

Carbon pathways to zooplankton: insights from the combined use of stable isotope and fatty acid biomarkers

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SUMMARY

1. Numerous studies have quantified the relative contribution of terrestrial- and phytoplankton-derived carbon sources to zooplankton secondary production in lakes. However, few investigated the pathways along which allochthonous and autochthonous carbon (C) was actually conveyed to consumers.
2. We suggest that the combined use of fatty acid and stable isotope biomarkers could solve this issue. We conducted a field study on two oligotrophic lakes, in which primary production increased significantly between 2002 and 2004. We used modelling to estimate the contribution of terrestrial- and phytoplankton-derived C to particulate organic C (POC) and zooplankton production from their $\delta^{13}\text{C}$ values in 2002 and 2004.
3. According to the isotope model, phytoplankton-derived C accounted for a major part of the POC pool in both lakes and supported more *Daphnia* sp. production in 2004 than in 2002. Fatty acid data revealed that increased contribution of algal-C to *Daphnia* production, although common between both lakes, was achieved through C pathways that were different. In one lake, *Daphnia* grazed more intensively on phytoplankton, whereas in the other there was greater grazing on bacteria. In the latter case, the increased primary production resulted in greater release of algal-derived dissolved organic C (DOC), which may have supported extra bacterial and eventually *Daphnia*, production.
4. This is the first study illustrating that the combination of fatty acid and stable isotope biomarkers could further our understanding of the factors controlling the relative magnitude of food webs pathways conveying organic matter to zooplankton.

Keywords: allochthonous carbon, bacteria, $\delta^{13}\text{C}$, phytoplankton, zooplankton feeding behaviour

Introduction

Terrestrial ecosystems export organic matter to freshwater or estuarine ecosystems, where it may contribute to the productivity of aquatic consumers. In lakes, it has been repeatedly demonstrated that zooplankton secondary production is supported by both phytoplankton- and terrestrial-derived carbon (Salonen &

Hammar, 1986; Hessen, Andersen & Lyche, 1989; Carpenter *et al.*, 2005). Zooplankton can graze on a wide range of food sources (i.e. phytoplankton, detritus, bacteria, ciliates and flagellates), depending on species composition of the community and environmental conditions (Burns & Gilbert, 1993; Kamjunke *et al.*, 1999). As a result, phytoplankton- and terrestrial-derived C may be conveyed to zooplankton and hence to higher trophic levels, along several direct and indirect pathways. For instance, zooplankton could obtain allochthonous C by grazing directly on particulate organic C (POC) from terrestrial inputs (Hessen *et al.*, 1989) or indirectly via bacterial or other micro-

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bial heterotrophic production supported by terrestrial dissolved organic C (DOC; Gifford, 1991; Cole *et al.*, 2002). Similarly, zooplankton can acquire autochthonous C by grazing directly on phytoplankton or by consuming bacteria or microbial heterotrophs whose production was itself supported by phytoplankton exudates and detritus (Baines & Pace, 1991).

Evidence for zooplankton relying on allochthonous C has been provided in several earlier studies (Salonen & Hammar, 1986; Hessen *et al.*, 1989), but stable isotope analyses (SIA) have improved the quantification of zooplankton allochthony and have revealed its large variability among systems and seasons (Grey, Jones & Sleep, 2001; Karlsson *et al.*, 2003; Carpenter *et al.*, 2005). However, SIA usually fails to separate the pathways that actually convey autochthonous and allochthonous C to zooplankton and to identify their relative importance among systems (Carpenter *et al.*, 2005). The involvement of the microbial loop in conveying C adds trophic steps to the food chain, thus resulting in higher trophic positioning for zooplankton. However, data on the natural abundance of ^{15}N may not be sufficiently sensitive to determine the trophic position of zooplankton unambiguously (Karlsson *et al.*, 2004) and therefore to detect the role played by the microbial loop. In contrast, fatty acids (FA), as source indicators of zooplankton diets, could further our understanding of such dietary pathways. Lipids in zooplankton are primarily derived from their diet, as very little *de novo* synthesis occurs (Goulden & Place, 1990). In addition, FA of algae [polyunsaturated fatty acids (PUFA); as reviewed by Napolitano (1999)] and of bacteria, [odd and/or branched C chain fatty acids (BAFA); Desvillettes *et al.* (1997)], have been used to infer feeding success and diet selectivity by zooplankton (Arts & Wainmann, 1999). This paper demonstrates that a combination of stable isotope and fatty acid biomarkers is a powerful tool to investigate C pathways to zooplankton in lakes. We studied two oligotrophic lakes, in which primary production increased between 2002 and 2004, resulting in an altered carbon supply to the pelagic food web. We employed SIA to identify whether the C that supported *Daphnia* sp. secondary production was significantly affected by changes in primary production and analysed FA biomarkers to detect whether and how *Daphnia* sp. feeding behaviour was modified between 2002 and 2004.

Methods

Study sites and sampling

The study was conducted between October 2001 and October 2002 and between October 2003 and October 2004. Sooke Reservoir (SOL; 48°33'N, 123°41'W) is a coastal lake on Vancouver Island (British Columbia, Canada) that is oligotrophic, temperate, humic, warm-monomictic and rarely covered with ice. The storage capacity of Sooke reservoir was increased by raising the dam by 6 m in autumn 2002. The elevation of the dam resulted in the flooding of 131 ha of previously clear-cut forest (corresponding to an increase of 78% of the initial lake surface area) that was probably responsible for the higher primary production. Shawnigan Lake (SHL; 48°33'N, 123°38'W) is morphologically similar to the nearby SOL and also oligotrophic. Nutrient concentrations have increased since 2003 and it remains unclear whether this pattern is the result of climate variability or anthropogenic activity.

Integrated epilimnetic water samples were taken every month, at the deepest spot of lake basins at SOL (20 m) and SHL (22 m), using a 5 cm diameter, 10 m length of Tygon tubing with a weight attached at one end. Epilimnetic water samples were taken on the whole epilimnion if it was <10 m deep or otherwise on the top 10 m. The standard limnological parameters [total phosphorous (TP), total organic carbon (TOC) and DOC, chlorophyll *a* (Chl *a*)] were measured [see Davies, Nowlin & Mazumder (2004) for details].

Benthic mussels (*Anodonta kennerlyi* Lea 1860) were collected by scuba diving near the deepest point of both lakes in August 2002 and August 2004 and frozen at -80 °C. Zooplankton was collected once or twice per month in both lakes by vertical hauls on the entire water column using a 64- μm mesh, 50 cm diameter Wisconsin net. Samples were immediately frozen at -80 °C.

Stable isotope

The mantle and foot tissues of mussels were dissected and freeze-dried. For some samples, the foot tissue did not provide enough material for stable isotope analysis (1 mg). However, both mantle and foot tissue $\delta^{13}\text{C}$ could be measured on 19 individuals. No significant difference was found between foot and mantle tissue $\delta^{13}\text{C}$ values (paired *t*-test, $P = 0.46$, $n = 19$).

Zooplankton samples were thawed and *Daphnia* spp. were separated manually (*D. rosea* in SOL and *D. pulex* in SHL). Samples were then freeze-dried, and subsequently weighed (1 mg) into tin capsules. Samples were analysed for $\delta^{13}\text{C}$, percent C and N on a Finnigan Delta Plus Advantage stable isotope ratio mass spectrometer. The analytical errors (reported as 1 SD) associated with $\delta^{13}\text{C}$ measures in *Daphnia* sp. and mussel mantle were 0.16‰ (calculated on 17 pairs of replicates) and 0.08‰ (calculated on seven pairs of replicates), respectively.

Body composition, assessed from C : N ratios, can affect zooplankton $\delta^{13}\text{C}$ in these study lakes (Matthews & Mazumder, 2005). Hence, we normalised the $\delta^{13}\text{C}$ values of zooplankton to a zero lipid content by calculating lipid extracted $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{LE}}$) values using a stoichiometric model elaborated by Matthews (2006). This model uses zooplankton C : N ratios and an empirical average difference between the $\delta^{13}\text{C}$ in lipids and carbohydrates/proteins (i.e. lipid-extracted tissue; $\Delta_{\text{C-L}}$). The model parameters were measured from an annual study at SOL in 2002 for *Daphnia* sp. We used $\Delta_{\text{C-L Daphnia}} = 5.1$ (SE = 0.33, $n = 14$). All the $\delta^{13}\text{C}$ values for zooplankton were reported as lipid-normalised values. Therefore, patterns of variation in *Daphnia* sp. $\delta^{13}\text{C}$ between 2002 and 2004 were independent of changes in body composition.

Fatty acid analysis

For the 2001–2002 period, zooplankton was collected in October 2001 and February, June and September 2002 and size-fractionated using Nitex mesh sizes (200- and 500- μm ; see Kainz, Arts & Mazumder (2004). Counts performed on the 500 μm size-fractions for each sampling date revealed that *Daphnia* sp. accounted for $\geq 75\%$ of individuals, while *Holopedium gibberum* Zaddach 1855 accounted for the rest of individuals. We thus used the 500 μm -size fraction as a proxy for *Daphnia* sp. FA composition.

For the 2003–2004 period, FA concentrations were measured in October 2003 and January, July and October 2004. On these occasions, approximately 500 *Daphnia* individuals were picked up from the sample that was briefly thawed, to minimise possible lipolytic degradation. Samples were then rinsed with filtered (0.45 μm) lake water to remove adhered matter. All samples were kept frozen at -80°C until freeze-

drying and then stored again at -80°C until FA analysis.

Fatty acids from freeze-dried zooplankton samples (4–7 mg) were analysed as described by Kainz *et al.* (2004). Briefly, the lipids were extracted using a 4 : 2 : 1 chloroform : methanol : water mixture. Fatty acids, measured as methyl esters, were prepared by trans-esterifying ($\text{BF}_3\text{-CH}_3\text{OH}$ at 85°C for 1 h) the lipid extract and subsequently analysed by gas chromatography (GC; Varian CP-3800, Varian, Inc., Palo Alto, CA, U.S.A.), using a Supelco 2560 Capillary Column (100 m, 0.25 mm inner diameter and 0.2 μm film thickness) and measured by a flame ionisation detector. Fatty acid methyl esters were identified by comparison of their retention times with known standards (37-component FAME mix, Supelco 47885-U) and quantified with reference calibration curves derived from 2.5, 50, 100, 250, 500, 1000 and 2000 $\text{ng } \mu\text{L}^{-1}$ solutions of the fatty acid methyl esters standard.

Essential FA (EFA; i.e. the sum of linoleic, LIN; α -linolenic, ALA; arachidonic, ARA; eicosapentaenoic, EPA and docosahexaenoic, DHA, acids) were used as algal-derived FA compounds, whereas the odd-saturated and branched-chain FA (i.e. the sum of C15:0 and C17:0 and their iso- and anteiso-series) as bacterial-derived FA (BAFA) compounds (Sun, Shi & Lee, 2000; Kainz & Mazumder, 2005). In addition to the absolute concentrations, the BAFA/EFA ratio was used to indicate zooplankton grazing on bacteria versus phytoplankton (Kainz & Mazumder, 2005).

Assessment of autochthonous C contribution to POC and zooplankton production

Carbon stable isotope signatures of POC and *Daphnia* may have been modified between 2002 and 2004 as a result of changes in phytoplankton-derived C contribution and/or changes in phytoplankton $\delta^{13}\text{C}$ values. For instance, an increase in terrestrial-derived C contribution to *Daphnia* production between 2002 and 2004 would result in a higher $\delta^{13}\text{C}$ value for *Daphnia* in 2004. However, a greater contribution from phytoplankton that became ^{13}C -enriched because of changes in primary production between 2002 and 2004 would generate the same pattern of isotope results. Hence, the interpretation of stable isotope results required modelling.

Assessment of autochthonous C contribution to POC. Total POC pool concentration was calculated as the difference between TOC and DOC concentrations monitored in the lakes (annual averages).

$$\text{POC} = \text{TOC} - \text{DOC} \quad (1)$$

If we assume that the concentration of phytoplankton POC ($\text{POC}_{\text{phyto}}$) is the product of the Chl *a* concentration and a constant parameter of C : Chl *a*, then the contribution of phytoplankton POC to the total POC pool ($\alpha_{\text{phyto/POC}}$) can be assessed as follows:

$$\alpha_{\text{phyto/POC}} = \frac{\text{POC}_{\text{phyto}}}{\text{POC}} = \frac{\text{Chl}a \times (\text{C} : \text{Chl}a)}{\text{POC}} \quad (2)$$

Assessment of phytoplankton $\delta^{13}\text{C}$ values. Particulate organic carbon is a mixture of C from phytoplankton, microbial heterotrophs and terrestrial POC. The carbon stable isotope value of POC thus depends on the relative biomass proportion of each end-member and on their $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{phyto}}$, $\delta^{13}\text{C}_{\text{bact}}$ and $\delta^{13}\text{C}_{\text{terr}}$). If $\alpha_{\text{phyto/POC}}$ and $\alpha_{\text{bact/POC}}$ are the contribution of phytoplankton ($\text{POC}_{\text{phyto}}$) and bacterial C (POC_{bact}), respectively, to the total POC pool, then

$$\begin{aligned} \delta^{13}\text{C}_{\text{POC}} &= \alpha_{\text{phyto/POC}} \times \delta^{13}\text{C}_{\text{phyto}} \\ &+ \alpha_{\text{bact/POC}} \times \delta^{13}\text{C}_{\text{bact}} \\ &+ [1 - (\alpha_{\text{phyto/POC}} + \alpha_{\text{bact/POC}})] \times \delta^{13}\text{C}_{\text{terr}} \quad (3) \end{aligned}$$

with

$$\alpha_{\text{bact/POC}} = \frac{\text{POC}_{\text{bact}}}{\text{POC}} \quad (4)$$

Bacterial and phytoplankton biomasses are usually well related (Simon, Cho & Azam, 1992). If γ is the ratio of bacterial to phytoplankton C biomass, then:

$$\text{POC}_{\text{bact}} = \gamma \times \text{POC}_{\text{phyto}} \quad \text{and} \quad \alpha_{\text{bact/POC}} = \gamma \times \alpha_{\text{phyto/POC}} \quad (5)$$

The long-term average of pelagic POC $\delta^{13}\text{C}$ value (August 2001 to August 2002) at SOL was -30.2‰ (SD = 0.9; Matthews & Mazumder, 2005) and not significantly different from the $\delta^{13}\text{C}$ values of mussels collected in August 2002 (*t*-test assuming unequal variances, $P = 0.33$). Mussel $\delta^{13}\text{C}$ values were then used as a proxy for $\delta^{13}\text{C}$ of POC (Post, 2002).

The $\delta^{13}\text{C}$ value of the bacterial end-member ($\delta^{13}\text{C}_{\text{bact}}$) was assessed assuming that a proportion β of the bacterial production was supported by phytoplankton-derived C while the remaining was pro-

duced from terrestrial C. We neglected methanotrophic bacterial production as the hypolimnion is never anoxic in either lake.

$$\delta^{13}\text{C}_{\text{bact}} = \beta \times \delta^{13}\text{C}_{\text{phyto}} + (1 - \beta) \times \delta^{13}\text{C}_{\text{terr}} \quad (6)$$

The contribution of autochthonous C ($\alpha_{\text{auto/POC}}$) to the total POC pool is consequently calculated as the sum of the proportion of phytoplankton C and that of bacterial C produced from phytoplankton-derived C.

$$\alpha_{\text{auto/POC}} = \alpha_{\text{phyto/POC}}(1 + \beta\gamma) \quad (7)$$

Thus, according to eqns 3, 5 and 7, $\delta^{13}\text{C}_{\text{phyto}}$ could be assessed as follows

$$\delta^{13}\text{C}_{\text{phyto}} = \frac{\delta^{13}\text{C}_{\text{POC}} - \delta^{13}\text{C}_{\text{terr}}[1 - \alpha_{\text{phyto/POC}} \times (1 + \beta\gamma)]}{\alpha_{\text{phyto/POC}} \times (1 + \beta\gamma)} \quad (8)$$

Assessment of autochthonous C contribution to *Daphnia* sp. secondary production. To assess autochthonous C contribution to *Daphnia* production ($\alpha_{\text{auto/Daphnia}}$), we used a two-source mixing model, with phytoplankton ($\delta^{13}\text{C}_{\text{phyto}}$) and terrestrial C ($\delta^{13}\text{C}_{\text{terr}}$) as end-members. Fractionation of C isotopes during a trophic transition was assumed to be negligible.

$$\alpha_{\text{auto/Daphnia}} = \frac{\delta^{13}\text{C}_{\text{Daphnia}} - \delta^{13}\text{C}_{\text{terr}}}{\delta^{13}\text{C}_{\text{phyto}} - \delta^{13}\text{C}_{\text{terr}}} \quad (9)$$

Terrestrial organic matter $\delta^{13}\text{C}$ values were measured in the SOL catchment ($-27.5\text{‰} \pm 0.8$ SD; $n = 44$; B. Hawkins, University of Victoria, unpubl. data).

Parameters and sensitivity. This model had three unmeasured parameters. The C : Chl *a* ratio was first set at 40 (Kritzberg *et al.*, 2004). The proportion of bacterial C produced from autotrophic C (β) was set at 0.35, which was within the range of values assessed by Kritzberg *et al.* (2004) in oligotrophic humic lakes. The ratio of bacterial to phytoplankton C biomass is greater in oligotrophic than in eutrophic lakes (Simon *et al.*, 1992). However, on the range of productivity of our study lakes, we assumed γ to be constant. It was set at 0.50, which is consistent with the γ value assessed by Biddanda, Ogdahl & Cotner (2001) on a set of oligo-mesotrophic lakes. Sensitivity analyses were then conducted, in order to identify to which of these parameters the model outputs were most sensitive.

Data analysis

To detect potential changes in the limnological variables, FA and stable isotope ratios between the two study periods, average values of the variables were calculated for the 2001–2002 and 2003–2004 periods. All values were weighted on a monthly basis, so that each month eventually counts equally in the average value. Potential disparities in the values of the variables between the two periods were tested using *t*-tests, either assuming or not assuming equal variances, depending on the results of the Levene tests. To compare the values of the BAFA/EFA ratio between the two study periods, we performed nonparametric Mann–Whitney tests, as these variables might not be Gaussian. The level of significance was set at $\alpha = 0.05$ for all tests. Statistical analyses were performed on SPSS (version 10, SPSS Inc., Chicago, IL, U.S.A.).

Results

In both study lakes, TOC, DOC and Chl *a* concentrations increased significantly between 2002 and 2004. Chl *a* concentrations were twice as high at SHL and three times as high at SOL in 2004 than in 2002. Total phosphorus concentration increased significantly only at SOL (Table 1; Fig. 1).

Mussel $\delta^{13}\text{C}$ values remained similar at SHL (*t*-test, $P = 0.19$) but significantly decreased between 2002 and 2004 at SOL (*t*-test, $P = 0.004$). These changes were not related to any modification in the mussel lipid content (*t*-test, $P = 0.141$), assessed from C/N ratios (Mc Connaughey & Mc Roy, 1979), or size (*t*-test, $P = 0.242$). *Daphnia* $\delta^{13}\text{C}$ values remained fairly consistent at SHL (*t*-test, $P = 0.99$) but increased significantly by 1.4‰ at SOL (*t*-test, $P = 0.02$; Fig. 2).

Modifying the value of the C : Chl *a* parameter by 25% generated a 25% change in the estimated contribution of autochthonous C to *Daphnia*. In contrast, modifying the input values of γ (the bacterial to

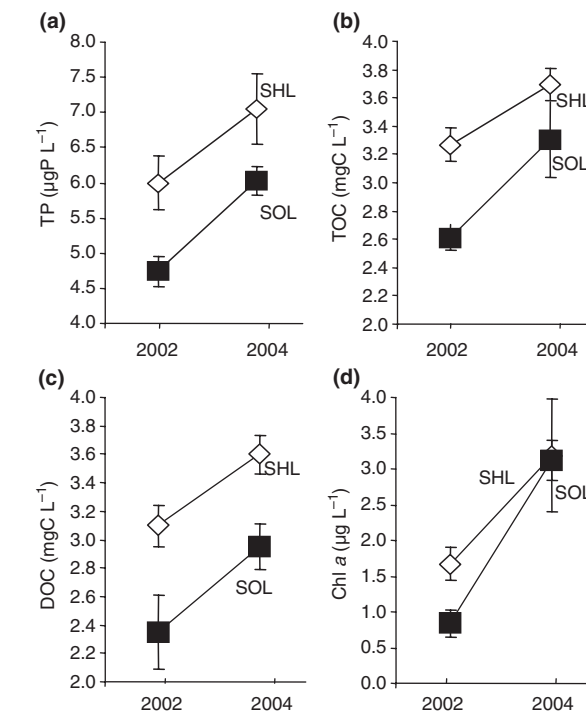


Fig. 1 Average (\pm SE) values of (a) TP, (b) TOC, (c) DOC and (d) Chl *a* concentrations in SHL (open diamonds) and SOL (closed squares) in 2002 and 2004.

phytoplankton C biomass ratio) or β (the proportion of bacterial biomass produced from autochthonous C) parameters by 25% only resulted in a 2–4% change in the output values. The model is consequently essentially sensitive to the value attributed to the C : Chl *a* parameter while only weakly sensitive to the value attributed to the other parameters. The C : Chl *a* ratios in phytoplankton have been reported to range from 25 to 60 in freshwater lakes (Andersson & Rudehall, 1993; Berseneva, Churilova & Georgieva, 2004). We thus ran the model for this range of C : Chl *a* values, keeping β and γ at their initial values (i.e. 0.35 and 0.50, respectively). We constrained the upper boundary of the C : Chl *a* range based on when $\alpha_{\text{auto/Daphnia}} = 100\%$.

Table 1 Average values (\pm SE) of chemical data and *t*-tests for differences between 2002 and 2004 values

	SHL				SOL			
	2002	2004	<i>t</i> -test	<i>P</i> -value	2002	2004	<i>t</i> -test	<i>P</i> -value
TP ($\mu\text{g P L}^{-1}$)	5.9 ± 0.4	7.0 ± 0.5	1.63	0.11	4.5 ± 0.2	5.9 ± 0.2	4.30	<10 ⁻³
TOC (mg C L^{-1})	3.3 ± 0.1	3.7 ± 0.1	2.55	0.02	2.6 ± 0.1	3.3 ± 0.3	2.28	0.03
DOC (mg C L^{-1})	3.0 ± 0.1	3.5 ± 0.1	2.53	0.02	2.3 ± 0.3	2.9 ± 0.2	2.04	0.05
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	1.6 ± 0.2	3.1 ± 0.8	2.17	0.04	0.8 ± 0.2	3.0 ± 0.3	6.90	<10 ⁻³

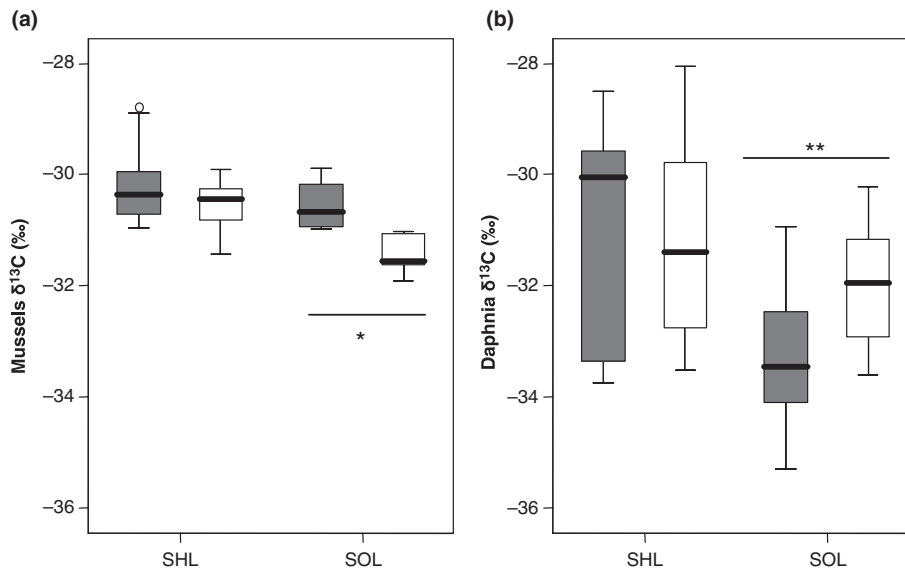


Fig. 2 Boxplots of the $\delta^{13}\text{C}$ values of (a) mussels and (b) *Daphnia* sp. SHL and SOL in 2002 (grey bars) and 2004 (open bars). * and ** indicate differences between 2002 and 2004 $\delta^{13}\text{C}$ values significant at $\alpha = 0.05$ and 10^{-2} .

In SHL, autochthonous C accounted for 21% to 49% of POC in 2002 and 56% to 83% in 2004. In SOL, phytoplankton C contribution to POC ranged between 8% and 20% in 2002 and between 37% and 90% in 2004. Thus, regardless of the C : Chl *a* parameter for both years and lakes, the autotrophic contribution to POC might have increased as a result of the increase in primary production between 2002 and 2004 (Table 2; Fig. 3a,b). The $\delta^{13}\text{C}$ values of phytoplankton in SHL ranged from -41‰ to -33‰ in 2002 and from -33‰ to -31‰ in 2004. In SOL, phytoplankton $\delta^{13}\text{C}$ values were estimated in the range -64‰ to -43‰ in 2002 and -38‰ to -32‰ in 2004. Hence, regardless of the C : Chl *a* parameter for both years and lakes, phytoplankton might have been enriched in ^{13}C in 2004 compared with 2002 (Table 2; Fig. 3c,d). Finally, the contribution of autochthonous C to *Daphnia* in SHL increased from between 28% and 68% in 2002 to between 67% and 100% in 2004. In SOL, the contribution of phytoplankton-derived C to *Daphnia* production also

increased, from 16–38% in 2002 to 42–100% in 2004 (Table 2; Fig. 3e,f).

At SHL, EFA concentrations in *Daphnia* were twice as high as in 2004 than in 2002, suggesting higher grazing on phytoplankton (*t*-test, $P = 0.01$). At SHL, concentrations of BAFA did not change (*t*-test, $P = 0.19$), nor the BAFA/EFA ratio (Mann–Whitney, $P = 0.11$). At SOL, in contrast, EFA concentrations did not change significantly between 2002 and 2004 (*t*-test, $P = 0.46$), implying that *Daphnia* grazing on phytoplankton altered little between the study periods. Moreover, the values of BAFA/EFA in *Daphnia* were significantly higher in 2004 than in 2002 (Mann–Whitney, $P = 0.04$), suggesting that the relative bacteria-to-phytoplankton consumption was more intense in 2004 (Fig. 4).

Discussion

The common discrepancy observed between the $\delta^{13}\text{C}$ values of particulate organic matter (POM) and

	SHL		SOL	
	2002	2004	2002	2004
POC (mg L^{-1})	0.3	0.2	0.3	0.4
$\alpha_{\text{auto/POC}}$	21 → 49%	56 → 83%	8 → 20%	37 → 90%
$\delta^{13}\text{C}_{\text{phyto}}(\text{‰})$	$-41 \rightarrow -33\text{‰}$	$-33 \rightarrow -31\text{‰}$	$-64 \rightarrow -43\text{‰}$	$-38 \rightarrow -32\text{‰}$
$\alpha_{\text{auto/Daphnia}}$	28 → 68%	67 → 100%	16 → 38%	42 → 100%

Table 2 Range of estimates for POC concentration, autochthonous carbon contribution to POC ($\alpha_{\text{auto/POC}}$), phytoplankton $\delta^{13}\text{C}$ and autochthonous carbon contribution to *Daphnia* production ($\alpha_{\text{auto/Daphnia}}$) assessed from the model, ran for 2002 and 2004

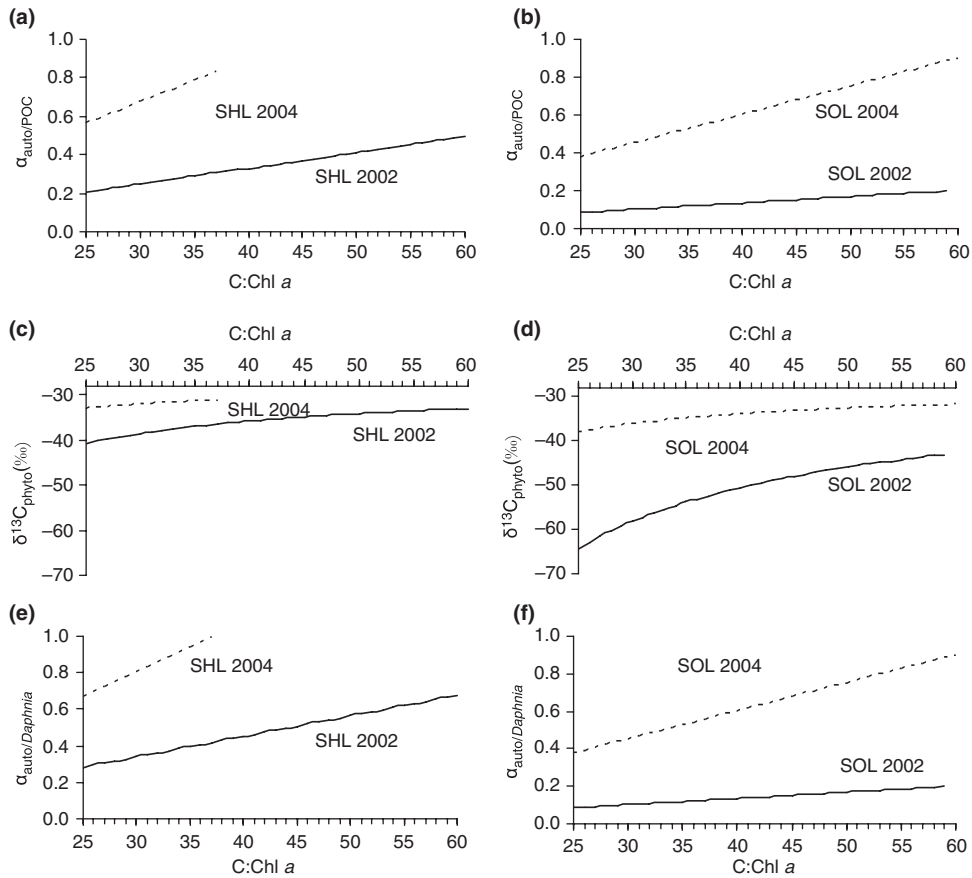


Fig. 3 Outputs of the model in relationships with the value of the C : Chl *a* parameter. Contribution of phytoplankton-derived carbon to POC ($\alpha_{\text{auto/POC}}$) depending on C : Chl *a* in (a) SHL and (b) SOL in 2002 (solid line) and 2004 (dotted line). Phytoplankton $\delta^{13}\text{C}$ depending on C : Chl *a* in (c) SHL and (d) SOL in 2002 (solid line) and 2004 (dotted line). Contribution of phytoplankton-derived carbon to *Daphnia* sp. production ($\alpha_{\text{auto/Daphnia}}$) depending on C : Chl *a* in (e) SHL and (f) SOL in 2002 (solid line) and 2004 (dotted line).

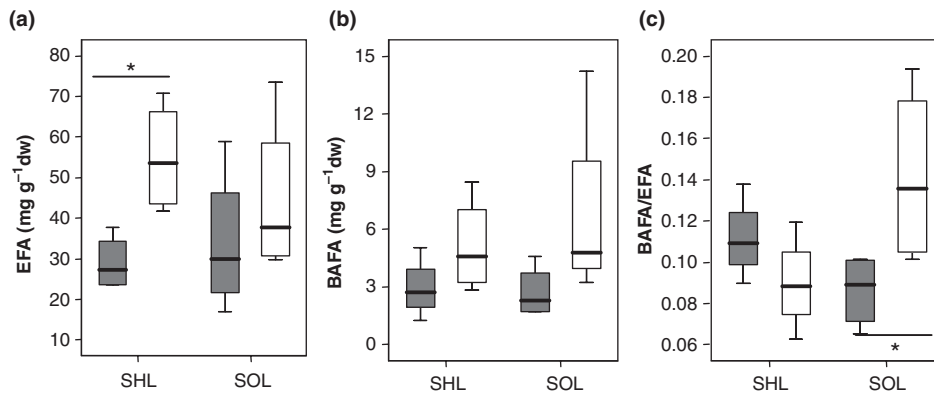


Fig. 4 Boxplots of the (a) essential fatty acids (EFA) concentrations, (b) bacterial fatty acids (BAFA) concentrations and (c) BAFA/EFA ratio in *Daphnia* sp. in 2002 (grey bars) and 2004 (open bars) in the two lakes (SHL and SOL). *Indicate differences between 2002 and 2004 values significant at $\alpha = 0.05$.

zooplankton in lakes is usually attributed to POM dilution by non-phytoplanktonic sources of organic C (Del Giorgio & France, 1996). If zooplankton feed

selectively within POM, then POM $\delta^{13}\text{C}$ is not a reliable baseline for studying sources of C for zooplankton. However, measuring phytoplankton

$\delta^{13}\text{C}$ is challenging as phytoplankton is difficult to separate from the POM pool in lake water. To our knowledge, phytoplankton $\delta^{13}\text{C}$ values have only rarely been measured in stratified lakes (Grey *et al.*, 2001; Vuorio, Meili & Sarvala, 2006). Assessments were obtained essentially using mechanistic models in which phytoplankton $\delta^{13}\text{C}$ was calculated as the difference between the $\delta^{13}\text{C}$ values of CO_2 and a fractionation factor ϵ (Karlsson *et al.*, 2003; Kritzberg *et al.*, 2004). Methods vary in the way they deal with the ϵ parameter. It was estimated either by fitting the model to the data (Pace *et al.*, 2004) or by using experimental models relating ϵ to the algae specific growth rate and CO_2 concentrations in the water (Karlsson *et al.*, 2003; Kritzberg *et al.*, 2004). Although these alternative methods provided conclusive results, a major shortcoming of this approach is that it requires a large dataset, including CO_2 concentrations, DIC $\delta^{13}\text{C}$ and primary productivity measurements. Unfortunately such data were not available for the two study lakes. We thus resorted to a different approach, based on the assessment of relative proportions of bacterial, phytoplankton and terrestrial C to POC. Among the three unmeasured parameters included, the model is essentially sensitive to the C : Chl *a* ratio. The C : Chl *a* ratio is usually set at 40 (Kritzberg *et al.*, 2004). However, marine studies have shown that it varies widely, depending on taxonomic composition, physiological acclimation and growth rates (Geider, MacIntyre & Kana, 1998). Studies of C : Chl *a* variability in freshwaters are scarce. C : Chl *a* ratios in marine systems range from 25 to 170 (Andersson & Rudehall, 1993; Reul *et al.*, 2005). The higher values and the highest seasonal variability occur in tropical and subtropical gyres (Maranon, 2005) as photoacclimation and nutrient limitation are thought to be the main factors in the variability of C : Chl *a* ratios. However C : Chl *a* ratios measured in temperate systems usually range between 25 and 60 (Andersson & Rudehall, 1993; Berseneva *et al.*, 2004), which is the range we adopted here. Estimates for phytoplankton $\delta^{13}\text{C}$ fell close to values reported previously (Grey *et al.*, 2001; Karlsson *et al.*, 2003), except in SOL in 2002, where -60‰ , obtained from the lowest C : Chl *a* values, might be an unrealistically low value. However, C : Chl *a* values are high in ultra-oligotrophic, nutrient-limited marine stations, as a result of low algae growth rates (Riemann, Simonsen & Stensgaard, 1989). Thus, values for the C : Chl *a*

ratio in the ultra-oligotrophic SOL, which displayed Chl *a* concentrations as low as $0.8\ \mu\text{g L}^{-1}$ in 2002, were probably high and closer to 60 than to 25. Assessments of phytoplankton $\delta^{13}\text{C}$ values obtained from C : Chl *a* ratios close to 60 (approximately -43‰) are consistent with the previous results of Karlsson *et al.* (2003), who estimated phytoplankton $\delta^{13}\text{C}$ values as low as -46‰ . Despite the high range of C : Chl *a* used as a model input, the contrast between 2002 and 2004 was still clear so that the model could show that phytoplankton-derived C accounted for a higher part of zooplankton production in 2004 in both lakes. Our approach would clearly benefit from a more accurate knowledge of C : Chl *a* ratios in freshwater ecosystems. However, it requires less information and the input data required are routinely collected in the biomonitoring of lakes.

In both lakes, primary production doubled between 2002 and 2004. As a result, autochthonous C contributed more to POC in 2004. Another consequence of the higher primary production was the higher $\delta^{13}\text{C}$ values for phytoplankton (Gu, Schelske & Brenner, 1996). Considering a C : Chl *a* ratio of 40, this increase in Chl *a* concentrations ($+1.5$ to $2.2\ \mu\text{g L}^{-1}$ at SHL and SOL, respectively) would result in an increase in POC concentrations of approximately 0.06 – $0.09\ \text{mg C L}^{-1}$. This increase would account for $<25\%$ of the eventually observed increase in TOC concentrations (i.e. $0.4\ \text{mg C L}^{-1}$ in each lake), thus suggesting that TOC concentration increased mainly as a result of DOC inputs. Baines & Pace (1991) estimated phytoplankton extracellular release to be 13% of total fixation. Therefore, higher phytoplankton exudates resulting from higher primary production may not account for the main part of the increase in DOC concentration. Thus, in both systems, TOC increased following higher terrestrial inputs of DOC and, consequently, a higher contribution of allochthonous C to the DOC pool. Hence, the nature of the C pool changed in both lakes, with a higher contribution of allochthonous C in DOC and autochthonous C in POC in 2004.

Stable isotope analysis coupled with the model provides evidence that phytoplankton-derived C sources contributed more to zooplankton production when primary production was higher for both lakes. Fatty acid concentrations, however, revealed contrasting patterns between SOL and SHL. In SHL, EFA concentrations in *Daphnia* almost doubled between

2002 and 2004, suggesting a higher consumption and retention of algal-derived FA, thus consistent with the higher phytoplankton-derived C contribution to zooplankton production detected by SIA. However, some heterotroph or mixotroph protists, common in oligotrophic lakes, were shown to be rich in EFA, as a result of trophic upgrading (Klein Breteler *et al.*, 1999). Thus, part of the higher EFA concentration in *Daphnia* in 2004 may have resulted from increased ingestion and/or retention of protist-derived EFA by *Daphnia*. In SOL, the higher reliance of *Daphnia* on autochthonous C came along with increased BAFA/EFA ratios. Considering branched fatty acids as markers for bacterial consumption, these results would imply a higher grazing on the microbial loop, suggesting that a greater part of phytoplankton-derived C was transferred through microbial pathways in 2004 compared with 2002. Recently, however, cyanobacteria have been shown to have a substantial content of branched fatty acids (Rezanka *et al.*, 2003). Higher BAFA/EFA ratios in *Daphnia* could also result from a higher grazing on cyanobacteria in 2004. In SHL, no major changes in phytoplankton community structure occurred between 2002 and 2004. In SOL, in contrast, the proportion of cyanobacteria in phytoplankton biomass increased from 5% (2002) to 15% (2004; data not published). Cyanobacteria are usually considered to be low quality food for *Daphnia* (Gulati & De Mott, 1997). Cyanobacterial carbon is transferred inefficiently to herbivorous zooplankton, which leads to a decoupling of primary and secondary production and the accumulation of cyanobacterial biomass. In addition, in these two lakes, cyanobacteria are only poorly ingested by *Daphnia* as evidenced by recent pigment analysis (M.-E. Perga, unpubl. data). Although higher cyanobacteria ingestion by *Daphnia* cannot be totally excluded, the BAFA/EFA increase in 2004 is more likely to result from a greater grazing of *Daphnia* on the microbial loop.

We attributed the increased DOC concentrations in both study lakes to increased terrestrial inputs. If terrestrial DOC is metabolised by bacteria, it would be mostly respired, which may explain why, even at SOL where *Daphnia* bacterial consumption might have been enhanced, terrestrial-derived C did not contribute more to *Daphnia* production in 2004. Bacterial production is consistently correlated with autochthonous C sources (assessed from Chl *a* concentrations), even in very heterotrophic systems, as a consequence

of higher preference and greater growth efficiency of bacteria on autochthonous DOC (Kritzberg *et al.*, 2005). In 2004, bacterial production of both lakes was probably more highly supported by phytoplankton-derived C than was the case in 2002. Thus, even if *Daphnia* relied more on bacteria in 2004 than in 2002, the contribution of phytoplankton-derived C to *Daphnia* production increased in SOL. Consumption of bacteria by *Daphnia* probably increased as a result of a higher proportion of bacterial particles within the edible fraction of POM.

Although the nutrient increase resulted in similar changes in origins of carbon to *Daphnia* in both lakes, the pathways through which phytoplankton-derived C was transferred to *Daphnia* differed between the two lakes. One reason for these differences could be that the phytoplankton community structure differs between the two lakes. This highlights the need for further investigations concerning factors controlling C pathways in food webs. The quantification of terrestrial subsidies that can support lake food webs is a central issue (Carpenter *et al.*, 2005), especially as current trends in climate changes and land-use are predicted to increase the input of terrestrial organic matter to aquatic systems (Evans, Monteith & Cooper, 2005). However, very few studies have so far provided direct information on organic matter pathways in lakes. In conclusion, this study demonstrates that stable isotopes and fatty acid biomarkers are complementary. The use of SIA alone would have suggested that the contribution of phytoplankton-derived C increased in both cases, but would not have detected such a disparity in the pathways passing phytoplankton-derived C to zooplankton between the two systems. In contrast, FA would have identified differing *Daphnia* feeding behaviour, but not that their ultimate C-sources were the same. Thus, the combined use of these two types of biomarkers furthers our understanding of the factors controlling the relative magnitude of each of the pathways conveying organic matter to zooplankton and hence to organisms higher in the food webs.

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