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Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in *Daphnia*

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Zooplankton transfer ecologically important fatty acids (FA) from their diets to upper trophic levels. We used diet-switching experiments with ^{13}C-labeled food sources to determine the time scale at which dietary uptake is manifested in the FA profiles of *Daphnia magna*. *Daphnia* dramatically shifted their FA composition in response to diet change within only four days, however *Daphnia* switched from a high quality (i.e. *Cryptomonas*) to a moderate quality (*Scenedesmus*) diet retained the most physiologically important FA from their original diet source even after 14 days. In particular, *Daphnia* exhibited long-term retention of eicosapentaenoic (EPA; 20:5ω3) and arachidonic acid (ARA; 20:4ω6) when switched from *Cryptomonas* to *Scenedesmus*. Similarly, when switched from *Scenedesmus* to *Cryptomonas*, *Daphnia* took up a high proportion of EPA and ARA after only two days. The phospholipid fatty acid (PLFA) fraction in *Daphnia* was preferentially enriched with stearic (18:0), oleic (18:1ω9), and linoleic acid (LIN; 18:2ω6). In contrast with studies of marine copepods, dietary FA also strongly affected the PLFA composition (structural lipids) of *Daphnia*. Results of $\delta^{13}C$ signatures of individual FA provided evidence of elongation and desaturation of α-linolenic (ALA; 18:3ω3) or stearidonic acid (SDA; 18:4ω3) to EPA 10 days after a diet switch to EPA-deficient *Scenedesmus*. Differences in the ARA content of *Daphnia* fed *Cryptomonas* and *Scenedesmus* suggest *Daphnia* consuming *Cryptomonas* synthesized ARA via retroconversion of ω6-docosapentaenoic acid (ω6-DPA; 22:5ω6). *Daphnia* preferentially accumulate and retain, as well as bioconvert, those FA that are also most physiologically important for fish production. Our results also indicate *Daphnia* FA composition responds to their diet on a short temporal scale and analyses of lipid biomarkers in zooplankton provide strong insights into the food sources that support their production.

Understanding which food sources support the production of any particular organism or trophic level in a food web is one of the most important challenges in ecological studies. For large organisms, it is often possible to determine what they have consumed by examining their gut contents, but such analyses have well known biases towards less easily digested organisms or structures (Iverson et al. 2004). For smaller organisms, such as many zooplankton, gut content analyses may be impractical if not impossible. Even if an unbiased estimate of the food consumed is obtained, this does not indicate which food resources make the most important contribution to an organism's somatic tissue production. Therefore to gain insights into predator-prey relationships and the flow of materials through food-webs, one can use diet specific biomarkers. An ideal biomarker would be unique to each diet source, easily identified, metabolically stable and non-harmful to the organism (Dalsgaard et al. 2003), however, all of these conditions are seldom meet simultaneously. The fatty acid (FA) composition of zooplankton has been shown to meet several of these conditions much of the time and therefore has considerable potential as a tool to disentangle trophic relationships and energetic pathways in marine and freshwater food webs (Dalsgaard et al. 2003, Brett et al. 2009b).

The need for diet specific biomarkers is urgent because prospective dietary items (e.g. terrestrial detritus, bacteria, phytoplankton and protozoa) can have very similar stable isotope signatures making it difficult or impossible to separate different diet sources when using the classic stable isotope approach (Pace et al. 2004). However, the major marine and freshwater phytoplankton taxa have very different FA profiles and there are a wide variety of taxa-specific FA biomarker molecules (Volkman et al. 1989, Ahlgren et al. 1992, Viso and Marty 1993). When compared with the stable isotope approach, the FA biomarker approach has the disadvantage that some FA are not always transferred from the diet to the consumer in a conservative fashion. For example, FA molecules can be synthesized de novo (Lee et al. 2006), or elongated, desaturated and/or retroconverted from one FA molecule to another (Dalsgaard et al. 2003, Brett et al. 2009b). However, when dietary target FA are readily available it is generally assumed that many aquatic consumers acquire dietary FA profiles (Dalsgaard et al. 2003).

Fatty acids are already used qualitatively as biomarkers in zooplankton, fishes, seabirds, and marine mammals (Dalsgaard et al. 2003, Kainz and Mazumder 2005, Richoux and Froneman 2008). However, there can be considerable variation in FA metabolism among organisms, tissues, lipid classes and FA groups. Before FA biomarkers are widely adopted for food-web assessments, many of the questions regarding organism FA metabolism will need to be resolved. For example, it is currently not known how long it takes for zooplankton to turnover their FA pools (i.e. what is the time-scale of the information stored in their lipids) (Graeve et al. 2005), how different lipid pools (i.e. neutral storage lipids and polar structural lipids) are influenced by diet, and whether particular FA molecules are more likely to be retained in zooplankton diets or alternatively synthesized from other FA (Brett et al. 2009b).

Freshwater zooplankton FA studies mostly focus on total lipids whereas marine zooplankton studies usually separate lipids into neutral lipid FA (NLFA) and polar phospholipids FA (PLFA). Previous research has indicated that non-polar storage lipids (especially triacylglycerols) reflect diet better than do membrane PLFA (Lee et al. 1971). In fact, several recent studies have concluded the FA profiles of freshwater zooplankton are not correlated with their seston diets (Persson and Vrede 2006, Smyntek et al. 2008). However, these studies are contradicted by laboratory studies of the cladoceran *Daphnia* spp. (Brett et al. 2006, 2009a, Burns et al. 2011), the cladoceran *Ceriodaphnia* and the copepod *Boeckella* (Burns et al. 2011), as well as a field study of the copepod *Diaptomus* (Ravet et al. 2010).

Understanding how and at what temporal scale the FA composition of zooplankton responds to their diets is especially important because a subset of these molecules (i.e. the highly unsaturated FA: eicosapentaenoic acid; EPA, arachidonic acid; ARA and docosahexaenoic acid; DHA) are known to be particularly important for fish physiology (Sargent et al. 1999, Tocher 2003). Furthermore, field research has shown that this group of FA is highly retained at the seston-zooplankton interface in freshwater food webs, with both cladocerans and copepods retaining ARA, cladocerans retaining EPA and copepods retaining DHA (Kainz et al. 2004, Ravet et al. 2010). Because these molecules are also important for zooplankton growth and reproduction, they have the potential to enhance zooplankton production and improve the nutritional quality of zooplankton for fish, thereby doubly enhancing fisheries production (Brett et al. 2006).

Because FA biomarkers appear to have considerable potential as dietary tracers, the first objective of this study was to determine the time-scale of the dietary information stored in *Daphnia* FA. That is, does the FA profile of zooplankton reflect the food they ingested during the last day or alternatively the last month? Because *Daphnia* have fast somatic growth rates, we hypothesize their FA will indicate food consumed within the last week (Brett et al. 2006). Secondly, this study tested how the structural phospholipid FA (PLFA) differed in their composition, dietary relationships, and turnover times compared to total lipids in *Daphnia*, with the hypothesis being the polar lipid fraction (i.e. membrane lipids) should be less dependent on diet and thus more conserved when diets are changed (Brett et al. 2009b). Finally, we tested the hypothesis that polyunsaturated FA (PUFA), the most important FA for *Daphnia* nutritional ecology, turnover at different rates than physiologically less critical FA, e.g. saturated and monounsaturated FA (SAFA and MUFA, respectively). We hypothesize that when *Daphnia* are switched from a high to a lower quality diet, they will preferentially retain the most physiologically important FA from their initial diet and rapidly replace less important FA. The objectives of this study were addressed by feeding *Daphnia* phytoplankton diets which differed greatly in their FA composition. Juvenile *Daphnia* were initially fed monoculture diets comprised of *Scenedesmus* or *Cryptomonas* for seven days and then switched to ^{13}C-labeled diets (e.g. from unlabeled *Cryptomonas* to labeled *Scendesmus*) for an additional 14 days.

Methods

Zooplankton and phytoplankton cultures

All experiments were conducted using a clone of *Daphnia magna* maintained on *Scenedesmus obliquus*. The phytoplankters *Scenedesmus* (obtained from the Max Planck Inst. for Limnology, Plön, Germany) and *Cryptomonas ozolinii* LP 2782 (obtained from the UTEX Culture Collection) were cultivated using the synthetic growth medium L16 (Lindström 1983) supplemented with B-vitamins. Both phytoplankton species were grown in an environmental chamber with a constant temperature (18°C) and light:dark cycle (14 h:10 h). To achieve a target ^{13}C-enrichment of 30‰, 3% of the $NaHCO_3$ in the L16 growth media was replaced with $NaH^{13}CO_3$ when culturing labeled *Scenedesmus* and *Cryptomonas*.

Experimental design

Approximately 6-h old *D. magna* juveniles were used for these experiments, and juveniles from specific adults were divided equally between the diet treatments to minimize maternal affects (Brett 1993). Twenty juveniles were placed as a group into 500 ml glass jars with 300 ml media and phytoplankton, and 15 groups were started for both treatments. Fresh food (at 5 mg dry wt l^{-1}) was provided to the *Daphnia* daily, and the experiment was carried out in a dark room to prevent phytoplankton growth. After seven days, *Daphnia* fed the initial *Cryptomonas* diet were switched to a ^{13}C-labeled *Scenedesmus* diet, and *Daphnia* initially fed *Scenedesmus* were switched to labeled *Cryptomonas* (see Graeve et al. 2005 for a similar experimental design). The experiments were conducted at 19 ± 1°C. *Daphnia magna* samples for fatty acid and FA ^{13}C determinations were taken before the diet switch (i.e. day zero) as well as 2, 4, 6, 10 and 14 days after the switch to ^{13}C-enriched diets. These *Daphnia* were placed into 1.5 ml Eppendorf vials, freeze-dried and stored at –80°C.

Fatty acid analyses

Lipids from freeze dried phytoplankton and zooplankton samples (2–6 mg) were extracted with a 4:2:1 chloroform:methanol:water mixture (Parrish 1999), and after sonicating and vortexing three times, the organic phases were removed and pooled. Phospholipids (PL) were separated

from total lipids using a Sep-Pak Light Silica cartridge (6cc; WAT023537) separation method. First, the resin of these cartridges was conditioned using methanol and chloroform (both 5 ml). Subsequently, total lipids (1 ml) were applied onto the resin, rinsed using methanol and then washed under vacuum using chloroform and a chloroform/methanol mixture (49:1). PL were collected after final resin washes using methanol. Toluene (1 ml) and a sulphuric acid–methanol solution (2 ml; 1% v/v) was added to the evaporated lipid extracts to form fatty acid methyl esters (FAME); samples were then vortexed and stored for 16 h at 50°C. Subsequently, $KHCO_2$ (2 ml, 2% v/v) and BHT (5 ml) were added, shaken and CO_2 released. After centrifugation, the top layer was removed and BHT (5 ml) was added again. CO_2 was released and after centrifugation the top layer was removed once again. The solution containing FAME was dried under N_2 and re-dissolved in hexane. The FAME were analyzed using a gas chromatograph (detector: FID 260°C, carrier gas: H_2:40 ml min^{-1}, N_2: 45 ml min^{-1}, air: 450 ml min^{-1}, temp. ramp: 140°C (5 min) – 4°C/min-240°C (20 min) = 50 min) equipped with a temperature-programmable injector and an autosampler. A Supelco SP-2560 column (100 m, 25 mm inner ø, 0.2 μm film thickness) was used for FAME separation. For calculation and, if necessary, manual resetting of the chromatograms we used Excalibur 1.4. Fatty acid concentrations were calculated using calibration curves based on known standard concentrations (i.e. 2.5, 50, 100, 250, 500, 1000 and 2000 ng μl^{-1} solutions of the FAME standard mixture, $r^2 = 0.996$, $n = 196$).

The $\delta^{13}C$ values of individual FA were determined using a GC-C connected to an isotope ratio mass spectrometer in the stable isotope laboratory at the Dept of Chemical Ecology and Ecosystem Research, Faculty of Life Sciences, Univ. of Vienna. Fatty acids were separated using a 30 m Agilent DB-23 column (0.25 mm × 0.15 μm) and then oxidized to carbon dioxide in an oxidation reactor at a temperature of 940°C with the reduction reactor kept at 630°C. The injector temperature was kept at 250°C with the splitless mode used for FA injections. The temperature program of the GC column started at 70°C for 1.5 min, after which the temperature was raised by 30°C min^{-1} to 150°C, and then by 4°C min^{-1} to 230°C and held at that temperature for 15 min. The total run time was 39.12 min. The samples were run against a mixture of internal standards that contained saturated fatty acids, linoleic acid, as well as C_{16} and C_{18} MUFA. These standards were first run with an EA-SIRMS and then calculated for every GC-C-SIRMS sample run. This standard mix was used for drift and linear corrections. For linear correction, three different concentrations of the standards were run after which a correction equation was calculated. For this standard mix, the calculated precision was 0.6‰ and the accuracy was 0.3‰. The calculated accuracy of these samples was 0.9‰, and the precision also 0.6‰. Only peaks with a total height of 50 mV at mass 44 were counted. The $\delta^{13}C$ values of the individual FA were manually calculated using background values. After drift and linear correction individual $\delta^{13}C$ FA values were corrected for the methanol used with the following equation: $\delta^{13}C_{FA}$ (corrected value) = $\delta^{13}C_{FAME} \times (N + 1 - \delta^{13}C_{methanol}/N$, where N is the number of carbon atoms in the individual fatty acid molecules and $\delta^{13}C_{FAME}$ is the initial $\delta^{13}C$ value of the fatty acid methyl ester.

Data analysis

We used linear regression analysis between the proportional FA composition of the *Daphnia* and their diets to quantify their similarity to their diets, and t-tests to identify those FA that were different between *Daphnia* and their diets, or were different between the total (TFA) and phospholipid (PLFA) fractions for the same diet, or to identify those FA that were most characteristic for a particular diet. All data for these statistical analyses were arcsine (square root)-transformed.

We calculated the proportion of *Daphnia* FA in the food treatments after diet switching that derived from the original and new food sources by comparing the actual *Daphnia* FA profiles in the 'switched' samples to hypothetical *Daphnia* FA profiles obtained by 'mixing' the actual *Daphnia* FA profiles obtained for the two initial diets to find the proportion of the initial diet that maximized the fit between the observed and predicted profiles. Specifically, we used the following algorithm: predicted switched diet FA profile = X*(initial FA profile) + (1 – X)*(subsequent FA profile), where X equals the hypothetical proportion of the original dietary FA retained by *Daphnia*. We then used the Solver function in Excel to find the value of X that maximized the correlation coefficient (*r)* between the observed and predicted FA profiles. For example, if *Daphnia* was switched from an initial diet of *Cryptomonas* to a subsequent diet of *Scenedesmus*, then the FA profiles of the *Daphnia* that only consumed *Cryptomonas* were used to represent the initial condition and the FA profiles of *Daphnia* that only consumed *Scenedesmus* were used to represent the subsequent condition. These mixing models generally resulted in very strong fits (i.e. $r^2 \approx 0.94$) between the observed and hypothetical FA profiles.

Results

Algae fatty acid composition

Cryptomonas ozolinii and *Scenedesmus obliquus* differed greatly in their FA composition (Table 1, Fig. 1). *Cryptomonas* had considerably higher proportions of stearic acid (18:0) and the PUFAs stearidonic acid (SDA) and EPA. *Cryptomonas* also had 2–3% (of TFA) ω6-docosapentaenoic acid (ω6-DPA) and DHA, whereas these FA were not found in *Scenedesmus*. *Scenedesmus* had a considerably higher relative contribution of 16:4ω3, linoleic acid (LIN) and α-linolenic acid (ALA) than *Cryptomonas*, and ≈ 4% more 18:1ω9 and 18:1ω7 in proportional terms.

Daphnia fatty acid composition

Daphnia fed *Cryptomonas* during the initial and asymptotic periods of these experiments had very different FA composition than *Daphnia* fed *Scenedesmus* (Table 1, Fig. 1). Specifically, *Daphnia* fed the *Cryptomonas* diet had considerably more EPA, 18:0, and SDA. *Daphnia* consuming *Cryptomonas* also had a 2–3% greater ARA content in proportional

Table 1. Fatty acid composition (%) of total lipids (total FA) and phospholipids (PLFA) in the primary producers *Cryptomonas ozolinii* and *Scendesmus obliquus* and the herbivorous consumer *Daphnia magna*. Initial: fatty acids composition of *D. magna* fed on either *C. ozolinii* or *S. obliquus* for 7 d. Asymptotic: fatty acid composition of *D. magna* 6–14 d after the diet switch. All values presented are the mean ± 1 SD. 14:0 = myristic acid; 16:0 = palmitic acid; 17:0 = margaric acid; 18:0 = stearic acid; 16:1ω7 = palmitoleic acid; 18:1ω9 = oleic acid; 18:1ω7 = *cis*-vaccenic acid; 18:2ω6 = linoleic acid (LIN); 18:3ω6 = γ-linolenic acid; 20:4ω6 = arachidonic acid (ARA); 22:5ω6 = ω6-docosapentaenoic acid; 16:4ω3 = hexadecatetraenoic acid; 18:3ω3 = α-linolenic acid (ALA); 18:4ω3 = stearidonic acid (SDA); 20:5ω3 = eicosapentaenoic acid (EPA); 22:5ω3 = docosahexaenoic acid (DHA).

Sample ID Food source Fraction sample size	Diet Crypto. Tot. Fas 2	Diet Scene. Tot. Fas 2	Initial Crypto. Tot. Fas 2	Initial Crypto. PLFAs 2	Initial Scene. Tot. FAs 2	Initial Scene. PLFAs 2	Asymptote Crypto. Tot. Fas 6	Asymptote Crypto. PLFAs 5	Asymptote Scene. Tot. Fas 6	Asymptote Scene. PLFAs 5
Saturated fatty acids										
14:0	1.2 ± 0.1	0.45 ± 0.0	1.0 ± 0.0	0.1 ± 0.1	0.9 ± 0.0	0.1 ± 0.1	0.8 ± 0.8	0.4 ± 0.4	1.2 ± 0.1	0.2 ± 0.1
16:0	17.1 ± 0.1	18.0 ± 0.2	15.8 ± 0.4	15.5 ± 0.3	14.3 ± 0.2	15.0 ± 0.9	17.0 ± 1.3	16.6 ± 2.0	16.5 ± 1.2	17.6 ± 0.4
17:0	0	0	1.0 ± 0.0	1.4 ± 0.1	0	0.5 ± 0.0	0.8 ± 0.4	1.2 ± 0.1	0.1 ± 0.2	0.4 ± 0.0
18:0	9.6 ± 0.4	3.6 ± 0.3	12.7 ± 1.1	17.3 ± 1.1	6.4 ± 0.6	13.7 ± 2.2	10.4 ± 3.3	16.5 ± 3.7	5.5 ± 2.1	8.2 ± 1.3
Monounsaturated fatty acids										
16:lω7	0	0.4 ± 0.0	1.4 ± 0.3	0.4 ± 0.1	1.1 ± 0.0	0.2 ± 0.1	1.0 ± 0.5	0.4 ± 0.2	1.2 ± 0.7	1.5 ± 0.6
18:lω9	3.3 ± 0.0	7.3 ± 0.1	7.6 ± 1.0	14.3 ± 3.7	8.4 ± 0.0	14.9 ± 0.3	12.7 ± 3.5	17.0 ± 1.7	12.8 ± 2.1	18.8 ± 0.7
18:lω7	0.6 ± 0.1	4.7 ± 0.2	3.7 ± 0.1	4.5 ± 0.6	4.4 ± 0.1	3.0 ± 0.3	3.8 ± 0.5	4.4 ± 0.2	4.8 ± 0.5	3.7 ± 0.4
ω6 Polyunstaturated fatty acids										
18:2ω6	3.3 ± 0.0	15.1 ± 0.2	4.6 ± 0.2	5.6 ± 0.2	10.6 ± 0.0	14.3 ± 0.5	6.1 ± 0.5	6.3 ± 0.6	12.1 ± 0.4	20.4 ± 2.1
18:3ω6	0	1.2 ± 0.0	0	0.1 ± 0.2	0.9 ± 0.0	0.7 ± 0.0	0	0.2 ± 0.1	1.3 ± 0.2	0.6 ± 0.2
20:4ω6	0	0	3.5 ± 0.0	4.0 ± 0.9	0	0.8 ± 0.2	3.2 ± 0.4	3.8 ± 0.4	1.1 ± 0.6	1.5 ± 0.5
22:5ω6	3.0 ± 0.0	0	0	0	0	0	0	0	0	0
ω3 Polyunstaturated fatty acids										
16:4ω3	0	15.3 ± 0.2	0.8 ± 0.1	0	12.5 ± 0.3	0.6 ± 0.0	0.5 ± 0.4	0	6.9 ± 2.5	0.1 ± 0.1
18:3ω3	21.1 ± 0.2	30.0 ± 0.3	15.6 ± 1.0	12.3 ± 1.7	37.6 ± 0.5	34.1 ± 2.0	15.0 ± 2.0	11.8 ± 2.0	29.4 ± 1.7	17.6 ± 0.4
18:4ω3	27.9 ± 0.5	4.1 ± 0.1	13.7 ± 0.8	5.8 ± 0.7	2.8 ± 0.1	1.7 ± 0.1	12.6 ± 4.7	5.2 ± 1.5	5.5 ± 1.1	2.7 ± 0.4
20:5ω3	13.5 ± 0.0	0	18.5 ± 0.2	18.6 ± 2.3	0	0.4 ± 0.1	16.2 ± 0.9	16.3 ± 2.2	1.6 ± 0.4	4.9 ± 1.4
22:6ω3	2.3 ± 0.2	0	0	0	0	0	0	0	0	1.7 ± 1.1
Sums										
SAFA	27.9 ± 0.4	21.9 ± 0.5	30.5 ± 1.5	34.2 ± 1.3	21.6 ± 0.8	29.2 ± 3.1	29.0 ± 3.4	34.7 ± 4.8	23.3 ± 3.1	26.4 ± 1.6
MUFA	4.0 ± 0.1	12.4 ± 0.2	12.7 ± 0.8	19.3 ± 4.4	13.9 ± 0.1	18.1 ± 0.8	17.5 ± 3.5	21.8 ± 1.9	18.8 ± 2.0	24.0 ± 1.0
ω6 PUFA	6.3 ± 0.0	16.3 ± 0.2	8.1 ± 0.2	9.8 ± 0.9	11.6 ± 0.1	15.8 ± 0.2	9.2 ± 0.9	10.3 ± 0.9	14.6 ± 0.4	22.5 ± 1.7
ω3 PUFA	64.8 ± 0.5	34.0 ± 0.4	47.9 ± 2.0	36.7 ± 4.7	40.4 ± 0.6	36.3 ± 2.0	43.8 ± 7.3	33.2 ± 5.0	36.5 ± 2.6	27.0 ± 3.1
ω3:ω6 ratio	10.4 ± 0.2	2.1 ± 0.0	5.9 ± 0.1	3.7 ± 0.1	3.5 ± 0.0	2.3 ± 0.1	4.8 ± 1.2	3.2 ± 0.3	2.5 ± 0.2	1.2 ± 0.2

terms (i.e. their diet contained no ARA, but 2–3% of their TFA and PLFA was ARA). *Daphnia* fed *Scenedesmus* had considerably more ALA, 16:4ω3, and LIN. Across the entire FA profile, the FA composition of *Cryptomonas* was strongly correlated with TFA of *Daphnia* that consumed *Cryptomonas* ($r^2 = 0.73$, $p < 0.001$), and moderately correlated with the PLFA ($r^2 = 0.49$, $p < 0.01$) of these *Daphnia*. The FA content of *Scendesmus* was very strongly correlated with the TFA of *Daphnia* that consumed *Scenedesmus* ($r^2 = 0.95$, $p < 0.0001$) and strongly correlated with the PLFA composition ($r^2 = 0.74$, $p < 0.001$). The TFA composition of *Daphnia* on both diets was strongly correlated with the PLFA composition of *Daphnia* on the corresponding diets ($r^2 \approx 0.80$, $p < 0.001$).

Consistent with a very strong dietary impact on *Daphnia* FA composition, there were few systematic differences between the relative FA composition of the *Daphnia* and phytoplankton. These modest differences ($p < 0.05$) included *Daphnia* had on average 1.1% and 3.0% more 16:1ω7 and 18:0, respectively, in relative terms and 2.5% less 16:0. However, within either monospecific diet, there were much more pronounced differences between the FA of the phytoplankton and *Daphnia*. In most cases, the *Daphnia* had less of whatever FA the phytoplankton had the most of. For example, *Daphnia* fed *Cryptomonas* had a ω3:ω6 ratio that was 43% less than their diet, whereas *Daphnia* that consumed *Scenedesmus* had a ω3:ω6 ratio that was 67% greater than their diet ($p < 0.05$). *Daphnia* fed *Scenedesmus* had less 16:0, 16:4ω3, and LIN than their diet. However, in contrast to this pattern, *Daphnia* fed *Scenedesmus* had more ALA than their diet despite the fact that ALA was the most prevalent FA in *Scenedesmus*. *Daphnia* fed *Cryptomonas* had much less SDA, ALA, ω6-DPA, and DHA, yet more LIN and ARA than their diet ($p < 0.05$). Despite the fact that EPA was the most characteristic FA in *Cryptomonas*, *Daphnia* fed this diet actually had a greater proportion of EPA.

PLFA versus total FA composition of *Daphnia*

We compared the TFA and PLFA composition of *Daphnia* for both the initial diet samples (i.e. immediately prior to the diet switch) as well as for the asymptotic period (i.e. days 6–14) after the diet switch (Fig. 1). In general, the PLFA lipid fraction for *Daphnia* contained significantly ($p < 0.01$) more 18:0 and 18:1ω9, and significantly less ALA than did the FA of the total lipid pool. For the asymptotic period, which had a larger sample size and greater statistical power, LIN was also significantly ($p = 0.02$) more prevalent in the PLFA, and 16:4ω3 and SDA were significantly ($p < 0.01$)

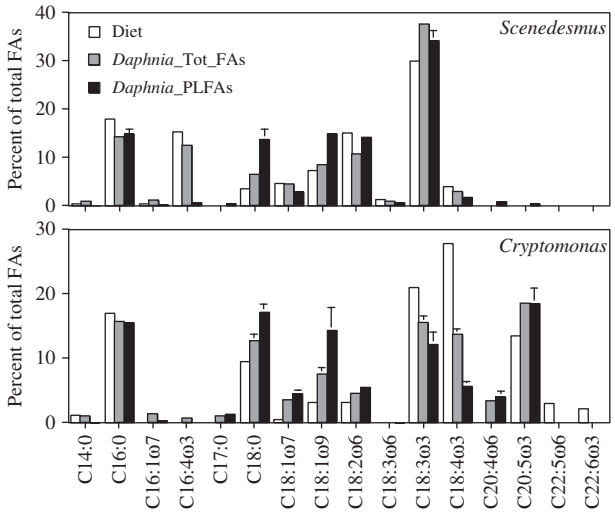

Figure 1. The FA composition of the experimental diets, and the *Daphnia* total and phospholipids FA during the initial period. The values presented are the mean ± 1 SD.

less prevalent. Overall, the PLFA had systematically lower ω3:ω6 ratios than the corresponding TFA ($p < 0.01$).

Daphnia fatty acid turnover time

In both diet-switching scenarios, *Daphnia* adjusted their fatty acid composition to their new diets within only a few days (Fig. 2). On average for both diet scenarios and lipid fractions, 57 ± 10% of the FA reflected the new diet after only two days and by day 6 the *Daphnia* FA composition in both treatments reached asymptotic values. When switching from a moderate food quality *Scenedesmus* diet to a very high quality *Cryptomonas* diet *Daphnia* almost entirely replaced their original FA with new FA from the second phase diets

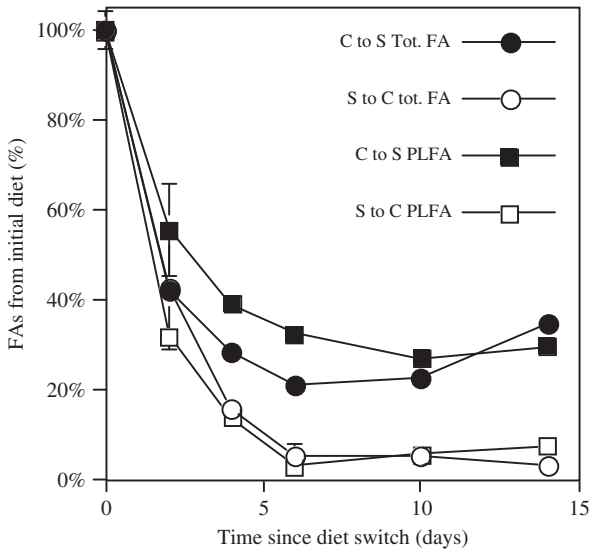

Figure 2. Shift in *Daphnia* FA composition from the initial to the asymptotic condition for the two diet scenarios and the two lipid fractions assessed (mean ± 1 SD). The values were obtained from a mixing model that obtained the best fit between the observed FA profile and a hypothetical mixed profile derived using end members from the initial diets (see Methods). S = *Scenedesmus*, C = *Cryptomonas.*

(i.e. during days 6–14 only 5 ± 2% of *Daphnia* FA appeared to originate for their initial diets). Conversely, when *Daphnia* were switched from *Cryptomonas* to *Scenedesmus*, our calculations suggest 28 ± 5% of their FA derived from their initial diet during the asymptotic period. In particular, the TFA of *Daphnia* in this treatment had on average significantly less 16:4ω3 and ALA, and significantly more ($p < 0.01$) 18:1ω9, LIN, SDA, ARA and EPA than expected compared to *Daphnia* fed *Scenedesmus* during the initial period. Most of these differences are consistent with the expected impact of a *Cryptomonas* diet on the FA composition of *Daphnia*, suggesting some extended carryover of FA from their initial diet, but the higher than expected proportions of LIN was counter to this explanation.

We found that the PLFA were on average 48 ± 11% of the TFA. Using this value and lipid partitioning calculations, we found only 26 ± 10% of SDA and 38 ± 11% of ALA was found in the PLFA fraction in both diet scenarios. The PUFA 16:4ω3 was only found when the *Daphnia* consumed *Scenedesmus*, and even then this FA constituted <5% of the PLFA fraction. In contrast, 75 ± 13% of the oleic acid (18:1ω9) and 79 ± 9% of the stearic acid (18:0) was in the PLFA fraction regardless of diet scenario. There was no difference in lipid partitioning for the ω6 FAs LIN and ARA between treatments, i.e. 60 ± 11% of the LIN and 74 ± 11% of the ARA was found in the PLFA fraction during the asymptotic stage in both diet scenarios. However, the calculations for EPA indicate that during the asymptotic stage, nearly all of the EPA in the *Cryptomonas* to *Scenedesmus* scenario was found in the PLFA fraction, and 58 ± 11% of EPA was found in the PLFA fraction in the *Scenedesmus* to *Crptomonas* scenario.

Of the most prevalent FA, the SAFA 16:0 had similar prevalence in the two diet types (i.e. 17–18% of total FA), as well as similar prevalence in the *Daphnia* that consumed these phytoplankton (i.e. 14–16% of TFA and PLFA) (Table 1), and therefore did not show a response to the diet switching treatments. The SAFA 18:0 was more prevalent in *Cryptomonas* than *Scenedesmus*, as well as more prevalent in the *Daphnia* that consumed *Cryptomonas* (Table 1). This SAFA also showed a modest response to diet switching, with an increasing trend when switched from *Scenedesmus* to *Cryptomonas*. The MUFA 18:1ω9 was twice as prevalent in the *Scenedesmus* as in *Cryptomonas*, however, this FA had nearly identical proportions in the FA of *Daphnia* consuming either diet. Accordingly, this FA did not show a response to the diet switch, but instead showed an increasing trend with *Daphnia* age, irrespective of diet. The MUFA 18:1ω7 constituted 4–5% of *Scenedesmus* FA and only 0.6% of *Cryptomonas* FA, but this FA accounted from 3–5% of *Daphnia* TFA and PLFA regardless of diet. As previously noted, both 18:0 and 18:1ω9 were considerably more prevalent in the PLFA than the TFA of *Daphnia* in all scenarios (Table 1, Fig. 1).

In contrast to the SAFA and MUFA, all of the major ω3 and ω6 PUFA in *Daphnia* responded rapidly to the diet switch in both the TFA and PLFA fractions. Asymptotic levels were achieved by day 2 for 16:4ω3, SDA and ALA; by day 4 for EPA and ARA; and by day 6 for LIN (Fig. 3). When *Daphnia* were switched from *Cryptomonas* to *Scenedesmus*, their 16:4ω3, LIN and ALA content increased sharply. Conversely, when *Daphnia* were switched from *Scenedesmus* to *Cryptomonas* their composition of SDA, ARA and EPA

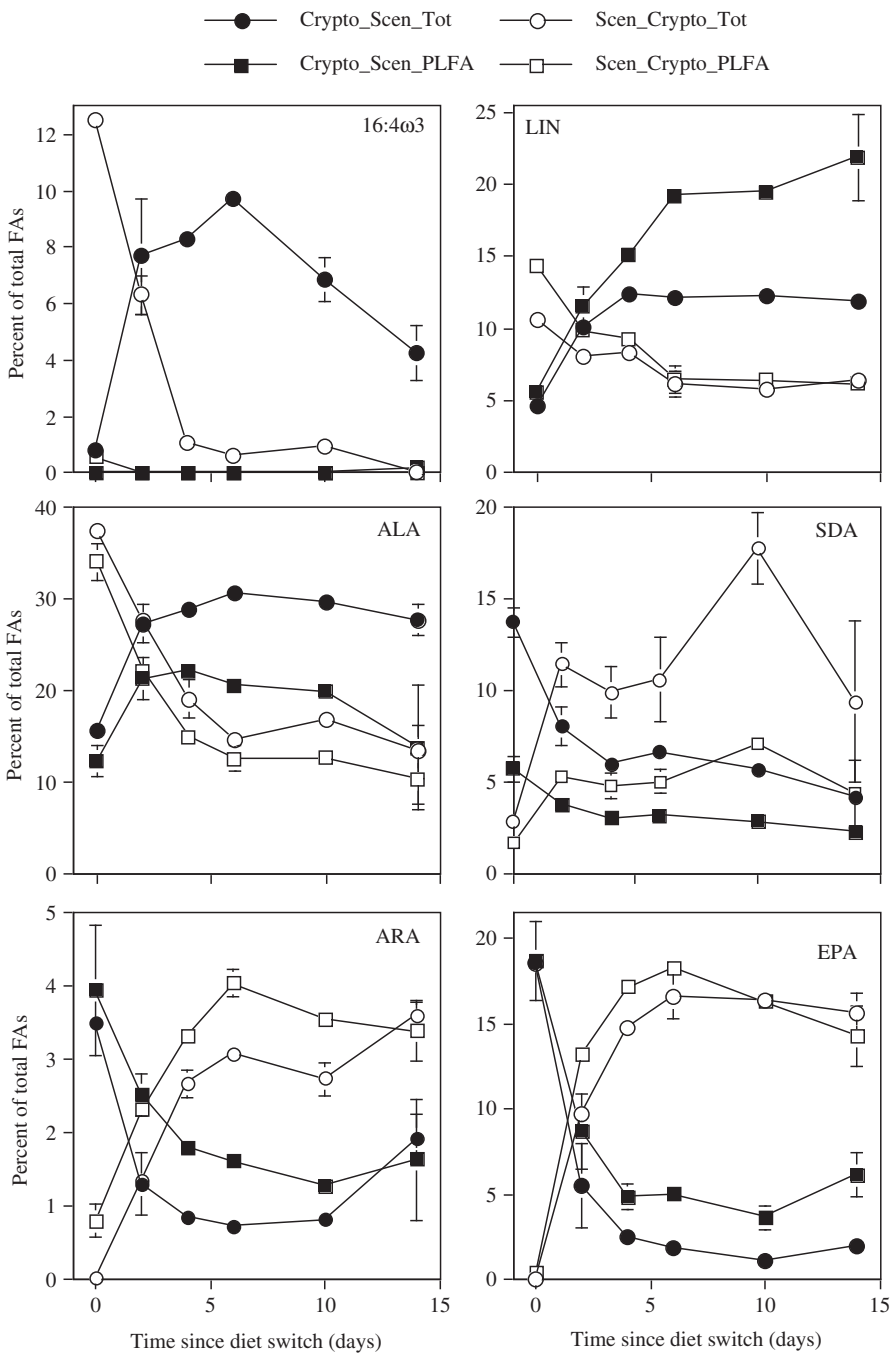

Figure 3. The shift in the lipid composition of *Daphnia* for individual FA for the two diet scenarios and the two lipid fractions. The values presented were for the major FA (Table 1), which showed the strongest dietary responses (mean ± 1 SD).

increased dramatically (Fig. 3). As expected, based on the fact that 16:4ω3 only occurred in *Scenedesmus* and EPA only occurred in *Cryptomonas*, these two FA were the most definitive biomarkers for these phytoplankters in *Daphnia*. The time series responses provide evidence for strong preferential retention for LIN and ALA (when switched to dietary *Scenedesmus*) and SDA, ARA, and EPA (when switched to dietary *Cryptomonas*; Fig. 3).

Fatty acid stable carbon isotope signatures

Fatty acid ^{13}C-enrichment after diet switching was seen for the key PUFA in the TFA and PLFA fractions after only two days and this enrichment was maintained through the remainder of this experiment (Fig. 4). The δ^{13}C value of those FA, which the initial diet did not contain (e.g. 16:4ω3 for *Cryptomonas* and EPA for *Scenedesmus*), was highly enriched immediately after the diet switch. This enrichment was clearly evident in the δ^{13}C values of 16:4ω3 (not shown), ALA and SDA in both lipid fractions two days after the diet switch to labeled *Scenedesmus* (Fig. 4). The very rapid replacement of unlabeled ALA and SDA with labeled FA when *Daphnia* were switched to a *Scenedesmus* diet suggests these FA may have been preferentially catabolized. Conversely, when *Daphnia* were fed labeled *Scenedesmus* the δ^{13}C value of EPA (which was not present in *Scenedesmus*) only became enriched after

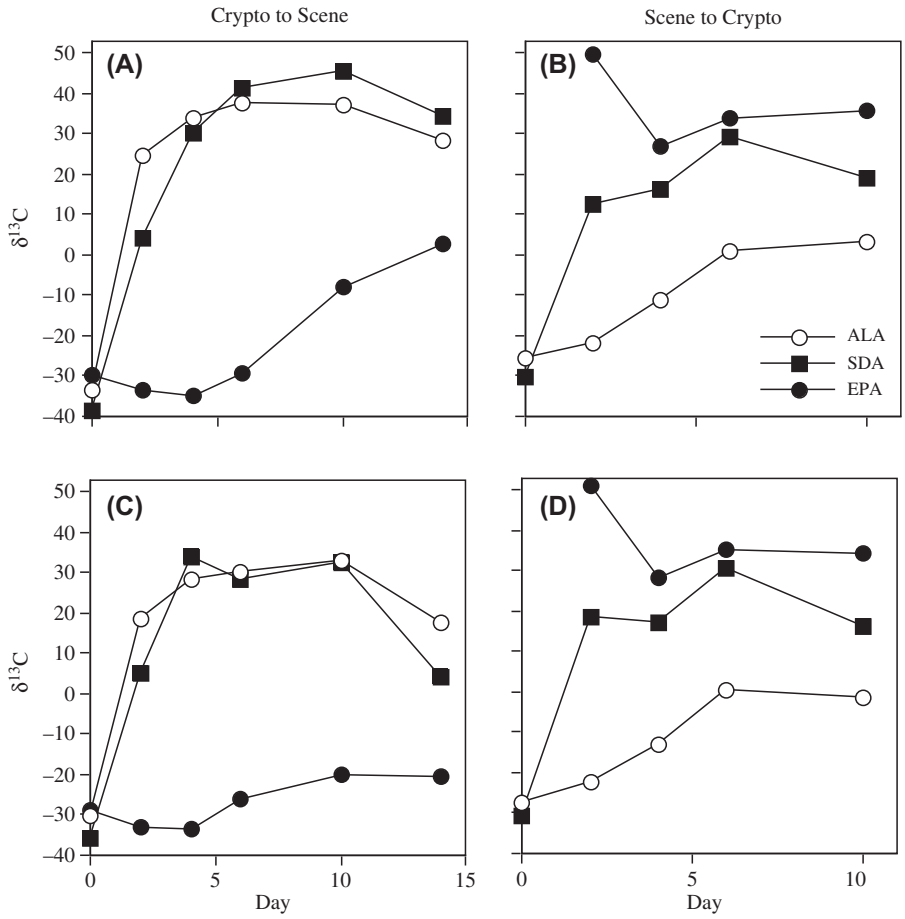

Figure 4. Changes in the δ^{13}C-ratios for the dominant ω-3 PUFA (i.e. ALA, SDA, and EPA) within *Daphnia* during the course of this experiment. (A) depicts the TFA results for the *Cryptomonas* to *Scenedesmus* scenario, (B) depicts the TFA results for the *Scenedesmus* to *Cryptomonas* to scenario, (C) depicts the PLFA results for the *Cryptomonas* to *Scenedesmus* scenario, and (D) depicts the PLFA results for the *Scenedesmus* to *Cryptomonas* scenario. We ran out of labeled *Cryptomonas* before day 14 of this experiment.

ten days in the TFA and the PLFA were only slightly enriched (Fig. 4). This suggests these *Daphnia* did not begin synthesizing EPA, from either ALA or SDA, until their EPA dropped to low levels (Fig. 3) and most of this recently synthesized EPA was found in the storage lipids. Conversely, the most enriched δ^{13}C values for both the TFA and PLFA fractions were for the EPA in *Daphnia* fed ^{13}C-labeled *Cryptomonas*. These results suggest EPA is aggressively retained when not present in the diet and rapidly taken up when it is.

Discussion

Our experiments demonstrate that only two days after diet switching, *Daphnia* replaced more than 50% of their FA and after only six days they reached new stable TFA and PLFA profiles which strongly expressed the FA composition of their new diets. Dietary uptake of the newly supplied diet was further proven by isotopic ^{13}C enrichment of the TFA and PLFA in *Daphnia*. These results strongly confirm the use of FA biomarkers as a tool in food-web studies for the herbivorous consumer *Daphnia*. When the diet was switched from high to moderate quality, *Daphnia* strongly preferentially retained EPA and ARA. The δ^{13}C results indicate that EPA was synthesized via elongation and desaturation of ALA and/or SDA. Comparisons of the ARA content of *Daphnia* fed *Scenedesmus* and *Cryptomonas* suggests *Daphnia* synthesized ARA via retroconversion of ω6-DPA present *Cryptomonas*. Our results suggest lipid transfer across the plant–animal interface in aquatic food webs can be extremely plastic. For this reason, strong dietary signals can transfer up from primary producers through zooplankton to fish in pelagic food webs (Danielsdottir et al. 2007).

Knowing the temporal scale for material transfer from primary producers to secondary consumers via key herbivores such as *Daphnia* in aquatic food webs is essential for our understanding of how quickly changes in the species composition and biomass of primary producers affect pelagic food webs. Our results suggest the energy and lipids contained in cladocerans preyed upon by zooplanktivorous fish in lakes would in many cases have originated from carbon fixed by phytoplankton within the last week. Conversely, studies of boreal marine calanoid copepods and euphausiids (Lee et al. 2006) suggest they have the capacity to store large lipids reserves derived from phytoplankton consumed six or more months prior. In fact, some marine zooplankton are known to store lipid reserves from one vernal phytoplankton bloom for subsequent use to support recruitment prior to the following year's bloom (Lee et al. 2006). Conversely, *Daphnia* are prone to boom-bust cycles (McCauley et al.

1999), causing them to in some cases release large juvenile cohorts at inopportune times such as the spring clear-water phase (Sommer et al. 1986). Based on these r versus K strategies, we suggest food webs with lipid and non-lipid storing zooplankton should function in a completely different manner. A short time scale for essential biochemical and energy transfer across the phyto–zooplankton interface would make food web interactions prone to boom-bust cycles, whereas zooplankton lipid storage could considerably dampen ecosystem oscillations.

The rapid FA uptake and retention as TFA and PLFA strongly suggests that newly acquired dietary FA reflect the FA profile of its diet source. Our studies showed that the *Daphnia* FA profile response was similar for the TFA and PLFA fractions and confirms that *Daphnia* PLFA can also be used to indicate dietary lipid sources of zooplankton (Taipale et al. 2009). These results suggest an absence of dietary impacts on PLFA composition may not be as widespread as previously thought (Lee et al. 1971, Brett et al. 2009b). However, our results also suggest the TFA have greater utility as dietary biomarkers because a broader array of producer specific FA can be detected in total lipids and because the statistical association between diet and *Daphnia* TFA composition is stronger. In general, our results confirm the value of phytoplankton biomarkers for *Daphnia*, especially ω3 and ω6 FA (Brett et al. 2009b).

There were notable differences in the relative abundance of certain FA between the TFA and PLFA fractions of *D. magna*, with the SAFA 18:0, the MUFA 18:1ω9, and the PUFA LIN significantly more prevalent in the PLFA lipid fraction. The SAFA 16:0 was prevalent in the PLFA, but this FA was equally important in the TFA fraction, suggesting that this SAFA is not preferentially retained in one specific lipid class. Depending on the diet, ALA and EPA could also be prevalent, although ALA was significantly less prevalent in the PLFA. Furthermore, the PUFA 16:4ω3 and SDA were much less prevalent in the PLFA than the TFA fraction. By comparison, the PLFA of marine calanoid copepods are dominated by DHA (≈ 35%), 16:0 (≈ 25%), and EPA (≈ 20%) (Scott et al. 2002), and not influenced by diet (Lee et al. 1971). Overall, these results indicate freshwater *Daphnia* have a very different, and much more diverse and flexible PLFA composition than do marine copepods.

The FA profiles of *Daphnia* showed a more complex response when the diet was switched from high to moderate food quality algae. Physiologically this indicates that *Daphnia* preferentially retain those FA that are most integral to their membranes and nutrition. Specifically *Daphnia* retained EPA and ARA already in their membranes when switched to a lower quality diet lacking these PUFA, and/or synthesized these molecules when physiologically required. The ability to convert the precursor ω3 PUFA ALA and SDA to EPA and DHA varies greatly from one organism to another (Kanazawa et al. 1979), and may determine competitive outcomes between different animals when essential fatty acid availability is limiting. Our results showed that *Daphnia* not only retained, but also bioconverted EPA from other molecules, such as ALA or SDA, as demonstrated by the initially very low and then subsequently increasing ^{13}C values of EPA when *Daphnia* were fed labeled *Scenedesmus*.

Arachidonic acid seems to be a key PUFA for *Daphnia* or zooplankton in general. Although its physiological role is not yet clearly understood, there is evidence that ARA is critical for zooplankton membranes as this ω6 PUFA is highly retained in fasting (Schlechtriem et al. 2006) and also feeding (Kainz et al. 2004) zooplankton. Because ARA is synthesized by very few phytoplankton, zooplankton need to bioconvert this molecule by elongating and desaturating precursors such as LIN or alternatively by retro-converting ω6-DPA (Hastings et al. 2001). Although not one of the original hypotheses proposed for this study, we expected *Daphnia* fed *Scenedesmus* would have much more ARA because this alga had ≈ 4.5 times more LIN than does *Cryptomonas* (Table 1). Unexpectedly, our results actually showed *Daphnia* fed *Cryptomonas* contained considerably more ARA than those fed *Scenedesmus* (i.e. 3.5% vs 0%, respectively, in the TFA, and 4.0% vs 0.8% in the PLFA). We suspected this unexpected outcome might be because our *Cryptomonas* contained ω6-DPA, which was subsequently confirmed via GC-MS (C. C. Parrish pers. comm.) at 3% of TFA. Thus our results suggest *Daphnia* preferentially retroconvert ARA from ω6-DPA as opposed to elongating and desaturating this molecule from shorter precursors. These results also tentatively suggest cryptophytes, which generally have very high ω3:ω6 ratios and no ARA (Brett et al. 2009b), are a much better source for ARA than are green algae due to the presence of ω6-DPA at 1–5% of TFA in six cryptophyte species (Taipale unpubl.).

Food web FA biomarker studies are promising because they offer insights into the source of consumer lipids in general and more importantly the origin of those FA that are most nutritionally critical (Richoux and Froneman 2008). For zooplankton and fish production broadly, the most critical FA are the ω3 PUFA EPA and DHA, and the ω6 PUFA ARA and perhaps ω6-DPA as well (Brett and Müller-Navarra 1997, Sargent et al. 1999, Parrish et al. 2007). Past research has shown cladocerans do not retain DHA even when their diet contains this PUFA (Brett et al. 2009b), which our results confirm. Similar to DHA, this study also suggests *Daphnia* do not accumulate ω6-DPA (i.e. 22:5ω6), which is a novel observation, but not unexpected since ω6-DPA is an ω6 analogue to DHA and ω3 and ω6 FA are processed via very similar biosynthetic pathways (Tocher 2003). Our results showed *Daphnia* rapidly acquired and aggressively retained both EPA and ARA, which is consistent with the known physiological importance of EPA for *Daphnia* (Müller-Navarra et al. 2000, Ravet et al. 2003). Although ARA has not yet been shown to be a growth limiting resource for zooplankton, Kainz et al. (2004) showed ARA was the most strongly accumulated FA across the seston–zooplankton interface in a series of Canadian lakes. These results indicate *Daphnia* sequester high quality EPA and ARA for the fish that consume them. Based on the selective retention patterns we observed and the FA composition of the structural PLFA, we suggest the SAFA 18:0, the MUFA 18:1ω9, and the PUFA LIN, ARA and EPA are the fundamental FA 'building-blocks' of *Daphnia* cell membranes.

Our results indicate analyses of zooplankton FA composition hold considerable promise for untangling the energetic basis of secondary production in aquatic food webs. Important questions such as the contribution of terrestrial

(Brett et al. 2009a), bacterial (Ederington et al. 1995, Taipale et al. unpubl.) or even different algae groups (Turner and Rooker 2006, Ravet et al. 2010) can be addressed using this approach. Our results suggest the FA composition of *Daphnia* was strongly modified by diet after only a few days. We also provided evidence that EPA and ARA were synthesized by *Daphnia* from precursor FA in their diets. Finally, our study showed *Daphnia* preferentially accumulate those highly unsaturated FA (i.e. EPA and ARA) that are important for their physiology and the nutrition of upper trophic level fish.

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