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# A low ω-3:ω-6 ratio in *Daphnia* indicates terrestrial resource utilization and poor nutritional condition

**SAMI J. TAIPALE[1]*, MARTIN J. KAINZ[2] AND MICHAEL T. BRETT[3]**

[1]DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCE, UNIVERSITY OF JYVÄSKYLA, PL 35 (YA), JYVÄSKYLÄ 40014, FINLAND, [2]WASSERCLUSTER – BIOLOGICAL STATION LUNZ, DANUBE UNIVERSITY KREMS, DR. CARL KUPELWIESER PROMENADE 5, LUNZ AM SEE A-3293, AUSTRIA AND [3]DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING, UNIVERSITY OF WASHINGTON, BOX 352700, SEATTLE, WA 98195, USA

*CORRESPONDING AUTHOR: samit@u.washington.edu

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It has been hypothesized that terrestrial particulate organic matter (t-POM) makes important contributions to *Daphnia* production in some lakes. We conducted a series of feeding experiments to explore the fatty acid responses in *Daphnia* to diets comprised of different terrestrial resources (i.e. *Alnus rubra*, *Phragmites australis*, *Betula nana* and *Betula pendula*) and mixed diets with terrestrial and phytoplankton (*Scenedesmus* or *Cryptomonas*) resources. When fed 100% phytoplankton, *Daphnia* had very similar ($r^2 > 0.80$) fatty acid profiles to their diets, whereas *Daphnia* that consumed t-POM diets had weak correlations ($r^2 = 0.002–0.56$) with the corresponding diet sources. Unusual 16 carbon chain polyunsaturated fatty acids (16:2ω6, 16:3ω3 and 16:4ω3), linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3) were diagnostic fatty acids for *Scenedesmus* and *Daphnia* that consumed this alga. Stearidonic acid (18:4ω3) and eicosapentaenoic acid (20:5ω3) were diagnostic for *Cryptomonas* and *Daphnia* that consumed this diet. All of the t-POM resources were characterized by a high content of saturated fatty acids (SAFA; 79 ± 12%), especially the diagnostic long-chain SAFA (20:0, 22:0, 24:0, 26:0, 28:0). *Daphnia* that consumed t-POM assimilated very little of these terrestrial biomarkers, but the shorter chain SAFA 16:0 and 18:0 were very prevalent in juvenile and adult *Daphnia* that consumed terrestrial plant matter. The ω-3:ω-6 ratios were distinctive between terrestrial (0.3–1.6) and phytoplankton resources (≈3–15), and this ratio in *Daphnia* was strongly associated with their diets ($r^2 = 0.88$). These results suggest that *Daphnia*, and perhaps zooplankton in general, preferentially retain algae-derived ω3 fatty acids, and low ω-3:ω-6 ratios in *Daphnia* indicate a mainly terrestrial diet or poor nutritional condition.

KEYWORDS: lipids; biomarkers; fatty acids; *Daphnia*

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## INTRODUCTION

There is considerable debate about the extent to which terrestrial carbon subsidies support the production of herbivorous invertebrates and fish in aquatic ecosystems. Several studies have concluded that consumer production in aquatic systems is preferentially supported by phytoplankton primary production (Francis *et al.*, 2011; Kankaala *et al.*, 2013) because terrestrial carbon is a factor of 10× lower in food quality than algae (Brett *et al.*, 2009; Taipale *et al.*, 2014). Furthermore, most of the terrestrial particulate organic matter (t-POM) transported to lakes is much too large for zooplankton to ingest, and transport is extremely episodic with most inputs occurring during a few rain and wind events (Preston *et al.*, 2008). However, other studies have concluded that t-POM, whether directly or via the microbial loop, supports ≈30−70% of consumer production in many oligotrophic lakes (Pace *et al.*, 2004; Jansson *et al.*, 2007; Cole *et al.*, 2011).

Until now, most studies of terrestrial contributions to secondary production in lakes are based on analyses of the carbon, nitrogen and hydrogen stable isotope ratios in zooplankton and particulate organic matter (i.e. seston). These calculations are simple, and work very well in laboratory circumstances when the required estimates for end member stable isotope ratios, e.g. phytoplankton and t-POM, can be easily directly measured. However, it is often impossible to directly measure phytoplankton $\delta^{13}C$ and $\delta^{15}N$ values in natural systems because the phytoplankton are mixed with the terrestrial, detrital, bacterial and protozoan components of the lake seston. Direct determination of stable isotopes of phytoplankton is possible for lakes with monospecific blooms or for large colonial forms (Vuorio *et al.*, 2006). Therefore, a variety of indirect approaches have been employed to estimate the stable isotope values of phytoplankton, but all of these have well-identified limitations (Marty and Planas, 2008). Furthermore, phytoplankton are not one homogenous group, but vary in their biochemical content (Ahlgren *et al.*, 1992; Taipale *et al.*, 2013). Thus, there can also be a high variation in stable isotope values among phytoplankton taxa (Vuorio *et al.*, 2006) because their bulk carbon isotope value is an average $\delta^{13}C$ value of all carbon-containing biomolecules. In an optimal situation, stable isotope values could be directly measured from biomolecules from distinct diet sources.

Fatty acids, sterols, amino acids, nucleic acids and carbohydrates are key biomolecules for all life on earth. For zooplankton, certain dietary polyunsaturated fatty acids (PUFA), such as arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to support optimal somatic growth and reproduction (Brett and Müller-Navarra, 1997). Fish also require these molecules for disease resistance, neural tissue and eye development, pigmentation and reproduction (Sargent *et al.*, 1999). The total lipid, ω-3 fatty acid and sterol content of t-POM is very low compared with phytoplankton (Taipale *et al.*, 2014). This is because t-POM is mainly comprised of recalcitrant lignin cellulose (Lynd *et al.*, 2002) and most perennial plants resorb 50% of the nitrogen and phosphorus from their leaves when they senesce (Arts, 1996).

Daphnids and calanoid and cyclopoid copepods are the main zooplankton taxa in freshwater systems. Copepods feed selectively based in part by taste (DeMott, 1986), and there is no direct evidence that copepods feed directly on t-POM. However, previous laboratory studies (Brett *et al.*, 2009; Wenzel *et al.*, 2012; Taipale *et al.*, 2014) have shown that daphnids, which feed nonselectively (DeMott, 1986), can directly ingest t-POM. Some studies also revealed that *Daphnia* with pure t-POM diets have much lower somatic growth and reproduction compared with *Daphnia* fed phytoplankton (e.g. Brett *et al.*, 2009). Simultaneous measurement of carbon isotopes and fatty acids revealed that *Daphnia* assimilated less fatty acids than other carbon-based biomolecules from t-POM (Taipale *et al.*, 2014).

The fatty acid biomarker approach has been widely applied in marine food web studies and is based on the premise that consumers often acquire fatty acid profiles that are strongly influenced by their diets (Dalsgaard *et al.*, 2003). The PUFA content of consumers also provides important information regarding their nutritional state. Recent research has shown that the fatty acid composition of freshwater zooplankton is very strongly related to that of their diets (Brett *et al.*, 2006; Ravet *et al.*, 2010; Burns *et al.*, 2011; Taipale *et al.*, 2011). *Daphnia* in particular have an extremely plastic fatty acid composition that is more strongly related to their diets compared with conspecifics consuming different resources (Brett *et al.*, 2006). Taipale *et al.* (Taipale *et al.*, 2011) showed that, within 4 days of diet switching, *Daphnia magna* had strongly modified their fatty acid composition and after 6 days 95% of their fatty acids were replaced. Both algal and terrestrial diet sources have a wide range of diagnostic fatty acid biomarkers that can be readily traced, even to particular algal groups (Brett *et al.*, 2009).

In the present study, we explored the utility of using the fatty acid composition of *D. magna* to infer terrestrial and algae resource utilization in more detail than previously studied. The overall objective of this study was to determine the extent to which the consumption of terrestrial resources can be inferred from the fatty acid profiles of *Daphnia*. We tested the hypothesis that, for physiological reasons (i.e. somatic growth and reproduction), herbivorous zooplankton preferentially retain mostly algae-derived

fatty acids, whereas fatty acids from terrestrial particulate diets, rich in long-chain saturated fatty acids (SAFA), are less utilized and thus less retained in consumers. To test this hypothesis, we conducted several feeding experiments to examine the influence of terrestrial resources on *Daphnia* fatty acid profiles by feeding them defined algal and terrestrial diets and determining their lipid composition. Moreover, we studied the terrestrial fatty acid fingerprints in *Daphnia magna* by feeding them $^{13}$C-labeled *Scenedesmus gracilis* and then switching their diets to terrestrial sources to test how quickly the terrestrial signal would manifest in *Daphnia*. Finally, we used fatty acid-based mixing models to calculate the relative contributions of terrestrial and phytoplankton basal resources to *Daphnia* lipids and also determined which fatty acid molecules were the most promising biomarkers for allochthonous and autochthonous resources in *Daphnia*.

## METHOD

### Experiment 1: effect of gradual increase of t-POM supply on *Daphnia* fatty acids

Life table experiments were used to calculate the relative contributions of dietary terrestrial and phytoplankton resources to *Daphnia*. These experiments started with ≈6-h-old neonates that were isolated from five separate broods (i.e. different females of *D. magna*) to avoid monoclonal bias and distributed evenly with 12 replicates (one *Daphnia* in 40 mL) in each of the six diet treatments. *Daphnia* were fed a gradient of red alder t-POM and *Cryptomonas* diets varying by 20% increments, i.e. 100% t-POM; 80:20 t-POM and *Cryptomonas* etc. This experiment was carried out in the dark (to prevent algal growth) in 40-mL vials at 19 ± 0.5°C and lasted 14 days, with survival and reproduction monitored daily. Identical daily food rations were offered to each treatment, but these were sequentially increased from 5 to 14 mg dry weight (DW) food $L^{-1}$ $day^{-1}$ during the experiment as the *Daphnia* grew and grazed increasing amounts of food. Food suspensions were prepared and replaced daily. During this experiment, 71 of the 72 *Daphnia* survived. Linear regression analysis was used to explore how dietary fatty acids in these resources predicted the fatty acid variability in the consumers.

### Experiment 2: effect of terrestrial and algal diet mixtures on *Daphnia* fatty acids

In this experiment, we tested how 100% t-POM (red alder), a mix of 50% t-POM and 50% of different phytoplankton (*Cryptomonas* or *Scenedesmus*), and 100% of each of these phytoplankton affected the diet specific fatty acid retention in *Daphnia*. This experiment was carried out at 19 ± 0.5°C in the dark in 2-L Erlenmeyer flasks filled with 1.5-L synthetic growth medium (L16) and initiated with a *D. magna* population (equivalent to 7.7 ± 3.0 mg DW $L^{-1}$) with four replicates. These *Daphnia* were fed consistent ratios of either 100% red alder t-POM, *Cryptomonas* and *Scenedesmus* or 50:50% mixtures of t-POM and the two algae. Every 2 days for 28 days a 300-mL sample was taken from these flasks for *Daphnia* enumeration. This 10% harvest rate $day^{-1}$ corresponds to the maximum sustainable yield for this clone of *D. magna* when fed moderate food quality phytoplankton (Brett *et al.*, 2009). After the *Daphnia* samples were collected, fresh food was added to these flasks at an amount equivalent to 3.2 ± 0.8 mg DW $L^{-1}$ $day^{-1}$. *Daphnia* samples were collected for 28 days for the 100% phytoplankton and the mixed t-POM and phytoplankton treatments, but only for 22 days for the 100% t-POM treatment because the populations in this treatment were near collapse. The growth efficiency was calculated by taking the mean daily yield of *Daphnia* during the second half of the experiment, to minimize the influence of initial conditions, and dividing this yield by the amount of food added to these bottles (DW to DW).

The methods we used to culture phytoplankton, prepare milled t-POM, determine sample fatty acid composition, estimate *Daphnia* mass and production were described previously in Brett *et al.* (Brett *et al.* 2009). We used principal component (PC) analysis (PCA) and nonmetric dimensional scaling (nMDS), in SPSS Version 11.0.4 for MAC, to assess the differences in the fatty acid composition of the various diets and *Daphnia* consuming these diets in both experiments. These multivariate statistical tests used arcsine square root transformed data, and the PCA used a covariance matrix and Varimax rotation. Those fatty acids that were encountered at greater than 0.5% of total fatty acids in any of our samples were used to classify these data. The significant PCs obtained from the PCA were then correlated against the original fatty acid data to obtain loadings. We also used a series of two-factor ANOVAs to test whether the phytoplankton type or presence/absence of t-POM in the batch experiment had the greatest influence on *Daphnia* fatty acids.

### Experiment 3: effect of diet switching on *Daphnia* fatty acids

In this experiment, we tested how quickly *Daphnia magna* start assimilating fatty acids from t-POM after a diet switch from phytoplankton to t-POM. *Daphnia magna* (DK-35−9) were initially cultured for 7 days with *Scenedemus gracilis* (obtained from the Institute of Zoology, University of Basel)

that had been cultured in a labeled medium comprised of 3% $H^{13}CO_3^-$. After 1 week, these *Daphnia* were switched to diets comprised of 100% birch (*Betula pendula*), dwarf birch (*Betula nana*) or common reed (*Phragmites australis*). Additionally, in one treatment, *Daphnia* were not given any food after the diet switch. This experiment was maintained in 1 L beakers with 30−50 individuals with two replicates per treatment. *Daphnia* were fed a t-POM food concentration of >5 mg $L^{-1}$ and sampled 3, 5 and 8 days after the diet switch. The experiment was carried out in the dark at a water temperature of 18 ± 2°C. Birch and dwarf birch were ground to fine particles using a Retch ZM 100 GWB ultra centrifugal Mill and common reed were ground using a Fritsch Planetary Mono Mill Pulverisette. For this experiment, ground t-POM was diluted into modified Woods Hole (WC) medium (Guillard, 1975) and filtered through a 50-μm screen and incubated 1 month in the dark with continuous shaking at 120 rpm. *Scenedesmus* cultures were enriched with $^{13}C$ (3% of the $NaHCO_3$) in WC medium. *Scenedesmus* was grown in an experimental chamber at a constant temperature (20°C) and light:dark cycle (14:10 h).

## Lipids and fatty acid analysis

Lipids were extracted with chloroform:methanol:water (4:2:1) from freeze-dried, homogenized *Daphnia* (0.3−0.7 mg) or t-POM/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 tubes. 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 (FAMEs), samples were trans-methylated in a water bath at 50°C overnight. The FAMEs formed were then extracted twice with *n*-hexane, and excess *n*-hexane was evaporated under $N_2$ and stored at −20°C until analysis.

FAMEs were analyzed using a gas chromatograph (Shimadzu Ultra) equipped with mass detector (GC-MS) at the University of Jyväskylä (Finland). An Agilent DB-23 column (30 m × 0.25 mm × 0.15 μm) was used with the following temperature program: 60°C for 1.5 min, then the temperature was increased at 10°C $min^{-1}$ to 100°C, followed by 2°C $min^{-1}$ to 140°C and 1°C $min^{-1}$ to 180°C and finally heated at 2°C $min^{-1}$ to 210°C and held for 6 min. Helium gas was used as a carrier gas with an average velocity of 34 cm $s^{-1}$. Fatty acid concentrations were calculated using calibration curves based on known standard solutions of a FAME standard mix (Taipale *et al.*, 2013). The Pearson correlation coefficient was >0.99 for each individual fatty acid calibration curve.

For fatty acid-specific stable isotope analyses, two replicates of each sample were combined to obtain the required intensity for the isotope ratio mass spectrometer (IRMS). In this analysis, FAME were injected into an Agilent 6890N GC with an Agilent DB-23 column (30 m × 0.25 mm × 0.15 μm). The GC was equipped with a Finnigan Delta Plus XP IRMS via the GC-III combustion interface (Thermo-Finnigan). The temperature in the oxidation reactor was 940°C and in the reduction reactor 630°C. The column temperature program was as follows: initial oven temperature 60°C was maintained 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 2°C $min^{-1}$ to 210°C that was held for 6 min. Hexadecanoic acid methyl ester (Indiana University, Arndt Schimmelmann), with a $\delta^{13}C$ value of −30.74‰, was used as the internal standard for calibration and drift correction.

We calculated the proportions (mean ± standard deviation; SD) of the initial and switched fatty acid sources in *Daphnia* after 3, 5 and 8 days post switch by comparing the actual *Daphnia* fatty acid profiles to hypothetical *Daphnia* fatty acid profiles (Brett *et al.*, 2009). A hypothetical fatty acid profile for a switched diet was calculated = $X \times$ (the percentage of total fatty acids for a particular fatty acid in the 100% *Scenedesmus* diet) + $(1 - X) \times$ (the percentage of fatty acids for a particular fatty acid in the 100% t-POM diet). We then compared this hypothetical fatty acid profile to that in *Daphnia* for the t-POM and used the Solver function in Microsoft Excel to find the value of $X$ that minimized the Error Sum of Squares between these two profiles. We also used Excel Solver to find the value of $X$ that maximized the fit ($r^2$) between the predicted and observed fatty acid profiles.

The contribution of t-POM and *Cryptomonas* (re-calculated according to Taipale *et al.*, 2011) as specific fatty acid sources to *Daphnia* after the diet switch from *Scenedesmus* was calculated using the measured $\delta^{13}C$ values of individual fatty acids in *Daphnia* 3, 5 and 8 days after the diet switch. The contribution of individual fatty acids to *Daphnia* was calculated with IsoError software (version 1.04; Phillips and Gregg, 2001). In all cases, we used only two diet sources and, thus, the uncertainty caused by variability of both sources was taken into account. When available, replicate results for *Daphnia* in different treatments were used for the calculations (pure *Scenedesmus* $n = 2$, most t-POM diets $n = 2$). Calculations were carried out using measurements of the *Daphnia* fatty acid composition just before the diet switch and *Daphnia* with pure *Cryptomonas*, birch or common reed diets. The SD for the mixture, *Scenedesmus* and t-POM/*Cryptomonas* in the IsoError calculation was 0.7, 3 and 5, and 0.7, 2 and 2 for the t-POM and *Cryptomonas* calculations, respectively.

*Table I: Two-way ANOVA results for the batch experiments that tested the effect of phytoplankton (Phyto) type (i.e.* Cryptomonas *or* Scenedesmus*) and the presence of 50%* Alnus rubra *derived t-POM in* Daphnia *diets on the fatty acid composition in* Daphnia

| Fatty acid | Source | df | SS | *F*-test | *P* value | Variance exp. (%) |
|---|---|---|---|---|---|---|
| 16:0 | Phyto (A) | 1 | 0.0039 | 49.74 | 0.0001 | 33.5 |
| | t-POM (B) | 1 | 0.0065 | 83.11 | 0.0001 | 55.9 |
| | AB | 1 | 0.0003 | 3.82 | 0.0742 | 2.6 |
| | Error | 12 | 0.0009 | | | 8.0 |
| 18:0 | Phyto (A) | 1 | 0.0021 | 17.05 | 0.0014 | 25.1 |
| | t-POM (B) | 1 | 0.0044 | 36.64 | 0.0001 | 53.9 |
| | AB | 1 | 0.0003 | 2.26 | 0.1582 | 3.3 |
| | Error | 12 | 0.0014 | | | 17.6 |
| 18:1ω9 | Phyto (A) | 1 | 0.0292 | 200.84 | 0.0001 | 87.2 |
| | t-POM (B) | 1 | 0.0015 | 10.51 | 0.0071 | 4.6 |
| | AB | 1 | 0.0010 | 6.93 | 0.0219 | 3.0 |
| | Error | 12 | 0.0017 | | | 5.2 |
| LIN | Phyto (A) | 1 | 0.0828 | 3658.42 | 0.0001 | 98.8 |
| | t-POM (B) | 1 | 0.0006 | 24.11 | 0.0004 | 0.7 |
| | AB | 1 | 0.0002 | 7.38 | 0.0187 | 0.2 |
| | Error | 12 | 0.0003 | | | 0.3 |
| ARA | Phyto (A) | 1 | 0.0387 | 433.21 | 0.0001 | 91.7 |
| | t-POM (B) | 1 | 0.0024 | 27.32 | 0.0002 | 5.8 |
| | AB | 1 | 0.0000 | 0.00 | 0.9482 | 0.0 |
| | Error | 12 | 0.0011 | | | 2.5 |
| ALA | Phyto (A) | 1 | 0.4148 | 1056.01 | 0.0001 | 97.0 |
| | t-POM (B) | 1 | 0.0052 | 13.35 | 0.0033 | 1.2 |
| | AB | 1 | 0.0030 | 7.63 | 0.0172 | 0.7 |
| | Error | 12 | 0.0047 | | | 1.1 |
| SDA | Phyto (A) | 1 | 0.2325 | 250.90 | 0.0001 | 91.5 |
| | t-POM (B) | 1 | 0.0074 | 7.98 | 0.0153 | 2.9 |
| | AB | 1 | 0.0032 | 3.48 | 0.0869 | 1.3 |
| | Error | 12 | 0.0111 | | | 4.4 |
| EPA | Phyto (A) | 1 | 0.6746 | 4167.54 | 0.0001 | 97.1 |
| | t-POM (B) | 1 | 0.0043 | 26.58 | 0.0002 | 0.6 |
| | AB | 1 | 0.0137 | 84.75 | 0.0001 | 2.0 |
| | Error | 12 | 0.0019 | | | 0.3 |
| ω-3:ω-6 ratio | Phyto (A) | 1 | 29.038 | 117.98 | 0.0001 | 70.9 |
| | t-POM (B) | 1 | 6.966 | 28.30 | 0.0002 | 17.0 |
| | AB | 1 | 1.984 | 8.06 | 0.0149 | 4.8 |
| | Error | 12 | 2.954 | | | 7.2 |

t-POM consumption was the main determinant of *Daphnia* SAFA (16:0 and 18:0) composition, whereas the content of 18:1ω9, LIN, ARA, ALA, SDA and EPA in *Daphnia* was mainly determined by the type of phytoplankton consumed. The *Daphnia* ω-3:ω-6 ratio was determined mainly by phytoplankton type and less by t-POM consumption. df, degree of freedom; SS, sum of squares. See text for fatty acid abbreviations.

# RESULTS

## Experiments 1 and 2

*Daphnia* that consumed phytoplankton diets in these experiments had fatty acid profiles that were very significantly similar to their resources (linear regression analysis): $r^2 = 0.97$ ($P < 0.0001$) between the fatty acid profiles of *Scenedesmus* and *Daphnia* that consumed *Scenedesmus* and $r^2 = 0.82$ ($P < 0.0001$) between the profiles of *Cryptomonas* and *Daphnia* that consumed *Cryptomonas*. Conversely, *Daphnia* that consumed red alder had a fatty acid composition that was only weakly related to their diet ($r^2 = 0.46$, $P < 0.001$).

*Daphnia* in Experiment 2 included both juveniles and adults, and many of the individuals present in the t-POM treatment at the end of this experiment appeared to be those that had merely survived because there was very little reproduction in this treatment. Because we only had one replicate for *Daphnia* that exclusively consumed t-POM (red alder) in this treatment, we could not determine differences between replicates. However, several differences were qualitatively very pronounced; first, *Daphnia* fed 100% red alder had much less of the SAFA 16:0 and 18:0 and much more of the monounsaturated fatty acids (MUFA) 16:1ω7 and 18:1ω9 than their diets. These *Daphnia* also did not contain the long-chain SAFA 20:0, 22:0 and 24:0 although these fatty acids comprised 11–12% of the lipids in their red alder diet. Second, these *Daphnia* were also considerably enriched in the ω3 and ω6 PUFA α-linolenic acid (ALA) and linoleic acid (LIN) compared with their diets.

The phytoplankton component of *Daphnia* diets very strongly influenced their lipid composition, whereas the influence of the 50% alder-enriched diets was much more modest (Table I; Fig. 1, 2). For example *Daphnia* that consumed *Cryptomonas* had much higher proportions of EPA,

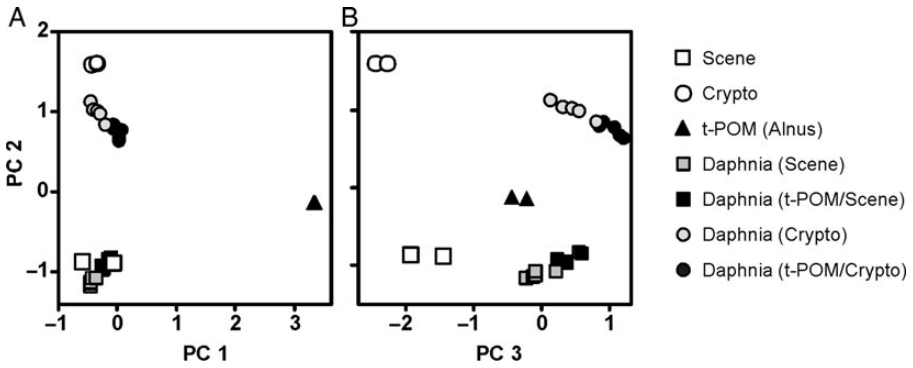


**Fig. 1.** Principal components analysis (PCA) with, (**A**) the first (PC1) and second (PC2), and, (**B**) the second and third (PC3) principle component for the diets used and *Daphnia* collected from the batch experiment. Diet samples are the fatty acid profiles of the food *Scenedesmus gracilis* (Scene; open square), *Cryptomonas* sp. (Crypto; open circle) or *Alnus* (t-POM, black triangle) used in this experiment. Samples labeled "Dph" represent *Daphnia* fatty acids after consuming *S. gracilis* (gray square), *Cryptomonas* sp. (gray circles), or a diet mix of Alnus-*Scenedesmus* (black squares) or Alnus-*Cryptomonas* (black/white circles).

stearidonic acid (SDA) and ARA than those fed *Scenedesmus*. Similarly, *Daphnia* fed *Scenedesmus* had much more 18:1ω9, LIN, and ALA than those fed *Cryptomonas*. However, *Daphnia* that consumed a 50% alder diet had significantly more of the SAFA 16:0 and 18:0 than *Daphnia* that consumed either phytoplankton diet exclusively. *Daphnia* that consumed 50% alder also had significantly more 18:1ω9 and ARA, and a lower ω3:ω6 fatty acid ratio than *Daphnia* feeding exclusively on phytoplankton.

The results of PCA and nMDS were very similar and thus only the PCA results are reported here. The PCA of the FA composition of the diets and *Daphnia* fed these diets from Experiments 1 and 2 separated the samples according to a terrestrial versus phytoplankton axis (PC1), phytoplankton type (PC2) and *Daphnia* versus diet (PC3) (Fig. 1). The first PC (21.7% of variability explained) separated the red alder from the phytoplankton diets and all *Daphnia*. This PC was very strongly positively correlated ($r \geq 0.94$, $P < 0.01$) with the long-chain SAFA 20:0, 22:0 and 24:0 as well as the shorter chain SAFA 16:0 ($r = 0.92$, $P < 0.01$) and 14:0 ($r = 0.82$, $P < 0.01$). These *r* values represent the loadings of these fatty acids on this PC. The first PC was very distinct from the other PCs, but it only distinguished a small number of t-POM samples (from all of the other samples). This resulted in the unusual situation of the first PC explaining less overall variability than the second PC. The second PC (53.8% of variability) separated the two phytoplankton diets as well as *Daphnia* consuming these diets, and was positively correlated with LIN ($r = 0.97$, $P < 0.01$), 18:1ω9 ($r = 0.85$, $P < 0.01$) and ALA ($r = 0.76$, $P < 0.01$), and negatively with SDA ($r = -0.85$, $P < 0.01$), EPA ($r = -0.83$, $P < 0.01$) and DHA ($r = -0.74$, $P < 0.01$). The third PC (12.0% of variability) separated *Daphnia* from their diets and was positively correlated with ARA ($r = 0.86$, $P < 0.01$). Although difficult to visualize (Fig. 2), the replication within Experiment 2 was very consistent, in most cases, the error bars above the means were smaller than the plot symbols and the average SD value for all treatments was $\pm 0.1$. The PCA (Fig. 1) almost perfectly superimposed the *Daphnia* fatty acid profiles when they were fed pure *Cryptomonas* and mixtures of *Cryptomonas* and alder in Experiment 1 over the fatty acid profiles of individuals fed pure *Cryptomonas* or 50:50 *Cryptomonas* and alder in Experiment 2.

We also compiled the experimental outcomes for the ω3:ω6 ratios from the two experiments where *Daphnia* were fed pure diets (Fig. 3). The ω3:ω6 ratios in *Daphnia* were strongly influenced by their diets, with lower ratios when their diets were solely comprised of terrestrial resources.

## Experiment 3 (diet switch)

Fatty acid analyses of ground common reed, birch and dwarf birch revealed that long-chain SAFA (20−28 carbon chain molecules) were diagnostic for these resources (Table II). Common reed had the highest contribution of these long SAFA (67 ± 8% of all fatty acids), whereas birch (47 ± 5% of all fatty acids) and dwarf birch (29 ± 3% of all fatty acids) had a lower contribution of long-chain SAFA. The contribution of ω-3 fatty acids was highest in dwarf birch and birch (17 ± 6 and 8 ± 1% of all fatty acids), whereas only trace amounts of ω-3 fatty acids were detected from common reed (0.1 ± 0.1% of all fatty acids). There were also only trace amounts of ω-6 fatty acids in common reed (0.2 ± 0.3% of all fatty acids), whereas both birch and dwarf birch had 4 ± 2% of ω-6 fatty acids. Additionally, all allochthonous diets had only trace amounts of EPA, but the EPA contribution was 1−7% in those *Daphnia* that solely consumed these allochthonous diets.

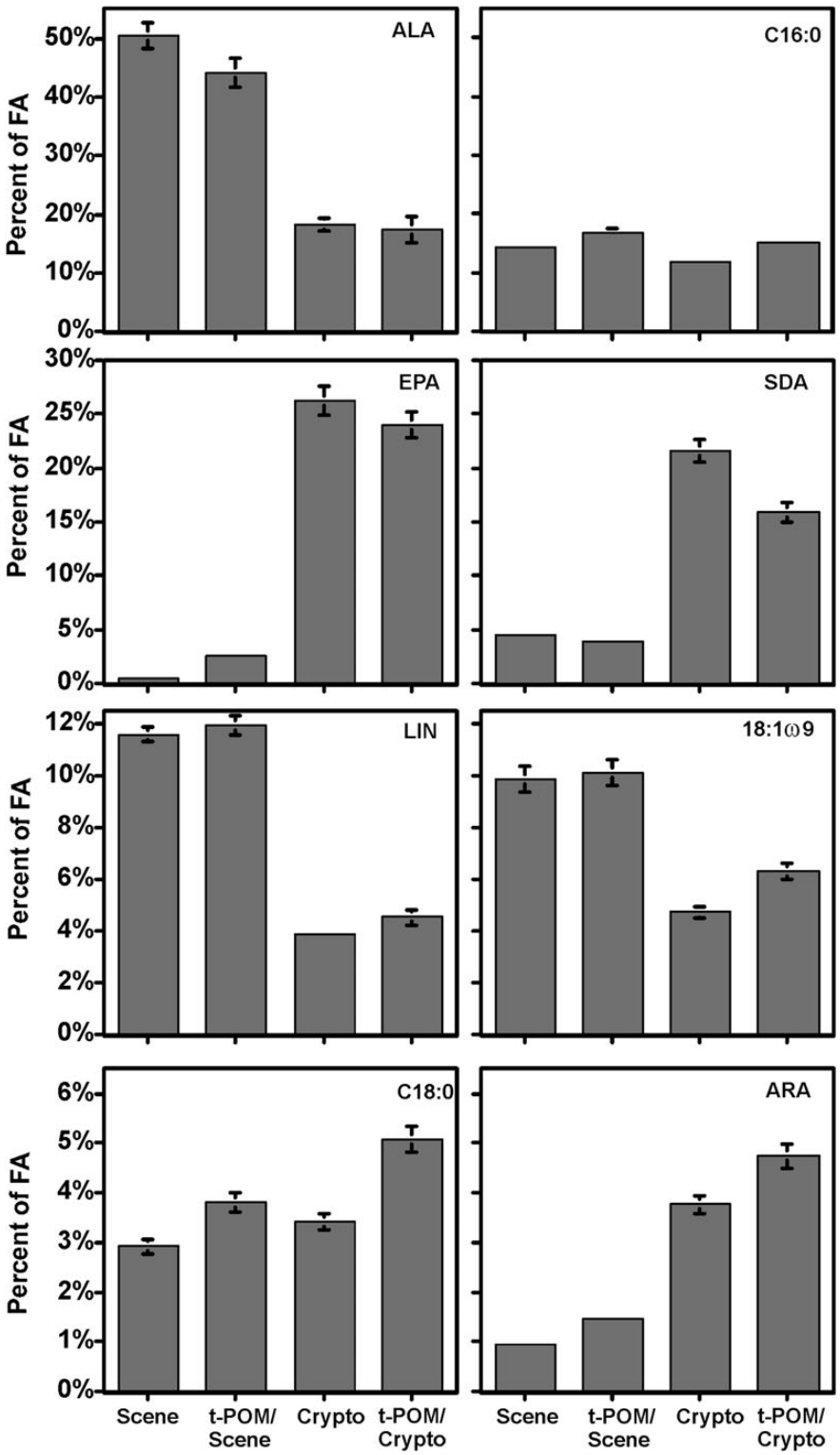


**Fig. 2.** The fatty acid composition of *Daphnia* consuming either pure phytoplankton or mixed (i.e. *Alnus*-POM + Phytos) diets from the batch experiment. The values presented are the means ± 1 SD ($n = 4$). ALA, α-linolenic acid; EPA, eicosapentaenoic acid; SDA, stearidonic acid; LIN, linoleic acid; ARA, arachidonic acid.

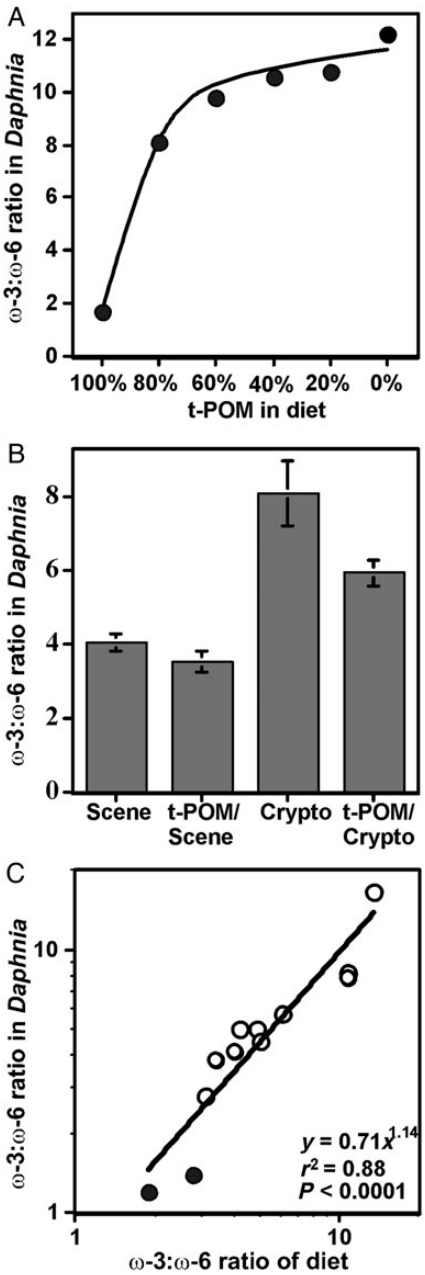


**Fig. 3.** Changes of ω-3:ω-6 ratios in *Daphnia*; (**A**) after feeding on different amounts of t-POM; (**B**) after feeding on pure *Scenedesmus* (Scene) or *Cryptomonas* (Crypto) and on mixture of t-POM with *Scenedesmus* or *Cryptomonas*; and (**C**) as a function of dietary ω-3:ω-6 ratios. The latter panel C is a compilation of results from regression analysis for the pure diet comparisons reported in this study and Brett *et al.* (Brett *et al.* 2009).

The fatty acid profiles of consumed *Scenedesmus* or *Cryptomonas* were strongly reflected in *Daphnia* ($r^2 = 0.90$ and $r^2 = 0.80$, respectively). Results of regression analysis between different t-POM and *Daphnia* consuming them were weaker, but still statistically significant for birch ($r^2 = 0.36$, $P = 0.01$) and dwarf birch ($r^2 = 0.54$, $P < 0.0001$), excluding common reed ($r^2 = 0.002$, $P = 0.817$). Three days after the diet switch, *Daphnia* had already obtained 64 ± 2% of their fatty acids ($r^2 = 0.98$; sum of squares for error, SSE = 21) from *Cryptomonas*, and 57 ± 9% of their fatty acids ($r^2 = 0.65$, SSE = 464) from common reed, whereas *Daphnia* only obtained 12 ± 4 and 23 ± 2% of their fatty acids from dwarf birch ($r^2 = 0.97$, SSE = 58) and birch ($r^2 = 0.94$, SSE = 88), respectively (Fig. 4a). Five and 8 days after the diet switch *Daphnia* obtained more fatty acids from common reed (91 ± 7%) than from dwarf birch (66 ± 3%) or birch (63 ± 1%). However, *Daphnia* fatty acid turnover was fastest with the *Cryptomonas* diet, where 88 ± 1 and 96 ± 2% originated from *Cryptomonas* 5 and 8 days after the diet switch, respectively. Nevertheless, the fit between the predicted and observed fatty acids was weak for the common reed treatment ($r^2 < 0.65$, SSE >466) due to the higher than expected 16:1ω7 content in *Daphnia* than for the *Daphnia* fed on the *Scenedesmus* or common reed diets (i.e. SEE ~253) than for the other treatments ($r^2 > 0.70$, SSE <329).

The ω-3:ω-6 ratios in *Daphnia* were highest (15 ± 1.7) after feeding on *Scenedesmus*, whereas much lower ω-3:ω-6 ratios were observed in *Daphnia* that consumed the t-POM diets (0.5 ± 0.2, Table II). After the diet switch from *Scenedesmus* to t-POM, the ω-3:ω-6 ratio in *Daphnia* dropped from an initial ratio of 15 down to a ratio of 1–2 within 8 days (Fig. 4b). A slight drop in the ω-3:ω-6 ratio was also observed for fasting *Daphnia* after 3, 5 and 8 days (Fig. 4b) and, after eight days, the ω-3:ω-6 ratio (1.4 ± 0.2) in *Daphnia* was similar for those fasting or consuming t-POM. The ω-3:ω-6 ratio in *Daphnia* fed on *Cryptomonas* was ~5.3 8 days after the diet switch and similar compared with a ω-3:ω-6 ratio of ~6 for juvenile and adult *Daphnia* fed only on *Cryptomonas*.

During this 8-day experiment, *Daphnia* retained as much SAFA as was initially supplied in all diet treatments (31 ± 3% of all fatty acids, Fig. 5). However, the contribution of MUFA to *Daphnia* dramatically increased after the diet switch for *Daphnia* with t-POM diets and especially *Daphnia* with the common reed diet (from 10 ± 3 to 44 ± 3%), whereas the contribution of MUFA remained low (23 ± 11%) in *Daphnia* that fasted. The contribution of ω-6 was similar in *Daphnia* with t-POM (9 ± 5%), which fasted (8 ± 4%), or consumed *Cryptomonas* (10 ± 1%), but the contribution of ω-3 was lower in *Daphnia* with t-POM diets (25 ± 9%) in comparison to *Daphnia* which fasted (37 ± 17%) or *Daphnia* that consumed the *Cryptomonas* diet (46 ± 3%).

The $\delta^{13}C$ values of total fatty acids were −31 ± 2.5, −35 ± 1.8, −34 ± 1.9‰ for common reed, birch and dwarf birch, respectively. Conversely, the $\delta^{13}C$ values for total fatty acids were 14 ± 10 and 45 ± 13‰ for *Scenedesmus* and for *Daphnia* fed *Scenedesmus*, respectively, just before the

*Table II: Fatty acid composition (in %) of terrestrial food sources as well as* Daphnia *consuming these diets after 8 days of diet switch from* Scenedesmus gracilis

| | *Scenedesmus gracilis* | *Daphnia Scenedesmus* | *Betula nana* | *Daphnia B. nana* | *Betula pendula* | *Daphnia B. pendula* | *Phragmites australis* | *Daphnia Phragmites* | *Daphnia* fasted |
|---|---|---|---|---|---|---|---|---|---|
| SAFA | | | | | | | | | |
| C14:0 | 0.4 ± 0.0 | 1.5 ± 0.2 | 2.7 ± 0.6 | 2.1 | 2.1 ± 0.1 | 1.4 | 1.7 ± 0.3 | 3.0 ± 0.2 | 1.7 ± 0.7 |
| C15:0 | 0.4 ± 0.0 | 0.8 ± 0.1 | 0.5 ± 0.3 | 1.1 | 0.3 ± 0 | 1.4 | 0.2 ± 0.2 | 2.0 ± 0.0 | 0.2 ± 0.2 |
| C16:0 | 17.3 ± 0.0 | 23.6 ± 0.4 | 30.5 ± 0.3 | 20.3 | 25.9 ± 0.5 | 16.6 | 18.6 ± 1.2 | 19.0 ± 0.0 | 20.8 ± 2.5 |
| C17:0 | 0.3 ± 0.1 | 1.2 ± 0.2 | 0.4 ± 0.1 | 1.1 | 0.4 ± 0.0 | 1.4 | 0.8 ± 0.2 | 1.4 ± 0.0 | 0.5 ± 0.1 |
| C18:0 | 0.6 ± 0.0 | 6.2 ± 0.1 | 5.4 ± 1.0 | 4.3 | 5.6 ± 0.1 | 5.0 | 7.8 ± 1.3 | 4.0 ± 0.5 | 8.1 ± 2.2 |
| C20:0 | 0.1 ± 0.0 | 0.6 ± 0.1 | 5.4 ± 1.0 | 0.4 | 5.1 ± 0.0 | 0.1 | 11.2 ± 2.0 | 0.2 ± 0.1 | 0 |
| C22:0 | 1.4 ± 0.0 | 0.3 ± 0.1 | 7.4 ± 0.2 | 0.4 | 6.0 ± 0.2 | 0.4 | 4.8 ± 0.1 | 0.2 ± 0.1 | 0 |
| C23:0 | 0 | 0 | 0.8 ± 0.1 | 0.0 | 0.9 ± 0.1 | 0.0 | 1.2 ± 0.3 | 0.2 ± 0.1 | 0 |
| C24:0 | 0 | 0 | 5.6 ± 1.5 | 0.0 | 5.6 ± 0.3 | 0.0 | 6.4 ± 0.8 | 0.2 ± 0.1 | 0 |
| C26:0 | 0 | 0 | 5.0 ± 2.8 | 0.0 | 14.6 ± 0.3 | 0.1 | 20.2 ± 3.9 | 0.2 ± 0.1 | 0 |
| C28:0 | 0 | 0 | 5.0 ± 3.6 | 0.0 | 13.5 ± 0.4 | 0.1 | 22.8 ± 0.7 | 0.2 ± 0.1 | 0 |
| C30:0 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0 | 0 |
| Σ SAFA | 21 ± 0.0 | 0.0 | 69 ± 7.3 | 29.7 | 80 ± 1.9 | 26.6 | 96 ± 0.7 | 30 ± 0.5 | 31 ± 0.9 |
| MUFA | | | | | | | | | |
| C16:1ω9 | 0.6 ± 0.1 | 2.3 ± 0.3 | 0.3 ± 0.1 | 2.5 | 0.3 ± 0.0 | 1.8 | 0.6 ± 0.5 | 2.4 ± 0.1 | 4.6 ± 2.0 |
| C16:1ω7 | 0.6 ± 0.1 | 2.0 ± 0.3 | 3.6 ± 1.1 | 16.7 | 2.2 ± 0.1 | 12.0 | 1.2 ± 0.3 | 25.9 ± 0.1 | 1.0 ± 0.1 |
| C16:1ω5 | 2.5 ± 0.1 | 0.2 ± 0.2 | 0 | 0.4 | 0 | 0 | 0 | 0 | 0 |
| C17:1ω7 | 0 | 0 | | 0.0 | 0 | 0 | 0 | 0 | 0 |
| C18:1ω9 | 1.8 ± 0.0 | 3.7 ± 0.4 | 4.1 ± 1.7 | 4.3 | 2.8 ± 1.5 | 5 | 0.8 ± 0.2 | 4.3 ± 0.0 | 10.2 ± 0.4 |
| C18:1ω7 | 0.8 ± 0.0 | 1.5 ± 0.1 | 2.5 ± 0.3 | 5.0 | 2.8 ± 0.1 | 10 | 1.1 ± 0.2 | 12 ± 0.0 | 4.3 ± 0.4 |
| Σ MUFA | 7 ± 0.1 | 10 ± 1.0 | 11 ± 0.2 | 28.9 | 8 ± 1.3 | 28.7 | 4 ± 0.0 | 46.4 ± 0.3 | 20.1 ± 0.3 |
| $C_{16}$ PUFA | | | | | | | | | |
| 16:2ω6 | 0.1 ± 0.0 | 0.1 ± 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4 ± 0.0 |
| 16:2ω4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16:3ω6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16:3ω3 | 0.6 ± 0.0 | 1.5 ± 0.2 | 0 | 0 | 0 | 0 | 0 | 0 | 1.1 ± 0.2 |
| 16:4ω3 | 16.4 ± 0.1 | 5.0 ± 0.2 | 0 | 0 | 0 | 0 | 0 | 0 | 1.4 ± 0.7 |
| Σ $C_{16}$ PUFA | 17 ± 0.1 | 7 ± 0.2 | 0 | 0 | 0 | 0 | 0 | 0 | 2.9 ± 0.3 |
| ω-6 PUFA | | | | | | | | | |
| C18:2ω6 | 2.2 ± 0.0 | 2.9 ± 0.2 | 3.9 ± 1.6 | 4.3 | 3.8 ± 0.1 | 6.9 | 0.2 ± 0.3 | 2.0 ± 0.0 | 15.7 ± 1.7 |
| C18:3ω6 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0 | 0 |
| C20:4ω6 | 0 | 0.3 ± 0.0 | 0 | 10.3 | 0.1 ± 0 | 13.1 | 0 | 5.4 ± 0.0 | 3.1 ± 0.5 |
| C22:2ω6 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0 | 0 |
| C22:5ω6 | 0 | 0 | 0 | 0.0 | 0 | 0.0 | 0 | 0 | 0 |
| Σ ω-6 PUFA | 2 ± 0.0 | 3 ± 0.3 | 4 ± 1.6 | 14.6 | 4 ± 1.6 | 20.0 | 0.2 ± 0.2 | 7.5 ± 0.0 | 20.5 ± 2 |
| ω-3 PUFA | | | | | | | | | |
| C18:3ω3 | 48.9 ± 0.3 | 40.3 ± 0.9 | 5.2 ± 4.7 | 16.7 | 7.3 ± 0.4 | 18 | 0.1 ± 0.1 | 8.3 ± 0.9 | 22.8 ± 1.4 |
| C18:4ω3 | 4.4 ± 0.1 | 2.6 ± 0.2 | 0.9 ± 0.4 | 0 | 0 | 0 | 0 | 0 | 2.0 ± 0.2 |
| C18:5ω3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C20:3ω3 | 0 | 0 | 0 | 0 | 0.1 ± 0 | 0 | 0 | 0.1 ± 0 | 0.1 ± 0 |
| C20:5ω3 | 0 | 0.3 ± 0.0 | 3.1 ± 2.9 | 3 | 0 | 3 | 0 | 1.6 ± 0.0 | 0 |
| C22:6ω3 | 0 | 0 | 2.2 ± 2.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| Σ ω-3 PUFA | 53 ± 0.1 | 27 ± 7.7 | 12 ± 10 | 19.5 | 8 ± 0.4 | 20.5 | 0.1 ± 0.1 | 11.6 ± 1 | 24.9 ± 1.2 |
| ω-3:ω-6 -ratio | 30.4 ± 0.4 | 15.1 ± 1.7 | 4.4 ± 0.4 | 1.4 | 2.0 ± 0.0 | 1.1 | 0.2 ± 0.2 | 1.6 ± 0.1 | 1.3 ± 0.1 |
| Sum μg FA $mg^{-1}$ | 71 ± 2 | 92 ± 24 | 13 ± 7 | 32 | 25 ± 13 | 32 | 5 ± 4 | 38 ± 10 | 22 ± 5 |

*Daphnia* fasted refers to fatty acid profile of *Daphnia* which were not given any food for 8 days after diet switch. *Daphnia Scenedesmus* is the fatty acid profile of *Daphnia* fed on *Scenedesmus gracilis* just before the diet switch. Total fatty acids are also presented as mass ratios (μg FA $mg^{-1}$ DW) for diets and *Daphnia*. SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

diet switch to t-POM (Appendix 1). After the diet switch from *Scenedesmus* to t-POM, or to *Cryptomonas*, the contribution of the switched diet to *Daphnia* increased with time. However, *Daphnia* assimilated fatty acids from the t-POM and *Cryptomonas* diets differently (Fig. 6). Three days after the diet switch to *Cryptomonas*, the isotopic signatures of fatty acids assimilated by *Daphnia* changed most rapidly for EPA (97 ± 4% from the switch diet) and ARA (91 ± 3% from *Cryptomonas*). A high proportion of 16:1ω7 (77 ± 6%) in *Daphnia* originated from *Cryptomonas* and t-POM diets only 3 days after the diet switch. SAFA and $C_{18}$ MUFA were more highly transferred to *Daphnia* from *Cryptomonas* than from the

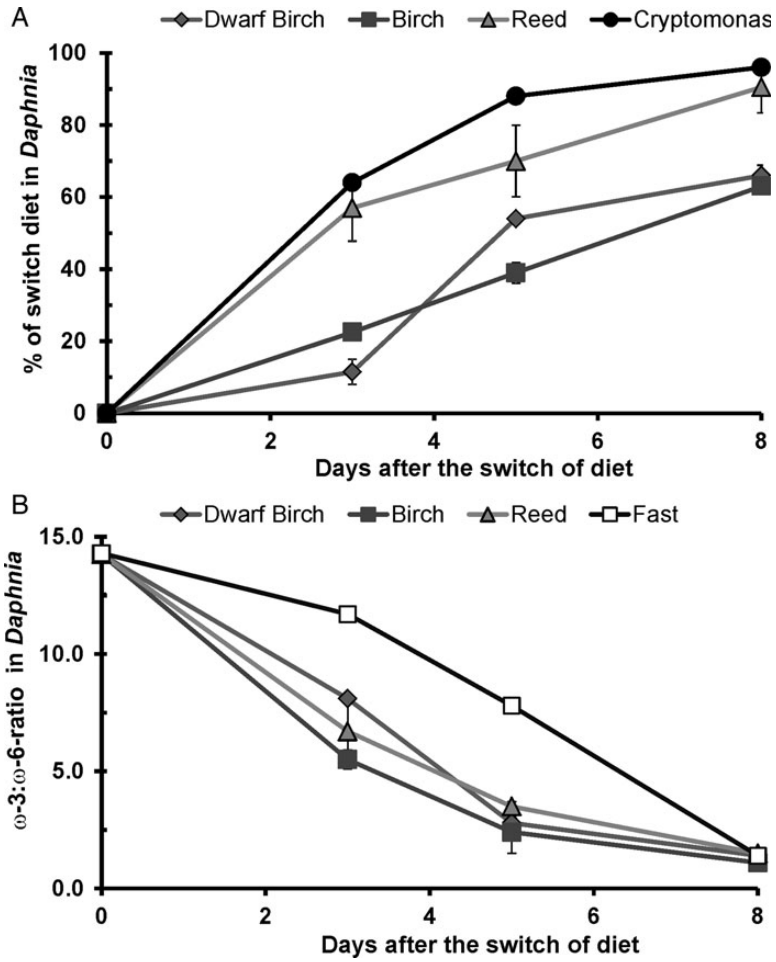


**Fig. 4.** (**A**) The contribution of dietary t-POM or *Cryptomonas* after 3, 5 and 8 days of diet switch from *Scenedesmus*. (**B**) Omega-3:ω-6 ratios in *Daphnia* after diet switch from *Scenedesmus* to t-POM diets (common reed, birch, dwarf) and fasting during 8 days.

t-POM diets. Furthermore, the *Cryptomonas* and t-POM diets moderately supported (<50%) the assimilation of ALA in *Daphnia* after the diet switch.

## DISCUSSION

We tested the hypothesis that herbivorous zooplankton preferentially retain algae-derived fatty acids. Conversely, fatty acids from t-POM (which is rich in long-chain SAFA) are less utilized by consumers. This rationale was based on the fact that zooplankton have a high physiological requirement for PUFA as a cellular constituent (e.g. Koussoroplis *et al.*, 2014) as well as for their somatic growth and reproduction. Our three separate *Daphnia* feeding experiments with various terrestrial resources (*Alnus rubra*, *P. australis*, *B. nana* and *B. pendula*) and phytoplankton (*Scenedesmus* or *Cryptomonas*) suggest that *Daphnia*, and perhaps many zooplankters in general, preferentially retain algae-derived ω-3 fatty acids and do not assimilate long-chain SAFA. We also found that low ω-3:ω-6 ratios in *Daphnia* indicate a mainly terrestrial diet or poor nutritional condition.

The fatty acid profiles of *Daphnia* fed *Scenedesmus* or *Cryptomonas* were very similar to the fatty acid profiles of the phytoplankton diets they consumed. Weaker, but still statistically significant (excluding common reed), relationships were found with *Daphnia* and the terrestrial resources. However, the fatty acid profiles of *Daphnia* clearly differed when they were fed pure diets of terrestrial or algal resources, or mixed terrestrial-algal diets.

Long-chain SAFA (20:0, 22:0, 24:0, 26:0 and 28:0) are characteristic fatty acids for various kinds of terrestrial plants and are prevalent in temperate and boreal deciduous trees including birch, alder, cottonwood, maple and willow (Brett *et al.*, 2009). In addition to deciduous trees, these fatty acids are also prevalent in common reed and peat (Wenzel *et al.*, 2012). Long-chain SAFA can contribute up to 67% of plant fatty acids, but only trace amounts (<1%) of 20:0 were found in *Daphnia* and longer chained

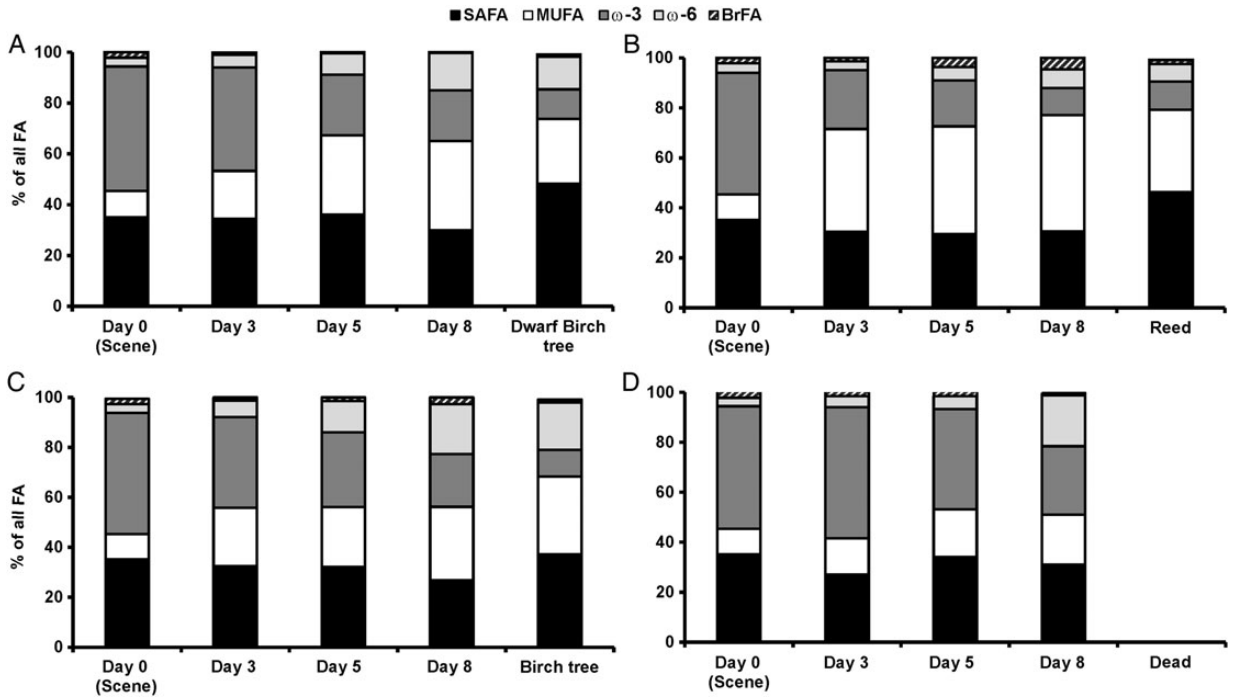


**Fig. 5.** The contribution of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), ω-3 polyunsaturated fatty acids (ω-3), ω-6 polyunsaturated fatty acids (ω-6) and branched fatty acid (BrFA) in *Daphnia* fed (**A**) dwarf birch, (**B**) common reed, (**C**) birch or (**D**) during fasting after 3, 5 and 8 days of the diet switch from *Scenedesmus*.

molecules were not detected. Trace amounts of the long-chain SAFA 20:0 and 22:0 may also be present in Chlorophyceae (Taipale *et al.*, 2013), which, if retained in herbivorous consumers, could be confused with terrestrial resources. However, when feeding on Chlorophyceae, only very small amounts of 20:0 and 22:0 were found in *Daphnia* (Table II), suggesting that *Daphnia* do not retain these long-chain SAFA, but may instead shorten them to 16:0 and 18:0. Our life table experiment supports the latter scenario because *Daphnia* feeding on red alder leaves had more 16:0 or 18:0 than did *Daphnia* fed on phytoplankton. However, when our terrestrial resources were fed to adult *Daphnia*, the contribution of long-chain SAFA in these *Daphnia* did not increase, but remained at the same level in all treatments, including fasting *Daphnia*. Therefore, long-chain SAFA cannot be used to track terrestrial fluxes via *Daphnia* to upper trophic levels.

In all the t-POM treatments (*Alnus*, *Betula* and *Phragmites*), *Daphnia* had higher levels of 16:1ω7 and LIN than found in the terrestrial or phytoplankton diets. *Daphnia* fed ground birch or common reed also had more $C_{18}$ MUFA than did the dietary sources. An especially high contribution of 16:1ω7 to *Daphnia* was detected after the diet switch from *Scenedesmus* to common reed. However, this fatty acid was poorly correlated between the diet and *Daphnia*, even though it was the most prevalent fatty acid in *Daphnia* after the diet switch. The fatty acids 16:1ω7 and 18:1ω7 are common in gram-negative heterotrophic bacteria, whereas LIN and oleic acid are dominant in fungi (Ratledge and Wilkinson, 1988). The compound-specific $\delta^{13}C$ measurements of 16:1ω7 in *Daphnia* indicated a bacterial dietary contribution to *Daphnia*. This suggests that bacteria growing on t-POM supplied *Daphnia* with 16:1ω7 after the diet switch because this fatty acid was not found in t-POM. Moreover, it is likely that bacteria and fungi increased during the incubation of dietary t-POM (especially in *Phragmites*), and consequently also in *Daphnia*, because the contribution of these source-specific fatty acids remained low in fasting *Daphnia*.

After the diet switch from *Scenedesmus* to t-POM the contribution of ω-6 in *Daphnia* increased slowly, whereas ω-3 decreased rapidly. Similar changes in PUFA were also seen in fasting *Daphnia*, in which ω-3 declined dramatically and ω-6 fatty acids increased just before those *Daphnia* died (on Day 8). The $\delta^{13}C$ values of LIN in *Daphnia* revealed LIN uptake was faster from t-POM, but slower from *Cryptomonas*. However, the $\delta^{13}C$ values indicated *Daphnia* obtained >90% of their ARA from *Cryptomonas* only 3 days after the diet switch. This suggests *Daphnia* preferentially retroconvert docosapentaenoic acid (22:5ω-6) rather than elongate and desaturate the precursor LIN (Strandberg *et al.*, 2014). Nevertheless, when fed t-POM *Daphnia* retained more ω-6 than ω-3 fatty acids, which indicates that dietary ω-6 from t-POM is efficiently tracked in this herbivorous consumer.

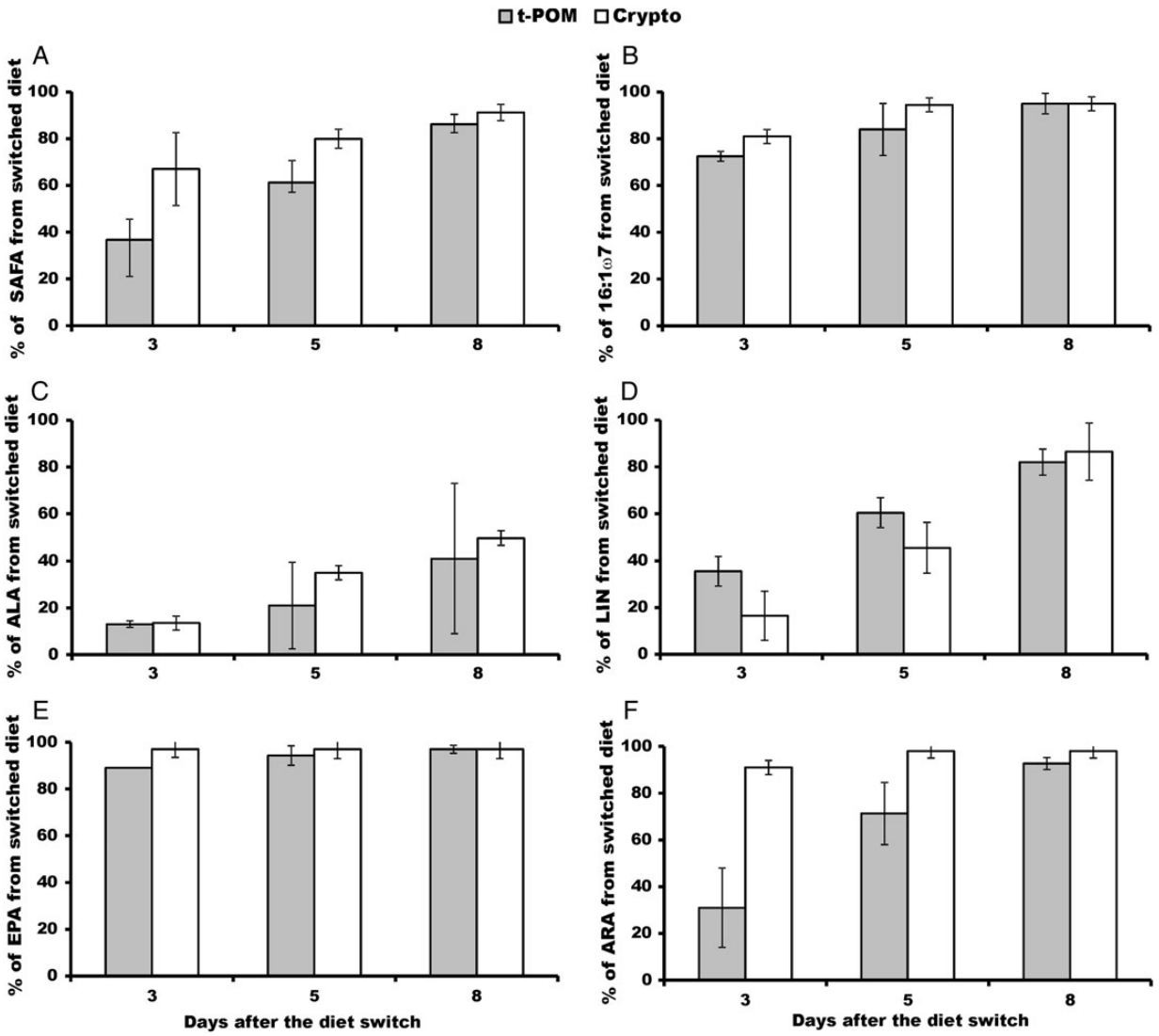


**Fig. 6.** The percentage (%) of t-POM (mean ± SD of common reed, birch, dwarf birch) and *Cryptomonas* (mean ± SD) originated (**A**) saturated fatty acids (SAFA; 14:0, 16:0, 18:0), (**B**) 16:1ω7c, (**C**) α-linolenic acid (ALA; 18:3ω3), (**D**) linoleic acid (LIN; 18:2ω6), (**E**) eicosapentaenoic acid (EPA; 20:5ω3) and (**F**) arachidonic acid (ARA; 20:4ω6) in *Daphnia* after 3, 5 and 8 days of the diet switch from *Scenedesmus*. Percentages were calculated by measuring $\delta^{13}C$ values of each fatty acid from *Daphnia* with distinct diets and using IsoError for mixing model calculations.

Moreover, we conclude that rapid changes in *Daphnia* ω-3:ω-6 ratios are more related to changes in fatty acid assimilation from recent feeding (see also high predictive power of diet for ω-3:ω-6 in *Daphnia*; $r^2 = 0.89$, Brett *et al.*, 2009) than fasting because ω-6 increased less in fasting *Daphnia* than in *Daphnia* fed t-POM diets. Previous studies (Taipale *et al.*, 2014) of *Daphnia* fed on common reed also showed that the ω-3:ω-6 ratio better reflects the assimilation of t-POM than the total dietary fatty acid composition.

It is now well known that EPA is a very important fatty acid for *Daphnia*, which have a limited ability to bioconvert the precursor ALA to EPA (von Elert, 2002; Taipale *et al.*, 2011). *Daphnia* feeding on the terrestrial diets contained up to 7% EPA, although EPA was under the detection limit in all of the terrestrial diets. The depleted $\delta^{13}C$ values of EPA in *Daphnia* confirmed that >89% of EPA originated from t-POM after 3 days of the diet switch and not from *Scenedesmus*. It is thus likely that *Daphnia* can either effectively retain EPA from t-POM or incubating t-POM stimulated the growth of heterotrophic nanoflagellates that produced and supplied EPA (Park *et al.*, 2003). However, it should be emphasized that due to the very low total fatty acid content in terrestrial resources in relation to algae, EPA in *Daphnia* fed on t-POM is too low to enhance *Daphnia* reproduction.

In freshwater systems, seston never consists entirely of t-POM, but is always a mixture of algae, protozoa,

bacteria and detritus. The contribution of t-POM to seston is higher in polyhumic or mesohumic lakes in relation to more productive/autotrophic lakes. While the flux of allochthonous carbon to zooplankton can be >50% in some polyhumic lakes, it is <10% in most lakes (Brett *et al.*, 2012). If ~80% of seston is t-POM, it was recently estimated that only ~30% of the FA in *Daphnia* (based on ω-3:ω-6 ratio) would be terrestrially derived (Taipale *et al.*, 2014). This high consumption of terrestrial resources was also evident by the low lipid content and low ω-3:ω-6 ratio in *Daphnia*. Nevertheless, more studies of ω-3:ω-6 ratios from natural phytoplankton assemblages are needed to use *Daphnia* ω-3:ω-6 ratios to quantify t-POM consumption in different lacustrine systems.

We conclude that even though there is no specific fatty acid that can be used as a diagnostic tracer for terrestrial resources in *Daphnia*, this study provides experimental evidence that the consumption of t-POM has a pronounced impact on *Daphnia* fatty acid composition. First, *Daphnia* fed terrestrial diets did not assimilate long-chain SAFA >20:0, which were diagnostic for the terrestrial resources. Second, *Daphnia* fatty acid composition differed considerably among different pure and mixed phytoplankton and terrestrial diets, and it was evident that *Daphnia* consuming t-POM had lower ω-3 fatty acid content. There were strong correlations between the ω-3:ω-6 ratios of *Daphnia* and their diets, and low ω-3:ω-6 ratios were the best indicators of the dietary influence of terrestrial diets and poor nutritional condition of *Daphnia*. Finally, fatty acid analyses can be used to infer terrestrial and algal contributions in *Daphnia*. Further experimental and observational work is required to expand the usability of ω-3:ω-6 ratios as proxies for terrestrial inputs to zooplankton, especially selectively feeding copepods.

## ACKNOWLEDGEMENTS

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# APPENDIX

*Table A1: The* $\delta^{13}C$ *values of* Scenedesmus gracilis *and allochthonous diets and corresponding* $\delta^{13}C$ *valus of* Daphnia *after 3, 5 and 8 days of diet switch from* Scenedesmus gracilis

| | Diet | Before experiment | | Day 3 | Day 5 | Day 8 | Diet | Day 3 | Day 5 | Day 8 | Diet | Day 5 | Day 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Scenedesmus gracilis* | *Daphnia Scenedesmus gracilis* | *Betula pendula* | *Daphnia B. pendula* | | | *Betula nana* | *Daphnia B. nana* | | | *Phragmites australis* | *Daphnia Phragmites* | |
| SAFA | | | | | | | | | | | | | |
| 14:0 | 29.4 | 52.0 | −34.3 | 10.9 | −9.0 | −24.1 | −34.9 | 11.9 | 12.1 | −28.1 ± 0.5 | −32.9 | −19.3 | −27.0 |
| 15:0 | 21.9 | | | 2.9 | −9.0 | −22.8 | −33.2 | 10.4 | 10.6 | −28.9 ± 1.5 | | −22.5 | −29.0 |
| 16:0 | 16.8 | 50.9 | −35.1 | 23.3 | 6.7 | −16.9 | | 23.3 | 23.4 | −24.5 ± 0.1 | −28.2 | −7.5 | −20.3 |
| 17:0 | | | | 13.5 | 5.7 | −9.7 | | 13.3 | 13.5 | −20.60 | −31.3 | −20.5 | −20.3 |
| 18:0 | 1.26 | 45.5 | −31.6 | 22.4 | 0.0 | −14.5 | −34.5 | 19.0 | −7.7 | −18.7 ± 0.3 | −32.9 | −10.6 | −25.1 |
| 20:0 | | | −35.0 | | | | −33.8 | | | | −33.8 | | |
| 22:0 | | | −34.9 | | | | −35.7 | | | | −33.7 | | |
| 24:0 | | | −36.1 | | | | −33.6 | | | | −34.3 | | |
| MUFA | | | | | | | | | | | | | |
| 16:1ω7 | 30.03 | 55.8 | −35.8 | −9.2 | −19.5 | −26.1 | −31.7 | −8.2 | −8.0 | −29.3 ± 1.1 | −29.1 | −25.2 | −26.9 |
| 16:1ω9 | | 50.1 | −32.6 | 24.3 | 6.6 | −13.8 | −31.1 | −1.6 | −1.4 | −18.6 ± 0.9 | | 1.2 | −13.8 |
| 18:1ω7 | 19.40 | 57.0 | −31.6 | 27.8 | 0.4 | −15.1 | −33.1 | 29.7 | −5.9 | −22.5 ± 1.5 | −31.7 | −9.0 | −19.1 |
| 18:1ω9 | 13.65 | 55.0 | −33.5 | 27.8 | 7.9 | −13.5 | −35.9 | 33.8 | −6.2 | −18.4 ± 0.8 | −32.3 | 6.1 | −15.8 |
| ω-6 PUFA | | | | | | | | | | | | | |
| 18:2ω6 | 12.95 | 60.0 | −36.8 | 21.6 | −1.4 | −20.6 | −35.3 | 30.6 | −2.0 | −22.7 ± 2.2 | | 11.2 | −10.6 |
| 20:4ω6 | | 20.7 | | −4.1 | −25.5 | −33.7 | | 10.1 | 10.3 | −31.2 ± 0.1 | | −22.1 | −29.2 |
| ω-3 PUFA | | | | | | | | | | | | | |
| 16:4ω3 | 2.94 | 37.5 | | 22.6 | 10.3 | | | 26.9 | 27.1 | | | −7.8 | 2.3 |
| 18:3ω3 | 11.85 | 37.9 | −37.1 | 29.1 | 22.5 | 7.2 | −37.9 | 27.1 | 7.8 | −16.8 ± 0.3 | −28.2 | 35.7 | 31.9 |
| 18:4ω3 | 6.69 | 22.8 | | | | | | | | | | | |
| 20:4ω3 | | | | 6.1 | −20.3 | −43.1 | | −19.1 | −19.0 | −32.6 ± 3.5 | | −27.4 | −36.5 |
| 20:5ω3 | | | | −29.2 | −30.9 | | | −28.5 | −31.8 | | | −32.1 | −25.6 |