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Ferox Trout (*Salmo trutta*) as 'Russian dolls': complementary gut content and stable isotope analyses of the Loch Ness foodweb

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SUMMARY

1. Conventional collection methods for pelagic fish species (netting, trawling) are impractical or prohibited in Loch Ness, U.K. To investigate trophic relationships at the top of the Loch Ness food web, an alternative strategy, angling, provided samples of the top predator, the purely piscivorous ferox trout (*Salmo trutta*).
2. The gut contents of these fish provided further samples of prey-fish, and subsequent examination of prey-fish guts revealed their dietary intake, analogous to the famous nested 'Russian dolls'. Each trophic level separated by gut content analysis provided further complementary samples for stable isotope analysis and thus information on the longer term, assimilated diet.
3. Ferox trout exhibited considerable cannibalism to supplement a diet of Arctic charr (*Salvelinus alpinus*). However, conspecifics stemmed from a lower isotopic baseline in relation to charr, so ferox trout exhibited a lower trophic level than predicted (4.3) by using the $\delta^{15}N$ values. Charr displayed dietary specialisation with increasing length, and isotopic values supported by the gut data placed the charr at a trophic level of 3.5. The isotope data also indicated that charr carbon was primarily autochthonous in origin.

Keywords: arctic charr, brown trout, Loch Ness, stable isotopes, trophic level

Introduction

An understanding of trophic relationships is fundamental to investigations of ecosystem processes, yet such relationships can prove difficult to determine in the natural environment (Polis & Winemiller, 1996 and references therein). A plethora of techniques are available to ecologists studying terrestrial ecosystems (e.g. direct observation of predation or ingestion, and faecal or pellet examination). However, the nature of the aquatic environment effectively limits the number of techniques available to aquatic ecologists and accurate determination of predator–prey relationships may be virtually impossible (Hobson & Welch, 1995).

Study of the diet based on gut content analysis (GCA) has been a standard practice in fish ecology (reviewed by Hyslop, 1980). Gut content analysis allows the stomach contents of a predator to be quantified in terms of specific taxa ingested, but not necessarily assimilated. It provides only information about feeding immediately prior to capture, unless the predator in question exhibits little diet heterogeneity. The trophic ecology of many fish species in cold-temperate lakes is characterised by adaptability or opportunism forged by environmental conditions that change seasonally (Dill, 1983). Therefore, unless GCA is also conducted seasonally, the technique is of limited value. Moreover, ingested items can often be masticated or digested beyond recognition, and softer bodied components of the diet may be significantly

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underestimated (Hyslop, 1980; Burns *et al*., 1998). Laboratory feeding experiments may not truly reflect dietary behaviour in the presence of natural food assemblages, because it is often impossible to recreate the complexity of the natural environment in a laboratory situation, and also incur problems of extrapolation from laboratory conditions to those *in situ*. Thus, many conventional techniques provide only limited information on trophic relationships.

An alternative approach is the analysis of stable isotope ratios in consumer tissues, a technique used increasingly as a powerful ecological tool to assess trophic relationships in a wide variety of ecosystems (reviewed by Peterson & Fry, 1987; Hobson, 1999). Typically, natural abundances of isotopes of carbon and nitrogen are used, and the measurement of both concurrently yields more information on feeding relationships than either element alone. As fractionation of carbon isotopes from food source to consumer is relatively conservative (0 to +1‰, DeNiro & Epstein, 1978), the $\delta^{13}C$ signature of an organism generally reflects the isotopic composition of the diet and provides information of the source of carbon to the food web. The abundance of ^{15}N in consumer tissues tends to increase relative to their prey because of preferential excretion of the lighter isotope during protein transamination and deamination. Enrichment of $\delta^{15}N$ is usually about 3.4‰ and can thus be used to delineate trophic structure in a food web (Minagawa & Wada, 1984). Both nitrogen and carbon in consumer tissues are derived exclusively from the diet and therefore trophic estimates using an isotopic technique rely on food that has been assimilated rather than just ingested (Gearing, 1991).

Stable isotope analysis (SIA) should be utilised to augment rather than replace conventional dietary investigation techniques. Although it has a number of advantages over GCA, especially regarding long-term assimilated diet, it lacks the taxonomic resolution that GCA can achieve. Consequently, a number of recent studies from a variety of ecological fields have used both techniques (Sydeman *et al*., 1997; Whitledge & Rabeni, 1997; Vander Zanden, Cabana & Rasmussen, 1997; Burns *et al*., 1998; Beaudoin *et al*., 1999).

Previous studies of the pelagic food web of Loch Ness have used SIA to assess the relative importance of allochthonous organic carbon to the crustacean zooplankton (Jones *et al*., 1998; Grey, Jones & Sleep, 2001). The relatively simple food web of Loch Ness is amenable to investigation by SIA. Preliminary isotopic work included only limited data on the fish species present, particularly Arctic charr (*Salvelinus alpinus* Linnaeus) because of its dietary relationship with zooplankton (Jones *et al*., 1998). Arctic charr are typically sympatric with brown trout (*Salmo trutta* Linnaeus) in large Scottish lochs (Campbell, 1979). Ecological relationships between trout and charr have been well documented, especially from Scandinavian waters (Aass, 1990; Langeland *et al*., 1991; L'Abée-Lund, Langeland & Sægrov, 1992), but little is known about the long-term feeding behaviour of the two fish species in Loch Ness (George & Winfield, 2000). Thus a more detailed investigation of the higher trophic levels utilising both GCA and SIA was prompted.

Although Arctic charr are the dominant pelagic fish in oligotrophic Loch Ness, the population is extremely sparse (recorded maximum, 36.7 individuals ha^{-1}, Bean, Winfield & Fletcher, 1996) and most reside at around 30 m depth. There is no commercial fishery on Loch Ness and permission to net for charr was refused by the local fisheries board because of concerns about interference with the local salmon fishery. Therefore, an alternative sampling strategy was required. Brown trout can often achieve considerable size in sympatry with Arctic charr by switching to piscivory and preying upon the smaller charr (Campbell, 1979). These purely piscivorous trout are known as ferox trout and are specifically sought by anglers, so ferox trout viscera and muscle samples could be obtained as by-products from a sport fishery at Loch Ness. As salmonines ingest prey intact with no mastication, it was hypothesised that trout gut contents could be examined for prey-fish, whose own gut contents might themselves yield zooplankton prey. In this way, we aimed to test whether: (1) GCA of large predatory fish could provide samples from several trophic levels; (2) such samples could then be further prepared for SIA whilst assessing any detrimental effects the gut environment may have on the quality of SIA values produced; (3) short- and long-term dietary information provided by GCA and SIA provided comparable data; (4) ferox trout are unselective in prey-fish choice because of the paucity of the Loch Ness pelagic; and (5) to utilise the isotopic data produced to place the Arctic charr and ferox trout in context with the rest of the Loch Ness pelagic food web, previously characterised by Grey *et al*. (2001).

Methods

Fish samples

Samples were made available by anglers between the months of April and October. Trout were caught during daylight hours by trolling assorted lures 2–30 m below the surface of water, within a 5 km radius of Urquhart Bay, Loch Ness. Fish wet weight (±5.0 g) and snout to tail fork length (±5.0 mm) were recorded upon capture. In the laboratory, a small portion of muscle tissue was taken from either side of the dorsal fin above the lateral line or from the pair of pectorals. One was treated to remove lipids according to the protocol of Bligh & Dyer (1959), whilst the other remained untreated. Lipid synthesis favours the lighter carbon isotope so tissues containing lipid stores tend to be more ^{13}C-depleted (DeNiro & Epstein, 1977), but the protocol used to remove the lipid fraction may have an adverse effect on the nitrogen isotope ratios (Pinnegar & Polunin, 1999). Both samples were placed in pre-combusted glass vials, oven dried at 65 °C, pulverised and stored frozen for SIA. The total alimentary canal was dissected from individual fish and preserved frozen until analysis.

Prey-fish found within the gut contents were identified, weighed and measured if the state of digestion allowed. Muscle tissue was removed as above and treated in the same manner. When possible, the fore-gut was also removed from the prey-fish and dissected. Invertebrates from gut contents were washed repeatedly with Milli Q water and separated into two fractions with an 800 μm mesh filter. Most zooplankton material passed through the mesh leaving macroinvertebrate material trapped. Macroinvertebrates were examined wet using a Leica stereozoom microscope and identified to species level if possible. Total zooplankton, or subsamples thereof, were examined microscopically in a Bogorov chamber (Newell & Newell, 1963). In samples of advanced digestion, recalcitrant indicator parts of organisms, such as the caudal spine of *Bythotrephes longimanus* Leydig, were used to indicate presence and estimate abundance (Thackeray, Grey & Jones, 2000). Macroinvertebrate and crustacean zooplankton samples were collected onto precombusted Whatman GF/F filters, oven dried at 65 °C and stored frozen for subsequent SIA.

Stable isotope analysis

Carbon and nitrogen isotopic analysis 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 were peeled away from the glass fibre on drying and combusted. Larger samples were ground under liquid nitrogen in a freezer mill (Spex Industries Inc.). Results are given using the δ notation,

$$d = [(X_{sample}/X_{reference}) - 1] \times 1000$$

expressed in units of per mil (‰) where $X = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.. The reference materials used were atmospheric nitrogen, and secondary standards of known relation to the international standard of Pee Dee belemnite for carbon. Typical precision for a single analysis was ± 0.1‰ for carbon and ± 0.3‰ for nitrogen.

Results

Morphometrics and gut analyses

Data come from the examination of 31 trout which had either consumed fish or had completely empty stomachs. These fish caught by rod and line exhibited a weight–length relationship, consistent with the few previous records of Loch Ness ferox trout (Martin & Shine, 1993). However, the current study included 12 fish over 600 mm in length and weighing up to 5.4 kg (larger than sampled by Martin & Shine, 1993). Twenty-eight prey-fish were extracted from the guts of ferox trout: 19 Arctic charr, five brown trout and two salmon parr (*Salmo salar* Linnaeus). Two prey-fish were too digested to enable identification. The majority of ferox trout contained a single prey-fish, but individuals were found with three or four prey, usually of similar size.

Twelve Arctic charr, four brown trout and one salmon parr were sufficiently intact to allow investigation of their gut contents. The cladocerans, *B. longimanus* and *Daphnia hyalina* Sars dominated the gut contents of most of the Arctic charr (Table 1), but benthic organisms (ostracods and chironomid larvae) and aerially derived insects were present in some stomachs. The number of prey species found within the gut was inversely proportional to charr size

Table 1 The abundance of aquatic prey species found within gut contents of Arctic charr dissected from ferox trout stomachs

Fish no.	Fish Length (mm)	*Bythotrephes*	Daphnia	Leptodora	Copepods	*Bosmina*	Chironomid pupae	Chironomid larvae	Ostracods
1	170	–	–	–	–	–	25	–	–
2	175	121	4	1	–	–	–	–	–
3	135	150	42	4	–	–	–	1	–
4	110	98	45	–	–	–	7	–	–
5	160	188	49	–	–	–	17	2	–
6	229	16	44	–	–	–	–	–	–
7*	47	1	4	–	17	4	–	3	1
8*	68	49	93	3	3	1	–	1	–
9	170	112	17	3	–	–	–	–	–
10	120	36	47	–	9	–	3	–	–
11	64	4	7	2	2	5	–	–	–
12	95	13	21	6	4	–	–	–	–

*Fish also containing evidence of aerially derived invertebrates.

(Fig. 1). Brown trout and salmon parr contained a variety of aerial insects and littoral-benthic macroinvertebrates, with a low percentage abundance of zooplankton contributing to the trout gut contents (<10% of ingested prey individuals).

Stable isotope analyses

Fish muscle $\delta^{13}C$ values were derived from the samples with lipids removed whilst $\delta^{15}N$ values were derived from untreated samples. Mean $\delta^{13}C$ and $\delta^{15}N$ signatures for each component are presented in Table 2. The mean $\delta^{13}C$ of ferox trout was –25.7 ± 1.2‰ (range –23.2 to –28.4‰). Smaller trout tended to be more ^{13}C-depleted but no significant enrichment was found with increasing size ($t = 0.9$, $P > 0.05$). Less variation was found in $\delta^{15}N$ which had a mean of 13.5 ± 1.0‰ (range 11.3–14.5‰) and no significant enrichment with increasing size ($t = 1.23$, $P > 0.05$).

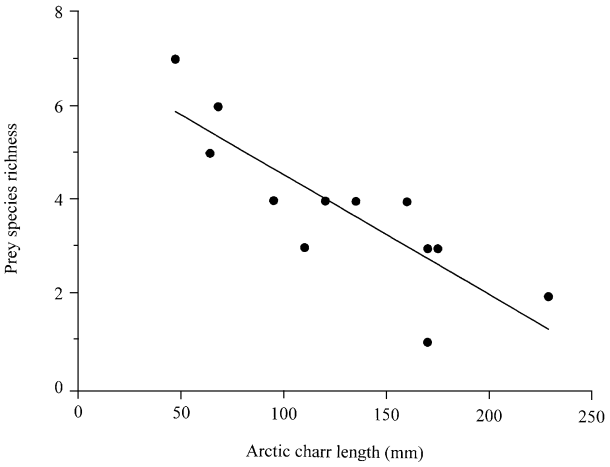

Fig. 1 Relationship between species richness in gut contents of Arctic charr and fish length. Fitted line is a first order regression, $y = 7.1–0.03x$, $r^2 = 0.7$.

Arctic charr dissected from guts of ferox trout exhibited less variability in isotopic ratios than brown trout. Salmon parr exhibited more ^{13}C-enrichment (–20.5 ± 0.1‰) and ^{15}N-depletion (8.2 ± 0.1‰) than other prey-fish. The gut contents of Arctic charr were generally ^{13}C-depleted relative to those of brown trout, whilst those of salmon parr were considerably enriched. The $\delta^{15}N$ values for prey fish gut contents were inversely related to their corresponding $\delta^{13}C$ values. Zooplankton from Arctic charr were ^{15}N-enriched compared with macroinvertebrates from the salmonids.

However, digestive processes may have had a detrimental effect on isotope sample quality. Acidification has been reported to affect the $\delta^{15}N$ of samples (Pinnegar & Polunin, 1999) and thus the digestive tract is potentially a hostile environment for nitrogen isotope ratio stability. Samples from prey-fish and gut contents were compared statistically (*t*-test) with muscle and prey specimens collected directly from the loch. Crustacean zooplankton $\delta^{13}C$ and $\delta^{15}N$ values derived from fish gut samples were within the inherent natural variability exhibited by plankton-net caught specimens from the same location ($t = 0.95$, $t = 0.35$, respectively, $P > 0.05$ for both). Similarly, Arctic charr muscle taken from ferox trout gut

Table 2 Mean carbon and nitrogen isotopic signatures (±1 SD) exhibited by each component. Gut contents are labelled by numerical dominance

Component	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Ferox trout	−25.7 ± 1.2	13.5 ± 1.0
Arctic charr	−28.0 ± 1.0	11.7 ± 0.6
Brown trout	−25.4 ± 1.7	10.7 ± 1.8
Salmon parr	−20.5 ± 0.1	8.2 ± 0.1
Arctic charr gut contents (zooplankton)	−29.2 ± 1.3	9.6 ± 0.3
Brown trout gut contents (macroinvertebrates)	−27.7 ± 0.9	7.2 ± 0.3
Salmon parr gut contents (macroinvertebrates)	−22.7	6.0

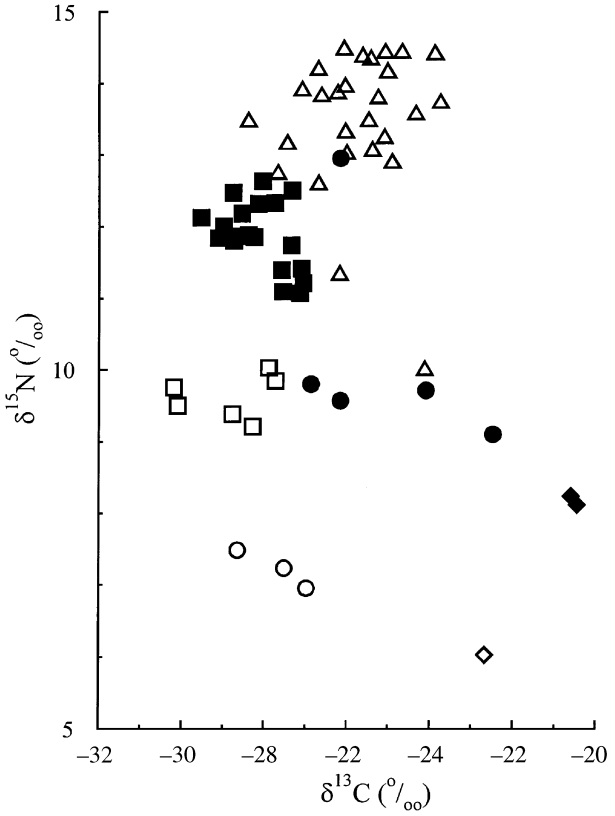

Fig. 2 Carbon and nitrogen isotope composition of fish and their gut contents from Loch Ness. Open triangles – ferox trout; filled squares – Arctic charr; open squares – Arctic charr gut contents (zooplankton); filled circles – brown trout; open circles – brown trout gut contents (macroinvertebrates); filled diamonds – salmon parr; open diamond – salmon parr gut contents (macroinvertebrates).

samples were not significantly different ($t = 0.75$, $t = 0.36$ for $\delta^{13}C$ and $\delta^{15}N$, respectively, $P > 0.05$ for both) from the few charr samples collected by Martin & Shine (1993).

When the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures for ferox trout and their prey-fish were plotted against each other (Fig. 2), separation of the salmonine species was achieved. The majority of brown trout prey-fish appeared to be at the same trophic level as the zooplankton prey of Arctic charr, with one outlying fish exhibiting a signature more typical of ferox trout. Prey-fish gut contents were more distinct, with the $\delta^{15}N$ values clearly separating zooplankton or macroinvertebrate dominance.

Discussion

The predatory nature of ferox trout in Loch Ness was used to provide samples of a number of trophic levels from one source. Despite 42% of ferox trout having empty stomachs, 28 prey-fish were extracted from the remainder and 17 of these yielded discernible stomach contents. Thus, information from three trophic levels was extracted from single fish. Although the sample number was small compared with typical net or trawl fish surveys in more productive environments, our approach produced valuable samples for analysis in circumstances under which other collection methods were impractical or prohibited.

Twelve Arctic charr were sufficiently intact to allow GCA. These fish had ingested mostly crustacean zooplankton, primarily the larger cladocerans consistent with other charr dietary studies (Maitland *et al.*, 1984). The inverse relationship between prey species richness in the gut and Arctic charr size (Fig. 1), suggests increasing specialisation with age. Smaller charr had ingested a variety of prey (benthic and aerially derived invertebrates), although crustacean zooplankton was predominant, indicative of pelagic or nearshore foraging. In contrast, smaller charr have been found to be epibenthic in other studies (Langeland *et al.*, 1991). Certainly, most Arctic charr from the pelagic zone of Loch Ness have been recorded acoustically between 10 and 30 m (George & Winfield, 2000). Arctic charr recorded from the Loch Ness profundal (220 m) tend to be larger individuals, mostly >200 mm (Shine & Martin, 1988). The larger charr in the current study were planktivorous and contained pelagic crustacean zooplankton species. The appearance of chironomid larvae in the guts of larger charr from Loch Ness is not necessarily indicative of benthic feeding, as late instar chironomid

larvae have been recorded frequently in the epilimnetic waters of Loch Ness (Hewitt, 2000). If larger charr were truly feeding on benthos, one might expect higher numbers of larvae of differing instars in gut samples. Supplementary evidence provided by charr morphology indicates that the specimens examined in the current study were pelagic and not typical benthic morphs described from Loch Ness (Martin & Shine, 1993).

Variability in zooplankton $\delta^{15}N$ from Arctic charr guts was likely a consequence of two taxa (*Bythotrephes* and *Daphnia*) that occupy different trophic levels comprising the gut contents in different ratios. Variability in zooplankton $\delta^{13}C$ from charr guts may also reflect a temporal shift in isotopic ratios in the smaller fractions of the plankton, because charr were taken over a number of months. The diet of Loch Ness zooplankton shifts from one derived from allochthonous inputs during winter and spring, to one dominated by autochthonous sources during summer (Grey *et al*., 2001). As zooplankton body size is small and metabolic turnover high, zooplankton isotopic ratios can alter within days (Grey, 2000a). The lack of variability in isotopic signatures of charr muscle (coefficient of variation, 3.4 and 6.6% for $\delta^{13}C$ and $\delta^{15}N$, respectively) indicates specialist diet selection and assimilation of zooplankton. An alternative explanation could be slower elemental turnover in tissues of larger fish (Grey, 2000a). However, brown and ferox trout are of comparable size (and larger) and exhibit substantial variability.

In contrast to Arctic charr, $\delta^{13}C$ of brown trout removed from ferox stomachs indicated a diet of macroinvertebrates. These littoral and aerial macroinvertebrates were assumed to be deriving the bulk of their carbon from allochthony because the basin morphometry of Loch Ness precludes significant contribution of organic matter from littoral sources (Jones *et al*., 1998). However, the catholic taste of brown trout is well documented (Elliott, 1994) and indeed supported here by the variation in trout $\delta^{13}C$. The mean $\delta^{15}N$ of brown trout prey-fish was lower than that of charr reflecting a food chain stemming from a different basal resource. The distinct signatures of the salmon parr partly reflect an initial parental input of isotopes derived from marine material, and a diet of ^{13}C-enriched, early instar lotic macroinvertebrates.

One hypothesis to be tested in this study was whether ferox trout would be unselective in the choice of prey because of the paucity of the Loch Ness pelagic food web. Although Arctic charr constitute 95% of the Loch Ness open water fish community (Bean *et al*., 1996) they represented only 73% of prey. Furthermore, brown trout accounted for 19% of prey fish ingested, contrary to the findings of L'Abée-Lund *et al*. (1992) who suggested that cannibalism was not significant in their Scandinavian systems. Substituting values for brown trout from the current study into Ivlev's Index ($E = 0.58$) suggests that brown trout are actively selected (Ivlev, 1961). Yet this may reflect migration of ferox between pelagic and nearshore waters to feed where relative dominance of prey species is likely to differ.

Trout that become purely piscivorous (i.e. ferox trout), tend to switch when 30–35 cm in length (Greer, 1995). The smallest trout included in the current study (290 mm) contained four prey fish as well as macroinvertebrates and zooplankton. Such a mixed diet indicates this trout may have been in the process of 'switching' to a ferox state; its $\delta^{15}N$ signature was still similar to that of other brown trout prey-fish suggesting little contribution from piscivory to muscle tissue signature prior to capture. The outlying trout prey fish which exhibited elevated $\delta^{15}N$, had a completely empty stomach and was probably a small ferox trout itself. Defining the exact transition period from brown to ferox trout is difficult because the switch is likely to occur gradually, if it occurs at all. Thus, one would expect some overlap of ferox and brown trout isotopic signatures as indeed was found (Fig. 2).

Trophic relationships

To relate Arctic charr and ferox trout nitrogen isotope values to the concept of trophic levels, an isotopic baseline or primary trophic level (TL1) was assigned (Fig. 3). The majority of samples considered in the current study were obtained during summer when the zooplankton community derived most of its biomass from phytoplankton production (Grey *et al*., 2001). Primary trophic level was assigned a $\delta^{15}N$ value of 3.1‰ (mean value from phytoplankton analyses weighted according to chlorophyll a concentration, Grey *et al*., 2001). Incremental, expected trophic levels were then assigned to grazing and predatory zooplankton assuming a $\delta^{15}N$ increase of 3.3‰ per

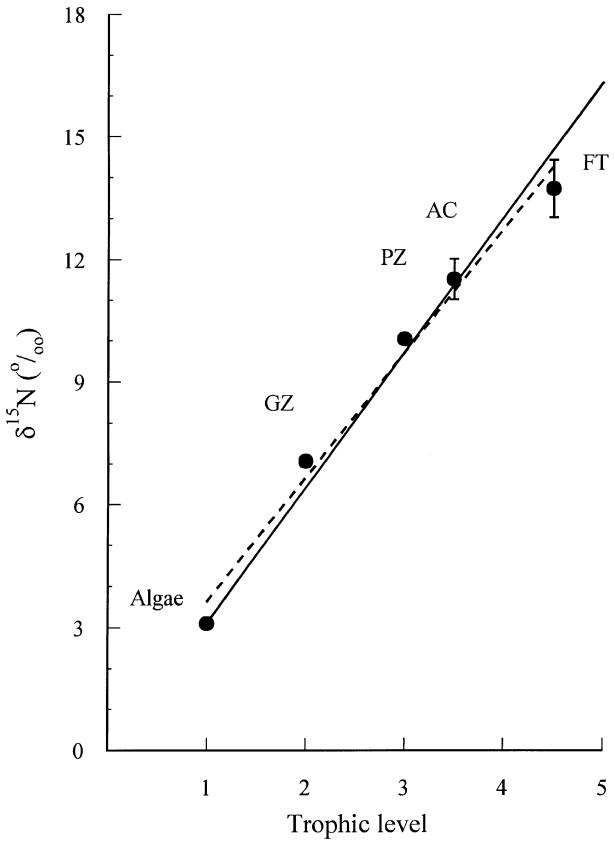

Fig. 3 Relationship between $\delta^{15}N$ and trophic level for the Loch Ness pelagic food web. Trophic levels were assigned from the knowledge of each animal's feeding habits: algae = TL1; GZ = grazing zooplankton (TL2); PZ = predatory zooplankton (TL3); AC = Arctic charr (TL3.5); FT = ferox trout (TL4.5). Solid line represents model fit assuming 3.3‰ increase in $\delta^{15}N$ per TL. Dashed line represents first order regression, $\delta^{15}N = 3.0$ (TL) + 0.6, $r^2 = 0.99$. Means ± 1 SD.

trophic level (Gorokhova & Hansson, 1999). Charr were assigned an increase representing an equal dietary mix of both grazing and predatory zooplankton based on GCA, and ferox trout assumed to be one further trophic level above charr. Ferox trout were lighter in ^{15}N than might be expected from a pure charr diet (Fig. 3) indicative of a proportion of diet being sourced from other fish species. Thus, both GCA and SIA of ferox trout provide comparable results.

The observed and expected nitrogen isotope data for zooplankton and charr were well matched, suggesting the GCA and SIA results were complementary. Therein lies a paradox, because zooplankton are only abundant seasonally in Loch Ness (Grey, 2000b) and alternative dietary sources (e.g. chironomids and other macroinvertebrates) are isotopically distinct (e.g. chironomid $\delta^{13}C$–35 to –40‰, $\delta^{15}N$ 0.8–2.0‰, Grey, 2002). Charr caught in February and March yielded no gut contents (Martin & Shine, 1993). It appears that charr in Loch Ness may not feed at all or simply subsist during winter on a meagre ration of littoral-benthic organisms (I.J. Winfield, personal communication), and little, if any, may be incorporated into biomass. The lack of variation in charr isotopic signatures suggests biomass is accumulated only during summer when the food web is driven by autochthonous production. To estimate the contribution of autochthonous carbon to charr biomass, mean $\delta^{13}C$ values for autochthonous (phytoplankton – 30.7‰) and allochthonous (POM –25.4‰) sources (Grey *et al.*, 2001) were used as end members in a two-source mixing model (Peterson & Fry, 1987). Carbon trophic enrichment factors of 0.43 and 1.21‰ (zooplankton and Arctic charr, respectively, Grey, 2000a), were applied to the mean Arctic charr $\delta^{13}C$ value of –27.8‰, assuming charr to be 2.4 trophic levels above an algal baseline (suggested by $\delta^{15}N$ values). Of these 2.4 trophic levels, 1.4 were assigned to zooplankton (a mix of grazers and predators), whilst the remaining 1.0 was assigned to charr. The model indicated that charr were 80% reliant upon autochthonous production. During summer, zooplankton derive about 80% carbon from autochthonous sources and 20% from allochthonous sources (Grey *et al.*, 2001). Therefore, despite 40% of zooplankton carbon being derived from allochthonous sources over an entire annual cycle (Grey *et al.*, 2001), charr biomass appears to be mainly accumulated during summer, and therefore is predominantly autochthonous in origin.

To conclude, GCA of ferox trout from Loch Ness provided sets of samples, analogous to nested Russian dolls, which not only yielded information on ingested prey, but also material for SIA. The effects of digestive processes on nitrogen isotope ratio stability within the gut environment were found to be negligible. Both GCA and SIA indicated the importance of zooplankton to the diet of Arctic charr. However, despite charr being the dominant pelagic fish in Loch Ness, charr density was too low to support ferox trout on its own. Gut content analysis and SIA indicated that the deficit was supplemented by cannibalism, probably from the nearshore zone. The isotope data generated provides valuable information regarding sources and fluxes of production through the Loch Ness food web to fish

species of conservation or commercial value, with implications for fisheries management in larger, oligotrophic lake systems.

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References

Aass P. (1990) Management of Arctic charr (*Salvelinus Alpinus L.*) and brown trout (*Salmo trutta* L.) fisheries in Lake Tunhovdfjord, a Norwegian hydroelectric reservoir. In: *Management of Freshwater Fisheries* (Eds W.L.T. van Densen, B. Steinmetz & R.H. Hughes), pp. 382–389. Pudoc, Wageningen.

Bean C.W., Winfield I.J. & Fletcher J.M. (1996) Stock assessment of the Arctic charr (*Salvelinus Alpinus*) population in Loch Ness, UK. In: *Stock Assessment in Inland Fisheries* (Ed. I.G. Cowx), pp. 206–223. Blackwell.

Beaudoin C.P., Tonn W.M., Prepas E.E. & Wassenaar L.I. (1999) Individual specialization and trophic adaptability of northern pike (*Esox lucius*): an isotope and dietary analysis. *Oecologia*, **120**, 386–396.

Bligh E.G. & Dyer W.J. (1959) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.

Burns J.M., Trumble S.J., Castellini M.A. & Testa J.W. (1998) The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. *Polar Biology*, **19**, 272–282.

Campbell R.N. (1979) Ferox trout, *Salmo trutta* (L.) and charr, *Salvelinus alpinus* (L.) in Scottish lochs. *Journal of Fish Biology*, **14**, 1–29.

DeNiro M. & Epstein S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science*, **197**, 261–263.

DeNiro M. & Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica Cosmochimica Acta*, **42**, 495–506.

Dill L.M. (1983) Adaptive flexibility in the foraging behaviour of fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, **40**, 398–408.

Elliott J.M. (1994) *Quantitative ecology and the brown trout*. Oxford Series in Ecology and Evolution, Oxford.

Gearing J.N. (1991) The study of diet and trophic relationships through natural abundance ^{13}C. In: *Carbon Isotope Techniques* (Eds D.C. Coleman & B. Fry), pp. 201–218. Academic Press, London.

George D.G. & Winfield I.J. (2000) Factors influencing the spatial distribution of zooplankton and fish in Loch Ness, UK. *Freshwater Biology*, **43**, 557–570.

Gorokhova E. & Hansson S. (1999) An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 2203–2210.

Greer R.B. (1995) *Ferox Trout and Arctic Charr: A Predator, its Pursuit and its Prey*. Swan Hill Press, Shrewsbury, UK.

Grey J. (2000a) The effects of diet switch on isotopic signatures of pelagic consumers. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, **27**, 3187–3191.

Grey J. (2000b) Tracing the elusive *Holopedium gibberum* the plankton of Loch Ness. *Glasgow Naturalist*, **23**, 29–34.

Grey J. (2002) A chironomid conundrum: questions arising from stable isotope analysis. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, **28** (in press).

Grey J., Jones R.I. & Sleep D. (2001) Seasonal changes in the importance of allochthonous organic matter to the diet of zooplankton in Loch Ness indicated by stable isotope analysis. *Limnology and Oceanography*, **46**, 505–513.

Hewitt D.P. (2000) *Factors influencing the spatial distribution of crustacean zooplankton in Loch Ness*. M.Phil. Thesis, Lancaster University, Lancaster.

Hobson K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120**, 314–326.

Hobson K.A. & Welch H.E. (1995) Cannibalism and trophic structure in a high Arctic lake: insights from stable-isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 1195–1201.

Hyslop E.J. (1980) Stomach contents analysis – a review of methods and their application. *Journal of Fish Biology*, **17**, 411–429.

Ivlev V.S. (1961) *Experimental Ecology of the Feeding of Fishes*. Yale University Press, New Haven, CT.

Jones R.I., Grey J., Quarmby C. & Sleep D. (1998) An assessment using stable isotopes of the importance of allochthonous organic carbon sources to the pelagic food web in Loch Ness. *Proceedings of the Royal Society of London B*, **265**, 105–111.

L'Abée-Lund J.H., Langeland A. & Sægrov H. (1992) Piscivory by brown trout *Salmo trutta* (L.) and Arctic charr *Salvelinus alpinus* (L.) in Norwegian lakes. *Journal of Fish Biology*, **41**, 91–101.

Langeland A., L'Abée-Lund J.H., Jonsson B. & Jonsson N. (1991) Resource partitioning and niche shift in Arctic charr *Salvelinus alpinus* and brown trout *Salmo trutta*. *Journal of Animal Ecology*, **60**, 895–912.

Maitland P.S., Greer R.B., Campbell R.N. & Friend G.F. (1984) The status and biology of the Arctic charr. *Salvelinus Alpinus* L. in Scotland. In: *Biology of the Arctic charr: Proceedings of the International Symposium on Arctic charr* (Eds Johnson & Burns), pp. 193–215. University of Manitoba Press, Winnipeg.

Martin D.S. & Shine A.J. (1993) The food and feeding relationships of pelagic fish in Loch Ness. *Scottish Naturalist*, **105**, 149–174.

Minagawa M. & Wada E. (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, **48**, 1135–1140.

Newell G.E. & Newell R.C. (1963) *Marine Plankton: A Practical Guide*. Hutchinson Biological Monographs.

Peterson B.J. & Fry B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecological Systems*, **18**, 293–320.

Pinnegar J.K. & Polunin N.V.C. (1999) Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. *Functional Ecology*, **13**, 225–231.

Polis G.A. & Winemiller K.O. (1996) *Food Webs: Integration of Patterns and Dynamics*. Chapman & Hall, London.

Shine A.J. & Martin D.S. (1988) Loch Ness habitats observed by sonar and underwater television. *Scottish Naturalist*, 111–199.

Sydeman W.J., Hobson K.A., Pyle P. & McLaren E.B. (1997) Trophic relationships among seabirds in central California: combined stable isotope and conventional dietary analysis. *Condor*, **99**, 327–336.

Thackeray S.J., Grey J. & Jones R.I. (2000) Feeding selectivity of brown trout (*Salmo trutta*) in Loch Ness, Scotland. *Freshwater Forum*, **13**, 47–59.

Vander Zanden M.J., Cabana G. & Rasmussen J.B. (1997) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}N$) and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1142–1158.

Whitledge G.W. & Rabeni C.F. (1997) Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2555–2563.

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