

Comparison of methods to determine algal $\delta^{13}\text{C}$ in freshwater

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Abstract

To accurately assess the flux of mass and energy to higher trophic levels in a food web using stable isotopes, the isotopic signature of basal sources is required. When studying aquatic food webs, it is difficult to obtain a signature for algae because of challenges associated with isolating small organisms from a bulk sample. In this study, we compared freshwater algal $\delta^{13}\text{C}$ values obtained using five approaches from the literature. Results indicated that the signatures derived from a primary consumer such as *Daphnia* sp., from particulate organic carbon with a correction for algal biomass, and from isolated algal samples were comparable. By contrast, algal $\delta^{13}\text{C}$ values based on the signature of carbon dioxide and algal carbon fractionation were significantly lower than those of the other approaches. The inconsistent values produced by this method were likely due to problems in determining fractionation values based on current models and were potentially related to bicarbonate uptake by algae.

Introduction

Stable isotopes analysis (SIA) is a popular tool used by ecologists to address a wide range of questions related to food sources of organisms, the length of food chains, or the transfer of contaminants (Cabana and Rasmussen 1994, Post et al. 2000, Grey et al. 2000, Pace et al. 2004). These applications are possible because the stable isotopic composition of a consumer's tissues is related to that of its food (Rounick and Winterbourn 1986, Vander Zanden and Rasmussen 2001). To address these questions, however, it is necessary to first ascertain the baseline signatures (e.g., algal signatures) supporting the food web.

To determine the relative contributions of various food sources to a consumer, mass balance equations are used to combine the isotopic signatures of the various food sources (Phillips and Gregg 2003). These mixing models require robust isotopic signatures of potential food sources for use as end

members. In streams and littoral habitats, macrophytes and macroalgae are often used to determine the baseline carbon signature of primary producers (Keough et al. 1998, Finlay 2004), with the assumption that a small number of taxa are representative of an entire community. In the case of microalgae, which are typical of pelagic environments, our ability to determine algal $\delta^{13}\text{C}$ values tends to be limited because of difficulties associated with isolating small-sized organisms from a bulk sample. The sample purity problem is a major concern in freshwater systems, particularly those rich in humic substances, including dissolved organic compounds and particulate detrital material (del Giorgio and France 1996, Jones et al. 1999). Although direct measurements of pelagic algal $\delta^{13}\text{C}$ are possible for large-sized taxa (e.g., *Volvox*, see Rautio and Vincent 2007), there is little evidence to suggest that a single algal genus could be used to represent the carbon signature of a complex community. Despite promising efforts to develop new techniques that refine isotopic signatures of particular groups of organisms (Boschker and Middelburg 2002, Pel et al. 2004, Hamilton et al. 2005, Pond et al. 2006), the majority of studies still rely on indirect methods to infer algal signatures. The most common methods are based on particulate organic carbon (POC) or dissolved inorganic carbon (DIC).

Bulk POC represents a mixture of live and detrital organic matter of terrestrial and aquatic origin. Terrestrial organic carbon signatures exhibit little variation in the boreal region (Junger and Planas 1994, Jones et al. 1999); thus, most of the variance in the $\delta^{13}\text{C}$ of POC is related to variation in the algal signatures. Because of the difficulty in separating living from

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nonliving organisms, POC signatures have been considered equivalent to those of algae, which is likely a valid assumption in systems dominated by phytoplankton production, including clear (Keough et al. 1996) and eutrophic (Gu et al. 2006) waters. In many colored, oligotrophic freshwater ecosystems, however, terrestrial organic matter often represents a significant portion of bulk POC (Jones et al. 1999, Finlay 2001, Pace et al. 2004, Carpenter et al. 2005) and, therefore, must be considered when calculating algal signatures by including the ratio of algal carbon to total POC in mixing models. Inconsistencies in the interpretation of algal signatures between various aquatic studies based on POC approaches (Hamilton and Lewis 1992, France et al. 1996) have led to using the signatures of primary consumer organisms as baseline food web signatures. Primary consumer signatures have been used as proxies for algal isotopic ratios (Finlay 2004), as tools to assess the influence of habitat on the diet of higher food web levels (Vander Zanden et al. 1999, Matthews and Mazumder 2003) and as baselines to infer food chain length (Post 2002).

Algal $\delta^{13}\text{C}$ can also be determined based on the signature of the particular DIC form the algae uses by accounting for the amount of fractionation that occurs during photosynthesis (ϵ_p). The signature and concentration of DIC are related to biotic (photosynthesis, respiration) and abiotic (inflowing waters, methane oxidation, photolysis) processes that, in turn, are reflected in the carbon signatures of autotrophs. In addition, the variability in algal $\delta^{13}\text{C}$ reflects the abilities of different algal species to incorporate carbon dioxide (CO_2) or bicarbonate (HCO_3^-) (Maberly and Spence 1983), which are isotopically distinct by a 10‰ difference (Mook et al. 1974). Finally, algal $\delta^{13}\text{C}$ variations may also be related to photosynthetic fractionation, which varies over a broad range due to the influences of DIC supply and demand and of phytoplankton physiological characteristics, such as growth rate and cell geometry (Laws et al. 1997, Popp et al. 1998, Burkhardt et al. 1999). As most oligotrophic freshwaters are supersaturated in CO_2 (Cole et al. 1994, Duarte and Prairie 2005), a number of studies (Karlsson et al. 2003, Pace et al. 2004, Pulido-Villena et al. 2005) have based their estimations of pelagic algal $\delta^{13}\text{C}$ on the assumption that CO_2 is the only form of carbon incorporated into algae due to its availability and the lower energy costs associated with its uptake by passive diffusion (Burkhardt et al. 1999).

To our knowledge, little work has been performed to compare the various approaches used in determining algal $\delta^{13}\text{C}$, despite reports of inconsistencies in data produced by different approaches and the extreme caution exercised in interpretations based on these tools (Raven et al. 1994, France 1996, del Giorgio and France 1996). The determination of accurate basal signatures is crucial to the interpretation of a consumer's isotopic composition, given the ecological implications of such findings. For example, most recent studies using $\delta^{13}\text{C}$ analysis to determine the carbon source for freshwater aquatic consumers have continued to generate mixed results concerning the presence of allochthonous carbon in the food web

(Jones et al. 1998, Bunn et al. 2003, Karlsson et al. 2003, Martineau et al. 2004, Pace et al. 2004, Marty and Planas 2005). It is therefore essential to assess if conclusions from SIA studies reflect differences in the functioning of communities or are instead attributable to the methods used, perhaps leading to biased conclusions. Further, a comparison of the various methods used to determine carbon algal signatures is important to identify the factors that generate differences between them.

The purpose of this study was to determine and compare algal stable carbon isotope ratios based on several approaches used in the literature. Specifically, the algal $\delta^{13}\text{C}$ value was (1) considered as the bulk POC signature; (2) calculated from the POC signature with a correction for algal biomass; (3) calculated from the carbon dioxide signature and algal fractionation; (4) derived from the signature of grazer organisms such as *Daphnia* sp.; and (5) considered the $\delta^{13}\text{C}$ of isolated algal material from particulate organic matter bulk. For approaches based on mathematical calculations, sensitivity analyses were performed to determine the effect of variations in parameters entered into the mixing models. Comparison of algal $\delta^{13}\text{C}$ values obtained from the five approaches revealed important discrepancies between methods, with significantly different signatures obtained when using CO_2 . Further, differences between methods raise questions with regard to the assumptions supporting calculations, especially those concerning the form of carbon incorporated by algae and the fractionation factor (ϵ_p).

Materials and procedures

Study area and sampling methods—Samples were collected between 2001 and 2003 from 13 pristine lakes and 6 reservoirs, located in 3 areas on the Canadian Shield (James Bay, Manicouagan, and Ste-Marguerite). Sampling included several stations per reservoir depending on its surface area (286 to 2646 km²), and one station per lake (deepest point). Data presented in this study were collected once, during mid-summer. Temperature, oxygen, and pH profiles were measured in situ with a YSI-6600 multiprobe. Integrated water samples (60 L) were collected using a 4-L Van Dorn bottle from the euphotic zone of the water column (Li-Cor LI-193SA and LI-190SA) or from the epilimnion of stratified water columns when it was deeper than the photic zone. This water was used to determine the concentration of particulate organic matter (see below), chlorophyll *a* (chl. *a*) concentration (Nusch 1980), and primary production (PP). The methods and results for the PP measurements are reported in Planas et al. (2005). Phytoplankton and zooplankton samples were collected from the entire water column (maximum 30 m depth) by vertical tows of a plankton net with a 110- μm mesh size. Zooplankton were kept alive in filtered water until arrival at the laboratory to allow gut evacuation. Algal species found in these systems were typical of oligotrophic ecosystems, and consisted predominantly of nanoflagellates and diatoms. The CO_2 concentration was measured in the field using a nondispersive infrared analyzer (Li-Cor LI-7000) and a gas chromatograph in the case of LG-2 reservoir (Varian

Table 1. Physical, chemical, and biological characteristics of 13 lakes (L) and 6 reservoirs (R) in Northern Canada for the summers of 2001–2003

Sites	Stations	pH	Temperature, °C	DIC, $\mu\text{mol} \cdot \text{L}^{-1}$	CO_2 , $\mu\text{mol} \cdot \text{L}^{-1}$	$\delta^{13}\text{C}_{\text{DIC}}$, ‰	Algal growth		Algal fractionation (ϵ_p), ‰	POC, $\mu\text{g} \cdot \text{L}^{-1}$	Chl. <i>a</i> , $\mu\text{g} \cdot \text{L}^{-1}$
							rate (μ), d^{-1}	$\mu/[\text{CO}_2]$			
L	Berté	6.1	16.0	43.5	25.7	—	0.6	22.9	16.6	86.9	0.6
L	Desaulnier	6.8	18.3	91.3	25.4	—	1.0	37.9	9.9	262.9	1.5
L	Duchaunay	—	16.5	—	—	—	0.3	—	—	116.9	0.8
L	Aux Cèdres	—	18.0	—	—	—	1.0	—	—	340.5	1.1
L	Germain	—	16.0	—	—	—	0.8	—	—	431.2	0.6
L	Jean-Marie	6.8	18.2	68.4	19.3	-28.9	0.3	13.5	20.8	453.9	2.1
L	Km 12	7.5	16.6	259.8	19.2	-16.6	0.5	27.3	14.7	214.8	1.3
L	Km 17	6.8	17.7	112.6	32.9	-34.3	0.5	13.8	20.7	430.5	1.9
L	Km 380	7.5	18.0	246.0	19.5	-17.0	1.0	49.9	4.6	320.4	1.2
L	Matonipi	—	11.0	—	—	—	0.3	—	—	202.5	0.9
L	Patukami	6.9	16.6	95.2	26.6	—	0.9	34.2	11.6	213.0	1.2
L	Polaris	6.5	17.8	35.4	16.6	-33.0	0.6	34.0	11.7	288.4	1.1
L	Yasinsky	6.9	17.5	130.0	33.3	—	1.0	31.0	13.0	102.9	1.8
R-LA1	2	6.4	15.4	74.3	38.5	-18.7	0.7	18.2	18.7	424.5	2.0
R-LA1	3	6.3	15.3	54.7	31.4	-32.8	0.7	20.7	17.6	394.5	2.1
R-LA1	4	6.4	16.2	78.5	41.3	-31.8	0.5	10.9	22.0	501.2	2.6
R-LA1	5	6.5	16.7	59.7	27.9	-30.6	0.5	18.8	18.5	385.0	2.1
R-LA1	43C (2001)	6.3	19.2	35.2	24.7	—	0.9	36.6	10.5	689.1	3.9
R-LA1	43C	6.8	14.9	109.7	34.1	-19.0	0.6	17.8	18.9	428.1	2.2
R-LA1	903 (2001)	6.4	17.9	48.7	31.5	—	0.5	16.1	19.6	281.1	2.4
R-LA2	1	6.3	11.8	41.6	25.6	-25.7	0.5	20.9	17.5	455.0	2.2
R-LA2	2	6.5	12.6	59.3	27.9	-29.0	0.5	18.0	18.8	367.2	2.3
R-LA2	3	6.3	12.9	44.7	27.3	-24.7	0.6	23.1	16.5	383.8	2.3
R-LA2	4	6.0	12.7	43.8	32.3	-30.3	0.5	14.2	20.5	358.6	1.9
R-LA2	5	6.1	12.9	42.0	29.4	-35.9	0.4	14.8	20.2	368.9	2.3
R-LG2	1	6.4	13.8	33.0	35.7	—	0.6	16.0	19.7	195.3	1.6
R-LG2	406	6.2	7.2	71.6	64.3	—	1.6	24.5	15.9	130.2	0.9
R-LG2	39	6.5	15.9	54.1	32.5	—	0.6	18.5	18.6	—	1.9
R-LG2	509	6.6	14.4	64.9	37.6	—	0.7	19.3	18.2	246.9	1.9
R-LG2	18	6.1	18.5	60.1	50.5	—	0.6	11.5	21.7	252.3	2.2
R-LG2	336	6.8	16.2	69.8	27.0	—	1.0	36.3	10.6	114.6	1.6
R-LG2	604	6.4	15.7	55.7	31.1	—	0.9	27.4	14.6	153.9	1.5
R-LG2	610	6.6	17.2	—	—	—	0.5	—	—	218.4	1.6
R-LG2	615b	6.4	12.0	79.9	70.4	—	0.5	6.8	23.8	461.5	1.1
R-LG4	1	6.1	11.4	56.7	39.4	-31.1	0.8	20.8	17.5	314.4	1.0
R-LG4	2	6.2	11.6	58.6	39.2	-33.9	0.4	11.1	21.9	265.6	1.4
R-LG4	3	6.1	11.2	55.8	38.8	-28.3	0.6	15.4	20.0	342.0	1.4
R-LG4	4	6.2	12.0	63.5	42.4	-29.8	0.4	9.9	22.4	357.8	1.7
R-LG4	5	7.6	15.9	413.8	26.0	-25.8	0.7	25.2	15.6	305.0	1.2
R-MA5	400	6.2	11.5	55.7	38.8	—	0.4	10.1	22.3	98.4	0.7
R-MA5	600	6.6	16.0	87.6	35.2	—	0.5	15.3	20.0	149.6	1.0
R-MA5	800	6.3	17.0	65.2	37.1	—	0.5	12.8	21.1	105.2	1.1
R-MA5	1200	6.6	14.0	87.1	35.6	—	0.4	11.0	21.9	206.3	1.3
R-SM3	5057	5.9	18.0	73.1	56.0	—	0.5	9.5	22.6	266.0	4.4
R-SM3	5107	—	13.0	—	—	—	0.4	—	—	196.4	2.1
R-SM3	5121	6.1	15.0	70.7	48.3	—	0.5	10.5	22.1	179.7	1.7
R-SM3	5124	6.0	12.5	94.5	69.7	—	0.6	8.1	23.2	335.2	4.3
R-SM3	5146	6.0	16.5	69.8	50.7	—	0.3	6.3	24.1	183.9	1.3

Star-3400), following the headspace technique described in Cole et al. (1994). The concentration of each carbon form was calculated based on carbonate thermodynamic equilibrium as a function of pH (Stumm and Morgan 1996). The isotopic signature of DIC was also determined for 20 stations by collecting water samples in glass bottles from 1-m depths, preserving the samples with HgCl_2 , sealing the bottles, and keeping them at 4°C until analysis. A summary of the main physical, chemical, and biological characteristics used in this study are presented in Table 1.

Methods to determine algal $\delta^{13}\text{C}$ —Method 1: Carbon algal signature as particulate organic carbon ($\delta^{13}\text{C}_{\text{alga}(1)}$). Particulate organic matter (POM) was collected on precombusted glass fiber filters (GF/C-Whatman), by filtering 0.5 to 1 L water sampled as described above. Filters were stored frozen in liquid nitrogen and dried at 45°C before analysis for carbon and nitrogen concentrations and carbon stable isotopes, which was performed on a GV Instruments Isoprime mass spectrometer coupled with a Carlo Erba Elemental Analyser (NA 1500 series 2).

Method 2: Carbon algal signature based on particulate organic matter and algal proportion ($\delta^{13}\text{C}_{\text{alga}(2)}$). A variation of the first method involved considering POM as a mixture of algae and detrital material in the calculation of the phytoplankton carbon signature. The following mixing model was used:

$$\delta^{13}\text{C}_{\text{POM}} = x \cdot (\delta^{13}\text{C}_{\text{alga}(2)}) + (1 - x) \cdot (\delta^{13}\text{C}_{\text{terr}}) \quad (1)$$

and modified to determine the phytoplankton signature as follows:

$$\delta^{13}\text{C}_{\text{alga}(2)} = [\delta^{13}\text{C}_{\text{POM}} - (1 - x) \cdot (\delta^{13}\text{C}_{\text{terr}})]/x \quad (2)$$

where x represents the proportion of algal carbon in the particulate organic matter pool, calculated from the ratio between phytoplankton biomass and POC concentration, which were both expressed in $\mu\text{gC} \cdot \text{L}^{-1}$. The ratio of organic carbon to chl. a was derived from the same mixing model, using algal signatures obtained from isolated phytoplankton samples ($\delta^{13}\text{C}_{\text{alga}(5)}$, methods described below):

$$\text{C:chl. } a = [(\delta^{13}\text{C}_{\text{POM}} - \delta^{13}\text{C}_{\text{terr}}) \cdot (\text{POC})] \cdot [(\delta^{13}\text{C}_{\text{alga}(5)} - \delta^{13}\text{C}_{\text{terr}}) \cdot (\text{chl. } a)]^{-1} \quad (3)$$

The terrestrial signature was determined based on the relationship between $\delta^{13}\text{C}_{\text{POM}}$ and chl. a , assuming that POC contains only terrestrial carbon when the chl. a concentration is zero.

Method 3: Carbon algal signature based on $\delta^{13}\text{C}$ of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{alga}(3)}$). DIC stable isotope compositions were determined using a TIC-TOC analyzer (1010 O-I-Analytical) connected to a Finnigan Mat Delta Plus Mass Spectrometer, following the methods described in St Jean (2003). The phytoplankton signature was considered a function of the carbon dioxide signature and the photosynthetic fractionation parameter epsilon (ϵ_p):

$$\delta^{13}\text{C}_{\text{alga}(3)} = \delta^{13}\text{C}_{\text{CO}_2(\text{aq})} - \epsilon_p \quad (4)$$

In this mixing model, carbon dioxide was assumed to be the main form of DIC incorporated during photosynthesis, and the

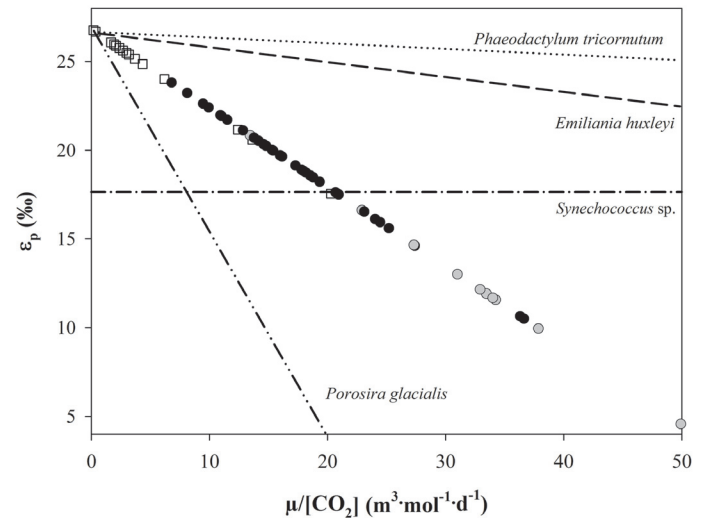


Fig. 1. Phytoplankton fractionation (ϵ_p , ‰), as a function of the ratio between phytoplankton growth rate and CO_2 concentration ($\mu/[\text{CO}_2]$, $\text{m}^3 \cdot \text{mol}^{-1} \cdot \text{d}^{-1}$), for reservoir stations (black circles) and lakes (gray circles), as modified after Karlsson et al. (2003) (open squares). Dashed lines indicate regressions obtained for the marine species described in Popp et al (1998).

signature of this carbon form was calculated according to Mook et al. (1974). Phytoplankton fractionation (ϵ_p) was calculated from the relationship between ϵ_p and phytoplankton growth rate divided by carbon dioxide concentration ($\mu/[\text{CO}_2]$), as described by Laws et al. (1995) and Popp et al. (1998). Phytoplankton growth rate (μ) was estimated as the ratio between algal biomass and primary production, the samples for which were collected in each ecosystem at the same time as those collected for SIA (see data in Planas et al. 2005). The maximum ϵ_p was set at -26.8‰ (Goericke et al. 1994) and the minimum ϵ_p was determined according to Karlsson et al. (2003), assuming zooplankton $\delta^{13}\text{C}$ signatures were autochthonous signatures for the highest $\mu/[\text{CO}_2]$ ratio (Lake Km. 380, $\mu/[\text{CO}_2] = 50 \text{ m}^3 \cdot \text{mol}^{-1} \cdot \text{d}^{-1}$ and calculated $\epsilon_p = 4.1\text{‰}$; Fig. 1).

Method 4: Carbon algal signature as primary consumer signature ($\delta^{13}\text{C}_{\text{alga}(4)}$). In this study, *Daphnia* sp. was considered as the primary consumer organism. It was isolated manually from the bulk zooplankton sample under a stereoscope and preserved using the same method as for the POC samples. Carbon SIA was conducted according to methods developed for small-sized samples (Limén and Marty 2004).

Method 5: Carbon algal signature based on isolated phytoplankton samples ($\delta^{13}\text{C}_{\text{alga}(5)}$). Sufficient algal material for running SIA was collected from a limited number of stations (2 lakes and 5 reservoir stations). Nonalgal organisms visible under a stereoscope were manually removed from the phytoplankton samples. Organisms were then concentrated onto a Nitex filter of $28\text{-}\mu\text{m}$ mesh size and stored in cryotubes in liquid nitrogen. The samples were centrifuged (1 min, $10\,000 \text{ g}$) to further separate the algal fraction from other organic particles. The

top, green fraction of the samples was then isolated, observed under a stereoscope to remove nonalgal material, and processed for SIA following the same protocol as for zooplankton. Additional microscopic observations revealed that sampled phytoplankton consisted mainly of large diatoms such as *Tabellaria* sp.

All particulate (POM, isolated phytoplankton, and zooplankton) SIA were performed in triplicate at the GÉOTOP-UQAM laboratory. It was not necessary to acidify the samples before combustion due to the relatively low concentration of inorganic carbonates in the circumneutral waters of the Canadian Shield. One sample per station was analyzed for the determination of $\delta^{13}\text{C}_{\text{DIC}}$ at G.G. Hatch Isotopes Laboratory (University of Ottawa, Canada). Results are given using the standard delta (δ) notation with $\delta = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \cdot 1000$, expressed in per mil (‰), where $R = {}^{13}\text{C}/{}^{12}\text{C}$ (Verardo et al. 1990). A secondary standard (leucine), known to relate to the international standard of Pee Dee Belemnite, was used as a reference material. The stable isotope measurements were precise to 0.08‰, on average.

Assessment

Determination of $\delta^{13}\text{C}_{\text{terr}}$, C:chl. *a* ratio, and ϵ_p —The isotopic signature of terrestrial organic carbon ($\delta^{13}\text{C}_{\text{terr}}$) was determined, using ANCOVA on a larger data set (Marty 2007), as the intercept of the relationship between $\delta^{13}\text{C}_{\text{POM}}$ and chl. *a*, assuming that POC is 100% terrestrial when chl. *a* reaches zero. Results of the ANCOVA model ($n = 65$; $r^2 = 0.65$; $df = 3, 61$), indicated that chl. *a* ($F = 108.2$; $df = 1$; $P < 0.0001$) and regions ($n = 65$; $F = 19.8$; $df = 2$; $P < 0.0001$) both had highly significant effects on predicted values of $\delta^{13}\text{C}_{\text{POM}}$. The interaction between chl. *a* and regions was not significant ($n = 65$; $F = 0.7$; $df = 2$; $P = 0.5$), indicating that the slope of the relationship was the same for all regions. Therefore, $\delta^{13}\text{C}_{\text{POM}}$ values of a given region could be predicted as:

$$\delta^{13}\text{C}_{\text{POM}} = \begin{pmatrix} I_{\text{JB}} = 0.97 (\pm 0.15) \\ -28.01 (\pm 0.20) + I_{\text{SM}} = -0.26 (\pm 0.15) \\ I_{\text{MAN}} = -0.70 (\pm 0.17) \end{pmatrix} - 1.45 (\pm 0.14) \cdot \text{chl. } a \quad (5)$$

where I is the intercept correction (\pm SE) specific to each region [James Bay (JB), Ste-Marguerite (SM), and Manicouagan (MAN)].

The C:chl. *a* ratio, calculated for stations where algal samples were collected, ranged from 37 to 103. The mean value (80) was applied to calculate the proportion of algal carbon in POC at each station. Based on this value, algal carbon exceeded POC concentration at four sites and, in these cases, POC was considered as 100% algal. Algal carbon represented, on average, 51% of POC, and ranged from 10% to 100%.

Phytoplankton fractionation (ϵ_p), obtained as a function of $\mu/[\text{CO}_2]$, was in the range of values observed for marine species (Popp et al. 1998) and followed the same line as values calculated for other lakes (Karlsson et al. 2003) (Fig. 1). It was generally lower in lakes than at reservoir stations (means 13.7‰ and

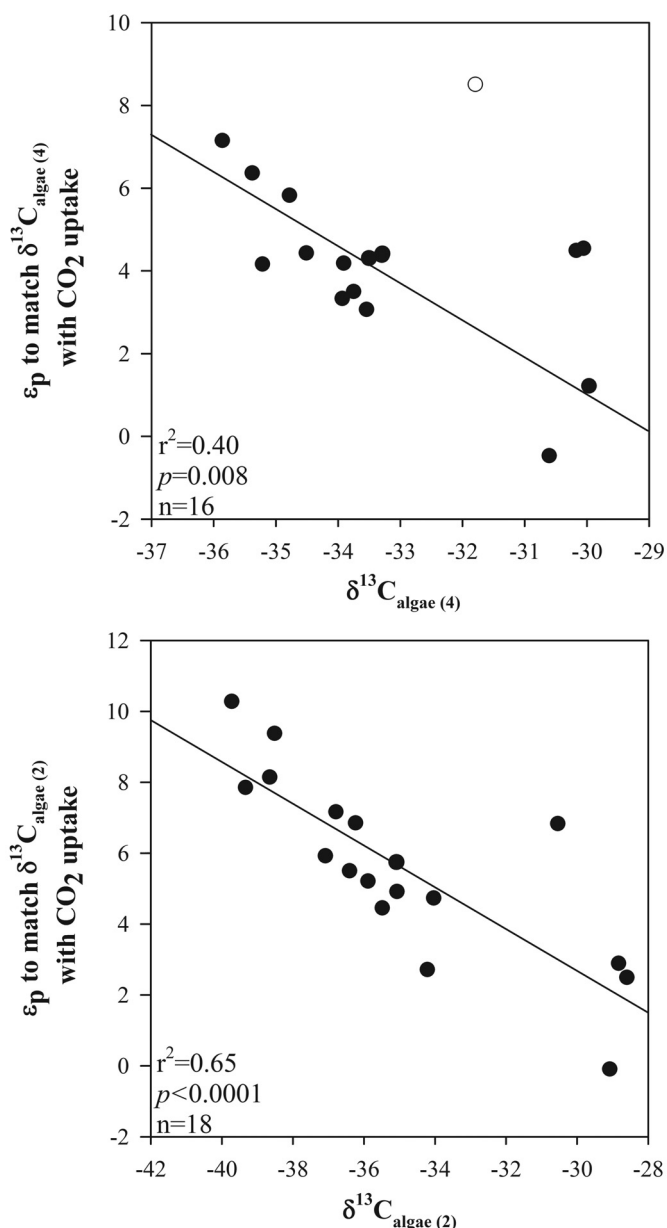


Fig. 2. Fractionation values (ϵ_p , ‰) calculated to match $\delta^{13}\text{C}_{\text{algae}(4)}$ signatures and $\delta^{13}\text{C}_{\text{algae}(2)}$, assuming CO_2 as the only source of carbon. One outlier (open circle) was excluded from analysis.

18.9‰, respectively) as a result of both higher CO_2 concentrations and lower growth rates in reservoirs (Table 1). Eq. 4 was also used to calculate the fractionation value corresponding to $\delta^{13}\text{C}_{\text{algae}(2)}$ and $\delta^{13}\text{C}_{\text{algae}(4)}$, assuming that CO_2 was the only carbon form assimilated by algae in these systems in which CO_2 is abundantly available (Fig. 2). Based on these calculations, estimates of ϵ_p ranged from almost 0‰ to 10‰ and were lower than those obtained with the $\mu/[\text{CO}_2]$ approach (mean ϵ_p 4.3‰ and 5.6‰ for $\delta^{13}\text{C}_{\text{algae}(2)}$ and $\delta^{13}\text{C}_{\text{algae}(4)}$, respectively).

Algal $\delta^{13}\text{C}$ for each approach—Stable isotope data suggested that particulate organic matter comprised both algae and

Table 2. Stable carbon isotopic composition ($\delta^{13}\text{C}$, ‰) of algae, according to the five methods compared in this study.

Site	Station	Method 1 $\delta^{13}\text{C}_{\text{POM}}$	Method 2 $\delta^{13}\text{C}_{\text{algae POM}}$	Method 3 $\delta^{13}\text{C}_{\text{algae DIC}}$	Method 4 $\delta^{13}\text{C}_{\text{Daphnia sp.}}$	Method 5 $\delta^{13}\text{C}_{\text{algae samples}}$
L	Berté	-29.3	-29.5	—	-29.6	—
L	Desaulnier	-29.3	-31.6	—	—	—
L	Duchaunay	-28.5	-28.1	—	-31.1	—
L	Aux Cèdres	-28.0	—	—	-31.2	—
L	Germain	-28.5	-31.2	—	-30.3	—
L	Jean-Marie	-29.4	-32.8	-52.3	-30.6	—
L	Km 12	-28.2	-29.1	-40.6	-30.1	—
L	Km 17	-28.2	-29.6	-49.9	-30.0	-32
L	Km 380	-28.1	-29.5	-30.7	-30.2	—
L	Matonipi	-29.2	-29.6	—	-29.3	—
L	Patukami	-29.6	-32.3	—	—	—
L	Polaris	-28.2	-29.7	-45.0	—	-29.2
L	Yasinsky	-29.1	-30.7	—	—	—
R-LA1	2	-30.1	-34.3	-48.4	-33.5	-33.3
R-LA1	3	-30.8	-35.3	-48.1	-34.5	-33.6
R-LA1	4	-29.9	-33.1	-51.3	-33.3	-32.7
R-LA1	5	-30.2	-33.9	-49.4	-33.5	-33.3
R-LA1	43C (2001)	-29.9	-32.8	—	-32.5	—
R-LA1	43C	-29.5	-32.5	-48.2	-33.3	—
R-LA1	903 (2001)	-30.6	-32.0	—	—	—
R-LA2	1	-30.8	-35.9	-49.0	-35.2	—
R-LA2	2	-30.3	-33.2	-49.9	-33.9	-34.6
R-LA2	3	-30.7	-34.2	-47.7	—	—
R-LA2	4	-29.9	-33.1	-50.7	-33.9	—
R-LA2	5	-30.4	-33.5	-50.9	-33.8	—
R-LG2	1	-29.6	-30.8	—	-30.9	—
R-LG2	406	-28.6	-29.5	—	-32.0	—
R-LG2	39	—	—	—	-30.7	—
R-LG2	509	-29.2	-30.3	—	-30.7	—
R-LG2	18	-32.3	-34.4	—	-33.1	—
R-LG2	336	-28.3	-28.2	—	-30.5	—
R-LG2	604	-29.9	-30.5	—	-31.0	—
R-LG2	610	-29.3	-30.5	—	—	—
R-LG2	615b	-28.4	-32.2	-53.1	-31.2	—
R-LG4	1	-29.3	-34.7	-46.9	-34.8	—
R-LG4	2	-29.9	-33.4	—	-35.1	—
R-LG4	3	-30.5	-36.3	-49.4	-35.4	—
R-LG4	4	-30.5	-35.4	-51.6	-35.9	—
R-LG4	5	-28.5	-30.7	-39.3	-31.8	—
R-MA5	400	-30.1	-30.9	—	-32.5	—
R-MA5	600	-30.3	-31.6	—	-33.8	—
R-MA5	800	-30.0	-30.2	—	-33.3	—
R-MA5	1200	-31.2	-34.5	—	-33.3	—
R-SM3	5057	-34.6	-33.0	—	-38.1	—
R-SM3	5107	-33.1	-34.0	—	-39.6	—
R-SM3	5121	-30.6	-31.4	—	-31.2	—
R-SM3	5124	-34.5	-34.3	—	-38.2	—
R-SM3	5146	-32.3	-35.6	—	-38.4	—

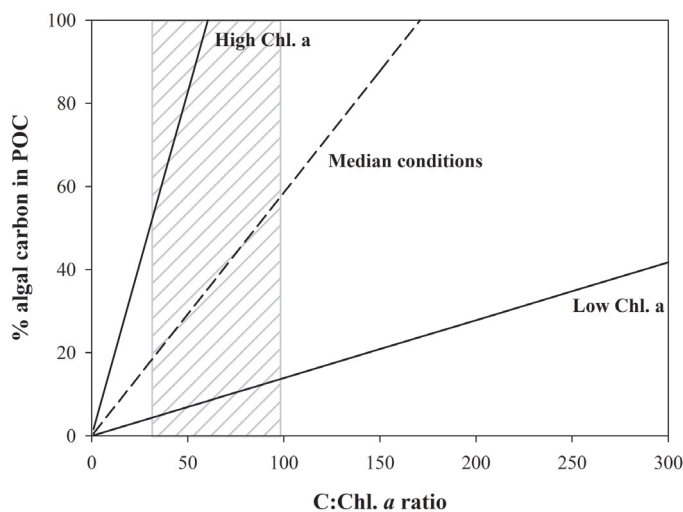


Fig. 3. Percentage of algal carbon in POM based on minimum chl. a (chl. $a = 0.6 \mu\text{g} \cdot \text{L}^{-1}$; POC = $431.2 \mu\text{g} \cdot \text{L}^{-1}$), maximum chl. a (chl. $a = 4.4 \mu\text{g} \cdot \text{L}^{-1}$; POC = $266 \mu\text{g} \cdot \text{L}^{-1}$), and median conditions (chl. $a = 1.6 \mu\text{g} \cdot \text{L}^{-1}$; POC = $273.5 \mu\text{g} \cdot \text{L}^{-1}$) (dashed line), as a function of C:chl. a ratio. Grey area indicates range of C:chl. a ratio calculated from isolated phytoplankton samples.

detritus. The $\delta^{13}\text{C}_{\text{algae}(1)}$ value ranged from -34.6‰ to 28.0‰ (mean -30.0‰) and, after accounting for the proportion of algal carbon in POM, $\delta^{13}\text{C}_{\text{algae}(2)}$ averaged lower than that of bulk POC (mean -32.2‰), ranging from -36.3‰ to -28.0‰ (Table 2). Dissolved inorganic carbon signatures ranged from -35.9‰ to -16.6‰ . Based on CO_2 signatures and fractionation values calculated as a function of $\mu/[\text{CO}_2]$ (Table 1, Fig. 1), the mean $\delta^{13}\text{C}_{\text{algae}(3)}$ was -47.0‰ and varied between -52.3‰ and -30.7‰ . The $\delta^{13}\text{C}_{\text{algae}(4)}$ values of *Daphnia* sp. ranged from -39.6‰ to -29.2‰ (mean -32.9‰), and the isolated phytoplankton samples had $\delta^{13}\text{C}_{\text{algae}(5)}$ values ranging from -34.6‰ to -29.2‰ (mean -32.7‰).

Effect of variation in C:chl. a ratio on proportion of algal carbon in POM—The effect of variations in the C:chl. a ratio on the proportion of algal carbon in POM was examined for maximum, median, and minimum chl. a concentrations (Fig. 3). The C:chl. a ratio was positively correlated with the proportion of algal carbon in POM. Under low chl. a concentrations, the majority of POM was terrestrial in origin, regardless of the C:chl. a ratio. However, increases in chl. a positively influenced the slope of this relationship, underscoring the importance of the C:chl. a ratio in the calculation of the proportion of algal carbon (and, in turn, that of $\delta^{13}\text{C}_{\text{algae}(2)}$). The slope obtained for the median chl. a value was 0.6, indicating that estimates of the algal carbon proportion were generally sensitive to C:chl. a ratios. This led to the examination of the effects that variations in the C:chl. a ratio have on $\delta^{13}\text{C}_{\text{algae}(2)}$, for a range of particulate organic carbon signatures (Fig. 4). When $\delta^{13}\text{C}_{\text{POM}}$ was similar to $\delta^{13}\text{C}_{\text{terr}}$ (i.e., close to -28‰), $\delta^{13}\text{C}_{\text{algae}(2)}$ exhibited little sensitivity to variations in the proportion of algal carbon in POM when the proportions were higher than 20% to 30%. By contrast, $\delta^{13}\text{C}_{\text{algae}(2)}$ was highly sensitive to changes in the algal carbon proportion when POM

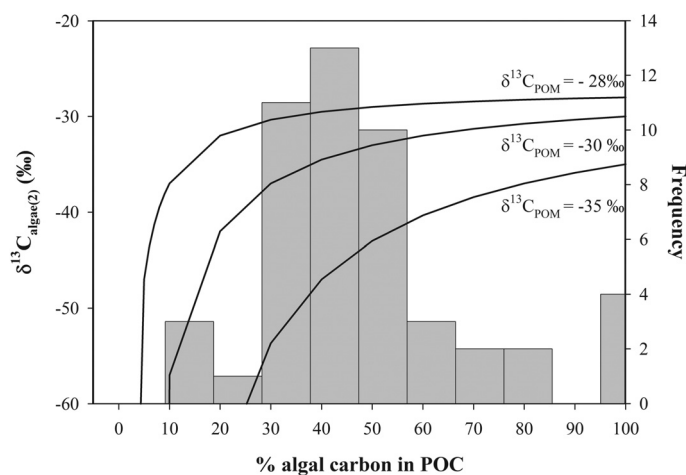


Fig. 4. The relationship between $\delta^{13}\text{C}_{\text{algae}(2)}$ and algal carbon proportion in POM. The left axis is the theoretical $\delta^{13}\text{C}_{\text{algae}(2)}$ as a function of the proportion of algal carbon in particulate organic carbon, for a range of $\delta^{13}\text{C}_{\text{POM}}$ values ($\delta^{13}\text{C}_{\text{terr}}$ was set at -27‰). The right axis is the frequency of the algal carbon proportion in POM (C:chl. $a = 80$).

was mostly terrestrial (algal carbon in POM $< 20\%$). Similar trends were observed for lighter values of $\delta^{13}\text{C}_{\text{POM}}$, with higher sensitivity of algal signatures over a larger range of algal carbon proportions. In short, with about 50% of POC originating from algae (Fig. 4) and mean $\delta^{13}\text{C}_{\text{POM}}$ at -30‰ , $\delta^{13}\text{C}_{\text{algae}(2)}$ signatures resulting from the calculations in this study were strongly influenced by the estimate of the proportion of algal carbon in POM.

$\delta^{13}\text{C}_{\text{algae}(2)}$ and mixing model assumptions—The reliability of mixing models depends primarily on the difference in the isotopic signatures of end members entered into the model. In this study, a mixing model was used to determine $\delta^{13}\text{C}_{\text{algae}(2)}$, based on the assumption that POC is composed of algal carbon associated with chl. a and terrestrial carbon. Nonalgal POC was considered of terrestrial origin in our study since macrophytes were not present in reservoirs and were very scarce in the oligotrophic lakes sampled. Because $\delta^{13}\text{C}_{\text{terr}}$ is rather uniform in the boreal ecoregion, the proportion of algal carbon in POM had more influence on algal signatures and therefore must be accurately determined to obtain correct algal signatures. As the sensitivity analysis showed, $\delta^{13}\text{C}_{\text{algae}(2)}$ varied widely with changes in the algal carbon proportion when POM was dominated by terrestrial organic carbon. Thus, algal signatures must be extremely light to account for a depletion in $\delta^{13}\text{C}_{\text{POM}}$ when terrestrial carbon dominates the bulk POC.

Two main sources of error can influence the proportion of algal carbon. First, chl. a concentration was used as a proxy in the calculation of algal signatures, assuming that algal carbon present in POM contains chl. a . Although chl. a is generally related to the signatures of various organic fractions, such as POC (this study, Gu et al. 1996), zooplankton (Jones et al. 1999, Pulido-Villena et al. 2005), and sediments (Gu et al. 1996), POC can include dead autochthonous carbon that does not contain

chlorophyll, which leads to the underestimation of algal signatures. This bias will, however, particularly affect algal signatures when POC is primarily of terrestrial origin and thus will have little consequence on most of the signatures compared in this study.

In addition to the bias arising from the presence of dead algae, the proportion of algal carbon in POC and, therefore, in the algal signature, is ultimately influenced by the ratio of organic carbon to chl. *a*, which varies greatly (20 to 300) according to season, irradiance, and productivity (Leavitt and Carpenter 1990). Because it was not possible to measure the ratio in situ, a constant value of 80 was assumed for all stations based on calculations from the isolated phytoplankton samples. Additionally, the limited amount of material available for isotopic analyses restricted the ability to measure the chl. *a* content of isolated phytoplankton samples, which would have provided a more direct measurement of C:chl. *a* than calculations from mixing models. Nonetheless, a C:chl. *a* of 80 is a realistic value for oligotrophic ecosystems (Westlake 1980, Leavitt and Carpenter 1990) and, if this was an underestimation, sensitivity analysis shows it would have had only a small effect on algal signatures. The concordance of signatures obtained for algal POC, *Daphnia* sp., and isolated algal samples in this study provides additional evidence that the ratio used was appropriate. However, similar studies should be conducted in more productive ecosystems to accurately determine the C:chl. *a* ratio for these systems, as it will likely be lower than in oligotrophic ones (Leavitt and Carpenter 1990).

Is $\delta^{13}\text{C}_{\text{algal}(5)}$ the best estimate of algal $\delta^{13}\text{C}$?—Ideally, the best estimate of algal signatures would be obtained on pure algal material separated from POM because it represents a direct measurement, independent of all other variables. A few samples of pure algae were successfully collected for this study, and this was possible only because large algal organisms dominated the community at these sites, allowing the use of a simple net tow and separation of nonalgal material from bulk POM. The separation of algae from POM was further simplified in these samples because a single species dominated the algal community. This method is difficult to apply in all systems, however: the collection of smaller algae requires a net of smaller mesh-size, also resulting in the collection of other particles of various sizes that are difficult to separate from the algae. In addition, separation is more complicated as algal communities become more diverse. To address these problems, improved separation techniques and compound-specific analyses are currently being developed to obtain direct measurements of algal community signatures. For example, combining fluorescent activated cell sorting (FACS) and $\delta^{13}\text{C}$ measurements of cellular fatty acids (FAs), Bontes et al. (2006) successfully determined the signature of different phytoplankton groups in a eutrophic lake. Nonetheless, the carbon signatures of specific compounds are often variable for a given algal group (Pel et al. 2003, Finlay 2004, Boschker et al. 2005, Bontes et al. 2006), and the results are currently limited

because of low data availability and poor knowledge of processes influencing the $\delta^{13}\text{C}$ of specific biomarker molecules (Pond et al. 2006). Also, such new techniques require equipment (gas chromatograph) and sample preparation not used for other approaches evaluated in this study, which ultimately translates into higher costs per analysis.

Comparisons between methods—To avoid the transformation of data that was not normally distributed, a nonparametric correlation (Spearman's ρ) was used to test the relationship between each pairing of the approaches for determining $\delta^{13}\text{C}_{\text{algal}}$ (Fig. 5). Also, the slopes and intercepts were compared to the 1:1 line by entering equality line parameters into a custom test. All correlations between pairs of approaches, except pairs involving $\delta^{13}\text{C}_{\text{algal}(3)}$, were significant. In addition, all relationships between approaches differed significantly from the 1:1 line, with the exception of that between $\delta^{13}\text{C}_{\text{algal}(4)}$ and $\delta^{13}\text{C}_{\text{algal}(2)}$ (Fig. 5). The $\delta^{13}\text{C}_{\text{algal}(1)}$ values were enriched compared to the $\delta^{13}\text{C}_{\text{algal}(2)}$ ones, as well as to the carbon signatures of isolated algae and *Daphnia* sp. The relatively depleted carbon signatures of *Daphnia* sp. compared with those of POC illustrate selective zooplankton feeding on isotopically light phytoplankton rather than on terrestrial POM. This applies to ecosystems in which POM is homogeneous over the entire water column, limiting differential feeding behavior according to depth. Previous studies have reported on the importance of methane-oxidizing bacteria as a source of isotopically light carbon (Kankaala et al. 2006), but such a source is unlikely to affect the results of this study, since anoxia was not observed in the water columns at our sites. The tight coupling between *Daphnia* sp. and its algal food source was supported by the strong correlation obtained between $\delta^{13}\text{C}_{\text{algal}(2)}$ and $\delta^{13}\text{C}_{\text{algal}(4)}$ and the similarity of this relationship to the 1:1 line.

Although the number of isolated algal samples was small, and despite the large mesh size of the net (110 μm), algal samples included the most abundant taxa in terms of biomass (Marty, unpublished data) and were thus representative of the phytoplankton community in our ecosystems. ANOVA (Welch) revealed significant differences between the means of the five approaches ($r^2 = 0.79$, $df = 161$, $P < 0.0001$), and a Tukey-Kramer HSD test on each pair showed that the mean values for $\delta^{13}\text{C}_{\text{algal}(2)}$, $\delta^{13}\text{C}_{\text{algal}(4)}$, and $\delta^{13}\text{C}_{\text{algal}(5)}$ were statistically similar (Table 3). The mean $\delta^{13}\text{C}_{\text{algal}(1)}$ was also similar to that of $\delta^{13}\text{C}_{\text{algal}(5)}$, but the mean $\delta^{13}\text{C}_{\text{algal}(3)}$ differed significantly from all others.

Discussion

The determination of algal signatures is essential for interpreting the signatures of higher trophic levels. Several approaches to determining algal signatures are available to aquatic ecologists, but little effort has been made to compare them. In this study, we have found consistent algal $\delta^{13}\text{C}$ values between three methods ($\delta^{13}\text{C}_{\text{algal}(2)}$, $\delta^{13}\text{C}_{\text{algal}(3)}$, and $\delta^{13}\text{C}_{\text{algal}(4)}$).

Algal signatures based on $\delta^{13}\text{C}_{\text{CO}_2}$ were lighter than those of any other approach, and, to our knowledge, no other studies have reported such low signatures. The depletion of $\delta^{13}\text{C}$ in

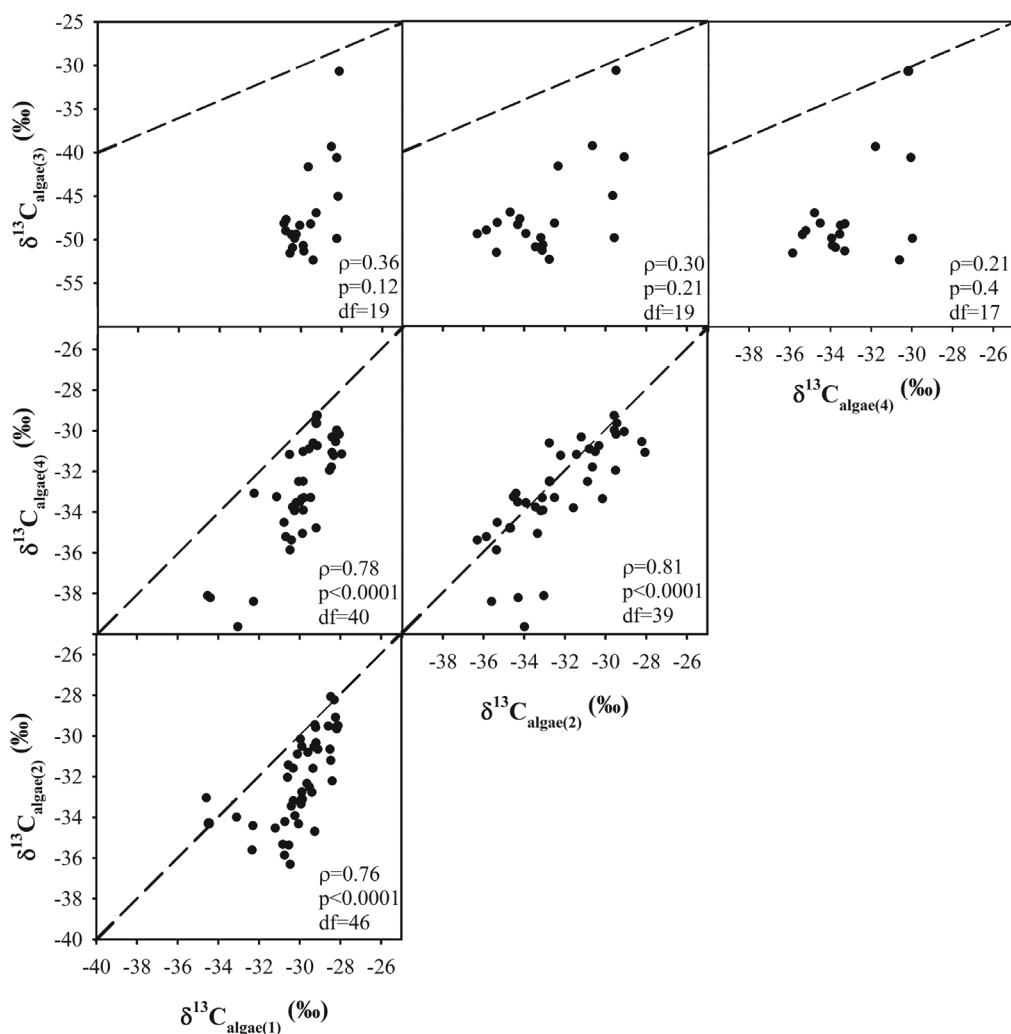


Fig. 5. Correlation matrix between each method used to determine algal $\delta^{13}\text{C}$. Dashed line indicates equal $\delta^{13}\text{C}$ values for the methods compared (1:1 line).

these algal signatures was related to the light signatures obtained for DIC. Although this study was not designed to determine sources of variation in $\delta^{13}\text{C}_{\text{DIC}}$, several processes or sources may be responsible for light DIC signatures in freshwaters. For example, methane oxidation (Prokopenko and Williams 2005), respiration (Bade et al. 2004), and intrusion of DIC inputs from peatlands or spring melt (Striegl et al. 2001) are all likely to influence the carbon dynamics of northern boreal lakes and reservoirs.

The results of this study clearly indicate that the algal carbon signatures calculated based on the signature of carbon dioxide and algal carbon fractionation were not comparable to those determined by the other four approaches. Three potential explanations that could account for such a discrepancy are discussed below.

Snapshot measurement of $\delta^{13}\text{C}_{\text{DIC}}$ for determining algal carbon signatures—Determining $\delta^{13}\text{C}_{\text{DIC}}$ for each site at a discrete depth (surface) and a single point in time may have introduced bias into the calculation if algal carbon fixation occurred under different

DIC conditions than those of surface water. In freshwaters, spatial variation in the isotopic composition of DIC is generally observed in relation to water stratification, with heavier signatures in the mixing layer and progressively lighter signatures as depth increases below the thermocline (Bernasconi et al. 1997, Lehmann et al. 2004). Vertical variations in DIC signatures are unlikely in northern ecosystems in which water stratification is nil or weak due to shallow lake depths and mixing in reservoirs.

Table 3. Mean carbon signature (in ‰) for each approach and approach comparisons based on Tukey-Kramer HSD test.

Variables	<i>n</i>	Mean	SE	HSD test
$\delta^{13}\text{C}_{\text{algae}(1)}$	47	-30.0	0.4	A
$\delta^{13}\text{C}_{\text{algae}(5)}$	7	-32.7	1.0	AB
$\delta^{13}\text{C}_{\text{algae}(4)}$	41	-32.9	0.4	B
$\delta^{13}\text{C}_{\text{algae}(2)}$	46	-32.2	0.4	B
$\delta^{13}\text{C}_{\text{algae}(3)}$	19	-47.0	0.6	C

Variables not connected by same letter are significantly different.

In this study, DIC signatures were similar in both stratified (lakes and LG-4 reservoir) and nonstratified (LA-1 and LA-2 reservoirs) ecosystems, indicating limited vertical variation in $\delta^{13}\text{C}_{\text{DIC}}$. When stratification was observed, the photic zone depth was similar to or deeper than that of the mixing layer; therefore, measurement of the surface water DIC signature was representative of the overall photic zone in which carbon fixation occurred.

Similarly, temporal variation in DIC signatures could be responsible for the discrepancy between DIC and algal $\delta^{13}\text{C}$ if the carbon incorporated into the algae had a different signature than that of the DIC sample. Temporal variations in the isotopic composition of DIC are mostly observed between seasons, due to the influence of inflowing waters in spring (Striegl et al. 2001), rather than over the course of a given season (Lehmann et al. 2004, Bade et al. 2004). Consequently, although the summer DIC data may not be representative of long-term isotopic conditions, they are likely valid for the time period during which the sampled algal biomass was produced.

Prediction of freshwater algal fractionation (ϵ_p)—The fractionation calculation is the most probable source of the discrepancy between estimates of algal signatures. The ϵ_p values were calculated based on the linear relationship between ϵ_p and $\mu/[\text{CO}_2]$, as described by Laws et al. (1997) for a series of cultured marine algae species. The relationships obtained for these four species were characterized by different slopes and, in the case of *Synechococcus* sp., a different intercept (Laws et al. 1995), as a result of variations in cell geometry (Popp et al. 1998) and potential effects of irradiance cycles, light intensity, and nutrient limitation (Burkhardt et al. 1999). Therefore, although the fractionation values determined in this study related to $\mu/[\text{CO}_2]$ in the same manner as in Karlsson (2003) and are in the same range as values obtained for single species (Laws et al. 1995), it is unlikely that a single relationship could be applied to a series of ecosystems characterized by different multispecies assemblages. The unrealistic $\delta^{13}\text{C}_{\text{algae}(3)}$ values obtained in this study confirm that the fractionation approach based on $\mu/[\text{CO}_2]$ cannot be applied outside a taxa-species context. This is supported by the lack of relationship observed between ϵ_p values obtained using $\mu/[\text{CO}_2]$ and ϵ_p values corresponding to algal signatures based on either *Daphnia* sp. or corrected POM (Fig. 2). These findings are similar to those reported in other recent studies on midlatitude lakes and rivers (Bade et al. 2006, Finlay 2004, Pace et al. 2004), which have found that fractionation could be much lower than 20‰, an average value used for many systems (Schindler and Lubetkin 2004). Although experimental time series studies were able to model fractionation (Bade et al. 2006, Pace et al. 2004), the results of this research on northern ecosystems, combined with fractionation values recently reported for other systems, highlight the necessity of exercising extreme caution in using a general fractionation value and of developing a better understanding of fractionation values for freshwater algae.

Source of carbon incorporated during photosynthesis—Another source of discrepancy between algal $\delta^{13}\text{C}$ values based on CO_2 signature and fractionation and those based on the other

methods may be related to the source of carbon fixed during photosynthesis. Quantifying the relative contributions of bicarbonate and CO_2 uptake by algae is difficult because of our limited ability to distinguish between fractionation and carbon source effects. If CO_2 is not the only source of carbon for algae, the algal signature will be based on a mixture of carbon forms, with CO_2 and bicarbonate signatures differing by approximately 10‰ (Mook et al. 1974). Considering the range of pH typically observed in lakes [6 to 9 (Kalf 2002)], bicarbonate may be the main form of carbon within DIC and may also be the primary carbon source for aquatic plants (Talling 1976, Allen and Spence 1981, Maberly and Spence 1983), even in slightly acidic mediums with abundant CO_2 (Findenegg 1976). Although there is little evidence of bicarbonate uptake under abundant CO_2 conditions, it is worth noting that bicarbonate was the most abundant carbon form at a number of sites, since pH ranged from 5.9 to 7.6 (mean 6.5). Furthermore, phytoplankton assemblages were mostly composed of diatoms, which have been reported to favor bicarbonate uptake (Allen and Spence 1981, Tortell et al. 1997, Keller and Morel 1999).

Conclusions and recommendations

Because of problems with current models that estimate phytoplankton fractionation, which also fail to consider the possibility of bicarbonate uptake, the use of inorganic carbon stable isotopes to determine algal $\delta^{13}\text{C}$ produced unrealistic values in this study. If these values were used to interpret data obtained for other compartments of the food web, they would lead to erroneous conclusions. Therefore, we caution against applying general rules of isotopic fractionation to all aquatic ecosystems, since, in the case of basal algal signatures, this would have tremendous consequences for studies of aquatic food webs. The application of algal signatures based on $\delta^{13}\text{C}_{\text{DIC}}$ to calculations determining the relative contributions of allochthonous and autochthonous carbon to organisms will lead to an overestimation of terrestrial inputs incorporated into organisms, which may partly explain the variation found on this topic in the literature. The discrepancy between $\delta^{13}\text{C}_{\text{algae}}$ values generated by the DIC approach and those generated by other methods highlights the need for further studies on carbon isotope fractionation and the form of carbon taken up by natural phytoplankton populations in freshwaters.

Ideally, the best estimate of algal signatures would be obtained using isolated algal material separated from POM. Although simple separation of algae from POM is feasible when large algal organisms and few species are present, such methods will benefit from the development of separation techniques or compound-specific analyses. Alternative approaches that could provide reliable estimates of algal signatures are adjusting the $\delta^{13}\text{C}$ of POC for the proportion of algal carbon in bulk POM and using the $\delta^{13}\text{C}$ of a primary consumer, such as *Daphnia* sp. Because $\delta^{13}\text{C}_{\text{algae}(2)}$ is derived from a mixing model involving several measurements, determining

the carbon signature of a primary consumer is the easiest and least expensive approach to determining algal carbon signatures. However, if a basal signature is required to determine carbon sources for higher trophic levels, then the $\delta^{13}\text{C}_{\text{algal}(2)}$ approach should be employed to avoid circularity that arises from using zooplankton signatures to infer zooplankton carbon sources.

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