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Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis

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Abstract

Seasonal variations in the stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of crustacean zooplankton and their putative food sources in oligotrophic Loch Ness were recorded during 1998. Bulk particulate organic matter (POM) showed $\delta^{13}C$ values consistent with a terrestrial plant origin from the catchment and exhibited little seasonal variation, whereas POM $\delta^{15}N$ was more variable, probably due to associated microbial action. In contrast, phytoplankton $\delta^{13}C$ was relatively light and showed some seasonal variation, but $\delta^{15}N$ values were more constant. The isotopic signatures of both POM and phytoplankton remained sufficiently distinct from each other throughout the period of study to allow their relative contributions to zooplankton diet to be assessed. Zooplankton isotopic signatures shifted seasonally, reflecting a dietary switch from a reliance on allochthonous carbon derived from POM during winter and early spring to heavy dependence on algal production during summer. Annually, crustacean zooplankton in Loch Ness derive approximately 40% of their body carbon from allochthonous sources, likely mediated via microbial links. Separate determination of isotope ratios for the main zooplankton species allowed a more detailed trophic investigation. The most abundant zooplankton species in the loch, *Eudiaptomus gracilis*, incorporated appreciable allochthonous carbon even during the peak of phytoplankton productivity. By contrast, *Daphnia hyalina* grew mainly in late summer and autumn and derived almost 100% body carbon from algal sources. This study is the first to quantify such a seasonal switch in zooplankton dependence between allochthonous and autochthonous sources of organic matter in a large lake.

The ecological importance of terrestrially derived material to lake food webs is receiving increasing attention. Much of the impetus for this has come from studies of the dark stained waters of small, boreal forest lakes of Scandinavia resulting from humic matter originating in the catchment (Meili 1992). The staining restricts light penetration into the water column and presents an unfavorable environment for photosynthetic fixation of carbon in situ by autotrophic phytoplankton (Jones 1992). Thus, in these systems, autochthonous production is usually very low. Humic material is capable of supporting bacterial metabolism (Hessen 1992) and associated heterotrophic organisms in the microbial loop (Tranvik 1992); consequently, a significant contribution to the humic lake food web may be derived from allochthonous matter via detritus-driven food chains. Jones (1992) hypothesized that the relative importance of allochthonous organic matter to the pelagic food web of lakes should increase with decreasing lake trophy, and a survey of the balance between planktonic production : respiration ratios lent support to this hypothesis (del Giorgio and Peters 1993, 1994). In oligotrophic lakes where community respiration equals or exceeds phytoplankton production (del Giorgio et al. 1997), the heterotrophic plankton must receive external subsidies of organic carbon to support their metabolism. There is a growing realization that such external subsidies of carbon can also be important to the food webs of some large lakes (e.g., Jansson et al. 1999). However, direct evidence for the contributions of allochthonous material remained elusive until recent applications of stable isotope analysis proved valuable in helping to quantify the relative importance of autochthonous and allochthonous carbon sources to lake plankton (Meili et al. 1996; Jones et al. 1998).

The stable isotope analysis approach requires the different basal resources available to a food web to exhibit distinct and robust isotopic ''signatures.'' The most frequently used signatures are those derived from the naturally occurring abundance ratios of $^{13}C : ^{12}C$ and $^{15}N : ^{14}N$ (Fry and Sherr 1984). These signatures can then be traced through the food web because the isotope ratios of organisms reflect those of the diet in a dependable manner. The $\delta^{13}C$ of an animal generally reflects that of its diet with minor enrichment ($<1‰$), while the greater enrichment of $\sim 3‰$ in $\delta^{15}N$ reflects the preferential excretion of the lighter isotope during metabolization (DeNiro and Epstein 1981; Fry and Arnold 1982; Minagawa and Wada 1984). Therefore, information on an animal's putative food sources and trophic level can be deduced by utilizing a combination of carbon and nitrogen isotope signatures. Stable isotope analysis offers advantages over conventional methods such as gut content analysis by representing the assimilated diet, as opposed to ingested material (Kling et al. 1992); incorporates dietary information over longer time periods as opposed to momentary ''snap-

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shots'' (Fry and Arnold 1982); and may even identify sources that are not detectable by inspection of ingested material. The latter advantage makes the stable isotope technique particularly useful for studies of planktonic organisms.

In a preliminary stable isotope study of the Loch Ness pelagic food web, Jones et al. (1998) confirmed that the particulate and dissolved organic matter (POM and DOM) in the loch water column originated predominantly from terrestrial detrital material. Autochthonous phytoplankton exhibited a carbon isotope signature significantly distinct from that of bulk POM and DOM. These were assumed to be the only food sources available to the pelagic food web because the basin morphometry of Loch Ness precludes a significant contribution of organic matter from littoral sources. From their preliminary study based mainly on samples collected during September 1996, Jones et al. (1998) suggested that ~50% of zooplankton carbon in the loch was probably derived from terrestrial sources but that this was likely to be seasonally variable.

Studies of seasonality of isotopic signatures in aquatic systems are still relatively rare, especially in freshwater (e.g., Gearing et al. 1984; Zohary et al. 1994; Yoshii et al. 1999), but they are crucial when considering organisms of small size with the potential to turn over assimilated isotopes quickly and thus exhibit differing isotopic signatures over a relatively short temporal scale (Grey in press). The aims of the current study were therefore (1) to assess seasonal variability in the isotopic signatures of the two putative food sources in Loch Ness to ensure that each remained distinct from the other; (2) to assess the relative importance of allochthonous material to zooplankton body carbon over an annual cycle and thus estimate the reliance of the pelagic food web on terrestrial organic matter imported from the catchment; and (3) to examine interspecific differences in dependence on allochthonous material related to different life history and feeding strategies among the zooplankton species in Loch Ness.

Methods

Samples were collected approximately monthly between February 1998 and February 1999. Bulk water samples were collected from Loch Ness at depths of 0, 10, 20, and 30 m using a 5-liter Friedinger sampler and were mixed in equal parts to produce a single integrated sample, as well as from a depth of 100 m. All water samples were stored in acid-washed 10-liter aspirators. Chlorophyll *a* (Chl *a*) was determined fluorometrically after filtering 1-liter subsamples of loch water through Whatman GF/C filters and extracting the chlorophyll into cold industrial methylated spirit (99% IMS: 95% ethanol, 4% methanol). POM was concentrated using a Minitan tangential flow ultrafiltration apparatus fitted with multiple 0.2-μm Durapore polyvinylidene fluoride filter plates. The concentrate was then collected on precombusted 25-mm Whatman GF/F filters and oven dried at 60°C. The ultrafiltrate was retained and concentrated for DOM analysis by freeze drying.

Crustacean zooplankton abundance was determined from vertical hauls from a depth of 30 m to the surface, using a plankton net of mesh size 110 μm. Samples were preserved with 70% IMS. Plankton was collected in bulk by horizontal tows through the surface waters (0–10 m) using nets of between 30 and 400 μm. These samples were stored without preservation. Crustacean zooplankton were later sorted manually using a fine pipette, with individual species being separated when numbers permitted, although a mixed zooplankton sample was also retained on each occasion. Zooplankton were maintained alive in filtered water to allow gut evacuation before further preparation when sufficient individuals of each species were collected onto precombusted 25-mm Whatman GF/F filters, rinsed with Milli-Q water, and oven dried at 60°C. Zooplankton samples were not acidified to remove inorganic carbonates, because previous investigations using Loch Ness zooplankton revealed negligible differences between acidified samples and controls (Grey unpubl. data), and acidification may have detrimental effects on nitrogen values (Pinnegar and Polunin 1999). From May to December, phytoplankton dominated by large siliceous diatom species could be separated from the other particulate matter by repeated sedimentation (Jones et al. 1998). Settled material was examined microscopically for purity, and subsamples of the diatom complex were then collected onto filters, as for zooplankton. Triplicate samples were collected for each parameter when possible, and all material was stored frozen until analyzed for stable isotopes.

Carbon and nitrogen isotopic analysis was carried out using a Roboprep-CN continuous flow analyzer coupled to a Tracermass single-inlet triple-collector mass spectrometer (both instruments by Europa Scientific). Samples collected on Whatman GF/F papers that remained firmly embedded in the fibers of the filter paper were cut into strips, and sample and glass fiber were combusted together. In cases where the weight of sample was greater, the sample peeled away from the glass fiber on drying and could be combusted on its own. Larger samples were ground under liquid nitrogen in a freezer mill (Spex). Results are given using the δ notation where

$$\delta = [(R_{sample}/R_{reference}) - 1] \times 1{,}000$$

expressed in units of per thousand (‰) and where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The reference materials used were secondary standards of known relation to the international standard of Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Typical precision was ± 0.1‰ for carbon and ± 0.3‰ for nitrogen. Further details of the isotopic analyses can be found in Jones et al. (1998).

Results

Algal and zooplankton biomass in Loch Ness exhibited the distinct seasonality typical of an oligotrophic temperate lake (Fig. 1) and consistent with that shown by previous studies of Loch Ness plankton (Maitland et al. 1981; Jones et al. 1996*b*). Algal biomass, indicated by concentration of Chl *a*, was extremely low during winter, grew steadily during spring and summer to a single peak in late July and early August, and then declined steadily. Zooplankton biomass was dominated for most of the year, and particularly during spring and early summer, by the copepods *Eudiaptomus gracilis, Cyclops abyssorum,* and their associated naupliar

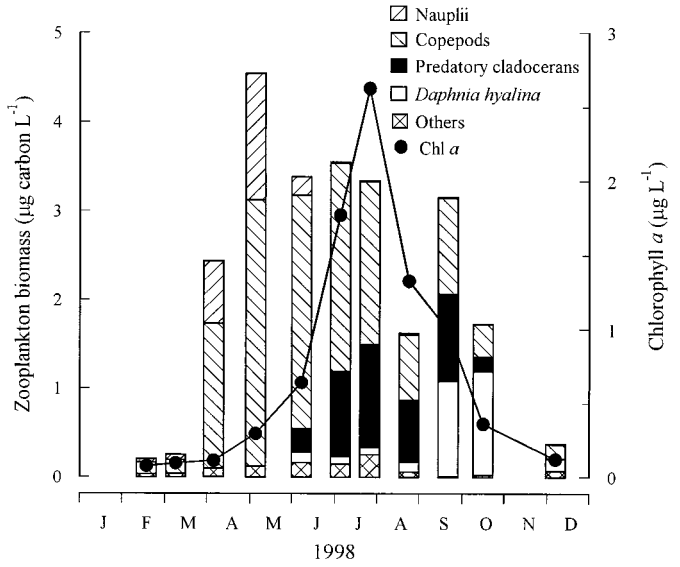

Fig. 1. Seasonality of crustacean zooplankton biomass (μg carbon L^{-1}) and Chl *a* (μg L^{-1}) in Loch Ness, 1998.

stages. Most of their population development thus occurred before the late summer peak in Chl *a* in the surface waters. Copepod biomass decreased during the later summer period, and by late September–October, the grazing cladoceran, *Daphnia hyalina*, constituted a large proportion of total zooplankton biomass as Chl *a* concentrations declined. *Bosmina coregoni* were present throughout the study at low densities, and *Holopedium gibberum* appeared in the water column briefly during late July and August. The predatory cladocerans, *Bythotrephes longimanus*, *Polyphemus pediculus*, and *Leptodora kindti*, were never numerically abundant but, because of their larger size, constituted 30–40% of total zooplankton biomass during August and September.

The δ^{13}C of POM from the loch surface waters (0–30 m) showed little variation during the study period (Fig. 2a) and was not significantly different from the δ^{13}C of POM from 100 m (paired *t*-test, $P > 0.05$). Surface-water POM δ^{13}C values ranged from -24.0 to -26.6‰ (annual mean = -25.5‰), suggestive of carbon derived primarily from the river inflows and thus of terrestrial detrital origin (see Jones et al. 1998). DOM δ^{13}C values were consistently lighter than POM and exhibited little seasonal variation (annual mean = ± 1 SD, -27.6 ± 0.4‰). Phytoplankton samples were appreciably ^{13}C-depleted relative to the total POM throughout those months when the two could be separated (May–December). Phytoplankton δ^{13}C ranged from -29.0 to -32.2‰ and became seasonally ^{13}C-enriched during the most productive months of late summer when Chl *a* values were >1 μg L^{-1} in the mixed surface waters. However, there was always sufficient distinction in isotopic signature between the autochthonous and allochthonous sources of carbon throughout the period of study to allow assessment of their relative contributions to the diets and body carbon of the crustacean zooplankton.

The δ^{13}C values of the mixed zooplankton samples (Fig. 2a) initially were closely associated with that of the loch POM. However, with increasing Chl *a* levels in June, zooplankton δ^{13}C rapidly became more ^{13}C-depleted, reflecting the phytoplankton δ^{13}C. As Chl *a* concentrations fell later in the year, the zooplankton δ^{13}C signature gradually became more ^{13}C-enriched and, by the following February, was again close to the POM δ^{13}C value. Therefore, when amalgamated into a single trophic functional group, the zooplankton in Loch Ness switched from a heavy reliance on POM-derived allochthonous carbon during winter to utilizing mainly algal-derived autochthonous carbon during the more productive summer months.

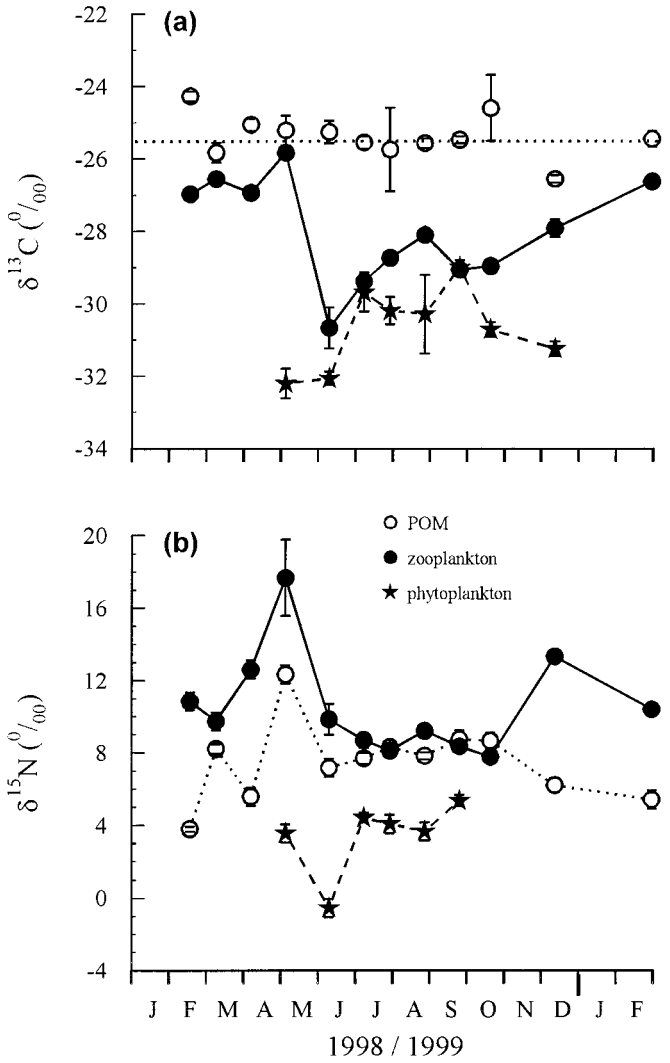

Fig. 2. Seasonal variation of (a) δ^{13}C values, and (b) δ^{15}N values of POM, mixed crustacean zooplankton, and phytoplankton from Loch Ness, 1998–1999 (mean $\pm$ SD). In (a), the dotted line indicates the annual mean δ^{13}C value for POM.

In contrast to the relatively invariable POM carbon isotope ratios, the δ^{15}N of POM (Fig. 2b) exhibited wide fluctuations during the early part of the year (3.8–12.3‰, February–May), was more consistent at ~8‰ during the summer, and became more ^{15}N-depleted during the winter (~6‰). Sufficient phytoplankton were collected on six occasions between May and September to allow nitrogen isotope analysis. The δ^{15}N of phytoplankton was lighter than that of POM (~4‰)

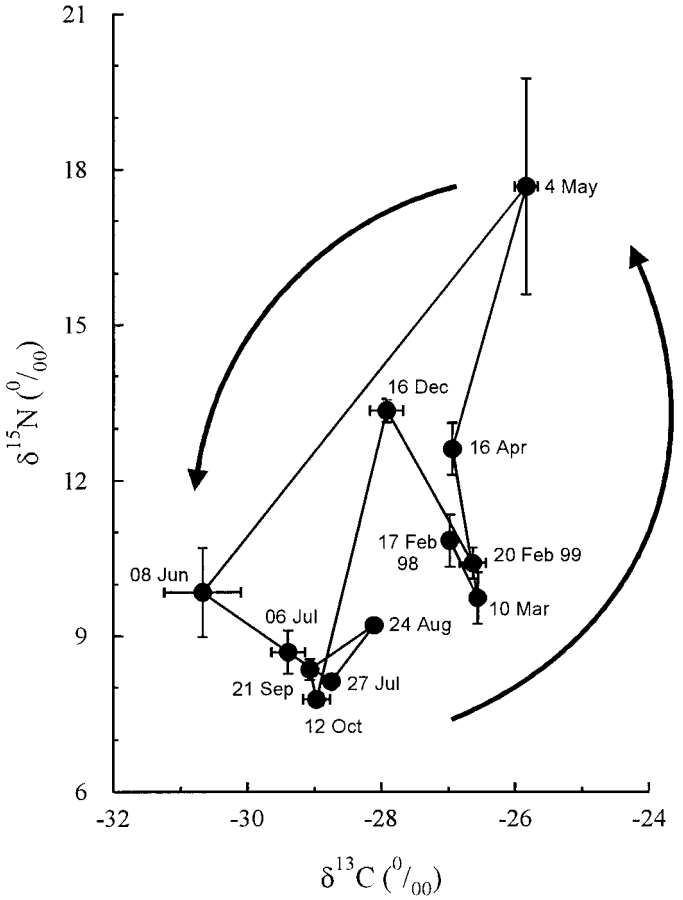

Fig. 3. $\delta^{13}C$ versus $\delta^{15}N$ plot of mixed crustacean zooplankton from Loch Ness, 1998 (mean ± SD). The arrows indicate the broad seasonal cycle suggested by the data.

and consistently distinct when the two could be separated physically. The mixed zooplankton $\delta^{15}N$ values were enriched relative to the POM at the beginning and end of the study period and exhibited simultaneous proportionate fluctuations. However, the enrichment from putative diet to consumer tissues varied between 2 and 7‰. The switch from allochthonous to autochthonous dietary sources during the summer suggested by the carbon signatures was again seen in the nitrogen signatures. The $\delta^{15}N$ of the mixed zooplankton became lighter between June and October, consistent with incorporation of the algal signature.

Plotting the mixed zooplankton $\delta^{13}C$ against $\delta^{15}N$ values revealed an apparent cyclical seasonal pattern (Fig. 3). The zooplankton signatures from June to October were clustered with relatively light carbon and nitrogen values compared to those from December to April in a second cluster. The values recorded during May were the heaviest for both isotope signatures, making the dietary switch between May and June very pronounced. There was a striking consistency between February values from successive years.

The percentage of carbon of allochthonous origin in the mixed zooplankton assemblage (Fig. 4) was determined using a simple mass balance equation.

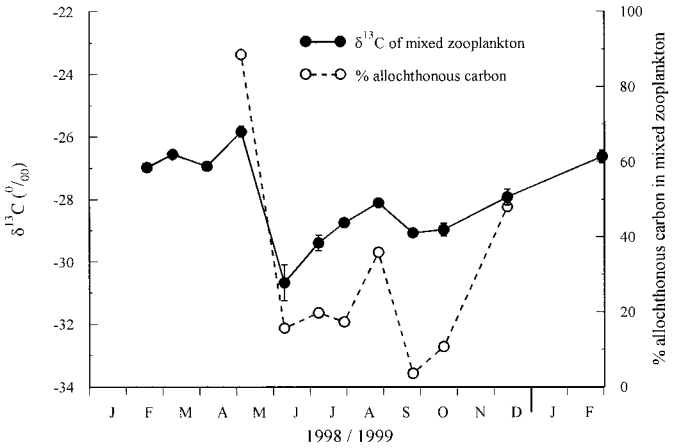

Fig. 4. Seasonal variation in the percentage contribution of allochthonous carbon to zooplankton body carbon and $\delta^{13}C$ of mixed zooplankton from Loch Ness, 1998.

$$\%\ \text{allochthonous carbon} = \frac{(\delta^{13}C_{Zoo} - F - \delta^{13}C_{Phyt})}{(\delta^{13}C_{POM} - \delta^{13}C_{Phyt})} \times 100$$

where $\delta^{13}C_{Zoo}$, $\delta^{13}C_{Phyt}$, and $\delta^{13}C_{POM}$ are the isotope signatures of the mixed zooplankton, the phytoplankton, and the POM, respectively, and F is the isotopic fractionation between a consumer and its food. We used the value of $\delta^{13}C_{Zoo}$ obtained for a particular sampling date, but we used the mean $\delta^{13}C_{Phyt}$ for that date and the previous date better to reflect the value the zooplankton had been exposed to during feeding and growth in the period before sample collection. In the case of POM, we used the annual mean $\delta^{13}C$ for bulk POM, which we considered would provide a better representation of the allochthonous $\delta^{13}C$ signature than individual values in view of the small variations in POM $\delta^{13}C$ (Fig. 2a). Fractionation of dietary isotopes to the tissues of a consumer typically results in enrichment in ^{13}C on the order of 0.5–1.0‰ (Michener and Schell 1994). However, for this study, a slightly lower mean enrichment factor of 0.43‰ was applied, derived from empirical data from concurrent laboratory experiments on fractionation in crustacean zooplankton, using the same genera as those found in Loch Ness (Grey 2000 in press, unpubl. data).

According to these mass balance calculations, allochthonous carbon accounted for 89% of zooplankton carbon in May, but the contribution declined during the summer months to 4% in September and, by December, had risen again to ~50%. Seasonal variation in the total zooplankton carbon biomass is shown in Fig. 5. From the calculated percentage of zooplankton carbon of allochthonous origin (Fig. 4) and the total zooplankton carbon biomass, the absolute amount of zooplankton carbon of allochthonous origin on each sample date could also be calculated (Fig. 5). Comparison of the integrated areas defined by the two curves showed that, on an annual basis, 38% of the total zooplankton body carbon in Loch Ness was apparently derived from allochthonous sources. Integration of the curves assumed that the concentrations at either end of the year were equal to the first and last sampling values. This assumption is justified because fluctuation in zooplankton biomass during the

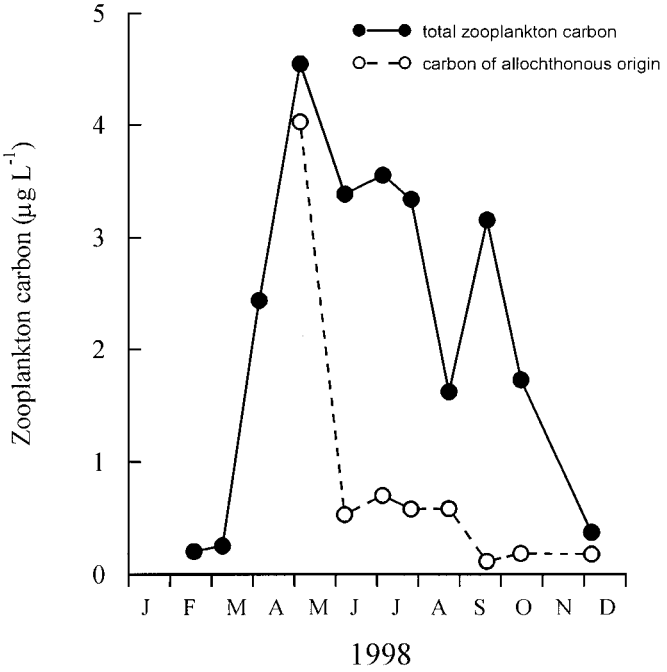

Fig. 5. Seasonal variation in total zooplankton carbon and in that part of allochthonous origin in Loch Ness, 1998.

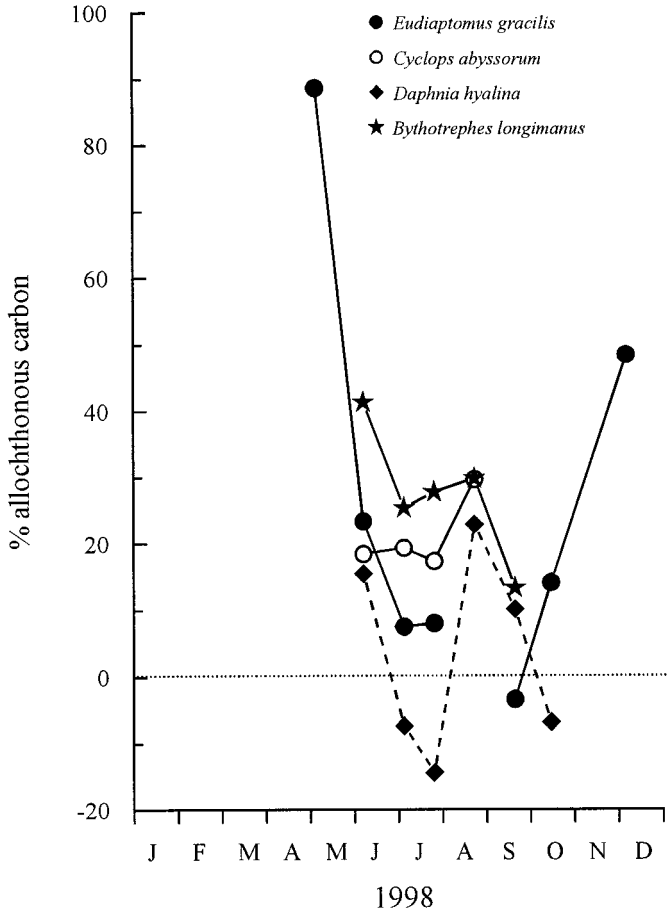

Fig. 6. Percentage contribution of allochthonous carbon to total body carbon of individual zooplankton species from Loch Ness, 1998.

winter months is negligible in Loch Ness (Maitland et al. 1981).

Four of the zooplankton species were sufficiently abundant during the summer months to allow individual assessments of the contribution made during this period to their total body carbon by carbon of allochthonous origin (Fig. 6). *E. gracilis* was the most abundant zooplankton species throughout the year and could be separately determined on most occasions. Thus, the switch from a high percentage of allochthonous carbon to a diet based on autochthonous algal production was the most marked in this species. The other zooplankton species could only be separated for stable isotope analysis during the summer months when the availability, and hence potential use of autochthonous carbon sources, would anyway be expected to be greatest. During this period, the reliance of *C. abyssorum* on allochthonous carbon was consistently ~20–30%, while that of *D. hyalina* was low and occasionally even negative when the δ^{13}C signature of the daphnids was depleted relative to measured δ^{13}C values of both POM and phytoplankton. A twofold enrichment factor of 0.86‰ was used in the calculation of the contribution of carbon to *B. longimanus* because it is a predatory species and hence two trophic levels above the basal resource. Approximately 40% of *B. longimanus* body carbon was apparently of allochthonous origin at the beginning of the summer, but this proportion declined to ~15% by late September.

Because both carbon and nitrogen signatures of the mixed zooplankton assemblage suggest a marked dietary switch to phytoplankton production during the summer, the mean Chl *a* weighted phytoplankton δ^{15}N value of 3.1‰ was assigned as the base trophic level (i.e., trophic level 1; Fig. 7). An average 3.2‰ enrichment in δ^{15}N per trophic level (TL) was then applied from comparable dietary studies on mysids (Toda and Wada 1990; Gorokhova and Hansson 1999). The nitrogen signatures of *D. hyalina* were consistent with a position at TL2, while those of *E. gracilis* were between TL2 and TL3 and actually reached TL3 in September. *C. abyssorum* δ^{15}N values followed a similar pattern to those of *E. gracilis* but were generally slightly heavier. The predatory *B. longimanus* was consistently above TL3.

Discussion

A striking feature of Loch Ness is how the spring increase in zooplankton carbon (predominantly copepods and their nauplii) precedes the increase in phytoplankton biomass as Chl *a* (Fig. 1). This observation provides circumstantial evidence for a heavy dependence by zooplankton on nonalgal carbon at certain times of the year. Although we did not determine seasonal variation in POM during this study, we have previously shown that total POC in Loch Ness is seasonally variable, with highest concentrations during winter and early spring when hydrological inputs of allochthonous organic matter are greatest (Jones et al. 1996*a*). Moreover, even during the summer peak of phytoplankton, biomass carbon in Loch Ness represents only ~12% of total POC within

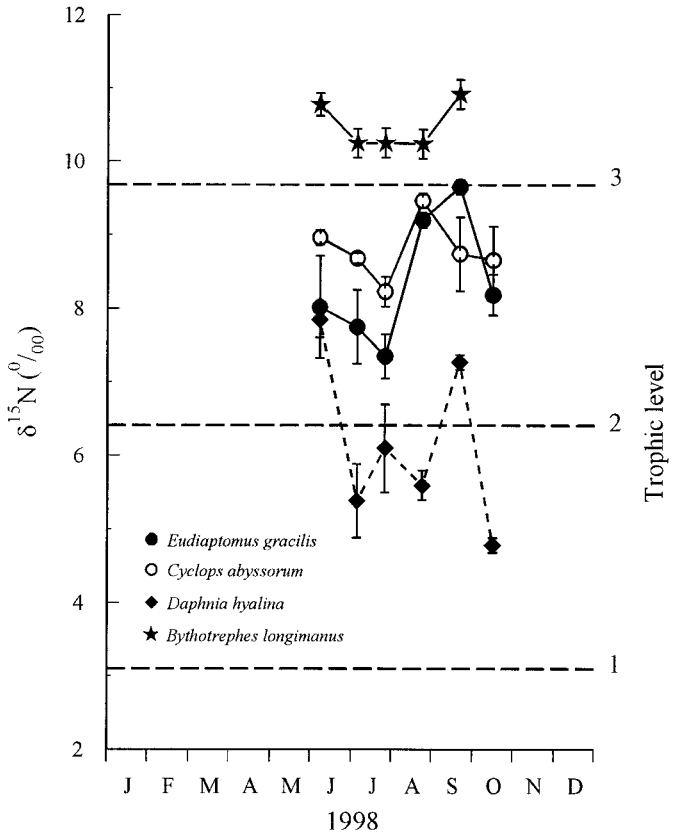

Fig. 7. Apparent trophic level of individual zooplankton species from Loch Ness (1998), relative to a phytoplankton $\delta^{15}N$ baseline (trophic level 1).

the upper 30 m of the water column (Jones et al. 1996*a*). Hence, of the two putative carbon sources to the pelagic food web in the loch, allochthonous detrital carbon is vastly more available than algal carbon at all times of year. From preliminary stable isotope analysis of the pelagic food web of Loch Ness, Jones et al. (1998) suggested that ~50% of zooplankton body carbon was derived from allochthonous matter. However, that estimate was based on detailed sampling on a single date in September and hence took no account of seasonal variability. The current study has addressed the issue of seasonality and therefore enables a proper assessment of the annual contribution of allochthonous organic carbon to the pelagic food web.

Putative carbon sources to the pelagic food web were necessarily grouped into two categories, algal production and bulk POM, even though it is recognized that each must comprise many components that current methodology does not allow us to separate. The carbon isotopic signature of bulk POM varied little on a seasonal basis, and the contribution of phytoplankton to that signal was so small as to be completely masked. Such consistency is hardly surprising in view of the overwhelming dominance of detrital POM and DOM to total organic carbon in Loch Ness (Jones et al. 1996*a*) and the stability of the $\delta^{13}C$ value for C_3 terrestrial vegetation (Lajtha and Marshall 1994). The isotopic signature for phytoplankton was derived from samples of siliceous diatoms separated by sedimentation, and it was assumed that this was representative of the phytoplankton community as a whole. The phytoplankton carbon biomass in Loch Ness is mainly from large diatoms, although cryptomonad flagellates are numerically abundant in the loch (Bailey-Watts and Duncan 1981; Jones unpubl. data). These naked flagellates are a preferred food for zooplankton and, with their potentially faster growth rates, are probably of greater importance than the diatoms to the diet of zooplankton (Ahlgren et al. 1990). Phytoplankton carbon and nitrogen signatures are known to vary spatially and temporally according to prevailing environmental conditions (Wada 1980). However, Loch Ness is an oligotrophic system of large volume and great depth of mixing, and, with a pH ~6.2, a high proportion of the total DIC is always available as free CO_2. Therefore, it is likely that the different phytoplankton species will all use the same carbon source with similar physiological fractionations and consequently will exhibit rather similar isotopic signatures (Zohary et al. 1994). The observed fluctuations in the $\delta^{13}C$ of the phytoplankton corresponded with changes in the $\delta^{13}C$ of DIC (Jones et al. in prep.). However, different boundary layer and CO_2 diffusion conditions may apply to large nonmotile diatoms and the smaller, motile flagellates, which could lead to diatoms being more ^{13}C-enriched than flagellates. Gearing et al. (1984) showed estuarine diatoms to be ~2‰ ^{13}C-enriched compared with nanoplankton. Nevertheless, until we are able to determine values separately for different taxa, those obtained from the large diatoms must remain our best estimate of the $\delta^{13}C$ of the phytoplankton in Loch Ness.

Both carbon and nitrogen zooplankton signatures indicated a reliance on POM during winter and early spring. Hessen et al. (1990) demonstrated that zooplankton can use detrital sources of organic matter directly but with only low assimilation efficiency and that this use is probably mediated through a series of microbial links. However, investigation of the actual food web structure at this level by stable isotope analysis is difficult due to inadequate separation of the potential components: bacteria, protozoa, and detrital particles (Jones et al. 1998). Purity of samples of these smaller components from natural food webs for isotopic analysis is a particular hindrance. If zooplankton were reliant on the organic content of the detrital POM directly, then from the literature, one might expect the isotopic signatures to be enriched by ~1 and 3‰ for carbon and nitrogen, respectively. In fact, the mixed zooplankton were never ^{13}C-enriched relative to the POM, whereas the mean nitrogen enrichment was 5.5‰. Such high $\delta^{15}N$ diet-tissue fractionation has been reported in daphnids maintained on an algal diet with high C : N ratios (Adams and Sterner 2000). We do not have data on inorganic nitrogen concentrations in Loch Ness, but Bailey-Watts and Duncan (1981) reported inorganic nitrogen concentrations ~100 $\mu g\ L^{-1}$ with little seasonal fluctuation, which would not suggest nitrogen limitation of algae and associated high C : N ratios. In fact, Jones et al. (1996*b*) reported that phytoplankton in the loch are probably light limited for most of the time. Alternatively, the high fractionation could represent a number of masked trophic steps (TL3 relative to a POM basal resource). Our results do not permit discrimination between these two possible explanations for

the resultant ^{15}N enrichment; indeed, the two are not mutually exclusive.

The annual mean $\delta^{13}C$ of DOM was $-27.6 \pm 0.4‰$ with low variability throughout the year, and the likely utilization of this carbon source by bacteria would help account for the zooplankton signature never being enriched relative to that of POM. Microbial fractionation of carbon from diet to consumer is thought to be <1‰, and much recycling of material may occur at this level (Sirevåg et al. 1977). The importance of organic nitrogen assimilated from dissolved inorganic nitrogen (DIN) by microbial heterotrophs has been identified in aquatic systems that are heavily subsidized by terrestrial organic matter (e.g., Findlay and Tenore 1982). Microbially assimilated DIN (MAD) was investigated in the Hudson River by Caraco et al. (1998), and their findings suggest that MAD can significantly elevate values of $\delta^{15}N$ in systems with sufficient residence time. The most enriched nitrogen values for both zooplankton and POM in the pelagial of Loch Ness were recorded in May, around the time when the water column began to increase in temperature (2°C, April–May; Grey 2000), and the first flush of microbial activity has been recorded previously (Laybourn-Parry and Walton 1998). The mixed zooplankton nitrogen isotope signatures were ~6‰-enriched relative to the POM during this period, suggesting that zooplankton were utilizing the microbial fraction rather than assimilating the POM per se. During the same period, the zooplankton community comprised ~30% nauplii and ~65% mixed copepod and copepodites, so a large proportion of the assemblage consisted of rapidly growing and molting organisms. The molts would provide an ideal substrate for bacterial production and associated protozoan grazers, and coprophagy of relatively $\delta^{15}N$-rich fecal pellets has been suggested in Lake Baikal (Yoshii et al. 1999). Thus, there was also likely to be an element of recycling of zooplankton exuviae and excreta. A further potentially confusing factor arises from the simultaneous presence of differing life stages of the same organism, because naupliar, copepodite, and adult stages can exhibit different feeding strategies at a microscale (Fryer 1998). Kankaala (1988) deduced from in situ grazing experiments that while algae are the principal food of adult *Daphnia longispina* in small humic lakes, bacterial productivity was important to the juveniles. Such ontogenetic shifts in diet have been observed to affect $\delta^{15}N$ values of the pelagic amphipod *Themisto japonica* collected from the Sea of Japan (Sugisaki et al. 1991).

The considerable difference (~4‰) between the May and June mixed zooplankton carbon signatures indicates how rapidly the switch in diet was incorporated into the consumer tissues. It also perhaps emphasizes the importance of the phytoplankton as a high-quality diet, because the concentration of Chl *a* at the time was $<0.5\ \mu g\ L^{-1}$. The zooplankton assemblage was dominated by the filter-feeding *E. gracilis*, yet it must have been exerting considerable selection of ingestion, or assimilation from ingested material, to have exhibited a phytoplankton-biased isotopic signature when the ratio of algal to detrital material was so low. Because the growth stages of copepods undergo frequent molting, turnover and apportioning of carbon from diet directly into new tissues are high. Consequently, the dietary signature is exhibited more readily in these life stages than in adults where turnover is the only agent of isotopic ''renewal'' (Gorokhova and Hansson 1999; Grey in press). Thereafter, the mixed zooplankton signatures shadowed the varying phytoplankton signatures closely until they diverged again in October with falling algal production. The slight deviations from July to September were influenced by the high predatory cladoceran biomass, which was assumed to have twice the fractionation factor representative of their higher trophic status.

The results of the seasonal survey at Loch Ness reveal that for 5 months of the year, >50% of the mixed zooplankton community biomass was derived from allochthonous organic matter. Even during the relatively productive summer period in this oligotrophic system, allochthonous matter accounted for ~20% of zooplankton biomass. Over the annual cycle, 38% of the zooplankton carbon of Loch Ness was derived from terrestrial sources. Other stable isotope studies of food webs in large lakes such as Lake Kinneret, Israel, or Lake Baikal, southern Siberia, have highlighted the importance of algal productivity as the primary carbon source (Yoshioka et al. 1994; Zohary et al. 1994; Yoshii et al. 1999). Thus, our detailed monthly stable isotope analysis study of Loch Ness appears to be the first to reveal marked seasonal changes in importance of allochthonous and autochthonous contributions to zooplankton body carbon throughout the year.

Of course, these conclusions depend on the validity of the parameters used in the mixing model. The model is particularly sensitive to the isotope fractionation factor, F. We used an empirically determined value for F of 0.43‰ obtained from experimental work with appropriate organisms (Grey in press, unpubl. data). However, a fractionation factor of 1‰ is frequently cited as a rough ''rule-of-thumb'' for ^{13}C enrichment between trophic levels (e.g., Fry and Sherr 1984). Using this value in our mixing model yielded a lower figure of 28% for the annual contribution of allochthonous carbon to zooplankton in Loch Ness. Conversely, France and Peters (1997) reported an average trophic ^{13}C enrichment of only 0.2‰ for freshwater food webs, compared with 0.5‰ for estuarine food webs and 1.1‰ for open-ocean food webs. Applying their value of 0.2‰ to our mixing model yielded a slightly higher figure of 43% for the annual contribution of allochthonous carbon to zooplankton in Loch Ness. We also had to make assumptions about the $\delta^{13}C$ value for the phytoplankton consumed by zooplankton (*see* above). If the zooplankton in the loch mainly grazed flagellates that were 2‰ ^{13}C-depleted compared to the large diatoms we analyzed, this would actually increase the annual dependence of zooplankton in Loch Ness on allochthonous carbon to 54%. This kind of sensitivity analysis does not fundamentally alter our conclusions about the importance of allochthonous carbon to zooplankton in Loch Ness. However, it does illustrate the importance of using appropriate parameters in mixing models to analyze stable isotope data, and it also emphasizes the need for more empirical determinations of trophic fractionation factors from a wider range of aquatic organisms and environments.

Different species within the community exhibited varying reliance on the two potential carbon sources, a phenomenon often overlooked in stable isotope studies by the amalgam-

ation of zooplankton into a single trophic functional group (Grey and Jones 1999; Grey et al. 2000). Both the carbon and nitrogen isotopic signatures of *D. hyalina* suggest virtually no assimilation from allochthonous sources during summer in Loch Ness, this species apparently being almost completely reliant on algal production. Indeed, daphnid biomass increased substantially following the observed Chl *a* peak, so these filter feeders must have been the most selective in dietary assimilation. However, it is also possible that the juvenile daphnids exploited a brief flush of bacterial production associated with the demise of the phytoplankton peak (Laybourn-Parry and Walton 1998). Certainly, the δ^{15}N of grazing zooplankton species increased relative to the phytoplankton signature during September (Fig. 7), indicative of a microbial fraction in the diet. The following decrease in grazing zooplankton δ^{15}N during October supports this hypothesis, because the return to a diet based primarily on POM-derived material would have led to further elevation in the nitrogen signature. Ojala et al. (1995) suggested that bacteria could serve as a ''life-support system'' to *D. longispina* but that detrital material was of a limited value to the nutrition of *Daphnia* in a polyhumic lake where >75% of the carbon was to be found in detrital form. The inclusion of some allochthonous material into the diet of *E. gracilis* apparently elevated the trophic status of this species above *D. hyalina*, but this is likely an artifact of incorporation of two differing basal resources. The *Daphnia*:*Eudiaptomus* relationship in humic lake Blacksåstjärn (central Sweden) was very different, in that the cladocerans were evidently dependent upon detrital terrestrial food sources, while the copepods were reliant upon pelagic primary production (Meili et al. 1996). However, Meili et al. (1996) also attributed the difference between the δ^{15}N of the two grazers to separate primary food sources rather than to a trophic interaction. Further elevation of δ^{15}N in *C. abyssorum* from Loch Ness was consistent with the omnivorous nature of this species and its predatory relationship with *E. gracilis* (Fryer 1957). Because the δ^{15}N of *B. longimanus* was constantly above TL3 during the summer, copepods may then have been contributing a small proportion to a diet primarily of daphnids, although in experiments to examine the effect of *Bythotrephes* on zooplankton densities, neither cyclopoid nor calanoid copepod biomass was depleted (Wahlström and Westman 1999). The extremely low densities of zooplankton in Loch Ness may force a more opportunistic approach upon the crustacean predators. Of course, such interpretations of trophic steps are dependent on the figure assumed to represent trophic enrichment in ^{15}N and highlight the need for more controlled laboratory studies of ^{15}N fractionation by components of pelagic food webs in lakes.

In conclusion, detailed sampling of the components of the Loch Ness pelagic food web has revealed considerable seasonal variation in the δ^{13}C and δ^{15}N of crustacean zooplankton, reflecting a seasonal switch in reliance from allochthonous to autochthonous organic matter sources. The tracing of this switch was made possible by the simplicity of the Loch Ness food web and the robust isotopic signatures of the putative sources. On an annual basis, ~40% of zooplankton production was based on allochthonous carbon of terrestrial origin. The results demonstrate that stable isotope analysis can be a powerful tool for quantifying the contribution of allochthonous material to lake production, the importance of which is being increasingly indicated by more conventional lake productivity studies (Hessen 1998; Arvola et al. 1999).

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