Stable isotope analyses document intraguild predation in wolf spiders (Araneae: Lycosidae) and underline beneficial effects of alternative prey and microhabitat structure on intraguild prey survival

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Intraguild predation (IGP) is common among generalist predators and an important issue in food web theory, because IGP may destabilise communities by increasing extinction of species. Also, IGP may interfere with the effectiveness of generalist predators as biological control agents. In general, occurrence of IGP in laboratory or field studies is inferred from abundance data or direct observations only. We investigated if tracing stable isotopes allows distinction between different types of predation and confirmation of IGP. Wolf spiders were chosen as model organisms for generalist predators; IGP of third instar *A. cuneata* on second instar *P. palustris* was investigated in a laboratory experiment. The availability of alternative prey and the complexity of the microhabitat were manipulated, since both factors are thought to facilitate coexistence of predators.

Stable isotope analysis documented predation of *A. cuneata* on *P. palustris* and predation on alternative prey by both juveniles. Both the presence of alternative prey and the availability of shelter reduced mortality of juvenile *P. palustris* during the first week. During the second week mortality increased in complex structure without alternative prey presumably due to enhanced activity and cannibalism among starving *P. palustris*. Thus, microhabitat complexity and prey abundance may foster coexistence of wolf spiders in the field.

In conclusion, stable isotope analysis was proven a powerful tool to investigate animal behaviour without direct observation. The method allowed disentangling predator feeding behaviour when more than one type of prey was present.

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Intraguild predation (IGP) is common in food webs and predators often coexist in stable natural communities (Polis and McCormick 1986, Arim and Marquet 2004) even though in theory IGP destabilises communities due to species exclusion (Holt and Polis 1997, McCann and Hastings 1997, McCann et al. 1998). Consequently, generalist predators have been ignored as biocontrol agents for a long time, since IGP may disrupt pest control (Symondson et al. 2002, Denno et al. 2003, Koss and Snyder 2005). Therefore it is crucial to elaborate factors that foster co-occurrence of predatory species in order to increase their effectiveness as biocontrol agents.

Age-/stage-restricted predation, interactions with other species, and spatial heterogeneity are promising

mechanisms favouring the coexistence of predator species (Holt and Polis 1997). The vulnerability to predation can change during development of animals due to size-refugia (Chase 1999), which is why IGP is often restricted to particular ages or stages (Ebenman and Persson 1988, Wissinger 1992). Since IGP systems are part of complex food webs, the tritrophic cascades are linked to other species, fostering the existence of IG prey populations through alternative resources (Holt and Polis 1997). Cannibalism being a special case of IGP is strongly reduced when alternative prey is available (Dong and Polis 1992, Wagner and Wise 1996, 1997, Rickers and Scheu 2005) and accordingly, IGP decreases in presence of alternative prey (Hodge 1999, Chen and Wise 1999, Meyhöfer and Hindayana 2000). Furthermore, IG prey escape predation in spatial refuges reducing the probability of species exclusion (Diehl 1993, Finke and Denno 2002, Lewis and Eby 2002, Langellotto and Denno 2004).

Nevertheless, unexpected effects of both prey availability and habitat structure on predation are frequently encountered in field (Moran and Hurd 1997) and laboratory experiments (Koss and Snyder 2005, Rickers and Scheu 2005), which is why studies manipulating both factors in a straightforward way are important. Until now, the occurrence of IGP in the field has largely been concluded indirectly based on animal density (Wise and Chen 1999, Meyhöfer and Hindayana 2000, Finke and Denno 2002, Lensing and Wise 2004). Using stable isotope techniques we aim to directly demonstrate IGP and to add to the usefulness of stable isotope analyses. We chose wolf spiders as model organisms for generalist predators which are easy to study in laboratory experiments and are promising agents for biological control (Riechert 1999, Snyder and Wise 1999).

Alopecosa cuneata (Clerck) and *Pardosa palustris* (L.) are common wolf spiders on grasslands. Female *A. cuneata* are almost twice the size of *P. palustris* (Roberts 1995) and in July, third instar *A. cuneata* co-occur with second instar *P. palustris* on grasslands near Darmstadt. This is a useful system for the study of IGP, which is strongly asymmetric and is determined by relative body-size of IG predator and IG prey (Snyder and Hurd 1995, Lucas et al. 1998, Woodward and Hildrew 2002, Balfour et al. 2003). Since smaller individuals of wolf spiders are more sensitive to starvation (Tanaka 1992, Walker et al. 2003) and more vulnerable to predation (Tanaka 1992), we hypothesised that the larger instars of *A. cuneata* prey on the smaller *P. palustris* when alternative prey is rare and habitat structure serving as shelter is limited.

We established four treatments in the laboratory, manipulating prey availability (with, without *Drosophila melanogaster*) and microhabitat structure (simple, complex). A single individual of *A. cuneata* was placed together with six juveniles of *P. palustris*. In order to compare weight gain and stable isotope ratios without intraguild prey and/or alternative prey, four more treatments without *P. palustris* were established. *D. melanogaster* provided as food during the experiment was enriched in the heavier carbon isotope to document differential predation of *A. cuneata* on *P. palustris* and alternative prey using stable isotope analysis.

Material and methods

The experiment was carried out in a climate-controlled green house at natural light conditions and constant temperature of 21°C.

Study species

Adult female *A. cuneata* and *P. palustris* were collected from xeric grasslands in May and June 2004 at the "Ehemaliger August-Euler-Flugplatz" close to Darmstadt, Hesse (49°51′ N, 8°35′ E). Spiders were kept separately in covered plastic containers (Ø 6.5 × 4.5 cm). Female *A. cuneata* weighed 55.8 ± 21.6 mg; female *P. palustris* weighed 23.8 ± 7.7 mg. Spiders either carried eggsacs or produced eggsacs during captivity. *A. cuneata*, the larger species, were fed 10 living *D. melanogaster* (Diptera; curly; b.t.b.e Insektenzucht GmbH, Schnürpflingen, Germany) daily, while *P. palustris* were fed only five flies per day. After production of eggsacs, females of *A. cuneata* retreated to a web burrow and stopped feeding, while females of *P. palustris* actively exposed the eggsacs to the light and continued feeding. The eggsacs of *A. cuneata* hatched after 20.0 ± 2.8 (n = 10) days, while eggsacs of *P. palustris* hatched already after 18.1 ± 1.7 (n = 31) days. *A. cuneata* produced 40.8 ± 13.5 juveniles, while eggsacs of *P. palustris* contained only 33.3 ± 10.1 spiderlings. Directly after dispersal from their mothers after three to four days, spiderlings were kept separately in small transparent plastic containers (Ø 3.5 × 2.5 cm). Juveniles of *A. cuneata* were fed fruit flies (*D. melanogaster*) and the springtail (Collembola) *Heteromurus nitidus* (laboratory culture) ad libitum until they reached the third instar. Since second-instar juveniles of *P. palustris* were too small to prey on fruit flies, they were fed solely *H. nitidus* ad libitum to exclude starvation and to minimize cannibalism during the experiment. All spiderlings were weighed shortly before the experiment started: weight of *A. cuneata* ranged from 0.9 to 2.0 mg and weight of *P. palustris* ranged from 0.4 to 0.8 mg.

Study design and procedures

Intraguild predation was investigated in a two factorial design including four treatments. Factors were availability of alternative prey (without, with *D. melanogaster*)

and habitat structure (simple, complex). One *A. cuneata* was placed with 6 juvenile *P. palustris*. Neither weight of *A. cuneata* ($F_{7,72} = 0.01$, p = 1.000) nor average weight of *P. palustris* per container ($F_{3,36} < 0.01$, p = 1.000) differed between treatments. Furthermore, the six *P. palustris* were similar in size (SD 0.00–0.02 mg). Juvenile *A. cuneata* were 2.5 ± 0.0 times heavier than the selected juveniles of *P. palustris* in order to exclude differential predation probability due to different size ratios of predator and IG prey. To ensure uniform predation rates, juvenile *A. cuneata* were starved for four days before the experiment started. In treatments with alternative prey, a constant prey level of three individuals of *D. melanogaster* was established, resembling prey in excess. Since we could not exclude predation on *D. melanogaster* or conspecifics by juvenile *P. palustris* during the experiment, we established four more treatments without *P. palustris* in order to compare weight gain and isotopic ratio of *A. cuneata* with and without IGP prey. Thus, the experiment was designed orthogonally and consisted of three factors, two levels of each (alternative prey, habitat structure and IGP). All treatments were replicated 10 times and survival of spiderlings and fruit flies was checked every other day; missing fruit flies were replaced accordingly. After two weeks all juveniles of *P. palustris* in the treatment without alternative prey in the simple habitat had died and the experiment was ended: surviving spiders were frozen at $-80°C$ for 24 h, weighed and dried for stable isotope analysis.

Study container

Plastic containers (Ø 6.5×4.5 cm) contained either 0.1 g (simple habitat structure) or 0.3 g (complex habitat structure) of *Hypnum cupressiforme* (L.), a moss that covers large areas of the ground at the study site. Plants were collected in the field, frozen at $-20°C$ for 48 h and dried at $60°C$ for another 48 h to exclude possible prey. The amount of moss mimicked that of microsites with low and high cover of moss in the field. Moss in plastic containers was moistened daily to ensure sufficient humidity.

Fruit fly culture

Since an average of three weeks is needed to detect changes in isotopic composition in juvenile spiders (Oelbermann and Scheu 2002b), drastic differences in carbon stable isotope ratio of IG prey and alternative prey are necessary to allow earlier detection of differences in diet. Consequently, *D. melanogaster* was reared on an agar-sugarcane-maize-semolina medium with D-glucose-1-[13]C (Campro Scientific GmbH, Berlin, Germany) that was highly enriched in [13]C (min. 99 atom%).

The calculated proportion of ^{13}C was 1.28 atom%, an enrichment of 188‰ over the isotopic ratio of *D. melanogaster* raised on a C3 plant resource (1.09 atom%).

Stable isotope analysis

Animals (spiders, fruit flies, springtails) were dried at $60°C$ for six days. At least three measurements per treatment were done. For *P. palustris* two sets of samples were generated to document possible differences in feeding during the experiment; the heaviest juveniles per treatment were selected and analysed separately, while the lightest juveniles per treatment had to be combined to ensure sufficient biomass for stable isotope measurements. Thus, whole animals (9–1200 µg) were enclosed in tin capsules, which had been cleaned with acetone previously. Capsules were analysed in a system consisting of an elemental analyser (NA 1500, Carlo Erba, Milan) coupled with a mass spectrometer (MAT 251, Finnigan; Reineking et al. 1993). Acetanilide (C_8H_9NO: C 71.1%; H 6.7%; N 10.4%; O 11.8%; Merck, Darmstadt) was used for internal calibration. The ratio of ^{13}C to ^{12}C was expressed relative to that in Pee Dee Belemnite and that of ^{15}N to ^{14}N relative to that in air. Ratios [‰] were calculated according to the following formula (Peterson and Fry 1987): $\delta X = (R_{sample} - R_{standard})/(R_{standard}) \times 1000$ with X representing the heavier isotope (^{15}N or ^{13}C), and R the ratio of heavy to light isotope (^{15}N/^{14}N respectively ^{13}C/^{12}C).

Statistical analyses

Data on survival of *P. palustris* was analysed using repeated measures GLM (general linear model). For differences in weight of *A. cuneata* and *P. palustris* GLM was applied. GLM was also used to detect differences in C:N ratio, $\delta^{13}C$ and $\delta^{15}N$ of spiderlings. In order to explain high standard deviations in $\delta^{13}C$ ratios of *P. palustris*, single measurements of ^{13}C were correlated to final biomass of juveniles. Regressions were performed in Statistica 6.0 (Stat Soft). All other statistical analyses were calculated using SAS 8.02 (SAS Institute Inc., Cary, USA). To calculate differences in mortality of *A. cuneata* between treatments, G-tests of independence with Williams' correction were used (Sokal and Rohlf 2001).

Results

Mortality and biomass

Mortality of *P. palustris* was highest at simple habitat structure without alternative prey (Fig. 1). Generally, mortality of second instar *P. palustris* was affected by the
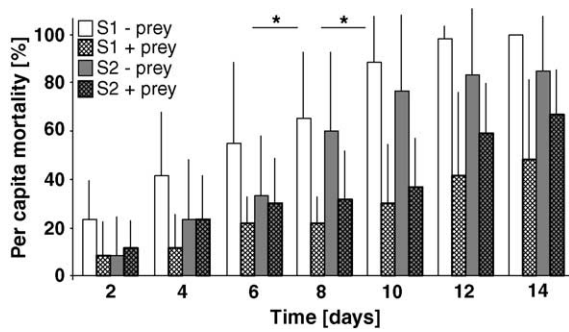
Fig. 1. Cumulative mortality ($\pm$SD) of juvenile *Pardosa palustris* as affected by habitat structure (S1: simple structure; S2: complex structure) and availability of alternative prey during two weeks. Asterisks indicate significant differences between time intervals.

availability of alternative prey as well as the interaction of alternative prey and habitat structure (Table 1). The effect of alternative prey changed with time: during the first 6 days, absence of alternative prey increased mortality of *P. palustris* only at simple habitat structure (prey $\times$ habitat structure interaction), while after eight days, mortality increased also at complex habitat structure without alternative prey (Table 1, Fig. 1). After 10 days mortality of juvenile *P. palustris* increased strongly when alternative prey was missing (Fig. 1).

In treatments with IGP and alternative prey, all individuals of *A. cuneata* survived until the end of the experiment. Without prey, *A. cuneata* started to die after 10 days and mortality was pronounced in simple habitat structure ($G_{1,20} = 6.97$, $p < 0.01$) (Fig. 2). Bodyweight of *A. cuneata* also differed between treatments ($F_{7,69} = 60.31$, $p < 0.001$; Fig. 3). In treatments with IGP and in those with alternative prey, weight gain of spiders was significantly increased from 1.1 mg to 2.0 mg ($F_{1,69} = 50.70$, $p < 0.001$) and from 0.4 mg to 2.7 mg ($F_{1,69} = 357.27$, $p < 0.001$), respectively. In treatments with alternative prey the increase in body weight was more pronounced at complex habitat structure (habitat structure $\times$ alternative prey interaction; $F_{1,69} = 8.88$, $p = 0.004$) and also when both alternative prey and IGP were available (habitat structure $\times$ prey $\times$ IGP interaction; $F_{1,69} = 4.58$, $p = 0.036$).

Even though juvenile *P. palustris* seemed to gain weight in treatments with alternative prey, changes in
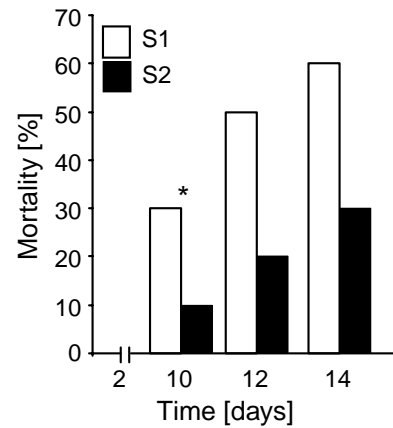

Fig. 2. Cumulative mortality of starving juvenile *Alopecosa cuneata* as affected by habitat structure (S1: simple structure; S2: complex structure). Asterisks indicate significant differences between treatments.
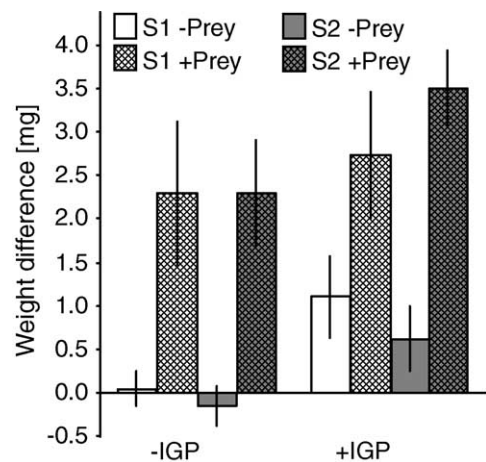

Fig. 3. Weight differences ($\pm$ SD) of juvenile *Alopecosa cuneata* as affected by IGP, alternative prey and habitat structure (S1: simple structure; S2: complex structure) before and after the experiment.

body weight of individual spiderlings within treatments were very high, resulting in high standard deviations in simple habitat structure with alternative prey (0.2 ± 0.4 mg) and in complex structure with alternative prey (0.2 ± 0.3 mg). Consequently, differences between treatments were not significant ($F_{2,19} = 0.80$, $p = 0.463$).

Table 1. Within and between-subjects effects of a repeated-measures GLM; influence of habitat structure (simple; complex) and alternative prey (absence; presence) on mortality of *Pardosa palustris*.

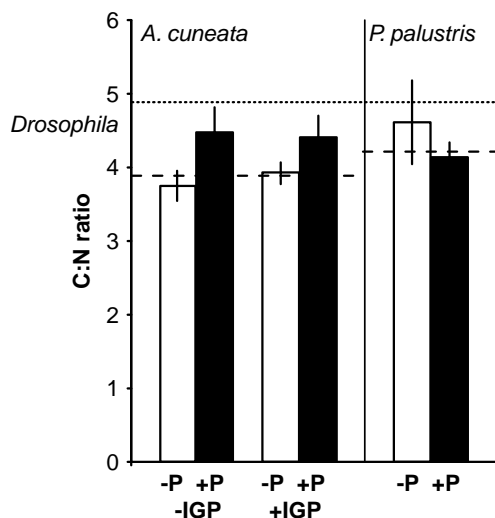|	Treatment	Df model	Df error	F	p
Between subject factors	structure	1	35	0.07	0.789
	alternative prey	1	35	23.58	<0.001
	structure $\times$ alternative prey	1	35	4.96	0.035
Within subject factors	time	6	210	102.16	<0.001
	time $\times$ structure	6	210	0.67	0.676
	time $\times$ alternative prey	6	210	11.47	<0.001
	time $\times$ structure $\times$ alternative prey	6	210	0.70	0.648

Fig. 4. C:N ratio ($\pm$SD) of juvenile *Alopecosa cuneata* and *Pardosa palustris* as affected by IGP and/or alternative prey (−P: without prey; +P: with prey). Initial C:N ratios of *A. cuneata*, *P. palustris* and *Drosophila melanogaster* are indicated by horizontal lines.

C:N ratio and stable isotopes

The C:N ratio of juvenile *A. cuneata* was significantly increased when alternative prey was available ($F_{1,44} = 59.34$, $p < 0.001$) (Fig. 4). Furthermore, the C:N ratio of juvenile *P. palustris* was significantly increased by 0.5 at complex habitat structure without alternative prey as compared to complex habitat structure with alternative prey ($F_{2,19} = 4.14$, $p = 0.027$). The $\delta^{13}C$ ratios of *A. cuneata* varied with the availability of intraguild prey (*P. palustris*) and alternative prey (*D. melanogaster*) (Fig. 5a), as feeding on alternative prey resulted in strongly increased $\delta^{13}C$ ratios ($\Delta^{13}C = 98.1‰$; $F_{1,44} = 2526.90$, $p < 0.001$) with the increase being most

pronounced without intraguild prey ($F_{1,44} = 24.12$, $p < 0.001$).

Feeding on intraguild prey (*P. palustris*) and alternative prey (*D. melanogaster*) affected $\delta^{15}N$ ratios of *A. cuneata* differently (significant interaction of alternative and intraguild prey: $F_{1,44} = 6.15$, $p = 0.017$) (Fig. 5b). With alternative prey the $\delta^{15}N$ ratios of *A. cuneata* were similar irrespective of the presence of intraguild prey. In contrast, without alternative prey, but in presence of *P. palustris*, $\delta^{15}N$ ratio of spiderlings was significantly increased. The $\delta^{15}N$ ratios of *P. palustris* did not differ between treatments ($F_{2,19} = 1.93$, $p = 0.171$) (Fig. 5b). In contrast, $\delta^{13}C$ ratios of *P. palustris* were significantly increased in treatments with alternative prey ($F_{2,19} = 5.95$, $p = 0.010$) and differed strongly within these treatments resulting in pronounced standard deviations (Fig. 5a). $\delta^{13}C$ ratios were positively correlated with the body weight of juvenile *P. palustris* at the end of the experiment at simple habitat structure with alternative prey ($r^2 = 0.72$, $F_{1,4} = 10.41$, $p = 0.032$) (Fig. 6), but not at complex habitat structure with alternative prey ($r^2 = 0.20$, $F_{1,4} = 1.02$, $p = 0.370$). Habitat structure did not affect C:N ratios or stable isotope ratios of *A. cuneata* and *P. palustris*.

Discussion

Effects of alternative and intraguild prey

In general, survival of *P. palustris* was enhanced when alternative prey was available. This is in line with earlier findings that IGP is reduced when prey is abundant (Lucas et al. 1998, Chen and Wise 1999, Meyhöfer and Hindayana 2000). Feeding solely on *D. melanogaster*, juvenile *A. cuneata* increased almost threefold in weight. As indicated by the enrichment in ^{13}C spiderlings

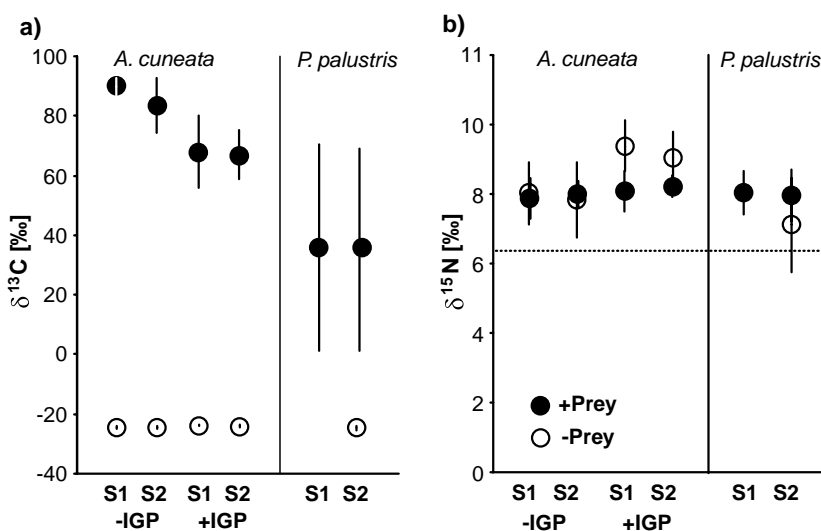Fig. 5. $\delta^{13}C$ ratio (a) and $\delta^{15}N$ ratio (b) ($\pm$SD) of juvenile *Alopecosa cuneata* and *Pardosa palustris* as affected by IGP, alternative prey and habitat structure (S1: simple structure; S2: complex structure). $\delta^{15}N$ ratio of *Drosophila melanogaster* is indicated by a horizontal line.
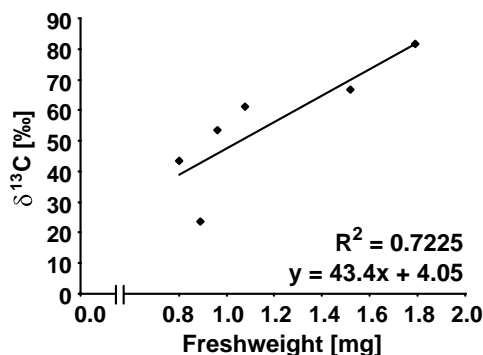
Fig. 6. Correlation between weight of juvenile *Pardosa palustris* and $\delta^{13}C$ ratio at simple habitat structure with alternative prey.

incorporated a substantial amount of carbon from their prey. Presumably due to the high C:N ratio of *D. melanogaster*, the C:N ratios of *A. cuneata* also increased. The enrichment in ^{13}C was less pronounced when intraguild prey was available. Either consuming both fruit flies and spiderlings led to a lower increase in ^{13}C due to additional ingestion of spiderlings with a lower $\delta^{13}C$ ratio, or *P. palustris* captured some of the fruit flies, lowering the number of fruit flies available to *A. cuneata*. Indeed, $\delta^{13}C$ ratios of juvenile *P. palustris* suggest that most of the surviving individuals had preyed on *D. melanogaster*. This conclusion is supported by the pronounced weight gain of *P. palustris* when alternative prey was available. Furthermore, the strong correlation between $\delta^{13}C$ ratios and final weight of juvenile *P. palustris* in simple structure with alternative prey indicates that juvenile growth mainly resulted from predation on *D. melanogaster*. In contrast, $\delta^{13}C$ ratios and the final weight of *P. palustris* were not related in simple structure with alternative prey, suggesting a higher frequency of cannibalism since spiders gained similar weight as compared to those in complex structure.

Feeding on juvenile *P. palustris* instead of *D. melanogaster* resulted in lower C:N ratios and lower increase in biomass of *A. cuneata* due to lower C:N ratios of juvenile *P. palustris*. Furthermore, feeding on *P. palustris* caused an increase in $\delta^{15}N$ and this was more pronounced when alternative prey was absent, suggesting that predation of *A. cuneata* on *P. palustris* was reduced in presence of alternative prey. Initially, *A. cuneata* were enriched in ^{15}N by 0.9‰ compared to *P. palustris*, increasing to an enrichment of 1.5‰ at the end of the experiment. IGP results in increased $\delta^{15}N$ ratios due to the consumption of prey on the same trophic level (Hobson and Welch 1995, Ponsard and Arditi 2000, McNabb et al. 2001). Fractionation was 1.4‰ at complex habitat structure and 1.8‰ at simple habitat structure, indicating increased predation on *P. palustris* at simple habitat structure. Interestingly, juvenile *A. cuneata* were depleted in ^{15}N when feeding on *D.*

melanogaster. Initially, juveniles of *A. cuneata* were enriched in ^{15}N by 1.1‰ and 4.3‰ compared to *H. nitidus* and *D. melanogaster*, respectively. The initial stable isotope ratio reflected the feeding of *A. cuneata* on *H. nitidus* with a high $\delta^{15}N$ ratio of 7.5‰ prior to the start of the experiment. Feeding on *D. melanogaster* ($\delta^{15}N = 6.3‰$) resulted in lower $\delta^{15}N$ ratios ($\Delta^{15}N = 1.8‰$) of *A. cuneata* approaching the trophic level fractionation of 2.5–3.4‰, which has been postulated in many food web studies (Post 2002, Vanderklift and Ponsard 2003). *P. palustris* fed *H. nitidus* only were only slightly enriched in ^{15}N ($\Delta^{15}N = 0.2‰$) at the beginning of the experiment, suggesting that only a small fraction of body nitrogen of *P. palustris* was replaced during feeding on *H. nitidus* for almost two weeks before the experiment. This is in line with trophic level fractionation of ca 3‰ of juvenile *P. lugubris* after 11 weeks of feeding (Oelbermann and Scheu 2002b). The $\delta^{15}N$ ratio of *P. palustris* only slightly increased when fed both *D. melanogaster* and conspecifics suggesting that the enrichment in ^{15}N due to cannibalism was levelled out by feeding on *D. melanogaster* depleted in ^{15}N.

Overall, feeding on a mixed diet of *D. melanogaster* and *P. palustris* resulted in a higher gain of biomass in *A. cuneata* at complex habitat structure. Beneficial effects of dietary mixing on growth and survival of generalist feeders is a common phenomenon (Wallin et al. 1992, Bernays et al. 1994, Acharya et al. 2004, Scheu and Folger 2004) that has been documented for spiders (Uetz et al. 1992, Toft 1999, Oelbermann and Scheu 2002a). Furthermore, spiders can increase their nitrogen intake and performance when supplementing their diet with IG prey (Matsumura et al. 2004). Yet, it remains unclear why this did not occur at simple habitat structure.

Effects of habitat structure

During the first week, mortality of juvenile *P. palustris* was only high at simple habitat structure without alternative prey. This indicates that both alternative prey and complex habitat structure served as refuge for the smaller spiderlings of *P. palustris*. The latter is supported by the lower gain in biomass of *A. cuneata* in complex habitat structure without alternative prey and less enrichment in ^{15}N in this treatment. Habitat heterogeneity is known to lessen the strength of predator-prey interactions (Diehl 1993, Marshall and Rypstra 1999, Lewis and Eby 2002) and this likely was the case in our study. The finding that starving juveniles of *A. cuneata* experienced higher mortality in simple habitat structure is enigmatic, since loss in biomass and changes in stable isotope ratios of *A. cuneata* were similar regardless of habitat structure, suggesting similar activity of starving spiders.

The positive effect of complex habitat structure on survival of *P. palustris* only was evident during the first week; later mortality increased strongly in complex habitat structure without alternative prey. After one week of starvation, cannibalism among *P. palustris* likely increased in treatments without alternative prey. Cannibalism is prevalent when food resources are limited and reduces competition for these resources (Wagner and Wise 1996, 1997, Samu et al. 1999). Starvation enhances cannibalism in juvenile *P. palustris* and complex habitat structure does not serve as shelter for juveniles of similar size (Rickers and Scheu 2005). Weight differences and high variability of δ^{15}N ratios of *P. palustris* at complex habitat structure without alternative prey also reflected starvation and/or cannibalism among *P. palustris*: some spiders lost weight due to starvation, while others were able to only slightly gain in weight due to cannibalism, since in wolf spiders conspecifics are known to be of poor food quality (Toft and Wise 1999, Oelbermann and Scheu 2002a). Highly variable δ^{15}N ratios of juvenile *P. palustris* add to this conclusion: some juveniles were slightly enriched in ^{15}N due to the consumption of conspecifics, while on the other hand starving *P. palustris* were depleted in ^{15}N similar to *A. cuneata*. This finding is surprising since starvation usually results in an enrichment of ^{15}N in juvenile wolf spiders (Oelbermann and Scheu 2002b, Rickers et al. 2006) due to preferential excretion of the lighter stable nitrogen isotope (Vanderklift and Ponsard 2003). Depletion in ^{15}N of juveniles presumably resulted from using up a major part of their body proteins with the remaining tissue consisting mainly of the exoskeleton, which is depleted in ^{15}N compared to other body tissues (Bunn et al. 1995). Finally, starvation may enhance activity and searching behaviour of wolf spiders (Walker et al. 1999) increasing the likelihood for predation by *A. cuneata* during the second week.

Overall, results of this experiment enhance the importance of alternative prey and habitat complexity for the occurrence and likelihood of IGP. Therefore, management practices in agroecosystems such as no-till practices or polyculture as well as the availability of litter or mulch, which increases alternative prey densities from the detritivore food web, may foster biological control by natural enemies such as wolf spiders. Stable isotope analyses successfully documented the occurrence of IGP and provided intriguing information about predation among juvenile *P. palustris*, proving very helpful in interpreting survival and biomass data. Consequently, stable isotope analysis is a powerful tool to investigate animal behaviour without direct observation which allows conclusions about trophic interactions when more than one predator is considered.

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