Temporal host-parasite relationships of the wild rabbit, *Oryctolagus cuniculus* (L.) as revealed by stable isotope analyses

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SUMMARY

Natural abundances of the stable isotopes, $^{15}N/^{14}N$ ($\delta^{15}N$) and $^{13}C/^{12}C$ ($\delta^{13}C$), were used to study temporal host-parasite relationships of the wild rabbit, *Oryctolagus cuniculus* (L.). During the 12-month sampling period, temporal isotopic shifts in $\delta^{15}N$ were noted for dietary vegetation, host rabbit faeces and fur, but not for muscle or stomach contents. $\delta^{15}N$ varied temporally for the parasitic cestode species, *Mosgovoyia pectinata* but not for *Cittotaenia denticulata*. Similarly, intestinal parasitic nematodes had apparent species-specific $\delta^{15}N$ patterns. Only rabbit fur and intestinal parasitic nematodes did not exhibit temporal shifts in $\delta^{13}C$. Overall, host faeces and stomach contents were isotopically indistinct as a likely consequence of coprophagy. Relative to their host, parasitic nematodes were ^{15}N-enriched, consistent with an increase in trophic level status. Conversely, cestodes were ^{15}N-depleted. Isotopically, each parasite reflected a species-specific relationship with their rabbit host. This technique could be utilized to integrate parasites into food-web studies.

Key words: host-parasite interactions, rabbit, stable isotopes, trophic interactions.

INTRODUCTION

The European rabbit (*Oryctolagus cuniculus*) was most likely introduced into the United Kingdom in the late twelfth century (Lever, 1977). Due to its rapid reproductive cycle and changes in land management (Pollard, Hooper & Moore, 1974), the European rabbit is now one of the major agricultural pests with an estimated loss of revenue of > £100 million to UK agriculture (Mills, 1986). Consequently, the ecology of the rabbit has been well studied (Thompson & King, 1994). However, there is a paucity of data on both the temporal trophic dynamics of the rabbit and the trophic-level interactions between the rabbit host and its parasites.

Naturally occurring stable isotopes of carbon (C) and nitrogen (N) have been utilized by ecologists and zoologists as a tool to determine trophic relationships within a community (e.g. Tieszen *et al*. 1979; Hobson & Welch, 1992; Scrimgeour *et al*. 1995; Neilson *et al*. 1998; Neilson, Boag & Smith, 2000). Furthermore, hitherto unknown temporal changes in diet have also been identified using stable isotope analyses (Ramsay & Hobson, 1991; Ben-David, Flynn & Schell, 1997; Godley *et al*. 1998; Hobson, Drever & Kaiser, 1999). Natural abundances of stable isotopes are effectively an integrated record of assimilated elements such as C and N (Peterson & Fry, 1987; Robinson, 2001) and, as such, are a better representation of the recent biochemical and dietary past of an organism than traditional snapshot methods, e.g. gut content analyses. Changes in the ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (expressed as $\delta^{13}C$ and $\delta^{15}N$) in animal tissue are indicative of dietary sources and trophic grouping, respectively (DeNiro & Epstein, 1978, 1981; Wada, Kabaya & Kurihara, 1993). Animal tissues such as muscle, hair (fur) and liver have different turnover rates of carbon and nitrogen due to differences in their metabolic activity (Tieszen *et al*. 1983; Hobson & Clark, 1992; Pinnegar & Polunin, 1999) and have been used as a proxy to indicate short and long-term dietary sources in birds (Hobson & Sealy, 1991; Hobson & Clark, 1992; Hobson, 1993), mammals and reptiles (Tieszen *et al*. 1983; Ames, van Vleet & Sackett, 1996; Hilderbrand *et al*. 1996; Godley *et al*. 1998; Hobson *et al*. 1999). Previously, Boag *et al*. (1998) from a small dataset ($n = 10$), reported isotopic differences between rabbit tissue (e.g. fur, muscle, embryos and faeces) and also noted that coprophagy had little effect on the isotopic discrimination between faeces and stomach contents.

Although parasites are an integral component of any trophic system, their inclusion in food-web studies is infrequent (Marcogliese & Cone, 1997). Similarly, the utilization of stable isotope techniques in host-parasite studies is rare although a few studies have investigated host-parasite interactions in plants

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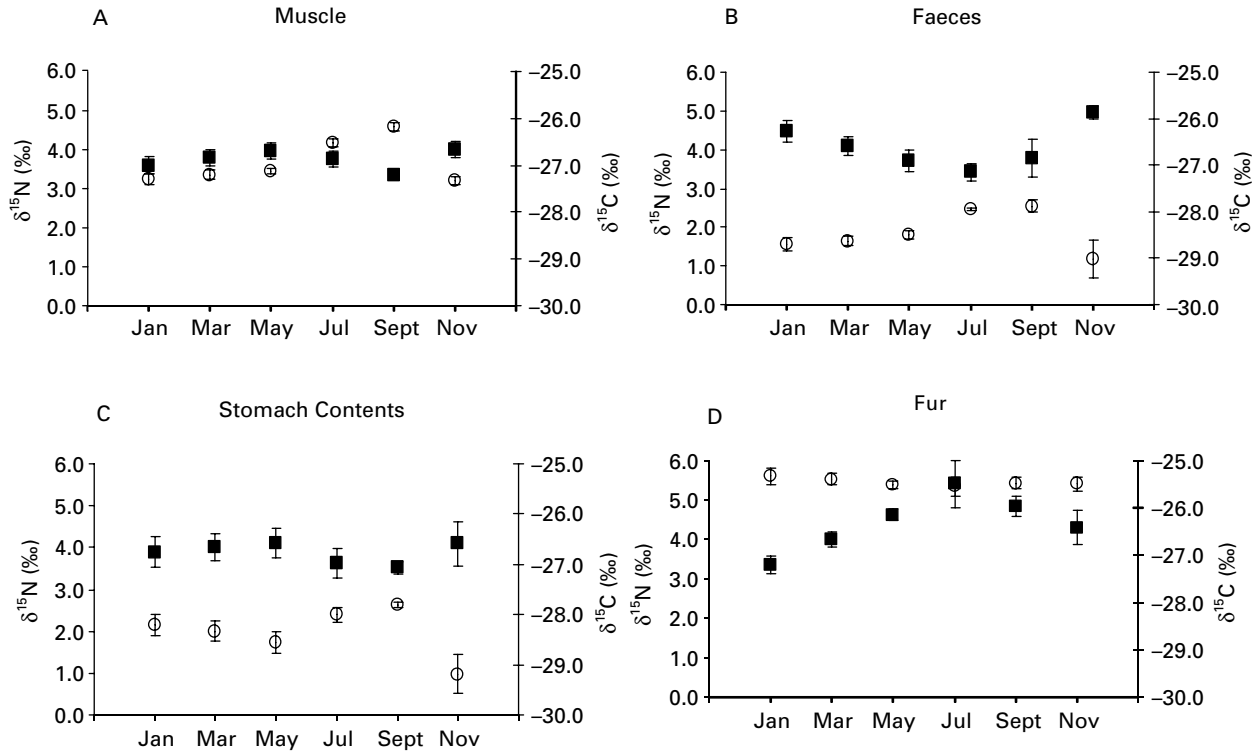

Fig. 1. Temporal mean δ^{15}N (closed squares) and δ^{13}C (open circles) for (A) rabbit muscle; (B) rabbit faeces; (C) rabbit stomach contents and (D) rabbit fur.

(Neilson & Brown, 1999, 2000), chironomids (Doucett, Giberson & Power, 1999) and fish (Iken *et al*. 2001; Pinnegar, Campbell & Polunin, 2001; Deudero, Pinnegar & Polunin, 2002). Data from Boag *et al*. (1998) suggested that different trophic relationships existed between parasitic intestinal nematodes and parasitic cestodes and their host, the European rabbit.

The objective of this paper is to report for the first time, the temporal trophic dynamics of a host-parasite system using the natural abundances of δ^{13}C and δ^{15}N, and to compare the isotopic discriminations between tissues to that reported by Boag *et al*. (1998) from a different wild rabbit population.

MATERIALS AND METHODS

Ten wild rabbits were captured using box traps from a study site located in the Scottish Borders on 6 separate occasions (January, March, May, July, September and November) during a 12-month period. The rabbits were humanely dispatched and post-mortems undertaken to collect tissue samples and intestinal parasites (Boag, 1972). Samples of fur, muscle, stomach contents, faeces and when present, parasitic cestodes (*Mosgovoyia pectinata* and *Cittotaenia denticulata*) and parasitic nematodes (*Graphidium strigosum*, *Passalurus ambiguus* and *Trichostrongylus retortaeformis*) were collected from the rabbits. Samples of vegetation, predominantly graminaceous plants, from within the home range of the sampled rabbits were removed at ground level by secateurs on each sampling occasion. Samples were processed and analysed by continuous-flow isotope ratio mass spectrometry (CF-IRMS) as described by Boag *et al*. (1998).

Isotope natural abundances are reported as:

$$\delta_{\text{sample}} = R_{\text{sample}} - R_{\text{standard}} / R_{\text{standard}} \times 1000‰$$

where R_{sample} and R_{standard} are the heavy/light isotope ratios of sample and standard. Analytical precision was $\leqslant 0{\cdot}2$‰ for δ^{13}C and $\leqslant 0{\cdot}4$‰ δ^{15}N.

A one-way analysis of variance using Minitab (Minitab, Pennsylvania, USA) was done to identify significant temporal differences for host and parasite samples.

RESULTS

$\delta^{15}N$

Muscle and stomach content δ^{15}N varied little ($P = 0{\cdot}365$ and $P = 0{\cdot}829$, respectively) during the sampling period (Fig. 1A, C). In contrast, faeces, fur and vegetation δ^{15}N exhibited statistically significant temporal trends (Figs 1B, D and 2A). Vegetation showed the greatest temporal variation ($P = 0{\cdot}021$) by becoming 2·9‰ less ^{15}N-enriched during the period January to September, thereafter, becoming 2·5‰ ^{15}N-enriched from September to November (Fig. 2A). Faeces also became less ^{15}N-enriched between January and July, although by only 1·0‰ before

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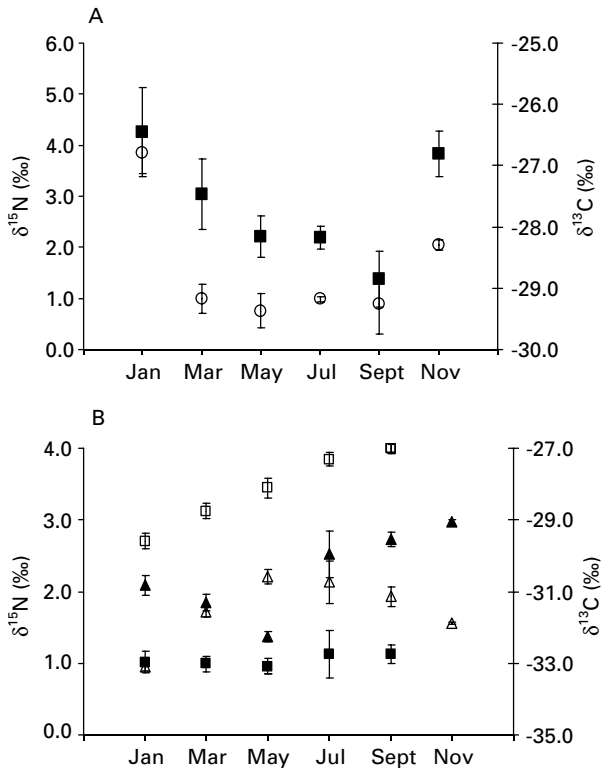

Fig. 2. (A) Temporal vegetation mean δ^{15}N (closed squares) and δ^{13}C (open circles) and (B) Temporal mean δ^{15}N (closed symbols) and δ^{13}C (open symbols) for the parasitic cestodes, *Cittotaenia denticulata* (squares) and *Mosgovoyia pectinata* (triangles).

following the same pattern as vegetation and becoming more ^{15}N-enriched (1·5‰) towards the end of the sampling period ($P=0{\cdot}045$; Fig. 1B). In contrast, between January and July fur became 2·1‰ more ^{15}N-enriched, thereafter gradually becoming less ^{15}N-enriched during the sampling period ($P=0{\cdot}000$; Fig. 1D).

The 3 species of intestinal nematode were only found on the first 3 sampling dates, indicative of their known biology when their intensity is greatest (e.g. *G. strigosum*) or when they are prevalent (Boag, 1988). Consequently, it was not possible to statistically test any observed isotopic temporal differences. Intestinal parasitic nematode δ^{15}N exhibited discordant temporal patterns (Table 1). Between January and May *G. strigosum* became 2·7‰ more ^{15}N-enriched, whereas *T. retortaeformis* became 1·7‰ less ^{15}N-enriched during the same period (Table 1). In contrast, both *P. ambiguus* and the cestode *C. denticulata* ($P=0{\cdot}988$) exhibited no δ^{15}N temporal patterns (Table 1; Fig. 2B). A second cestode species, *M. pectinata*, exhibited significant ($P=0{\cdot}013$) temporal variation in δ^{15}N initially becoming slightly less ^{15}N-enriched during January–May and thereafter 1·6‰ ^{15}N-enriched from May to November (Fig. 2B).

$\delta^{13}C$

Muscle ($P=0{\cdot}000$), faeces ($P=0{\cdot}000$), vegetation ($P=0{\cdot}000$) and stomach content ($P=0{\cdot}036$) δ^{13}C varied temporally during the sampling period (Figs 1A–C and 2). Between September and November, all three tissues became 1·1–1·4‰ more ^{13}C-depleted (Figs 1A–C and 2). In contrast, fur δ^{13}C was uniform throughout the sampling period (Fig. 1D).

Both cestode species became less ^{13}C-depleted during the sampling period ($P=0{\cdot}000$) although the temporal trend of both species differed (Fig. 2B). During the period January–May, intestinal nematode δ^{13}C showed no obvious temporal trends (Table 1).

Trophic interactions

Figure 3 summarizes the isotopic data, based on an average value for each component or parasite group across the entire sampling period. Both rabbit tissues, muscle and fur, were 1·1 and 1·7‰ ^{15}N-enriched, respectively, relative to the host (rabbit) diet (C_3-grass). Similarly, stomach contents and faeces were 1·2 and 1·4‰ ^{15}N-enriched relative to dietary material (Fig. 3). Compared to rabbit muscle, levels of ^{15}N-enrichment differed among intestinal nematode species: *G. strigosum* (5·7‰), *P. ambiguus* (5·3‰) and *T. retortaeformis* (3·1‰)(Table 1, Fig. 3). It should be noted that the nematode data pertained only to the period January–May and, in contrast, the parasitic cestodes were less ^{15}N-enriched than rabbit muscle and as with the nematodes this differed among cestode species, *C. denticulata* (2·7‰) and *M. pectinata* (1·5‰).

Mean faeces and stomach content δ^{13}C were similar to vegetation δ^{13}C (Fig. 3). Overall, both rabbit tissues sampled were less ^{13}C-depleted than the dietary material by 1·7‰ (muscle) and 3·2‰ (fur), respectively (Fig. 3). Unlike δ^{15}N, mean δ^{13}C for *G. strigosum* and *T. retortaeformis* was similar to that for host muscle (Fig. 3). In contrast, *P. ambiguus* was 1·6‰ more ^{13}C-depleted (Fig. 3). Similarly, both cestode species were more ^{13}C-depleted than host muscle by 1·9‰ (*C. denticulata*) and 5·5‰ (*M. pectinata*), respectively (Fig. 3).

DISCUSSION

Information regarding the trophic dynamics of herbivores is maximized by isotopic analysis of a combination of tissue, faecal and stomach content samples (Tieszen *et al.* 1983), providing information on the average long-term, short-term and immediate diet. Dietary information derived from bone collagen is typically used as an indicator of long-term diet as it is considered to be an integrated value over the life-term of the animal (Stenhouse & Baxter, 1979; Tieszen *et al.* 1983; Hobson & Clark, 1992), although Mizutani, Hasegawa & Wada (1986) reported that

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Table 1. Mean δ^{13}C (‰) and δ^{15}N (‰) values for 3 intestinal nematodes recorded from the European rabbit (*Oryctolagus cuniculus*)

(Values in parentheses are standard errors.)

	Graphidium strigosum		*Passalurus ambiguus*		*Trichostrongylus retortaeformis*	
	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
January	−26·7 (0·42)	7·5 (0·61)	−28·7 (0·05)	9·0 (0·31)	−27·4 (0·18)	7·3 (0·36)
March	−26·6 (0·25)	8·7 (0·40)	−28·5 (0·10)	8·9 (0·25)	−27·5 (0·15)	6·8 (0·40)
May	−26·5 (0·15)	10·2 (0·19)	−27·9 (n/d)	8·9 (N.D.)	−27·7 (0·29)	5·6 (0·32)
Mean	−26·6 (0·16)	9·4 (0·45)	−28·6 (0·15)	9·0 (0·25)	−27·5 (0·15)	6·8 (0·39)

N.D., not determined as $n=1$.

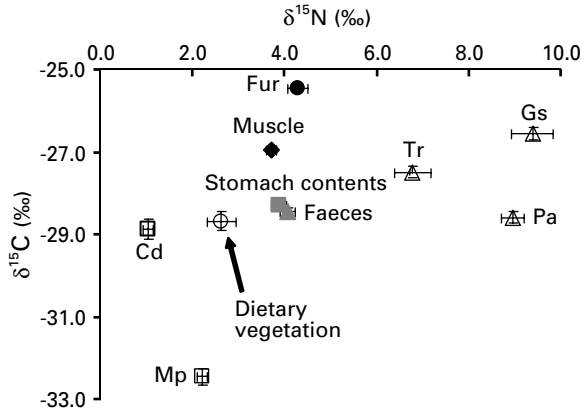

Fig. 3. Seasonal mean δ^{13}C *vs* δ^{15}N for all samples. Cd, *Cittotaenia denticulata*; Gs, *Graphidium strigosum*; Mp, *Mosgovoyia pectinata*; Pa, *Passalurus ambiguus* and Tr, *Trichostrongylus retortaeformis*. In some instances error bars are smaller than the symbols.

collagen isotope values could be indicative of diet primarily during developmental stages.

In this study, sampling was done approximately every 60 days; therefore collagen analysis was considered to be ineffective in measuring any isotopic shifts in diet during the sampling period. Under controlled experiments, isotopic turnover in muscle has been reported as 12·4 days for quails (Hobson & Clark, 1992) and 27·6 days for gerbils (Tieszen *et al*. 1983). Consequently, in the context of this study, muscle was used as a potential indicator of any isotopic shifts in 'long-term' diet.

Samples of vegetation, predominantly grass species, taken from within the home range of the sampled rabbits exhibited temporal isotopic shifts in both δ^{13}C and δ^{15}N, likely to represent temporal water availability and internal translocation of plant N, respectively, similar to that previously reported for a range of plants (Farquhar & Richards, 1984; Stewart *et al*. 1995; Handley & Scrimgeour, 1997; Neilson *et al*. 1998; Chang & Handley, 2000) including *Lolium perenne* (perennial ryegrass) and *Poa* species (Neilson *et al*. 1998, 2002) typical of Scottish uplands such as the study site.

Similarly, rabbit faeces δ^{13}C and δ^{15}N varied temporally. Temporal shifts in rabbit faeces δ^{13}C were opposite to that of dietary δ^{13}C. Whilst the pattern of temporal variation of rabbit faeces δ^{15}N mimicked that of dietary δ^{15}N, the relative changes in ^{15}N-enrichment at each sampling date were less than that measured for dietary material as a likely consequence of fractionation along biochemical pathways (Robinson, 2001). In contrast, rabbit muscle and sampled stomach contents only exhibited temporal isotopic shifts in δ^{13}C but not in δ^{15}N, whereas the opposite was true for rabbit fur, i.e. a temporal shift in δ^{15}N but not δ^{13}C. The temporal shift in fur δ^{15}N may reflect the moulting and regenerating process that commences in March, peaks in July and terminates in October (Cowan, 1991). Selective assimilation of isotopically distinct compounds derived from the same dietary source (Macko *et al*. 1987; Hare *et al*. 1991) and/or differential metabolism of C and N between diet and muscle, fur, and stomach contents may have masked smaller temporal isotopic shifts (Boag *et al*. 1998).

It is unclear why faecal δ^{15}N should have a temporal trend similar to dietary δ^{15}N but stomach content δ^{15}N did not. Rabbits are known to produce two faeces types, soft and hard (Madsen, 1939; Taylor, 1939), the soft being immediately re-ingested by the rabbit directly from the anus during excretion, thus faeces in this study refers to hard faeces that are excreted at night. A detailed description of faeces production by rabbits has been presented by Björnhag (1994) and Hirakawa (2001). Hirakawa (2001) reported that rabbits not only re-ingest soft faeces but also regularly re-ingest hard faeces. Thus a mix of isotopic sources derived from raw dietary material, soft and hard faeces in the stomach may have diluted the overall isotopic value for stomach content.

Although temporal trends of δ^{15}N differed between faecal and stomach content, there was no overall statistical seasonal difference. Based on similar results, Boag *et al*. (1998) suggested that the discriminating branch-point for nitrogen metabolism for lagomorphs occurred within the animal not the gut. Given that in this study there was a clear temporal

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trend in faecal δ^{15}N reflecting dietary material, it is unclear whether this is true. It is possible that the discrepancy in δ^{15}N between faecal material and stomach content indicates that coprophagy may be an isotopic fractionation process contrary to that noted by Boag *et al*. (1998). However, the assertion made by Boag *et al*. (1998) was based on a limited dataset (single sampling date, $n=10$) and it is clear from our data that if sampling of the rabbits used in that study had occurred between May and September, the interpretation by Boag *et al*. (1998) was valid.

The lack of any significant ^{15}N-enrichment in muscle during the sampling period suggests that the sampled rabbit population were not under any nutritional stress (Hobson, Alisaukas & Clark, 1993; Scrimgeour *et al*. 1995).

Intestinal nematodes were recovered from their rabbit hosts only between January and May. Consequently, no comment can be made about temporal isotopic variation during the complete sampling period. However, during the period January–May it was clear that the three parasitic nematode species exhibited different isotopic trends. This may reflect differences in their epidemiology, derivation of dietary sources and/or bioavailability of certain compounds (Neilson & Brown, 1999). Both cestode species were recovered throughout the sampling period. *Cittotaenia denticulata* δ^{15}N was consistent throughout whereas *M. pectinata* δ^{15}N became less ^{15}N-enriched during the first three sampling dates and thereafter became ^{15}N-enriched during the latter half of the sampling period. Similarly, there were different trends in δ^{13}C for both cestode species during the sampling period. The isotopic variation between both cestode species may reflect inter-specific differences in (a) metabolic pathways, (b) lipid content that is derived directly from the host and/or (c) membrane digestion that directly effects which molecules are absorbed (Smyth, 1994). Such differences between nematode species may also indicate differing metabolisms, selective absorption of isotopically distinct compounds derived from either dietary material or the host (Neilson & Brown, 1999).

Averaged over the sampling period, fur was less ^{13}C-depleted than muscle, consistent with that reported by Boag *et al*. (1998) but contradictory to Hilderbrand *et al*. (1996) who noted that a range of rabbit tissues including 'hair' (=fur) were isotopically similar for both δ^{13}C and δ^{15}N, although the rabbits in that study were domesticated and fed a constant diet. Muscle and fur of wild rabbits in this study had similar δ^{15}N values consistent with that previously reported for wild rabbits (Boag *et al*. 1998).

The trophic relationships between intestinal parasites and their host rabbits reported from a small dataset (Boag *et al*. 1998) from an upland pasture in north central Scotland was similar to that reported here from a more extensive dataset from an upland pasture in southern Scotland. All three parasitic intestinal nematode species were ^{15}N-enriched relative to the rabbit host (muscle) with a mean ^{15}N-enrichment of 4·8‰ similar to that reported by Boag *et al*. (1998) and within the range (0–6‰) of a single step increase in putative trophic level (Minagawa & Wada, 1984; Wada *et al*. 1993; Scrimgeour *et al*. 1995; Neilson *et al*. 2000). A similar increase in putative trophic level between parasitic chironomid larvae and stonefly nymphs was reported by Doucett *et al*. (1999), whereas Neilson & Brown (1999) noted that putative trophic level increases appeared to be species specific when 5 different longidorid plant-parasitic nematode species were studied. In contrast, nematode parasites of fish have been reported to be less ^{15}N-enriched relative to their respective hosts (Iken *et al*. 2001; Pinnegar *et al*. 2001; Deudero *et al*. 2002). Furthermore, Deudero *et al*. (2002) reported species-specific δ^{15}N values for nematode parasitic taxa from 10 different fish hosts. Similarly, our nematode δ^{15}N data suggest specific host-parasite relationships that may reflect the derivation of N from different host sources, for example, *T. retortaeformis* from villi, *G. strigosum* from blood and *P. ambiguus* from intestinal bacteria (Barker & Ford, 1975) that in turn affect the bioavailability of certain compounds and/or bio-molecules (Neilson & Brown, 1999).

As with the parasitic nematode species, the cestodes *C. denticulata* and *M. pectinata* were isotopically different. In contrast to nematodes, cestodes were ^{15}N-depleted relative to host, concurring with previous studies on rabbit and fish hosts (Boag *et al*. 1998; Pinnegar *et al*. 2001; Deudero *et al*. 2002) but in complete disagreement with that expected of a consumer relative to its host.

With few exceptions, total lipid content of cestodes (20–35% *vs* 5–12% of dry tissue) is greater than that of nematodes (see Table 1, Frayha & Smyth, 1983; Köhler & Voight, 1988) and consequently cestodes are typically significantly more ^{13}C-depleted than nematodes (DeNiro & Epstein, 1978; Focken & Becker, 1998; Pinnegar & Polunin, 1999). This was true for *M. pectinata* but not for *C. denticulata*. The former being 3·9–5·9‰ more ^{13}C-depleted than any of the three parasitic nematode species recovered from the rabbit host, whereas the latter had a similar δ^{13}C value to the parasitic nematode, *P. ambiguus*. The apparent species-specific difference in δ^{13}C between *C. denticulata* and *M. pectinata* noted both in this study and Boag *et al*. (1998) may be as a result of either selective absorption of fatty acids and subsequent lipid fraction incorporation (Jacobsen & Fairburn, 1967; Barrett, 1981) or the source of fatty acid (Smyth, 1994), as studied extensively in the cestode, *Hymenolepis diminuta* (Frayha & Smyth, 1983).

This study provides additional information on the trophic relationships of a closed host-parasite system and strongly suggests that there are both temporal and species-specific differences between parasites and their relationship with the host. Furthermore,

isotopic analyses can provide opportunities to characterize the role of parasites within food webs (Marcogliese & Cone, 1997) and compound-specific stable isotope analysis (Evans *et al*. 2003) could be utilized to study specific metabolic pathways in parasites, e.g. lipid metabolism by analysing isotopic variation in cholesterol (Chamberlain *et al*. 2004).

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