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Katja Oelbermann · Stefan Scheu

Stable isotope enrichment ($\delta^{15}N$ and $\delta^{13}C$) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality

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Abstract Analysis of the natural variations in stable isotope ratios in animal tissue may be a powerful tool to reveal the trophic position and feeding preferences of generalist predators such as lycosid spiders. In the present study, changes in $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios in the lycosid spider *Pardosa lugubris* (Araneae: Lycosidae), fed with prey of different quality, were investigated. Experimental food chains included three trophic levels: prey media, prey organism and predator. In order to analyse the time course of stable isotope enrichment, different life stages of *P. lugubris* feeding on *Drosophila melanogaster* were studied. The ^{15}N content of hatchlings of *P. lugubris* was significantly lower than that of their mothers, indicating the existence of nitrogen pools with different ^{15}N signatures in female *P. lugubris*. With duration of feeding and progressing development, age and body weight, the ^{15}N content in spiderlings increased. Starvation resulted in ^{15}N and ^{13}C enrichment in *P. lugubris*. Being fed with prey of different quality resulted in varying patterns of stable isotope enrichment. ^{15}N but not ^{13}C content consistently increased when fed on high quality prey (*Heteromurus nitidus*, *D. melanogaster* and a mixed diet consisting of *H. nitidus* and *D. melanogaster*). In contrast, low quality prey (*Rhopalosiphum padi*, *Folsomia candida* and a mixed diet consisting of *H. nitidus*, *D. melanogaster* and *F. candida*) resulted in deviations from postulated patterns of stable isotope enrichment with ^{15}N content being similar to that of starving spiderlings. When fed on high quality prey, ^{15}N enrichment of *P. lugubris* was close to ca. 3‰. It is concluded that the analysis of variations in the natural abundance of stable isotopes is particularly helpful in revealing the trophic structure of terrestrial food webs in which polyphagous feeders predominate, as is the case in litter and soil.

K. Oelbermann (✉) · S. Scheu
Darmstadt University of Technology, Institute of Zoology, Schnittspahnstrasse 3, 64287 Darmstadt, Germany
e-mail: oelb@bio.tu-darmstadt.de
Tel.: +49-6151-165521, Fax: +49-6151-166111

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Introduction

Spiders are typical generalist predators feeding on a multitude of prey species. Lycosid spiders like *Pardosa lugubris* (Araneae: Lycosidae) are widespread polyphagous predators in forest litter communities. Due to methodological difficulties, rather little is known about predators such as lycosid spiders. Their wide food spectrum is considered to consist predominantly of qualitatively equivalent prey (Edgar 1969; Wise 1993; Kajak 1995). However, laboratory feeding experiments with juvenile *P. lugubris* revealed that different prey species result in marked variations in survival, growth and development of spiderlings (Oelbermann and Scheu, in press).

The analysis of the natural variations in stable isotope ratios in animal tissue has proven to be a powerful tool for analysing the diet of predators (Hobson and Clarke 1992; Angerbjöorn et al. 1994; Koch et al. 1995), for determining the physiological condition of birds (Hobson et al. 1993) and for following the path of assimilated carbon and nitrogen in animals (Tieszen et al. 1983; Tieszen and Fagre 1993). In contrast to more direct methods such as gut content analysis, stable isotope analysis looks at the diet over a longer period of time and therefore reflects the long-term feeding behaviour of animals (Peterson and Fry 1987; Schmidt et al. 1998). The method may be particularly helpful in revealing the trophic position of species with extra-intestinal digestion like spiders, since gut content analysis in these species is difficult.

Animal tissue is enriched in ^{15}N relative to food resources. On average, the $^{15}N/^{14}N$ ratio of predators is increased by 3–4‰ compared with their prey (DeNiro and Epstein 1981; Minagawa and Wada 1984; Owens 1987; Peterson and Fry 1987; Cabana and Rasmussen 1994). In top predators, therefore, the concentration of ^{15}N is at a maximum (Cabana and Rasmussen 1994). Due to the stepwise ^{15}N enrichment with increasing trophic level,

the ^{15}N content of predators can be used as a time-integrated indicator of their trophic position (Fry 1988; Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 1999). The enrichment in ^{15}N in predator tissue is caused by preferential enzymatic processing of ^{15}N compounds, resulting in their excreta being depleted and their tissue being enriched in ^{15}N.

Variations in nitrogen stable isotope ratios may also be used to analyse the nutritional status of animals. Starvation results in an enrichment of ^{15}N in animal tissue (Hobson et al. 1993). However, the ratio of stable isotopes in predator tissue is not only affected by the isotopic composition of the food, but also by food quality. Compared with food of high quality, low-quality food results in an increase in ^{15}N enrichment (Webb et al. 1998). High levels of ^{15}N in starving or nutritionally stressed animals are thought to be a consequence of recycling of body nitrogen (Hobson et al. 1993; Webb et al. 1998; Adams and Sterner 2000).

In contrast to ^{15}N, ^{13}C is only slightly enriched in upper trophic levels; on average the increase per trophic level is only 0.5–1‰ (DeNiro and Epstein 1978; Macko et al. 1982; Minagawa and Wada 1984; Ostrom and Fry 1993; Lajtha and Michener 1994). The content of ^{13}C in the tissue of predators, therefore, resembles that of their food (DeNiro and Epstein 1978) and may be used to trace the relative contribution of food materials to animal nutrition (Magnusson et al. 1999; Vander Zanden and Rasmussen 1999).

Variations in stable isotope ratios have been used to trace food relationships in animal communities; however, the factors influencing the enrichment in ^{15}N and ^{13}C per trophic level are little understood (Scrimgeour et al. 1995; Gannes et al. 1997). This applies in particular to terrestrial arthropods. In this study the enrichment in stable isotopes (^{13}C and ^{15}N) in predators relative to their prey was investigated under controlled laboratory conditions. Second instars of the lycosid spider *P. lugubris*, a widespread species in central Europe, were reared for 11 weeks on diets of single and mixed prey species. Prey species of different food quality, i.e. differentially affecting survival, growth and development of spiderlings, were studied (Oelbermann and Scheu, in press). Changes in stable isotope ratios in simple experimental food chains were analysed to test the validity of the stepwise enrichment of ^{15}N with increasing trophic level (Minagawa and Wada 1984). In particular, we investigated whether changes in $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios depended on prey quality. We hypothesized that variations in stable isotope enrichment in predator tissue are due, at least in part, to food of different quality. We also tested whether starvation increased ^{15}N and ^{13}C contents of spiderlings, and whether contents differed between different life stages of *P. lugubris*.

Materials and methods

Changes in $\delta^{15}N$ and $\delta^{13}C$ in *P. lugubris* with time

In order to analyse the time course of enrichment in ^{15}N in predator tissue, different life stages were studied: adult female *P. lugubris*; hatchlings of the female (second instars which were removed from the mother's opisthosoma in order to exclude food intake); and spiderlings fed for 3, 6 and 11 weeks with *Drosophila melanogaster*. Adult cocoon-carrying female *P. lugubris* were collected in the field, transferred to the laboratory and fed with *D. melanogaster* until juveniles hatched.

Changes in $\delta^{15}N$ and $\delta^{13}C$ with trophic level and starvation

Juvenile *P. lugubris* were reared on single-species diets of different food quality (cf. Oelbermann and Scheu, in press): two Collembola species, *Heteromurus nitidus* (high quality) and *Folsomia candida* (very low quality); the dipteran *D. melanogaster* (intermediate quality); and the aphid *Rhopalosiphum padi* (low quality). In addition, two mixed-species diets were investigated, one of low food quality (mix 1, consisting of *F. candida*, *H. nitidus* and *D. melanogaster*) and one of high food quality (mix 2, consisting of *H. nitidus* and *D. melanogaster*) (Table 1). Spiderlings kept without prey were included in the analysis to investigate the effect of starvation on tissue stable isotope ratios. Prey organisms were taken from laboratory cultures. They were fed with standard media of constant stable isotope ratios (yeast 1 for *F. candida*, yeast 2 for *H. nitidus*, smashed bananas for *D. melanogaster* and wheat plants for *R. padi*) (Table 1). By comparing the stable isotope ratios between three trophic levels – standard media, prey species and predators – changes in $\delta^{15}N$ and $\delta^{13}C$ with trophic level were investigated. Spiderlings fed on *H. nitidus*, *D. melanogaster* and mix 2 were sampled after 11 weeks at instar stages 4–6 and a body weight of 6.3–9.3 mg. Starving spiderlings and spiderlings fed on *F. candida*, *R. padi* and mix 1 were analysed after they had died. Due to the low-quality food they did not develop beyond the second instar and weighed only 0.6–0.7 mg.

Samples for stable isotope analysis (animals and standard media) were frozen at –22°C and then dried at 60°C. Whole organ-

Table 1 Prey media, prey organisms and predators of the experimental food chains studied. 0: starvation treatment, H: single diet of *Heteromurus nitidus*, D: single diet of *Drosophila melanogaster*, R: single diet of *Rhopalosiphum padi*, F: single diet of *Folsomia candida*, mix 1: mixed diet consisting of *H. nitidus*, *D. melanogaster* and *F. candida*, mix 2: mixed diet consisting of *H. nitidus* and *D. melanogaster*

Food chain	Prey medium	Prey organism	Predator
0	–	–	*Pardosa lugubris*
H	Yeast 2, (*Saccharomyces* sp.)	*Heteromurus nitidus* (Collembola, Entomobryidae)	*P. lugubris*
D	Smashed banana	*Drosophila melanogaster* (Diptera, Drosophilidae)	*P. lugubris*
R	Wheat plants	*Rhopalosiphum padi* (Homoptera, Aphididae)	*P. lugubris*
F	Yeast 1, (*Saccharomyces* sp.)	*Folsomia candida* (Collembola, Isotomidae)	*P. lugubris*
Mix 1	Yeast 1 and 2, smashed banana	*H. nitidus*, *F. candida* and *D. melanogaster*	*P. lugubris*
Mix 2	Yeast 2, smashed banana	*H. nitidus* and *D. melanogaster*	*P. lugubris*

isms including the gut were analysed. Gut contents may influence the overall content in stable isotopes in animal tissues. Therefore, animals fed high-quality diets were starved for 2 days before being prepared for analysis. Three replicates were analysed except for adult female *P. lugubris*, second-instar stages and wheat, which were analysed with just two replicates.

Dried samples were weighed into tin capsules and stored in a desiccator until measurement. Isotope ratios were determined by a coupled system of an elemental analyser (NA 1500, Carlo Erba, Milan) and a mass spectrometer (MAT 251, Finnigan). The system is computer-controlled, allowing on-line measurement of ^{15}N and ^{13}C. Stable isotope abundance is expressed using the δ notation with $\delta X\ (‰)=(R_{sample}-R_{standard})/R_{standard}\times 1{,}000$, with X representing ^{15}N or ^{13}C, and R_{sample} and $R_{standard}$ representing the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratios of the sample and standard, respectively. For ^{15}N, atmospheric nitrogen served as the primary standard and acetanilide (C_8H_9NO, Merck, Darmstadt) for internal calibration. For ^{13}C, Peedee belemnite marine limestone was used as the standard (Lajtha and Michener 1994). The mean standard deviation of samples of 10–200 µg N is 0.2‰ and for those containing 200–500 µg N it is 0.1‰ (Reineking et al. 1993).

Statistical analysis

Changes in $\delta^{15}N$ and $\delta^{13}C$ with trophic level were analysed by univariate analysis of variance (ANOVA). Prior to ANOVA, homogeneity of variances was checked by the Levene test. The $\delta^{15}N$ and $\delta^{13}C$ values of spiderlings of different treatments (diets) and the nitrogen content (%) of prey organisms were also analysed by ANOVA. Changes in ^{15}N enrichment in *P. lugubris* with time in the *D. melanogaster* treatment were analysed by ANOVA using sampling date as factor. For comparison of means, Tukey's HSD (equal cell size) or the Scheffé test (unequal cell size) was used.

Results

Changes in $\delta^{15}N$ and $\delta^{13}C$ in *P. lugubris* with time

Adult female *P. lugubris*, their second-stage instars, and spiderlings which had fed for 3, 6, and 11 weeks on *D. melanogaster* differed significantly in their ^{15}N content ($F_{4,12}$=104.3, P<0.001). With progressing development, age and body weight, $\delta^{15}N$ values increased (Fig. 1). Second-stage instars had 2.14‰ lower $\delta^{15}N$ values than their mothers (P=0.02, Tukey's HSD). Then, within the following 3 weeks, $\delta^{15}N$ values increased strongly, by 5.09‰, reaching the level of *D. melanogaster*. During the next few weeks, $\delta^{15}N$ values of *P. lugubris* further increased; compared with *D. melanogaster*, by 1.36‰ and 2.16‰ after 6 and 11 weeks, respectively.

The ^{13}C content of adult female *P. lugubris*, their second-stage instars and spiderlings which had fed for 3, 6 and 11 weeks on *D. melanogaster*, differed significantly ($F_{5,15}$=142.4, P<0.001; Fig. 1). Second-instar stages had significantly higher $\delta^{13}C$ values ($\Delta(\delta^{13}C)$=1.34‰) than their mother (P=0.001, Tukey's HSD). Within the following 3 weeks, $\delta^{13}C$ values decreased strongly, by 3.86‰, reaching the level of *D. melanogaster* ($\Delta(\delta^{13}C)$= 0.44‰) and remained at that level until the end of the experiment.

Changes in $\delta^{15}N$ and $\delta^{13}C$ with trophic level

In the yeast 2–*H. nitidus*–*P. lugubris* food chain, $\delta^{15}N$ values increased significantly with trophic level ($F_{2,8}$= 135.9, P<0.001). Collembolans were enriched in ^{15}N by 5.19‰ relative to yeast, and spiderlings by 2.53‰ compared with their prey (Fig. 2). $\delta^{13}C$ values of the three trophic levels were almost identical ($F_{2,8}$=0.113, P=0.895). The difference between the $\delta^{13}C$ value of *P. lugubris* and that of *H. nitidus* was only 0.02‰.

In the smashed banana–*D. melanogaster*–*P. lugubris* food chain, $\delta^{15}N$ values also significantly increased with trophic level ($F_{2,8}$=550.1, P<0.001). *D. melanogaster* differed from banana medium by 3.25‰ and *P. lugubris* from *D. melanogaster* by 2.16‰ (Fig. 2). $\delta^{13}C$ values also differed significantly ($F_{2,8}$=65.2, P<0.001) with *D. melanogaster* and *P. lugubris* having lower values than the banana medium, (–1.54‰ and –1.16‰, respectively, P<0.001, Tukey's HSD), but $\delta^{13}C$ values of *D. melanogaster* and *P. lugubris* differed only slightly (0.38‰, P=0.08, Tukey's HSD).

In contrast to the previous food chains, in the wheat–*R. padi*–*P. lugubris* food chain, $\delta^{15}N$ values did not consistently increase with trophic level, but trophic

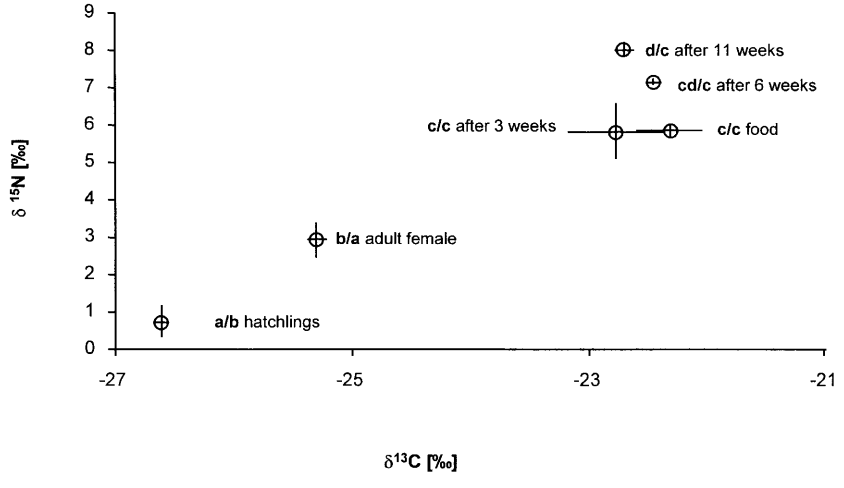

Fig. 1 $\delta^{15}N$ and $\delta^{13}C$ values (±SD) of adult female *Pardosa lugubris*, second-instar stages right after hatching out of the cocoon and juveniles fed for 3, 6 and 11 weeks on *Drosophila melano-gaster*. Significant differences are indicated by different letters (Tukey's HSD, P<0.05) with the first and second letters representing significant differences in $\delta^{15}N$ and in $\delta^{13}C$ values, respectively

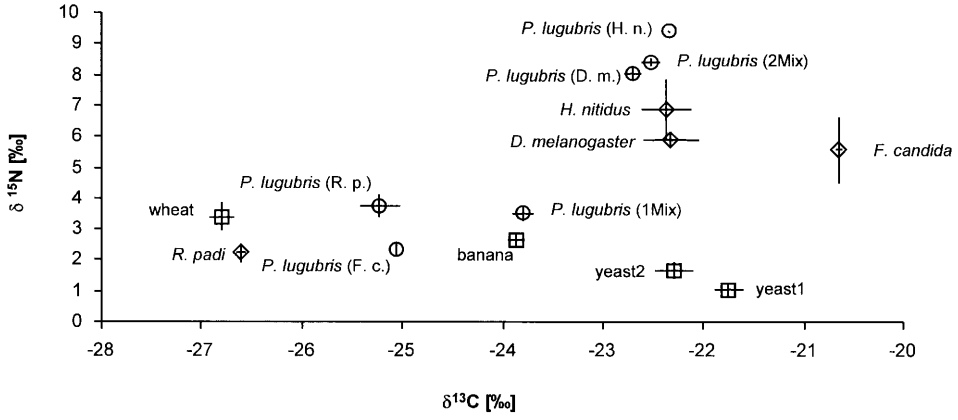

Fig. 2 $\delta^{15}N$ and $\delta^{13}C$ values (±SD) of the three trophic levels studied (prey media, prey organism and predator) of the yeast–*Heteromurus nitidus*–*Pardosa lugubris*, the banana–*Drosophila melanogaster*–*P. lugu-bris*, the wheat–*Rhophalosiphum padi*–*P. lugubris*, the yeast–*Folsomia candida*–*P. lugubris*, the mix 1 (*H. nitidus*, *D. melanogaster* and *F. candida*) and mix 2 (*H. nitidus* and *D. melanogaster*) food chains (see text for details). *Brackets* indicate the respective food chains of *P. lugubris*

levels differed significantly ($F_{2,7}$=13.4, P=0.01). *R. padi* had lower $\delta^{15}N$ values than wheat (–1.16‰, P=0.047, Tukey's HSD), but $\delta^{15}N$ values of *P. lugubris* were increased by 1.50‰ compared with *R. padi* (P=0.01, Tukey's HSD). Thus, $\delta^{15}N$ values of *P. lugubris* (3.73‰) resembled that of wheat (3.39‰, P=0.64, Tukey's HSD; Fig. 2). Trophic levels also significantly differed in ^{13}C content ($F_{2,7}$=86.8, P<0.001). *P. lugubris* was enriched by 1.38‰ compared with *R. padi* (P<0.001, Tukey's HSD), whereas aphids were not significantly enriched compared with wheat (0.18‰, P=0.458, Tukey's HSD).

In the yeast 1–*F. candida*–*P. lugubris* food chain, $\delta^{15}N$ values also did not consistently increase with trophic level, but differed significantly between trophic levels ($F_{2,8}$=41.8, P<0.001). *F. candida* was significantly enriched in ^{15}N compared with yeast (+4.54‰, P<0.001, Tukey's HSD), but $\delta^{15}N$ values of *P. lugubris* were significantly lower than those of *F. candida* (–3.22‰, P=0.002, Tukey's HSD; Fig. 2). $\delta^{13}C$ values also differed significantly between trophic levels ($F_{2,8}$=2043.8, P<0.001); *F. candida* was enriched in ^{13}C by 1.00‰ compared with yeast (P<0.001, Tukey's HSD) and *P. lugubris* was strongly depleted in ^{13}C compared with *F. candida* (–4.4‰, P<0.001, Tukey's HSD).

In the mix 1 treatment, differences in $\delta^{15}N$ values of trophic levels resembled those of the yeast 1–*F. candida*–*P. lugubris* food chain ($F_{6,20}$=47.4, P<0.001). On average, prey organisms were significantly enriched in ^{15}N by 4.92‰ relative to prey media (P<0.001, Tukey's HSD), and *P. lugubris* was significantly depleted, by 3.43‰, compared with prey organisms (P≤0.007, Tukey's HSD; Fig. 2). The $\delta^{13}C$ values also differed significantly between trophic levels ($F_{6,20}$=126.5, P<0.001). *P. lugubris* was depleted in ^{13}C by 1.49‰, 1.45‰ and 3.15‰ compared with *D. melanogaster*, *H. nitidus* and *F. candida*, respectively (P<0.001, Tukey's HSD). *F. candida* was significantly enriched in ^{13}C compared with all other components of this food chain (P<0.001, Tukey's HSD).

In contrast to the previous food chain, $\delta^{15}N$ values of trophic levels of the mix 2 treatment consistently increased with trophic level ($F_{4,14}$=114.6, P<0.001). On average, prey organisms were enriched in ^{15}N by 4.22‰ compared with prey media (P<0.001, Tukey's HSD), and

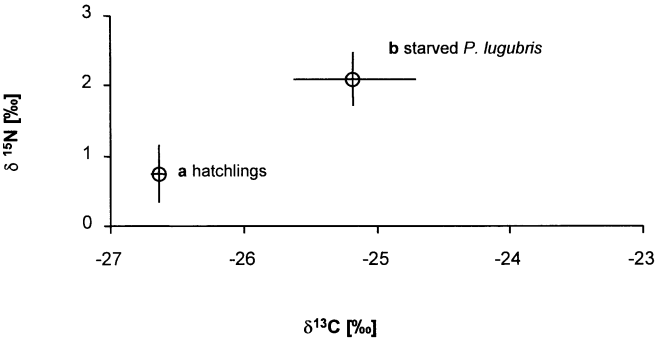

Fig. 3 Changes in $\delta^{15}N$ and $\delta^{13}C$ values (±SD) of *P. lugubris* due to starvation: data of second instar stages of *P. lugubris* right after hatching out of the cocoon and *P. lugubris* starved to death. Significant differences between $\delta^{15}N$ and $\delta^{13}C$ values are indicated by different letters (Tukey's HSD, P<0.05)

P. lugubris was enriched in ^{15}N by 2.05‰ compared with prey organisms (P≤0.014, Tukey's HSD; Fig. 2). $\delta^{13}C$ values differed significantly between trophic levels ($F_{4,14}$=36.2, P<0.001). The banana medium was depleted in ^{13}C compared with all other components (P<0.001, Tukey's HSD). $\delta^{13}C$ values of yeast, *H. nitidus*, *D. melanogaster* and *P. lugubris* differed by a maximum of only 0.23‰ (P≥0.623, Tukey's HSD).

Changes in $\delta^{15}N$ and $\delta^{13}C$ in *P. lugubris* due to starvation

$\delta^{15}N$ and $\delta^{13}C$ values of juvenile *P. lugubris* immediately after hatching out of the cocoon were 0.75±0.42‰ and –26.63±0.07‰, respectively. Starving hatchlings survived on average 12 days. Compared with their initial content, hatchlings starved to death were significantly enriched by 1.34‰ in ^{15}N and by 1.46‰ in ^{13}C ($F_{1,4}$=13.9, P=0.034 and $F_{1,4}$=18.4, P=0.023, respectively; Fig. 3).

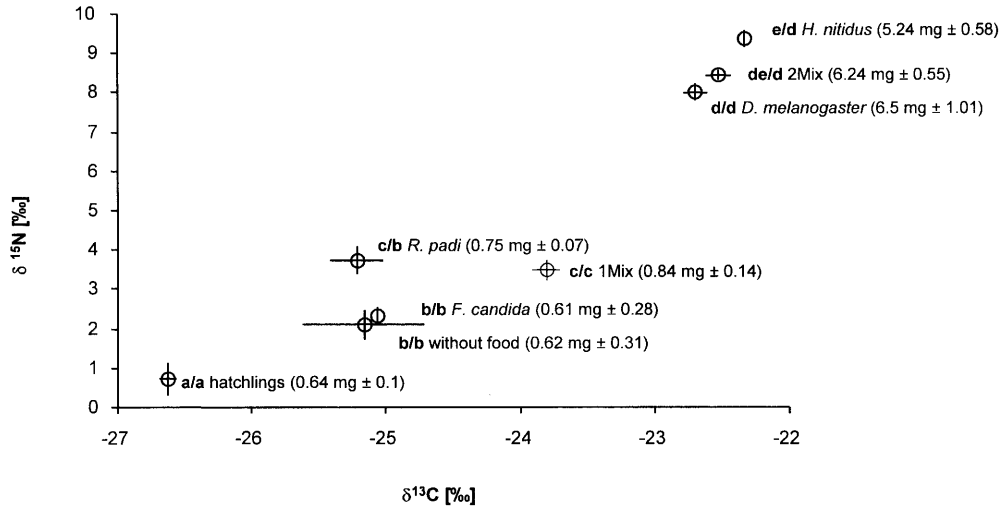

Fig. 4 $\delta^{15}N$ and $\delta^{13}C$ values (±SD) in juvenile *P. lugubris* reared on *Heteromurus nitidus*, mix 2 (*H. nitidus* and *Drosophila melanogaster*), *D. melanogaster*, *Rhopalosiphum padi*, mix 1 (*H. nitidus*, *D. melanogaster* and *Folsomia candida*) and *F. candida*, in starving *P. lugubris* and in second instar stages of *P. lugubris* immediately after hatching out of the cocoon. Significant differences between $\delta^{15}N$ values are indicated by different letters (Tukey's HSD, $P<0.05$) with the first and second letter representing significant differences in $\delta^{15}N$ and in $\delta^{13}C$, respectively. The average body weight (±SD) of the spiderlings at the time of the analysis is given in brackets

Discussion

Changes in stable isotope ratios in *P. lugubris* with time

It is a matter of debate whether or not ^{15}N content in animals increases with age (Owens 1987). Rau et al. (1981) and Spies et al. (1989) reported that ^{15}N content in sole increased with age. Adams and Sterner (2000) confirmed this correlation for cladocerans. In contrast, Minagawa and Wada (1984) and Kiriluk et al. (1995) found no correlation between age and $\delta^{15}N$ values of marine mussel species and sea trout; nevertheless, $\delta^{15}N$ values varied in early stages.

$\delta^{15}N$ values of spiderlings fed on *D. melanogaster* increased with age and time of consumption. Hatchlings of *P. lugubris* receive their initial nitrogen reserves from their mother. Nevertheless, adult female *P. lugubris* were enriched in ^{15}N by 2.1‰ compared with hatchlings. Based on ^{15}N analysis, Schmidt et al. (1998) proposed the existence of different nitrogen pools in earthworms with different ^{15}N signatures. It is probable that adult lycosid spiders also consist of different nitrogen pools with varying $^{15}N/^{14}N$ ratios. In the field, adult female *P. lugubris* increase their feeding rates before producing cocoons (Edgar 1971). Nitrogen from the prey consumed within that period may be allocated predominantly to nitrogen reserves for the brood without intense metabolization of molecules. Due to the preferential conversion of ^{14}N in metabolic reactions (DeNiro and Epstein 1981; Minagawa and Wada 1984), highly metabolized molecules should be enriched in ^{15}N. This may explain why hatchlings had higher ^{14}N content than their mothers. After 3 weeks' feeding on *D. melanogaster*, $\delta^{15}N$ values of spiderlings were markedly increased and approached that of prey, i.e. the highest ^{15}N enrichment through consumption took place in a relatively short time period. Also, Ostrom et al. (1997) reported that after a change of food, the isotopic composition of the predatory ladybird beetle (*Hippodamia variegata*) had changed within 21 days. Overall, after 11 weeks, $\delta^{15}N$ values appeared to approach a total ^{15}N enrichment in spiderlings of ca. 3‰ relative to *D. melanogaster*, corresponding to the trophic level ^{15}N enrichment postulated by Minagawa and Wada (1984).

In contrast to ^{15}N, ^{13}C in hatchlings of *P. lugubris* was replaced completely after 3 weeks of feeding on *D. melanogaster*, indicating different patterns of fractionation for ^{15}N and ^{13}C. Later, the ^{13}C content was independent of age and developmental stage, whereas the ^{15}N content further increased with progressing development of *P. lugubris*.

Food quality and stable isotope ratios in *P. lugubris*

Webb et al. (1998) and Adams and Sterner (2000) reported that food of low quality results in higher stable isotope enrichment compared with high-quality food. The enrichment in ^{15}N and ^{13}C in animals may also increase with nutritional stress and starvation (Ambrose and DeNiro 1986; Hobson et al. 1993; Lajtha and Michener 1994; Adams and Sterner 2000). In the present study, $\delta^{15}N$ values of *P. lugubris* also depended on food quality, but in contrast to previous studies, $\delta^{15}N$ values increased with prey quality. The food quality of the different prey species used has been investigated previously (Oelbermann and Scheu, in press). In respect of the $\delta^{15}N$ values of *P. lugubris*, four groups may be distinguished: (1) starving spiderlings and spiderlings fed on *F. candida*; (2) spiderlings fed on mix 1 (*H. nitidus*, *D. melanogaster* and *F. candida*) and on *R. padi*; (3) spiderlings fed on

Table 2 $\delta^{15}N$ values of juvenile *Pardosa lugubris* feeding on different diets (see Table 1) and ^{15}N enrichment in spiderlings relative to their prey ($\Delta(\delta^{15}N)$). Also, nitrogen contents of prey organisms and of corresponding spiderlings, and the food quality of diets are given. Judgement of food quality is based on survival, growth and development of spiderlings (Oelbermann and Scheu, in press). In mixed diets mean $\delta^{15}N$ values of prey organisms were taken to calculate $\Delta(\delta^{15}N)$ (*). For key to food chains, see Table 1

Food chain	$\delta^{15}N$ value of spiderlings (‰±SD)	$\Delta(\delta^{15}N)$ betweenprey spiderlings and their (‰)	N content of prey organisms (%±SD)	N content of spiderlings (%±SD)	Food quality
0	2.09±0.38	–	–	11.97±1.01	–
H	9.40±0.95	+2.53	11.29±0.91	12.13±0.30	Good
D	8.03±0.20	+2.16	9.76±0.03	9.81±1.00	Intermediate
R	3.73±0.32	+1.50	8.27±0.27	12.93±0.57	Low
F	2.33±1.05	–3.22	12.54±1.33	11.92±0.66	Very bad
mix 1	3.48±0.26	–2.00*	11.19*	12.48±0.12	Very bad
mix 2	8.42±0.12	+2.05*	10.53*	11.02±0.22	Very good

D. melanogaster and on mix 2 (*H. nitidus* and *D. melanogaster*); and (4) spiderlings fed on *H. nitidus* (Fig. 4). Spiderlings reared on food of intermediate (*D. melanogaster*), high (*H. nitidus*) and very high quality (mix 2) were strongly enriched in ^{15}N compared with their prey. The enrichment in ^{15}N in spiderlings was most pronounced in the *H. nitidus* (2.53‰) and *D. melanogaster* treatment (2.16‰).

Spiderlings fed on high-quality food were bigger and developed faster compared with those fed on low-quality food (cf. Fig. 4). Therefore, spiderlings of different treatments were in different stages and differed in size. The high ^{15}N enrichment in spiderlings fed on high-quality food was therefore most likely due to their more advanced development and higher body weight. The lower ^{15}N enrichment in spiderlings fed on mix 2 compared with *H. nitidus* was presumably caused by the lower food quality of *D. melanogaster*. Feeding on a single-species diet of *D. melanogaster* resulted in high mortality of spiderlings of later stages, probably through the lack of some essential nutrients.

Spiderlings fed on *R. padi*, *F. candida* and mix 1 did not grow and develop and therefore remained small. Correspondingly, their $\delta^{15}N$ values were low. When fed on low-quality food (*R. padi*), *P. lugubris* was little enriched in ^{15}N (1.50‰); in the *F. candida* and mix 1 treatment the ^{15}N content in spiderlings was even lower than that in their prey. Obviously, due to the low food intake, the nitrogen pool of *P. lugubris* hatchlings was insufficiently replaced by that of their prey. Thus, $\delta^{15}N$ values as well as $\delta^{13}C$ values suggest that *P. lugubris* refused *F. candida* as prey, confirming our previous assumption.

Nutritional stress and starvation result in a reduced nitrogen intake, while ^{14}N loss due to excretion continues, resulting in an increase in $^{15}N/^{14}N$ ratios in animal tissue (Ambrose and DeNiro 1986; Hobson et al. 1993; Lajtha and Michener 1994). Similarly, consumption of low-quality food is associated with reduced nitrogen intake (Adams and Sterner 2000). Therefore, starving animals and those feeding on low-quality food should be enriched in ^{15}N. In the present study, starving spiderlings were enriched in ^{15}N by 1.34‰ compared with hatchlings, and a similar enrichment occurred in spiderlings fed on *F. candida* and mix 1. Obviously, food of very low quality induced ^{15}N enrichment very similar to starvation.

As in the study of Adams and Sterner (2000), $\delta^{15}N$ values of spiderlings were correlated with the nitrogen (N) content of their prey except in the yeast 1–*F. candida*–*P. lugubris* food chain (Table 2). *F. candida* had the highest N content of the prey organisms studied; it was even higher than that of *P. lugubris*. The high N content of *F. candida* suggests high food quality for *P. lugubris*; however, when fed on *F. candida*, the N content of *P. lugubris* remained lower than that of the Collembola and was similar to that of starving spiderlings. Obviously, rejection of *F. candida* as prey by *P. lugubris* is not related to prey nitrogen content.

R. padi is of low quality as food for *P. lugubris* (Oelbermann and Scheu, in press) and aphids had about 5% lower N content than spiderlings. The low enrichment in ^{15}N in spiderlings fed on aphids suggests that spiderlings gained enough energy to sustain life functions, but did not have sufficient nutrients to develop or grow. *H. nitidus* had high N content and spiderlings were strongly enriched in ^{15}N. The N content of *D. melanogaster* was similar to that of *P. lugubris* and ^{15}N enrichment in spiderlings was also high. On average, the N content of prey in the mix 2 treatment (*H. nitidus* and *D. melanogaster*) was slightly lower than that of the *H. nitidus*-only treatment. Correspondingly, ^{15}N enrichment was also somewhat lower.

The enrichment in ^{13}C also depended on food quality. For high-quality prey, ^{13}C content of predator and prey differed only a little (<0.37‰), supporting the conclusion that $\delta^{13}C$ of predators resembles that of their diet (Minagawa and Wada 1984). With decreasing food quality, however, ^{13}C content increased. The enrichment was most pronounced in starving spiderlings and those fed low-quality food (*R. padi*, *F. candida* and mix 1). Enrichment in ^{13}C due to starvation and consumption of low-quality food has been documented previously by Webb et al. (1998).

$\delta^{13}C$ values can be used to determine carbon sources of predators (DeNiro and Epstein 1978; Peterson and Fry 1987; Lajtha and Michener 1994; Vander Zanden and

Rasmussen 1999). Due to weak ^{13}C variation at the base of the present food chains (different prey media), tracing the favoured prey components in the mixed species diets was not possible. DeNiro and Epstein (1978) stressed that the $\delta^{13}C$ values can only be used to determine the carbon sources of predators if the ^{13}C content of potential foods differs considerably.

Stable isotope enrichment in prey organisms

H. nitidus was strongly enriched in ^{15}N relative to yeast (+5.19‰), exceeding the proposed average per trophic level of 3.4‰ (Minagawa and Wada 1984) by a considerable amount. To a lesser extent this also applied to *F. candida*, which was enriched relative to yeast by 4.54‰. It is probable that the Collembola did not only feed on yeast, but also on fungi growing on the yeast medium. In addition, they may also have fed on exuvia or carcasses of conspecifics. *D. melanogaster* was enriched in ^{15}N by 3.25‰ compared with banana medium, confirming the postulated increase between predator and diet. The aphid *R. padi* was depleted in ^{15}N by 1.16‰ compared with its food source, wheat. Few differences in ^{15}N content between aphids and their hosts have been documented previously (Scrimgeour et al. 1995; Ostrom et al. 1997; Yoneyama et al. 1997).

Conclusions

This study indicates that the postulated stepwise trophic level enrichment in ^{15}N by ca. 3‰ normally applies to generalist predators like *P. lugubris*. In the field it is unlikely that generalist feeders like spiders survive for long on diets of very low quality. Deviations from postulated $\delta^{15}N$ patterns due to low quality prey are likely to be small. However, care is needed when hatchlings or early juveniles are included, since they may be considerably depleted in ^{15}N compared with adults. Overall, the analysis of variations in the natural abundance of stable isotopes appears to be a quick and reliable method for determining the trophic position of animal species in terrestrial food webs. The method might be particularly helpful in revealing the trophic structure of food webs in which polyphagous feeders predominate, as is the case in litter and soil.

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