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Eutrophication and browning influence *Daphnia* nutritional ecology

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Abstract

Climate change and land use practices can enhance lake eutrophication and browning, which influence phytoplankton composition by decreasing the availability of food high in nutritional quality (algae) and increasing the abundance of low-quality food (terrestrial detritus, bacteria) for herbivorous zooplankton. Nutritionally valuable algae for zooplankton are rich in essential biomolecules such as amino acids, polyunsaturated fatty acids (PUFA), sterols, and phosphorus. We performed laboratory experiments and showed a stronger positive relationship between zooplankton (*Daphnia*) cumulative offspring number and availability of high-quality algae (Cryptophytes: *Rhodomonas/Cryptomonas*; and Chrysophytes: *Mallomonas*) than with intermediate-quality (Chlorophytes: *Acutodesmus*) or poor-quality

(Dinoflagellates: *Peridinium*) algae. The higher cumulative offspring number of *Daphnia* was a result of higher amounts of total ω-3 and ω-6 PUFA, proteins, sterols, and amino acids in the algal diets. The experiments also showed that even a small addition of high-quality algae (*Rhodomonas*) to intermediate-quality (*Acutodesmus*) or low-quality diets (bacteria, heterotrophic nanoflagellates, or terrestrial organic matter) can enhance the *Daphnia* cumulative offspring production. Our carbon mass balance calculation for a eutrophic clearwater lake and an oligotrophic polyhumic lake showed that the abundance of high-quality phytoplankton (cryptophytes, chrysophytes, diatoms) among total particulate organic carbon was minor (8.7% [SD 2.4%] and 6.5% [7.0%]). We modeled *Daphnia* diets (i.e., resource assimilation) using a fatty acid mixing model. Our analyses showed that *Daphnia* were able to locate high-quality algae (cryptophytes, chrysophytes, and diatoms) more effectively during cyanobacteria blooms in a eutrophic lake (55% [SD 12%]) than in a polyhumic lake (25% [10%]). Nevertheless, our results show that intense eutrophication and browning diminish assimilation of high quality algae, limiting *Daphnia* biomass production.

Key words: amino acids, bacteria, fatty acids, heteronanoflagellates, phytoplankton, polyunsaturated sterols, zooplankton.

INTRODUCTION

Eutrophication due to extensive nutrient loading, especially phosphorus, from point and diffuse sources (Powers et al. 2016) is known to cause nuisance blooms of cyanobacteria in lakes (Schindler 2012). Climate change enhances precipitation and intensity of storms, especially in the northern hemisphere, which leads to increased nutrient loading; combined with increasing temperature, the consequence is a mutual intensification of eutrophication symptoms (Kernan et al. 2010, Moss et al. 2011, IPCC 2014, Anneville et al. 2015). The

resulting processes, such as nutrient metabolization and the dynamics of trophic networks, are likely to be modified by climate change and its interaction with anthropogenic activities (Le Moal et al. 2019).

Browning of surface waters has been observed in temperate and boreal regions in North America and North and Central Europe (e.g., Monteith et al. 2007, Couture et al. 2012, Räike et al. 2016). This phenomenon, caused by increased concentrations of colored terrestrial dissolved organic carbon (DOC), and also coupled with iron interactions (Weyhenmeyer et al. 2014), profoundly affects the physical and chemical environment that phytoplankton encounter (Thrane et al. 2014, Seekell et al. 2015). Both eutrophication and browning are known to cause alterations in biodiversity, ecosystem functioning, and energy flows in freshwater lakes (Karlsson et al. 2009, Vonlanthen et al. 2012, Grimm et al. 2013, Lindholm et al. 2018), but they also change dynamics of biochemical components in food webs, such as essential fatty acids (Müller-Navarra et al. 2004, Taipale et al. 2016a).

The quality of food for heterotrophs is determined by both its physical edibility and its nutritional value (Becker et al. 2004, Jonasdottir 2015). The nutritional requirements of herbivorous zooplankton, especially of the model organism *Daphnia*, have been well established: phosphorus (P), polyunsaturated fatty acids (PUFAs), sterols, and amino acids (AAs) are key factors regulating *Daphnia* growth and reproduction (e.g., Urabe et al. 1997, Ravet et al. 2003, Martin-Creuzburg et al. 2005, Fink et al. 2011, Peltomaa et al. 2017). For optimal performance, *Daphnia* requires high amounts of P in relation to nitrogen (N) and carbon (C; Hessen et al. 2013). The C:P ratio is an important factor controlling both somatic growth and reproduction (Ravet and Brett 2006, Peltomaa et al. 2017). In aquatic food webs, linoleic (LIN; 18:2ω6) and alpha-linolenic acids (ALA, 18:3ω3) are essential ω-6 and ω-3 fatty acids (FAs) serving as precursors for other physiologically active essential polyunsaturated FAs, such as arachidonic acid (ARA; 20:4ω6), eicosapentaenoic acid (EPA;

20:5ω3), and docosahexaenoic acid (DHA; 22:6ω3; Arts et al. 2009). Zooplankton, as well as other heterotrophs, need either to obtain EPA and DHA directly from their diet or to convert them from ALA or stearidonic acid (SDA; 18:4ω3) via elongation. However, the conversion efficiency is generally low in *Daphnia* (<1%; von Elert 2002, Taipale et al. 2011a) and also varies among different developmental stages (Tocher 2010). EPA is likely the most important essential FA supporting somatic growth and reproduction of *Daphnia,* whereas DHA seems to be the most important FA for copepods and many fish (Arts et al. 2009). In addition to ω-3 PUFA, zooplankton require cholesterol for reproduction (Martin-Creuzburg and von Elert 2009). *Daphnia* must convert diet-obtained phytosterols to cholesterol; however, phytosterols vary in their potential to support somatic growth of *Daphnia*, making some phytosterols more efficient in supporting somatic growth than others in relation to cholesterol (Martin-Creuzburg et al. 2014).

Amino acids are required building blocks for protein synthesis, precursors for some molecules (e.g., nucleic acids), and the part of coenzymes and signaling molecules for regulating mRNA translation (Pardee 1954, Fafournoux et al. 2000, Ronnestadt et al. 1999). Twenty of all known AAs are required for protein synthesis, of which 9 are called “essential” (EAA; histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine, and lysine) because consumers cannot synthesize them de novo. In fish, restricted availability of EAA can lead to starvation (Ketola 1982), but almost no results have been published on the importance of AA for zooplankton. Traditionally, AAs were not considered limiting components in freshwater food webs, but high AA and EAA content of phytoplankton have recently been shown to explain high growth and reproduction rates of *Daphnia*, respectively (Peltomaa et al. 2017).

Phytoplankton taxa differ in their ability to synthesize ω-3 PUFA, ω-6 PUFA, sterols, and AAs (Peltomaa et al. 2017), and the actual content (e.g., PUFA concentration in algae cells)

is also influenced by the environment (Guschina and Harwood 2009). Phytoplankton generally contain AA, sterols, and ω-3 PUFA (Taipale et al. 2016c), but different taxa differ in their EPA and DHA content, low- and high-efficiency sterols, and total EAA concentration (Taipale et al. 2013, Galloway and Winder 2015, Peltomaa et al. 2017), making them poor-, intermediate-, or high-quality diets for zooplankton (Ahlgren et al. 1992, Müller-Navarra 1995). Generally, cryptophytes, diatoms, and synyrophytes are considered to have high nutritional value for *Daphnia* because they synthesize EPA, DHA, and low threshold sterols, whereas green algae are considered intermediate because they lack EPA and DHA, and cyanobacteria are a poor-quality diet because they lack sterols (Peltomaa et al. 2017). Dinoflagellates are often rich in DHA and are the preferred diet for copepods (Santer 1996), but *Daphnia* do not grow well with *Peridinium*, a common taxon in lakes. Poor growth may be due to the armoring and low levels of sterols (Peltomaa et al. 2017). In addition to phytoplankton, *Daphnia* in the wild may also be supported by different types of bacteria (heterotrophic, photoautotrophic, and methane oxidizing bacteria), ciliates, heteronanoflagellates (HNFs), and terrestrial dissolved/particulate organic matter (tDOM/tPOM), which are generally considered poor-quality diets because they lack ω-3 PUFA and sterols (Gutseit et al. 2007, Martin-Creuzburg et al. 2011, Wenzel et al. 2012, McMeans et al. 2015, Taipale et al. 2012, 2016b).

Eutrophication may enhance phytoplankton biomass production but influence the community composition by promoting the non-DHA synthesizing taxa, resulting in, for example, lower amounts of DHA in carnivorous perch in humic than in oligotrophic lakes (Taipale et al. 2016b). Climate change can also enhance cyanobacteria blooms, which are of low nutritional value for zooplankton (Martin-Creuzburg et al. 2008, Deng et al. 2016, Ventelä et al. 2016). Increased loading of tDOM and tPOM from catchment areas (i.e., "browning") suppresses phytoplankton biomass production by reducing light attenuation

(Karlsson et al. 2015, Deininger et al. 2017). However, sestonic EPA content can be high in dystrophic lakes because of the high biomass of raphidophytes such as *Gonyostomum semen* (Gutzeit et al. 2007, Taipale et al. 2016a), which can be consumed only by large zooplankton such as *Eudiaptomus* and *Holopedium*, but not by cladocerans such as *Daphnia* or *Ceriodaphnia* (Johansson et al. 2013).

Copepods are able to actively select food particles based on their nutritional quality (Cowles et al. 1988) or toxicity (DeMott and Moxter 1991). Filter-feeding cladocerans have been thought to feed non-selectively (DeMott 1986, Lambert 1987, Butler et al. 1989); however, detailed analysis from scanning electron microscope observations have shown that *Daphnia* feed selectively on larger particles as a result of a series of complex behavioral–mechanistic processes (Hartman and Kunkel 1991). Furthermore, all ingested material is not necessarily assimilated, resulting in preferential retention of high-quality diets. *Daphnia* and other zooplankton feed preferentially on cryptophytes (Persson 1985, Knisely and Geller 1986), which are easily ingestible and have high nutritional value. Both juvenile and adult *Daphnia* are also able to locate regions with high-quality food (Schatz and McCauley 2007), possibly through perception of increased ingestion rate and odor (Jensen et al. 2001). Rapid detection of high nutritional value food patches can support rapid population growth because of the parthenogenetic reproduction strategy in *Daphnia* (Ebert 2005).

We conducted 2 laboratory experiments and analyzed the resource assimilation by *Daphnia* in a eutrophic clearwater lake and an oligotrophic polyhumic lake. Our goals were to (1) quantify the influence of eutrophication and browning on assimilation of high nutritional quality algae in a key-herbivorous zooplankton (*Daphnia*) throughout the open-water season and (2) define how these changes influence the population growth and dynamics of herbivorous zooplankton (*Daphnia*). We conducted laboratory experiments to examine the thresholds for nutritionally distinct algal diets and growth and reproduction rates for *Daphnia*

in boreal lakes and to study the effects of diminishing nutritional value of diets as a result of eutrophication and browning. We analyzed FAs, P, and sterols from the diets and defined which biomolecules are potentially limiting in lakes experiencing browning or eutrophication. Finally, we modeled *Daphnia* resource assimilation in a eutrophic and a polyhumic lake using an FA mixing model. We hypothesized that eutrophication and browning would increase the bottom-up regulation of production in pelagic food webs because of the limitation of high nutritional quality algae (essential biomolecules). Specifically, we hypothesized that *Daphnia* would be more limited on high-quality algae in the humic lake than in the eutrophic lake because cryptophytes are more abundant in eutrophic than in humic lakes.

METHODS

Laboratory experiments

Diet source cultures for experiments

Phytoplankton strains used in this study, originally isolated from freshwater systems, were cultured at 18–20 °C under a 14:10 h or 16:8 h light:dark cycle with a light intensity of 30–70 µmol m^{-2} s^{-1}. *Acutodesmus* sp. (University Basel), *Peridinium cinctum* (SCCAP K-1721), *Mallomonas kalinae* (SCCAP K-1875), *Rhodomonas minuta* (CPCC 344), and *Cryptomonas ozolinii* (UTEX LB 2782) were cultured in MWC medium (Guillard 1975). The heterotrophic gram-positive bacterium *Micrococcus luteus* (ATCC 4698) was cultivated using tryptic soy broth media in serum vials (150 mL) at 30 °C for 48–60 h, with new cultures started from plate colonies every second day. Birch leaves (*Betula pendula*) were collected from the yard of University of Jyväskylä (Finland) and milled to fine particles using a Retch ZM 100 GWB ultra centrifugal Mill, diluted with MWC medium (Guillard 1975), and filtered through a 50 µm screen. HNFs were isolated from a humic lake (Lake Pääjärvi, southern Finland, 60°04′N,

25°08′E) and cultured in darkness with *Micrococcus luteus* using filtered lake water as the medium, which also contained residues of algae (*Chlamydomonas*).

Daphnia culturing

All experiments were conducted using a clone of *Daphnia magna* (DK-35-9; hereafter *Daphnia*), which originated from a pond in northern Germany but has been maintained in the laboratory for several years. Prior to the experiments, *Daphnia* females were transferred into glass vials filled with 40 mL ADaM (Klüttgen et al. 1994; modified by using only one-twentieth of the SeO_2 concentration) and cultured at 18 (standard deviation [SD] 1) °C. *Daphnia* females were fed *Acutodesmus* sp. and synchronized to reproduce neonates every third day. For the experiments, we used the third or later clutches of neonates.

Experiment 1: threshold experiment with algae

In the 20 d life table experiment, *Daphnia* were cultured with *Acutodesmus*, *Peridinium*, *Mallomonas*, and *Rhodomonas* (*Rhodomonas* for days 0–12, and *Cryptomonas* for days 13–21) using a food C concentration gradient of 0.25, 1.25, 2.5, 3.75, 4.75, and 5 mg L^{-1} for each. The newly released neonates (~6 h old) from several *Daphnia* females were distributed individually into glass vials (40 mL of L16 media). Each treatment consisted of 6 replicates in *Mallomonas* and *Rhodomonas* experiments and 10 replicates in *Acutodesmus* and *Peridinium* experiments. *Daphnia* were fed every second day, and the growth medium was changed simultaneously. At the end of the experiment, individuals were placed into 1.5 mL Eppendorf tubes, freeze-dried, and stored at −80 °C, until analyzed for FA, sterol, P, C, and N. Total biomass (g) of pooled *Daphnia* for each treatment was used to calculate the growth rate as $g = (\ln Bt_{21} - \ln Bt_0)/t$, where B is biomass (dry weight) at the end (t_{21}) and beginning

(t_0) of the experiment. The cumulative offspring number was the sum of offspring in each treatment at the end of the experiment.

Experiment 2: mixed-diets

Daphnia were cultured with C content of 5 mg L^{-1} of bacteria (*Micrococcus*), birch, HNFs, *Acutodesmus*, or *Rhodomonas* for 14 d. In addition, bacteria, birch, HNFs, and *Acutodesmus* (75%; 3.75 mg L^{-1}) were mixed with *Rhodomonas* (25%; 1.25 mg L^{-1}) to examine reproduction of *Daphnia* under limited availability of high quality food. To characterize the benefit of mixed diets, *Daphnia* was also cultured with 25% (1.25 mg L^{-1}) *Rhodomonas*. Each treatment had 11 replicates (1 randomly chosen neonate, <12 h). The experimental vial size, medium change, and feeding were similar to experiment 1. The experiment lasted 14 d, but growth rates were also measured after 8 d. We used similar protocols for calculating somatic growth rate on days 8 and 14 and cumulative offspring number on day 14, as in experiment 1.

Field study

Both study lakes are located in southern Finland, only 40 km apart. Eutrophic Lake Vesijärvi (61°04′N, 25°32′E) is a medium-sized (110 km^2) clearwater lake (color ~20 mg L^{-1} platinum-cobalt units [Pt-Co], DOC 5–8 mg L^{-1}). The lake consists of several basins; the southernmost Enonselkä basin (area 26 km^2 and mean depth 6.8 m) became strongly polluted during the 1900s by municipal and industrial wastewater discharged from the surrounding city of Lahti. Sewage was diverted from the lake in 1976, but it remained eutrophic with frequent cyanobacterial blooms (Keto and Sammalkorpi 1988). After 15 years, the Enonselkä basin started to recover following a large-scale biomanipulation (i.e., mass removal of planktivorous fish; Kairesalo et al. 1999, Kairesalo and Vakkilainen 2004). However, the

improved water quality was only temporary; high cyanobacterial biomasses reappeared in the beginning of the 21st century, despite continuous management of fishing (Kairesalo and Vakkilainen 2004). To improve water quality, local water managers started hypolimnetic aeration in autumn 2009, which has continued yearly during stratification periods, with the aim to reduce internal loading of P from bottom sediments. In our field study year of 2016, Enonselkä basin was aerated between 28 June and 2 September. During the aeration, the average concentration of total P both in surface and near sediment water was 25 µg L^{-1}, and the concentration of chlorophyll *a* varied from 6 to 20 µg L^{-1}.

We sampled the Enonselkä basin within a 2-week interval between 5 July and 20 September 2016. Zooplankton samples were taken with a 1 m long Limnos tube sampler (volume 6.9 L) from the entire water column (0–30 m) in the deepest part of the basin. Subsamples were pooled into composite samples from the epilimnion (0–10 m) and hypolimnion (10–30 m). After filtering through a 50 µm mesh, the samples were preserved in ethanol. Phytoplankton samples were collected from 0 to 10 m and preserved with acid Lugol's solution. Phytoplankton abundance and community composition were determined using the Utermöhl (1958) method with an inverted microscope. Seston samples were taken also with Limnos tube sampler from 0 to 10 m, and 0.1–1 L of lake water was filtered through GF/C filters (size 47 mm) for sterol analysis and through 0.2 or 0.45 µm (mixed cellulose ester, Whatmann) for FA analysis. Additionally for FA analyses, zooplankton (*Daphnia*/*Bosmina*) were harvested with a 50 µm plankton net descended to 10 m and then lifted to the surface.

The polyhumic Lake Mekkojärvi (61°13′N, 25°8′E) is a small (area 0.35 ha), dark (water color 300–800 mg L^{-1} Pt-Co), oligotrophic (P: 12 [SD 3] µg L^{-1}) forest lake in the Evo forest area in southern Finland. Lake Mekkojärvi has a maximum depth of 4.3 m, a mean depth of 2 m, and is steeply stratified in summer when the oxic epilimnion is narrow (0.5–1 m).

Although Lake Mekkojärvi is small, it is a well-established model lake for this type of high-latitude boreal habitat (Salonen and Jokinen 1988, Jones 1992, Salonen and Lehtovaara 1992, Ojala and Salonen 2001, Taipale et al. 2008, 2009a, 2011b, 2016b, Kankaala et al. 2010). As is the case for many of these lakes, Lake Mekkojärvi is naturally acidic (pH 4–6) and has a high total DOC concentration (C: 20–45 mg L^{-1}). During this study, the concentration of DOC in epilimnion varied from 18 to 28 mg L^{-1}, P from 8 to 17 µg L^{-1}, and chlorophyll *a* from 2 to 14 µg L^{-1}. Methane oxidizing bacteria (MOB) and photosynthetic bacteria are major producers in Lake Mekkojärvi (Taipale et al. 2011b). The lake usually has a *Chlamydomonas* (Chlorophyte) bloom shortly after ice breakout in the spring and a *Mallomonas* (Chrysophyte) bloom early in June (Taipale et al. 2008). HNFs are the main grazers of bacteria and are eaten by *Daphnia longispina*, the major zooplankton species in the lake (Salonen and Lehtovaara 1992). Lake Mekkojärvi is fishless and thus optimal for studying dietary nutritional quality impact on zooplankton population dynamics. We used phytoplankton and *Daphnia* biomass measured during the open-water season in 2005 and 2006 for *Daphnia* diet analysis in Mekkojärvi (Taipale et al. 2008, Kankaala et al. 2010).

Modeling *Daphnia* resource assimilation

To generate estimates of the dietary assimilation of different basal resources by zooplankton, we used an FA-based mixing model analysis (Galloway et al. 2014, 2015), adapted for analysis of FAs from the isotope mixing models MixSIR (Moore and Semmens 2008) and SIAR (Parnell et al. 2010). The FA source tracking algorithm in R (FASTAR) model uses a "resource library" file consisting of mean (SD) FAs of a consumer, in this case *Daphnia*, fed a diversity of known basal monocultures in controlled laboratory feeding trials (Galloway et al. 2014). Here we used same resource library as Taipale et al. (2016c), which is similar but more applicable to the basal resources available in these lakes. We applied the mixing model

analysis to FA profiles of *Daphnia* from Lake Vesijärvi and Lake Mekkojärvi. Lake Vesijärvi data were collected in 2016, whereas Lake Mekkojärvi data were collected in 2005 and 2006 (Taipale et al. 2009a, 2016a). FASTAR was also used to test if the eutrophication and browning diminish assimilation of high-quality diets and whether that could explain decrease in *Daphnia* biomass.

Pelagic carbon mass balance

We compared the median values of each diet source from FASTAR analyses with the available proportion of that resource observed in the field for each diet to define which diet source *Daphnia* preferentially utilize. The observed proportion of each diet (actinobacteria, cryptophytes, chrysophytes, cyanobacteria, diatoms, dinoflagellates, green algae, golden algae, MOB, and tPOM) was calculated using following equation:

$$\text{Contribution of diet (\%)} = 100 \times \text{C biomass of diet component } (\mu g\ L^{-1}) / \text{POC } (\mu g\ L^{-1}). \quad (1)$$

We were able to calculate total C biomass for all sampling times in Lake Mekkojärvi, but only for 3 d (27 Jun, 19 Jul, 20 Sep) in Lake Vesijärvi. Biovolumes of phytoplankton classes were converted to C biomass ($\mu g\ L^{-1}$) according to the equations in Menden-Deuer and Lessard (2000). The C biomass ($\mu g\ L^{-1}$) of Actinobacteria, Polynucleobacter, MOB, and *Chlorobium* (green sulfur bacteria) for Mekkojärvi was obtained from our previous measurements (Taipale et al. 2011b) and calculated using the same FA biomarkers used for Lake Vesijärvi. Particulate organic C (POC) was measured by Salonen (1979) for Lake Mekkojärvi, whereas the difference between TOC and DOC was used for Lake Vesijärvi. The C biomass of tPOM was calculated as the difference between total POC ($\mu g\ L^{-1}$) and the sum of C biomass of other diets.

We also calculated the FA concentration (µg L^{-1}) and FA content (µg mg^{-1} C) of ω-3 and EPA in the seston of Lake Vesijärvi in 2016 and in seston of Lake Mekkojärvi in 2005. FAs were quantified using an internal standard (Sigma-Aldrich PLFA 15:0) for Lake Mekkojärvi and an external standard mix (Nu-Check Prep 566c Fame Standard Mix) for Lake Vesijärvi. For correlation analyses, we used the average values of phytoplankton biomass and ω-3 PUFA concentration and content of the epilimnion and metalimnion in the lakes.

Biochemical analysis

Amino acid analysis

Proteins from 1–2 mg of freeze-dried sample were hydrolyzed with 1 mL of 6 M HCl at 110 °C for 20 h for AA analysis. After hydrolysis, the samples were diluted with 5 mL of deionized water and purified with Bio-Rad Poly-Prep Prefilled Chromatography Columns (Phenomenex, Torrance, CA, USA; cat # 731-6213). Salts and organic compounds were removed by adding 10 mL deionized-water (ion-free) to the cartridge, which after AAs were eluted from the column with 6 mL of 2 M of NH_4OH. Samples were then dried under N flow on a heat block at 60 °C. AAs were run as their propyl chloroformates using EZ:faast kit for preparation (Phenomenex). Samples were run with a GC-MS (Shimadzu Ultra, Kyoto, Japan) using ZB-AAA column (9.5 m × 0.25 µm × 0.25 mm) using the following temperature program: a rise from an initial temperature of 110 °C to 320 °C at a rate of 30 °C min^{-1}, after which the temperature was held for 7 min at 320 °C. The injection temperature was 300 °C and the interface was 290 °C. The total column flow was 2.35 mL min^{-1}, and linear velocity was 71.2 cm s^{-1}. AA identification was based on specific ions included by the EZ:faast library. For quantification we used the Sigma-Aldrich AA-18 standard mix, of which we made a 4-point calibration curve (0.005, 0.05, 0.1, and 0.2 µg $µL^{-1}$), derivatized using an EZ:faast kit. Based on the properties of the EZ:faast kit, we were able to analyze 8 EAAs

(valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, and histidine) but not tryptophan. In addition to EAAs, we were able to quantify 10 non-EAAs (NEAA: alanine, asparagine, aspartate, glutamate, glycine, glycine-proline, ornithine, proline, serine, and tyrosine).

Fatty acid and sterol analysis

Fatty acid samples were transmethylated with 1% H_2SO_4 in methanol, and FA methyl esters were run with a gas chromatograph equipped with a mass spectrometer (GC-MS, Shimadzu Ultra, Kyoto, Japan). The instrument was equipped with an Agilent DB-23 (Santa Clara, CA, USA) column (30 m × 0.25 mm × 0.25 μm) using the same temperature program, identification and quantification as Taipale et al. (2016c) .

Sterols were analyzed according Taipale et al. (2016c). Briefly, the extracted lipids were saponified with KOH at 70 °C and then silylated with *N,O-bis*[trimethylsilyltrifluoro-acetamide] (BSTFA), trimethylchlorosilane and pyridine at 70 °C. Trimethylsilyl derivatives of sterols were analyzed with GC-MS equipped with a Phenomenex ZB-5 Guardian column (30 m × 0.25 mm × 0.25 μm). Sterols were identified using characteristic ions (Taipale et al. 2016c).

Carbon, nitrogen, and phosphorus analysis

The proportions of C (C%) and N (N%) content of phytoplankton were analyzed with a Flash 1112 series Element Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Protein content (Protein%) was analyzed by multiplying the elemental N content by 6.8, which is the N content of phytoplankton proteins (Lourenco et al. 2004). Phytoplankton P content (P%) was determined from filtered samples (20–25 mL, on 47 mm GF/C glass microfiber filters; Whatman, UK), oven dried at 105 °C for 4 h. *Daphnia* were freeze-dried and 0.1–0.7 mg

weighed for P analyses. The filters and *Daphnia* were placed in glass jars, and P was extracted with sulfuric acid. Samples were analyzed with the automated discrete photometric analyzer Gallery Plus (Thermo Fisher Scientific).

Data analysis

For field data, the interactions between *Daphnia* biomass (C: µg L^{-1}) and distinct biochemical and biological factors were tested using Pearson correlation coefficients, but Spearman rank order correlation analysis was used when data did not meet normality assumptions. The tested factors were total P of seston (µg L^{-1}), total sum of ω-3 and EPA concentration (FA: µg L^{-1}) and content (µg mg^{-1} C) in the seston (same day, 4 d earlier, 7 d earlier), the contribution of assimilated high-, intermediate-, and poor-quality phytoplankton according to the FASTAR results, total phytoplankton biomass (C: µg L^{-1}), and biomass of high-quality (cryptophytes, chrysophytes, diatoms), intermediate-quality (green algae, Conjugatophyceae), and poor-quality (cyanobacteria, raphidophytes) phytoplankton (C: µg L^{-1}). Because *Daphnia*'s reproduction cycle is 3–4 d, and because they exhibit a change of FA in their tissues in 6 d (Ebert 2005, Taipale et al. 2011a), we also compared seston 4 d earlier and 7 d earlier with *Daphnia* biomass.

The growth response and dietary thresholds of *Daphnia* in laboratory experiment 1 were estimated with nonlinear regression analysis following Taipale et al. (2016b). The Kruskal-Wallis *H* test was used to test differences in the timing of *Daphnia* first clutch or cumulative offspring number between different algae and food concentrations in laboratory experiment 1, and the differences in the growth on day 14 and in the cumulative offspring number between different diets in laboratory experiment 2. The differences in growth on day 8 could not be tested because the number of replicates was too low. The interactions between the biochemical composition of the diet and of *Daphnia*, and the growth and cumulative

offspring number of *Daphnia* were examined with Spearman rank order correlation. All statistical analyses were conducted using SPSS 24.0 (IBM Corp., Armonk, NY, USA). The FASTAR analysis (Galloway et al. 2015) was run using R (R Development Core Team 2017) following the assumptions described in Taipale (2016b). We summarized the mixing model results by summing the median proportion of each item in the mixing model into the food quality categories. This form of data reduction must be interpreted with caution because it may oversimplify or mask the uncertainty captured in the Bayesian model posterior distributions. However, we used the approach here to generate an index of relative food quality dynamics in the lake through time and to generate the hypotheses tested in the feeding experiments (figures for all posterior distribution analyses are in Supplemental Material).

RESULTS

Laboratory experiment 1: the effect of diet quantity and biochemical quality on Daphnia growth and reproduction

Biochemical composition of algal diets

Rhodomonas had the highest protein content (72% [SD 1%] of dry weight [DW]) and AAs (307 [51] µg mg C^{-1}) among all algae (Table 1). The concentration of nonessential AAs was 3 times higher in *Rhodomonas* than in any other algae. Leucine (19% [1%] of all AAs) and alanine (11% [1%] of all AAs) were major AAs in *Rhodomonas*. Protein and AA contents of *Cryptomonas*, *Peridinium*, *Acutodesmus*, and *Mallomonas* were similar, whereas lysine (~30% of all AAs) was the major AA. The total concentration of ω-3 PUFA was 2 times higher in *Rhodomonas* than in any other algae, which all had similar ω-3 PUFA contents (76 [10] µg mg^{-1} C). Highly unsaturated FAs (HUFA) content (EPA+DHA) was highest in *Peridinium* (56 [11] µg mg^{-1} C) and in *Rhodomonas* (45 [5] µg mg^{-1} C), but only low amounts were found in *Mallomonas* (4 [1] µg mg^{-1} C), and the HUFA content was under the

detection limit (0.05 µg mg^{-1} C) in *Acutodesmus*. Trimethyl-sterols were the main sterol group in *Peridinium*, which also contained cholesterol (20% [2%] of total sterols) and a small amount of campesterol (1.5% [0.5%]). *Rhodomonas* contained brassicasterol (89% [3%]) and stigmasterol (10% [3%]), whereas *Mallomonas* contained ß-sitosterol (22% [1%]) in addition to stigmasterol (74 [SD 1%]). *Acutodesmus* had mainly Δ7 (fungisterol, dihydrochonrillasterol) and Δ7, 22 sterol (chondrillsterol) but also contained fucosterol.

Daphnia growth and reproduction

The *Daphnia* somatic growth and cumulative offspring number varied among the 4 algal diets. *Daphnia* survival was highest with *Rhodomonas* (100%), followed by *Mallomonas* (87%), *Acutodesmus* (81%), and *Peridinium* (55%). The maximum somatic growth (W_{max}) was highest with *Acutodesmus* (0.21 [SD 0.00] mg d^{-1}), intermediate with *Mallomonas* (0.18 [0.00] mg d^{-1}) and *Rhodomonas* (0.17 [0.01] mg d^{-1}), and lowest with *Peridinium* (0.15 [0.01] mg d^{-1}; Fig. 1). However, the amount of C needed by *Daphnia* to achieve the growth threshold (90% of W_{max}) was lowest with *Rhodomonas* (0.9 mg C), whereas with *Mallomonas*, 2.2 mg of C was required (Fig. 1). The cumulative offspring number was also highly variable among algae, but no offspring was produced at lowest food concentration (C: 0.25 mg L^{-1}) in any algae treatment. The cumulative offspring number with the 2 highest food concentrations (C: 3.8 and 5 mg L^{-1}), during 20 d was higher with *Rhodomonas* than with *Peridinium* (Kruskal Wallis *H* test, pairwise comparisons, $p < 0.05$), but otherwise did not differ ($p > 0.05$) among diets. Cumulative offspring number with C concentration of 1.25 and 2.5 mg L^{-1} was lower (Kruskal Wallis *H* test, pairwise comparisons, $p < 0.05$) in *Daphnia* fed with *Peridinium* than with *Rhodomonas* or with *Acutodesmus*. In addition, *Daphnia* fed with *Rhodomonas* had more offspring than *Daphnia* fed with *Mallomonas* ($p < 0.05$).

In all food concentrations, *Daphnia* produced the first clutch earlier when fed with *Rhodomonas* or *Mallomonas* than when fed with *Acutodesmus* or *Peridinium* (Kruskal Wallis *H* test, pairwise comparisons, $p < 0.05$). With *Rhodomonas*, *Daphnia* produced the first offspring on day 8 with all food concentrations and continued to produce offspring every second day, excluding days 12 to 14 when the diet was switched to *Cryptomonas*. With *Mallomonas*, *Daphnia* also had the first offspring on day 8, but clutch size was half that with *Rhodomonas*. When the *Mallomonas* food concentration decreased to 25% (C: 1.25 mg L^{-1}), the first offspring was produced on day 10. With *Acutodesmus* the first clutch was generally produced on day 10, and with *Peridinium* the first offspring was produced on average on day 14.

Biochemical thresholds and correlations

The concentrations of AAs, ω-3 PUFA (µg L^{-1}), and sterols corresponding to the amount of C at the growth threshold varied among the algal diets (Fig. 1). The threshold concentration of EAA was 216 µg L^{-1} for *Mallomonas* and other algal diets had considerable lower threshold concentrations (109 µg L^{-1} for *Acutodesmus*, 116 µg for *Peridinium*, and 94 µg L^{-1} for *Rhodomonas*). The threshold concentration of ω-3 PUFA was 178 µg L^{-1} for *Mallomonas* but 96 µg L^{-1} for *Acutodesmus* and 90 µg L^{-1} for *Peridinium* and for *Rhodomonas*. *Acutodesmus* had no HUFA, but HUFA concentration was 26 µg L^{-1} in *Rhodomonas*, 58 µg L^{-1} in *Peridinium*, and 9 µg L^{-1} in *Mallomonas* when 90% of the maximum growth rate was achieved. The threshold sterol content was low for *Peridinium* (8 µg L^{-1}) and for *Rhodomonas* (14 µg L^{-1}) but was higher for *Acutodesmus* (49 µg L^{-1}) and for *Mallomonas* (119 µg L^{-1}). *Daphnia* did not achieve the maximum cumulative offspring number with either of these diets. According to the correlation matrix, growth correlated most strongly with the short-chain ω-3 PUFA, total ω-6 PUFA, high threshold sterols, NEAA, and high

protein content of algae. Large offspring size correlated positively with the total ω-3 PUFA, the short-chain ω-3 PUFA, total AA, EAA, NEAA, and high protein content of algae.

Laboratory experiment 2: the effect of small addition of high-quality diets on Daphnia growth and reproduction

Biochemical composition of diets

Among all diets, *Rhodomonas* had the highest ω-3 PUFA, HUFA, sterol, and P content (µg mg^{-1}), whereas *Micrococcus luteus* had the highest AA content and *Acutodesmus* had the highest ω-6 content (Table 2). *Rhodomonas* was the only diet that contained high amounts of HUFA (EPA+DHA). Compared to *Rhodomonas*, *Acutodesmus* contained less than half of total ω-3 PUFA, sterol, and P. *Micrococcus luteus* contained no ω-3 PUFA or sterols, whereas tPOM (birch) and HNFs contained small amounts in ω-3 PUFA and sterols (Table 2). The ß-sitosterol (45% [SD 1%]), campesterol (20% [1%]), and stigmasterol (17% [1%]) were the main sterols in birch, whereas HNFs contained mainly stigmasterol and trace amount of cholesterol (<2%). The 25% addition of *Rhodomonas* in mixed diets remarkably increased the availability of AAs, P, ω-3 PUFA, HUFA, and sterols for *Daphnia*. In the tPOM and *Rhodomonas* mixed diets, 68–100% of AAs, P, ω-3 PUFA, HUFA, and sterols originated from *Rhodomonas*. *Micrococcus luteus* contained high amounts of AAs and P, and thus only 15–27% of AAs and P originated from *Rhodomonas* in the mixed diet, whereas 100% of PUFA and sterols originated from *Rhodomonas* in mixed diet. The mix of HNFs and *Rhodomonas* sp. was similar to bacteria, but 77% and 54% of sterols and ω-6 PUFA originated from *Rhodomonas*, respectively. The mix of 75% of *Acutodesmus* and 25% of *Rhodomonas* enhanced the amount of EAA, ω-3 PUFA, sterols, and P compared to 100% of *Acutodesmus*; however, the most significant increase was in the availability of EPA in the diet.

Survival and somatic growth of *Daphnia*

Survival was 90–100% in most treatments, but all *Daphnia* fed with pure *Micrococcus* died between days 4 and 10. *Daphnia* growth was highest after 8 d with 100% concentration of pure *Rhodomonas* or *Acutodesmus* or with the mixed *Rhodomonas* or *Acutodesmus* diets. After 14 d, the growth of *Daphnia* fed with either *Rhodomonas* or *Acutodesmus* diets differed significantly from *Daphnia* fed with 25% *Rhodomonas* or 100% tPOM. In addition, *Daphnia* fed with *Acutodesmus* grew faster than *Daphnia* fed with 100% HNFs, mixed HNFs and *Rhodomonas*, or mixed tPOM and *Rhodomonas* (Fig. 2a). The cumulative offspring number was highest with mixed *Rhodomonas* and *Acutodesmus* (49 [SD 4] ind.$^{-1}$), or pure *Rhodomonas* (47 [17] ind. $^{-1}$), but because of the high variation, they did not differ statistically from 100% *Acutodemus*, the mixed HNFs and *Rhodomonas*, or mixed *Micrococcus* and *Rhodomonas*, even though the last 3 had much lower average offspring numbers (Fig. 2b). Among all mixed diets, the mix of tPOM (birch) and *Rhodomonas* resulted in the lowest cumulative offspring number (12 [5] ind.$^{-1}$).

The *Daphnia* growth rate and reproduction correlated positively with the total dietary sterol content, ALA+SDA, total ω-3, and ω-6 PUFA, but not with EPA, AA, or P content (Fig. 3). Furthermore, growth and reproductive output were high when the total ω-3 PUFA, ALA+DA content, and C:P in *Daphnia* were high, whereas they were not related to the sterol, EPA, or ω-6 PUFA content of *Daphnia*.

Field study: Daphnia *food quality in eutrophic and humic lakes*

Phytoplankton community structure and *Daphnia* population dynamics in Lake Vesijärvi

During the open-water season in 2016, cyanobacteria blooms prevailed for most of the growing season from early June until early August, and thus poor-quality diets were the most abundant available food during that period (Fig. 4a). During this bloom, cyanobacteria (*Woronichia*, *Microcystis*, *Snowella*, and *Planktothrix*) consisted of 66–95% of the total phytoplankton biomass. When moderate-quality dinoflagellates were included in the analysis, poor-quality phytoplankton composed 69–95% of the total phytoplankton biomass. By contrast, high-quality diets 10–20% of total phytoplankton were composed of primarily diatoms and cryptophytes in June and July. The abundance of diatoms (*Aulacoseira*, *Fragilaria*, *Asterionella*, and *Tabellaria*) increased in in August, when they accounted for 96% of all phytoplankton biomass. Cyanobacteria (mostly *Woronichinia* and *Planktothrix*), diatoms (mostly *Cyclotella* sp.) and cryptophytes composed 39%, 29%, and 6.2% of all phytoplankton biomass at the end of September, respectively. *Daphnia* and *Bosmina* biomass was lowest during cyanobacteria blooms in June and July but started to increase at the end of July. In August, the biomass of *Daphnia* and *Bosmina* was doubled compared to June and July (Fig. 4a).

The concentration of fatty acids and sterols in seston and *Daphnia* in Lake Vesijärvi

The contribution of all ω-3 PUFA varied from 9% to 25% of total FAs in the seston of Lake Vesijärvi. The concentration of ALA, SDA, EPA, and DHA in the seston was highly variable during the open-water season in 2016 (Fig. 5a). The FA concentration of ω-3PUFA and HUFA (EPA+DHA) was 0.27 (SD 0.25) and 0.04 (0.02) µg L^{-1} in July (during cyanobacteria blooms), respectively, and increased 11- and 27-fold in early August. The highest concentrations of sestonic ω-3 PUFA and HUFA were measured during the clearwater stage on 23 August, when they were 80- and 250-fold higher, respectively, than during the

blooming period in July. The concentration of ω-3 PUFA and HUFA remained high in September.

The concentration of sterols in the seston did not follow a clear pattern but reached maximum in early July. The high-threshold sterols (desmosterol, campesterol, b-sitosterol) were dominant (>50% of all sterols) in the seston during cyanobacteria blooms, whereas low-threshold sterols (brassicasterol, stigmasterol, and cholesterol) were dominant in August and September, largely resulting in an increase of brassicasterol, which formed up to 40% of all August sterols. In contrast to FAs, total sterol content was higher during cyanobacteria blooms than in late August or September (Fig. 5a). The total sterol content of *Daphnia* was similar in July and September (4.3 [SD 0.01] µg mg^{-1} DW) but was half of that in July (1.4 [0.3] µg mg^{-1} DW). Surprisingly, we found equal amounts of desmosterol and cholesterol from *Daphnia*, which together formed 70–90% of all sterols in *Daphnia*.

The contribution of high-quality algae correlated positively (Pearson: $r > 0.92$, $p < 0.05$, $n = 5$) with the concentration of ALA, SDA, EPA, and DHA but not with the concentration of sterols.

Daphnia diet composition in Lake Vesijärvi

The diet assimilated by *Daphnia* and *Bosmina* estimated by the FA mixing model analysis consisted mainly of phytoplankton (69% [SD 12%] in all diets), with the dominant contributors being diatoms (23% [12%] in all diets) and cryptophytes (20% [8%] in all diets; Fig. 6a). Chrysophytes were important only in late July, when they accounted for 27% of *Daphnia* assimilated resources. Dinoflagellates were the most important intermediate-quality diet (12 [5%] of all diets), whereas both green algae (intermediate-quality) and cyanobacteria (poor-quality) formed 4% (3%) of all diets. The *Daphnia* assimilated diet consisted of more poor-quality items (Actinobacteria, cyanobacteria, MOB, and tPOM) during cyanobacteria

blooms in July than in the other months. However, even during this period, the contribution of cyanobacteria in *Daphnia* diet did not exceed 11%.

Pelagic carbon mass balance and preferential utilization of diets

Phytoplankton formed 43% [SD 3%] of all POC in July and September, when quantitative community data were available for Lake Vesijärvi. However, because cyanobacteria were the most abundant phytoplankton, the high- and intermediate-quality phytoplankton formed only 11 (5%) of all POC. Because Actinobacteria, *Polynucleobacter,* and MOB together formed only a small proportion of total POC (<3%), most of the POC had to be detritus, including terrestrial particles. We calculated *Daphnia* diet preference by comparing the relative abundance of diet source consumed by *Daphnia* to its availability in the lake, where a positive value indicates preference and a negative value indicates avoidance of the diet source. Our comparison between available and consumed diet sources showed that in both lakes, *Daphnia*/*Bosmina* preferred primarily cryptophytes (difference between consumed and available: 14.4% [3.2%]) and diatoms (difference: 26.2% [11.6%]), and then dinoflagellates (8.1% [4.9%]) and chrysophytes (5.4% [5.4%]; Fig. 7). The difference between assimilated and available of detritus/tPOM (−29.3% [1.6%]) and cyanobacteria (−13.2% [3.6%]) was strongly negative, indicating these resources were the least desired diets for *Daphnia*/*Bosmina*.

Phytoplankton composition and *Daphnia* biomass in Lake Mekkojärvi

Cyanobacteria (mostly *Microcystis* sp. and *Snowella* sp.) formed 69% (SD 15%; as C biomass) of all phytoplankton in 2005 (Fig. 4b). The contribution of cryptophytes varied widely between 0.8% and 29% of all phytoplankton, with the lowest contribution measured in spring and highest in autumn 2006. Chrysophytes were <4% of total phytoplankton

biomass throughout the open-water season (Fig. 4b), except on 6 June when *Mallomonas akrokomos* formed 67% of phytoplankton biomass. *Chlamydomonas* sp. was most abundant in spring 2005 and 2006, whereas diatoms (*Asterionella*, *Aulacoseira*, *Fragilaria*, and *Tabellaria*) formed 24% and 44% of phytoplankton biomass in October 2005 and 2006, respectively. The biomass of *Daphnia* was highest on 5 June (Taipale et al. 2009b) and was generally higher in autumn than spring or summer.

The concentration of fatty acids and sterols in seston and *Daphnia* in Lake Mekkojärvi

The contribution and concentration of ω-3 PUFA, EPA, and DHA in the seston of the whole water column (epilimnion–hypolimnion; Fig. 5b) in Lake Mekkojärvi varied widely throughout the open-water season. Generally, the contribution of ω-3 PUFA was higher in spring (33% [SD 15%]) and autumn (32% [22%]) than in summer (13% [6%]). Whole water column sestonic fractions (FA) of ω-3 content (µg mg^{-1} C) and phospholipid-derived FA (PLFA) were 38 (22) µg mg^{-1} C and 19 (14) µg L^{-1}, respectively. Correspondingly, sestonic content and concentration of HUFA (EPA+DHA) of the PLFA fraction were highest during autumn (7.9 [2.5] µg mg^{-1} C and 3.3 [1.6] µg L^{-1}), but the content and concentration of HUFA was one-third of autumnal values in summer and spring. Sestonic EPA concentration increased significantly with increasing biomass of cryptophytes and diatoms (Pearson: r = 0.726, p = 0.011, n = 11), but also with total biomass of high-quality algae (cryptophytes, diatoms, and chrysophytes; Pearson: r = 0.683, p = 0.020, n = 11). *Daphnia* biomass was not related to seston P (Spearman: r = −0.27, p = 0.60, n = 6) or N (r = 0.371, p = 0.47, n = 6) content, or to the total phytoplankton biomass (p > 0.08), but was correlated with the biomass of high-quality phytoplankton (Pearson; r = 0.58, p < 0.05, n = 12). *Daphnia* biomass was not related to the HUFA concentration of the same day seston but followed the seston HUFA

content measured at 4 d (Pearson: $r = 0.59$, $p = 0.03$, $n = 14$) and 7 d (Pearson: $r = 0.57$, $p = 0.04$, $n = 14$) before.

Daphnia diet composition and population dynamics of Lake Mekkojärvi

During open-water seasons 2005 and 2006, the nutritional quality of the assimilated diet of *Daphnia* varied widely (Fig. 6b) according to the FA-based mixing model analyses. *Daphnia* assimilated resources from multiple dietary sources but mainly from poor (45% [SD 16%]) and intermediate (26% [16%]) quality diets (Fig. 6b). The contribution of the high-quality resources (cryptophytes, chrysophytes, and diatoms) was on average 20% (1%) of all assimilated resources. During the spring *Chlamydomonas* bloom, the model results indicate that *Daphnia* are supported by ~50% green algae. The biomass of *Daphnia* was highest in early June when high-quality phytoplankton was most abundant. According to linear regression ($F_{10, 11}= 8.73$, $p = 0.014$, $r^2=0.68$; $y = 1075.2x - 20.3$), ~47% of the *Daphnia* biomass (y: µg C L^{-1}) was explained by the proportion of assimilated high-quality algae (x: proportion) during summer stratification. *Daphnia* had 2 smaller biomass peaks during the autumn turnover, which was result of high assimilation of MOB and diatoms. According to the mixing model analysis, the contribution of tPOM to *Daphnia* was lowest in spring and highest during summer and autumn and did not correlate with the DOC concentration in the epilimnion.

Pelagic carbon mass balance and preferential utilization of diets

All phytoplankton combined consisted of 28% (SD 15%) of total POC, but high- and intermediate-quality phytoplankton formed only 6.6% (6.7%; range 2.2–22.8%) of POC in Lake Mekkojärvi during the open-water season 2006. Taipale et al. (2011b) previously showed that *Chlorobium* (53% [31%] of total POC) and MOB (29% [18.5%]) accounted for

the highest proportion of POC, along with tPOM/detritus (37% [22.8%]) while Actinobacteria and *Polynucleobacter* formed only a minor proportion of total POC (<5%). Our comparison between the relative abundance of assimilated diet source in *Daphnia* and its availability in the lake revealed that *Daphnia* preferred, in declining order, green algae (difference between consumed and available: 14.7% [13.8%]), diatoms (12.3% [9.1%]), cryptophytes (6.3% [2.8%]), dinoflagellates (1.5% [0.8%]), and chrysophytes (3.5% [3.5%]; Fig. 7b). The MOB (−17.1% [19.7%]), detritus/tPOM (−16.0% [26.1%]), and cyanobacteria (−10.8% [12.2%]) were consumed at a lower proportion than their overall availability, indicating they were the least preferred in diets of *Daphnia*/*Bosmina*. We found that daphnids in Lake Mekkojärvi were more flexible in their food consumption compared to *Daphnia* in Lake Vesijärvi, as evidenced by higher variation in the utilization of green algae, cyanobacteria, and tPOM/detritus.

DISCUSSION

Our first laboratory experiment with 4 algae showed that the cumulative offspring number of *Daphnia* increased substantially with food quantity; the steepest rate was with *Rhodomonas*/*Cryptomonas* and *Mallomonas.* This explains why zooplankton are known to preferentially feed on high nutritional quality algae (Persson 1985, Knisely and Geller 1986). Our second experiment showed that the cumulative offspring number of *Daphnia* is multiplied when even a small amount of high-quality diet (*Rhodomonas*) is added to poor- and intermediate-quality diets. Furthermore, *Daphnia* achieved a lower cumulative offspring number when *Rhodomonas* was mixed with tPOM than with any other diet. Biochemical analysis of the diets showed that tPOM is low in all essential biomolecules, whereas bacteria and HNFs contained high amounts of AAs and P.

Eutrophication and browning can drive freshwater ecosystems toward a low abundance of nutritionally valuable phytoplankton. We found that high-quality phytoplankton (cryptophytes, chrysophytes, and diatoms) formed only 8.7% (SD 2.4%) of total POC in a eutrophic clearwater lake (TP: 20–30 µg L^{-1}, DOC: 5–8 mg L^{-1}) and 6.5% (7.0%) of total POC in an oligotrophic polyhumic lake (TP: 8–14 µg L^{-1}, DOC: 20–40 mg L^{-1}), which is only half of that previously found in eutrophic or humic lakes (Taipale et al. 2016b). The analysis of the lake FA data allowed us to document the seasonal shifts in zooplankton resource assimilation. The high-quality algae consisted of 55% (12%) and 26% (9%) of *Daphnia* diet in eutrophic and humic lakes, respectively, as estimated with the FA mixing model. Moreover, the comparison between assimilated and available diets demonstrates that in both lakes, *Daphnia* preferentially consume patches of high nutritional quality algae. This strategy of obtaining nutritionally valuable algae enabled *Daphnia* to build high biomasses during summer stratification in a humic lake. However, high assimilation of diatoms during fall mixing did not result in a high biomass of *Daphnia.* We speculate that increasing mixing depth was probably unfavorable to vertically migrating *Daphnia* because it increases mortality, accompanying a reduction in hypolimnetic refuge volume (Sastri et al. 2014). Another option for the negligible response of *Daphnia* to enhanced food resources was the shift to diapause, evidenced as males and ephippial females in the population. Our study thus demonstrates how eutrophication and browning may diminish high nutritional quality resources and challenge herbivorous zooplankton to locate high nutritional quality algae, especially in highly humic lakes.

Enhancement of the nutritional value of poor-quality diets

Zooplankton must obtain the essential biomolecules (AAs, FAs, sterols, and P) from their diet. The purpose of our laboratory experiment was to define maximum offspring production

when fed dinoflagellates, chrysophytes, and green algae in relation with cryptophytes, a well-known superior diet for *Daphnia* (Brett et al. 2009, Peltomaa et al. 2017). Previously, we showed that *Daphnia* have difficulty ingesting cyanobacteria, euglenoids, and dinoflagellates, and hence these are poor-quality diets (Peltomaa et al. 2017). However, in this study, *Daphnia* had higher growth and cumulative offspring numbers when fed the dinoflagellate *Peridinium* than previously observed (Peltomaa et al. 2017). The generally low cumulative offspring number with *Peridinium* diet may be a result of the thick cell wall of dinoflagellates, or of *Daphnia* having difficulties utilizing trimethyl sterols to cholesterol, similar to dihydrocholesterol or lanosterol (Martin-Creuzburg and von Elert 2004). *Daphnia* generally grows well with green algae and can have a relatively high reproduction rate with *Acutodesmus*; however, in this study *Daphnia* biomass did not follow the assimilated proportion of green algae in the eutrophic or in the humic lake, indicating that the green algae lacked some biomolecules (e.g., sterols or EPA) essential for *Daphnia* offspring production. In our laboratory experiment, *Daphnia* fed with *Mallomonas* (chrysophyte) had the second highest cumulative offspring number, but variation was high between individuals. In our previous experiment, *Daphnia* fed with cryptophytes and diatoms had a similar reproduction rate (Peltomaa et al. 2017). These 2 taxa were also preferentially consumed in eutrophic and humic lakes, but again exhibited high variation, possibly a result of difficult ingestion of some species of diatoms (filamentous, colonial) and chrysophytes (spines), or temporal variation in nutritional value due to environmental factors such as temperature, light intensity, and nutrients (Guschina and Harwood 2009), and algal growth stage (Jonasdottir 1994, Boelen et al. 2017).

Our second laboratory experiment demonstrated that even a small addition of high-quality diet can improve the nutritional quality of the poor-quality diets, with a resulting synergistic effect on growth and reproduction of the zooplankton consumer. This improvement can be

explained by limitation of 1 or 2 essential biomolecules; when zooplankton obtain these biomolecules above a threshold, they can utilize the non-limiting components of the poorer-quality diet. For example, the bacteria or bacteria–HNF diet did not contain or had only low amounts of ω-3 PUFA and sterols, respectively, which obviously hindered reproduction of *Daphnia* fed these diets. When a small amount of *Rhodomonas* was added (25%), *Daphnia* were no longer limited with respect to ω-3 PUFA and sterols and could therefore beneficially utilize the high P and AA concentrations provided by the bacteria and HNFs. This process also explains the higher cumulative offspring number of *Daphnia* with bacteria than with the tPOM diet, in which all the biomolecules exist in low concentrations.

Eutrophication and browning impact on assimilation of high-quality resources

Our field study and laboratory studies demonstrated that nutritionally high-quality diets are crucial for supporting *Daphnia* production. Among all phytoplankton, cryptophytes are a superior diet for *Daphnia* (Peltomaa et al. 2017), but they alone do not explain the changes in *Daphnia* biomass, which was also found to be related to the abundance of diatoms and chrysophytes, indicating that a constant high availability of high-quality algae is important for successful zooplankton biomass development. The seasonal succession of different nutritionally valuable phytoplankton taxa is important for supporting zooplankton biomass and the function of lake food webs. Diatoms are usually abundant during spring and autumn turnover times, whereas the abundance of cryptophytes and chrysophytes fluctuates during the open-water seasons (Stewart and Wetzel 1989). We found that the factors influencing phytoplankton succession were quite different in studied eutrophic and humic lakes. Grazing pressure on phytoplankton was high in the humic fishless lake but relatively low in the eutrophic lake because of the intense top-down regulation in Lake Vesijärvi (Anttila et al. 2013). The population of smelt (*Osmerus eperlanus*), the dominant pelagial planktivorous

fish in Lake Vesijärvi, reached extremely high abundance in 2015–2017, with almost exclusively 0+ individuals (Ruuhijärvi et al. 2018). The heavy top-down control by smelt is, furthermore, probably enhanced by hypolimnetic aeration that eradicated the dark, hypoxic hypolimnetic refuge for large-bodied cladocerans, evidenced as a remarkable decline in the body size of filter-feeding cladocerans following the increase of smelt population (KK, unpubl. data). This mixing, and thus destratification, of water layers throughout the summer may have also favored cyanobacteria and diatoms over motile cryptophytes and chrysophytes. The water color of Lake Mekkojärvi (437 [SD 49] mg L^{-1} Pt-Co) was exceptionally high in summer 2005, resulting in low light attenuation into the water column, possibly favoring mixotrophic algae adapted to low light and considered a common diet for zooplankton in humic lakes (Arvola and Salonen 2001). Both chrysophytes and cryptophytes were found to be abundant in summer, but no higher assimilation of either of these taxa by *Daphnia* was found. Generally, the assimilation rates of cryptophytes were higher in the eutrophic lake than in the humic lake. Although moderate eutrophication can increase the biomass of cryptophytes (genus *Cryptomonas*; Trifonova 1993, Taipale et al. 2016a), and cryptophytes can make up to 57% (SD 2%) of *Daphnia* diet in the absence of cyanobacteria blooming (Taipale et al. 2016b), our finding of cryptophytes accounting for 20% (8%) of assimilated *Daphnia* diet in Lake Vesijärvi shows that cyanobacteria blooms can significantly suppress utilization of this superior diet. Furthermore, our present seasonal study of Lake Mekkojärvi showed that intense browning diminishes utilization of cryptophytes by *Daphnia* to 8% (2%). In 2016, the cyanobacterial bloom in Lake Vesijärvi began in early June and lasted until the end of July. This extended bloom period probably prevented cryptophytes and chrysophytes from increasing in biomass. After the cyanobacterial bloom collapsed, diatoms (centric; *Cyclotella*) became more abundant, and sestonic EPA content increased 10-fold, resulting in increase in *Daphnia* population biomass. In Lake Mekkojärvi, the *Daphnia* diet

consisted of diatoms in the early summer and during the autumnal turnover, which is also when the highest sestonic EPA content was measured. Altogether, these results from both lakes studied suggest that, in the absence of the raphidophyte *Gonyostomum semen*, sestonic EPA content is a reliable proxy of availability of high nutritional quality algae.

Compensation under deficiency of high nutritional quality algae

Because eutrophication and browning diminish the abundance of high nutritional quality algae, compensation by utilizing intermediate- and poor-quality diet is crucial for zooplankton. The seasonal succession of the diet composition of *Daphnia* differed between the eutrophic lake and the humic lake. Green algae, especially *Chlamydomonas*, blooms after ice-break in small ponds, including humic lake Mekkojärvi, but forms only ~20% of all *Daphnia* diet until August, even though it formed <1% of available POC. This result may be biased from the FASTAR mixing model analysis, which may be confounded by cyanobacteria (e.g., *Aphanothece* and *Aphanizomenon*) and green algae because of the relatively similar FA profiles between these groups. Our mixing model results did not indicate green algae as a significant dietary resource for *Daphnia* in Lake Vesijärvi, whereas dinoflagellates formed 12% (SD 5%) of *Daphnia* diet. By contrast, the model estimated that dinoflagellates formed <2% of the *Daphnia* diet in Mekkojärvi. This result may be explained by differences in the digestibility and ultimately the assimilation of dinoflagellates in these lakes; the dinoflagellates in Lake Vesijärvi were soft-shield *Gymnodinium*, whereas in Lake Mekkojärvi this taxa consisted of armored *Peridinium*.

Because of the narrow oxygen layer and low primary production in humic lakes, *Daphnia* are forced to feed on bacteria and terrestrial detritus in addition to phytoplankton (Ojala and Salonen 2001, Taipale et al. 2008, Kankaala et at. 2010). Our previous study on multiple humic lakes showed that the contribution of assimilated tPOM is not related to lake DOC

content (Taipale et al. 2016a), whereas our present study emphasizes high seasonal variation (2–44%) in utilization of tPOM by *Daphnia*, according to the mixing model analyses. The relatively high values of tPOM estimated here can also be related to poor nutritional status of *Daphnia*, which have similar FA signatures when fed tPOM and experimentally starved (Galloway et al. 2014). In contrast to the humic lake, tPOM formed only 4–10% of all assimilated diets in *Daphnia* in the eutrophic Lake Vesijärvi in 2016. Furthermore, bacteria (Actinobacteria or MOB) did not seem to be an important food (<10%) for *Daphnia* in the eutrophic Lake Vesijärvi, where *Daphnia* is mostly supported by diatoms and cryptophytes. In the humic lake, bacteria formed up to 35% of all assimilated diets; thus, *Daphnia* seems to prefer photosynthetic bacteria and MOB, presumably because of their high P content (Salonen and Lehtovaara 1992), which together with the high-quality algae can support high offspring production and population growth rates in *Daphnia* (Taipale et al. 2012).

We found that the role of heterotrophic bacteria is relatively small in *Daphnia* diets. The trophic upgrading of bacteria by ciliates or HNFs has been proposed in some studies (Sanders et al. 1996, Bec et al. 2003, 2006), whereas others have reported the low nutritional quality of these diets because of the absence of sterols (Martin-Creuzburg et al. 2006). Ciliates are able to ingest sterols from their diet (Martin-Creuzburg et al. 2006), and thus ciliates feeding on the residues of algal detritus can contain some algal-originated sterols. The cumulative offspring number did not differ when *Daphnia* were fed with either *Actinobacteria* directly or HNFs that were feeding on *Actinobacteria*. Therefore, even if HNFs could upgrade the nutritional value of bacteria to *Daphnia* in laboratory experiments (Bec et al. 2003, 2006), it would not likely provide a significant benefit for *Daphnia* fitness in the field conditions, where high-quality algae are always present.

In conclusion, we showed that zooplankton growth rate and offspring production can vary depending on which phytoplankton classes are available as food, and this, in turn, is affected

by eutrophication and browning in 2 representative, yet contrasting, boreal lakes. Thus, forthcoming studies should focus on the impact of these disturbances on phytoplankton composition at the genus level to improve understanding of how they might modify the interactions between zooplankton and phytoplankton. Major environmental changes such as eutrophication and browning can have cascading negative effects from primary produces via herbivorous zooplankton to fish, as previously showed in a laboratory experiment (Taipale et al. 2018), a finding supported by our field detection of diminished availability and assimilation of high nutritional quality algae in zooplankton.

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Figure 1. Somatic growth rate and cumulative offspring number (reproduction) of *Daphnia* in relation to sterol (STE) and ω-3 content during a 20 d laboratory experiment, fed with (a)

Rhodomonas, (b) *Acutodesmus*, (c) *Mallomonas*, and (d) *Peridinium*. Dashed lines refer to growth rate threshold.

Figure 2. *Daphnia* growth rate (mean [SD]) and cumulative offspring number on day 14 in laboratory experiment 2. The dark gray bar denotes for the proportion of 25% *Rhodomonas* (Rhodo) addition in the mixed diets, the gray bar the proportion of the diet, and the light bar the "unknown" proportion. tPOM = terrestrial particulate organic matter; HNF = heteronanoflagellate. Letters above zones are results of non-parametric multiple comparison post hoc test.

Figure 3. Correlation plot describing the interactions between the biochemical composition of the diet and of *Daphnia* (Dph), and the growth and cumulative offspring number (offspring) of *Daphnia*. Only significant correlations are shown. The areas of circles show the absolute value of corresponding correlation coefficients. Colors (shades) show the sign of the correlation: red (black) indicates a negative correlation, and blue (light grey) a positive correlation. HUFA = highly unsaturated fatty acids, BrFA = Branched fatty acids, MUFA = monounsaturated fatty acids, SAFA = saturated fatty acids, CHL = cholesterol, STE = phytosterols, w3 = the sum of all ω-3FA, AA = amino acids, NEAA = non-essential amino acids, EAA = essential amino acids.

Figure. 4. The contribution of distinct phytoplankton (%) of total phytoplankton biomass (a) during open-water season in 2016 in Lake Vesijärvi (eutrophic lake), and (b) during open-water season in 2005 and 2006 in Lake Mekkojärvi (humic lake). Abbreviations: Crypto = cryptophytes, Golden = golden algae, Dino = dinoflagellates, Green = green algae, Cyano = cyanobacteria.

Figure. 5. The concentration (FE/STE: µg L^{-1}) of alpha-linolenic acids (ALA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) of seston (primary y-axis) and *Daphnia* (secondary y-axis) biomass in (a) Lake Vesijärvi (eutrophic lake) and (b) Lake Mekkojärvi (humic lake). The concentration (C: µg L^{-1}) of low threshold sterols (LTS: brassicasterol, cholesterol, stigmasterol) and high threshold sterols (HTS: ß-sitosterol, campesterol, desmosterol) of seston (primary y-axis) are also represented for Lake Vesijärvi.

Figure. 6. Dietary resources assimilated by *Daphnia* on (a) Lake Vesijärvi in 2016 and (b) Lake Mekkojärvi in 2005 and 2006 based on fatty acid-based mixing model analysis. Crypto = cryptophytes, Golden = golden algae, Dino = dinoflagellates, Green = green algae, Cyano = cyanobacteria, Actino = Actinobacteria, MOB = methane oxidizing bacteria, tPOM = terrestrial particulate organic matter.

Figure. 7. Boxplot analysis of the diet preference of *Daphnia* based on the difference between the contribution of assimilated and available 5 phytoplankton groups, Actinobacteria, methane oxidizing bacteria, and particulate organic matter together with detritus in Vesijärvi and Mekkojärvi. Positive values indicate high preference (more assimilated than available), whereas negative values cite low preference (more assimilated than used). Abbreviations: Crypto = cryptophytes, Golden = golden algae, Dino = dinoflagellates, Green = green algae, Cyano = cyanobacteria, Actino = Actinobacteria, MOB = methane oxidizing bacteria, tPOM = terrestrial particulate organic matter.

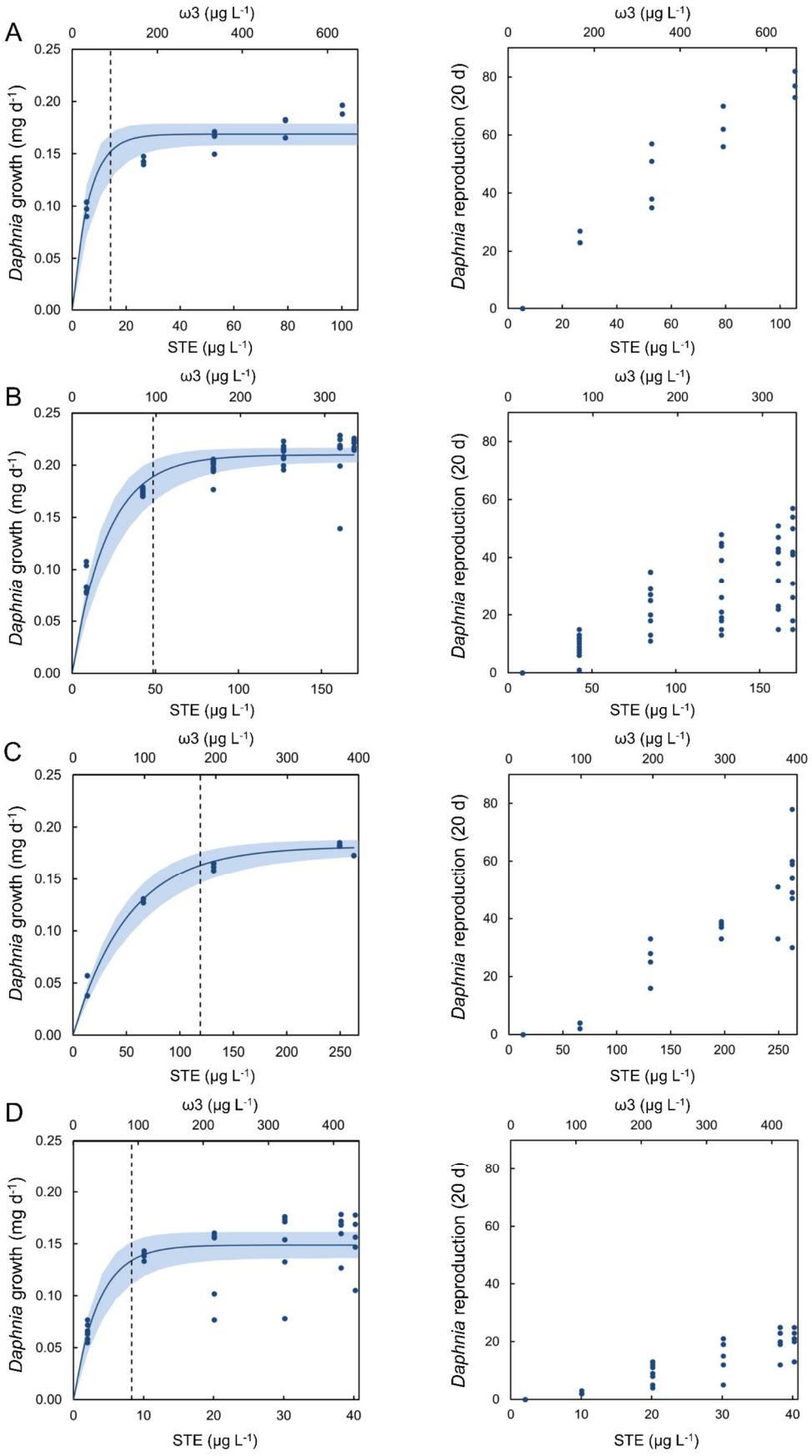
A
B
C
D
ω3 (µg L-1)
Daphnia growth (mg d-1)
Daphnia reproduction (20 d)
STE (µg L-1)

Figure 1.

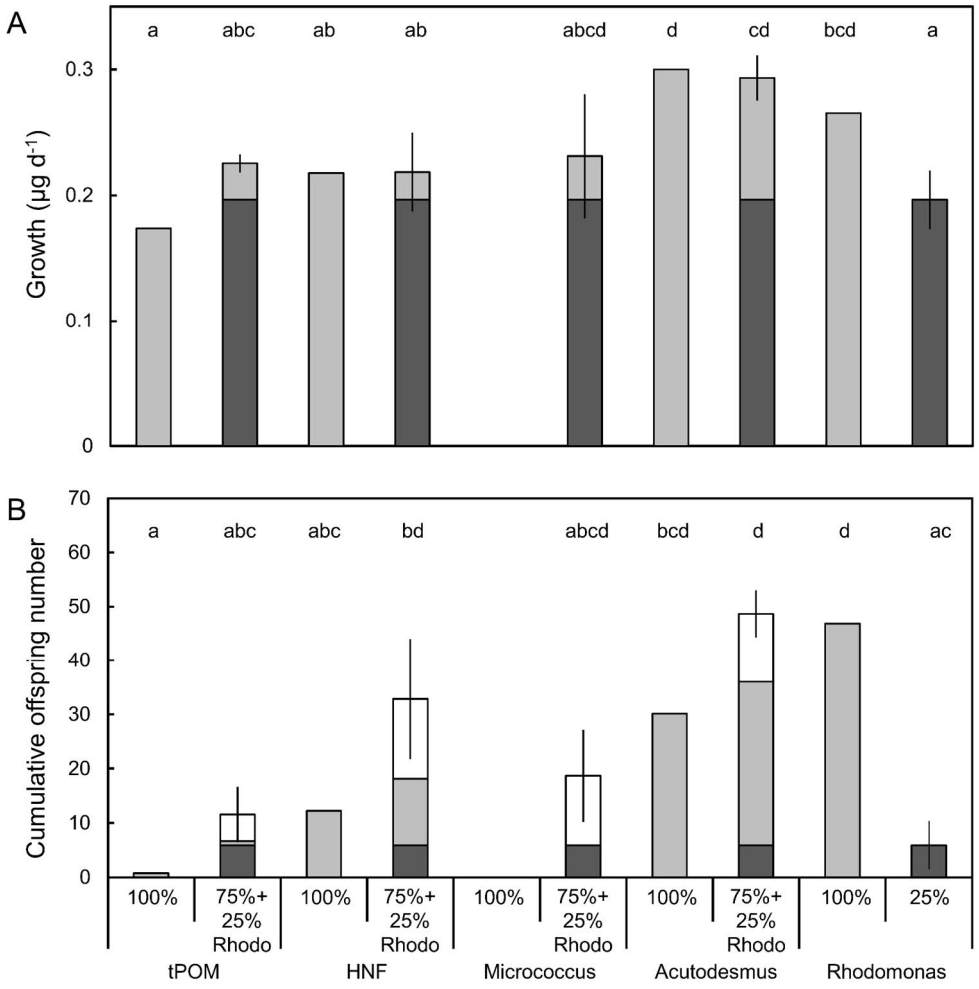
A
a
abc
ab
ab
abcd
d
cd
bcd
a
Growth (µg d-1)
0.3
0.2
0.1
0
B
a
abc
abc
bd
abcd
bcd
d
d
ac
Cumulative offspring number
70
60
50
40
30
20
10
0
100%
75%+ 25% Rhodo
100%
75%+ 25% Rhodo
100%
75%+ 25% Rhodo
100%
75%+ 25% Rhodo
100%
25%
tPOM
HNF
Micrococcus
Acutodesmus
Rhodomonas

Figure 2

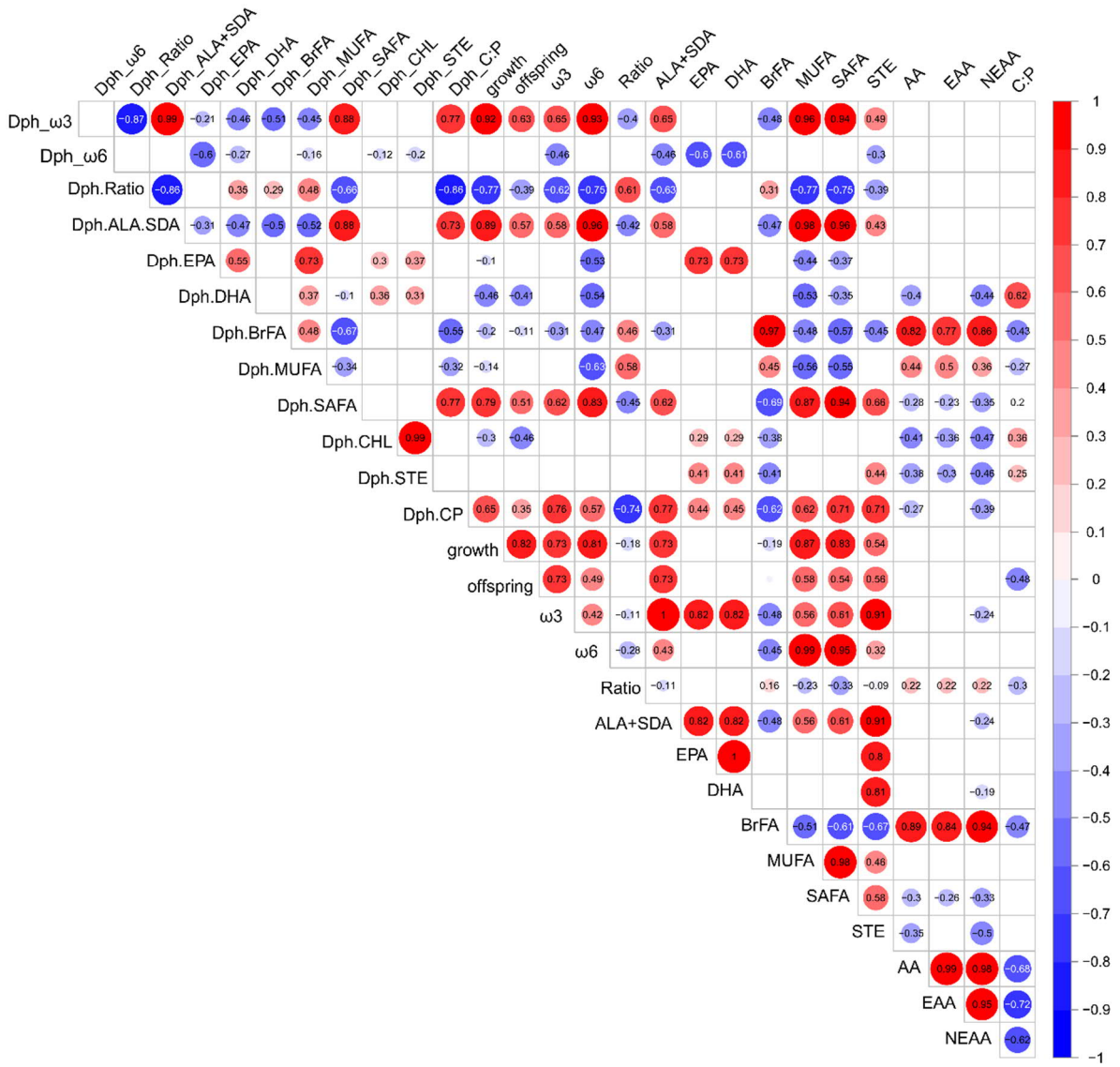
Dph_ω6
Dph_Ratio
Dph_ALA+SDA
Dph_EPA
Dph_DHA
Dph_BrFA
Dph_MUFA
Dph_SAFA
Dph_CHL
Dph_STE
Dph_C:P
growth
offspring
ω3
ω6
Ratio
ALA+SDA
EPA
DHA
BrFA
MUFA
SAFA
STE
AA
EAA
NEAA
C:P
Dph_ω3
Dph_ω6
Dph.Ratio
Dph.ALA.SDA
Dph.EPA
Dph.DHA
Dph.BrFA
Dph.MUFA
Dph.SAFA
Dph.CHL
Dph.STE
Dph.CP
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
−0.1
−0.2
−0.3
−0.4
−0.5
−0.6
−0.7
−0.8
−0.9
−1

Figure 3.

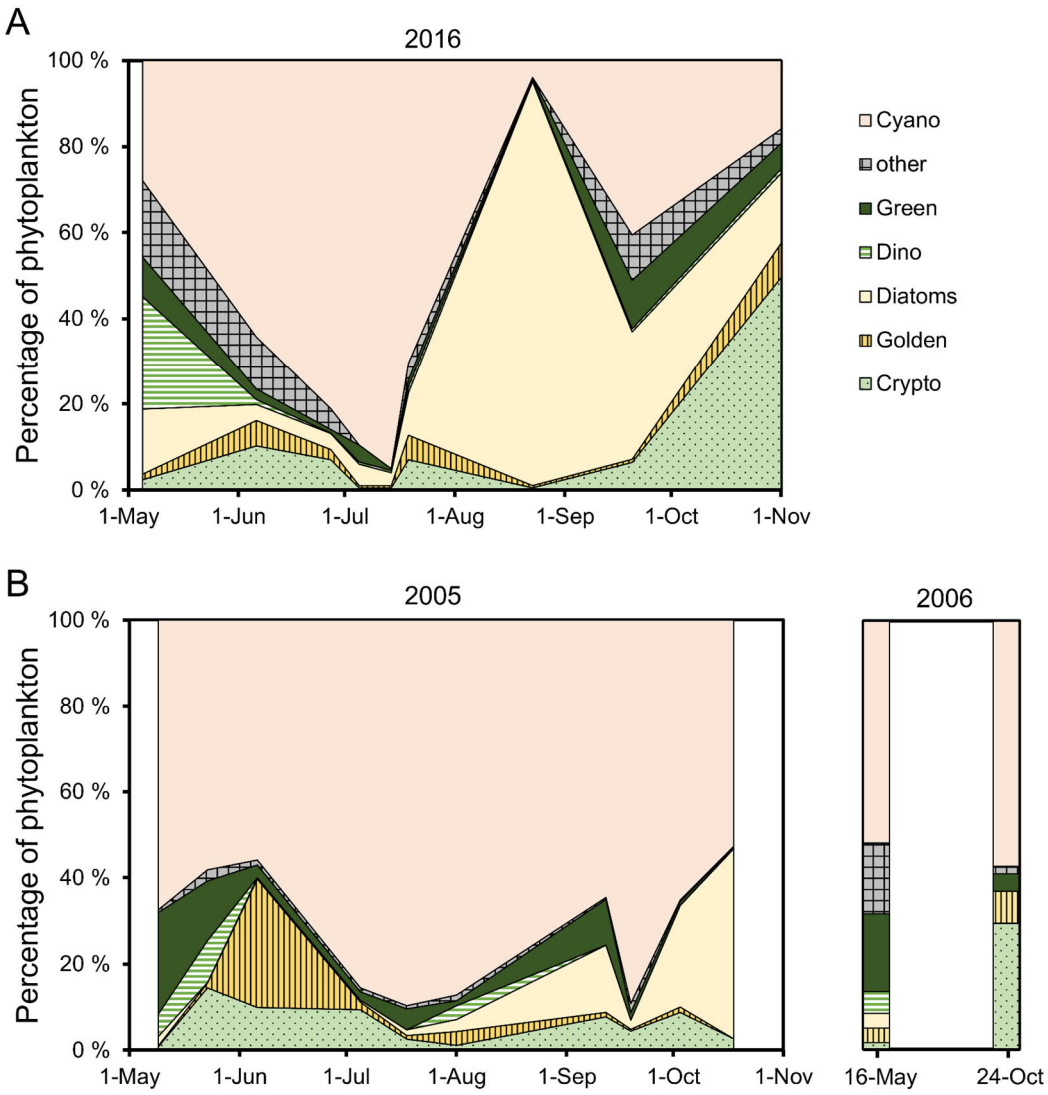
A
2016
100 %
80 %
60 %
40 %
20 %
0 %
Percentage of phytoplankton
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
1-Nov
Cyano
other
Green
Dino
Diatoms
Golden
Crypto
B
2005
2006
16-May
24-Oct

Figure 4.

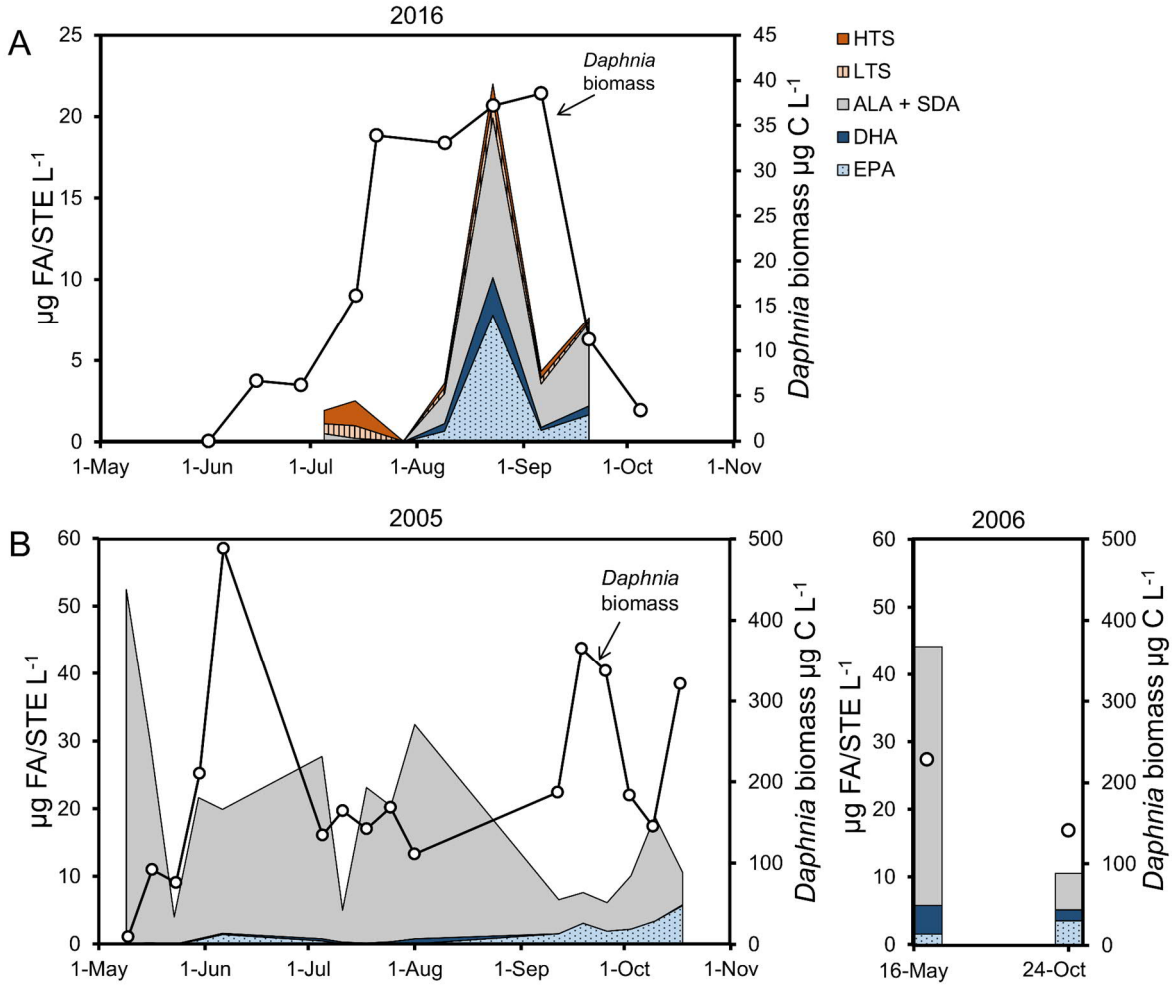
A
2016
Daphnia
biomass
HTS
LTS
ALA + SDA
DHA
EPA
µg FA/STE L-1
Daphnia biomass µg C L-1
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
1-Nov
B
2005
2006
16-May
24-Oct

Figure 5.

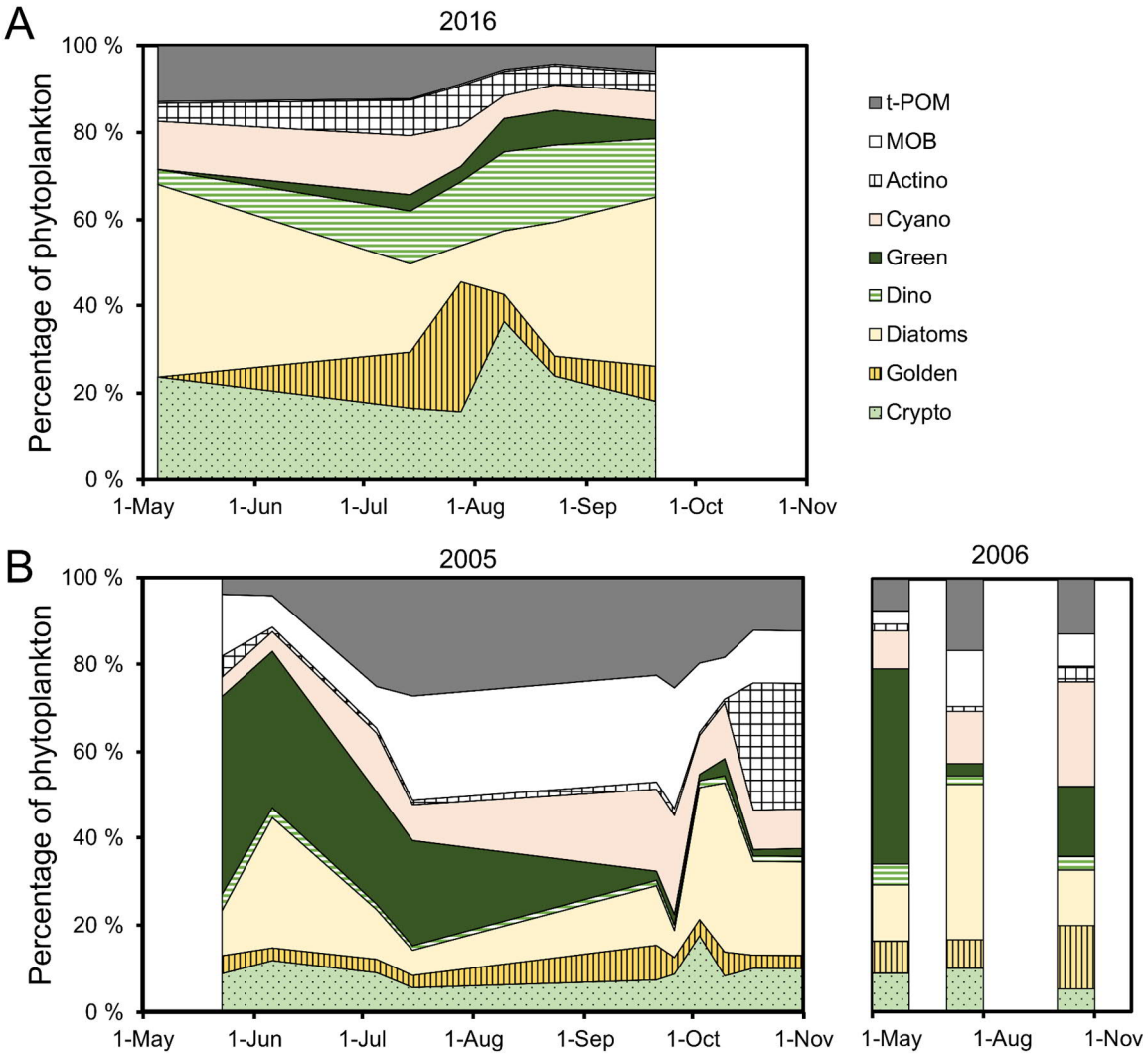
A
2016
100 %
80 %
60 %
40 %
20 %
0 %
Percentage of phytoplankton
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
1-Nov
t-POM
MOB
Actino
Cyano
Green
Dino
Diatoms
Golden
Crypto
B
2005
2006
1-May
1-Aug
1-Nov

Figure 6.

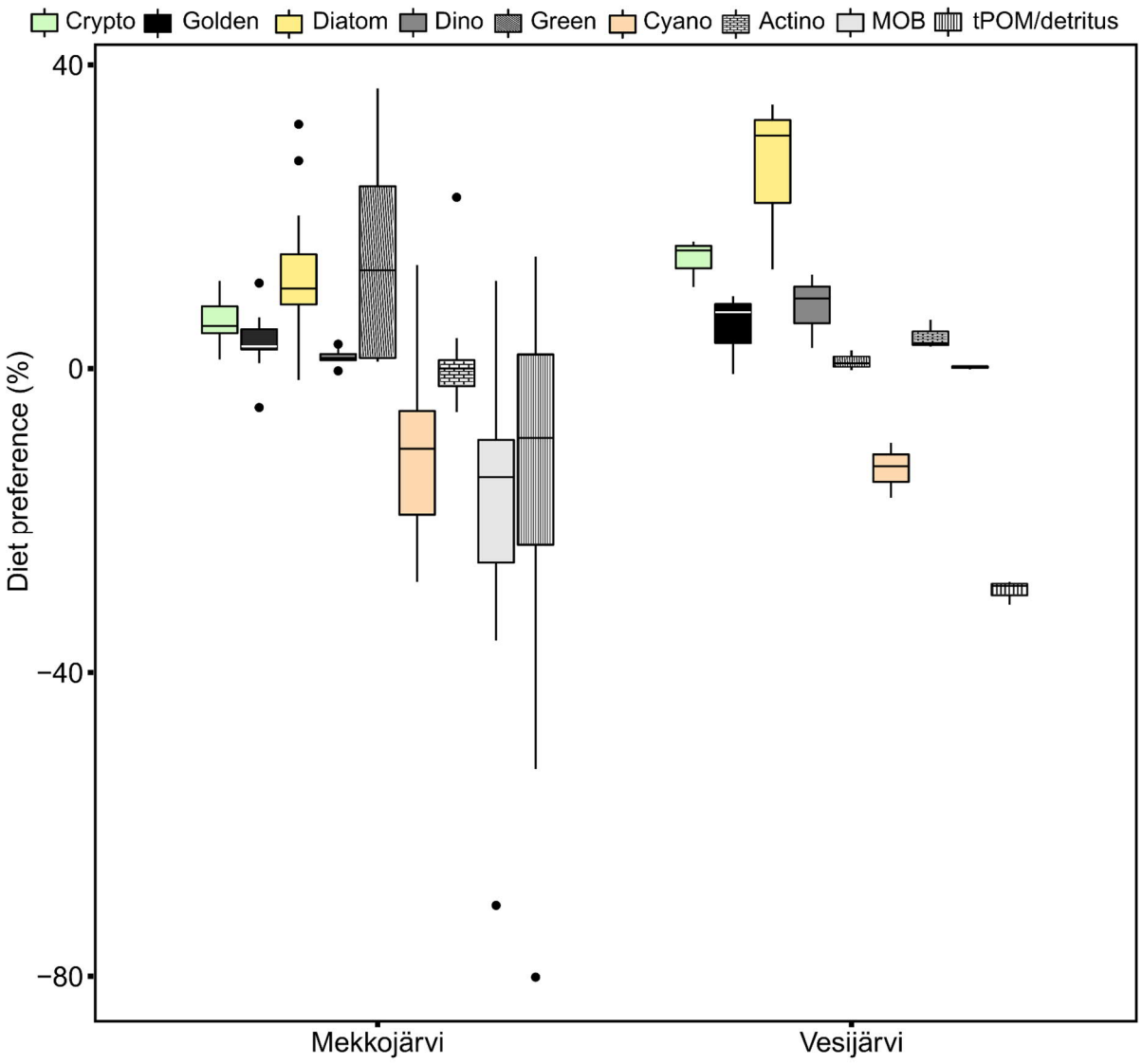
Crypto
Golden
Diatom
Dino
Green
Cyano
Actino
MOB
tPOM/detritus
40
0
−40
−80
Diet preference (%)
Mekkojärvi
Vesijärvi

Figure 7.

Table 1. Biochemical composition of the algae in the threshold experiment (experiment 1). Protein% is the percentage of protein of dry weight (DW). Amino acids are divided into essential amino acid (EAA) and non-essential amino acids (NEAA). Sterol content is the sum of all sterols, whereas sterol index <1.0 indicates potential sterol limitation. PUFA is the sum of all ω-3 and ω-6 polyunsaturated fatty acids. ALA = α-linolenic acid (18:3ω3), SDA = stearidonic acid (18:4ω3), EPA = eicosapentaenoic acid (20:5ω3), DHA = docosahexaenoic acid (22:6ω3). Standard deviation in parentheses.

Algae	Protein%	EAA	NEAA	Sterols	Sterol	ω-3 PUFA	ω-6 PUFA	ALA + SDA	EPA	DHA
		µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C	index	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C
Acutodesmus sp.	55 (0.1)	102 (54)	47 (13)	8 (1.4)	0.5	67 (2)	12 (3)	67 (2)	<0.1	<0.1
Rhodomonas lacustris	72 (0.1)	163 (28)	144 (23)	18 (0.4)	3.1	170 (55)	8 (3)	124 (40)	34 (12)	12 (3)
Cryptomonas ozolinii	57 (4)	117 (12)	52 (4)	24 (2.2)	3.4	97 (26)	10 (1)	67 (17)	24 (7)	5 (2)
Mallomonas kalinae	48 (1.7)	95 (43)	37 (11)	53 (1.8)	5.9	79	15	75	0.4	3.2
Peridinium cinctum	41 (5.1)	112 (3)	43 (2)	8 (0.1)	0.4	87 (11.7)	0.4 (0.1)	31 (4.2)	23 (3.0)	34 (4.5)

Table 2. Dietary nutrient contents (average [SD]; µg mg^{-1} C) of the high-quality diet mixture experiment (experiment 2). The experiment has 5 different dietary sources: *Rhodomonas* (cryptophytes), *Micrococcus luteus* (Actinobacterium), milled leaves of *Betula pendula* (birch), heteronanoflagellate (HNF), and *Acutodesmus* (green algae). Details on amino acids, sterols, fatty acids, and the abbreviations used follow Table 1. HUFA is the sum of the highly unsaturated fatty acids EPA+DHA.

Treatment	EAA	NEAA	sterols	ω-3 PUFA	ω-6 PUFA	ALA + SDA	HUFA	P
	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C	µg mg^{-1} C
Rhodomonas	115 (4)	46 (2)	18 (2)	101 (13)	1 (0.2)	68 (7)	33 (6)	0.21 (0.02)
Actinobacterium	164 (4)	133 (7)	0 (0)	0	0 (0)	0 (0)	0 (0)	0.18 (0.03)
Birch	10 (3)	8 (2)	6 (1)	0.6 (0.1)	1.2 (0.1)	0.6 (0.1)	0 (0)	0.019 (0.003)
HNF	114 (3)	93 (3)	2 (0.3)	0.2 (0.01)	0.4 (0.02)	0.03 (0.01)	0.16 (0.03)	0.19 (0.01)
Acutodesmus	73 (14)	58 (21)	8 (1)	33 (2)	14 (0.5)	167 (8)	0 (0)	0.069 (0.004)