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Differing *Daphnia magna* assimilation efficiencies for terrestrial, bacterial, and algal carbon and fatty acids

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Abstract. There is considerable interest in the pathways by which carbon and growth-limiting elemental and biochemical nutrients are supplied to upper trophic levels. Fatty acids and sterols are among the most important molecules transferred across the plant–animal interface of food webs. In lake ecosystems, in addition to phytoplankton, bacteria and terrestrial organic matter are potential trophic resources for zooplankton, especially in those receiving high terrestrial organic matter inputs. We therefore tested carbon, nitrogen, and fatty acid assimilation by the crustacean *Daphnia magna* when consuming these resources. We fed *Daphnia* with monospecific diets of high-quality (*Cryptomonas marssonii*) and intermediate-quality (*Chlamydomonas* sp. and *Scenedesmus gracilis*) phytoplankton species, two heterotrophic bacterial strains, and particles from the globally dispersed riparian grass, *Phragmites australis*, representing terrestrial particulate organic carbon (t-POC). We also fed *Daphnia* with various mixed diets, and compared *Daphnia* fatty acid, carbon, and nitrogen assimilation across treatments. Our results suggest that bacteria were nutritionally inadequate diets because they lacked sterols and polyunsaturated omega-3 and omega-6 (ω-3 and ω-6) fatty acids (PUFAs). However, *Daphnia* were able to effectively use carbon and nitrogen from Actinobacteria, if their basal needs for essential fatty acids and sterols were met by phytoplankton. In contrast to bacteria, t-POC contained sterols and ω-6 and ω-3 fatty acids, but only at 22%, 1.4%, and 0.2% of phytoplankton levels, respectively, which indicated that t-POC food quality was especially restricted with regard to ω-3 PUFAs. Our results also showed higher assimilation of carbon than fatty acids from t-POC and bacteria into *Daphnia,* based on stable-isotope and fatty acids analysis, respectively. A relatively high (>20%) assimilation of carbon and fatty acids from t-POC was observed only when the proportion of t-POC was >60%, but due to low PUFA to carbon ratio, these conditions yielded poor *Daphnia* growth. Because of lower assimilation for carbon, nitrogen, and fatty acids from t-POC relative to diets of bacteria mixed with phytoplankton, we conclude that the microbial food web, supported by phytoplankton, and not direct t-POC consumption, may support zooplankton production. Our results suggest that terrestrial particulate organic carbon poorly supports upper trophic levels of the lakes.

Key words: bacteria; Daphnia magna; *fatty acids; nitrogen;* Phragmites australis; *phytoplankton; sterols; terrestrial organic carbon.*

Introduction

Strong linkages between aquatic and terrestrial ecosystems have been noted in various systems. For example, arthropods are more abundant on islands in the Gulf of California with seabird colonies than without, due to marine inputs to the terrestrial food web (Polis and Hurd 1996). Similarly, when Pacific salmon return to their spawning sites, marine-derived resources are supplied to freshwater systems, providing an important resource for both the freshwater, as well as terrestrial plants and animals (Schindler et al. 2003). Freshwater systems can also be closely linked to terrestrial systems by inputs of terrestrial organic matter from their catchment basins. In some boreal lakes, the annual input of organic carbon from the catchment is estimated to be greater than that produced by phytoplankton (e.g., Berggren et al. 2010, Einola et al. 2011, Wilkinson et al. 2013). Moreover, during recent decades a trend of increasing concentrations of terrestrial organic carbon has been observed in many aquatic ecosystems in boreal and temperate regions (e.g., Monteith et al. 2007, Lepistö et al. 2008, Couture et al. 2012). Bacterial production in lakes is supported

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by both autochthonous (phytoplankton origin) and allochthonous (terrestrial origin) dissolved organic carbon (DOC; Tranvik 1988, Kritzberg et al. 2006, Kankaala et al. 2013). Therefore, it is plausible that herbivorous zooplankton production is not only based on primary production, but is also supported by a microbial pathway (DOC–bacteria–protozoa) or even directly by terrestrial particulate organic carbon (t-POC; Hessen et al. 1990*a*, Jones et al. 1998, Cole et al. 2006).

The magnitude of the transfer of terrestrial organic carbon to higher trophic levels in pelagic food webs has recently been the subject of debate (Brett et al. 2009, 2012, Cole et al. 2011, Francis et al. 2011). Experimental investigations on zooplankton assimilation of carbon, nitrogen, and essential biochemicals from bacterial sources and t-POC are limited (but see Brett et al. 2009, Taipale et al. 2012). Some field studies suggest high zooplankton reliance on terrestrial organic matter. Cole et al. (2011) and Karlsson et al. (2012) concluded, based on stable isotopes of carbon, nitrogen, and hydrogen, that ~20–60% of lake zooplankton carbon originated from terrestrial sources. Similarly, carbon and nitrogen stable-isotope studies have suggested high proportions of non-phytoplankton dietary sources for zooplankton in oligotrophic and mesotrophic lakes with varying DOC concentration (Grey et al. 2001, Kankaala et al. 2010*b*). However, other recent research has questioned the feasibility of such high t-POC contributions on the basis of food quality. For example, Francis et al. (2011) estimated that a negligible proportion of zooplankton production was supported by terrestrial inputs in 25 Pacific Northwest lakes, when phytoplankton below the mixed layer was taken into account. The feasibility of the high allochthonous carbon utilization hypothesis advanced by Cole et al. (2011) has been questioned due to the very low food quality of t-POC to zooplankton and the low availability of t-POC in comparison to phytoplankton and autochthonous carbon fluxes (Brett et al. 2009, 2012).

In addition to essential elements such as carbon, nitrogen, and phosphorus, the growth and reproduction of zooplankton and fish requires some essential biochemical compounds, i.e., fatty acids (FAs; Parrish 2009), sterols (Martin-Creuzburg et al. 2009), and amino acids (Wilson 2003). In aquatic food webs, FAs are synthesized by phytoplankton and bacteria, and then transferred via zooplankton to higher trophic levels. It has been shown that some polyunsaturated fatty acids (PUFAs) are essential for animals (i.e., mollusks, crustaceans, and fish, as well as humans; see Parrish 2009) because animals lack the enzymes to synthesize these molecules de novo (Vance and Vance 1985). PUFAs can be divided into omega-3 and omega-6 (ω-3 and ω-6) families, which cannot be interconverted in zooplankton or in fish. For zooplankton and fish, the most critical FAs are eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA; Sargent et al. 1999, Arts et al. 2001, Ravet and Brett 2006). Because *Daphnia* are inefficient at bioconverting, for example, the C_{18} ω-3, α-linolenic acid (ALA) to the longer, C_{20} ω-3 EPA de novo (von Elert 2002, Taipale et al. 2011), phytoplankton species with high EPA concentration (e.g., cryptomonads or diatoms) are very high-quality resources for *Daphnia* (Brett et al. 2006). Diets with high total concentrations of essential FAs without EPA (e.g., green algae) are intermediate quality for *Daphnia* (Brett et al. 2006), whereas diets with low concentrations of ω-3 FAs and sterols (e.g., t-POC or cyanobacteria; see Brett et al. 2009, Martin-Creuzburg et al. 2009) are biochemically low-quality resources for zooplankton.

Bacteria alone are known to be incomplete dietary resources for zooplankton (Martin-Creuzburg et al. 2011, Taipale et al. 2012, Wenzel et al. 2012), in part because they do not synthesize PUFAs (excluding some marine bacteria; Russell and Nichols 1999) or sterols (Volkman 2003). However, recent studies have revealed that *Daphnia* feeding on bacteria–phytoplankton mixtures can obtain similar somatic growth and reproduction as on pure phytoplankton cultures (Taipale et al. 2012, Wenzel et al. 2012). This is presumably related to the fact that zooplankton benefit from the high phosphorus content of bacteria (Hessen et al. 1990). Even though t-POC can be utilized by bacteria (Jones 1992, Tranvik 1998), efficient direct assimilation of t-POC by *Daphnia*, for example, is unlikely (Brett et al. 2009). Furthermore, carbon and nitrogen assimilation rates of t-POC and bacteria into zooplankton have not been experimentally demonstrated using stable isotopes, the most common method applied in pelagic food web studies.

In addition to phytoplankton primary production, bacterial production and direct input of t-POC are, thus, alternative pathways for elemental and biochemical resources for zooplankton. The objective of this study was to compare *Daphnia* assimilation efficiencies on several phytoplankton, bacteria, and t-POC diets based on their FA, sterol, carbon, nitrogen, and phosphorus contents. Our experiment was designed to specifically evaluate the quality of t-POC (a riparian grass and a terrestrial tree) and bacteria (two heterotrophic strains) both alone and combined with different amounts of three types of phytoplankton diets for *Daphnia*. Because *Daphnia* have been previously shown to be dependent on algal FAs and sterols, we hypothesized that growth rates, reproduction, and survival of *Daphnia* would be reduced with increased proportion of bacterial and/or terrestrial resources increases in the diet relative to phytoplankton.

Materials and Methods

Zooplankton culture

All experiments were conducted using a clone of *Daphnia magna* (DK-35-9; hereafter *Daphnia*) initially grown and maintained on *Scenedesmus gracilis*.

Phytoplankton cultures

Scenedesmus gracilis (obtained from the Institute of Zoology, University of Basel, Basel, Switzerland) and *Cryptomonas marssonii* (CCAP 979/70; obtained from the University of Helsinki, Helsinki, Finland), and *Chlamydomonas* sp. (isolated from Lake Musta-Kotinen, Evo, Finland) were cultivated using L16 culture medium (Lindström 1983) supplemented with biotin and cyanocobalamin (B_{12}). All phytoplankton species were grown in an experimental chamber at a constant temperature (20°C) and light : dark cycle (14 h : 10 h). To obtain differences in carbon isotope signals between the diets, *Scenedesmus* and *Cryptomonas* cultures were enriched with ^{13}C (3% of the $NaHCO_3$ in the L16 medium consisted of $NaH^{13}CO_3$ [99%]; Cambridge Isotope Laboratories, Cambridge, UK).

Bacterial cultures

We used two heterotrophic bacterial strains representing typical lake bacteria; Actinobacterium *Candidatus* Rhodoluna limnophila MWH-VicMua1 (originating from tropical Lake Victoria, east Africa [Hahn 2009*a*]) and Betaproteobacterium *Polynucleobacter necessarius* ssp. *asymbioticus* MWH-Mekk-D6 (originating from the boreal, polyhumic Lake Mekkojärvi, Evo, Finland [Hahn et al. 2009*b*]). These strains were grown in liquid nutrient broth soyotone yeast extract (NSY) medium (Hahn et al. 2004) on a rotary shaker at room temperature. Average cell length (and cell volume) of the Actinobacterium VicMua1 and the Betaproteobacterium Mekk-D6 were 0.87 μm (0.060 μm^3) and 0.73 μm (0.089 μm^3), respectively. These are typical cell sizes for small planktonic bacteria (cf. Hessen 1985, Arvola et al. 1992).

Terrestrial carbon source

We used leaf particles of common reed (*Phragmites australis* (Cav.) Trin. ex Steud), as a terrestrial particulate food resource for zooplankton. Common reed is a very persistent species that grows in a wide range of conditions and has also invaded wetlands, rivers, lakes, and coastal zones of all continents except Antarctica (Chambers et al. 1999, Lambertini et al. 2012). After dying, *Phragmites* is degraded by various microbes and fungi (Kominkova et al. 2000), and detrital particles can potentially be consumed by zooplankton. We collected *Phragmites australis* (hereafter called t-POC) from the shore of Lake Pyhäselkä (eastern Finland), and ground it to small particles using a Fritsch Planetary Mono Mill Pulverisette 6. The particles were then diluted into the L16 medium directly (t-POC L16 diet) or into filtered (GF/F Whatman, nominal pore size ~0.7 μm) water from Lake Pyhäselkä and incubated for two months in the dark to simulate decay by a natural microbial assemblage (t-POC Lake diet). Before the feeding experiments, the suspensions were filtered through a 48-μm mesh in order to obtain an optimal particle size for *Daphnia*. The quality of t-POC from leaves of a terrestrial deciduous tree, red alder (*Alnus rubra*), as a diet source for *D. magna* was previously tested (Brett et al. 2009). Thus, we also used these results for a more comprehensive analysis of terrestrial FA assimilation by *Daphnia*.

Life table experiments

Daphnia neonates (~6 h old) were used for all experiments. Neonates from specific adults were divided equally between treatments to minimize maternal effects (Brett 1993) and distributed individually into glass vials (40 mL of L16 medium) with each treatment consisting of 10 replicates. The medium was changed and the *Daphnia* fed every other day with total food concentrations of 1.5, 2, and 5 mg C/L for ages 2, 4, and 6+ days, respectively. These food concentrations were above the incipient limiting level for ingestion (see Lampert 1987). In addition to the pure (100%) diets of t-POC and each taxon of phytoplankton and bacteria, we also used diets consisting mainly (98% or 75%) of bacteria or t-POC and mixed with (2% or 25%) high-quality phytoplankton (*Cryptomonas*), respectively, to evaluate the quantity of FAs and sterols of phytoplankton origin needed to achieve positive *Daphnia* somatic growth and reproduction (see Appendix A). We also tested a mixture of four different diets (58% of t-POC diluted with L16, 20% of Actinobacterium VicMua1, 20% of Betaproteobacterium Mekk-D6, and 2% of *Cryptomonas*) to mimic t-POC- and bacteria-dominated conditions in nature. Due to the high number of juveniles needed, the experiments with pure bacteria, *Scenedesmus* and *Chlamydomonas* diets, and the second life table experiment with *Cryptomonas* diet were started at different times than the other experiments. Life table experiments lasted 14 d, after which the size of *Daphnia* was measured and the number of eggs enumerated under a microscope, and the individuals were then placed into 1.5-mL Eppendorf tubes, freeze-dried, and stored at −80°C. Preserved individuals were randomly divided between FA and stable-isotope analyses. Total biomass growth rate (g) of pooled *Daphnia* for each treatment were calculated as $g = (\ln Bt_{14} - \ln Bt_0)/t$, where B is biomass (dry mass) at the end (t_{14}) and at the beginning (t_0) of the experiment.

Batch experiments

Due to the high mortality rate of *Daphnia* in some treatments, we were unable to measure carbon and nitrogen stable isotopes in all cases. We therefore also carried out two batch experiments to obtain more biomass for stable-isotope analyses. In the first experiment, we used intermediate-quality (100% *Scenedesmus*) and high-quality (100% *Cryptomonas*) diets, which were labeled with ^{13}C and mixed with t-POC (95% of total carbon) diluted either with L16 medium or lake water at a food concentration of 5 mg C/L. In the second batch

experiment, we used an intermediate quality diet (100% *Scenedesmus*) mixed with t-POC (50%, 25%, and 5% of total carbon) and diluted with L16 medium. For the batch experiment, nine *Daphnia* neonates (~6 h old) were placed into 200-mL glass beakers with each treatment including three replicates. The medium was changed and the *Daphnia* fed every 2 d. The batch experiment lasted 10 d for *Cryptomonas* and *Cryptomonas* mixed diets and 8 d for the *Scenedesmus* and *Scenedesmus* mixed diets. The size of *Daphnia* was measured and number of eggs quantified under a microscope, freeze-dried, and stored at −80°C.

Total phosphorus, total nitrogen, and organic carbon content of food suspensions

Total phosphorus concentration was analyzed with ammonium molybdate spectrometric method (ISO 6878:2004, according to the Finnish Standards Association; more information *available online*).[7] Total nitrogen and organic carbon concentrations were analyzed with a multi N/C instrument (Analytik Jena, Jena, Germany).

Fatty acid analyses

Lipids from freeze-dried phytoplankton, bacteria, t-POC (1–2 mg of each), and zooplankton (0.3–0.4 mg) samples were extracted with a 4:2:1 chloroform : methanol : water mixture (Parrish 1999). These samples were then sonicated and vortexed two times and the organic phases removed and pooled. FAs were transmethylated at 50°C overnight using 1% sulfuric acid as a catalyst. FA methyl esters were analyzed with a gas chromatograph (6890N; Agilent Technologies, Santa Clara, California, USA) with mass spectrometric detection (Agilent 5973N). An Agilent DB-23 column (30 m × 0.25 mm × 0.15 μm) was used with the following temperature program: 60°C was maintained for 1.5 min, then the temperature was increased at 10°C/min to 100°C, followed by 2°C/min to 140°C, and 1°C/min to 180°C, and finally heated up at 2°C/min to 210°C, and then held for 6 min. Helium was used as carrier gas with an average velocity of 34 cm/sec.

Sterol analysis

Sterols were analyzed in 3–4 mg of freeze-dried material of all diets. Total lipids were extracted three times from the freeze-dried material with dichloromethane : methanol (2:1, v:v). Pooled cell-free extracts were evaporated to dryness under N_2-atmosphere and saponified with methanolic KOH (0.2 mol/L, 70°C, 1 h). Subsequently, the neutral lipids were partitioned into iso-hexane : diethyl ether (9:1, v:v). The lipid-containing fraction was evaporated to dryness under N_2 and resuspended in iso-hexane (10–20 μL). Sterols were analyzed using a gas chromatograph (GC; HP 6890; Hewlett-Packard, Palo Alto, California, USA) equipped with a flame ionization detector (FID) and a HP-5 (30 m × 0.25 mm inner diameter × 0.25 μm film; Agilent Technologies) capillary column. Details of GC configurations are given elsewhere (Martin-Creuzburg et al. 2009). Sterols were quantified by comparison to the internal standard 5α-cholestan, considering response factors determined previously with lipid standards (Sigma, Steraloids, Newport, Rhode Island, USA) and identified by their retention times and their mass spectra, which were determined with a gas chromatography (GC)–mass spectrometer (5975C; Agilent Technologies) equipped with a fused-silica capillary column (DB-5MS, Agilent; GC configurations as described for FID). Sterols were analyzed in their free form and as their trimethylsilyl derivatives, which were prepared by incubating 20 μL of iso-hexane sterol extract with 10 μL of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) for 1 h at room temperature. Spectra were recorded between 50 and 600 atomic mass units (amu) in the electron impact (EI) ionization mode. The limit for quantitation of FAs and sterols was 20 ng.

Stable-isotope analyses

Approximately 0.6 mg of zooplankton and ~1.0 mg of phytoplankton and bacteria were weighed in tin cups for $\delta^{13}C$ and $\delta^{15}N$ analyses, which were carried out on a Carlo-Erba Flash 1112 series Element Analyzer connected to a Thermo Finnigan Delta Plus Advantage IRMS at the University of Jyväskylä, Finland. These samples were compared to the NBS-22 standard using fish muscle as a laboratory-working standard. The precision of the $\delta^{13}C$ and the $\delta^{15}N$ analyses were 0.2‰ and 0.3‰, respectively, for all samples.

Data analyses

The contribution of ingested bacteria, phytoplankton, and t-POC in *Daphnia* was calculated using $\delta^{13}C$ and $\delta^{15}N$ and FA measurements of the diet components and *Daphnia* in both life table and batch experiments. Mean (±SE) carbon and nitrogen assimilation, based on $\delta^{13}C$ and $\delta^{15}N$ values, was calculated with IsoError software (version 1.04; Phillips and Gregg 2001). In all cases, we had 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*, $n = 3$; pure *Cryptomonas*, $n = 2$; some mixed diets, $n = 2$). If only a single result per treatment was available, the variability was estimated by using ±SD values of $\delta^{13}C$ and $\delta^{15}N$ of the diet sources (0.4‰ and 0.6‰), which included the uncertainty caused by the stable-isotope analyses (95% probability).The mean (±SE) fractional contributions of the diet sources were based on the intercepts of linear Keeling plot regression equations (Phillips and Gregg 2001). In these mixing model calculations, the $\delta^{13}C$ and $\delta^{15}N$ of the phytoplankton

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and t-POC diet components were measured directly from *Daphnia* fed on either phytoplankton or t-POC diet, thus including isotopic fractionation. The fractionation of bacterial carbon and nitrogen to *Daphnia* was estimated to be 0.5‰ and 2.0‰, respectively, as observed for *Daphnia* fed on *Micrococcus luteus* for six days (S. J. Taipale, *unpublished data*). The same fractionation values were assumed to be valid for t-POC diluted to the L16 medium and lake water.

We calculated the proportions (mean ± SD) of different FA sources in *Daphnia* in the mixed-diet treatments originating from t-POC, bacteria and phytoplankton by comparing the actual *Daphnia* FA profiles to hypothetical *Daphnia* FA profiles (see Brett et al. 2009). A hypothetical FA profile for a mixed diet was calculated $= X \times$ (the percentage of total FAs for a particular FA in the 100% *Cryptomonas* diet) $+ (1 - X) \times$ (the percentage of FAs for a particular FA in the 100% bacterial or t-POC diet). We then compared this hypothetical FA profile to the *Daphnia* FA profile for the t-POC or bacteria and *Cryptomonas* diet 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 FA profiles. Due to the different ω-3 : ω-6 FA ratios of the t-POC and phytoplankton diets, we were also able to calculate the FAs assimilated (mean±SD) by *Daphnia* using a two source mixing model for the t-POC and phytoplankton mixed diets (Brett et al. 2009).

Statistical tests

Statistical analyses were conducted using IBM PASW (version 18.0; SPSS 2009) or IBM SPSS (version 19.0; IBM 2010) software. Differences in the survival of *Daphnia* individuals during the life table experiments were analyzed with Cox regression analysis using food treatments as categorical covariates. Kruskal-Wallis Test with stepwise step-down multiple comparisons was used to compare the treatment means for *Daphnia* size and the number of juveniles because the treatment variances were not equal. Parametric Pearson correlation (r) or nonparametric Spearman's rho (Appendix D) correlation (r_S) calculations were applied to show how *Daphnia* response variables were related to the various diet components.

The relationships between the proportion of non-phytoplankton (bacteria or t-POC) carbon and FAs in the diets and those assimilated by *Daphnia* were analyzed using linear ($y = bx$) or power function ($y = ax^b$) regression models. Thus, we assumed that when bacteria or t-POC was not available in the diet, these could not be incorporated by *Daphnia*. However, it was not likewise assumed that diets consisting 100% of bacteria or t-POC would result in a corresponding 100% incorporation of these diets in carbon or FA contents of *Daphnia*, because of high observed rates of *Daphnia* mortality on pure bacteria and t-POC diets. For the linear regression analysis, we used the least squares approach for the highest fits (r^2). The parameters a and b were estimated using iterative least squares method in SPSS software. The judgment whether the relationship deviated significantly (risk level $\alpha = 5\%$) from linear ($b = 1$) was based on assessing whether the value $b = 1$ was excluded from the bootstrapped 95% trimmed range of the estimate of b.

Results

Quality of diet sources

Nitrogen and phosphorus content.—Actinobacterium VicMua1 and Betaproteobacterium Mekk-D6 had the highest nitrogen and phosphorus concentration amongst all diets. In contrast, nitrogen and phosphorus concentrations were lowest in the t-POC diets (see Table 1 and Appendix A). Mixed treatments with bacteria therefore contained more nitrogen and phosphorus than treatments with pure phytoplankton/t-POC or mixed t-POC and phytoplankton diets.

Fatty acid composition of phytoplankton, bacteria, and t-POC.—The three phytoplankton taxa, the two bacterial taxa, and the two t-POC diets had very distinct FA profiles that also varied in their PUFA concentrations (see Appendix B). In the phytoplankton taxa *Scenedesmus* and *Chlamydomonas*, the ω-3 PUFAs (ALA and stearidonic acid [SDA]), constituted 30–37% of all FAs. *Cryptomonas* was also rich in ALA and SDA (~40–45%), but also contained EPA, and DHA (3–4%). *Cryptomonas* had the highest concentration of ω-3 PUFAs in relation to carbon content (Fig. 1D). The bacterial strains did not contain PUFAs. The FAs of Actinobacterium VicMua1 were dominated by *iso*-and *anteiso*-branched FAs, whereas in the Betaproteobacterium Mekk-D6 monounsaturated fatty acids (MUFA) formed the highest proportion. Cyclopropane FAs were found only in Betaproteobacterium Mekk-D6.

Up to ~90% of t-POC FAs consisted of saturated fatty acids (SAFA), and the proportion of PUFAs was <1%. However, the proportion of PUFAs was slightly higher in the t-POC Lake diet than that in the t-POC L16 diet (Appendix B). In phytoplankton, the ratio between ω-3 and ω-6 FAs was between 3 and 10, whereas in t-POC this ratio was 0.2. The ratio of the sum of total FAs to organic carbon (totFA : C) varied between 0.022–0.039 in algae and 0.031–0.036 in the two bacterial strains, whereas in t-POC this ratio was approximately 10-,fold lower (Table 1).

Sterol content of phytoplankton, bacteria, and t-POC.—The total concentration of sterols was similar in all three phytoplankton taxa. The sterol content of t-POC was ~22% of that in phytoplankton, whereas bacteria did not contain any sterols. Details of sterol profiles of the different food sources are provided in the supplementary material (Appendix B).

TABLE 1. Diet sources and their characteristics in the experiments with *Daphnia magna*: particle length, percentage of carbon (C) and nitrogen (N) by dry mass, C:N ratio, carbon : phosphorus (C:P) ratio (by moles), ω-3 : ω-6 ratio of polyunsaturated fatty acids, total fatty acids to carbon ratio (totFA : C), and stable carbon ($\delta^{13}C$) and nitrogen and ($\delta^{15}N$) values (mean ± SD).

Diet source	Abbreviation for diet	Length (μm)	C (%)	N (%)	C:N	C:P	ω-3 : ω-6	totFA : C	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Phragmites australis diluted in L16 medium	t-POC L16	2–120†	42.3	1.7	25	201	0.2	0.003	−28	−0.87
Phragmites australis diluted in lake water	t-POC Lake	1–50†	42.2	2.2	19	nd	0.2	0.003	−29	1.89
Candidatus Rhodoluna limnophila (MWH-VicMua1)	VicMua1	<1.2	41.8	10.2	4	46	nd	0.031	−22.0 ± 0.1	5.6 ± 0.2
Polynucleobacter necessarius ssp. *Asymbioticus* (MWH-Mekk-D6)	Mekk-D6	<2	43	10.9	4	21	nd	0.036	−22.0 ± 0.1	−0.25 ± 0.1
Chlamydomonas sp.	Chlamydo	7–15	44.6	7.4	6	137	5	0.039	−21.7 ± 0.5	−9.5 ± 0.3
Scenedesmus gracilis	Scene	12–17	44.5	5.7	8	116	3.5	0.022	44.1 ± 0.6	−12.5 ± 0.1
Cryptomonas marssonii	Crypto	14–80	44.2	6.2	7	59	9.8	0.025	25.3 ± 0.6	−9.7 ± 0.1

Notes: Particles of *Phragmites australis* (t-POC) were either diluted in L16 medium or lake water and incubated for two months. Heterotrophic bacteria were Actinobacterium *Candidatus* Rhodoluna limnophila VicMua1 and Betaproteobacterium *Polynucleobacter necessaries* ssp. *Asymbioticus* Mekk-D6. Phytoplankton species were *Chlamydomonas* sp., *Scenedesmus gracilis*, and *Cryptomonas marssonii*. "No data" is indicated with nd.

† Approximately 60% were 2–5 μm.

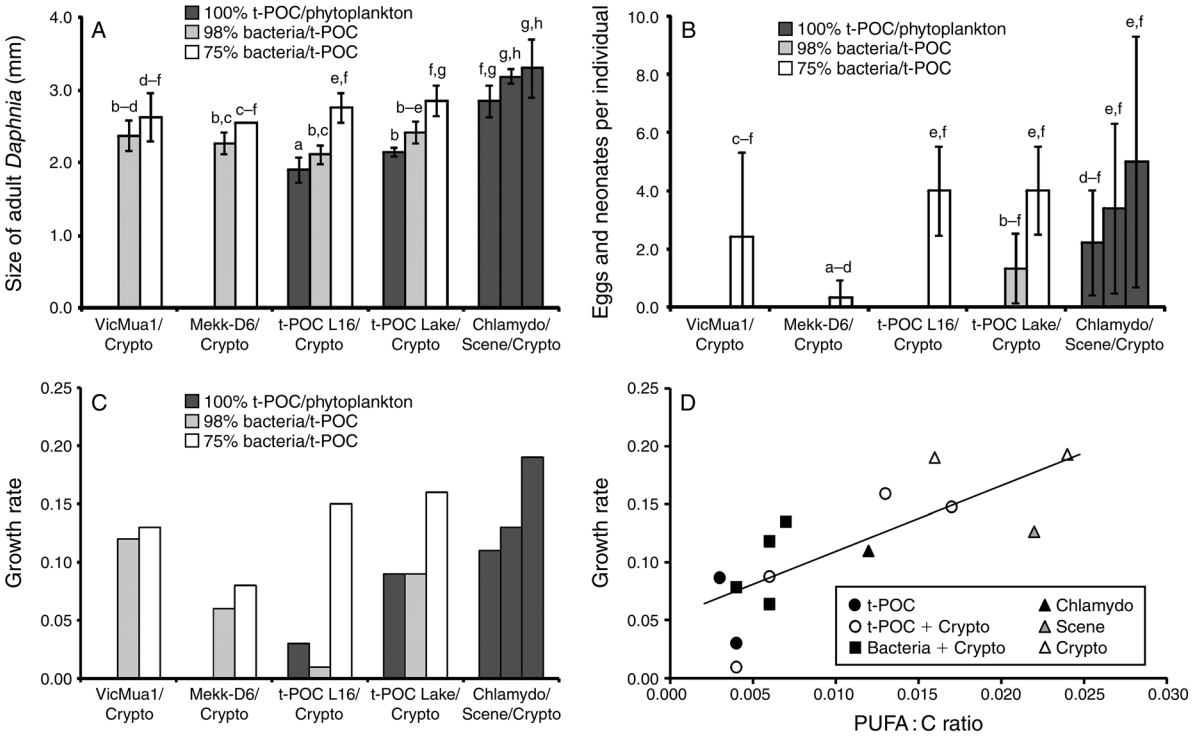

FIG. 1. (A) Size (mean ± SD) of adult *Daphnia magna* (mm) and (B) number (mean ± SD) of offspring (eggs and neonates per individual) at the end of 14 days, and (C) daily growth rate of *Daphnia* in the life table experiment with the different diets: 100% common reed (*Phragmites australis* (Cav.) Trin. ex Steud) diluted in L16 culture medium (t-POC L16) or in lake water (t-POC Lake), *Cryptomonas marssonii* (Crypto), *Scenedesmus gracilis* (Scene), and *Chlamydomonas* sp. (Chlamydo), and with diets consisting of 98% and 75% bacteria (Actinobacterium *Candidatus* Rhodoluna limnophila VicMua1 or Betaproteobacterium *Polynucleobacter necessaries* ssp. *asymbioticus* Mekk-D6) or t-POC and 2% and 5% of *Cryptomonas*. Different letters (a–h for size, and a–f for the number of offspring) represent treatments that were not significantly different from one other at the 0.05 significance level. (D) Biomass growth rate of *Daphnia* with different diets related to the ratio of polyunsaturated fatty acids to carbon (PUFA : C) of *Daphnia* bodies at the end of the life table experiment.

Daphnia response to the diet sources

Survival, growth rate, and offspring production in life table experiments.—All *Daphnia* fed pure Actinobacterium VicMua1 or Betaproteobacterium Mekk-D6 died after eight or six days, respectively; therefore, *Daphnia* in these treatments differed significantly from the others (Cox regression survival analysis; Appendix C). *Daphnia* fed pure *Cryptomonas* had the highest survival rate, but this treatment did not differ significantly from the other treatments consisting of pure *Scenedemus*, *Chlamydomonas,* t-POC, mixed bacteria–phytoplankton, or mixed t-POC–phytoplankton diets.

After 14 days, *Daphnia* fed on *Cryptomonas* had the largest body size (3.3 ± 0.4 mm; Fig. 1A), followed by *Daphnia* fed *Scenedesmus* (>3.0 mm). Among all diets consisting of 25% of phytoplankton and 75% of non-phytoplankton sources, *Daphnia* fed t-POC Lake had the largest body size, whereas *Daphnia* fed 75% Betaproteobacterium Mekk-D6 had the smallest body size. *Daphnia* fed a 100% t-POC L16 diet were significantly smaller than *Daphnia* fed any other diet.

The reproduction of *Daphnia* was highly variable among the treatments. The experimental duration of 14 days was not long enough for *Daphnia* in all treatments, particularly when fed diets consisting of only 2% *Cryptomonas*. The greatest reproduction occurred when *Daphnia* were fed either *Cryptomonas* or *Scenedesmus* (Fig. 1B). No reproduction within 14 days occurred when *Daphnia* were fed 100% of either type of t-POC or 98% mixed diets excluding t-POC diluted in lake water. The body size and offspring number of *Daphnia* significantly correlated with the concentrations of ω-3 and ω-6 PUFAs and sterols in the diets, but not with diet nitrogen and phosphorus concentrations (Appendix D).

The biomass growth rate of *Daphnia* was highest with the pure *Cryptomonas* diet (0.18 d^{-1}) and lowest when fed t-POC in L16 medium (Fig. 1C). The t-POC mixed with lake water (100% and 98%) diet yielded higher growth rates than that mixed in L16 medium. When 25% of the diet consisted of *Cryptomonas*, the growth rate with t-POC was higher than with pure algal diets consisting of *Chlamydomonas* and *Scenedesmus*. The diets consisting of 2% and 25% *Cryptomonas* and mixed with Actinobacterium VicMua1 yielded higher growth rates than the diets with similar proportions of *Cryptomonas* and Betaproteobacterium Mekk-D6.

Survival and somatic growth of Daphnia *in the batch experiments*.—Survival of *Daphnia* was similar for all *Scenedesmus* and mixed-diet treatments (94–100%), whereas *Daphnia* feeding on pure *Cryptomonas* had a higher survival rate (94%) than *Daphnia* fed on *Cryptomonas* and t-POC mixed diets (61–89%). Ten-day-old *Daphnia* raised on *Scenedesmus* (body size of 3.0 ± 0.3 mm) were bigger than *Daphnia* grown on t-POC mixed diets (2.0 ± 0.2 mm). *Daphnia* fed *Cryptomonas* (body size of 2.4 ± 0.8 mm) for eight days were only slightly bigger than *Daphnia* fed t-POC mixed diet, t-POC L16 (2.1 ± 0.2 mm), or t-POC Lake (2.2 ± 0.0 mm).

Fatty acid content and composition of Daphnia.—Total FA content of *Daphnia* was influenced by the diet (Fig. 2) and was highest in *Daphnia* either fed with pure phytoplankton (e.g., *Cryptomonas*, 70 ± 3 μg FA/mg dry mass [DM]) or the mixed diet of Actinobacterium VicMua1 and *Cryptomonas* (63 ± 0 μg FA/mg DM). In contrast, the total FA concentration of *Daphnia* was low on pure or mixed t-POC diets or the bacterial diets, Betaproteobacterium Mekk-D6. Furthermore, the total FA concentration increased when the contribution of *Cryptomonas* increased in the t-POC diet (Fig. 2A, B). Principal component analysis of the diet sources and *Daphnia* (Appendix E) demonstrated low incorporation of t-POC FAs to *Daphnia*. The highest contribution, proportionally, of ω-6 in *Daphnia* was measured in the pure t-POC treatments, while the total concentration of ω-6 FAs was similar among all treatments (Fig. 2A, B).The total concentration of ω-3 FAs in *Daphnia* increased, along with the increased proportions of added *Cryptomonas* in the diet-mixing life table experiments. EPA concentrations in *Daphnia* were highest on the pure *Cryptomonas* diet (7.2 ± 0.9 μg EPA/mg DM). Similar EPA concentrations were found in *Daphnia* fed the mixed diet of 75% of t-POC and 25% of *Cryptomonas* (5.8 ± 0.1 μg EPA/mg DM), but *Daphnia* in other mixed diets had much lower total EPA concentration. Significant contribution of bacterial FAs to *Daphnia* was also detected in the VicMua1 bacteria and *Cryptomonas* mixed treatment (Fig. 2C and Appendix E).

Proportional FA composition of the diets influenced *Daphnia* FA composition. Total FA profiles of diets and *Daphnia* produced high fits ($r^2 = 0.55$–0.83; Table 3) between hypothetical diet FA profiles (see *Materials and methods*) and observed FA profiles of *Daphnia*. Tot-FA : C ratio of *Daphnia* was correlated with that of diets ($r = 0.66$, $P = 0.002$, $n = 18$). However, when compared with the totFA : C ratios of the diets (Table 1), the ratio was clearly higher in *Daphnia* fed with t-POC, and t-POC mixed with algae (Table 2). In life table experiments, the biomass growth rate, including both somatic growth and offspring production, was positively correlated with the totFA : C ratio ($r = 0.75$, $P = 0.001$, $n = 14$) and with the PUFA : C ratio of *Daphnia* ($r = 0.76$, $P = 0.001$, $n = 14$; see Fig. 1D).

Assimilation of carbon, nitrogen, and FAs.—The $\delta^{13}C$ and $\delta^{15}N$ values of the algal, bacterial, and t-POC diet sources differed clearly ($\delta^{13}C$ −21.7 to 44.1‰; and $\delta^{15}N$ −12.5 to 5.6‰; Table 1). *Scenedesmus* and *Cryptomonas* were clearly enriched with $\delta^{13}C$ due to uptake of inorganic ^{13}C from the culture medium. The differences were reflected in the $\delta^{13}C$ and $\delta^{15}N$ values of *Daphnia* fed with pure algal diets and those mixed with bacteria or t-POC (Table 2). Thus, the IsoError mixing model calculations of the different diet source proportions showed relatively low replicate variability for carbon

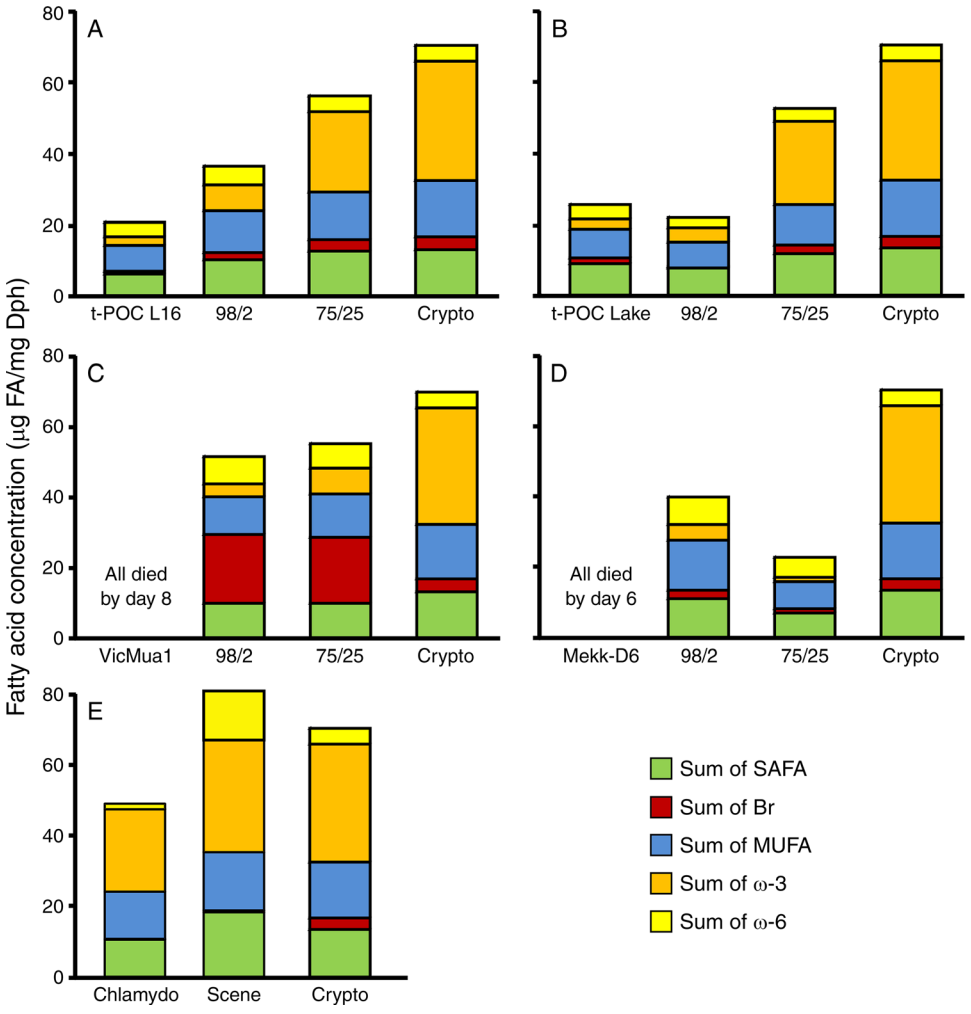

FIG. 2. (A–D) Fatty acid (FA) concentration of *Daphnia* (Dph; in dry mass) fed mixtures of experimental diets in comparison with the best diet, *Cryptomonas* (Crypto). (A) *Daphnia* fed with diets consisting t-POC diluted in L16 medium (t-POC L16), (B) t-POC diluted in lake water (t-POC Lake), (C) Actinobacterium VicMua1, and (D) Betaproteobacterium Mekk-D6. In panels (A–D), the FA concentrations of *Daphnia* fed with t-POC or bacteria, mixed with *Cryptomonas* in ratios of 98/2 and 75/25 are shown. Panel (E) shows the FA concentrations in *Daphnia* fed with algal monocultures (*Chlamydomonas* [Chlamydo], *Scenedesmus* [Scene], *Cryptomonas*). Abbreviations are: SAFA, saturated fatty acids; Br, branched fatty acids; MUFA, monounsaturated fatty acids; ω-3 and ω-6, omega-3 and omega-6 polyunsaturated fatty acids, respectively.

(Table 3). The assimilation of carbon and nitrogen from bacteria to *Daphnia,* fed with mixed diets of *Cryptomonas* and Actinobacterium VicMua1 or *Micrococcus luteus* (Taipale et al. 2012), matched well with the proportions of the bacteria in the diets (see r^2 values in Table 3). Carbon assimilation from Actinobacteria increased linearly in *Daphnia* with the proportion in the diet ($F_{1,5} = 3145.277$, $r^2 = 0.998$, $I < 0.0005$; Fig. 3A). The mixed ratio of 75% of VicMua1 bacteria resulted in 72% ± 3% of carbon and 88% ± 6% of nitrogen originating from bacteria (mean ± SD). In contrast, *Daphnia* in the 75% t-POC L16 diet treatment incorporated only 24% ± 7% of their carbon and 31% ± 10% of their nitrogen from the t-POC source. The incorporated proportions of the 75% t-POC Lake diet were 44% ± 5% for carbon and 9% ± 6% for nitrogen, respectively. However, when the proportion of t-POC was 95%, mixed with either *Scenedesmus* or *Cryptomonas,* the proportion of t-POC carbon in *Daphnia* increased up to 68–72% (t-POC L16) and 58–65% (t-POC Lake).

The contribution of actinobacterial FAs increased linearly in *Daphnia* with increasing proportion in their diet ($F_{1,5} = 322.87$, $r^2 = 0.984$, $I < 0.0005$; Fig. 3B). However, the proportion of bacterial FAs in *Daphnia* was much less than what was available in the diet, e.g., when the diet consisted of 75% of actinobacteria, *Daphnia* FAs were only 28–36% of bacterial origin. The contribution of assimilated FAs into *Daphnia* using total FA fits resulted in a slightly higher FA assimilation for the t-POC mixed diet than for bacteria mixed diets

TABLE 2. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values (mean ± SD) and total fatty acid to carbon ratio (totFA : C) of *Daphnia* at the end of the experiments.

Diets	Percentages in diet	^{13}C (‰)	^{15}N (‰)	totFA : C
Life table experiment				
Cryptomonas	100	37 ± 8.1	7.0 ± 0.1	0.033
Scenedesmus	100	−19.20	−8.10	0.033
Chlamydomonas	100	−25.20	−7.90	0.020
t-POC Lake	100	nd	nd	0.010
t-POC L16	100	nd	nd	0.018
t-POC L16/*Cryptomonas*	98/2	nd	nd	0.013
t-POC L16/*Cryptomonas*	75/25	21.3	−5.0	0.029
t-POC Lake/*Cryptomonas*	98/2	nd	nd	0.018
t-POC Lake/*Cryptomonas*	75/25	8.3	−6.2	0.024
VicMua1/*Cryptomonas*	98/2	−19.5	−6.3	0.025
VicMua1/*Cryptomonas*	75/25	−5.0	4.7	0.025
Mekk-D6/*Cryptomonas*	98/2	nd	nd	0.021
Mekk-D6/*Cryptomonas*	75/25	nd	nd	0.015
t-POC L16/VicMua1/ Mekk-D6/*Cryptomonas*	48/20/20/2	−17.7	3.6	0.016
Batch experiment				
Cryptomonas	100	9.7 ± 3.6	−6.5 ± 0.1	0.030
Scenedesmus	100	52.4 ± 0.8	−10.2 ± 0.7	0.032
t-POC L16/*Cryptomonas*	95/5	15.9	−5.0	0.015
t-POC L16/*Scenedesmus*	95/5	−5.7	−5.0	0.014
t-POC L16/*Scenedesmus*†	50/50	−12.7 ± 0.1	6.3 ± 0.2	nd
t-POC L16/*Scenedesmus*†	25/75	−13.4 ± 0.6	5.9 ± 0.4	nd
t-POC L16/*Scenedesmus*†	5/95	−14.6 ± 0.4	6.0 ± 0.2	nd
t-POC Lake/Cryptomonas	95/5	−12.2	−3.3	0.016
t-POC Lake/*Scenedesmus*	95/5	−0.1	−3.1	0.014

Notes: Because of the high mortality of *Daphnia* in some treatments, there was not enough material for all analyses (nd = no data).

† Results from additional batch experiments, in which of the $\delta^{13}C$ and $\delta^{15}N$ values of *Daphnia* fed ^{13}C unenriched *Scenedesmus* were −12.2 ± 0.1‰ and 6.2 ± 0.0‰, respectively.

TABLE 3. Assimilated proportions (mean ± SE) of carbon and nitrogen from t-POC or bacteria by *Daphnia* based on analyses of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (results of IsoError model) as well as assimilated proportions of t-POC and bacteria by *Daphnia* based on total FA profiles (TFA, mean ± SD; calculated according to Brett et al. 2009), and ω-3:ω-6 ratios (mean ± SD) of diets and *Daphnia* in different experiments.

Mixed diets	Percentages in diet	Assimilated (%, $\delta^{13}C$)	Assimilated (%, $\delta^{15}N$)	Assimilated (%, TFA)	r^2	Error SS	Assimilated (%, ω-3 : ω-6)
Life table experiment							
t-POC L16/*Cryptomonas*	98/2	nd	nd	48 ± 1	0.89	110	90 ± 6
t-POC L16/*Cryptomonas*	75/25	24 ± 7	31 ± 10	0 ± 1	0.95	45	13 ± 6
t-POC Lake/*Cryptomonas*	98/2	nd	nd	85 ± 1	0.97	27	90 ± 6
t-POC Lake/*Cryptomonas*	75/25	44 ± 5	9 ± 6	6 ± 1	0.97	31	31 ± 6
VicMua1/*Cryptomonas*	98/2	97 ± 1	100 ± 6	40 ± 6	0.55	391	nd
VicMua1/*Cryptomonas*	75/25	72 ± 3	88 ± 6	36 ± 1	0.73	199	nd
Mekk-D6/*Cryptomonas*	98/2	nd	nd	35 ± 1	0.82	219	nd
Mekk-D6/*Cryptomonas*	75/25	nd	nd	70 ± 8	0.83	302	nd
Batch experiment							
t-POC L16/*Cryptomonas*	95/5	68 ± 3	19 ± 8	24 ± 1	0.93	60	68 ± 6
t-POC L16/*Scenedesmus*	95/5	72 ± 1	45 ± 6	36 ± 1	0.84	166	47 ± 3
t-POC L16/*Scenedesmus*†	50/50	26 ± 3	25 ± 17	nd	nd	nd	nd
t-POC L16/*Scenedesmus*†	25/75	13 ± 5	33 ± 28	nd	nd	nd	nd
t-POC L16/*Scenedesmus*†	5/95	5 ± 1	0 ± 12	nd	nd	nd	nd
t-POC Lake/*Cryptomonas*	95/5	58 ± 3	31 ± 6	41 ± 1	0.95	47	75 ± 6
t-POC Lake/*Scenedesmus*	95/5	65 ± 1	50 ± 5	61 ± 1	0.94	56	39 ± 3

Notes: For total FAs we maximized total fit (r^2) and minimized error sum of squares (Error SS) between hypothetical FA profile and actual FA profile of *Daphnia*. In some treatments, *Daphnia* mortality was high, and there was not enough material for stable-isotope analyses. Due to lack of PUFAs in bacteria, ω-3 : ω-6 based analyses were not possible for bacteria-mixed treatments (nd = no data).

† Results from additional batch experiments.

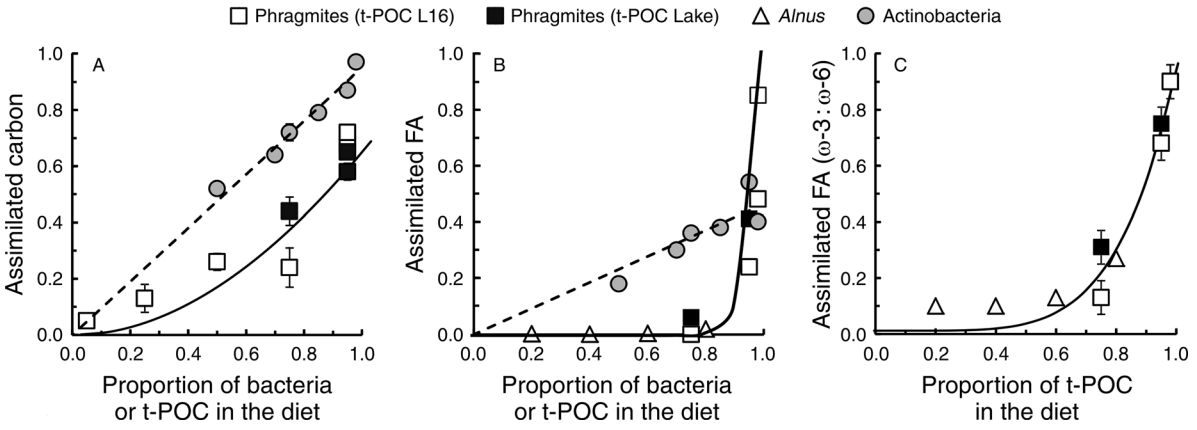

FIG. 3. Proportions (±SD) of assimilated bacteria and t-POC by *Daphnia* related to the proportions of bacteria and t-POC in the diets, which were mixed with algae (*Scenedesmus* or *Cryptomonas*). Actinobacteria consisted of either *Micrococcus luteus* or VicMua1, and t-POC consisted of red alder (*Alnus rubra*) or common reed (*Phragmites australis*), diluted with L16 culture medium or lake water. Linear or power function lines of regression equations: (A) Assimilated carbon originating from Actinobacteria ($y = 0.951x$) and from t-POC ($y = 0.71x^{1.86}$) based on $\delta^{13}C$ measurements, (B) assimilated FAs based on total FAs (Actinobacteria, $y = 0.473x$; t-POC, $y = 1.04x^{22.23}$), and (C) assimilated FAs based on the ω-3 : ω-6 ratio ($y = 0.97x^{5.23}$). The results for red alder are from Brett et al. (2009), and those for Actinobacteria *Micrococcus luteus* are from Taipale et al. (2012).

when mixed with 2% *Cryptomonas*. When t-POC was mixed with ≥25% *Cryptomonas*, the assimilation of FAs originating from t-POC was <6% (data also for the assimilation of *Alnus rubra* particles from Brett et al. 2009). Thus, the relationship between t-POC in the diet and assimilated carbon or FAs in *Daphnia* was not linear (Appendix F). However, when assimilation was estimated using the ω-3 : ω-6 FA ratio, the estimated assimilation was higher (13–31%) for t-POC origin FAs than calculated using the complete FA profiles (Fig. 3C). We also calculated the contribution of assimilated FAs for *Daphnia* fed with the four type mixed diets (58% of t-POC diluted with L16, 20% of Actinobacterium VicMua1, 20% of Betaproteobacterium Mekk-D6, and 2% of *Cryptomonas*), which resulted in a high fit ($r^2 = 0.85$). In this experiment, 59% of *Daphnia* FAs originated from t-POC, 32% from *Cryptomonas*, 8% from Actinobacterium VicMua1, and 0% from Betaproteobacterium Mekk-D6.

DISCUSSION

For the crustacean *D. magna*, the diet quality of terrestrial organic particles, two heterotrophic bacterial strains, and three phytoplankton species, differed greatly in terms of PUFA, sterol, carbon, nitrogen, and phosphorus content. As observed in previous studies (Martin-Creuzburg et al. 2011, Taipale et al. 2012), solely bacterial diets were not nutritionally adequate for *Daphnia* because they lacked PUFA and sterols. In contrast, t-POC contained sterols, ω-6 and ω-3 FAs, but in much lower levels than those in phytoplankton. This indicates that t-POC food quality was especially restricted with regard to ω-3 PUFAs. The phosphorus content was higher in bacterial diets than in phytoplankton or t-POC diets, but this did not improve the survival or growth of *Daphnia* in our experiments. Somatic growth rates and offspring production of *Daphnia* correlated positively with the concentration of dietary essential FAs and sterols and TotFA : C and PUFA : C ratios.

Here, we show that *Daphnia* is able to utilize different types of t-POC (e.g., milled foliage of deciduous trees or riparian grass) directly, but that these resources yielded much lower growth and net reproductive output compared with *Daphnia* raised on pure phytoplankton or phytoplankton mixed with the bacterial strains. The poor performance of *Daphnia* on t-POC diets found here are consistent with conclusions of Brett et al. (2009). The assimilation of FAs and carbon from t-POC was low and was related to the relative contribution of phytoplankton in the diet. However, the food mixture consisting of 75% t-POC and 25% *Cryptomonas* yielded better growth rates of *Daphnia* than the pure green algal (*Chlamydomonas* and *Scenedesmus*) diets (cf. Fig. 1). This might be due to the fact that the supply of *Cryptomonas* (25% of the diet, 0.3–1.25 mg C/L; see *Materials and methods*) in our experiment was high enough for *Daphnia*, supporting a higher growth rate than that by green algae monocultures, which do not contain EPA (Brett et al. 2006).

The assimilated proportion of carbon, nitrogen, and FAs in *Daphnia* increased linearly with an increasing proportion of bacteria in the diet. However, *Daphnia* assimilated bacterial FAs with lower efficiency than bacterial total carbon (Fig. 3A, B). Accordingly, the assimilation of FAs into *Daphnia* from t-POC, based on FA profiles, was 7–38% lower than carbon assimilation based on stable-isotope values. However, the ω-3 : ω-6 PUFA ratio model calculations resulted in similar estimates of assimilated terrestrial resources as modeled from stable isotopes (Table 3). Therefore, our calculations suggest that the ω-3 : ω-6 PUFA ratio is a good

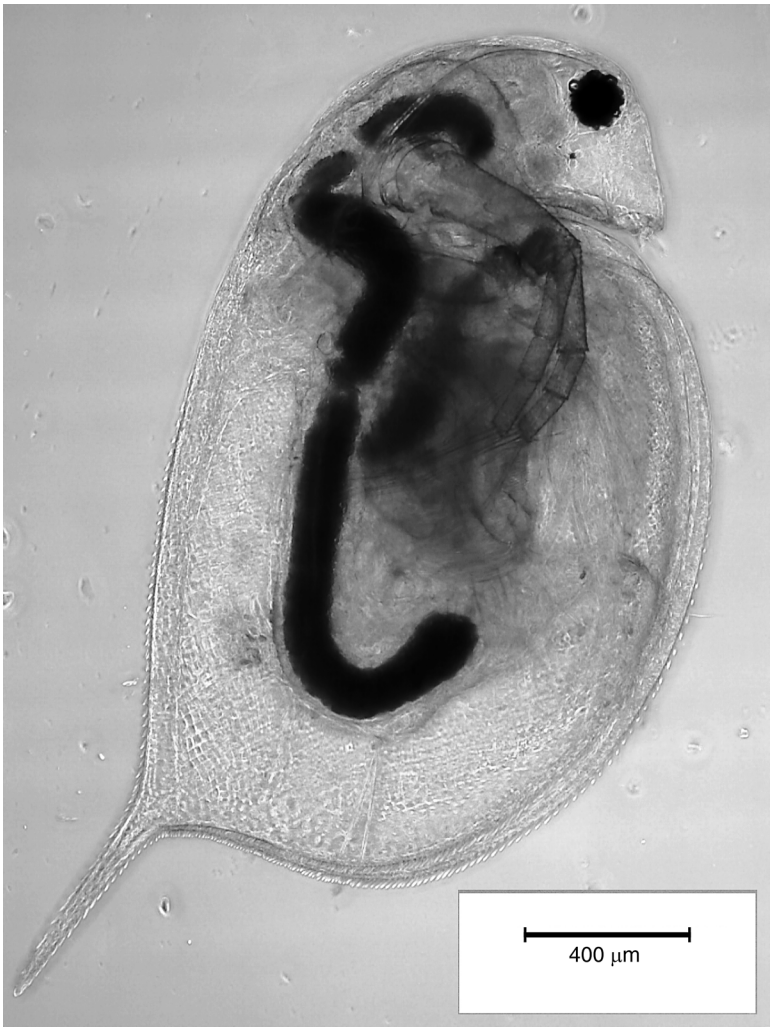

PLATE 1. A light-microscope image of freshwater crustacean *Daphnia magna* after feeding 10 days on a t-POC diet. Diet source can be seen as black pigment in *Daphnia* gut. Photo credits: S. J. Taipale.

indicator of terrestrial diet origin in the food web (cf. Torres-Ruiz et al. 2007). The differential assimilation of FAs and total carbon found here may, in part, explain the differences in the estimated contribution of allochthonous carbon in the pelagic food webs, based either on FAs or stable isotopes of carbon, nitrogen, and hydrogen (Brett et al. 2009, Cole et al. 2011).

Our direct assimilation measurements showed that the maximum contribution of t-POC into *Daphnia*, either as carbon or FAs, could be as high as 68–72%, but this only happened in treatments where only ~5% of particulate organic carbon consisted of phytoplankton (Table 3). However, these conditions poorly supported zooplankton growth. We found that with increasing totFA : C and PUFA : C ratios, the biomass growth rate simultaneously increased in *Daphnia*. PUFAs and sterols from algae were especially needed for reproduction (see Fig. 1B), whereas bacterial and terrestrial organic carbon and FAs were presumably mainly used for somatic growth and metabolism. Thus, it seems that high quantities of t-POC only partially can support zooplankton production and that the transfer of allochthonous particulate organic matter to upper trophic level is inefficient. The poor assimilation of t-POC might be due to its higher content of structural biochemicals that cannot be conveyed efficiently to the upper trophic levels of aquatic food webs (Kainz et al. 2002, Brett et al. 2009). The polymeric structure of terrestrial organic matter requires enzymatic cleavage via microbial extracellular enzymes or cleavage by photo-oxidation, both of which can allow bacterial utilization of terrestrial carbon (Münster and De Haan 1998). In our study, t-POC decayed for two months in lake water containing a natural microbial community yielded better growth and greater terrestrial carbon assimilation by *Daphnia* than t-POC diluted directly to synthetic algal growth medium. Thus, these results suggest primary importance of terrestrial carbon pro-

cessing by bacteria or transfer via a microbial link (bacteria–flagellate–ciliate) to *Daphnia*. Most previous studies also suggest that terrestrial carbon is mainly linked to zooplankton production via the microbial pathway (Karlsson et al. 2003, Jansson et al. 2007, Berggren et al. 2010).

Our laboratory experiment showed that *Daphnia* are able to assimilate bacterial carbon with similar efficiency as phytoplankton if the required essential FA and sterols are obtained from other sources (also see Taipale et al. 2012, Wenzel et al. 2012). *Daphnia* consuming strains of Actinobacterium VicMua1 and Betaproteobacterium Mekk-D6 bacteria were able to survive and to sustain positive growth rates with as little as 2% of the diet originating from *Cryptomonas*, which contained 0.2 μg sterols, and 0.2 μg ω-6 and 1 μg ω-3 PUFAs per mg C. However, *Daphnia* was not able to reproduce with only 2% *Cryptomonas* in their diet. Any reproduction on t-POC diets required at least 25% *Cryptomonas*, corresponding to 1.4 μg sterols, 1.4 μg ω-6 and 13.4 μg ω-3 PUFAs per mg C. Our results, therefore, also indicate that, for *Daphnia* reproduction, generally, higher levels of sterols, and ω-6 and ω-3 PUFAs are needed.

Freshwater systems have different types of bacteria that potentially transfer autochthonous and allochthonous carbon to upper trophic levels via zooplankton. Heterotrophic bacteria, such as Actinobacteria and Proteobacteria are typical in the oxic epilimnia of the lakes (Zwart et al. 2002, Jezberová et al. 2010) where they utilize organic carbon. Several studies have shown qualitative differences between bacterial taxa as diet sources for zooplankton (Deines and Fink 2011, Martin-Creuzburg et al. 2011, Taipale et al. 2012, Wenzel et al. 2012). The results obtained in the present study with Actinobacterium VicMua1 were similar with the previous results on *Daphnia* fed with heterotrophic *Micrococcus luteus* (Taipale et al. 2012), indicating that *Daphnia* can maintain high somatic growth rates when Actinobacteria is mixed with high-quality phytoplankton. *Daphnia* did not grow well with the Betaproteobacterium Mekk-D6 tested here, which might be toxic for *Daphnia* because of the presence of cyclopropanes, which may be enzyme inhibitors and possess insecticidal, antifungal, and herbicidal properties (for review see Salaün and Baird 1995).

Our results suggest that, although planktonic crustaceans could consume t-POC, the microbial food chain (DOC–bacteria–protozoa) is a more important route for transferring allochthonous organic carbon to zooplankton in most freshwater systems. However, a recent analysis of bacterial and primary production of boreal lakes suggests that bacteria are potentially important resources for zooplankton only in small lakes (Kankaala et al. 2013). Moreover, poor growth efficiency of bacteria utilizing allochthonous DOC, compared with autochthonous DOC (del Giorgio and Cole 1998, Kritzberg et al. 2006, Berggren et al. 2009), and the additional steps in the microbial food chain, from bacteria via protozoans and/or mixotrophic flagellates to crustacean zooplankton, may lead to large respiratory losses (cf. Blomquist et al. 2001, Berglund et al. 2007, Kankaala et al. 2010*a*). Our results show that essential biochemicals from phytoplankton are needed to support adequate zooplankton growth and reproduction, and that terrestrial organic carbon is a poor basal resource for the upper trophic levels of lakes.

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Supplemental Material

Appendix A

Detailed concentration information of added nutrients in batch and life table experiments (*Ecological Archives* E095-049-A1).

Appendix B

Fatty acid and sterol profiles of terrestrial particulate organic carbon (t-POC), bacteria, and phytoplankton diets (*Ecological Archives* E095-049-A2).

Appendix C

Results of Cox regression survival analysis of experiments (*Ecological Archives* E095-049-A3).

Appendix D

Correlations between different nutrients of diets and offspring and body size of *Daphnia* (*Ecological Archives* E095-049-A4).

Appendix E

Principal component analysis of diets and *Daphnia* (*Ecological Archives* E095-049-A5).

Appendix F

The parameter estimates of t-POC diets for power functions (*Ecological Archives* E095-049-A6).