Diet-specific biomarkers show that high-quality phytoplankton fuels herbivorous zooplankton in large boreal lakes

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SUMMARY

1. The zooplankton is a key link in the transfer of energy from primary producers up through aquatic food webs. Previous efforts to quantify the importance of basal resources to aquatic consumers have used stable isotopes (SI) and simple ternary models, including only 'bulk' phytoplankton, bacteria or terrestrial particulate organic matter (t-POM).

2. We used a novel Bayesian mixing model based on fatty acids (FA) to quantify the dietary assimilation of seven basal resources, including five phytoplankton groups, pelagic bacteria and t-POM, to Cladocera in large boreal lakes in Finland. To account for trophic enrichment of FA from the diet to consumers, we parameterised the model with a resource library, from many feeding trials, consisting of *Daphnia magna* fed 22 diverse basal taxa.

3. The results of the feeding trials show that the distinctive FA profiles of algal groups are transferred to consumers. Moreover, the large number of FA variables (n = 22) used in the model avoids the limitations of underdetermined mixing problems, common to SI modelling, in cases when the number of resources outnumbers the tracer variables.

4. We show that cladocerans were generally supported by phytoplankton (86–94%), with little use of t-POM (1–9%) and bacteria (1–3%). Cladocerans used primarily high-quality phytoplankton (cryptophytes, diatoms and dinoflagellates) in both summer (51 ± 22%) and autumn (79 ± 12%), and the relative importance of medium-quality resources (cyanobacteria, chlorophytes and chrysophytes) declined from 37 ± 23% in the summer to 8 ± 2% in the autumn.

5. High-quality resources, rich in essential biochemical compounds, are critical in fuelling food webs in large lakes, even those with high concentrations of allochthonous organic matter.

Keywords: allochthony, Bayesian mixing model, *Daphnia*, fatty acids, resource partitioning

Introduction

The zooplankton is an important link in the transfer of energy, in the form of organic carbon and essential biomolecules, from basal resources to consumers higher in the food web, especially to fish. Pelagic freshwater food webs are based predominantly upon primary production by phytoplankton, but consumers may also be subsidised by terrestrial organic matter, either by direct use of terrestrial particulate organic matter (t-POM) or via the dissolved organic matter (DOM)–bacteria–protozoa microbial food chain (Carpenter *et al.*, 2005; Jansson *et al.*, 2007; Kankaala *et al.*, 2013). Recent investigations into the importance of terrestrial organic matter for pelagic freshwater zooplankton have generated contrasting views about the general importance of allochthonous

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resources to zooplankton in lakes (Jones *et al.*, 1998; Brett *et al.*, 2009; Cole *et al.*, 2011; Francis *et al.*, 2011). The documented increase in the terrestrial DOM concentration in freshwater ecosystems in boreal and temperate areas (e.g., Monteith *et al.*, 2007; Couture, Houle & Gagnon, 2012) calls for detailed information on the effects of DOM on aquatic food webs in different lake ecosystems. Most studies have focussed on small- and medium-size lakes, and there is still limited understanding how increased loading of allochthonous carbon will affect pelagic trophic relationships in large (e.g. >100 km^2) lakes (Finstad *et al.*, 2014). Large lakes generally have longer food chains than smaller lakes (Post, Pace & Hairson, 2000) and yield greater harvests of commercially important fish exploited by humans. Thus, the composition and quality of the basal food web resources in the large lakes also have wide ecological and economic relevance.

The quality of dietary resources affects zooplankton assimilation efficiency, somatic growth and reproduction (Taipale *et al.*, 2014). High-quality resources, which contain essential phytosterols (Martin-Creuzburg & Von Elert, 2009), fatty acids (FA) and amino acids (Müller-Navarra, 2008), are likely to have disproportionately large effects on food webs (Brett *et al.*, 2009). In freshwater systems, several ω-3 and ω-6 polyunsaturated fatty acids (PUFA) such as 20 : 5ω3 (EPA), and 22 : 6ω3 (DHA), are essential compounds that are needed for growth and reproduction of fish and zooplankton (Sargent *et al.*, 1999; Müller-Navarra, 2008). An abundance of phytosterols and ω-3 PUFA generally infers that phytoplankton is of high food quality for zooplankton (Brett *et al.*, 2009). Previous studies with the filter-feeding cladoceran zooplankter *Daphnia* have shown that cryptophytes and diatoms are excellent food and chlorophytes and cyanobacteria are intermediate, while t-POM and bacteria are of poor quality due to an absence or low content of PUFA and sterols (Brett *et al.*, 2009; Taipale *et al.*, 2014). The FA profiles of *Daphnia* have been shown clearly to reflect that of their phytoplanktonic (Brett *et al.*, 2006) and bacterial (Taipale *et al.*, 2012) diets. The complex FA signatures of different phytoplankton lineages, bacteria and t-POM also have taxon-specific characteristics and differences (Gugger *et al.*, 2002; Kelly & Scheibling, 2012; Taipale *et al.*, 2012, 2013). Environmental conditions, such as light intensity, temperature and nutrient limitation (Piepho, Arts & Wacker, 2012), have a secondary influence on the FA profiles of algae, whereas the major differences in algae FA are explained by phylogenetic relationships (Galloway *et al.*, 2012; Taipale *et al.*, 2013).

Previous efforts to partition the relative importance of different energy sources to consumers in aquatic food webs (e.g., Pace *et al.*, 2004; Carpenter *et al.*, 2005; Taipale *et al.*, 2008) have mainly used a simple ternary model of basal resource groups, including 'bulk' phytoplankton, bacteria and allochthonous t-POM. Estimates of resource use for aquatic consumers have long relied upon stable isotope (SI) mixing models (Phillips & Gregg, 2003) of the carbon and nitrogen isotope compositions of putative resources and consumers. A limitation of this approach is that natural systems have many more potential dietary sources than there are SI tracers (Fry, 2013a). Recent studies have concluded that Bayesian mixing models are robust to limitations on the number of sources (Ward *et al.*, 2011; Semmens *et al.*, 2013), but the application of SI-based mixing models is clearly limited in cases where distinct resources have overlapping SI signatures. For example, even when primary producers are labelled *in situ* (C fixation) by the addition of ^{13}C-enriched bicarbonate to the water column (Cole *et al.*, 2006; Taipale *et al.*, 2008), mixing models cannot resolve varying contributions from specific phytoplankton groups to consumers. This is because it has not been possible to isolate and measure empirically SI ratios of components of the seston, which contains many groups (different phytoplankton phyla, bacteria, protozoa, detritus) consisting of dozens of species (see Vuorio, Meili & Sarvala, 2006) that may be highly dynamic in time and space (Wetzel, 2001).

New approaches for quantifying the contribution of various basal resources to species feeding higher in the food web are needed to account for both resource quality and quantity. Fatty acids are commonly used as trophic biomarkers (Dalsgaard *et al.*, 2003), but are rarely used for quantitative estimation of consumer diets. The fine phylogenetic resolution of the FA signatures of basal resources, combined with the predictable transfer of these biomarkers into zooplankton, makes mixing model analyses a promising approach for quantitative estimation of consumer diets in lakes. We used a Bayesian mixing model based on FAs to calculate the proportional use of basal resources by wild cladoceran zooplankters in six large boreal lakes, with differing dissolved organic carbon (DOC) content among lakes and dynamic phytoplankton community composition. We parameterised the mixing model with a resource library of FA profiles of *Daphnia magna* (hereafter *Daphnia*) raised on diverse basal diets. These feeding trials are necessary because certain FA in consumers may be preferentially retained or synthesised from dietary FA (Bell & Tocher, 2009; Taipale, Kainz & Brett, 2011). Our

analyses therefore account for such FA modification by cladocerans for each of the diets evaluated with the mixing model because we first measured and accounted for the retention of these FA in the experimental consumers. We used the model to ask (i) what is the relative use of bacteria, t-POM and five phytoplankton groups by Cladocera in large lakes?; (ii) is the modelled resource use correlated with observed lake DOC and phytoplankton community composition?; and (iii) does the assimilation of low-, medium- and high-quality diets by Cladocera differ seasonally?

Methods

Generating signatures of prey library and mixtures

Zooplankton diets. To generate a resource library relevant to the potential diets of cladocerans in the lakes under investigation, we prepared a taxonomically diverse assemblage of eight diets ('source groups'), including microalgae, bacteria and t-POM (Table 1), for subsequent feeding trials with *Daphnia*. Where possible, a number of taxa were included in this list from within each of the major source groups. Microalgae were cultured, following Taipale *et al.* (2013), as food for *Daphnia* in batch experiments (see below). When several taxa comprised a group signature (e.g. Cryptophyceae, where the group $n = 3$), each taxon FA profile was the mean of experimental replicates ($n = 2$–5) of *Daphnia* fed that taxon, and the standard deviation (SD) of the group was calculated across taxa. For groups with only one taxon represented (e.g. Dinophyceae), the mean ± SD was calculated from the experimental replicates for that taxon. We assigned (*a priori*) a food quality ranking to these groups (e.g. Brett & Muller-Navarra, 1997; Brett *et al.*, 2009) based on the relative abundance of long-chain essential FA (e.g., $\geq C_{20}$ ω-3 and ω-6 FA) in these resources as (Taipale *et al.*, 2013): 'high quality' (cryptophytes, diatoms and dinoflagellates), 'medium quality' (chlorophytes, cyanobacteria and chrysophytes) and 'low quality' (bacteria and t-POM).

The heterotrophic gram-positive lake bacterium Actinobacterium *Micrococcus luteus* (ATCC 4698) was cultured in liquid NSY medium on a rotary shaker at *c.* 20 °C (Taipale *et al.*, 2012). Actinobacterium was selected because it is a typical pelagic bacterium in large lakes (Newton *et al.*, 2011). The t-POM diets were prepared using leaf particles of three common boreal riparian species: common reed (*Phragmites australis*), birch (*Betula pubescens*) and dwarf arctic birch (*Betula nana*). Common reed and birch leaves were collected from the shore of Lake Pyhäselkä (eastern Finland), arctic birch was collected along Lake Kilpisjärvi (northern Finland), and leaves were ground into small particles using a Fritsch Planetary Mono Mill Pulverisette 6 (reed) or Retch ZM 100 GWB ultra centrifugal mill (birch) and sieved to obtain a realistic particle size distribution for *Daphnia* diets following Taipale *et al.* (2014). Briefly, ground birch was diluted in WC medium and incubated for one month in the dark with continuously shaking at 120 rpm. The reed profile used in the library is the mean of two experimental replicates of *Daphnia* fed either reed particles that were diluted directly into L16 media or diluted into lake water and incubated for 2 months in the dark, to simulate decay by a natural microbial assemblage (Taipale *et al.*, 2014).

Batch experiments. We conducted several 10- to 14-day *Daphnia* feeding trials between 2009 and 2013 to generate a resource library of *Daphnia* FA profiles on each pure monoculture diet (Table 1). *Daphnia* neonates (*c.* 6 h old) were first cultured for 2 days with *Scenedesmus gracilis* (Chlorophyceae) and then switched to the experimental diet (Table 1). *Daphnia* were cultured in 200-mL glass jars with ADAM medium at 20 ± 1 °C. The medium was changed, and the *Daphnia* fed every other day by bringing the total food concentration to >2 mg C L^{-1}. At the end of feeding trials, *Daphnia* were lyophilised and stored at −80 °C.

We also evaluated the FA profiles of *Daphnia* from a previous bacteria–cryptophyte mixture feeding trial (Taipale *et al.*, 2012) and a controlled *Daphnia* starvation experiment. In the mixture experiment, *Daphnia* were fed a blend of cryptophytes and Actinobacteria in a concentration gradient [mix number (ratios of % cryptophytes : Actinobacteria) in mixes: 1 (50 : 50); 2 (30 : 70); 3 (15 : 85); 4 (5 : 95)] to test four of the five mixtures of the bacterial FA assimilation relationships reported in Taipale *et al.* (2012). In the starvation experiment, *Daphnia* neonates were initially fed one of three representative phytoplankton genera from each of three lineages for 6 days (Cryptophyceae, *Cryptomonas ozolinii*; Fragilariophyceae, *Fragilaria crotonensis*; Chlorophyceae, *Scenedesmus obliquus*) and starved for 6 days prior to FA analyses.

Lake phytoplankton and cladoceran sampling

We collected seston (<50 μm) and zooplankton samples (net size 100 μm) from eight sampling stations in six large lakes (all >100 km^2, mean depth *c.* 8–10 m; see Table 2) in eastern Finland (Karjalan Pyhäjärvi 58, Orivesi 107, Orivesi 2, Paasivesi 5, Suvasvesi 29,

Table 1 Summary of microalgal, bacterial and t-POM diets cultured and fed to *Daphnia* in the life-table experiments to generate the biochemical signatures of the prey library. Source group naming follows the current high order designation in AlgaeBase (Guiry & Guiry, 2014) that is common to all members of the group. The Synurophyceae group is referred to throughout the paper as chrysophytes or the abbreviation 'Chryso' due to its common usage as such

	Group				
Source group	Abbreviation	Taxa *n*	Genus species	Order	Library *n**
Actinobacteria	Bacteria	1	*Micrococcus luteus*	Micrococcales	6
Chlorophyceae	Chloro	4	*Pediastrum sp.*	Sphaeropleales	1
			Scenedesmus ecornis	Sphaeropleales	1
			Chlamydomonas sp.	Chlamydomonadales	1
			Eudorina sp.	Chlamydomonadales	1
Cryptophyceae	Crypto	3	*Cryptomonas marsonii*	Cryptomonadales	1
			Cryptomonas ozolinii	Cryptomonadales	1
			Rhodomonas lacustris	Cryptomonadales	1
Cyanophyceae	Cyano	7	*Aphanizomenon sp.*	Nostocales	1
			Microcystis sp.	Chroococcales	1
			Microcystis aeruginosa	Chroococcales	1
			Planktothrix rubences†	Oscillatoriales	2
			Pseudanabaena limnetica	Pseudanabaenales	1
			Synechococcus elongatus	Synechococcales	1
Dinophyceae	Dino	1	*Peridinium aciculiferum*	Peridinales	2
Heterokontophyta	Diatom	4	*Fragilaria crotonensis*†	Fragilariales	2
			Asterionella formosa	Fragilariales	1
			Cyclotella meneghiniana	Thalassiosirales	1
Plantae	t-POM	3	*Phragmites australis*	Poales	1
			Betula pubescens	Fagales	1
			Betula nana	Fagales	1
Synurophyceae	Chryso	1	*Mallomonas caudata*	Synurales	2

*Number of taxa FA profiles used to build the library.
†Two strains.

Table 2 Location, total lake area, site depth, Secci depth and some chemical properties [dissolved organic carbon (DOC), total nitrogen (TotN), total phosphorus (TotP) and chlorophyll *a* concentration (fraction <50 μm)] of water in the uppermost 0–4 m layer (mean ± SD, June–September, n = 3–5) at sampling stations in the lakes in eastern Finland studied here. Lake Paasivesi is sub-basin of Lake Orivesi, but is presented here separately from the other parts of Orivesi. The full name of lake Karjalan Pyhäjärvi 58 is abbreviated as K. Pyhäjärvi 58

Lake	Latitude	Longitude	Area (km^2)	Depth (m)	Secchi depth (m)	DOC (mg C L^{-1})	TotN (μg L^{-1})	TotP (μg L^{-1})	Chl-*a* (μg L^{-1})
K. Pyhäjärvi 58	61°48.44N	29°52.74E	247	26	5.1 ± 0.3	5.3 ± 0.5	228 ± 20	4.9 ± 0.9	2.2 ± 0.3
Paasivesi 5	62°08.43N	29°26.70E	124	74	2.5 ± 0.5	8.4 ± 0.4	349 ± 27	5.7 ± 3.2	1.9 ± 0.5
Orivesi 107	62°10.51N	29°44.22E	477	11	2.1 ± 0.3	7.0 ± 0.3	304 ± 32	11.8 ± 1.0	6.2 ± 1.6
Orivesi 2	62°14.30N	29°26.82E	–	28	2.3 ± 0.4	8.7 ± 0.4	358 ± 30	8.2 ± 1.0	2.0 ± 0.4
Suvasvesi 29	62°39.95N	28°11.58E	233	85	3.1 ± 0.5	8.8 ± 0.4	490 ± 42	10.0 ± 7.3	2.6 ± 0.5
Pyhäselkä 5	62°27.89N	29°47.97E	361	68	1.9 ± 0.0	9.5 ± 0.5	399 ± 48	9.3 ± 0.6	1.5 ± 0.4
Suvasvesi 399	62°45.24N	28°01.74E	–	30	2.7 ± 0.5	9.9 ± 0.5	597 ± 84	10.8 ± 5.5	2.8 ± 0.5
Kallavesi 25	62°49.58N	27°52.21E	478	50	2.3 ± 0.6	10.2 ± 0.2	639 ± 107	12.1 ± 7.1	3.3 ± 0.6

Suvasvesi 399, Pyhäselkä 5, Kallavesi 25; station names follow those used by the water quality database of the Finnish Environment Institute, http://wwwp2.ymparisto.fi/scripts/oiva.asp) in the summer (early August) and autumn (late September) of 2011. Sampling of these lakes is part of an ongoing investigation assessing the importance of autochthonous and allochthonous resources in large boreal lake food webs along a gradient of total DOC concentration of *c*. 5–10 mg C L^{-1} (see Table 2). The DOC concentration of water was analysed with a Multi N/C (Analytic Jena) instrument. Total nitrogen, total phosphorus and chlorophyll *a* concentrations (pooled 0–4 m depth) were analysed with standard methods (www.sfs.fi).

Cladocera were separated from other zooplankton in the laboratory, and *Daphnia* spp. generally comprised >70% of the biomass of the pooled Cladocera group. We estimated phytoplankton community composition in

field samples using the Utermöhl method of settling a 25–50 mL seston subsample and with an inverted microscope counted and measured all the cells from 25 fields with two magnifications (500× small cells, 250× large cells). Phytoplankton data are presented as the per cent contribution of each group to the estimated biomass. The seasonal comparisons (see below) do not include results from either Karjalan Pyhäjärvi 58 (no autumn samples), or Paasivesi 5, due to insufficient cladoceran biomass for a FA sample.

Fatty acid analyses

Lipids from lyophilised *Daphnia* (0.3–0.5 mg) and Cladocera samples were extracted with a 2 : 1 : 0.75 chloroform : methanol : 1% NaCl mixture and methylated using sulphuric acid (1% v/v) in methanol. Experimental *Daphnia* FA methyl esters were analysed using a gas chromatograph (GC; Shimadzu Ultra) equipped with a mass spectrometer (MS) detector at the University of Jyväskylä (Finland), and FA methyl esters of wild Cladocera were analysed with GC-MS (Agilent 6890N and Agilent 5973N) at the University of Eastern Finland. *Daphnia* FA from the starvation experiment were analysed with GC flame ionisation detection (HP6890) at the University of Washington. All GC and MS methods followed Taipale *et al.* (2013).

Fatty acid mixing model

We used a Bayesian mixing model, 'FA Source Tracking Algorithm in R' (FASTAR), which is adapted from the SI-based MixSIR model of Moore and Semmens (2008), to calculate the proportional use of possible food resources to consumers that best reflect the FA profiles found in the wild consumers. The model uses a 'resource library' file consisting of mean ± SD FA profiles of *Daphnia* fed different primary producer taxa, and a consumer file of FA profiles of wild zooplankton. The general equation that describes the relative abundance of individual FA in a consumer as a result of mixing from multiple dietary sources with variable signatures is:

$$u_j = \sum_{i=1}^{n} p_i(m_{j,i} + f_{j,i})$$

where n represents the number of sources, $m_{j,i}$ and $f_{j,i}$ represent the mean and fractionation of source item i with respect to FA j, the vector p represents the estimated proportions or relative contributions for each source to the consumer (constrained to sum to 1.0), and u_j represents the mean of the mixture, as a derived parameter. The mixture variance can also be expressed in terms of the variances of the sources ($s^2_{j,i}$) and fractionation ($g^2_{j,i}$),

$$\sigma_j^2 = \sum_{i=1}^{n} p_i^2(S^2_{j,i} + g^2_{j,i}) \text{ (Moore Semmens, 2008).}$$

Because the resource library is from our feeding trials, which implicitly incorporate modification of dietary signatures by the consumer, we did not include trophic fractionation in the model.

The model results are summarised as posterior distributions that describe the probability of the proportional exploitation by the consumer of each resource. We used a multivariate uninformative prior for the proportions (Dirichlet) and an inverse gamma prior distribution on the residual variance parameter. We report the posterior distributions of the mixing solutions for each cladoceran FA profile (e.g. n = 14, where six stations were sampled in two seasons and two stations were sampled once), both graphically (as probability densities) and in tabular form as the median solution and the 95% credibility interval (CI) range (2.5–97.5 percentile range) for each resource. The posterior distributions were estimated using the Gibbs sampling algorithm of Markov Chain Monte Carlo (MCMC) implemented using the open-source Just Another Gibbs Sampler (JAGS) software (Plummer, 2003) within the R statistical software environment (R Development Core Team, 2013). Following previously published mixing models, we implemented the analysis using a normal likelihood function. MCMC chains were run for 100 000 iterations with a 50 000-iteration burn-in and a thinning rate of 50. Because the FA profiles of *Daphnia* fed chlorophytes and cyanobacteria overlapped in multivariate space (see below), we pooled these resources prior to FASTAR modelling. Prior to analyses, all branched FA (n = 6) were pooled, and the resulting 22 variable data set common to all samples was run on untransformed proportional FA data.

Data analyses

We used non-metric multidimensional scaling (NMDS; Euclidean distance) to visualise FA signatures of the resource library 'resource polygon' (e.g. Fry, 2013a), the experimental *Daphnia* mixtures, wild Cladocera and a subset of the taxa related to the *Daphnia* starving experiment in multivariate space. We used analysis of similarity (ANOSIM; one-way test, 9999 permutations; Clarke & Gorley, 2006) to test for seasonal differences in the phytoplankton community composition (Bray–Curtis

similarity). Multivariate plots used untransformed proportional data. Proportional consumer diet estimates from FASTAR (n = 14) were correlated (Pearson's correlations and associated P-values) with measured water chemistry variables (DOC, total nitrogen, phosphorus and chlorophyll *a*) and with proportions of each phytoplankton group estimated by microscopy from field samples of each sampling event. We also calculated a FA unsaturation index (UI; Logue *et al.*, 2000) to evaluate the potential confounding effects of water temperature in consumer FA content (e.g. Masclaux *et al.*, 2009). We correlated (Spearman's rho) measured lake water temperature with the UI to investigate whether cladocerans in colder water (e.g. Autumn samples) had a lower UI. In addition, we used a nonparametric Mann–Whitney U-test to compare summer and autumn lake Cladocera UI values. We used permutational multivariate ANOVA (PERMANOVA, Euclidean distance, 9999 permutations; Anderson, Gorley & Clarke, 2008) to compare the FA profiles of *Daphnia* starved or fed t-POM to those fed phytoplankton to test the significance (*post hoc*) of patterns observed in the starvation NMDS plot. We used the paired *t*-tests on arcsine-transformed proportional FASTAR results and observed phytoplankton abundance for each resource group across the two sampling events for lakes with both summer and autumn sampling. Univariate tests were conducted using R, SPSS v. 19.0 for Mac (IBM Inc., Armonk, NY, U.S.A.), and multivariate analyses were conducted using both PRIMER V.6.0+PERMANOVA (Primer-E Ltd., Plymouth, U.K.; Clarke & Gorley, 2006) and R.

Results

Resource library and cultured Daphnia

The FA profiles of *Daphnia* fed each of the members of the basal resource library were visualised in NMDS plots (Fig. 1a,b), and the FA profiles are summarised in Table S1. Each symbol on the plot is a species-level replicate of *Daphnia* fed a monoculture diet from that group, except for the dinoflagellate, chrysophyte and bacterial profiles, which are true within-strain experimental replicates (see Methods). With the exception of *Daphnia* fed chlorophytes and cyanobacteria, the FA profiles were clearly separated according to the different resource groups, as visualised with an NMDS ordination excluding bacterial diets (Fig. 1b). *Daphnia* fed Actinobacteria showed the greatest difference, grouping in the positive axis of NMDS1 (Fig. 1a) due to an abundance of branched FA and a general lack of PUFA. The *Daphnia* fed the *Cryptomonas* and Actinobacteria mixtures separated in the NMDS according to the mixture gradient (Fig. 1a).

The FA profiles of starved *Daphnia* that were fed initial diets of a common genus within each of the dominant phytoplankton groups plotted in multivariate space (negative axis of NMDS2) with animals experimentally fed t-POM diets (Fig. 2). The multivariate FA profiles of *Daphnia* fed algal diets (pooled as one group of 12) and

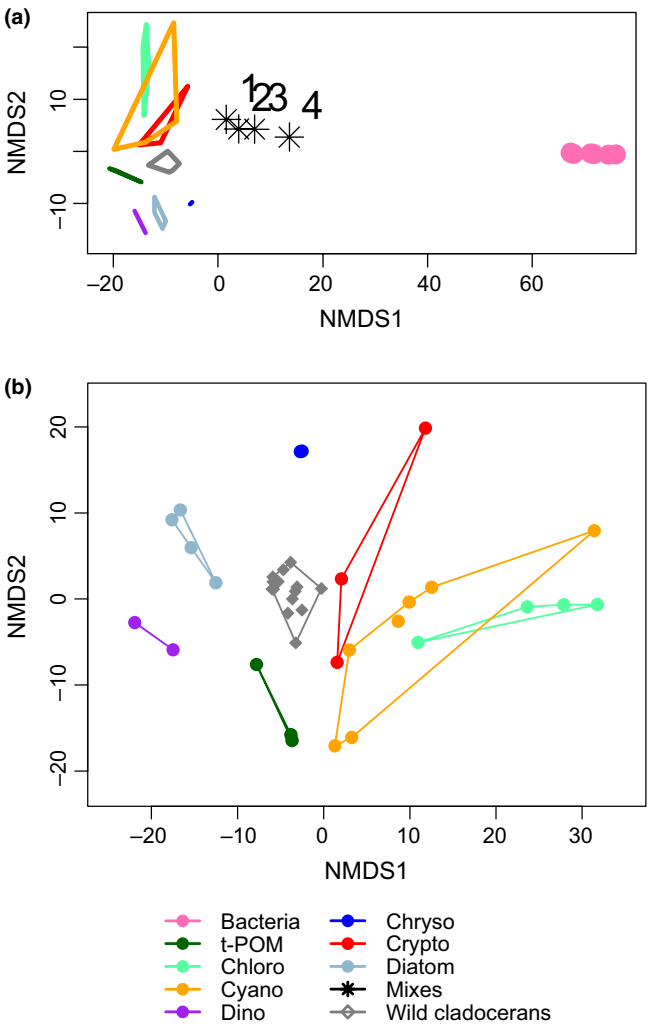

Fig. 1 Non-metric multiple dimension scaling (NMDS; Euclidean distance) plot showing the FA profiles of the end members, mixtures and wild Cladocera in multivariate space. (a) the NMDS ordination (stress = 0.07) is generated using all FA profiles to show the relation of the distinctive bacteria-fed *Daphnia* relative to other samples in 2D space. Numbers on mixtures refer to the gradient discussed in the text. For clarity, only the outer perimeter of each non-bacterial resource polygon is shown. (b) an NMDS ordination (stress = 0.11) of only the *Daphnia* fed non-bacterial diets, showing the positioning of each resource library end member and the 14 wild cladoceran samples from the study lakes in Finland (grey diamonds).

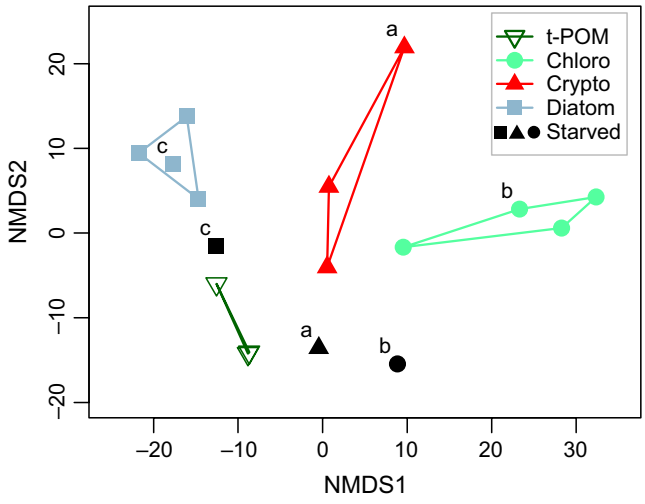

Fig. 2 NMDS plot of *Daphnia* starved and fed Cryptophyceae (red), Chlorophyceae (green), diatoms (light blue) and t-POM (dark green) diets (stress = 0.08). In the starvation treatments, *Daphnia* were initially fed one of the three representative phytoplankton genera from each of three lineages for 6 days [Cryptophyceae (filled triangles), *Cryptomonas*; Heterokontophyta (filled squares), *Fragilaria*; Chlorophyceae (filled circles), *Scenedesmus*] and starved for 6 days. The letter label indicates the genus of the library signatures that corresponds to the *Daphnia*, which were initially fed that same genus prior to starvation.

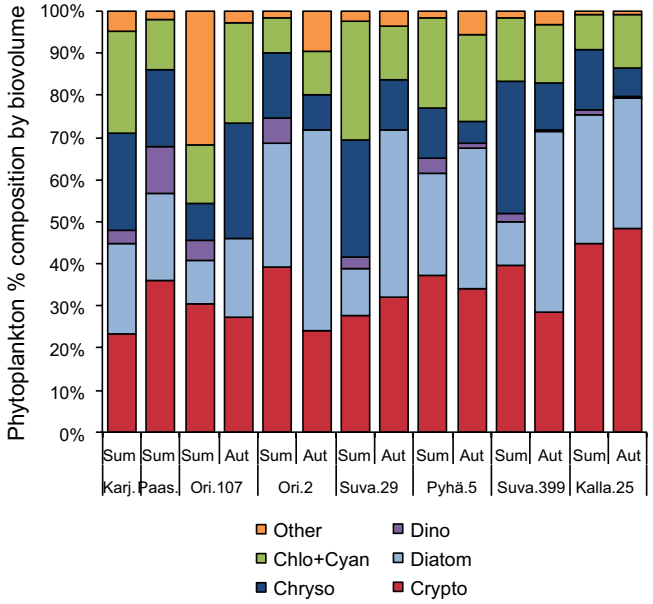

Fig. 3 Phytoplankton percentage community composition (by biovolume) in two seasons and across six large lakes in Finland. The five phytoplankton categories (excluding 'other') summarised here are the same as those used in modelling (abbreviations follow Table 1).

those fed t-POM and starved (pooled as one group of six) differed significantly (PERMANOVA, pseudo-F_1 = 3.66, P = 0.021).

Lake phytoplankton and cladocerans

The phytoplankton groups included in the resource library (Table 1) represented well the general phytoplankton composition in the oligotrophic and mesotrophic lakes studied. The mean ± SD of the 'other' phytoplankton category (i.e. those taxa that did not fall within our six initial phytoplankton groups; Table 1) was 5.1 ± 8.0%. Most of this variation was due to one sample from the Orivesi 107 station (summer) for which 31.8% of the total was 'other' (Fig. 3), due to the abundance of the raphidophyte [*Gonyostomum semen* (Ehrenberg) Diesing] in the sample. When this outlier sample was removed, the 'other' category accounted for 3.1 ± 2.4% of the total observed lake phytoplankton biomass across all lakes.

The observed phytoplankton community composition in the lakes (n = 14 samples) was positively correlated with FASTAR model estimate of cladoceran resource use for the diatom category (r^2 = 0.291, d.f. = 12, P = 0.046), but no significant correlations were found for other phytoplankton groups. The measured DOC, total nitrogen and total chlorophyll *a* content in the lakes did not correlate with any FASTAR category solution (Table 3). Total phosphorus was significantly positively correlated with the median FASTAR categories of pooled chlorophyte–cyanobacteria and chrysophytes and negatively correlated with the diatom category (Table 3). The ANOSIM test showed that the phytoplankton community composition (proportions of six groups) differed among seasons (ANOSIM, Global R = 0.234, P = 0.036). The per cent composition by biovolume of the phytoplankton groups is summarised for all study sites and dates in Fig. 3.

There was a slight negative but non-significant correlation between the highly variable FA unsaturation index of cladoceran consumers and lake water temperature, which ranged from *c*. 11 to 22 °C between the two sampling seasons (Spearman's rho = −0.388, n = 24, P = 0.061). The UI did not differ between the two sampling dates (Mann–Whitney U = 88.0, n = 24, P = 0.238).

Wild Cladocera FA profiles (Table S2) fitted within a central region of the NMDS ordination of the resource polygon and were surrounded by the end-member diet resources (Fig. 1b). Estimates of the dietary assimilation of each resource by the Cladocera are summarised in Table 4, as the median and 95% credibility intervals (CI) of the posterior model probability density for each diet, while Figure S1 represents all sample mixture distributions graphically. The sum of the median from all phyto-

	DOC		TotN		TotP		Chl-*a*	
FASTAR category	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Bacteria	0.11	0.713	0.27	0.344	−0.25	0.397	0.16	0.585
t-POM	−0.42	0.138	−0.49	0.076	0.12	0.674	0.14	0.640
Chlo+Cyan	−0.14	0.622	−0.32	0.263	0.63	**0.015**	0.44	0.117
Chryso	0.10	0.746	−0.21	0.480	0.61	**0.020**	0.17	0.564
Dino	−0.42	0.135	−0.19	0.521	0.17	0.556	0.45	0.106
Diatom	0.00	0.994	0.15	0.609	−0.56	**0.035**	−0.46	0.102
Crypto	0.40	0.154	0.49	0.078	−0.47	0.090	−0.34	0.232

Table 3 Pearson's product moment correlations (*r*) and associated *P*-values of median FASTAR model estimates of cladoceran use of seven resource categories and the four water chemistry variables at each station and season sampling event ($n = 14$). Degree of freedom for all tests is 12, and bold text indicates significant *P*-values

plankton taxa ranged from 86 to 95%, and the estimated median ± CI use of t-POM and bacterial diets across all samples (both seasons) were 4.5 ± 0–22% and 3 ± 0–6%, respectively (Table 4). Dinoflagellates and chrysophytes were generally of minor importance (all medians ≤9%) to cladocerans, except for in Orivesi 107 (Autumn; 20 ± 17–25%).

Cladocerans used primarily high-quality phytoplankton (cryptophytes, diatoms and dinoflagellates) in both summer [mean ± SD of the Σ of medians (51 ± 22%)] and autumn (79 ± 12%), and the contribution of medium-quality resources (cyanobacteria, chlorophytes and chrysophytes) declined from 37 ± 23% in the summer to 8 ± 2% in the autumn (Fig. 4a). Across all lakes for which there were both summer and autumn samples, FASTAR identified declines in the median usage by Cladocera of chrysophytes ($t = 3.52$, d.f. = 5, $P = 0.016$), chlorophytes–cyanobacteria ($t = 2.65$, d.f. = 5, $P = 0.045$) and t-POM ($t = 3.629$, d.f. = 5, $P = 0.016$), and increases in cryptophytes ($t = -3.456$, d.f. = 5, $P = 0.018$), bacteria ($t = -2.682$, d.f. = 5, $P = 0.047$) and diatoms (although not significant for this group) from the summer to the autumn (Fig. 4b). A summer and autumn comparison of the relative abundance of each observed phytoplankton group, corresponding to groups used in the FASTAR model, found significant declines in dinoflagellate abundance ($t = 3.740$, d.f. = 5, $P = 0.013$) and increased diatom abundance in the autumn ($t = -3.255$, d.f. = 5, $P = 0.023$), but no differences in other groups.

Discussion

We used a novel fatty-acid-based mixing model approach to quantify the use of five different phytoplankton groups, t-POM and Actinobacteria, by cladoceran consumers in large boreal lakes. In these lakes with rather

Table 4 FASTAR results showing estimated dietary use (%) of seven basal resource groups to wild cladoceran consumers in eight stations of six large boreal lakes in Finland. Abbreviations follow Tables 1 and 2. Due to overlap in FA profiles of *Daphnia* fed chlorophytes and cyanobacteria, for modelling purposes, these groups are combined (Chlo+Cyan; see Methods). Results are the medians (Mdn) followed by the 95% credibility interval (CI) from the probability densities of the FASTAR solutions of cladoceran utilisation of each resource

Season	Bacteria	t-POM	Chlo+Cyan	Chryso	Dino	Diatom	Crypto	ΣPhyto
Lake	Mdn (CI)	Mdn (CI)	Mdn (CI)	Mdn (CI)	Mdn (CI)	Mdn (CI)	Mdn (CI)	Mdn
Summer								
K. Pyhäjärvi 58	2 (1–3)	9 (1–19)	2 (1–18)	2 (0–8)	4 (3–6)	59 (49–66)	20 (12–31)	87
Paasivesi 5	1 (0–3)	4 (0–17)	49 (31–63)	5 (0–15)	0 (0–15)	27 (15–37)	9 (1–36)	90
Orivesi 107	3 (0–6)	9 (1–22)	36 (24–50)	5 (0–14)	0 (0–2)	33 (25–42)	12 (4–21)	86
Orivesi 2	1 (0–4)	7 (1–22)	42 (27–54)	6 (0–15)	0 (0–10)	35 (22–45)	5 (0–26)	88
Suvasvesi 29	1 (0–4)	5 (0–20)	66 (52–79)	9 (1–22)	0 (0–7)	11 (6–19)	3 (0–14)	89
Pyhäselkä 5	3 (1–3)	5 (0–16)	9 (0–30)	4 (0–11)	0 (0–22)	48 (30–63)	26 (2–51)	86
Suvasvesi 399	2 (0–3)	5 (0–16)	20 (2–34)	3 (0–11)	1 (0–21)	49 (32–62)	15 (3–31)	88
Kallavesi 25	2 (0–4)	6 (0–17)	33 (22–44)	6 (0–14)	2 (2–4)	30 (23–37)	20 (12–30)	90
Autumn								
Orivesi 107	3 (1–5)	1 (0–6)	36 (29–43)	2 (0–7)	20 (17–25)	25 (20–32)	12 (7–18)	94
Orivesi 2	3 (1–3)	3 (0–15)	10 (1–30)	4 (0–13)	0 (0–1)	57 (47–69)	18 (6–36)	90
Suvasvesi 29	3 (1–4)	3 (0–13)	4 (0–34)	2 (0–10)	0 (0–3)	54 (38–72)	26 (4–51)	87
Pyhäselkä 5	3 (2–3)	4 (0–13)	1 (0–19)	2 (0–8)	0 (0–1)	40 (24–71)	47 (9–66)	91
Suvasvesi 399	3 (3–4)	2 (0–9)	3 (1–11)	2 (0–7)	2 (1–3)	62 (51–71)	23 (15–35)	92
Kallavesi 25	3 (2–3)	3 (0–13)	2 (1–10)	2 (0–6)	1 (1–2)	36 (21–53)	50 (32–67)	91

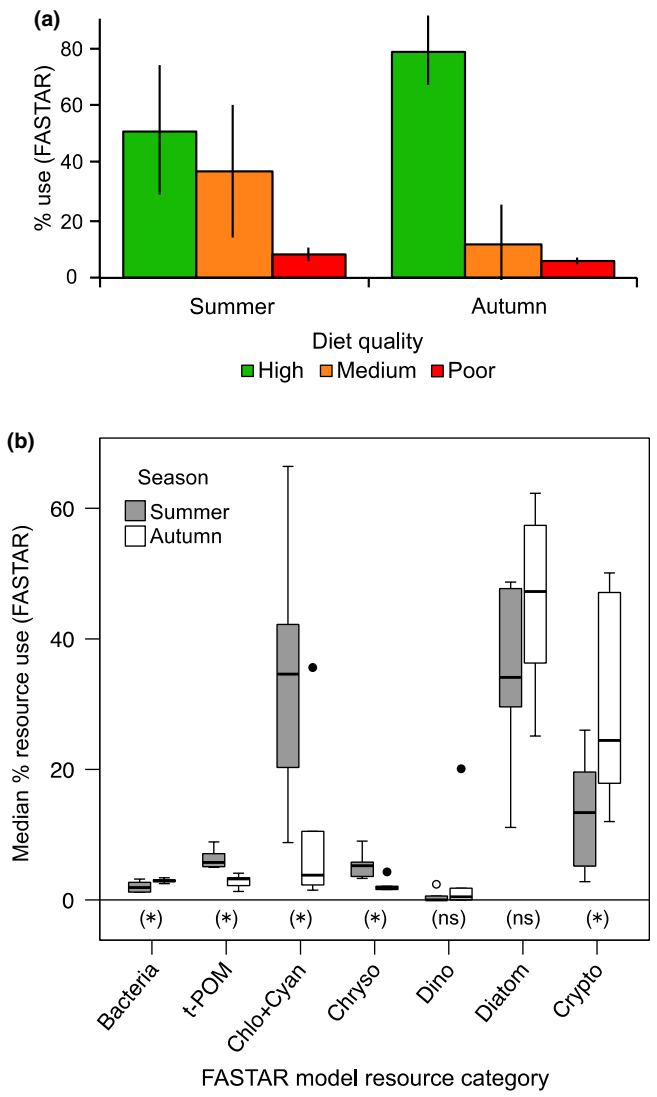

Fig. 4 Summary of FASTAR results. (a) FASTAR modelled proportional use (mean ± SD of the sum of medians) of high (cryptophytes, diatoms and dinoflagellates)-, medium (chlorophytes, cyanobacteria and chrysophytes)- and low (bacteria, t-POM)-quality resources to cladocerans in all 14 samples. (b) Boxplot showing the calculated median resource use of each of seven resource groups (abbreviations follow Table 1) to wild cladocerans in six stations where sampling occurred in both seasons. Median is heavy black line inside box, box are 25–75 percentiles, whiskers are 95% CI of the medians, and black dots are outliers beyond the CI. Significance of *t*-tests comparing each category (arcsine-transformed proportional data) across sampling events is shown along *x*-axis (*$P < 0.05$, ns = not significant).

high DOC concentrations (*c*. 5–10 mg C L^{-1}), cladocerans are predominantly supported by phytoplankton (*c*. 90%) with little use of either bacterial (*c*. 3%) or terrestrial (*c*. 5%) resources. Our results provide unprecedented taxonomic resolution of consumer diets and emphasise the importance of high-quality phytoplankton (especially cryptophytes and diatoms) in the diet of filter-feeding zooplankton, particularly in the autumn. Terrestrial organic matter is known to affect both physical and chemical conditions in lake ecosystems, and diverse conclusions have been drawn on its importance on supporting aquatic food webs (Jansson *et al*., 2007; Francis *et al*., 2011; Finstad *et al*., 2014; Kelly *et al*., 2014). Large contributions to lake food webs from terrestrial sources or from bacterial production to zooplankton have been shown only for small lakes (Kankaala *et al*., 2010; Wilkinson *et al*., 2013), but, due to constraints of the commonly used SI mixing model approach, the proportional ranges of putative dietary sources in such studies are generally quite wide. A predominance of terrestrial-derived OM in the seston does not necessarily mean that this material is an important resource to consumers (Lewis *et al*., 2001).

Mixing model analyses using FA are a promising quantitative tool for zooplankton because the previously documented class-level resolution of phytoplankton FA (Taipale *et al*., 2013) was clearly transferred to the *Daphnia* in our experiments (Fig. 1a,b), and the number of FAs used in the model ($n = 22$) outnumbers the number of resources the model resolves ($n = 7$). Until now, it has not been possible to investigate wild zooplankton diets at such a high taxonomic resolution due to limitations of the SI-based mixing model approaches commonly used. For example, whether conventional two- or three-variable SI mixing models can reliably resolve three or four basal resource groups (e.g. 'underdetermined' mixing model problem; Fry, 2013a) is unresolved and debated (Fry, 2013b; Semmens *et al*., 2013). Furthermore, different phytoplankton groups cannot be resolved with SI mixing models, as phytoplankton have overlapping and highly variable SI values (Vuorio *et al*., 2006). However, the major differences in FA profiles are well explained by phylogenetic relationships between the major primary producer taxa (Galloway *et al*., 2012; Taipale *et al*., 2013).

All biochemical mixing model analyses of consumer resource use assume that the imperfect transfer of raw biomarker values from dietary sources to consumer tissues (e.g., fractionation) is known and accounted for with 'trophic enrichment factors' (Parnell *et al*., 2010). The trophic enrichment factors used for SI are often assumed to be relatively stable, at *c*. 0–1‰ for $\delta^{13}C$, and *c*. 3–4‰ for $\delta^{15}N$, per trophic level (Post, 2002). However, trophic enrichment is rarely actually measured with feeding trials (del Rio *et al*., 2009), and has only recently been investigated for other isotopes such as deuterium (Graham, Harrison & Harrod, 2014). Stable isotope fractionation depends upon the consumer and specific tissue studied (Caut, Angulo & Courchamp, 2009), and both the physiological

state and diet of the consumer (McCutchan *et al*., 2003). It is therefore not surprising that mixing model results are highly sensitive to poorly constrained assumptions about fractionation (Bond & Diamond, 2011).

The FA profiles of dietary resources are also not unaltered during incorporation into consumer tissues, and the trophic modification of dietary FA will depend upon nutritional quality of the prey and energetic demands of the consumer (Taipale *et al*., 2011). Invertebrates may preferentially catabolise or modify FA molecules from dietary precursor FA (Bell & Tocher, 2009). Quantitative mixing model analyses, whether SI or FA based, therefore require that feeding trials are conducted with relevant consumers with a range of realistic diets to account for fractionation of the tracers of interest. Consequently, our modelling approach was designed to account for anticipated diet specificity in consumer FA modification observed in other experimental feeding trials with FA (Budge, Penney & Lall, 2012) and SI (McCutchan *et al*., 2003) by accounting for trophic modification of biomarkers in the resource library. The FASTAR model outputs are a measure of the proportional importance of different resources assimilated into the total lipid pool of the consumer. The model provides a quantitative index of the likely diet of the consumers examined by fitting the observed FA profile of the consumer to the combination of all the potential sources in the resource library.

Due to the high biochemical quality of phytoplankton, it was not surprising that the most probable solutions for the cladoceran diets in the studied boreal lakes consisted mainly of phytoplankton (range of the sum of phytoplankton medians across lakes = 86–94%) and only included a small subsidy from t-POM (median range = 1–9%) and bacterial (1–3%) basal resources. Our model estimated a rather high upper limit to the range within the 95% CI of solutions for t-POM (22%), which may be caused by the fact that the *Daphnia* fed the t-POM diets in our resource library have a FA signal from the *Scenedesmus* diets (Chlorophyceae) initially fed to *Daphnia* neonates or to the mothers in the previous generation. Additionally, higher FASTAR estimates of t-POM use may also indicate the generally poor nutritional condition of cladocerans. This hypothesis is supported by the *Daphnia* starvation FA profiles after consuming initial cryptophyte, chlorophyte and diatom diets; the starved animals all grouped in multivariate space with the animals fed t-POM diets (Fig. 2). The overlapping FA profiles of experimental *Daphnia* fed certain cyanobacteria and chlorophytes (Fig. 1b) may also be due to the fact that *Daphnia* grew poorly on cyanobacteria and thus retain certain FA that are common to both taxa [e.g., 18 : 3ω3 (ALA) and 18 : 2ω6 LIN)], obtained initially from *Scenedesmus* in the maternal diets. We therefore combined these resources into a single group for modelling. Future applications of the FASTAR model could also include either compound-specific ($\delta^{15}N$) amino acid values, which would probably separate cyanobacteria from eukaryotes (McCarthy, Lehman & Kudela, 2013), or sterols, as cyanobacteria are known to be depleted in essential phytosterols (Martin-Creuzburg & Von Elert, 2009) relative to phytoplankton. For example, previous work with an early version of the model used here showed that the use of both SI and FA variables concurrently improved the performance of the mixing model in resolving the theoretical diets of modelled consumers (Dethier *et al*., 2013).

The Actinobacteria–cryptophyte mixture experiment of Taipale *et al.* (2012; Fig. 1a) also demonstrates the importance of the proportionally less abundant, but also higher-quality, cryptophyte diets for *Daphnia*. For example, even when Actinobacteria comprised 95% of the available diet (label 4), the remaining, high-quality cryptophyte portion (5%) still strongly affects the similarity of FA profiles of the *Daphnia* fed different compositions in the gradient. A recent study by Taipale *et al.* (2014) showed that, relative to SI analysis, a strict FA approach will underestimate total carbon assimilation of terrestrial and bacterial resources by *Daphnia*, although the results of both methods were strongly correlated. To reconcile these approaches, future work is needed to compare quantitative resource estimates generated using FA and SI approaches in both laboratory experiments and lakes for which all resources are known and biochemical signatures are well parameterised. However, assuming doubled total carbon assimilation relative to that FA (Taipale *et al*., 2014), the maximum median estimate of the proportions of bacterial and t-POM diet sources for the cladocerans across all our study lakes would still have been relatively low (e.g. bacteria: 6% in summer, 6% in autumn; t-POM: *c*. 18% in summer, *c*. 8% in autumn).

High-quality phytoplankton was particularly important to zooplankton in large lakes: when pooled, cryptophytes and diatoms accounted for 36–79% (excluding Suvasvesi 29, 14%) of cladoceran resource use in the summer and 37–87% in the autumn. Except for one station (Orivesi 107 during the autumn), the role of high-quality dinoflagellates was generally small in the lakes studied here. The summed assimilation of high-quality diets to cladoceran consumers (defined as diatoms, cryptophytes and dinoflagellates) was 57–87%

in the autumn. The model indicated that in the summer, across all lakes, a substantial fraction of cladoceran diets originated from the pooled category of chlorophytes–cyanobacteria (across-lake median = 34.5%; Fig. 4b). The relative importance of this group to Cladocera declined considerably in the autumn (median = 3.5%), even though the relative abundance of this pooled group in the lakes did not differ among seasons (community proportion, $P = 0.951$). The decline in medium-quality diets is presumably due to the increased utilisation of higher-quality phytoplankton diets in the autumn. *Daphnia* can grow and reproduce on poorer-quality diets when high-quality resources are also present, even if only in small amounts (Taipale *et al*., 2012). Thus, even though chlorophytes–cyanobacteria contributed substantially to cladocerans in the summer, high-quality diets still account for a large fraction (51 ± 22%) of their resource use, which probably fuelled their somatic growth and reproduction.

Except for the diatom category, phytoplankton group abundance in the lakes was not statistically correlated with the FASTAR results of cladoceran resource use. This lack of direct correlation is likely to be due to the fact that phytoplankton community and cladoceran consumer samples were collected on the same day in each season. It is expected that FA turnover time for *Daphnia* is *c*. 7–10 days (Taipale *et al*., 2011), resulting in a delayed dietary signal in the consumer. It is also possible that the lack of correlation among the non-diatom groups indicates that cladocerans selectively forage on diatoms and other high-quality resources (Xuwang *et al*., 2011), or that high-quality FA are preferentially retained in zooplankton tissues, relative to other lower-quality groups during colder conditions (e.g. Sperfeld & Wacker, 2012). Future work could address this question by repeated, weekly sampling of zooplankton and phytoplankton community composition in one lake through multiple seasons. Of the water chemistry measurements assessed here, only total phosphorous correlated with the median of any FASTAR resource category (Table 3). The model indicated a positive correlation between phosphorus and both the chrysophytes and the pooled chlorophyte–cyanobacteria group. A positive correlation between total phosphorus and the abundance of cyanobacteria is typical for lakes. The negative correlation between phosphorus and diatom use by Cladocera was unexpected and may be explained by the preferential retention or use of diatom FA discussed above.

The conceptual model we use here generally assumes that the differences in FA content among Cladocera are driven primarily by diet and retention of lipids of these various resources into the lipids of the consumers. Alternative physiological mechanisms for these differences in consumer FA patterns between seasons include (i) that the differences in water temperature between seasons drive zooplankton FA composition (Masclaux *et al*., 2009) due to increased FA unsaturation in organisms in colder water (Sperfeld & Wacker, 2012) and (ii) that FA resources are differentially allocated to egg production or growth in different seasons (Vargas, Escribano & Poulet, 2006). We found no correlation with temperature or differences between seasons in Cladocera UI values, which would have provided support to the first alternative hypothesis. Many studies have demonstrated that high-quality diets (e.g. cryptophytes) result in increased growth and egg production in *Daphnia* (Brett *et al*., 2009; Taipale *et al*., 2014). In addition, for both high- and low-quality diets, the clutch size of the cladocerans *Daphnia manga* and *Simocephalus vetulus* has been shown to be greater at lower (12 °C) than at high (20 °C) temperature (Masclaux *et al*., 2009), which is relevant to the water temperatures observed during our two sampling periods. If increased egg production in the autumn was driving the patterns in FA content, this is also likely to be a consequence of an increased contribution of high-quality food to consumers. It is not possible to determine which mechanism (e.g. dietary or physiological constraints such as egg production) is primarily driving the FA profiles of the zooplankton in our field data. Future work could evaluate this question by separating egg-laden from non-egg-bearing zooplankton in the same samples and comparing FA composition or FASTAR model results for these subsamples.

Our results show that the cladoceran zooplankton in large lakes obtains the majority of its vital biochemical compounds, required for successful reproduction and growth, from high-quality phytoplankton, especially diatoms and cryptophytes. Due to the high resolution of the model, we were able to estimate the importance of distinct phytoplankton groups in wild zooplankton diets, which has not previously been possible using any other method. This is important due to the different dietary quality of various phytoplankters, where the fastest reproduction is found in cladocerans when they use diets with a high EPA content (Brett *et al*., 2009). The ecosystem scale effects of allochthonous organic matter on large lake food webs are likely to operate primarily via the combination of indirect factors, such as phosphorus, nitrogen and DOC load, and changes in light penetration, which affects primary production by phytoplankton, and less via direct use of allochthonous organic matter by consumers. For example, Finstad *et al*. (2014) recently showed that brown trout biomass in Norwegian lakes showed a unimodal response to total DOC content, dependent on lake size, indicating poten-

tial positive and negative effects of DOC loading on lakes. Their results suggested that, in deeper lakes, the effects of DOC inputs switched from positive to negative at lower values than in shallow lakes. Our results, which found no relationship between lake DOC concentration and the modelled cladoceran diets, support these conclusions. Our results also identify that high-quality phytoplankters are the key resources that fuel the base of the food web, and ultimately fish production, in these large boreal lakes.

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References

Anderson M.J., Gorley R.N. & Clarke K.R. (2008) *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*. PRIMER-E Ltd, Plymouth.

Bell M.V. & Tocher D.R. (2009) Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In: *Lipids in Aquatic Ecosystems*. (Eds M.T. Arts, M.T. Brett & M. Kainz), pp. 211–236. Springer, New York, NY.

Bond A.L. & Diamond A.W. (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications*, **21**, 1017–1023.

Brett M.T., Kainz M.J., Taipale S.J. & Seshan H. (2009) Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 21197–21201.

Brett M.T. & Muller-Navarra D.C. (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology*, **38**, 483–499.

Brett M.T., Muller-Navarra D.C., Ballantyne A.P., Ravet J.L. & Goldman C.R. (2006) *Daphnia* fatty acid composition reflects that of their diet. *Limnology and Oceanography*, **51**, 2428–2437.

Budge S.M., Penney S.N. & Lall S.P. (2012) Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. *Canadian Journal of Fisheries and Aquatic Sciences*, **69**, 1033–1046.

Carpenter S.R., Cole J.J., Pace M.L., Van De Bogert M., Bade D.L., Bastviken D. *et al.* (2005) Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. *Ecology*, **86**, 2737–2750.

Caut S., Angulo E. & Courchamp F. (2009) Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, **46**, 443–453.

Clarke K.R. & Gorley R.N. (2006) *PRIMER v6: User Manual/Tutorial*. PRIMER-E Ltd, Plymouth.

Cole J.J., Carpenter S.R., Kitchell J., Pace M.L., Solomon C.T. & Weidel B. (2011) Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 1975–1980.

Cole J.J., Carpenter S.R., Pace M.L., Van De Bogert M.C., Kitchell J.L. & Hodgson J.R. (2006) Differential support of lake food webs by three types of terrestrial organic carbon. *Ecology Letters*, **9**, 558–568.

Couture S., Houle D. & Gagnon C. (2012) Increases in dissolved organic carbon in temperate and boreal lakes in Quebec, Canada. *Environmental Science and Pollution Research*, **19**, 361–371.

Dalsgaard J., St John M., Kattner G., Muller-Navarra D.C. & Hagen W. (2003) Fatty acid trophic markers in the pelagic marine food environment. *Advances in Marine Biology*, **46**, 225–340.

del Rio C.M., Wolf N., Carleton S.A. & Gannes L.Z. (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews*, **84**, 91–111.

Dethier M.N., Sosik E.A., Galloway A.W.E., Duggins D.O. & Simenstad C.A. (2013) Addressing assumptions: variation in stable isotopes and fatty acids in marine macrophytes can confound conclusions of food web studies. *Marine Ecology Progress Series*, **478**, 1–14.

Finstad A.G., Helland I.P., Ugedal O., Hesthagen T. & Hessen D.O. (2014) Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters*, **17**, 36–43.

Francis T.B., Schindler D.E., Holtgrieve G.W., Larson E.R., Scheuerell M.D., Semmens B.X. *et al.* (2011) Habitat structure determines resource use by zooplankton in temperate lakes. *Ecology Letters*, **14**, 364–372.

Fry B. (2013a) Alternative approaches for solving underdetermined isotope mixing problems. *Marine Ecology Progress Series*, **472**, 1–13.

Fry B. (2013b) Minmax solutions for underdetermined isotope mixing problems: reply to Semmens et al. (2013). *Marine Ecology Progress Series*, **490**, 291–294.

Galloway A.W.E., Britton-Simmons K.H., Duggins D.O., Gabrielson P.W. & Brett M.T. (2012) Fatty acid signatures differentiate marine macrophytes at ordinal and family ranks. *Journal of Phycology*, **48**, 956–965.

Graham C.T., Harrison S.S.C. & Harrod C. (2014) Differences in the contributions of dietary water to the hydro-

gen stable isotope ratios of cultured Atlantic salmon and Arctic charr tissues. *Hydrobiologia*, **721**, 45–55.

Gugger M., Lyra C., Suominen I., Tsitko I., Humbert J.-F., Salkinoja-Salonen M.S. *et al.* (2002) Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology*, **52**, 1007–1015.

Guiry M.D. & Guiry G.M. (2014) *AlgaeBase. World-wide electronic publication, National University of Ireland, Galway.* Vol. 20-January-2014. National University of Ireland, Galway.

Jansson M., Persson L., De Roos A.M., Jones R.I. & Tranvik L.J. (2007) Terrestrial carbon and intraspecific size-variation shape lake ecosystems. *Trends in Ecology & Evolution*, **22**, 316–322.

Jones R.I., Grey J., Sleep D. & Quarmby C. (1998) An assessment, using stable isotopes, of the importance of allochthonous organic carbon sources to the pelagic food web in Loch Ness. *Proceedings of the Royal Society B-Biological Sciences*, **265**, 105–111.

Kankaala P., Bellido J.L., Ojala A., Tulonen T. & Jones R.I. (2013) Variable production by different pelagic energy mobilizers in boreal lakes. *Ecosystems*, **16**, 1152–1164.

Kankaala P., Taipale S., Li L. & Jones R.I. (2010) Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. *Aquatic Ecology*, **44**, 781–795.

Kelly J.R. & Scheibling R.E. (2012) Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series*, **446**, 1–22.

Kelly P.T., Solomon C.T., Weidel B.C. & Jones S.E. (2014) Terrestrial carbon is a resource, but not a subsidy, for lake zooplankton. *Ecology*, DOI: 10.1890/1813-1586.1891.

Lewis W.M. Jr, Hamilton S.K., Rodriguez M.A., Saunders J.F. & Lasi M.A. (2001) Foodweb analysis of the Orinoco floodplain based on production estimates and stable isotope data. *Journal of the North American Benthological Society*, **20**, 241–254.

Logue J.A., De Vries A.L., Fodor E. & Cossins A.R. (2000) Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure. *Journal of Experimental Biology*, **203**, 2105–2115.

Martin-Creuzburg D. & Von Elert E. (2009) Ecological significance in aquatic food webs. In: *Lipids in Aquatic Ecosystems* (Eds M.T. Arts, M.T. Brett & M.J. Kainz), pp. 43–64. Springer, New York, NY.

Masclaux H., Bec A., Kainz M.J., Desvilettes C., Jouve L. & Bourdier G. (2009) Combined effects of food quality and temperature on somatic growth and reproduction of two freshwater cladocerans. *Limnology and Oceanography*, **54**, 1323–1332.

McCarthy M.D., Lehman J. & Kudela R. (2013) Compound-specific amino acid $\delta^{15}N$ patterns in marine algae: tracer potential for cyanobacterial vs. eukaryotic organic nitrogen sources in the ocean. *Geochimica et Cosmochimica Acta*, **103**, 104–120.

McCutchan J.H. Jr, Lewis W.M. Jr, Kendall C. & McGrath C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378–390.

Monteith D.T., Stoddard J.L., Evans C.D., De Wit H.A., Forsius M., Høgåsen T. *et al.* (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, **450**, 537–541.

Moore J.W. & Semmens B.X. (2008) Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters*, **11**, 470–480.

Müller-Navarra D.C. (2008) Food web paradigms: the biochemical view on trophic interactions. *International Review of Hydrobiology*, **93**, 489–505.

Newton R.J., Jones S.E., Eiler A., McMahon K.D. & Bertilsson S. (2011) A guide to the natural history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews*, **75**, 14–49.

Pace M.L., Cole J.J., Carpenter S.R., Kitchell J.F., Hodgson J.R., Van De Bogert M.C. *et al.* (2004) Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature*, **427**, 240–243.

Parnell A.C., Inger R., Bearhop S. & Jackson A.L. (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE*, **5**, e9672.

Phillips D.L. & Gregg J.W. (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, **136**, 261–269.

Piepho M., Arts M.T. & Wacker A. (2012) Species-specific variation in fatty acid concentrations of four phytoplankton species: does phosphorus supply influence the effect of light intensity or temperature? *Journal of Phycology*, **48**, 64–73.

Plummer M. (2003) JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. In: *Proceedings of the 3rd International Workshop on Distributed Statistical Computing (DSC 2003). March*, pp. 1–10. Available at: http://www.r-project.org/conferences/DSC-2003/Proceedings/Plummer.pdf.

Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703–718.

Post D.M., Pace M.L. & Hairson N.G. Jr (2000) Ecosystem size determines food-chain length in lakes. *Nature*, **405**, 1047–1049.

R Development Core Team. (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/.

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

Semmens B.X., Ward E.J., Parnell A.C., Phillips D.L., Bearhop S., Inger R. *et al.* (2013) Statistical basis and outputs of stable isotope mixing models: comment on Fry (2013). *Marine Ecology Progress Series*, **490**, 285–289.

Sperfeld E. & Wacker A. (2012) Temperature affects the limitation of *Daphnia magna* by eicosapentaenoic acid, and the fatty acid composition of body tissue and eggs. *Freshwater Biology*, **57**, 497–508.

Taipale S., Brett M.T., Hahn M.W., Martin-Creuzburg D., Yeung S., Hiltunen M. *et al.* (2014) Differing *Daphnia magna* assimilation efficiencies for terrestrial, bacterial, and algal carbon and fatty acids. *Ecology*, **95**, 563–573.

Taipale S., Kankaala P., Tiirola M. & Jones R.I. (2008) Whole-lake dissolved inorganic ^{13}C additions reveal seasonal shifts in zooplankton diet. *Ecology*, **89**, 463–474.

Taipale S., Peltomaa E., Strandberg U., Galloway A.W.E., Ojala A. & Brett M.T. (2013) Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquatic Microbial Ecology*, **71**, 165–178.

Taipale S.J., Brett M.T., Pulkkinen K. & Kainz M.J. (2012) The influence of bacteria-dominated diets on *Daphnia magna* somatic growth, reproduction, and lipid composition. *FEMS Microbiology Ecology*, **82**, 50–62.

Taipale S.J., Kainz M.J. & Brett M.T. (2011) Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in *Daphnia*. *Oikos*, **120**, 1674–1682.

Vargas C.A., Escribano R. & Poulet S. (2006) Phytoplankton food quality determines time windows for successful zooplankton reproductive pulses. *Ecology*, **87**, 2992–2999.

Vuorio K., Meili M. & Sarvala J. (2006) Taxon-specific variation in the stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of lake phytoplankton. *Freshwater Biology*, **51**, 807–822.

Ward E.J., Semmens B.X., Phillips D.L., Moore J.W. & Bouwes N. (2011) A quantitative approach to combining sources in stable isotope mixing models. *Ecosphere*, **2**, art19.

Wetzel R.G. (2001) *Limnology: Lake and River Ecosystems*. Academic Press, San Diego, CA.

Wilkinson G.M., Carpenter S.R., Cole J.J., Pace M.L. & Yang C. (2013) Terrestrial support of pelagic consumers: patterns and variability revealed by a multilake study. *Freshwater Biology*, **58**, 2037–2049.

Xuwang Y., Shasha Z., Jian H. & Pengfei L. (2011) Effects of food quality and starvation on the optimal foraging behavior of *Daphnia magna* (Cladocera). *Acta Ecologica Sinica*, **31**, 328–333.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. FASTAR mixing model solutions for all samples.

Table S1. FA profiles (mean ± SD; % of total FA) of *Daphnia* raised on each of the 7 diets considered in the FASTAR mixing model resource library.

Table S2. FA profiles (% of total FA) of wild cladocera sampled in two seasons at Finnish boreal lakes and evaluated with the FASTAR mixing model.

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