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2016-08

Taipale , S J , Vuorio , K , Brett , M T , Peltomaa , E , Hiltunen , M & Kankaala , P 2016 , ' Lake zooplankton delta C-13 values are strongly correlated with the delta C-13 values of distinct phytoplankton taxa ' , Ecosphere , vol. 7 , no. 8 , 01392 . https://doi.org/10.1002/ecs2.1392

http://hdl.handle.net/10138/169844
https://doi.org/10.1002/ecs2.1392

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SPECIAL FEATURE: BIOMARKERS IN TROPHIC ECOLOGY

Lake zooplankton $\delta^{13}C$ values are strongly correlated with the $\delta^{13}C$ values of distinct phytoplankton taxa

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Citation: Taipale, S. J., K. Vuorio, M. T. Brett, E. Peltomaa, M. Hiltunen, and P. Kankaala. 2016. Lake zooplankton $\delta^{13}C$ values are strongly correlated with the $\delta^{13}C$ values of distinct phytoplankton taxa. Ecosphere 7(8):e01392. 10.1002/ecs2.1392

Abstract. Analyses of carbon stable isotopes are often used to estimate the contributions of allochthonous and autochthonous dietary resources to aquatic consumers. Most pelagic food web studies assume that all phytoplankton taxa have a similar $\delta^{13}C$ value. We studied pelagic food web compartments (dissolved inorganic carbon [DIC], phytoplankton, bacteria, seston, cladoceran zooplankton) in 12 small (< 0.1 km^2) lakes in southern Finland. These lakes were classified as oligotrophic, mesotrophic, eutrophic, and dystrophic based on their concentrations of total phosphorus and dissolved organic carbon. Additionally, we studied phytoplankton photosynthetic carbon fractionation (ε_p) in laboratory conditions. The photosynthetic fractionation in 28 phytoplankton cultures from nine different phytoplankton classes varied significantly at the class level, and fractionation correlated significantly with the DIC concentration of the growth media. In small boreal lakes, the $\delta^{13}C$ values of different phytoplankton taxa, as directly measured or estimated from the $\delta^{13}C$ values of biomarker fatty acids, varied greatly (−18‰ to −44.5‰). Phytoplankton $\delta^{13}C$ values varied significantly by lake type and were most depleted in dystrophic lakes even though the $\delta^{13}C$ values of the DIC was similar to mesotrophic lakes. Further within-taxa variation was found between lakes and between different depths within a lake. Vertical samples from dystrophic lakes also showed lower ε_p in the phytoplankton from meta- and hypolimnion, possibly as a result of reduced light intensity. Altogether, in nine of the 10 sampled lakes, the $\delta^{13}C$ values of cladoceran zooplankton were between the minimum and the maximum phytoplankton $\delta^{13}C$ value of each lake, and thus, phytoplankton alone could explain zooplankton $\delta^{13}C$ values. We conclude that stable isotope mixing models should take into account carbon variation among different phytoplankton taxa.

Key words: boreal lakes; carbon stable isotopes; photosynthetic fractionation; phytoplankton; Special Feature: Biomarkers in Trophic Ecology; zooplankton.

Received 16 October 2015; revised 23 March 2016; accepted 29 March 2016. Corresponding Editor: B. Hayden.

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Introduction

The fundamental goal of most ecosystem studies is to understand the energy and nutrient flows through food webs. There is also considerable interest in using novel approaches to better understand food web linkages. Stable isotopes have been used extensively during the last three decades to assess consumer dietary sources and trophic level in many ecosystems. In freshwater systems, one of the most actively studied topics has been determining the relative importance of autochthonous and allochthonous contributions in the diets of zooplankton and fish using stable isotope analyses (Karlsson et al. 2003, Kankaala et al. 2010, Cole et al. 2011, Francis et al. 2011, Rautio et al. 2011, Solomon et al. 2011, Berggren et al. 2014, Tanentzap et al. 2014). Recently, stable isotope studies have been extended to the analyses of hydrogen isotopes (Solomon et al. 2011, Berggren et al. 2014). Mixing models with one or two stable isotopes can unravel consumer diets in laboratory systems when consumers have two diets and when the diets' stable isotope fractionation is known. However, natural pelagic systems are much more complex and many assumptions are needed when stable isotope mixing models are used to estimate consumer diets. For example, many indirect methods have been used to estimate the phytoplankton stable isotope ratios, and consumer isotope fractionation is very difficult to characterize in field studies. This gives rise to considerable uncertainty in mixing models, and model outcomes are often highly dependent on assumptions (Brett 2014*a*).

Often pelagic food webs are simplified to ternary isotope models having allochthonous (t-POM, terrestrial particulate organic matter), autochthonous (phytoplankton), and bacterial sources (Pace et al. 2004) or alternatively terrestrial, benthic, and pelagic resources (Solomon et al. 2011, Berggren et al. 2014). These models try to resolve zooplankton diets using two (C and N) or three (C, N, and H) isotopes. In lakes, zooplankton often prefer to graze on phytoplankton of a certain size class, for example, high-quality cryptophytes, and avoid the smallest cells as well as large cells, filamentous and non-filamentous colonies (Kinsely and Geller 1986, Cyr and Curtis 1999). However, the $\delta^{13}C$ value of different phytoplankton taxa can easily vary by > 8‰ within the same lake (Vuorio et al. 2006). Therefore, if only a single phytoplankton $\delta^{13}C$ value is applied, the mixing model results of zooplankton diets may be erroneous. Furthermore, there are many challenges associated with accurately distinguishing phytoplankton $\delta^{13}C$ signatures from those of bacteria and t-POM in seston. Bulk seston represents not only phytoplankton, but also a significant proportion of terrestrial organic matter and different types of bacteria, and therefore, indirect methods have been mainly used to estimate the $\delta^{13}C$ values of distinct diet sources.

A common indirect method for algal $\delta^{13}C$ determination uses the $\delta^{13}C$ value of dissolved inorganic carbon (DIC) and an assumed fractionation factor (ε_p) between the DIC and bulk algal carbon taken from the literature (Karlsson et al. 2003, 2012, Taipale et al. 2009). However, this method has considerable uncertainty, because of wide variability in literature-based ε_p values, which can result in bias in the estimated $\delta^{13}C$ values of phytoplankton (Marty and Planas 2008, Brett 2014*a*). More precise estimates can be achieved by correcting the $\delta^{13}C$ of POM for the algal proportion based on the POM chlorophyll *a* content and an assumed particulate C-to-Chl *a* ratio for phytoplankton (Cole et al. 2004, Rautio et al. 2011). However, phytoplankton C-to-Chl *a* ratios are also highly variable due to the differences in taxonomic composition, light availability, and nutrient conditions for phytoplankton communities (Montagnes et al. 1994). Other authors (Pace et al. 2007, Kankaala et al. 2010, Berggren et al. 2014) have estimated the phytoplankton $\delta^{13}C$ signal by directly determining the $\delta^{13}C$ values of phytoplankton-specific fatty acids within the seston (typically ω-3 fatty acids) and assuming a phytoplankton lipid fractionation ($\Delta^{13}C$) based on the values from the literature (Schouten et al. 1998, Riebesell et al. 2000, Van Dongen et al. 2002). When Kankaala et al. (2010) used this approach, they assumed a ~8‰ isotopic difference between bulk biomass and biomarker fatty acid based on the results of Van Dongen et al. (2002) for various marine phytoplankton. Pace et al. (2007) and Berggren et al. (2014) assumed a ~3–4‰ isotopic difference between bulk phytoplankton C and their corresponding fatty acids. The empirical basis of their assumptions was unclear. Van Dongen et al. (2002) measured an ~8‰ isotopic difference between bulk carbon and lipid relative

to saturated fatty acids (SFAs; $C_{14:0}$, $C_{16:0}$, $C_{18:0}$) in phytoplankton monocultures; however, these authors did not determine the $\Delta^{13}C$ for ω-3 fatty acids (FAs), which can be diagnostic biomarkers for phytoplankton. A recent study showed that the carbon isotopic difference between freshwater phytoplankton and their taxon-specific biomarker fatty acids averaged ~7‰, and importantly that this lipid fractionation value varied among different phytoplankton groups (Taipale et al. 2015). These authors concluded that the group-specific differences enable the $\delta^{13}C$ value calculations for major phytoplankton classes and bacterial groups at a finer resolution. Because lipids are ^{13}C-depleted compared to bulk values, using smaller phytoplankton lipid fractionation values for these calculations results in more depleted phytoplankton estimates.

In most cases, it is assumed that heterotrophic bacteria have $\delta^{13}C$ signatures similar to the organic substrates that they metabolize, which makes it difficult to separate bacterial signatures from t-POM or algal signatures in zooplankton using carbon isotopes. The bacterial community of oligotrophic lakes predominately consists of heterotrophic bacteria, including gram-positive (e.g., *Actinobacteria*) and gram-negative bacteria (e.g., *Polynucleobacter* sp.; Zwart et al. 2002, Hahn 2006). However, in stratified lakes, distinct bacterial groups are often found in the metalimnion and hypolimnion, including methane-oxidizing bacteria (MOB; Kojima et al. 2009, Taipale et al. 2009), photosynthetic green sulfur bacteria (GSB; Selig et al. 2004), and other chemoautotrophic bacteria. Zooplankton can migrate to the oxic–anoxic boundary or even briefly into the anoxic hypolimnia to feed on bacteria while also avoiding zooplanktivorous fish and invertebrate predators (Salonen and Lehtovaara 1992). The estimated $\delta^{13}C$ values of these bacterial taxa have usually been based on substrate values (CH_4 or DIC) and assuming fractionation factors: −8‰ to −28‰ for MOB and −12‰ to −14‰ for photoautotrophic bacteria (Sirevåg et al. 1977, Templeton et al. 2006).

Although phytoplankton, bacteria, and t-POM $\delta^{13}C$ values could be known accurately, ternary stable isotope models (e.g., t-POM, phytoplankton, bacteria) have difficulties in calculating the relative contributions of these sources in zooplankton diets due to poor differentiation of $\delta^{13}C$ values for these resources. Therefore to better distinguish resource $\delta^{13}C$ values at a whole-lake scale, some studies have experimentally enriched phytoplankton by the additions of ^{13}C-labeled inorganic carbon (Cole et al. 2004, 2006, Taipale et al. 2008) or $\delta^{13}C$-enriched allochthonous organic carbon sources have been used (Peura et al. 2014, Scharnweber et al. 2014). However, when epilimnetic phytoplankton are clearly enriched, more depleted zooplankton ^{13}C values do not necessarily mean t-POM or bacteria utilization in zooplankton diets, because many phytoplankton species are able to migrate vertically and are capable of mixotrophy by feeding on bacteria or organic substrates in unenriched regions of the lake. As a consequence, the isotopic signals for similar diet organisms can vary vertically resulting in complex signals in consumers.

Here, we try to unravel some assumptions related to ternary isotope models for herbivorous zooplankton diets. We studied the $\delta^{13}C$ values of phytoplankton, terrestrial particulate organic carbon (t-POC), and bacteria in different types of boreal lakes using the $\delta^{13}C$ signals of phospholipid fatty acid (PLFA) and direct biomass measurements. We also cultured phytoplankton from nine different algal classes and studied how DIC concentrations influenced the carbon fractionation (ε_p) between DIC and phytoplankton cells and compared these results to those obtained in field studies. To study the vertical and horizontal distribution of phytoplankton $\delta^{13}C$, we sampled lakes with different dissolved organic carbon (DOC) and nutrient concentrations and took samples from the epi-, meta-, and hypolimnia. Finally, we compared the $\delta^{13}C$ values of different phytoplankton and herbivorous cladoceran zooplankton in lakes, to determine whether the variation in phytoplankton $\delta^{13}C$ values between and within lakes could explain the $\delta^{13}C$ values of the zooplankton.

Methods

Phytoplankton culturing

We studied photosynthetic carbon fractionation (ε_p) between phytoplankton and DIC by culturing 28 phytoplankton strains from nine different classes in optimal growth conditions. Most of the phytoplankton strains were grown at 18°C under a 14-h:10-h light/dark cycle with a light

intensity of 30–70 μmol·m^{-2}·s^{-1}. Each strain was cultured in a medium specific to that strain (Table 1). The strains of *Cryptomonas marssonii*, *Cryptomonas ovata*, *Synura* sp., *Gonyostomum semen*, *Euglena gracilis*, and *Selenastrum* sp. were cultured at 20°C with the light intensity of 40 μmol·m^{-2}·s^{-1} using a 16-h:8-h light/dark cycle. We used plastic or glass flasks, volume > 200 mL. Depending on the cell density, 0.5–3 mL of the phytoplankton stock was inoculated per 100 mL of fresh culture media. The samples for phytoplankton $\delta^{13}C$ analyses were harvested in the late phase of exponential growth, that is, 2–3 weeks after the inoculation. The concentrations and $\delta^{13}C$ values of DIC were measured from the same samples with isotope ratio mass spectrometry (IRMS) interfaced with gas chromatography (details in Taipale et al. 2008).

Phytoplankton fractionation experiment

We studied the influence of the DIC ($NaHCO_3$) concentration on carbon fractionation by culturing *Cryptomonas erosa*, *Diatoma tenuis*, *Peridinium cintum*, *Monoraphidium griffithii*, and *Pseudanabaena limnetica* (marked as † in Table 1) in 300-mL plastic culture bottles (Sarstedt) for four weeks with modified WC medium (MWC, see Table 1) and adding 0, 3.4, and 34 mg inorganic C/L at the beginning of the incubation and after two weeks. These strains were grown at 18°C under a 14-h:10-h light/dark cycle with a light intensity of 60–70 μmol·m^{-2}·s^{-1}. After the experiment, phytoplankton were centrifuged and the resulting pellets were freeze-dried for lipid and $\delta^{13}C$ analyses. DIC samples were also taken for $\delta^{13}C$ and concentration measurements as described above.

Sampling of small boreal lakes

We initially sampled five small boreal lakes (size range 0.003–0.145 km^2) with different DOC concentration (5–40 mg C/L) during May and September–October 2006. The $\delta^{13}C$ values of phytoplankton and bacteria were determined using compound-specific biomarkers and isotopic differences (Kankaala et al. 2010, Taipale et al. 2015). To have a broader perspective on the $\delta^{13}C$ values of the phytoplankton, bacteria, and t-POM, we sampled additional lakes (Table 2) in 2013, 2014, and 2015, including oligotrophic, eutrophic, and dystrophic lakes. This was performed because phytoplankton composition varies between lake types due to the different light and nutrient conditions (Lepistö and Rosenström 1998). The lakes were classified according to their total P and DOC concentration as oligotrophic (≤ 10 μg P/L, ≤ 5 mg C/L), mesotrophic (10–30 μg P/L, 10–20 mg C/L), eutrophic (> 35 μg P/L, < 20 mg C/L), and dystrophic (15–30 μg P/L, > 20 mg C/L) lakes.

In 2006, the samples were taken at the deepest point of each lake with a 30 or 60 cm long water sampler (Limnos Ltd, Komarow, Poland, volume 2 or 4.25 L) and filtered through a 100-μm mesh to remove zooplankton. The samples were taken separately from the epilimnion (Epi), metalimnion (Meta), and hypolimnion (Hypo) as determined by measuring oxygen and temperature profiles at 0.5-m intervals with a YSI 55 probe (Yellow Springs Instruments, Yellow Springs, Ohio, USA, accuracy ± 0.3°C, ± 0.3 mg O_2/L). The transition zone from hypoxia to anoxia was detected from the measurements of redox potential with a Schott redox-combination electrode (BlueLine 31 Rx, Schott, Mainz, Germany) connected to a WTW MultiLine P3 pH/conductivity meter (Weilheim, Germany). During the years 2013–2015, the samples were taken only from the epilimnion and all between-lake data comparisons are based on the values measured at this layer.

Phytoplankton, terrestrial, and bacteria collection

In the samples from 2006, the particles in 0.22- to 100-μm fraction were concentrated from ~8 to 28 L of water (two replicates) with Millipore tangential flow filtration (Pellicon P2GVPP05 cassette, Merck KGaA, Darmstadt, Germany, pore size 0.22 μm). Subsample concentrates (~100 mL) were freeze-dried for compound-specific $\delta^{13}C$ analyses. Distinct phytoplankton and bacteria classes were separated using compound-specific biomarkers.

During the years 2013–2015, seston samples were collected using 25- and 50-μm plankton nets and filtered through a series of sieves of different pore sizes (10, 20, 50, 100, 150, and 250 μm) to separate different phytoplankton taxa, bacteria, and detritus (Vuorio et al. 2006). We were able to separate at least one phytoplankton taxon at each lake (Table 4) as well as bacteria from the seston of Majajärvi, Nimetön, and Syrjänalunen in 2014. The final phytoplankton composition of each

Table 1. Class, order, species, and the strain code information of the studied freshwater phytoplankton.

Class/Order/Species	Number	Strain	Media	L/D	LI	Temp
Cyanophyceae (cyanobacteria)						
Chroococcales						
Microcystis sp.	1	NIVA-CYA 642	MWC[1]	14:10	50	20
Snowella lacustris	2	NIVA-CYA 339	MWC[1]	14:10	50	20
Synechococcales						
Synechococcus elongatus	3	UTEX LB 563	MWC[1]	14:10	50	20
Nostocales						
Anabaena flos-aquae	4	NIVA 138	MWC[1]	14:10	50	20
Oscillatoriales						
Phormidium tenue	5	NIVA-CYA 25	MWC[1]	14:10	50	20
Planktothriux rubescens	6	SCCAP K-576	MWC[1]	14:10	50	20
Pseudanabaenales						
Limnothrix planctonica	7	NIVA-CYA 107	MWC[1]	14:10	50	20
Pseudanabaena limnetica†	8	NIVA 276/11	MWC[1]	14:10	50	20
Cryptophyceae (Cryptophytes)						
Cryptomonadales						
Cryptomonas marssonii	9	CCAP 979/70	DY-V[2]	16:8	30	20
Cryptomonas ovata	10	SCCAP K-1876	AF6[3]	16:8	30	20
Cryptomonas ozolinii	11	UTEX LB 2782	MWC[1]	14:10	30	20
Cryptomonas erosa†	13	CPCC 446	MWC[1]	14:10	60–70	20
Dinophyceae (Dinoflagellates)						
Peridiniales						
Peridinium cintum† (n = 3)	14	SCCAP K-1721	MWC[1]	14:10	70	20
Synurophyceae (golden algae)						
Synurales						
Synura sp.	15	SCCAP K-1875	MWC[1]	16:8	30	20
Raphidophyceae (raphidophytes)						
Chattonellales						
Gonyostomum semen	16	LI21	MWC[1]	16:8	80	20
Diatomophyceae (diatoms)						
Thalassiosirales						
Cyclotella meneghiniana	17	CCAC 0039	MWC[1]	14:10	40	18
Fragilariales						
Asterionella formosa	18	NIVA-BAC-3	MWC[1]	14:10	40	18
Fragilaria crotonensis	19	UTEX LB FD56	MWC[1]	14:10	40	18
Synedra rumpens var.	20	NIVA-BAC 18	MWC[1]	14:10	40	18
Tabellarialales						
Diatoma tenuis†	21	CPCC 62	MWC[1]	14:10	60–70	20
Euglenophyceae (euglenoids)						
Euglenales						
Trachelomonas	22	SCCAP K-1380	MWC[1]	14:10	40	18
Chlorophyceae (green algae)						
Chlamydomonadales						
Eudorina sp.	23	K-1771	MWC[1]	14:10	70	20
Chlamydomonas reindhardtii	24	UWCC	MWC[1]	14:10	70	20
Sphaeropleales						
Monoraphidium griffithii†	25	NIVA-CHL 8	MWC[1]	14:10	70	20
Pediastrum sp.	26	SCCAP K-1033	MWC[1]	14:10	70	20
Acutodesmus sp.	27	University of Basel	MWC[1]	14:10	70	20
Selenastrum sp.	28	SCCAP K-1877	MWC[1]	16:08	70	20
Trebouxiophyceae						
Prasiolales						
Botryococcus sp.	29	SCCAP K-1033	MWC[1]	14:10	70	20

Table 1. Continued.

Class/Order/Species	Number	Strain	Media	L/D	LI	Temp
Conjugatophyceae						
Desmidiales						
Cosmarium reniforme	30	SCCAP K-1145	MWC[1]	14:10	50	18
Staurastrum sp.	31	SCCAP K-1349	MWC[1]	14:10	50	18

Notes: Different media and light cycle, that is, the light/dark period (L/D, h), light intensity (LI, $\mu mol \cdot m^{-2} \cdot s^{-1}$), and temperature (°C) were used for different strains. Strains 14, 16, and 29 were not cultured, but collected from lakes during their blooms. All the strains are numbered, and the same numbers are used in Fig. 2. Sources (as provided in superscript in the Media column) are as follows: (1) Guillard and Lorenzen (1972), Guillard (1975); (2) Andersen et al. (1997); (3) Watanabe et al. (2000).

† These strains represented each class in the bicarbonate carbon fractionation experiment.

sample was checked under a microscope. Birch (*Betula* spp.) and aspen (*Populus tremula*) leaves were collected on the shore of Lake Mekkojärvi on 2011 representing t-POM. Phytoplankton, bacteria, and terrestrial concentrates were freeze-dried and prepared for stable isotope analyses.

Lipid analyses for phytoplankton and bacteria $\delta^{13}C$ determination

Freeze-dried material was placed into extraction tubes containing 11.4 mL of a chloroform–methanol–50 mmol/L phosphate buffer solution, pH 7.4 (1 : 2 : 0.8 [volume ratio]). Lipids were extracted, fractionated, saponified, and methylated according to Bligh and Dyer (1959) and Taipale et al. (2009). For quantification, dipentadecanoylphostatidylcholine ($C_{38}H_{76}NO_8P$; Sigma) was added as an internal standard. Additionally, tridecanoic ($C_{14}H_{28}O_2$; Sigma) and nonadecanoic ($C_{20}H_{40}O_2$; Sigma, Sigma-Aldrich, Germany) acid methyl esters were used to quantify the methylation efficiency.

Table 2. Twelve boreal lakes with various concentrations of dissolved inorganic carbon (DIC mg/L), dissolved organic carbon (DOC mg/L), particulate organic carbon (POC mg/L), total nitrogen (tot N μg/L), phosphorus (tot P μg/L), chlorophyll *a* (Chl *a* μg/L), and color values (mg Pt/L) were sampled during the years 2006, 2013, 2014, and 2015.

Lake type/Lake/Year	Season	Method	DIC	DOC	POC	tot N	tot P	Chl *a*	Color
Oligotrophic									
Syrjänalunen 2014	Summer	Direct	4.5	3	0.4	373	10	1.6	6
Valkea-Mustajärvi 2006	Spring	PLFA	2.7	5	0.43	367	8	3	30
Valkea-Mustajärvi 2014	Summer	Direct	1.0	4	0.42	423	10	7.3	18
Mesotrophic									
Alinen-Mustajärvi 2006	Spring	PLFA	3.4	12	0.63	671	13	6.2	149
Valkea-Kotinen 2006	Spring	PLFA	3.8	13	nd	450	24	9.7	180
Valkea-Kotinen 2014	Summer	Direct	3.0	15	0.46	630	29	12.7	142
Eutrophic									
Kataloistenjärvi 2015	Summer	Direct	2.1	9	1.43	620	35	11.1	52
Kyynärö 2015	Summer	Direct	2.7	17	3.07	965	77	44.9	139
Köyhälampi 2014	Summer	Direct	4.6	>8	nd	>700	>35	nd	15
Lovojärvi 2015	Summer	Direct	3.3	14	1.6	785	49	24.4	105
Tuusulanjärvi 2015	Summer	Direct	4.1	9	1.09	940	69	29	76
Dystrophic									
Majajärvi 2014	Summer	Direct	2.0	25	0.56	724	30	10.8	263
Mekkojärvi 2006	Spring	PLFA	6.1	26	0.87	419	17	11.3	199
Nimetön 2006	Spring	PLFA	8.5	22	0.68	828	31	8.5	392
Nimetön 2013	Summer	Direct	nd	24	0.55	822	19	7.4	280
Nimetön 2014	Summer	Direct	2.6	30	nd	886	23	nd	469

Notes: The lakes were classified into different lake types according to their DOC and tot P contents. Phytoplankton and bacteria $\delta^{13}C$ values were measured using compound-specific biomarkers (PLFA, phospholipid fatty acid) in May and in October 2006, but measured directly from phytoplankton during the years 2013, 2014, and 2015. The results here represent the mean values for epilimnion (nd = no data). Results for meta- and hypolimnion of five boreal lakes (2006) can be found in Kankaala et al. (2010).

Gas chromatography combustion stable isotope ratio mass spectrometry (GC-C-SIRMS)

The $\delta^{13}C$ values of the fatty acids were determined using a GC-C TA III connected to an Isotope Ratio Mass Spectrometer (IRMS, DELTAPLUSXP; Thermo Co., Waltham, Maine, USA) at the Department of Environmental Sciences of the University of Eastern Finland. Fatty acids were separated using a 30-m FS DB-5 30 MTR column (0.25 mm thickness) and then oxidized to carbon dioxide in an oxidation reactor at a temperature of 940°C with the reduction reactor kept at 630°C. The temperature program of the GC column started at 50°C and was kept for 1 min at 50°C, after which the temperature was raised by 30°C/min to 140°C and then by 1°C/min to 220°C and finally 15°C/min to 300°C. The total run time was 94.3 min. The injector temperature was kept at 270°C. The samples were run against an internal standard, that is, hexadecanoic acid methyl ester ($C_{17}H_{34}O_2$, >99%, Indiana University, Arndt Schimmelmann), with a $\delta^{13}C$ value of −30.74‰. This hexadecanoic acid methyl ester standard was used for drift and linear correction. For linear correction, four different concentrations of hexadecanoic were run after which a correction equation was calculated. For the hexadecanoic acid standard, the calculated accuracy was ±0.3‰ and the precision was ± 0.6‰. To take into account possible $\delta^{13}C$ changes during methylation, precision and accuracy were also calculated using tridecanoic and nonadecanoic acid methyl esters standards, which were first run with an EA-SIRMS (DELTAPLUSXP; Thermo Co.) and then calculated for every GC-C-SIRMS sample series. The calculated accuracy of these samples was ± 0.9‰, and the precision was ± 0.6‰. Only peaks with a total height of 50 mV at mass 44 were counted. The $\delta^{13}C$ value of the fatty acids was manually calculated using individual background values. Because of co-elution of C_{16} monounsaturated fatty acids (MUFAs), it was not possible to separate 16:1ω9c, 16:1ω7c, 16:1ω8c, or 16:1ω6c, and these FAs were detected as one peak.

Biomarkers

Phytoplankton groups differ greatly in their FA composition (Ahlgren et al. 1992, Taipale et al. 2013), and taxonomical biomarker FA can also be used in compound-specific analyses (Taipale et al. 2015). However, the concentration of group-specific C_{16} polyunsaturated fatty acids (PUFAs) in these samples was too low for compound-specific analyses. Therefore, we used alpha-linolenic acid (ALA, 18:3ω3), stearidonic acid (SDA, 18:4ω3), eicosapentaenoic acid (EPA, 20:5ω3), and docosahexaenoic acid (DHA, 22:6ω3) to estimate the $\delta^{13}C$ values of the different phytoplankton groups (Table 3). Each lake was treated as a unique system and the biomarker fatty acid varied between lakes depending on their phytoplankton composition, and thus, commonly shared FAs among different classes, for example, ALA, could be used as taxonomic biomarkers. The isotopic difference between the bulk biomass and the biomarker

Table 3. Biomarker fatty acids (FAs) for different phytoplankton and bacterial groups used in the 2006 study and corresponding isotopic differences between biomarkers and biomass (from Taipale et al. 2015) used to calculate the $\delta^{13}C$ values of phytoplankton and bacteria.

Diet source	Biomarker FAs	Isotopic difference ($\Delta\delta^{13}C_{\text{biomarker FA–biomass}}$)
Phytoplankton group		
Chlorophyceae	18:3ω3	−6.5
Cryptophyceae	18:4ω3, 20:5ω3	−7.1
Chrysophyceae	18:4ω3, 20:5ω3	−7.1
Cyanophyceae	18:3ω3	−11.4
Diatomophyceae	20:5ω3	−6.2
Dinophyceae	22:6ω3	−2
Microbial group		
Heterotrophic bacteria (Actinobacteria)	a-15:0, i-15:0	−2.1
Methane-oxidizing bacteria (type I)	16:1ω5c/t	−8
Green sulfur bacteria (*Chlorobium*)	14:0, a-15:0, 16:1ω7,	−4.7

fatty acids was based on the results of Taipale et al. (2015, Table 2).

The iso- and anteiso-15:0 PLFAs from epilimnion were used to estimate the abundance and the $\delta^{13}C$ values of heterotrophic gram-positive bacteria (Actinobacteria). The 16:1ω8c, 16:1ω6c, and 16:1ω5t monounsaturated PLFAs were used to characterize the abundance of methane-oxidizing type I bacteria (Bowman et al. 1991), but only 16:1ω5t was used to estimate the $\delta^{13}C$ values of the MOB. For GSB (*Chlorobium* sp.), we used the $\delta^{13}C$ values of myristic acid (14:0), anteiso-15:0 and 16:1ω7 PLFAs from the meta- and hypolimnion of Lake Mekkojärvi, because *Chlorobium* sp. constituted more than 60% of bacterial DNA in these depths (Taipale et al. 2013). Total bacterial fatty acids were summed to include all fatty acids excluding 18:1ω9, PUFAs, and SAFAs. Because the contribution of diatoms was < 3% of the phytoplankton biomass in the epilimnion of all the lakes, it was assumed that 16:1ω7c came primarily from bacterial sources (heterotrophic and methane-oxidizing bacteria).

Additional laboratory analyses

Samples for water color, total N, total P, chlorophyll *a*, and POC concentration were analyzed at the Lammi Biological Station using standard methods. DOC concentrations (fraction < 0.22 μm) were determined with a SHIMADZU TOC-5000A (Kyoto, Japan) Total Organic Carbon Analyzer. DIC concentrations were measured from separate samples taken in glass-stoppered bottles from the same depths as the phytoplankton. The bottles were kept on ice and analyzed within 4 h according to Salonen (1981).

The species composition and biomass of phytoplankton samples were determined with an inverted microscope at 600× magnification using the Utermöhl (1958) technique. A 50 mL sample was settled for 24 h, and random quadrants were counted until the counts for most species reached 200. Every species observed was recorded with their cell number and size. The phytoplankton abundance and biomass were calculated based on the abundance, size, and geometric form of the phytoplankton. Biovolume was converted to carbon using a factor of 0.2 pg/μm^3 (Reynolds 2006).

Lake zooplankton was sampled with vertical plankton net (mesh size 100 μm) tows. Herbivorous cladocerans (mainly *Daphnia*) were picked into tin cups (5–80 individuals in each) in the laboratory and dried at +60°C or freeze-dried for $\delta^{13}C$ analyses with IRMS (details in Taipale et al. 2008).

Results

Photosynthetic carbon fractionation in laboratory cultures of phytoplankton

The average $\delta^{13}C$ value of all phytoplankton taxa, grown in media commonly used for their culturing (see Table 1), was −22.0‰ ± 5.2‰ (Fig. 1A), whereas the $\delta^{13}C$ value of the DIC at the time of the phytoplankton biomass harvesting was −11.1‰ ± 4.8‰. The concentration of DIC varied between 0.2 and 11.2 mg C/L in all phytoplankton cultures during phytoplankton growth. The $\delta^{13}C$ value of the cultured phytoplankton did not correlate significantly (r = −0.152, P = 0.399, n = 32) with the $\delta^{13}C$ value of the DIC.

Photosynthetic fractionation (ε_p) varied significantly between phytoplankton classes (−10.9‰ ± 7.6‰, ANOVA: $F_{7,25}$ = 7.401, P < 0.01) even though Tukey's post hoc test was not able to create homogenous subsets because the Dinophyceae, Euglenophyceae, and Raphidophyceae groups contained only one species each. Nevertheless, photosynthetic fractionation was higher in the cultures of Cryptophyceae, Chrysophyceae, Raphidophyceae, Dinophyceae, and Euglenophyceae (average of ε_p = 21‰ ± 7‰) than in the cultures of Chlorophyceae, Cyanophyceae, Charophyceae, or Diatomophyceae (average of ε_p = 8‰ ± 4‰). All strains of Chlorophyceae, Charophyceae, Cyanophyceae, and Dinophyceae seemed to have a high growth rate although we did not measure this directly. Also, all Diatomophycean taxa except *Asterionella* grew fast in the cultures, whereas Chrysophyceae, Cryptophyceae, Raphidophyceae, and Euglenophyceae grew quite slowly. Photosynthetic fractionation (ε_p) correlated significantly with DIC concentration (Pearson's correlation, r = −0.62, P < 0.01, n = 31) in all laboratory cultures (Fig. 1B). However, the DIC concentration at the time of harvesting was high (> 5 mg C/L) only among Cryptophyceae, Chrysophyceae, or Diatomophyceae; thus, this correlation is the result of only a few taxa.

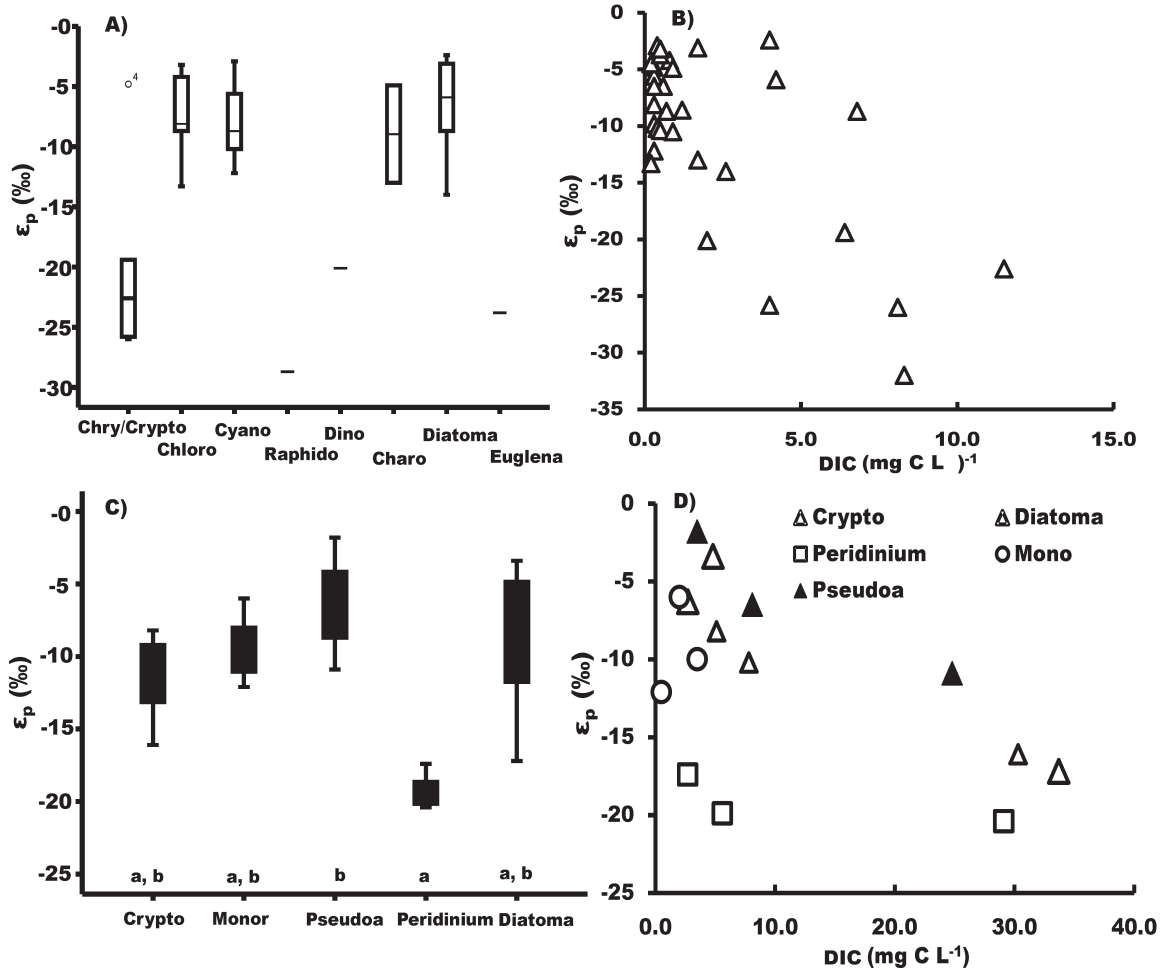

Fig. 1. (A) Photosynthetic carbon fractionation (ε_p) of different phytoplankton classes cultured in laboratory (boxplots of median values with 25th and 75th percentiles; see Table 1 for culture media). (B) In these cultures, fractionation correlated significantly ($r = -0.69$, $P < 0.01$, $n = 34$) with dissolved inorganic carbon (DIC) concentration of the medium. (C) In cultures with extra DIC addition, carbon fractionation differed significantly between phytoplankton taxa (letters a and b denote homogenous subsets by ANOVA Tukey's post hoc test), but the data pooled for all taxa (D) did not show a significant correlation between photosynthetic carbon fractionation (ε_p) and DIC concentration. Chry/Crypto, Chrysophyceae and Cryptophyceae; Chloro, Chlorophyceae; Cyano, Cyanophyceae; Raphido, Raphidophyceae; Dino, Dinophyceae; Charo, Charophyceae; Diatoma, Diatomophyceae; Euglena, Euglenophyceae; Pseuda, *Pseudanabaena*; Mono, *Monoraphidium*.

Impact of DIC addition on carbon fractionation in the laboratory experiment

Photosynthetic fractionation (ε_p) dependency on DIC concentration was studied by culturing five strains (*Monoraphidium*, *Cryptomonas*, *Peridinium*, *Pseudanabaena*, and *Diatoma*) that represent different phytoplankton classes. The average of photosynthetic fractionation (ε_p) was −11.1‰ ± 5.9‰ in all phytoplankton treatments. Photosynthetic fractionation varied significantly between the phytoplankton strains (ANOVA: $F_{5,15} = 3.504$, $P < 0.05$). Tukey's post hoc test created two homogenous subsets where *Pseudanabaena* did not belong to the first subset and *Peridinium* to second subset, while other strains belonged to the both subsets (Fig. 1C). This was due to the fact that DIC concentration did not influence the *Peridinium* photosynthetic

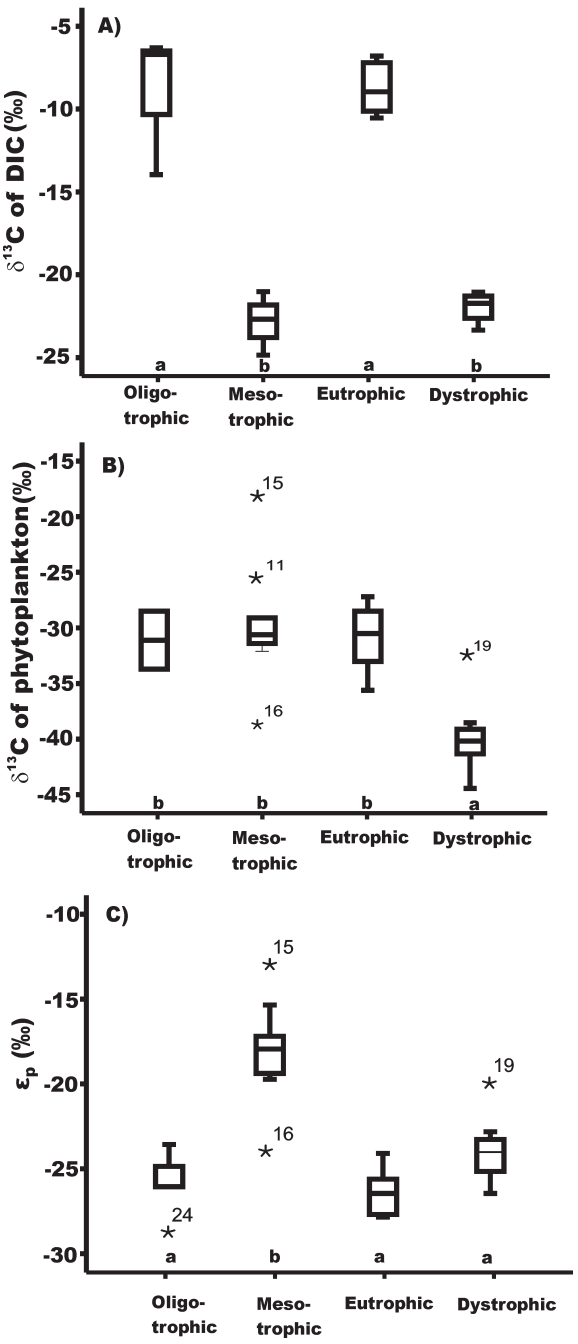

Fig. 2. The $\delta^{13}C$ values of (A) dissolved inorganic carbon and (B) phytoplankton as well as (C) photosynthetic fractionation (ε_p, ‰) in oligotrophic, mesotrophic, eutrophic, and dystrophic lakes (letters a and b denote homogenous subsets by ANOVA Tukey's post hoc test). (Numbers with asterisk represent extreme values within a lake type.)

fractionation (ε_p = −19.6‰ ± 1.6‰), which was the same in all treatments and differed from other cultures (ε_p = −9.1‰ ± 5‰). Although for *Cryptomonas*, *Pseudanabaena,* and *Diatoma* the greatest photosynthetic fractionation was observed at the highest DIC concentrations, in the data pooled for all taxa the fractionation (ε_p) was not significantly correlated with DIC concentration (Fig. 1D). The lowest fractionation (ε_p = −1.8‰ ± 0.3‰) was measured for *Pseudanabaena* with the lowest DIC treatment. The highest fractionation (ε_p = −12.1‰) among *Monoraphidium* treatments was measured at the lowest DIC concentration (0.5 ± 0.05 mg C/L). However, *Monoraphidium* grew so fast that DIC concentration at the time of harvesting in the highest DIC addition (34 mg C/L) was only 3.5 mg C/L. With the other taxa, in the same addition treatments (i.e., 34 mg C/L), the DIC concentration was 10 times higher (30.0 ± 3.7 mg C/L) at the time of sampling. The average DIC concentration was 2.9 ± 1.7 mg C/L in treatments with no addition of $NaHCO_3$, and 5.7 ± 2.5 mg C/L in the treatment where the addition was 3.4 mg C/L. The carbon content of cultured phytoplankton in all treatments was similar (51.1% ± 1.9%) excluding *Diatoma tenuis*, which had a carbon content of 34.8% ± 1.4%. The lipid content was higher in *Monoraphidium* (21% ± 4.8%) than in the other cultures (7.6% ± 1.2%).

Phytoplankton carbon fractionation in boreal lakes

The $\delta^{13}C$ values of the DIC varied significantly (ANOVA, $F_{3,15}$ = 50.1, P < 0.01) between the lake types and the Tukey's post hoc test formed two homogenous subsets: (1) oligotrophic and eutrophic and (2) mesotrophic and dystrophic lakes (Fig. 2A). Photosynthetic carbon fractionation of all phytoplankton was −21.7‰ ± 3.7‰, −17.9‰ ± 3.9‰, and −23.5‰ ± 3.1‰ in oligotrophic, dystrophic, and eutrophic lakes, respectively, but was only −6.2‰ ± 3.9‰ in mesotrophic lakes (Fig. 2C) due to lower fractionation values in the mesotrophic Lake Valkea-Kotinen (Table 4). Photosynthetic carbon fractionation did not correlate significantly with the DIC, DOC, total phosphorus, and total nitrogen concentrations of the lakes or phytoplankton class (P > 0.102). Phytoplankton

Table 4. The $\delta^{13}C$ values of bulk phytoplankton biomass consisting of different taxa as well as the $\delta^{13}C$ of dissolved inorganic carbon (DIC; ‰) and calculated photosynthetic fractionation factors (ε_p) in the studied lakes.

Lake type/Lake/Season	Method	Class	Genus/Species	$\delta^{13}C$ biomass	$\delta^{13}C$ DIC	ε_p
Oligotrophic						
Syrjänalunen						
Summer 2014	Direct	Charo, Chloro, Dino	*Cosmarium* cf. *bioculatum* (49%), *Botryococcus* cf. *braunii* (28%), *Isthmochloron lobulatum* (8%), *Dinobryon bavaricum* (6%)	−31.2	−13.9	−17.3
	Direct	Cyano		−33.8	−13.9	−19.9
Valkea-Mustajärvi						
Spring 2006	PLFA	Chryso, Crypto	*Mallomonas, Dinobryon, Pseudopedinalla, Cryptomonas, Rhodomonas*	−33.9	−6.7	−27.2
Summer 2014	Direct	Dino, Raphido	*Peridinium bipes* (95%), *Gonyostomum semen* (4%)	−28.6	−6.3	−22.3
	Direct	Cyano		−28.4	−6.3	−22.1
Mesotrophic						
Valkea-Kotinen						
Spring 2006	PLFA	Dino	*P. bipes* (>99%)	−31.1	−22.5	−8.6
Spring 2006	PLFA	Chryso, Cyano	*Dinobryon, Pedinella, Pseudopedinella, Aphanocapsa, Snowella*	−18.0	−22.5	4.5
Autumn 2006	PLFA	Dino	*P. bipes* (>99%)	−38.5	−20.9	−17.6
Summer 2014	Direct	Raphido	*G. semen* (92%), *Merismopedia* (6%)	−29.2	−24.7	−4.5
Summer 2014	Direct	Dino	*P. bipes* (>95%)	−25.5	−24.7	−0.8
Summer 2014	Direct	Raphido, Dino	*G. semen* (60%), *P. bipes* (40%)	−29.3	−24.7	−4.6
Summer 2014	Direct	Cyano	*Merismopedia warmingiana* (84%), Chlorophyceae (6%)	−30.7	−24.7	−6.0
Alinen-Mustajärvi						
Spring 2006	PLFA	Dino	*Peridinium*	−31.5	−22.6	−8.9
Spring 2006	PLFA	Chryso	*Chrysococcus, Dinobryon, Mallomonas*	−32.2	−22.6	−9.6
Eutrophic						
Köyhälampi						
Summer 2014	Direct	Dino	*Ceratium hirundinella* (100%)	−32.9	−7.2	−25.7
Summer 2014	Direct	Dino	*C. hirundinella* (96%)	−33.1	−7.2	−25.9
Tuusulanjärvi						
Summer 2015	Direct	Diatom	*Asterionella formosa, Aulacoseira* (99%)	−29.9	−6.8	−23.1
Summer 2015	Direct	Diatom	*Aulacoseira, A. formosa* (90%)	−28.2	−6.8	−21.4
Summer 2015	Direct	Euglen	*Colacium* (51%), detritus (49%)	−28.6	−6.8	−21.8
Lovojärvi						
Summer 2015	Direct	Cyano	*Worocnichia, Pseudanabaena*	−30.6	−10.5	−20.1
Summer 2015	Direct	Diatom	*Urosolenia* (90%)	−39.1	−10.5	−28.6
Kataloistenjärvi						
Summer 2015	Direct	Chloro	*Botryococcus* (85%), *Aulacoseira* (7%)	−27.3	−8.9	−18.4
Kyynärö						
Summer 2015	Direct	Dino	*C. hirundinella* (80%), *A. formosa* (8%)	−35.7	−10.1	−25.6
Summer 2015	Direct	Dino, Diatom, Cyano	*Ceratium* (40%), *Asterionella* (15%), *Aulacoseira* (7%), *Woronichia* (7%), *Microcystis* (6%)	−35.0	−10.1	−24.9
Dystrophic						
Nimetön						
Spring 2006	PLFA	Chloro	*Chlamydomonas, Chlorogonium*	−41.5	−20.9	−20.6
Spring 2006	PLFA	Chryso, Crypto	*Dinobryon, Mallomonas, Cryptomonas*	−41.3	−20.9	−20.4
Spring 2006	PLFA	Chryso, Crypto, Diatom	*Dinobryon, Mallomonas, Cryptomonas, Navicula, Tabellaria*	−40.8	−20.9	−19.9
Autumn 2006	PLFA	Crypto, Chryso	*Cryptomonas, Paraphysomonas*	−38.6	−21.8	−16.8
Summer 2013	Direct	Chryso	*Mallomonas caudata* (>99%)	−40.7		
Summer 2014	Direct	Chryso	*M. caudata* (>99%)	−44.0	−20.9	−23.1
Summer 2014	Direct	Cyano		−34.9	−20.9	−14.0

Table 4. Continued.

Lake type/Lake/ Season	Method	Class	Genus/Species	$\delta^{13}C$ biomass	$\delta^{13}C$ DIC	ε_p
Mekkojärvi						
Spring 2006	PLFA	Chloro	*Chlamydomonas*	−44.5	−21.4	−23.1
Spring 2006	PLFA	Cyano	*Microcystis*	−39.6	−21.4	−18.2
Spring 2006	PLFA	Chryso, Crypto, Diatom	*Mallomonas*, *Paraphysomonas*, *Pseudopedinella*, *Cryptomonas*, *Tabellaria*	−39.4	−21.4	−18.0
Majajärvi						
Summer 2014	Direct	Chloro	*Sphaerocystis schroeteri* (97%)	−39.8	−23.2	−16.6
Summer 2014	Direct	Chloro	*S. schroeteri* (96%)	−39.0	−23.2	−15.8
Summer 2014	Direct	Cyano		−32.7	−23.2	−9.5

Notes: The $\delta^{13}C$ of phytoplankton was obtained by using direct measurements of $\delta^{13}C$ signal (the proportion of different genera/species in the sample indicated by %) or calculated from biomarker phospholipid fatty acid (PLFA). For phytoplankton class abbreviations, see the legend of Fig. 1.

fractionation varied significantly ($F_{7,34}$ = 3.047, P < 0.05) at the phytoplankton class level; however, Tukey's post hoc test was not able to create homogenous subsets due to there being too few cases for some phytoplankton classes (Fig. 3). The photosynthetic fractionation was greater for the laboratory cultures of Charophyceae, Chlorophyceae, Diatomophyceae, and Cyanophyceae than for taxa of respective classes analyzed from lake samples. For the other classes, the difference in fractionation was not great between laboratory cultures and field samples.

Phytoplankton carbon isotope ratio in boreal lakes

Phytoplankton $\delta^{13}C$ values varied among lake types and were significantly lower in dystrophic lakes than in other lake types (ANOVA, $F_{3,33}$ = 16.9, P < 0.01, Fig. 2B). The average $\delta^{13}C$ value of all phytoplankton in the two oligotrophic lakes, Lake Syrjänalunen and Lake Valkea-Mustajärvi, was −31.2‰ ± 2.7‰, including classes of Chlorophyceae, Charophyceae, Cyanophyceae, Cryptophyceae, Chrysophyceae, Dinophyceae, and Raphidophyceae. The stable isotope sample of Lake Syrjänalunen consisted mainly of Charophyceae and Chlorophyceae, and

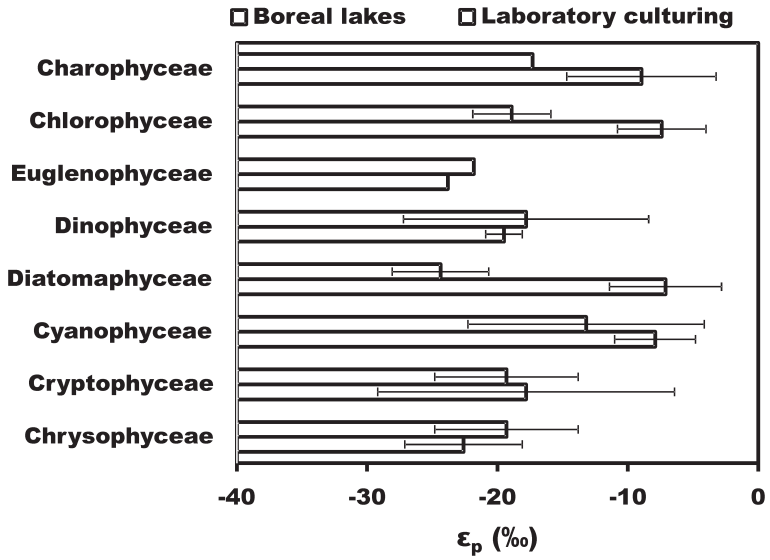

Fig. 3. Mean (± SD) photosynthetic carbon fractionation (ε_p, ‰) of different phytoplankton classes in 12 boreal lakes and in laboratory cultures of taxa in respective classes.

their combined $\delta^{13}C$ value was −31.2‰ ± 0.2‰ (Table 4). Cyanophyceae from the same lake had more depleted $\delta^{13}C$ values (−33.8‰ ± 0.2‰). In the 2006 Lake Valkea-Mustajärvi samples, the combined $\delta^{13}C$ value of Chrysophyceae (*Mallomonas* sp.) and Cryptophyceae (*Cryptomonas* spp.) was −33.9‰ ± 1.0‰, whereas in 2014 the $\delta^{13}C$ values of *Peridinium* and Cyanophyceae for this lake were more enriched, −28.6‰ ± 0.1‰ and −28.4‰ ± 0.2‰, respectively.

The phytoplankton $\delta^{13}C$ values of the two mesotrophic lakes (average ± SD = −29.6‰ ± 5.5‰) varied between the lakes but also between taxa. *Peridinium* sp. (Dinophyceae) dominated (97%) the phytoplankton community in Lake Valkea-Kotinen in the spring of 2006 and the summer of 2014. The $\delta^{13}C$ value was ~6‰ more enriched for *Peridinium* sp. in Lake Valkea-Kotinen in 2014 (−25.5‰ ± 0.3‰) than in 2006 (−31.1‰ ± 1.0‰). The spring $\delta^{13}C$ value of *Peridinium* in 2006 was similar to *Peridinium* in Lake Alinen-Mustajärvi of the same year. Furthermore, the spring sample of *Peridinium* from Valkea-Kotinen in 2014 contained some rotifers (*Polyarthra* and *Synchaeta*), which may have affected the results even though they were avoided in isotope analysis by selecting only green freeze-dried biomass. During fall sampling *Peridinium* sp. dominated (> 92%) the phytoplankton community as well, and we measured −38.5‰ ± 1.0‰ for *Peridinium* sp. in the epilimnion. *Gonyostomum semen* was also abundant in Lake Valkea-Kotinen in 2014 and had a $\delta^{13}C$ value of −29.2 ± 0.1‰. In Lake Alinen-Mustajärvi, the $\delta^{13}C$ value of Chrysophyceae (*Chrysococcus* sp., *Dinobryon* sp., *Mallomonas* sp.) was −31.5‰ ± 1.0‰ in the epilimnion in 2006.

The phytoplankton $\delta^{13}C$ values of the eutrophic lakes (average ± SD = −32.0‰ ± 3.4‰) were close to those of the oligotrophic lakes (−31.2‰ ± 2.7‰). Among the five eutrophic lakes sampled, Lake Kyynärö and Lake Köyhälampi both had high abundance of *Ceratium hirundinella.* The $\delta^{13}C$ value of *Ceratium* was −35.3‰ ± 0.7‰ in Lake Kyynärö and −33.0‰ ± 0.2‰ in Köyhälampi, respectively. The stable isotope sample for Lake Tuusulanjärvi contained Diatomophyceae (*Aulacoseira*, *Asterionella*) and Euglenophyceae (*Colacium* sp.). The $\delta^{13}C$ values of Diatomophyceae and Euglenophyceae were −29.0‰ ± 1.2‰ and −28.6‰ ± 0.1‰, respectively. Our isotope sample from Lake Kataloistenjärvi contained *Botryococcus* spp., and its $\delta^{13}C$ value was −27.3‰ ± 0.6‰. The sample from Lovojärvi contained mixed cyanobacteria (*Woronichinia*, *Pseudanabaena*) and the $\delta^{13}C$ value was −30.6‰ ± 0.2‰.

The most depleted phytoplankton $\delta^{13}C$ values (average ± SD = −39.8‰ ± 3.2‰) were measured for dystrophic lakes. Among the three dystrophic lakes, Mekkojärvi and Majajärvi were only sampled once, whereas Nimetön was sampled three times. Mekkojärvi consisted mainly of Chlorophyceae (*Chlamydomonas* sp.) in the spring 2006 when the $\delta^{13}C$ value was −44.7‰ ± 1.0‰ in the epilimnion. The other abundant phytoplankton groups were Cryptophyceae, Chrysophyceae, and Diatomophyceae, which had a combined $\delta^{13}C$ value of −39.4‰ ± 1.0‰. Cyanophyceae were a minor phytoplankton group in the spring and mainly consisted of *Chroococcus* sp. and *Snowella* sp., but *Microcystis* sp. were also present in the meta- and hypolimnion. The $\delta^{13}C$ value for Cyanophyceae in the metalimnion was −34.2‰ ± 1.0‰ in Lake Mekkojärvi. The other dystrophic lake (Lake Nimetön) was sampled in 2006, 2013, and 2014, and during every sampling event, *Mallomonas caudata* was the most abundant species. This phytoplankton had a $\delta^{13}C$ value of −41.7‰ ± 1.4‰ in the spring and summer, but was more enriched (−38.6‰ ± 0.4‰) in the autumn of 2006. During the summer of 2014, Majajärvi had a Chlorophyceae (*Sphaerocystis* sp.) bloom with the $\delta^{13}C$ value of −39.4‰ ± 0.6‰. The $\delta^{13}C$ value of the Cyanophyceae in Majajärvi was −32.7‰ ± 0.2‰.

Phytoplankton carbon isotopic signals by depth

Phytoplankton sampled in 2006 from the epi-, meta-, and hypolimnion of five lakes showed the differences in taxon-specific $\delta^{13}C$ values between the depths. In the two dystrophic lakes, the $\delta^{13}C$ value of Chlorophyceae was more depleted in the epilimnion than in the meta- and hypolimnion (~5.0‰ and 4.4‰). Also, the $\delta^{13}C$ of Cyanophyceae was 5.1‰ more depleted in the epilimnion than in the meta- or hypolimnion of Lake Mekkojärvi. The greatest within-taxa difference between the epi- and metalimnion was measured in Lake Valkea-Kotinen for *Peridinium*; the $\delta^{13}C$ value was ~9‰ more enriched in the epilimnion (−31.1‰ ± 0.6‰) than in the metalimnion (−40.5‰ ± 0.6‰).

Table 5. The $\delta^{13}C$ values (‰) of seston (size fraction > 0.2 μm), dissolved organic carbon (DOC), and three bacterial groups in the studied lakes (GBS, green sulfur bacteria; Actino, Actinobacteria; MOB, methane-oxidizing bacteria).

Lake type/Lake/Season	$\delta^{13}C$ seston	$\delta^{13}C$ DOC	$\delta^{13}C$ GBS	$\delta^{13}C$ Actino	$\delta^{13}C$ MOB
Oligotrophic					
Syrjänalunen					
Summer 2014	−36.5			−33.1	
Valkea-Mustajärvi					
Spring 2006	−30.6	−27.0		−27.8	−62.2
Summer 2014		−29.4			
Mesotrophic					
Alinen-Mustajärvi					
Spring 2006	−28.8	−26.1		−29.6	−53.2
Valkea-Kotinen					
Spring 2006	−27.9	−26.6		−29.2	
Summer 2014					−60.8
Eutrophic					
Kataloistenjärvi					
Summer 2015	−31.8 ± 0.6				
Kyynärö					
Summer 2015	−31.8 ± 0.1				
Köyhälampi					
Summer 2014	−29.0				
Lovojärvi					
Summer 2015	−32.2 ± 0.2				
Tuusulanjärvi					
Summer 2015	−27.8 ± 0.3				
Dystrophic					
Mekkojärvi					
Spring 2006	−31.7	−25.7	−31.9	−29.5	−52.9
Nimetön					
Spring 2006	−34.2	−25.7		−27.7	−54.9
Summer 2014	−38.4	−25.7		−27.7	
Majajärvi					
Summer 2014				−29.5	

Carbon isotopic signals of t-POC and bacteria

The $\delta^{13}C$ value of tree leaves (*Populus tremula*, *Betula* sp.) was measured for the samples collected from the shoreline of the Lake Mekkojärvi in fall 2011. Thus, the measured $\delta^{13}C$ value, −29.8‰ ± 0.4‰, represents t-POC from C3 vegetation entering boreal lakes. The $\delta^{13}C$ values of the DOC were similar (−26.0‰ ± 0.7‰) in the whole water column among the five lakes sampled in 2006 (Table 5), which is consistent with recalcitrant terrestrial DOC (t-DOC). Furthermore, the $\delta^{13}C$ value of *Actinobacteria* in 2006 (measured by $\delta^{13}C$ of PLFA) and all heterotrophic bacteria in 2014 (directly determined) was similar (−28.7‰ ± 1‰) among the different lakes excluding Syrjänalunen (−33.1‰ ± 0.1‰, Table 5), which is an oligotrophic ground water lake. GSB were found only in Lake Mekkojärvi in 2006 ($\delta^{13}C$ value of −31.9‰ ± 1‰). The $\delta^{13}C$ values of MOB varied greatly between lakes (−56.8‰ ± 4.4‰) as well as vertically within the lakes, with the MOB $\delta^{13}C$ values approximately 10‰ lighter in the hypolimnion than in the epilimnion.

Carbon isotopic variation between seston and ternary sources

We were able to measure the carbon signal from ternary sources (phytoplankton, terrestrial, bacteria) in oligotrophic, mesotrophic, and dystrophic lakes, but not in eutrophic lakes for which bacterial samples could not be separated from detritus. In all lakes, the $\delta^{13}C$ values of t-DOC and t-POC were the most enriched of

Table 6. The $\delta^{13}C$ values (‰) of herbivorous cladoceran zooplankton with trophic carbon fractionation in 10 studied lakes. Fractionation between *Daphnia* and diet, measured in previous (Taipale et al. 2014, Taipale et al. 2015) experiments (n = 10, each sample contained ~5 *Daphnia*), was −0.6‰ ± 0.3‰ for cryptomonas and 1.5‰ ± 0.4‰ for green algae.

Lake type/Lake/Season	$\delta^{13}C$ cladocerans	Cladoceran $\delta^{13}C$ with fractionation	$\delta^{13}C$ phytoplankton
Oligotrophic			
Syrjänalunen			
Summer 2014	−29.3	−28.3 to −31.3	−31.1 to −33.6
Valkea-Mustajärvi			
Spring 2006	−33.6	−31.3 to −34.6	−29.0 to −36.0
Mesotrophic			
Alinen-Mustajärvi			
Spring 2006	−28.4	−27.4 to 30.4	−31.5 to −32.2
Valkea-Kotinen			
Spring 2006	−32.2	−30.2 to −34.2	−18.0 to −31.1
Summer 2014	−25.8 ± 1.4	−24.8 to −27.8	−25.5 to −30.7
Eutrophic			
Köyhälampi			
Summer 2014	−30.9	−28.9 to −31.9	−33.1
Lovojärvi			
Summer 2015	−32.4 ± 1.0	−31.4 to −34.4	−30.6 to −39.4
Tuusulanjärvi			
Summer 2015	−28.1 ± 0.1	−27.1 to −30.1	−28.6 to −29.1
Dystrophic			
Mekkojärvi			
Spring 2006	−39.5	−37.5 to −40.5	−34.2 to −44.5
Nimetön			
Spring 2006	−36.7	−34.7 to −37.6	−39.4 to −41.5
Summer 2014	−40.7	−38.7 to −41.7	−34.9 to −44.0
Majajärvi			
Summer 2014	−39.8	−37.8 to −40.8	−32.7 to −39.5

Notes: Here, we used the range of minimum and maximum fractionation (−1‰ for high-quality and +2‰ for intermediate-quality diet). The range of phytoplankton $\delta^{13}C$ values (‰) during the time of sampling is also shown.

the ternary sources considered excluding Valkea-Kotinen, in which the $\delta^{13}C$ values of Cyanophyceae in 2006 and Dinophyceae in 2014 were even more enriched than terrestrial signals (Tables 4 and 5). Additionally, the $\delta^{13}C$ of heterotrophic Actinobacteria had a similar $\delta^{13}C$ value with fallen leaves (isotopic difference −0.5‰ ± 1.8‰), but their $\delta^{13}C$ value differed (−2.5‰ ± 1.2‰) from that of DOC in all lakes (Table 5). Nevertheless, the $\delta^{13}C$ value of the heterotrophic bacteria was closer to the $\delta^{13}C$ value of phytoplankton (isotopic difference −0.6‰ ± 1.8‰) than to the terrestrial signal (isotopic difference −3.3‰ ± 0.3‰) in the oligotrophic Lake Syrjänalunen. Carbon isotopic difference between the most depleted phytoplankton taxa and terrestrial signal (t-POC) was greatest in dystrophic lakes (−12.2‰ ± 2.1‰), but lower in mesotrophic (−3.1‰ ± 4.9‰), oligotrophic (−4.8‰ ± 1.2‰), and eutrophic lakes (−1.5‰ ± 0.8‰). However, the isotopic difference between Cyanophyceae and t-POC was only −4.1‰ ± 1.1‰ in dystrophic lakes and −1.3 ± 2.0 in the other lakes except for Valkea-Kotinen in 2006.

Carbon isotopic values of Daphnia *and ternary sources*

We were able to measure the $\delta^{13}C$ values of cladocerans from 10 lakes, some of which were sampled twice (Table 6). For two lakes (Kataloistenjärvi and Kyynärö), we did not obtain enough biomass for isotope analyses. The cladoceran $\delta^{13}C$ values did not correlate significantly with the $\delta^{13}C$ values of the seston, but correlated significantly with the $\delta^{13}C$ of the

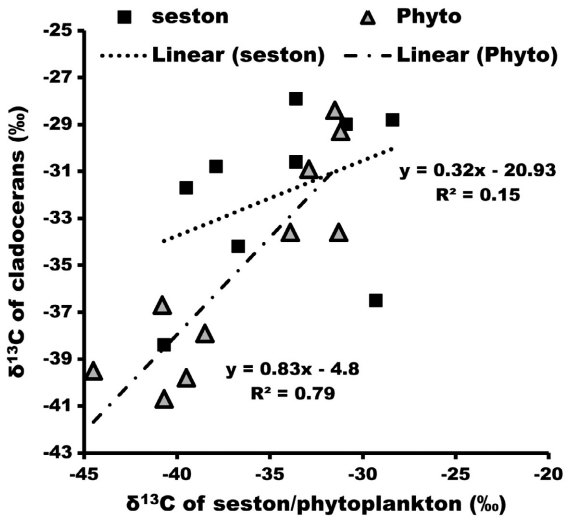

Fig. 4. Herbivorous cladoceran $\delta^{13}C$ values related to those of bulk seston and major phytoplankton classes in 10 lakes. Only the relationship between phytoplankton and cladoceran $\delta^{13}C$ values was significant.

major phytoplankton classes observed in each lake (r = 0.79, P < 0.01, n = 10, Fig. 4). The cladoceran $\delta^{13}C$ values including trophic fractionation were within the range of $\delta^{13}C$ values of simultaneously sampled phytoplankton taxa (Table 6) except for Alinen-Mustajärvi in 2006. The $\delta^{13}C$ value of cladocerans in this sample was between the $\delta^{13}C$ values of t-POC/Actinobacteria and t-DOC.

Discussion

The diets of herbivorous zooplankton consist of a combination of different types of phytoplankton, protozoa, bacteria, and terrestrial- and aquatic-derived detritus. However, the $\delta^{13}C$ values of these separate food sources are often impossible to measure directly. Therefore, isotope mixing models usually utilize indirect estimates of phytoplankton and bacterial stable isotope values. An often used indirect method for phytoplankton $\delta^{13}C$ value estimation is to measure the $\delta^{13}C$ value of DIC and assume a constant photosynthetic fractionation (ε_p) value (Brett 2014*a*). Our study of phytoplankton ε_p in laboratory cultures showed a clear correlation with DIC values in most phytoplankton taxa studied when other environmental factors were constant; however, these results were not validated in natural systems where phytoplankton fractionation did not correlate with DIC concentrations. Furthermore, our study of 12 lakes showed that the phytoplankton $\delta^{13}C$ values varied greatly by taxa and depth and thus assuming one carbon fractionation or stable isotope value for all phytoplankton is unwarranted. We found that cladoceran $\delta^{13}C$ values correlated strongly with the $\delta^{13}C$ values of the major phytoplankton taxa in each lake. We also found that variation in the phytoplankton $\delta^{13}C$ values by taxa and depth within lakes could fully explain the cladoceran $\delta^{13}C$ values in nine of the 10 lakes sampled.

Phytoplankton ^{13}C fractionation and DIC

The $\delta^{13}C$ value of DIC is easily measurable using an IRMS system, in samples stored in exetainers for up to six months (Taipale and Sonninen 2009). Therefore, a general model for photosynthetic carbon fractionation (ε_p) would be very useful, especially if this fractionation could be calculated for distinct phytoplankton taxa. Different conditions including temperature, DIC concentration, growth rate, and cell size have been shown to impact photosynthetic fractionation in laboratory cultures (Laws et al. 1997, Popp et al. 1998, Rost et al. 2002, Brutemark et al. 2009, Radabaugh et al. 2014), but these patterns have not been verified in natural systems (Bade et al. 2006). Our phytoplankton cultures were grown in similar light, temperature, and nutrient conditions, and only DIC concentration and growth rates varied. In these experiments, the DIC concentration had a clear impact on ε_p across the different phytoplankton classes. However, the study with DIC additions showed that DIC concentration did not impact phytoplankton taxa similarly perhaps due to the differences in cell size and surface-area-to-volume ratios (Rau et al. 1996, Popp et al. 1998). This is a possible explanation for the greater carbon fractionation by the dinoflagellate *Peridinium* (ε_p −19.2‰ ± 1.6‰) compared with the other taxa in the study. Furthermore, DIC concentration did not impact *Peridinium* carbon fractionation. This can be a result of high growth rate of *Peridinium*, because growth rate is also a key factor influencing ε_p (Laws et al. 1997). The results of our DIC addition experiment

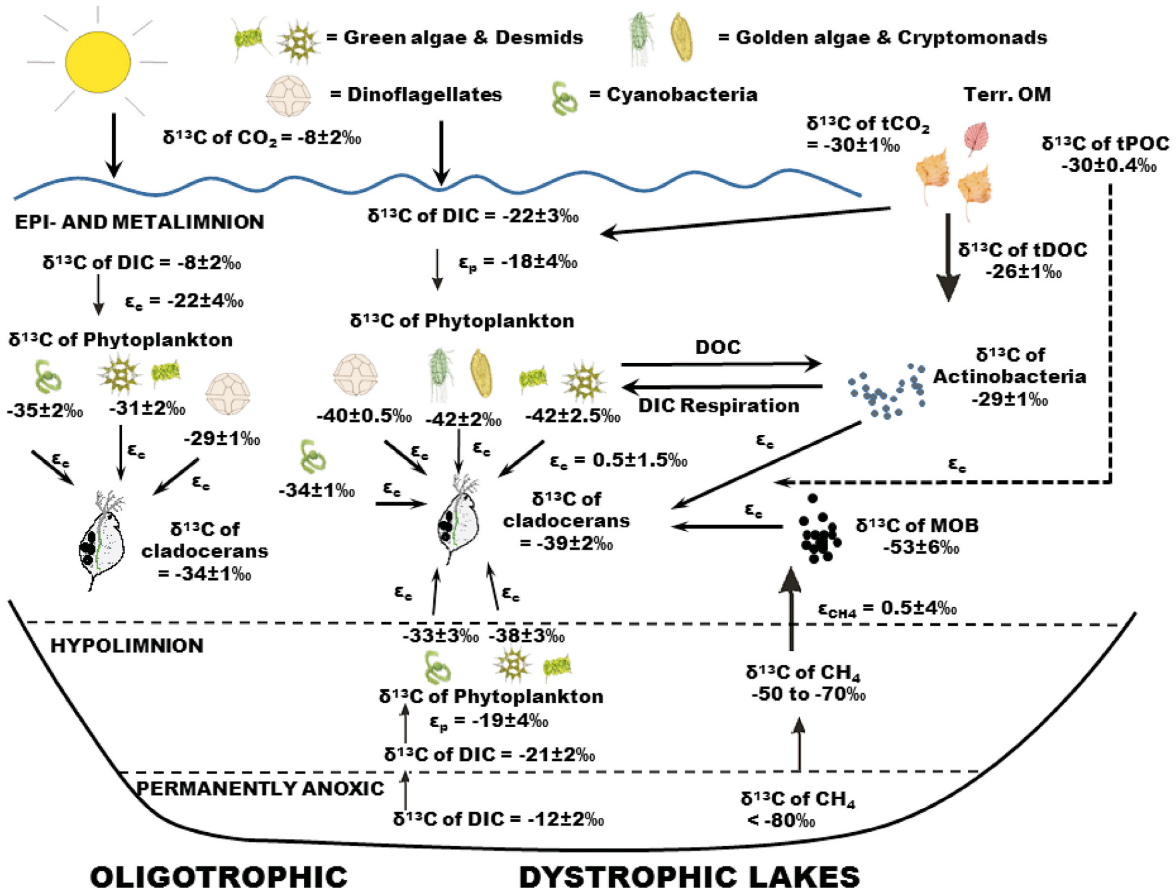

Fig. 5. The $\delta^{13}C$ values of dissolved inorganic carbon, terrestrial particulate organic carbon (t-POC), distinct phytoplankton and bacteria groups, and cladocerans in two clear water (Valkea-Mustajärvi and Syrjänalunen) and three dystrophic (Mekkojärvi, Nimetön, and Majajärvi) lakes. Photosynthetic fractionation (ε_p = mean ± SD) is given as an average for different phytoplankton groups in these lakes and calculated separately for hypolimnion. Permanently anoxic refers to bottom water column layer of Nimetön, which does not mix. Carbon fractionation (0.5‰ ± 1.5‰) estimate between herbivorous cladoceran and potential dietary sources is based on earlier measurements (Taipale et al. 2014).

confirm that various factors influence photosynthetic carbon fractionation and explain why it is poorly related to DIC concentration of the lakes, as observed also by Bade et al. (2006). Bade et al. (2006) found a relatively low (0‰ to −15‰) ε_p, which is similar to our laboratory cultures, while in the field data we found higher (more than −20‰) ε_p for oligotrophic, dystrophic, and eutrophic lakes.

In some cases, ε_p varied greatly between laboratory and lake measurements, but also between lakes, making it difficult to obtain even rough estimates of ε_p. For example, we found high ε_p for *Peridinium* (Dinophyceae) in laboratory cultures and also in oligotrophic (−22.3‰ ± 0.3‰) and eutrophic (*Ceratium*, −34.2‰ ± 1.4‰) lakes, but only −8.6‰ and −0.8‰ in the mesotrophic Lake Valkea-Kotinen during the years 2006 and 2014, respectively. Therefore if a lake in the same forested area were randomly chosen to determine the $\delta^{13}C$ value of Dinophyceae (*Peridinium*, *Ceratium*) by measuring $\delta^{13}C$ value of the DIC and assuming a photosynthetic fractionation value, the potential values for fractionation would vary from −1‰ to −34‰. Furthermore, these directly measured field ε_p values show higher (even 10‰) maximum isotopic fractionation (ε_p) than previously suggested (Popp et al. 1998).

Our results show that for modeling photosynthetic fractionation in natural systems, it is not enough to know the influence of one factor (e.g., DIC concentration), but more detailed understanding of other factors (light, temperature, and growth stage) and their combinations is also required. Due to these constraints, we conclude that direct measurements as well as compound-specific analyses should be used to determine the $\delta^{13}C$ values of phytoplankton and bacteria (Grosse et al. 2015, Taipale et al. 2015).

Variation in the $\delta^{13}C$ value of phytoplankton by taxa and depth

The laboratory experiment of Pel et al. (2003) as well as the conclusions by Vuorio et al. (2006) and Bade et al. (2006) reported that phytoplankton $\delta^{13}C$ values as well as carbon fractionation are taxon specific. This was also true in our laboratory cultures and lakes; however, $\delta^{13}C$ also varied within the same species in the same lake. Vuorio et al.'s (2006) study of eutrophic lakes showed that the $\delta^{13}C$ value of cyanobacteria could be as much as 20‰ enriched compared to the $\delta^{13}C$ values of other phytoplankton taxa, but their results also showed a considerable variation in the $\delta^{13}C$ values among different cyanobacteria species. The lakes from which we were able to measure $\delta^{13}C$ values from cyanobacteria showed more enriched $\delta^{13}C$ values (~9‰) in relation to the other phytoplankton in dystrophic and eutrophic lakes. In the oligotrophic and mesotrophic lakes, the $\delta^{13}C$ values of the cyanobacteria were not the most enriched of the phytoplankton and also had high variation among different cyanobacteria species and lakes (Vuorio et al. 2006).

We found the most depleted phytoplankton $\delta^{13}C$ values from dystrophic lakes and these are, so far, the most depleted directly measured phytoplankton values we know. For example, we measured $\delta^{13}C$ values of −44.0‰ and −41.0‰ for *Mallomonas caudata* at the same time of the year in two successive years from a dystrophic lake. Additionally, other phytoplankton taxa also had $\delta^{13}C$ values close to approximately −40‰ in the dystrophic lakes. These lakes are strongly stratified and light attenuation is strong, which can result in vertically heterogeneous $\delta^{13}C$ phytoplankton values because photosynthetically active radiation (PAR) can have an impact on ε_p (Radabaugh et al. 2014). We measured lower ε_p (−3.9‰ ± 2.1‰) and less depleted phytoplankton $\delta^{13}C$ values from the meta- and hypolimnion than in the epilimnion, which is similar when PAR was reduced by 50% from 40 to 20 $mol \cdot m^{-2} \cdot d^{-1}$ in the diatom experiment of Radabaugh et al. (2002). These findings show that the $\delta^{13}C$ value not only varies greatly between phytoplankton taxa (Vuorio et al. 2006), but can also vary within time and space in the same lake.

A high proportion of phytoplankton in dystrophic lakes has been reported to be mixotrophic and can ingest organic particles (Salonen and Jokinen 1988, Tranvik et al. 1989). It is not yet known how much mixotrophy influences ε_p and the $\delta^{13}C$ values of phytoplankton and how much of the variation in the distinct $\delta^{13}C$ values of *Mallomonas* in successive years (Nimetön) could be explained by different levels of mixotrophy because the $\delta^{13}C$ value of the DIC was similar in both years.

The $\delta^{13}C$ values of zooplankton and potential dietary sources

Stable isotope mixing models often simplify zooplankton diet sources down to three groups: phytoplankton, bacteria, and t-POM (Karlsson et al. 2003, Cole et al. 2011). Some studies also include methane-oxidizing bacteria and photoautotrophic bacteria (Taipale et al. 2009). If four or more sources are included in mixing models that use two isotopes, the mixing models will be underdetermined resulting in high misclassification error (Brett 2014*b*). Due to the depleted $\delta^{13}C$ value of methane (−50‰ to −80‰, Kankaala et al. 2007) and methane-oxidizing bacteria, methane-based sources can be easily tracked using their carbon isotope values (Kankaala et al. 2006, Taipale et al. 2009, Jones and Grey 2011, Sanseverino et al. 2012). However, the isotopic distance between terrestrial and phytoplankton dietary sources is often small. In our study, the isotopic distance between terrestrial (t-POC) and phytoplankton (excluding cyanobacteria) sources was the largest in the dystrophic lakes (approximately −12‰) and was more similar in oligotrophic, mesotrophic, and eutrophic lakes (approximately −3‰). However, the isotopic distance between cyanobacteria and terrestrial sources did not differ much in dystrophic lakes (approximately −4‰).

Our $\delta^{13}C$ values for t-POC differed by 3–4‰ from the $\delta^{13}C$ values of DOC in boreal lakes, where DOC is typically of terrestrial origin (Kortelainen 1993, Mattsson et al. 2005). Our measured $\delta^{13}C$ value for t-POC is similar to the previously measured $\delta^{13}C$ values of t-POC and t-DOC from a forested brook (Billet et al. 2012). Furthermore, the $\delta^{13}C$ values of Actinobacteria were closer to the $\delta^{13}C$ value of t-POC compared with t-DOC in all lakes excluding one oligotrophic lake, in which the bacteria $\delta^{13}C$ value was similar to that of phytoplankton. Because the $\delta^{13}C$ values of bacteria were systematically similar to the $\delta^{13}C$ value of t-POC in lakes, this could mean that bacteria utilize fresh t-POC or both phytoplankton- and terrestrial-origin organic carbon; however, in mixing model calculations, t-POC and heterotrophic bacteria cannot be separated by their carbon isotope signal.

One of the most problematic challenges with zooplankton mixing model calculations is accurately estimating phytoplankton $\delta^{13}C$ values. This is problematic because there is high variation in the $\delta^{13}C$ values among phytoplankton taxa as well as the variation caused by multiple environmental factors. Our data showed that the $\delta^{13}C$ values of cyanobacteria were more enriched compared with other phytoplankton taxa and that in most cases the values of cladocerans were between the minimum and maximum phytoplankton $\delta^{13}C$ values. Furthermore, herbivorous cladocerans, for example, *Daphnia*, are thought to feed non-selectively (DeMott 1986). However, in our study, the cladoceran $\delta^{13}C$ values did not correlate with seston $\delta^{13}C$ values and instead correlated with the $\delta^{13}C$ values of the different phytoplankton taxa, indicating that *Daphnia* selectively assimilate phytoplankton. Marty and Planas (2008) also suggested that phytoplankton $\delta^{13}C$ values could be calculated from the $\delta^{13}C$ values of *Daphnia*.

Even though bias in ternary isotope modeling is smaller when phytoplankton are labeled by ^{13}C, stable isotope labeling does not equalize the $\delta^{13}C$ values of different phytoplankton taxa, and these can still vary greatly as shown in laboratory cultures (Pel et al. 2003, Taipale et al. 2011) and field studies (Vuorio et al. 2006). Therefore, isotope mixing models should account for the different dominant phytoplankton taxa present in a system to take into account the actual isotope range of the phytoplankton. It is especially important to separate cyanobacteria from other phytoplankton due to the more enriched carbon signal. Colonial cyanobacteria can be easily separated from other phytoplankton by size separation filtration. In all cases, isotope signals should be measured directly from phytoplankton biomass or diagnostic biomarkers via compound-specific stable isotope analyses (Taipale et al. 2015).

The separation of phytoplankton taxa in consumer diets is presently possible when using a fatty acid-based Bayesian algorithm (Galloway et al. 2014, 2015), which has the advantage over stable isotopes of using many more dietary tracers (i.e., > 20 FA), many of which are characteristic for particular phytoplankton groups. Because the $\delta^{13}C$ value of bulk biomass represents the average of all organic compounds, it would be useful to combine $\delta^{13}C$ values and fatty acids in one mixing model to obtain even more precise understanding of herbivorous zooplankton diets.

Acknowledgments

This research was supported by Academy of Finland research grants 251665 awarded to S.J. Taipale, 276268 to E. Peltomaa, 263350 to Paula Kankaala, and 260797 to Marja Tiirola.

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