnivory in marine copepods (e.g., Graeve et al. 1997; Falk-Petersen et al. 2000). Interestingly, Cripps et al. (1999) suggested a high proportion of ω3 PUFAs (fatty acid abbreviations are explained in Table 1) in *Euphausia superba* was a sign of food limitation and perhaps carnivory. When studying the freshwater cladoceran *Daphnia galeata* von Elert (2002) showed the fatty acid profile of this zooplankter was enriched with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) when fed diets artificially enriched with these FAs. Becker and Boersma (2005) observed a similar relationship for *Daphnia magna* fed diets enriched with EPA.

In addition to the role FAs may play as biomarkers of lipid flow through food webs, certain FAs and especially ω3-PUFAs have been shown to be determinants of phytoplankton food quality for herbivorous zooplankton (Ahlgren et al. 1990; Müller-Navarra 1995; Ravet et al. 2003). Research on the nutritional requirements of a range of fish species has also shown ω3-PUFAs and especially DHA, as well as the ratios of ω3 to ω6 FAs, have critical impacts on fish growth, reproduction, and survival (Adams 1999; Olsen 1999; Sargent et al. 1999). Because these FAs or their precursors cannot be synthesized by animals, but are produced almost exclusively by plants, they are essential for animals and are called essential fatty acids (EFAs). EFA-rich fish like salmon, tuna, sardines, etc., merely bioaccumulate phytoplankton EFAs consumed in their diet (Arts et al. 2001).

Understanding the extent to which zooplankton dietary FAs accumulate conservatively within their somatic tissues is of great value for untangling zooplankton dietary composition and trophic relationships. Furthermore, knowing to what extent nutritionally critical FAs are transferred from phytoplankton to zooplankton has obvious implications for the nutritional ecology of fish. We tested the similarity of *Daphnia* fatty acid composition to that of their diets by feeding *Daphnia* monocultures of phytoplankton taxa representing a wide range of taxa (e.g., cryptophytes, chlorophytes, and cyanophytes) with very different fatty acid composition and nutritional value.

Methods

Experimental protocol—All experiments were conducted using a clone of *Daphnia pulex* originally isolated from Clear Lake, California, and subsequently maintained on the green alga *Scenedesmus obliquus* in a growth chamber with a constant temperature (18°C) and light : dark cycle

(14 h : 10 h). Nine phytoplankton monocultures were used, including three cryptophytes (*Cryptomonas ovata* 979/44, *C. ovata* 979/61, and *Rhodomonas minuta*), three chlorophytes (*Ankistrodesmus* sp., *Selenastrum capricornutum*, and *S. obliquus*), and three cyanophytes (*Microcystis aeruginosa* 2063, *M. aeruginosa* 2387, and *Synechococcus elongatus*). The cyanophyte cultures used were all non-toxic and single-celled. These cultures were used because they were all easily mass cultured, and we could therefore obtain sufficient phytoplankton food for these experiments. All phytoplankton (as well as *Daphnia*) cultures were maintained on L16 growth medium (Lindström 1983) supplemented with earth extract and B vitamins. The earth extract used in these experiments was sterile, but none of the phytoplankton cultures were axenic. During these experiments, a final phytoplankton concentration of 2.0-mg L^{-1} dry weight (i.e., the incipient limiting food concentration for *Daphnia*) was used, with biomass in the different monocultures determined daily using regression equations between phytoplankton biomass and in vivo fluorescence as determined on a Turner Designs 10-AU fluorometer. These experiments were conducted in a flow-through system (see Ravet et al. [2003] for a description) partially submerged in a 70-liter aquarium equipped with 12 120-mL chambers. The flow-through chambers were continuously supplied with stirred phytoplankton at a rate of 1.2 liters per day to minimize *Daphnia* overgrazing using a multichannel peristaltic pump.

Twelve hours before the start of each experiment, egg-bearing *Daphnia* were separated from stock cultures and placed into individual 20-mL scintillation vials with *S. obliquus* as food. At the beginning of each experiment, eight neonates from this ≈6-h old cohort were randomly selected and transferred to the flow-through chambers. These experiments lasted 6 days, i.e., the time required for the neonates to reach the primiparous instar. Because the slowest growing *Daphnia* in these experiments increased their mass by a factor of eight, these 6-day experiments were sufficient to assure that nearly the entire *Daphnia* fatty acid pool turned over simply because of somatic growth. Each *Daphnia* by phytoplankton taxa combination was originally carried out in triplicate, but these triplicates were subsequently pooled to form single samples to obtain sufficient material to conduct the fatty acid analyses in the experiments where *Daphnia* grew poorly (i.e., the cyanophyte treatments). At the conclusion of each experiment, *Daphnia* were collected and stored at −80°C for fatty acid determinations.

The phytoplankton used in these experiments were selected based on their very different fatty acid composition, known impacts on *Daphnia* growth and reproductive responses, and our ability to mass culture them. To demonstrate the food quality differences, a series of experiments feeding *Daphnia* mixtures of cryptophyte, chlorophyte, or cyanophyte phytoplankton was run. The cryptophyte mixtures were comprised of equal parts *C. ovata* 979/44, *C. ovata* 979/61, and *R. minuta*. The cyanophyte mixtures were equal parts *M. aeruginosa* 2063, *M. aeruginosa* 2387, and *S. elongatus*, and the chlorophyte mixtures were equal parts *S. obliquus* and *Ankistrodesmus* sp. Each phytoplankton mixture was run with eight replicates. At the conclusion of these experiments, *Daphnia* were measured individually for length and clutch size (eggs per individual) under a microscope, and then dried (24 h at 105°C) and weighed (in groups of four individuals from each replicate) with a Cahn Microbalance (model C33) to obtain the average individual weight per replicate. In other regards, the protocol for these experiments was virtually identical to that described in the preceding paragraphs.

Fatty acid analyses—Fatty acid methyl esters from the phytoplankton and zooplankton samples (Kattner and Fricke 1986) were analyzed with a gas chromatograph (HP6890) equipped with a programmable temperature vaporizer injector, a fused silica capillary column (DB-WAX, J&W Scientific; 30 m × 0.32 mm with 0.25-μm film thickness), and a flame ionization detector. We injected 5 μL of sample, and used helium as the carrier gas. We used the following temperature program: 40°C held for 5 min, then heated at 10°C per min to 150°C, held for 5 min, then heated at 1°C per minute to 220°C where it was kept for 20 min. Individual FAs were identified based on the retention times of fatty acid methyl ester standards (Supelco 37 component FAME mix) dissolved in n-hexane. Quantification was performed with an internal standard (21 : 0) and quantitative mixes to calculate response factors for each fatty acid analyzed. Because we did not have standards for C_{16} PUFAs, which can be markers for chlorophytes, we did not identify these FAs.

Statistical analyses—The *Daphnia* growth and reproductive responses to the cryptophyte, chlorophyte, and cyanophyte diets were assessed with a single factor analysis of variance (ANOVA) and Dunnett t-tests for post-hoc comparisons. To tease apart the relative contribution of diet and intrinsic characteristics on *Daphnia* fatty acid composition, we conducted a series of two-factor ANOVAs. These analyses used "phytoplankton or *Daphnia*" and "diet" as the two factors. According to this scheme, *Daphnia* consuming a cryptophyte diet would be classified as a Crypto/Daph, whereas a cyanophyte fed to *Daphnia* would be classified as Cyano/Phyto. The phytoplankton or *Daphnia* factor represented systematic differences between zooplankton and phytoplankton fatty acid composition, whereas the diet factor assessed the dissimilarity between the phytoplankton groups as well as the similarity between the *Daphnia* and their diet's fatty acid composition. This approach treated the three different phytoplankton species within each taxonomic group as replicates for statistical purposes. These ANOVAs were carried out for the following groups of FAs: sum of saturated FAs, sum of monounsaturated FAs, sum of C_{18} ω3 PUFAs, EPA plus DHA, sum of C_{18} ω6 PUFAs, arachidonic acid, and sum of bacterial FAs, as well as the ratio of ω3 to ω6 FAs. These eight fatty acid categories were selected a priori because they represent the key fatty acid functional groups in zooplankton and fish nutrition (as well as potential bacterial contaminants), and because grouping the data reduced the number of predictor variables to less than half

Table 2. Mean total fatty acid percentages for the three phytoplankton groups tested.

Fatty acid	Cryptophytes	Chlorophytes	Cyanophytes
14:0i	0.2	2.7	0.0
14:0	2.5	0.6	12.4
15:0i	2.7	0.2	5.5
15:0	0.8	0.2	1.0
16:0i	1.2	0.0	3.5
16:0	24.1	34.7	36.6
16:1	3.4	1.0	8.9
17:0i	1.8	4.3	1.4
17:0	0.0	0.6	3.3
17:0	0.7	0.1	2.5
18:0	3.8	1.5	5.2
18:1ω6/ω9	3.1	33.2	1.5
18:1ω7	8.7	3.4	9.2
18:2ω6	3.2	7.2	1.9
18:3ω6	0.2	0.6	0.6
18:3ω3	16.5	7.5	0.3
18:4ω3	13.0	1.7	0.7
20:0	1.9	0.0	0.3
20:1ω7	0.1	0.0	0.0
20:1ω9	0.4	0.4	0.8
20:2ω6	0.0	0.0	0.4
20:4ω6	0.0	0.0	2.5
20:5ω3	9.9	0.1	1.5
22:6ω3	1.7	0.0	0.0

the sample size, which is important for some of the statistical analyses. We also compared the zooplankton to the phytoplankton values for each of these fatty acid categories using least squares regressions to determine which FAs had the most potential to be used as dietary trophic markers for *Daphnia*. To aid in the visualization of these results, we used principle components analysis (PCA) to compare the fatty acid composition of the *Daphnia* to that of their diets using the eight fatty acid variables mentioned above. The three largest principal components (PCs) were rotated using the normalized varimax strategy, and new component coefficients were calculated (Richman 1986). Finally, we also used discriminant analysis to determine whether the FA composition of the *Daphnia* could be used to correctly identify their diet. These analyses were carried out using SPSS version 11.0 for Mac.

Results

Table 2 reports the average percent fatty acid composition for the phytoplankton groups used in these experiments. The saturated fatty acid palmitic acid (16:0) was the most common fatty acid for all three groups assessed. The fatty acid composition of the cryptophytes was dominated by palmitic acid, the monounsaturated fatty acid vaccenic acid (18:1ω7), as well as the ω3 PUFAs α-linolenic acid ([ALA] 18:3ω3), stearidonic acid ([SDA] 18:4ω3), and EPA (20:5ω3). The fatty acid composition of the chlorophytes was dominated by palmitic acid, the monounsaturate oleic acid (18:1ω9), the ω6 PUFA linoleic acid ([LIN] 18:2ω6) and the ω3 PUFA α-linolenic acid. The cyanophytes had high percentages of the saturated FAs myristic (14:0), isopalmitic (15:0i), palmitic acid, and stearic (18:0) acid, and the monounsaturates palmitoleic (16:1ω7) and vaccenic acid. The cyanobacteria also had a substantial amount of arachidonic acid ([ARA] 20:4ω6) and a surprisingly high EPA content. The high average cyanobacteria EPA content was primarily because the *S. elongatus* culture used in these experiments contained 3.8% EPA.

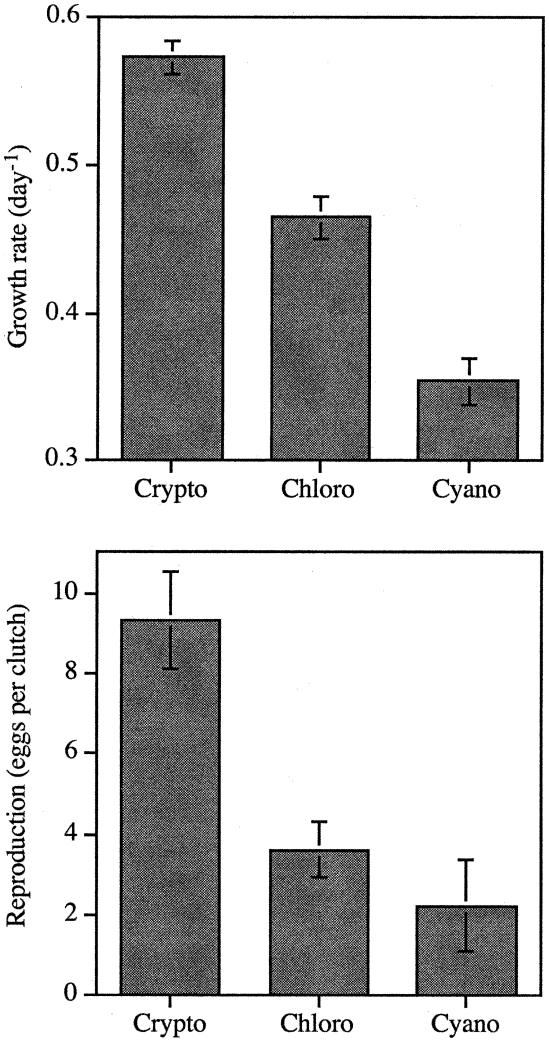

Fig. 1. *Daphnia pulex* instantaneous somatic growth rate and primiparous instar clutch size when fed mixtures of the same cyanophyte, chlorophyte, and cryptophyte taxa used in these experiments. See Methods for a detailed description of the protocol used for these experiments. The error bars represent $\pm$ 1 SD.

Major differences in growth and fecundity were evident for *Daphnia* feeding on the three groups of phytoplankton used for these experiments (Fig. 1). *Daphnia pulex* fed a mixture of cryptophytes grew at very high rates and had very large clutch sizes during their primiparous instar, whereas *Daphnia* fed a mixture of cyanophytes had both poor growth and reproduction. In contrast, *Daphnia* fed a mixture of chlorophytes had moderately good growth rates, but moderately poor reproduction. The difference in the reproductive response when consuming cyanobacteria

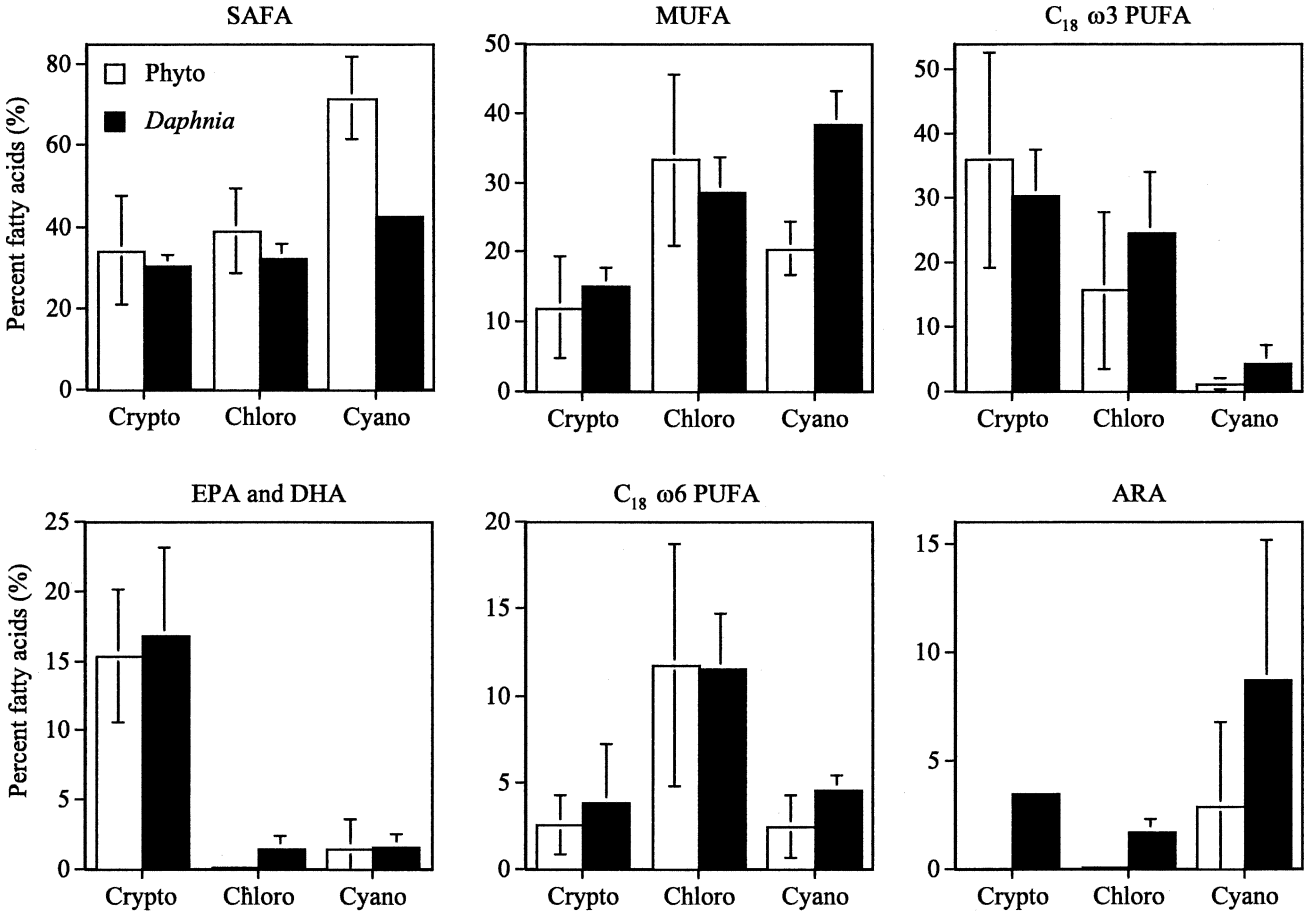

Fig. 2. Percent fatty acid composition for both the phytoplankton and the *Daphnia* that consumed these phytoplankton. See Methods for a list of the phytoplankton taxa used for these experiments. The error bars represent ± 1 SD. Refer to Table 1 for abbreviations.

and chlorophyte diets was significant at the $p < 0.05$ level as determined by a Dunnett *t*-test. All other treatment growth and reproductive rate comparisons were significantly different at the $p < 0.0001$ level.

Comparisons of phytoplankton and *Daphnia* fatty acid composition (*see* Fig. 2 and Table 3) show that, in general, *Daphnia* had less saturated FAs and more arachidonic acid than did their diets. These comparisons also suggest *Daphnia* consuming cyanobacteria either converted saturated FAs to monounsaturated FAs or preferentially metabolized saturated FAs, either or both of which caused these daphnids to have considerably less saturated FAs and considerably more monounsaturated FAs than their diets. Despite these trends, *Daphnia* fatty acid composition matched that of their diet much more closely than it did the fatty acid composition of *Daphnia* consuming other diets (Fig. 2; Table 3). This was especially the case for C_{18} $\omega 3$ and $\omega 6$ PUFAs, EPA plus DHA, and the ratio of $\omega 3/\omega 6$ FAs, where between 63% and 86% of the total sums of squares could be explained by the phytoplankton taxa factor.

The first three principal components of the rotated PCA explained 41%, 33%, and 22% of the variance, respectively, and 96% of the overall variance (Fig. 3). The first PC was most strongly associated with C_{18} $\omega 3$ PUFAs, SAFAs, and the $\omega 3/\omega 6$ ratio (Table 4). The second PC was most correlated with MUFAs, C_{18} $\omega 3$ PUFAs, EPA plus DHA, and the $\omega 3/\omega 6$ ratio. The third PC was most strongly correlated with C_{18} $\omega 6$ PUFAs. Figure 3 shows the phytoplankton and *Daphnia* fatty acid composition was interdispersed within the cryptophyte or chlorophyte clusters, but that *Daphnia* consuming cyanophytes separated somewhat from their diet along the axes of the second and especially the third PC. Overall, the cryptophytes and *Daphnia* consuming cryptophytes formed a distinct cluster, and there was only slight overlap between the chlorophyte and cyanophyte clusters.

When classifying the data by *Daphnia* versus phytoplankton (to test whether the *Daphnia* and phytoplankton used to feed them had systematically different fatty acid profiles), discriminant analysis correctly classified 94% of the cases when the specific cases being classified were used in the overall classification scheme and 72% of cases when cross validating these groups. Cross validation was done by deriving a classification scheme for all cases other than the case being tested (i.e., a leave-one-out scenario). In this

Table 3. ANOVA results for the eight fatty acid categories considered in this analysis.†

Source	df	MS	*F*-test	*p* Value	% Variance	*r*
			Saturated fatty acids			
Phyto. or *Daphnia*	1	789	11.60	0.0052	18.1	0.52*
Diet	2	1089	16.01	0.0004	50.1	
Interaction	2	284	4.18	0.0419	13.1	
Error	12	68			18.8	
			Monounsaturated fatty acids			
Phyto. or *Daphnia*	1	135	2.93	0.1126	6.1	0.05
Diet	2	559	12.18	0.0013	51.0	
Interaction	2	195	4.25	0.0402	17.8	
Error	12	46			25.1	
			C18 ω3 PUFAs			
Phyto. or *Daphnia*	1	21	0.22	0.6511	0.5	0.45*
Diet	2	1398	14.51	0.0006	67.6	
Interaction	2	82	0.85	0.4525	3.9	
Error	12	96			28.0	
			EPA and DHA			
Phyto. or *Daphnia*	1	4	0.35	0.5674	0.4	0.93**
Diet	2	449	39.03	0.0001	86.2	
Interaction	2	1	0.07	0.9293	0.2	
Error	12	11			13.3	
			C18 ω6 PUFAs			
Phyto. or *Daphnia*	1	4.9	0.38	0.5509	1.1	0.32
Diet	2	138.9	10.78	0.0021	62.9	
Interaction	2	2.0	0.16	0.8566	0.9	
Error	12	12.9			35.0	
			Arachidonic acid			
Phyto. or *Daphnia*	1	59.8	6.14	0.0291	21.9	0.94**
Diet	2	41.4	4.25	0.0403	30.3	
Interaction	2	6.6	0.68	0.5255	4.9	
Error	12	9.7			42.9	
			Bacterial fatty acids			
Phyto. or *Daphnia*	1	6.4	0.19	0.6680	1.1	0.52*
Diet	2	93.1	2.83	0.0982	30.9	
Interaction	2	7.5	0.23	0.7989	2.5	
Error	12	32.8			65.5	
			ω3/ω6 FA ratio			
Phyto. or *Daphnia*	1	0.05	0.46	0.5107	0.7	0.52*
Diet	2	2.86	25.06	0.0001	77.9	
Interaction	2	0.10	0.89	0.4346	2.8	
Error	12	0.11			18.6	

† The "Phyto. or *Daphnia*" factor represents systematic differences between the fatty acid composition of *Daphnia* and their diets. The "diet" factor represents similarity between the fatty acid composition of *Daphnia* and their diets. Percent variance is the percent of the total sum of squares explained by that term. The *r* value is the coefficient of determination for a comparison of phytoplankton and *Daphnia* fatty acid composition for the nine phytoplankton taxa considered. Refer to Table 1 for abbreviations.

* $p < 0.05$; ** $p < 0.01$.

analysis, ARA and SAFA showed the greatest differences between the *Daphnia* and their diets, however, the ARA data were not actually used for the classification because they were not normally distributed. When classifying the data by phytoplankton groups (crypto, chloro, and cyano) and lumping the *Daphnia* with their food (i.e., to test whether the phytoplankton groups had different fatty acid profiles and the *Daphnia* had similar fatty acid profiles compared to their diets), discriminant analysis correctly classified 100% of the cases when the specific cases being classified were used in the classification and 89% of cases when cross validating these groups. In this case, the cross validation error occurred between the chlorophyte and cyanobacteria groups. For this classification, the fatty acid categories EPA plus DHA, the $\omega 3/\omega 6$ ratio, C_{18} $\omega 6$ PUFAs, and C_{18} $\omega 3$ PUFAs were the most important for detecting differences between the different phytoplankton taxa as well as similarities between *Daphnia* and their diets.

To identify the FAs that have the most potential to serve as dietary trophic markers for *Daphnia*, the *Daphnia* fatty acid composition was compared to that of their diet for the eight fatty acid categories (Table 3). This showed that with the exception of MUFAs and C_{18} $\omega 6$ PUFAs, the fatty acid composition of the phytoplankton was significantly corre-

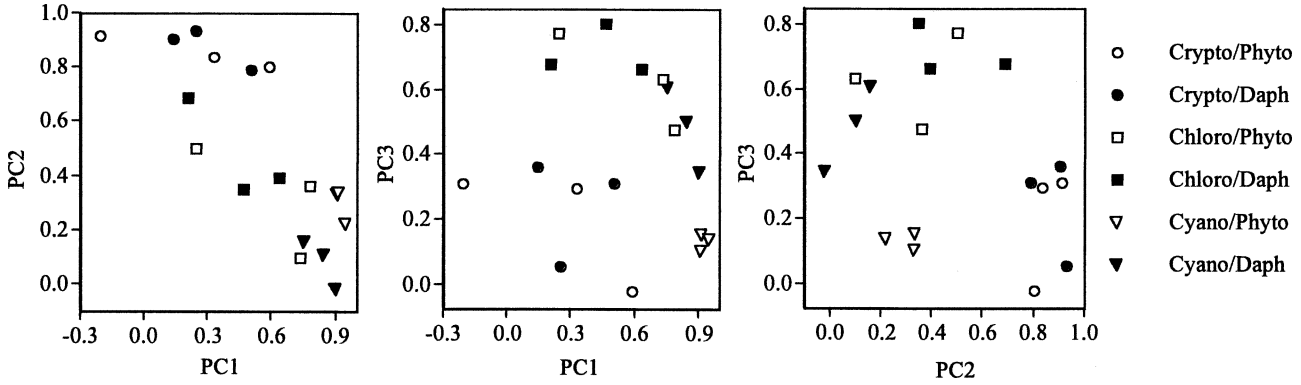

Fig. 3. Bivariate plots of phytoplankton and *Daphnia* fatty acid composition for the first principle component (PC1) versus PC2, PC1 versus PC3, and PC2 versus PC3. The first PC of the rotated PCA explained 41% of the variance and was most strongly associated with C_{18} ω3 PUFAs, SAFAs, and the ω3/ω6 ratio. The second PC explained 33% of the variance and was most correlated with MUFAs, C_{18} ω3 PUFAs, EPA plus DHA, and the ω3/ω6 ratio. The third PC explained 22% of the variance and was most strongly correlated with C_{18} ω6 PUFAs. Crypto/Phyto represents the fatty acid composition of the cryptophytes used as food in these experiments, and Cyano/Daph represents the fatty acid composition of *Daphnia* fed cyanobacteria.

lated with that of the *Daphnia* consuming them. In fact, for EPA plus DHA and ARA, the correlation between the fatty acid composition of *Daphnia* and their diets was quite striking ($r^2 \approx 0.93$) (Table 3). We combined the results for these two groups of PUFAs into a single figure (Fig. 4), which showed that in an absolute sense *Daphnia* had, on average, 4% more EPA, DHA, and ARA than did their diets but that the slope of this relationship was not significantly different from 1 (t-value = 0.78, n = 9).

Discussion

The results of this study have two important implications. First, they suggest the previously established benefit of having EFA-rich phytoplankton at the base of aquatic food webs (Müller-Navarra 1995; Brett and Müller-Navarra 1997) will be transferred efficiently via zooplankton to higher trophic levels. Second, the fact that the percentages of most major fatty acid groups were moderately correlated ($r^2 \approx 0.5$) and EPA, DHA, and ARA were strongly correlated ($r^2 \approx 0.9$) when comparing the fatty acid composition of *Daphnia* to that of their diets suggests zooplankton fatty acid analyses may be a powerful complement to more traditional stable isotope analyses when trying to infer trophic relationships in aquatic food webs. The fact that the sum of EPA, DHA, and ARA in *Daphnia* compared to their diets had a slope nearly identical to 1 and an intercept equal to 4% suggests these physiologically critical FAs were strongly conserved and, to some extent, enriched in *Daphnia*. This result also suggests *Daphnia* ω3 and ω6 highly unsaturated FAs have great potential to serve as dietary trophic markers.

Knowing the extent to which organisms modify their own FA profiles compared to those of their diets is essential when using the FATM approach to infer trophic relationships (Dalsgaard et al. 2003). Although the FA composition of *Daphnia* was quite similar to that of their diets, they did have systematically less saturated FAs and more ARA than did their diets. In addition, the fatty acid profiles of *Daphnia* consuming cyanobacteria indicated they converted saturated FAs to monounsaturated FAs. These systematic shifts in zooplankton FA composition probably vary substantially by zooplankton group (i.e., cladocerans, copepods, rotifers, ciliates, etc.) (Napolitano 1999; Dalsgaard et al. 2003; Persson and Vrede 2005) and taxa-specific FA composition shifts should be studied for more freshwater zooplankton groups before our results are extrapolated to other freshwater zooplankton.

Our observation that *Daphnia* HUFA content has the most potential to serve as a dietary trophic marker is supported by von Elert's (2002) observation that when *Daphnia galeata* were fed diets artificially enriched with EPA and DHA these FAs were enriched in their FA profiles. Similarly, Becker and Boersma (2005) observed a direct relationship between *Daphnia magna* EPA content and that of their diet when *S. obliquus* was supplemented with EPA. Follow-up experiments should examine the impact of diet on *Daphnia* FA composition when food concentrations are not saturating because research carried out on marine copepods and euphasids has shown food limitation can modify the FA profiles laid down in marine zooplankton (Dalsgaard et al. 2003).

Table 4. Correlation coefficients (r) between the three principal components and the eight fatty acid categories used to generate them. Refer to Table 1 for abbreviations.

	PC1	PC2	PC3
SAFAs	0.79	−0.48	−0.48
MUFAs	0.51	−0.82	0.65
C_{18} ω3 PUFAs	−0.98	0.86	0.08
EPA plus DHA	−0.46	0.78	−0.59
C_{18} ω6 PUFAs	−0.20	−0.14	0.80
Arachidonic acid	0.38	−0.45	−0.10
Bacteria FAs	0.59	−0.46	−0.30
ω3/ω6 FA ratio	−0.74	0.87	−0.24

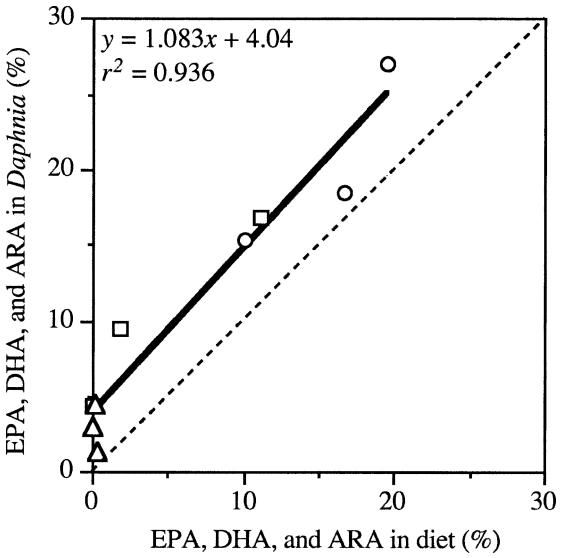

Fig. 4. A bivariate plot of *Daphnia* $\omega 3$ and $\omega 6$ highly unsaturated fatty acid composition (i.e., EPA, DHA, and ARA) against that of their diet. The dashed line represents a perfect one-to-one relationship. The cryptophyte treatments are represented by open circles, the chlorophyte treatments by open squares, and the cyanophyte treatments by open triangles.

The fact that *Daphnia* always had more arachidonic acid than their diets suggests they converted LIN and GLA to ARA and/or they preferentially accumulate this FA. This is consistent with Kainz et al.'s (2004) observation that of all the FAs assessed, macrozooplankton were most enriched with ARA relative to lake seston. Furthermore, the fact that *Daphnia* were enriched in ARA relative to their diets in these experiments intriguingly suggests arachidonic acid may be more important nutritionally for *Daphnia* than previously appreciated. If this is the case, it would also suggest cyanobacteria can, in small quantities, play a positive role in *Daphnia* nutrition (provided they are non-toxic and ingestible) because the cyanobacteria used here consistently had more ARA than either cryptophytes or chlorophytes. However, in this context it is worth noting that when Ravet et al. (2003) added small amounts of the same cyanobacteria (i.e., 20% of total biomass) to cryptophyte taxa mixtures they did not notice improved growth or reproduction.

These results also have important implications for aquatic food web interactions and especially zooplanktivorous fish nutrition because they suggest the nutritional benefits of consuming EFA-rich phytoplankton will transfer up through the food web. Considerable research on fish physiology from the aquaculture literature has demonstrated the critical roles that EPA, DHA in particular, and more recently ARA, play in fish nutrition, especially for juvenile fish (Adams 1999; Olsen 1999; Sargent et al. 1999; Izquierdo et al. 2000). Our results show the content of these critical FAs in *Daphnia* was strongly correlated with that of their diet, indicating these critical EFAs are highly conserved. Further, because the total proportion of these FAs in *Daphnia* was on average 4% higher in an absolute sense than in their diet these results also indicate these critical FAs were enriched via bioconversion from related C_{18} $\omega 3$ and $\omega 6$ precursors such as ALA and SDA for EPA and DHA, and LIN and GLA for ARA. Alternatively, this result could indicate other FAs were preferentially catabolized. The fact that HUFAs were enriched is particularly noteworthy because EPA, DHA, and ARA are known to be the most physiologically important EFAs for invertebrates (Stanley-Samuelson 1994). Similarly, our results also suggest the ratio of $\omega 3$ to $\omega 6$ FAs in *Daphnia* is strongly dependent on diet and ranges greatly from approximately 10 : 1 in *Daphnia* which consumed cryptophytes to only 2 : 1 or 1 : 1, respectively, in *Daphnia* that consumed chlorophytes or cyanophytes. These differences in the relative availability of $\omega 3$ and $\omega 6$ FAs may be especially important for larval and juvenile fish survival and growth (Sargent et al. 1999; Izquierdo et al. 2000). Ultimately these results also have implications for human health because dietary EFAs are known to influence a very wide range of human health outcome measures (Simopoulos 1999), and fish-derived EFAs (the quantitatively most important EFA source in human diets) originate from phytoplankton at the base of aquatic food webs (Arts et al. 2001).

Although FA bioaccumulation was not measured per se, the results are consistent with Kainz et al.'s (2004) finding that EPA and ARA were the most strongly accumulated (or retained) FAs when comparing the FA composition of seston to that of the macrozooplankton in the lakes they studied. Kainz et al. (2004) concluded EFA "transfer efficiency to higher trophic levels will likely vary with the size and taxonomic composition of planktonic organisms and perhaps the trophic status of the lake." Our results suggest the seston or phytoplankton taxonomic composition of zooplankton diets may have an especially important impact on fatty acid trophic transfer in aquatic food webs. Furthermore, because Müller-Navarra et al. (2004) showed the EFA composition of lake seston varies strongly with trophic state, our results also suggest EFA transfer efficiency may be strongly related to lake trophic status, with greatly reduced transfer efficiency in more phosphorus rich lakes. This is consistent with Ahlgren et al.'s (1996) finding that fish collected from oligotrophic lakes had a different lipid and FA composition compared to the same species collected from an eutrophic lake. The results of Ballantyne et al. (2003), and more recently Persson and Vrede (2006), also suggest different zooplankton taxa (especially cladocerans and copepods) may have quite different EFA composition. In fact, the taxonomic composition of the phytoplankton at the base of the food web and of the zooplankton consuming them may have a much stronger impact on EFA trophic transfer to higher trophic levels than zooplankton community size structure per se.

Overall, the results of this study indicate that analyses of *Daphnia* FA composition may be useful for inferring their diet composition and that the most nutritionally important EFAs in phytoplankton are transferred efficiently across the plant–animal interface in planktonic food webs. The next step in this research area is to show that the FA composition of freshwater zooplankton matches that of

their diet when consuming wild phytoplankton assemblages.

References

Adams, S. M. 1999. Ecological role of lipids in the health and success of fish populations, p. 132–160. *In* M. T. Arts and B. C. Wainman [eds.], Lipids in freshwater ecosystems. Springer-Verlag.

Ahlgren, G., I.-B. Gustafsson, and M. Boberg. 1992. Fatty acid content and chemical composition of freshwater microalgae. J. Phycol. **28:** 37–50.

———, L. Lundstedt, M. T. Brett, and C. Forsberg. 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. J. Plankton Res. **12:** 809–818.

———, L. Sonesten, M. Boberg, and I.-B. Gustafsson. 1996. Fatty acid content of some freshwater fish in lakes of different trophic levels—a bottom-up effect? Ecol. Freshw. Fish **5:** 15–27.

Arts, M. T., R. G. Ackman, and B. J. Holub. 2001. "Essential fatty acids" in aquatic ecosystems: A crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. **58:** 122–137.

Ballantyne, A. P., M. T. Brett, and D. E. Schindler. 2003. The importance of dietary phosphorus and highly unsaturated fatty acids for sockeye (*Oncorhynchus nerka*) growth in Lake Washington—a bioenergetics approach. Can. J. Fish. Aquat. Sci. **60:** 12–22.

Becker, C., and M. Boersma. 2005. Differential effects of phosphorus and fatty acids on *Daphnia* growth and reproduction. Limnol. Oceanogr. **50:** 388–397.

Brett, M. T., and D. C. Müller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic food-web processes. Freshw. Biol. **38:** 483–499.

Dalsgaard, J., M. St. John, G. Kattner, D. C. Müller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine food environment. Adv. Mar. Biol. **46:** 226–340.

Desvilettes, C., G. Bourdier, C. Amblard, and B. Barth. 1997. Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. Freshw. Biol. **38:** 629–637.

Falk-PetersenS., W. Hagen, G. Kattner, A. Clarke, and J. R. Sargent. 2000. Lipids, trophic relationships and biodiversity in Arctic and Antarctic Krill. Can. J. Fish. Aquat. Sci. **57:** 178–191.

Fraser, A. J., J. R. Sargent, J. C. Gamble, and D. D. Seaton. 1989. Formation and transfer of fatty-acids in an enclosed marine food-chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L) larvae. Mar. Chem. **27:** 1–18.

Gatten, R. R., J. R. Sargent, and J. C. Gamble. 1983. Diet-induced changes in fatty-acid composition of herring larvae reared in enclosed ecosystems. J. Mar. Bio. Assoc. UK **63:** 575–584.

Gillan, F. T., R. B. Johns, T. V. Verheyen, J. K. Volkman, and H. J. Bavor. 1981. Transmonounsaturated fatty acids in marine bacterial isolates. Appl. Environm. Microbiol. **41:** 849–856.

Graeve, M., W. Hagen, and G. Kattner. 1994. Herbivorous or omnivorous? On the significance of lipid composition as trophic markers in the Antarctic copepods. Deep-Sea Res. **41:** 915–924.

———, G. Kattner, and D. Piepenburg. 1997. Lipids in Arctic benthos: Does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biol. **18:** 53–61.

Heintz, R. A., B. D. Nelson, J. Hudson, M. Larsen, L. Holland, and M. Wipfli. 2004. Marine subsidies in freshwater: Effects of salmon carcasses on lipid class and fatty acid composition of juvenile coho salmon. Trans. Am. Fish. Soc. **133:** 559–567.

Izquierdo, M. S., J. Socorro, L. Arantzamendi, and C. M. Hernandez-Cruz. 2000. Recent advances in lipid nutrition in fish larvae. Fish Physiol. Biochem. **22:** 97–107.

Kainz, M., M. T. Arts, and A. Mazumder. 2004. Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnol. Oceanogr. **49:** 1784–1793.

Kattner, G., and H. S. G. Fricke. 1986. Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J. Chromat. **361:** 263–268.

———, and W. Hagen. 1995. Polar herbivorous copepods—different pathways in lipid biosynthesis. ICES J. Mar. Sci. **52:** 329–335.

Lindström, K. 1983. Selenium as a growth factor for plankton algae in laboratory experiments and in some Swedish lakes. Hydrobiologia **101:** 35–48.

Müller-Navarra, D. C. 1995. Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. Arch. Hydrobiol. **132:** 297–307.

———, M. T. Brett, S.-K. Park, S. Chandra, A. P. Ballantyne, E. Zorita, and C. R. Goldman. 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature **427:** 69–72.

Murata, N., H. Wada, and Z. Gombos. 1992. Modes of fatty-acid desaturation in cyanobacteria. Plant Cell Physiol. **33:** 933–941.

Napolitano, G. E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems, p. 21–44. *In* M. T. Arts and B. C. Wainman [eds.], Lipids in freshwater ecosystems. Springer-Verlag.

Olsen, Y. 1999. Lipids and essential fatty acids in aquatic food webs: What can freshwater ecologists learn from mariculture?, p. 161–202. *In* M. T. Arts and B. C. Wainman [eds.], Lipids in freshwater ecosystems. Springer-Verlag.

Parker, P. L., C. Van Baalen, and L. Maurer. 1967. Fatty acids in eleven species of blue-green algae: Geochemical significance. Science **155:** 707–708.

Perry, G. J., J. K. Volkman, R. B. Johns, and H. J. Bavor, Jr. 1979. Fatty acids of bacterial origin in contemporary marine sediments. Geochim. Cosmochim. Ac. **43:** 1715–1725.

Persson, J., and T. Vrede. 2006. Polyunsaturated fatty acids in zooplankton: Variation due to taxonomy and trophic position. Freshw. Biol. **51:** 887–900.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. **18:** 293–320.

Pohl, P., and F. Zurheide. 1979. Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors, p. 65–80. *In* H. A. Hoppe and T. Levring [eds.], Marine algae in pharmaceutical science. Walter de Gruyter.

Ravet, J. L., M. T. Brett, and D. C. Müller-Navarra. 2003. A test of the role of polyunsaturated fatty acids in phytoplankton food quality for *Daphnia* using liposome supplementation. Limnol. Oceanogr. **48:** 1938–1947.

Richman, M. B. 1986. Rotation of principal components. J. Climatol. **6:** 293–335.

Sargent, J., G. Bell, L. McEvoy, D. Tocher, and A. Estevez. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture **177:** 191–199.

Sargent, J. R., and R. J. Henderson. 1986. Lipids, p. 59–108. *In* E. D. S. Corner and S. C. M. O'Hara [eds.], The biological chemistry of marine copepods. Clarendon Press.

Simopoulos, A. P. 1999. Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr **70:** 560S–569S.

Stanley-Samuelson, D. W. 1994. Prostaglandins and related eicosanoids in insects. Adv. Insect Physiol. **24:** 115–212.

Vance, D. E. 1996. Glycerolipid biosynthesis in eukaryotes, p. 153–181. *In* D. E. Vance and J. E. Vance [eds.], Biochemistry of lipids, lipoproteins and membranes. Elsevier Science.

von Elert, E. 2002. Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. Limnol. Oceanogr. **47:** 1764–1773.

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