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Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state

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Abstract Carbon stable isotope analysis was carried out on zooplankton from 24 United Kingdom lakes to examine the hypothesis that zooplankton dependence on allochthonous sources of organic carbon declines with increasing lake trophy. Stable isotope analysis was also carried out on particulate and dissolved organic matter (POM and DOM) and, in 11 of the lakes, of phytoplankton isolates. In 21 of the 24 lakes, the zooplankton were depleted in ^{13}C relative to bulk POM, consistent with previous reports. $\delta^{13}C$ for POM showed relatively little variation between lakes compared to high variation in values for DOM and phytoplankton. $\delta^{13}C$ values for phytoplankton and POM converged with increasing lake trophy, consistent with the expected greater contribution of autochthonous production to the total organic matter pool in eutrophic lakes. The difference between $\delta^{13}C$ for zooplankton and that for POM was also greatest in oligotrophic lakes and reduced in mesotrophic lakes, in accordance with the hypothesis that increasing lake trophic state leads to greater dependence of zooplankton on phytoplankton production. However, the difference increased again in hypertrophic lakes, where higher $\delta^{13}C$ values for POM may have been due to greater inputs of ^{13}C-enriched organic matter from the littoral zone. The very wide variation in phytoplankton $\delta^{13}C$ between lakes of all trophic categories made it difficult to detect robust patterns in the variation in $\delta^{13}C$ for zooplankton.

Key words Carbon stable isotopes · Plankton · Allochthonous organic matter · Lake trophy

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Introduction

In lakes, crustacean zooplankton are low-order consumers and represent an important link between the base of the pelagic food web and those organisms at higher trophic levels which may have economic or conservation value. In recent years the traditional concept of zooplankton grazing predominantly on autochthonous primary production has been challenged. In more productive lakes, zooplankton communities certainly can graze phytoplankton production so efficiently that they are regarded as a biomanipulation tool (e.g. Moss 1992). However, recent studies have revealed zooplankton diets supported by planktonic heterotrophs and detritus via the microbial pathway in oligotrophic and humic lakes in which phytoplankton production is limited (Hessen et al. 1990; Jones 1992). Indeed, in such lakes bacterial respiration frequently outweighs phytoplankton production, indicating a net heterotrophic plankton (del Giorgio and Peters 1994) which must receive an organic carbon subsidy from the littoral zone or the catchment. Unfortunately, the contribution of non-algal sources is difficult to quantify directly and in many cases has had to be inferred (Laybourn-Parry et al. 1994).

The use of stable isotope analyses to investigate sources and pathways of organic matter in pelagic food webs (e.g. Fry and Sherr 1984; Peterson and Fry 1987) potentially can address the unquantified fraction directly, avoiding the need to infer relative contributions. It is a prerequisite of the method that the source end-points are sufficiently distinct and robust to allow discrimination and tracing of the isotope ratios (usually carbon) through the food web. Allochthonous sources of carbon derived from terrestrial vegetation generally exhibit a rather uniform $\delta^{13}C$ of around –26 to –27‰. In contrast, the range of values for phytoplankton carbon isotope ratios reported in the literature is greater than 20‰ (Gu et al. 1994; Yoshioka et al. 1994; Zohary et al. 1994). Phytoplankton isotopic signatures are determined by the source of CO_2 and the degree of fractionation during the uptake of dissolved inorganic carbon (DIC) and subsequent photosyn-

thesis. Thus the end-point may vary not only with specific algal physiology but also considerably between lakes due to differences in basin morphometry and catchment geology. Phytoplankton is difficult to isolate from other similarly sized microplankton and detritus, so particulate organic matter (POM) is routinely used as a surrogate end-point, which may mask the true isotopic signature and cause misinterpretation of relationships further up the food web (e.g. del Giorgio and France 1996). Despite these potential problems, stable isotope analysis of the pelagic foodweb of oligotrophic Loch Ness clearly revealed a heavy dependence on detrital carbon derived from terrestrial vegetation in the catchment (Jones et al. 1998).

Most stable isotope studies have focused on a single water body (Yoshioka et al. 1994; Gu et al. 1997; Jones et al. 1998). Attempts to incorporate a range of study lakes are relatively rare. Until the collation of a large number of literature data sets by del Giorgio and France (1996) and France et al. (1997), there had been little examination of systematic differences between lakes in the stable isotope signatures of plankton, and of the reasons for such differences. Here we report on the use of stable isotope analysis to test the hypothesis of Jones (1992) that the relative importance of allochthonous sources of organic carbon to lake plankton should decrease with increasing lake trophy. We selected examples from suites of lakes in different geographical areas of the United Kingdom exhibiting a range of trophy. Particular attention was paid to obtaining a pure phytoplankton sample for carbon stable isotope analysis from as many of the lakes as possible and thus determining algal contribution to POM, as this has been omitted in many studies. Zooplankton has generally been treated as a single fraction of the plankton in previous isotope studies (del Giorgio and France 1996; France et al. 1997). However, we determined separately the carbon stable isotope signatures of the different species of crustacean zooplankton on the supposition that raptorial feeders may be actively selecting food, whilst filter feeders may be more opportunistic and therefore exhibit distinct isotopic signatures. Moreover, predatory zooplankton are expected to show isotopic enrichment relative to grazing zooplankton. Thus, we also report on patterns between lakes for carbon isotope signature of different zooplankton types.

Methods

Samples were collected from 24 lakes distributed throughout the British Isles (Fig. 1). Lakes were sampled once in early summer (between May and July) and 11 of the lakes were sampled on a second occasion in late summer (during September and October). Six lakes are part of the Norfolk Broads complex, four are from the Shropshire and Cheshire plain, six from the English Lake District, two in Northern Ireland and the remainder lie in Scotland. The lakes selected vary widely in basin morphometry and trophic state (from oligotrophic to hypertrophic) and range from clear water to moderately coloured.

At each lake, vertical profiles for temperature and oxygen concentration were determined using a Yellow Springs Instruments

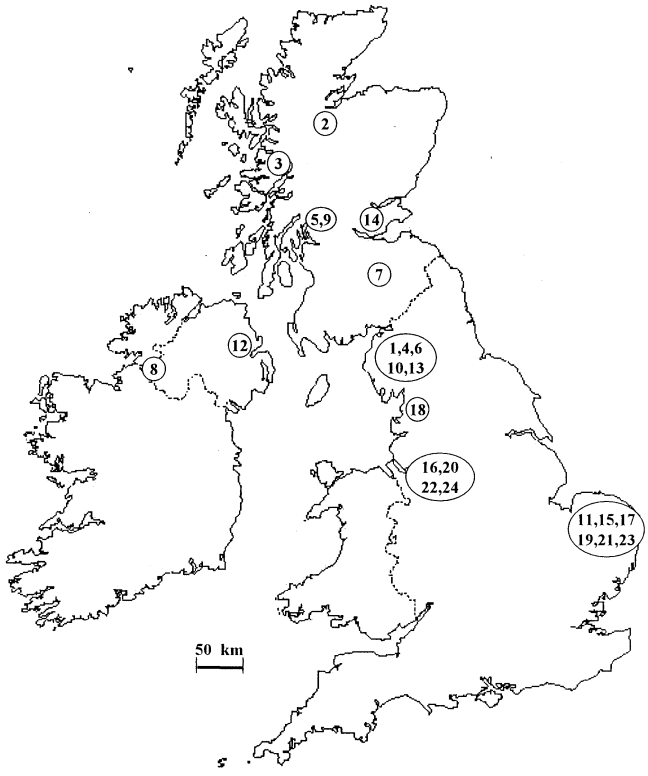

Fig. 1 Map of the British Isles showing location of lakes sampled during the study period. Numbers identify the lakes listed in Table 1

probe, and these were used to gauge the extent of the mixed layer. Integrated water samples for particulate and dissolved organic matter (POM, >0.2 µm and DOM, <0.2 µm) were then collected from the epilimnion of each lake using a 5 l Friedinger sampler. All water samples were stored in acid-washed 10-l aspirators. Aliquots were removed and analysed for total phosphorus (TP) and chlorophyll *a* using standard limnological techniques and preserved with acidified Lugol's iodine for phytoplankton identification. Particulate organic carbon (POC) was determined using a universal carbon analyser with high-temperature combustion (Salonen 1979). POM was concentrated using a Minitan tangential flow ultrafiltration apparatus fitted with multiple 0.2-µm Durapore (polyvinylidene fluoride) filter plates. The concentrated POM was then collected on precombusted Anodisc inorganic filters (13 mm, no polypropylene support ring) and dried at 60°C. The ultrafiltrate was collected, acidified and concentrated for DOM analysis by freeze drying. A small sample of ultrafiltrate was also used for colour determination from absorbance at 440 nm, according to Cuthbert and del Giorgio (1992).

Plankton from each lake was collected by vertical hauls through the epilimnion to the surface with a plankton net of mesh 110 µm and stored without preservation (one vertical haul was preserved with 70% industrial methylated spirit for zooplankton identification and determination of relative abundance). Crustacean zooplankton were later picked from the fresh samples by hand using a fine pipette, with different taxa being separated when numbers permitted. Separated zooplankton were maintained in Whatman glass fibre filter grade F (GF/F)-filtered water for 2 h to allow gut evacuation. Sufficient individuals of each species were then collected on precombusted GF/F filters, rinsed with Milli-Q water and dried at 60°C. In lakes with high phytoplankton concentrations, an attempt was made to separate phytoplankton from other particles, either by repeated sedimentation for diatoms (Jones et al. 1998) or by using the natural buoyancy of cyanobacterial species. Sub-samples of the phytoplankton concen-

trate were then collected on precombusted GF/F filters, rinsed and dried at 60°C.

Analysis for ^{13}C was carried out using a Roboprep-CN continuous flow analyser coupled to a Tracermass single-inlet triple-collector mass spectrometer (both instruments by Europa Scientific). Samples collected on GF/F papers and which remained firmly embedded in the fibres of the filter paper were cut into strips and sample and glass fibre combusted together. In cases where the weight of sample was greater, the sample peeled away from the glass fibre on drying and could be combusted on its own. Where Anodisc filters were used to collect samples, the aluminium oxide was ground to a fine powder between two small watch glasses. Larger samples were ground under liquid nitrogen in a freezer mill (Spex Industries Inc.). Results are given using the δ notation where $\delta=[(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{reference})-1]\times1000$, expressed in per mil units (‰). The reference materials used were secondary standards of known relation to the international standard (Pee Dee belemnite). The analytical precision is generally ±0.1‰. In most cases triplicate samples were prepared and analysed.

Results

Table 1 summarises the measured characteristics of the lakes during the period of sampling. Most lakes were sampled only once during the survey, but a few lakes were sampled twice and for these means of the two values are shown. The lakes were deliberately selected to provide examples from a wide range of trophic state over a large geographical area. When the lakes are ranked by TP and grouped according to the OECD scale of lake trophic state based on TP (e.g. Lampert and Sommer 1997) it can be seen that the lakes span a very wide range of trophic state from oligotrophic through to hypertrophic. Total phosphorus actually ranged from 7 to 780 µg l^{-1} with a high coefficient of variation (CV). Chlorophyll *a* concentration ranged from 0.1 to 49.9 µg l^{-1}, also with a high CV, but was not significantly correlated with TP. However, there was a strong relationship between chlorophyll *a* and POC ($P<0.001$, $r=0.806$) indicating a significant overall contribution from algal biomass to bulk POM. Water colour ranged from 15.9 in Loch Morar to 56.8 mg Pt l^{-1} in Oakmere and exhibited the lowest CV. There was no significant relationship between water colour and any of the other environmental parameters measured.

The $\delta^{13}C$ values of all seston and zooplankton samples from the 24 lakes ranged from –16.4 to –37.4‰ (Table 2). Bulk POM exhibited the smallest range, whilst phytoplankton and zooplankton samples exhibited the greatest ^{13}C-enrichment and ^{13}C-depletion respectively. There was significant ^{13}C-depletion of POM with increasing water colour ($P<0.05$; $r=0.44$), but the percentage variation attributable was low. In many of the lakes $\delta^{13}C$ values for DOM corresponded closely to those of POM (Fig. 2). However, in several lakes the DOM was appreciably ^{13}C-enriched compared to the DOM, and this was particularly characteristic of the hypertrophic lakes as well as some of the more eutrophic lakes. For

Table 1 Environmental characteristics recorded from the 24 study lakes, with the coefficient of variation (*CV%*) for each. Lakes have been ranked using total phosphorus (*TP*) as an indicator of increasing trophic state. Values in parentheses are the TP ranges (µg l^{-1}) used to define the lake trophic states; *indicates lakes for which values given are means from two sampling dates (Chl *a* chlorophyll *a*, *POC* particulate organic matter)

Lake	TP (µg l^{-1})	Chl a (µg l^{-1})	POC (mg C l^{-1})	Colour (mg Pt l^{-1})
Oligotrophic (5–10)				
1. Coniston*	7	0.4	0.75	25.7
2. Loch Ness*	10	0.1	0.3	51.0
3. Loch Morar*	10	0.1	0.22	15.9
Mesotrophic (>10–30)				
4. Ullswater*	14	0.7	0.48	26.9
5. Dubh Lochan	16	1.9	0.54	52.5
6. Bassenthwaite	18	8.7	0.84	41.7
7. Cauldshiels Loch	22	12.5	1.86	34.1
8. Lough Erne	23	6.2	0.78	52.1
9. Loch Lomond	24	2.2	0.36	39.8
10. Blelham Tarn	25	10.0	1.07	39.1
Eutrophic (>30–100)				
11. Upton Broad*	32	43.2	5.2	48.0
12. Lough Neagh	37	49.9	5.15	36.8
13. Esthwaite	44	20.7	1.49	33.8
14. Loch Leven	59	38.0	3.25	27.8
15. Ranworth Broad	80	43.75	2.91	42.2
16. Oak Mere*	94	12.3	2.92	56.8
17. Cromes Broad	95	7.0	1.88	43.0
18. Wyresdale Park Lake*	97	17.3	3.28	54.3
Hypertrophic (>100)				
19. Filby Broad*	146	4.7	1.3	38.0
20. Rostherne Mere	168	8.7	0.54	31.7
21. Ormesby Broad*	184	4.1	1.87	45.5
22. Colemere	282	16.7	1.45	41.3
23. Alderfen Broad*	529	12.0	2.13	46.4
24. White Mere*	780	34.7	1.02	32.5
CV%	157	104	82	26

Table 2 The $\delta^{13}C$ values (‰) of particulate and dissolved organic matter (*POM, DOM*), mixed grazing zooplankton (*GZ*), predatory zooplankton (*PZ*) and phytoplankton from the 24 study lakes, ranked as in Table 1. Values shown are means±1 SD for three replicate samples and, in some cases (*), for two sampling occasions

Lake	POM	DOM	GZ	PZ	Phytoplankton
Oligotrophic (5–10)					
Coniston*	–21.7±0.3	–23.2±0.4	–28.5±0.2	–27.5±0.2	
	–22.1±0.2		–28.3±0.3	–27.7±0.3	
Loch Ness*	–25.1±0.1	–28.1±0.1	–29.0±0.3	–30.0±0.2	–31.9±0.8
	–25.3±0.2	–28.3±0.1	–27.2±0.2	–27.0±0.2	–31.8±0.7
Loch Morar*	–20.1±0.4	–16.2±0.4	–30.5±0.1		
	–19.5±0.5	–16.0±0.3	–27.8±0.3		
Mesotrophic (>10–30)					
Ullswater*	–24.6±0.2	–26.7±0.3	–26.4±0.7	–24.5±0.2	
	–24.4±0.2		–27.6±0.8		
Dubh Lochan	–27.1±0.7	–27.3±0.2	–32.4±0.2		
Bassenthwaite	–26.9±0.1	–30.2±0.3	–32.4±0.4	–29.7±0.3	
Cauldshiels Loch	–24.0±0.9	–16.3±0.2	–19.5±0.5		–16.4±0.2
Lough Erne	–28.8±0.2	–28.1±0.1	–31.3±0.1		
Loch Lomond	–24.0±0.5	–26.1±0.3	–27.9±0.5		–26.5±0.2
Blelham Tarn	–29.7±0.2	–30.6±0.1	–32.3±0.4		
Eutrophic (>30–100)					
Upton Broad*	–21.9±0.3	–9.1±0.4	–34.0±0.6		
	–22.2±0.3	–9.3±0.4	–32.8±0.3		
Lough Neagh	–25.6±0.3	–28.2±0.3	–28.9±0.8		
Esthwaite	–29.7±0.2	–28.6±0.2	–35.7±0.9		–33.6±1.1
Loch Leven	–21.6±0.0	–15.2±0.8	–22.5±0.2		–20.1±0.1
Ranworth Broad	–30.8±0.4	0.9±0.6	–34.0±4.3		–30.1±0.2
Oak Mere*	–25.7±0.2	–24.9±0.3	–24.7±0.4		
	–25.5±0.3		–24.7±0.3		
Cromes Broad	–21.1±0.7	–12.0±0.3	–37.4±2.2		
Wyresdale Park Lake*	–33.1±0.4	–28.4±0.2	–35.1±0.7	–33.3±1.7	–35.2±0.2
	–35.0±1.0	–28.7±0.1	–35.2±0.6	–33.5±1.2	–35.2±0.4
Hypertrophic (>100)					
Filby Broad*	–20.6±0.2	–2.0±0.4	–31.2±0.2		–32.1±0.1
	–20.7±0.2		–31.2±0.1		–32.1±0.2
Rostherne Mere	–21.5±0.7	–2.3±0.5	–25.4±0.4		–18.4±3.1
Ormesby Broad*	–19.6±0.7	–2.3±0.4	–28.6±0.2		–32.2±0.2
	–20.7±0.6		–27.8±0.2		–32.2±0.1
Colemere	–21.8±0.2	–9.1±0.3	–25.3±0.7	–21.6±0.1	
Alderfen Broad*	–26.3±0.8	–15.2±0.1	–23.0±5.8		
	–24.2±1.2	–14.9±0.2	–23.9±4.7		
White Mere*	–20.8±0.4	–7.1±1.2	–27.5±0.4	–25.0±0.2	–21.0±1.2
	–19.9±0.5	–8.0±0.7	–26.7±0.4	–25.5±0.1	–21.8±1.1
Mean	–24.7	–18.2	–29.2	–27.2	–26.2
CV%	15.7	58.1	15.3	14.1	27.7

the 11 lakes from which pure phytoplankton samples could be isolated, $\delta^{13}C$ values ranged between –16.4 and –35.2‰ (Table 2). Phytoplankton $\delta^{13}C$ correlated only weakly with POM $\delta^{13}C$. The phytoplankton isolates generally comprised one or two predominant species in the hypertrophic lakes, such as the flake-like *Aphanizomenon flos-aquae* and globular colonial *Volvox* in Whitemere, or diatom complexes in Loch Ness and Loch Lomond. The relatively more coloured lakes tended to yield phytoplankton samples more depleted in ^{13}C ($P<0.01$; $r=0.74$, Fig. 3). In Wyresdale Park Lake (54 mg Pt l^{-1}), *Aphanizomenon flos-aquae* $\delta^{13}C$ was recorded at –35.2‰, whilst in Rostherne Mere (32 mg Pt l^{-1}) the same species had an isotope signature of –18.4‰.

There was no significant relationship between the $\delta^{13}C$ of bulk POM and that of grazing zooplankton (Fig. 4). Zooplankton were depleted in ^{13}C relative to bulk POM in over 85% of the lakes sampled with a mean isotopic difference (±1 SD) between zooplankton and POM of –4.8±4.6‰ (maximum –16.3‰ in Cromes Broad, Table 2). Only Cauldshiels Loch, Alderfen Broad and Oak Mere contained zooplankton communities enriched in ^{13}C relative to bulk POM (Fig. 4, Table 2). The degree of enrichment ranged between 1 and 4.5‰ in these three lakes. The zooplankton communities of many of the study lakes were dominated by a single grazing or herbivorous species, but in 12 lakes there were different species in sufficient numbers to allow separate analysis of their carbon isotopic content. Despite significant differences between species within the same lake (e.g. Alderfen Broad: *Cyclops viridis* –20.7‰; *Daphnia longispina* –30.1‰; *t*-test, $P<0.01$), there was no consistent trend of depletion in ^{13}C relative to

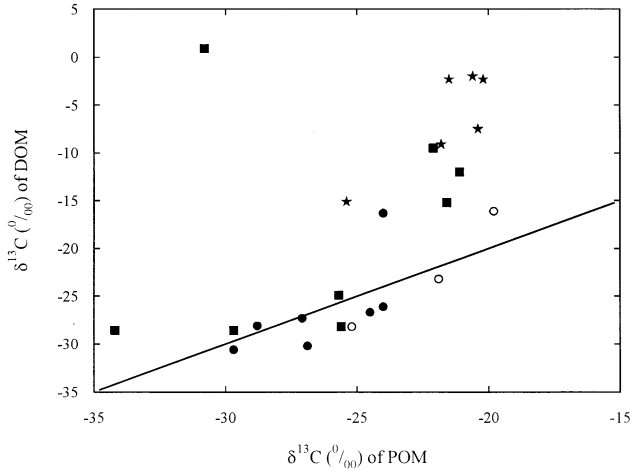

Fig. 2 Carbon stable isotope composition (expressed as δ13C‰) of dissolved organic matter (*DOM*) related to that of particulate organic matter (*POM*) in the study lakes. The *line of equality* is shown. Lake trophic states are indicated as oligotrophic (*open circles*), mesotrophic (*filled circles*), eutrophic (*squares*) and hypertrophic (*stars*) according to Table 1

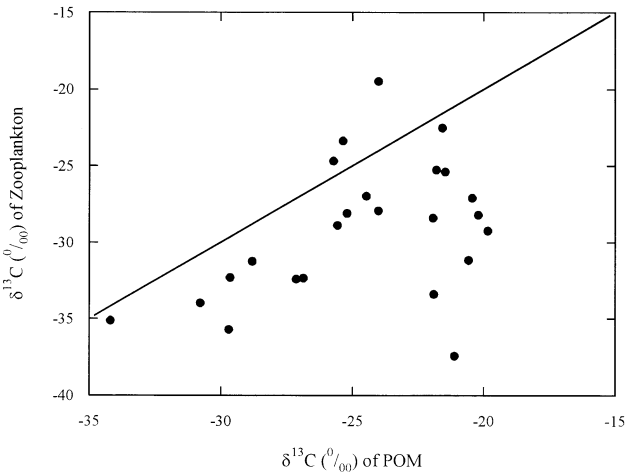

Fig. 4 Relationship between grazing zooplankton carbon stable isotope composition (expressed as δ13C‰) and that of POM in the study lakes. The *line of equality* is shown

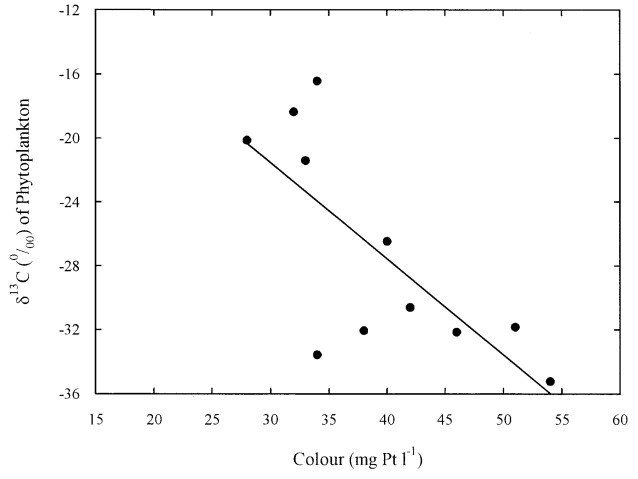

Fig. 3 Phytoplankton carbon stable isotope composition (expressed as δ13C‰) as a function of water colour in the study lakes. The *fitted regression* is $y=-0.6x-3.47$ ($r=0.74$, $P<0.01$)

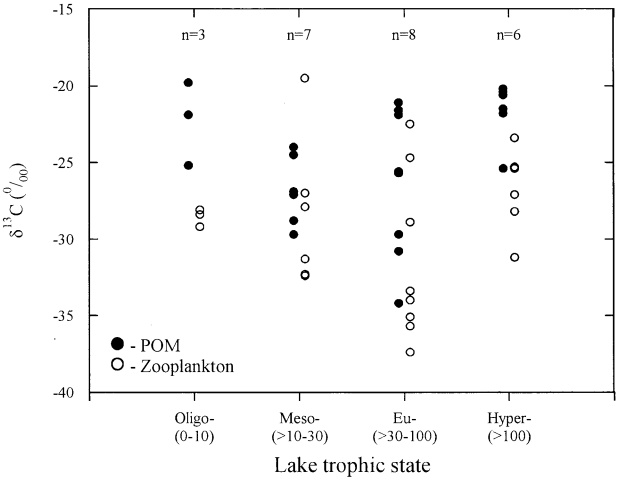

Fig. 5 Grazing zooplankton and POM carbon stable isotope composition (expressed as δ13C‰) grouped by lake trophic state according to OECD classification (*values in parentheses* are total phosphorus (*TP*) ranges in µg l-1)

bulk POM between grazing zooplankton species grouped as cladocerans and copepods. In Alderfen and Ranworth Broads, copepod species were observed to be 13C-enriched relative to POM whilst the cladoceran species present were 13C-depleted. The mean δ13C difference (±1 SD) between copepods and cladocerans in the other ten lakes was 0.9±1.8‰. Seven lakes contained zooplankton communities with at least one abundant, and therefore separable, predatory species, such as *Leptodora kindtii*, *Bythotrephes longimanus* or the larva of the phantom midge *Chaoborus*. For these lakes the relationship between predatory and grazing zooplankton δ13C was very strong ($P<0.005$; $r=0.94$), with six out of seven systems exhibiting predator 13C-enrichment of 1‰ or greater (mean 1.4‰, SD±1.3).

Grazing zooplankton δ13C values were also strongly correlated with those of phytoplankton in the 11 lakes where separation of a pure phytoplankton sample was possible ($P<0.001$; $r=0.87$). However, 6 of the 11 lakes contained zooplankton which were 13C-depleted relative to both bulk POM and phytoplankton and these were generally lakes of higher trophic status. When POM and grazing zooplankton δ13C data were grouped according to TP concentration and hence lake trophic state (Fig. 5), the values tended to converge with increasing TP from oligotrophic to eutrophic. No overlap of POM and zooplankton δ13C values was seen in oligotrophic lakes, whereas considerable overlap was evident in mesotrophic lakes and particularly in eutrophic lakes (Fig. 5). However, divergence recurred when lake tro-

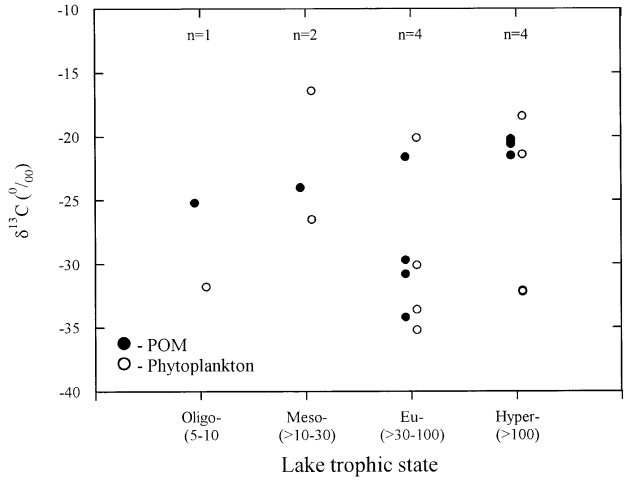

Fig. 6 Phytoplankton and POM carbon stable isotope composition (expressed as $\delta^{13}C‰$) grouped by lake trophic state according to OECD classification (*values in parentheses* are TP ranges in $\mu g\ l^{-1}$)

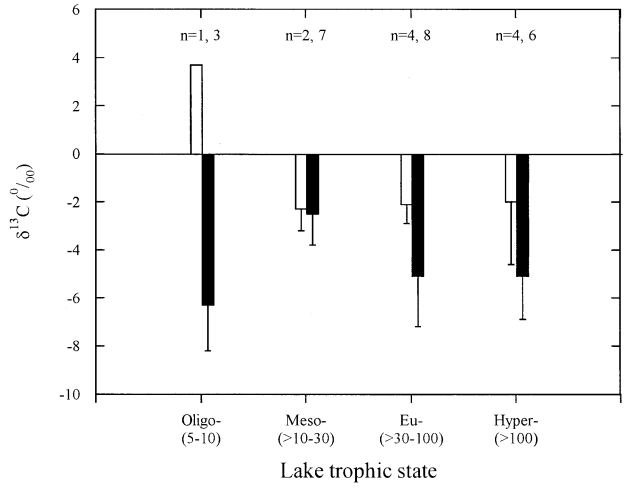

Fig. 7 Mean $\delta^{13}C$ difference (±SE) between grazing zooplankton and phytoplankton (*open bars*) and grazing zooplankton and POM (*filled bars*) grouped by of lake trophic state according to OECD classification (*values in parentheses* are TP ranges in $\mu g\ l^{-1}$)

phic state increased still further to hypertrophic. A similar trend was suggested in the extent of the difference between $\delta^{13}C$ values for POM and phytoplankton (Fig. 6), although the smaller number of samples renders the pattern more tenuous. The average $\delta^{13}C$ differences between grazing zooplankton and POM support this trend (Fig. 7), with the greatest difference in the oligotrophic lakes, reduced difference in the mesotrophic lakes, and greater divergence again evident in eutrophic and hypertrophic lakes. The average $\delta^{13}C$ difference between grazing zooplankton and phytoplankton (Fig. 7) was also greatest in an oligotrophic lake, reduced in mesotrophic lakes, but showed no tendency to increase again with higher trophic state.

Discussion

This study was intended to test the hypothesis that the relative importance of allochthonous sources of organic carbon to lake plankton should decrease with increasing lake trophy (Jones 1992). We focused on crustacean zooplankton since these organisms play a pivotal role in transfer of carbon through lake food webs. In order to obtain results with high generality we sampled lakes from around the United Kingdom encompassing a wide range of area and depth as well as trophic state. Water colour is a good indicator of the loading of allochthonous organic matter to lakes (Jones and Arvola 1984), but our lakes showed a restricted range of colour with low CV (Table 1). Thus the only marked gradient in our selected study lakes was of trophic state. Chlorophyll *a* concentration exhibited high CV, similar to that of POC and with which it correlated strongly, indicating a substantial overall contribution by algal biomass to the total organic carbon within the seston of the 24 lakes. The lack of correlation between chlorophyll *a* and TP (two separate indicators of lake trophic state) was probably an artefact of the single sampling occasions coinciding with algal population "crashes" in some lakes, particularly in the Norfolk Broads. Therefore, although both TP and chlorophyll *a* showed a large range and CV across the 24 lakes, TP was considered a more reliable indicator of trophic state and hence a more suitable parameter against which to investigate zooplankton-POM relationships in more detail.

For this synoptic survey it was only possible to sample most of the 24 lakes on a single occasion. We carried out the sampling in early to mid-summer when zooplankton populations in most northern temperate lakes are most productive and hence their carbon isotope signatures should best reflect their principal food sources. However, we were able to repeat sample 11 of the lakes on a second occasion in late summer. The results obtained from the two sampling occasions (Table 2) showed a very high degree of consistency. Thus we are confident that for this kind of synoptic survey using stable isotope analysis, a single sampling occasion does provide useful data, although within a particular lake some seasonal variability of stable isotope signatures can occur (e.g. Yoshioka et al. 1994; Zohary et al. 1994).

Crustacean zooplankton can graze a wide range of particulate matter, including phytoplankton, bacteria and detritus. Therefore the bulk, undifferentiated particulate organic matter (POM), for which we determined the stable isotope signature, can be considered the putative food source for the zooplankton, although the degree of food selection from within the bulk POM may vary between species and lake type. Allochthonous inputs of POM and DOM derived from terrestrial sources are expected to have $\delta^{13}C$ values close to those commonly reported for terrestrial C_3 vegetation (Lajtha and Marshall 1994; Jones et al. 1998). Lake phytoplankton is typically more ^{13}C-depleted than this and lake littoral vegetation more ^{13}C-enriched, mainly because of differences in in-

organic carbon sources. Mesotrophic and eutrophic lakes had mean POM $\delta^{13}C$ values (±1 SD) of –26.2±2.3‰ and 26.6±4.9‰ respectively (Table 2), which are close to values of –26 to –28‰ typical of terrestrial C_3 vegetation and soil organic matter (Peterson and Fry 1987). In contrast, the mean value from the hypertrophic lakes of –21.6±1.9‰ showed appreciable enrichment in ^{13}C. These results are consistent with a stronger influence of autochthonous production in lakes with greater nutrient enrichment. The more ^{13}C-enriched values from the oligotrophic lakes reflect the small sample size of predominantly clear-water lakes with little allochthonous loading. For many of the lakes the $\delta^{13}C$ for DOM approximately equated to that for POM, and the absolute values suggest that in these cases the dissolved organic carbon pools were mainly derived from allochthonous sources. However, in some lakes (and particularly in those lakes in which the POM was more ^{13}C-enriched), the DOM tended to be even more so, and in these cases the dissolved organic carbon pool was probably dominated by autochthonous material originating in the littoral zone.

The shift in relative contribution to POM across the trophic gradient away from allochthonous sources and towards phytoplankton might be assessed by the difference in $\delta^{13}C$ between phytoplankton and bulk POM (Table 2, Fig. 6). In eutrophic lakes the difference for individual lakes was generally <1‰, indicating systems driven by autochthonous production in which bulk POM was predominantly phytoplankton. Conversely, in oligotrophic Loch Ness phytoplankton constitutes only a minor part of the bulk POM, and the signatures of the two carbon pools were quite distinct (>5‰ difference), with that of POM much closer to that of allochthonous material of terrestrial origin (cf. Jones et al. 1998). However, this analysis is complicated by the high variability in $\delta^{13}C$ of phytoplankton. Variation in phytoplankton $\delta^{13}C$ reflects both the isotopic signature of the inorganic carbon source and the degree to which fractionation occurs during photosynthesis. In freshwaters, the source of DIC may have a particular impact on phytoplankton $\delta^{13}C$ (Raven et al. 1994) and it is a shortcoming of this study that a successful protocol for determination of the $\delta^{13}C$ of DIC was not in our use at the time of the survey. Literature $\delta^{13}C$ values for lakewater DIC are often around –5‰, although hypolimnetic DIC may be more ^{13}C-depleted during stratification (Keough et al. 1996). Lakes receiving a substantial input of allochthonous organic carbon which, when metabolised, releases ^{13}C-depleted DIC into the water column have lower $\delta^{13}C$ values of around –12‰ (Meili et al. 1996). Subsequent assimilation by phytoplankton can then produce a further ^{13}C-depleted algal signature as observed in the more coloured lakes (Fig. 3) and elsewhere by Rau (1978) and Jones et al. (1999). Of the less coloured lakes in the present study, many tended to be more productive. Plankton collected during periods of high biomass and primary productivity tend to exhibit ^{13}C-enrichment (Degens et al. 1968; Fry and Wainright 1991; Zohary et al. 1994; France et al. 1997) due to reduced isotopic fractionation at high cell densities or growth rates, or a switch to utilising HCO_3^- as CO_2 concentration is depleted (France et al. 1997). Algal samples from less coloured, more productive lakes like Loch Leven and Rostherne Mere illustrate this tendency (Table 2). Therefore, a combination of both algal physiology and environmental influences results in considerable inter-lake variability in phytoplankton $\delta^{13}C$, as it does in the oceans (Francois et al. 1993).

In 21 of the 24 lakes, the $\delta^{13}C$ of zooplankton was lower than that of POM (Fig. 4) indicating that zooplankton were actually depleted in ^{13}C relative to their putative food source. A review by del Giorgio and France (1996) of 76 published observations of $\delta^{13}C$ for lacustrine macrozooplankton and POM or microplankton revealed that freshwater data consistently fell below the line of equality (cf. Fig. 4), with a mean isotopic difference between zooplankton and POM in freshwaters of –2.6‰. From our survey of UK lakes, the mean ^{13}C depletion (±1 SD) was somewhat greater than this (–4.8±4.6‰), but not nearly so great as that found in a set of Finnish lakes (–8.2±4.0‰) by Jones et al. (1999). This zooplankton ^{13}C-depletion relative to POM might be explained by: (1) the accumulation in zooplankton of ^{13}C-depleted lipids (Kling et al. 1992); (2) spatial separation between where zooplankton are sampled and where they feed, with corresponding differences in carbon sources; or (3) selective feeding by zooplankton on isotopically light carbon sources which may be masked or diluted by a large detrital contribution to POM that is enriched in ^{13}C (del Giorgio and France 1996).

Lipids were not removed from zooplankton samples during this study. However, experiments on *Daphnia hyalina* (J. Grey, unpublished work) revealed no significant difference between control samples and those with lipids removed according to the protocol of Bligh and Dyer (1959). Recent findings by Zohary et al. (1994) also suggest that lipid accumulation is insufficient to account for zooplankton ^{13}C-depletion. Vertical migration of zooplankton to feed on distinct food sources has been demonstrated (e.g. Salonen and Lehtovaara 1992) and indicated in isotopic studies by separating epilimnetic and metalimnetic microplankton and relating them to epilimnetic zooplankton $\delta^{13}C$ (del Giorgio and France 1996). However, several of the lakes in our study (in particular the Norfolk Broads, c. 1 m mean depth) did not possess a stratified water column, although the Broads zooplankton $\delta^{13}C$ was the most depleted relative to POM (–8.9±6.0‰). Clearly, vertical migration through a structured water column cannot account for the marked ^{13}C-depletion in our lake set. The more general explanation offered by del Giorgio and France (1996) was that the phytoplankton isotopic signature is often masked by allochthonous and littoral detritus which is usually enriched in ^{13}C relative to phytoplankton (Meili et al. 1993; France 1995; Jones et al. 1998). In less productive lakes, detrital material dominates the organic carbon pool and plankton biomass may constitute very little (Meili 1992; Jones et al. 1997). Consequently, the isotope signature of allochthonous material not only predominates in the POM but may be traced up through the food chain, as demonstrated in oligotrophic Loch Ness by Jones et al.

(1998), and elsewhere in small humic lakes by Meili et al. (1996). With nutrient enrichment and greater phytoplankton production in lakes, autochthonous production increasingly dominates the organic carbon pool. Since 75% of the lakes included within our survey are eutrophic or hypertrophic and algal biomass is a large contributor to POM in these systems (see above), a close correlation between zooplankton and phytoplankton $\delta^{13}C$ would be predicted, this proved to be the case ($P<0.001$; $r=0.87$). Both phytoplankton and zooplankton $\delta^{13}C$ should, therefore, be expected progressively to converge with that of POM with increasing lake trophy, as observed by del Giorgio and France (1996).

When our 24 lakes were grouped according to trophic status, the divergence between zooplankton and POM signatures did decrease from oligotrophic to mesotrophic and eutrophic lakes in line with this prediction (Figs. 5, 7). In the oligotrophic lakes there was no overlap between $\delta^{13}C$ values for zooplankton and POM, whereas in the mesotrophic and eutrophic lakes there was considerable overlap in the spread of $\delta^{13}C$ values (Fig. 5). However, our study lakes encompassed a much wider range of TP than that of the Canadian Shield lakes studied by del Giorgio and France (1996), and recurring divergence between grazing zooplankton and POM $\delta^{13}C$ signatures was suggested in the hypertrophic lakes (Figs. 5, 7). Despite the much smaller sample size, there was also some indication of the same pattern for phytoplankton and POM signatures (Fig. 6). The range of divergence was actually greatest in hypertrophic lakes (from +6 to –12‰), due to the variability in phytoplankton $\delta^{13}C$ values. In contrast, the mean difference between $\delta^{13}C$ signatures for grazing zooplankton and phytoplankton (Fig. 7) changed with increasing lake trophic state from +3.7‰ in the single oligotrophic lake for which data were available to a mean of around –2‰ for the mesotrophic, eutrophic and hypertrophic lakes. In general, the very wide variability in phytoplankton $\delta^{13}C$ values across all the lakes together with the restricted number of lakes from which it was possible to adequately separate the phytoplankton made it difficult to identify patterns.

A more detailed examination of the $\delta^{13}C$ values obtained from the Norfolk Broads highlights some of the difficulties in interpreting stable isotope results from multiple-lake surveys. Two of the hypertrophic lakes, Ormesby and Filby Broads, are large, shallow and reed-fringed, and contain a limited amount of macrophyte growth. The ratio of littoral to pelagic is high in this morphometric lake type, and littoral production and emergent plants such as *Phragmites* and *Typha* are known to be ^{13}C-enriched relative to phytoplankton production (Keough et al. 1996; Schlacher and Wooldridge 1996; France 1995). Thus the observed ^{13}C enrichment of POM relative to phytoplankton in these lakes probably reflects a strong contribution of littoral detritus to POM. In Cromes Broad, the zooplankton were particularly depleted in ^{13}C relative to POM (16‰, Table 2). The phytoplankton was dominated by small flagellates (e.g. *Rhodomonas*), but Cromes Broad is generally a clear water lake with abundant, submerged macrophyte growth, which may account for the ^{13}C-enriched POM. The zooplankton community was dominated by *Bosmina* spp., small cladocerans particularly well adapted to bacterivory. Thus in Cromes Broad, *Bosmina* was evidently feeding extremely selectively on a constituent of the food web that was either inseparable from the POM or was entirely omitted from our sampling. The zooplankton community of Alderfen Broad consisted of three major species and was one of the few lakes to exhibit overall zooplankton ^{13}C-enrichment relative to POM (Table 2). However, whilst the raptorial *Cyclops viridis* and *Simocephalus vetulus* (a cladoceran associated with plant surfaces) were ^{13}C-enriched relative to POM by around 5‰, *Daphnia longispina* was ^{13}C-depleted by around 5‰ and was evidently selecting a very different carbon source. Periphyton and other biofilms tend to be enriched relative to their planktonic counterparts due to discrimination against ^{13}C in the boundary layer (France 1995). The isotopic signatures of *C. viridis* and *S. vetulus* suggest a periphytic diet, consistent with their known feeding habits, whereas *D. longispina* was probably feeding principally on phytoplankton. Thus an amalgamated $\delta^{13}C$ value for zooplankton from Alderfen Broad (–23.4‰) proves misleading and conceals two distinct carbon pathways.

The larger disparity between $\delta^{13}C$ of grazing zooplankton and POM observed in our study than that reported by del Giorgio and France (1996), may be partly explained by the amalgamation of zooplankton species. So far as we are aware, previous studies have mostly grouped species as one bulk zooplankton sample, whereas we separated predatory zooplankton species from grazing species. France and Peters (1997) observed that the average trophic fractionation of ^{13}C in freshwater systems was only in the order of +0.2‰. Although we found no consistent differences in stable isotope patterns between grazing zooplankton grouped as cladocerans and copepods across the 24 lakes, predatory zooplankton were consistently enriched in ^{13}C relative to their presumed grazing zooplankton prey by >1‰. Consequently when predatory species contribute a large proportion to zooplankton biomass they would considerably elevate a "single" amalgamated zooplankton $\delta^{13}C$ value. If we had included predatory zooplankton species in proportion to their biomass contribution from the seven lakes in which they were sufficiently abundant to be analysed separately, then the average ^{13}C-depletion for "bulk zooplankton" would be only –3.2‰ compared to the observed –4.1‰ for grazing zooplankton alone. In general it is desirable to separate zooplankton into feeding categories prior to stable isotope analysis rather than treating them as a single guild (cf. Grey and Jones 1999).

Overall, our results provide broad support for the hypothesis that the relative importance of allochthonous sources of organic carbon to lakes decreases with increasing lake trophy. France et al. (1997) suggested that the ^{13}C-enrichment of plankton in eutrophic lakes could be a serious impediment to the intended use of stable carbon isotope analysis because of the consequent lack of distinction between pelagic, littoral and terrestrial car-

bon source end-points, and our data partially support this view. Certainly there were difficulties evident in our study of detecting reliable patterns in the variation of $\delta^{13}C$ of trophic components across different lakes types. Nevertheless, we suggest that with the addition of further refinements, such as algal and zooplankton identification and especially improved methods for direct measurement of phytoplankton $\delta^{13}C$, discrimination and tracing of carbon pathways in eutrophic systems will be greatly assisted by stable isotope analysis.

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