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Prey killing rate of a generalist predator may be enhanced by macronutrient manipulation

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Abstract Generalist arthropod predators forage not only to gain energy and nutrients, but also to obtain a balanced intake of macronutrients (the intake target). We test two opposite hypotheses concerning the predation rate of nutritionally imbalanced predators: It will increase (1) if the prey is rich in macronutrients that the predator is short of, or (2) if the prey is low in macronutrients that the predator is short of. We used the wolf spider *Pardosa amentata* (Clerck) as the predator and nutritionally manipulated *Drosophila melanogaster* Meigen as prey. We completed a full factorial experiment with eight treatment groups, in which we measured predation, consumption and prey utilization of high-protein (HP) and high-lipid (HL) flies by spiders that were previously treated with either HP- or HL-flies for two or six days. The results supported hypothesis 2. Whether spiders had been previously fed HP-or HL-flies, those that were tested against the same type of fly killed more than those tested against the opposite type of fly. A likely explanation for this result is that the predator will be unable to reach its macronutritional intake target by continued feeding on the same prey. It will stay nutritionally imbalanced and continue to catch prey in an attempt to redress its imbalance. In natural systems, predation rates may thus be increased by the widespread mismatch between predators' nutritional demands and what is available in prey. In practical biological control, it suggests a beneficial effect of feeding the predator prior to release with the pest it is intended to control.

Keywords Araneae · Lycosidae · Nutritional imbalance · Predation enhancement

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Introduction

A plethora of approaches have been developed to enhance the efficiency of biological control of insect pests by predators (e.g., Madadi 2018). Several of these concern modification of the agricultural environment in order to increase predation via an increase of the whole community of predators. With respect to techniques that involve release of laboratory produced predators, strategies also include ways to enhance the efficiency of each single predator, for example by acclimation to the temperature prevailing at the release site (Terblanche

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2014; Sinclair et al. 2022), acclimation via the pre-release food, e.g., to the specific pest that the predator is intended to control (Ishii and Shimada 2010), or artificial selection for higher predation potential (Dumont et al. 2019). A possibility that has been little researched is macronutritional manipulation of the predator prior to release. Like many other animals, generalist predators are able to regulate their food intake so that they obtain a specific ratio of protein and lipid (Mayntz et al. 2005; Simpson and Raubenheimer 2012), and they maximize their fitness when they consume a diet of this specific nutrient composition (Jensen et al. 2012). An important goal of foraging for generalist predators is therefore to compose a diet which comes as close as possible to the optimal food composition, named the intake target (Simpson and Raubenheimer 2012). When defending their intake target, nutritionally imbalanced predators (whether fed into protein or lipid deficiency) subsequently consume lower amounts of prey that are poor in the deficient nutrient than of prey that are rich in the nutrients that the predator is short of (Mayntz et al. 2005). A question derived from these findings is, whether a high/low food consumption can be translated into a higher/lower predation rate, i.e., will the predator kill a higher number of prey that are able to satisfy its immediate nutritional needs? If the answer is positive, it leads to the hypothesis (hypothesis 1) that biocontrol can be enhanced by pre-feeding the predator with prey that are nutritionally complementary to the pest species that it is going to be released against. Thus, if the pest is rich in protein, the predator should be pre-fed a diet rich in lipid and; if the pest is rich in lipid, the predator should be pre-fed a protein-rich diet. In both situations, the predator is supposed to take advantage of the pest to regain its nutritional balance.

An alternative and opposite hypothesis (hypothesis 2) is possible, however. It can be imagined that the imbalanced predator will quickly redress its optimal body composition by feeding on a prey with a complementary composition, after which there will be no effect of the pre-treatment. If the predator is pre-fed into, say, a high protein demand by offering it only lipid-rich food, it will consume much of each prey if this is protein-rich, but little if the prey is lipid-rich (Mayntz et al. 2005). In the latter case, the predator might stay hungry and nutritionally imbalanced, continue to kill prey and thus increase predation rate.

The two scenarios offer different predictions not only regarding the best direction of pre-feeding the predator (same or opposite bias as the pest), but also regarding the duration of the presumed effect. This will again be determined in an interaction with the degree to which each prey will be utilized. In hypothesis 1, because the pest fulfils the predator's nutritional demands, each prey might be completely utilized. Thus, after an initial period with a high predation and consumption rate, the predator would become satiated and in a nutritionally balanced state, after which the pre-treatment effect will have vanished. In hypothesis 2, because the prey cannot fulfil the predator's demands, each prey will be only partly consumed, and the physiological state of hunger and imbalance induced by the pre-treatment will continue and perhaps even deepen. Thus, the intended effect of the pre-treatment will continue for a longer time, possibly even leading to an increasing rate of predation due to increasing level of hunger and nutritional imbalance. Eventually, the continued nutritional imbalance will probably weaken the predator. The described scenarios assume that the pest forms the majority of prey available. They also assume that the pest is palatable prey for the predator. If not, it is a bad choice for biological control.

These general predictions do not account for the possibility that the effects of imbalance to either side of the intake target may be asymmetrical. Generally, if the food is poor in lipid, predator species overconsume protein-rich food in an attempt to reach the lipid target. In contrast, if food is rich in lipid and poor in protein, little overconsumption of lipid takes place. Such a pattern has been documented for several arthropod predators (e.g., Jensen et al. 2011, 2012). Thus, we may expect a stronger response to nutritional imbalance in lipid limited than in protein limited predators.

Finally, the magnitude of effects on prey killing rate and consumption may depend on the duration of the nutritional pre-treatment prior to release, as that would determine the level of the predators' nutritional imbalance at the time of release. Thus, apart from the interaction between treatment and test prey, simulating the effect of nutritional pre-treatment on predation on a pest, our objectives included a possible effect of pre-treatment duration. Our experiment

therefore included the factors treatment diet (either high-protein (HP)- or high-lipid (HL)-flies), test diet (HP- or HL-flies), and treatment duration in a full factorial design. We confirm hypothesis 2 that predation can be enhanced by pre-feeding the predator with prey of same nutrient composition as the prey it is intended to control.

Materials and methods

Animals

The study was intended as a test of concept. Therefore, the predator (a wolf spider, *Pardosa amentata* (Clerck)) and prey (fruit flies, *Drosophila melanogaster* Meigen) species were not those of a specific biocontrol situation, but chosen for experimental suitability. However, in some agricultural situations wolf spiders are an important part of the generalist predator complex (Kiritani and Kakiya 1975; Symondson et al. 2002), and some species of fruit flies are real crop pests (Lee et al. 2022).

We collected subadults of *P. amentata* from a meadow at Tåstrup Sø, Denmark (56° 7' 40.8'' N, 9° 58' 1.2'' E). Spiders were collected by hand and stored separately in plastic vials (2×7.5 cm) with a plaster bottom to maintain a moist environment. They were kept in the refrigerator until the start of the experiments (2–5 days). After the experiment, the spiders were released at the site of capture.

As experimental prey, we used wild-type *Drosophila melanogaster* raised on two nutrient-modified media. Both media were based on Carolina Instant Drosophila Medium Formula 4–24, mixed with other ingredients: flies of high lipid content (HL-flies) were created by raising the flies on a medium composed of 80% basic medium and 20% sucrose, flies of high protein content (HP-flies) were made by raising the flies on a medium composed of 60% basic medium and 40% casein. According to Mayntz et al. (2005), HP-flies contain *ca*. 70% crude protein and *ca*. 8% lipid; HL-flies contain *ca*. 45% crude protein and *ca*. 28% lipid. Cultures on the enriched media were started with flies from the stock culture, i.e., raised on the plain Carolina medium. By using individuals of the same species as the alternative prey types, we avoided the series of confounding factors (size, behaviour, defensive chemistry, and others) that would emerge, if we had used different prey species. During a pre-experimental standardization treatment, all spiders were fed *D. melanogaster* raised on dog-food enriched Carolina medium, supplemented with similarly raised *D. sordidula* Kikkawa and Peng and the springtail *Tomocerus vulgaris* (Tullberg). Dog food enriches the Drosophila medium with multiple nutrients, which make the resulting flies of high food quality for spiders (Mayntz and Toft 2001). *Tomocerus* is of extremely high food quality to wolf spiders even if raised on non-enriched medium (Toft and Wise 1999).

Experimental procedure

All animals went through the standardization period which consisted of two days of *ad libitum* feeding with all the prey types indicated above, followed by seven days of starvation (Fig. 1). Starvation served to secure a large intake of the treatment diets during the first part of the experiment proper. The experiment had two phases: a treatment period of two or six days, followed by a test period of 15 days. Thus, following standardization the spiders were divided in two groups, which were subject to feeding treatments of different duration (two days or six days) and started concurrently. Each of the two groups was further divided in two, with one subgroup being fed HP-flies, the other HL-flies. During treatment periods, prey was offered daily in surplus to ensure *ad libitum* feeding. Finally, i.e., after the two or six days treatment periods, each of the resulting four treatment groups were divided in two for the test part of the experiment: one half of the spiders treated with HP-flies was tested with HP-flies, the other half was tested with HL-flies. The same was true for the spiders treated with HL-flies. At each division, female and male spiders (distinguished by the swollen pedipalps of males) were randomly allocated to the new groups. Thus, the final test groups contained an approximately equal number of males and females. Sample sizes for each of the eight tests groups were $n = 15–16$. The design (Fig. 1) is similar to that of Schmidt et al. (2012; their experiment 2) except that the treatment prey, test prey combination HL,HL was missing in that study.

During the test period, we offered ten flies to each spider daily during the first three days. These flies were either nutritionally the same or complementary

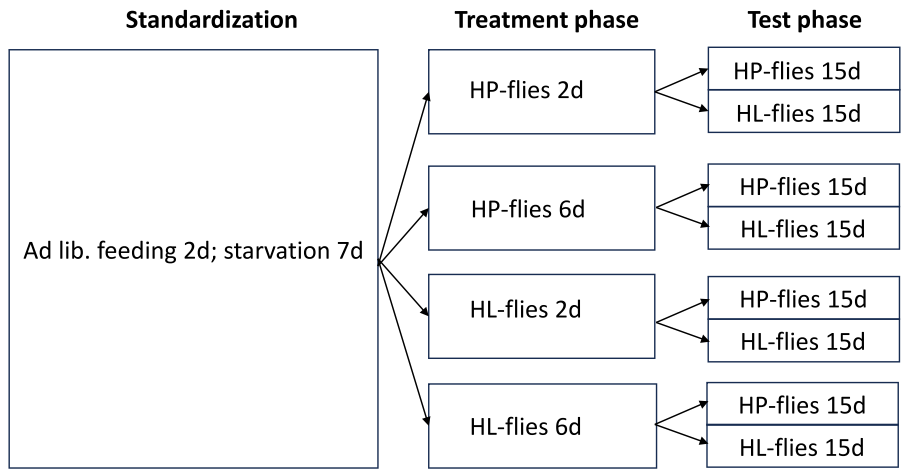

Fig. 1 Experimental design. Standardization (*ad libitum* feeding for two days; starvation for seven days) served to remove individual differences in feeding condition that might have existed in the field. Treatment phase: half of the spiders were fed high-protein (HP) flies, the other half were fed high-lipid (HL) flies; half of the spiders in each of these groups were treated for two days, the other half for six days. Test phase: half of the spiders of each of the four treatment groups were offered HP-flies, the other half HL-flies for 15 days

to what the spiders had received during pre-feeding. After 24 h, we counted the number of live flies remaining, the number of dead but uneaten flies, and the number of fly carcasses (sucked up flies). After the third day, we offered ten flies and recorded the same three fly categories at three-day intervals. This continued until day 15. We judged from the predation rates on the first three days that ten flies would be appropriate for the subsequent three-day periods. We had expected that predation/consumption would be highest in the early days of the experiment. Thus, we had not anticipated an increase in predation/consumption (see the Results section below). Thus, some individuals may have been prey limited at the last three-day periods, indicated by the fact that sometimes all ten flies had been killed/eaten. However, this limitation was not so severe that it prevented an increasing consumption during the experiment. All dead flies and remains were collected and preserved in a freezer. Subsequently, they were dried in a vacuum oven (VacuTherm VT6060M; Thermo Scientific, Langenselbold, Germany) at 60 °C for at least two days and weighed.

A measure of consumption was obtained by taking separate samples of flies ($n = 160$) from the cultures which were weighed individually (live weight). These flies were freeze-killed, dried in the vacuum-oven (as described above), and reweighed. As there was no significant difference in dry weight between HP- and HL-flies, these measurements gave a single value for the average dry weight of flies (0.2648 mg), from which we calculated the dry weight of flies offered assuming an equal sex ratio. Consumption during each test period was calculated by multiplying the average dry weight of flies with the number of flies killed and subtracting the dry weight of remnants (i.e., dead flies and carcasses of eaten flies). Finally, prey utilization was calculated as the amount consumed per fly killed.

Data analysis

The values of the three dependent variables (number of prey killed, amount of prey consumed, and prey utilization) at day 1 are the only test measures that are dependent solely of the treatments. All subsequent measures depend (increasingly) on previous predation/consumption during the test period, as spiders' hunger level and nutritional balance change. We therefore analyzed predation, consumption and utilization of test flies separately for day 1 as expression of spiders' short-term response to the treatment prey and test prey. These were analyzed with a Generalized Linear Model (GLM) including treatment prey (HP-flies/HL-flies), test prey (HP-flies/HL-flies), and treatment duration (two days/six days) as factors in a

full-factorial analysis; Poisson distributions with log link function were used for predation numbers, and normal distributions with identity link function for consumption and utilization. As some spiders killed no flies on the first test day, but all had killed some during the first three days, we also used the summed predation/consumption/utilization during the first three days as a measure of "early" response to pre-treatments. Similarly, we used the summed predation/consumption/utilization during the five three-day periods as a measure of the total effect of treatments. Additionally, we analysed the changes in predation/consumption/utilization over the first three days, and over the five three-day periods with a full-factorial repeated-measures MANOVA including the same factors as above. Here we used the within-subjects factor time and its interactions with the included factors to indicate how the responses changed over the course of the experiment. Contrasts were used for post-hoc comparisons ($\alpha = 0.05$). The graphs were drawn using the least squares means from the statistical tests. JMP Pro 16.0 (SAS Institute Inc., 1989–2019) was used for the analyses.

Spider sex was recorded because it could be easily recognized morphologically. As the experiment used subadult individuals, we did not expect any strong sex effects. In fact, when included as a fourth factor in the statistical analyses, it showed no significant main effects. As females and males were equally represented in all treatment combinations, we simplify the statistical output by neglecting the factor sex.

Results

Predation

Test prey and the interaction between test prey and treatment prey had strong effects on the number of flies killed, both at the start of the experiment (day 1 (Table 1, Fig. 2a) and days 1–3 summed (Fig. 2b)), and over the whole experiment (total predation days 1–15; Fig. 2c). Predation was higher on HP-flies than on HL-flies, but the extent of the difference depended on an interaction with treatment prey. At all stages of the experiment, predation on HP-flies was higher if the treatment prey was HP-flies, and predation on HL-flies was highest if HL-flies was the treatment prey (Fig. 2a-c). This treatment effect was marginally significant for predation on HL-flies for day 1 (contrast $p = 0.0584$), and significant for day 1-3 ($p = 0.0001$) and day 1-15 ($p = 0.0019$). For predation on HP-flies, it was significant on day 1 ($p = 0.0055$) and day 1–3 ($p = 0.0326$) and non-significant for day 1–15 ($p = 0.2779$).

Depending on the treatment prey/test prey combination, predation rate changed over the course of the experiment, slightly over the first three days and more strongly over the five three-day periods (Table S1 (time, time × test prey and time × treatment prey × test prey effects), Fig. 3a-b). During the first three days, predation on HP-flies increased the most if the treatment prey was HL-flies (Fig. 3a), but over the five three-day periods predation on HL-flies by spiders treated with HP-flies showed the largest increase (Fig. 3b). At the end of the experiment, the predation rate of the treatment prey-test prey combinations had become very similar (Fig. 3b), indicating that treatment effects had vanished.

Treatment duration affected predation, both as a main effect and in interactions both with treatment prey and test prey (Table 1). On day 1, predation on HP-flies by spiders of the two-day HP-fly treatment was significantly higher than that of all other treatment combinations (Fig. S1a). These effects were weakened over days 1–3 (Fig. S1b) and disappeared over days 1–15 (Table 1). However, because of the early effects, it affected the course of change in predation over the whole experiment (Table S1).

Consumption

During the first three days, consumption of flies was largely independent of all factors, i.e., there was no difference in consumption of HP- and HL-flies, and only a weak treatment prey × test prey interaction was present (Table 1, Fig. 2d-e). Total consumption of HP-flies was larger than that of HL-flies independently of other factors (Table 1, Fig. 2f). Over the whole experimental period, consumption of both types of flies increased independently of treatment prey, but consumption of HP-flies increased the most (Table S1, Fig. 3d). Consumption was hardly affected by treatment duration (Table 1 and S1).

Table 1 Statistical analysis (GLM) of predation (number of flies killed), consumption (mg eaten) and utilization (mg eaten per fly killed) on day 1, days 1–3 (summed) and days 1–15 (summed)

	Day 1			Days 1–3			Days 1–15		
	df	χ^2	*p*	*df*	χ^2	*P*	*df*	χ^2	*p*
Predation									
Full model	7	60.17	**0.0001**	7	67.17	**< 0.0001**	7	91.22	**< 0.0001**
Treatment prey (Trp)	1	0.28	0.5966	1	2.63	0.1050	1	2.55	0.1104
Test prey (Tep)	1	2.51	0.1132	1	40.10	**< 0.0001**	1	76.34	**< 0.0001**
Trp × Tep	1	10.80	**0.0010**	1	18.67	**< 0.0001**	1	9.25	**0.0024**
Treatment duration (Tdur)	1	10.09	**0.0015**	1	4.10	**0.0429**	1	0.08	0.7773
Trp × Tdur	1	3.92	**0.0477**	1	0.03	0.8532	1	0.12	0.7328
Tep × Tdur	1	9.28	**0.0023**	1	1.83	0.1767	1	3.21	0.0733
Trp × Tep × Tdur	1	1.49	0.2227	1	0.46	0.4987	1	1.04	0.3089
Consumption									
Full model	7	3.82	0.8005	7	13.79	0.0551	7	18.82	**0.0088**
Treatment prey (Trp)	1	0.41	0.5223	1	0.69	0.4054	1	2.97	0.0846
Test prey (Tep)	1	0.53	0.4658	1	2.30	0.1295	1	11.24	**0.0008**
Trp × Tep	1	1.46	0.2267	1	4.88	**0.0272**	1	1.36	0.2436
Treatment duration (Tdur)	1	1.40	0.2361	1	3.63	0.0567	1	0.30	0.5835
Trp × Tdur	1	0.00	0.9896	1	0.11	0.7396	1	0.01	0.9079
Tep × Tdur	1	0.03	0.8643	1	1.30	0.2550	1	3.87	**0.0490**
Trp × Tep × Tdur	1	0.05	0.8273	1	1.50	0.2209	1	0.35	0.5549
Utilization									
Full model	7	31.01	**< 0.0001**	7	74.44	**< 0.0001**	7	64.51	**< 0.0001**
Treatment prey (Trp)	1	4.06	**0.0440**	1	0.43	0.5125	1	0.25	0.6155
Test prey (Tep)	1	9.00	**0.0027**	1	68.01	**< 0.0001**	1	38.91	**< 0.0001**
Trp × Tep	1	0.48	0.4876	1	1.40	0.2362	1	4.62	**0.0316**
Treatment duration (Tdur)	1	1.42	0.2330	1	1.38	0.2397	1	3.16	0.0754
Trp × Tdur	1	1.05	0.3056	1	0.03	0.8735	1	0.00	0.9593
Tep × Tdur	1	13.44	**0.0002**	1	6.50	**0.0108**	1	25.93	**< 0.0001**
Trp × Tep × Tdur	1	1.82	0.1769	1	0.97	0.3251	1	0.12	0.7241

Spiders that captured no prey (zero-values) were included for predation and consumption but excluded for utilization. Significant *p*-values are in bold

Prey utilization

Utilization of prey (i.e., consumption (mg dry weight) per prey killed) was higher for HL-flies than for HP-flies over all five three-day periods (Table 1, Fig. 2g-i). A time × test prey interaction (Table S1) reflected that utilization of HL-flies was constant over the experiment, whereas utilization of HP-flies increased (Fig. 3f), so that at the end of the experiment, both fly types were utilized equally.

Treatment duration had some effects of prey utilization, but apart from day 1-3 these were rather weak (Table 1 and S1). On day 1, utilization of HP-flies treated for two days with HP-flies was significantly lower than that of other test prey × treatment duration groups (results not shown). This was a simple consequence of the predation effect of treatment duration and lack of a similar effect on consumption.

Discussion

Predation on both HP-flies and HL-flies were higher if test flies were the same as the treatment flies compared to predation on the same flies by spiders treated with the opposite type of flies (Fig. 2a-c).

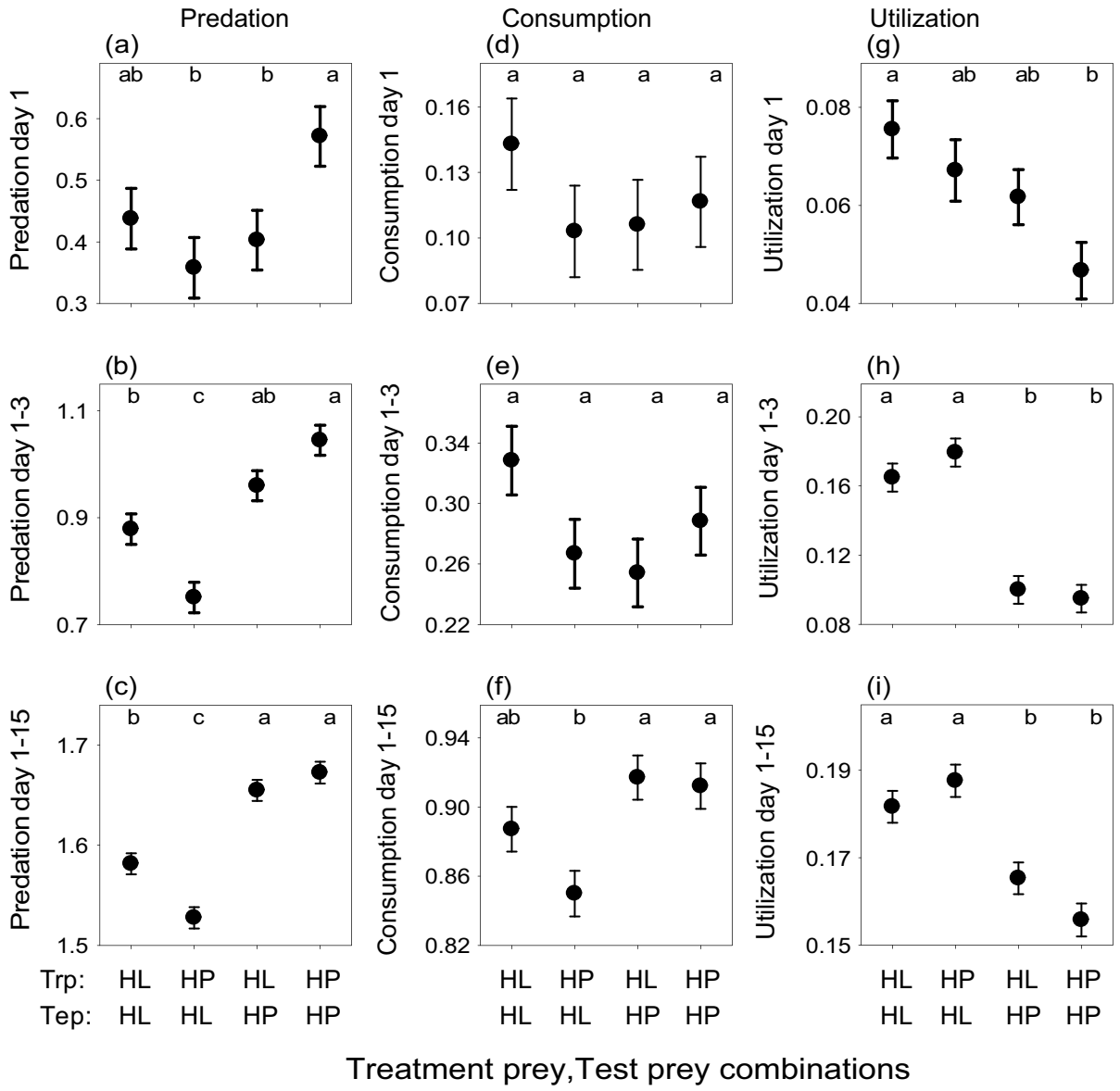

Fig. 2 Predation, consumption and utilization of test prey (HP-flies or HL-flies) by wolf spiders *Pardosa amentata*, dependent on treatment prey (HP-flies or HL-flies) on day 1 (**a**, **d**, **g**; units: predation, consumption, utilization per day), cumulated during days 1–3 (**b**, **e**, **h**; units; predation, consumption, utilization per three days), and cumulated during days 1–15 (**c**, **f**, **i**; units: predation, consumption, utilization per 15 days) of the experiment. Abscissae show treatment prey,test prey (Trp,Tep) combinations. Values shown are least squares means ± SE from full factorial 3-way GLMs. For statistics, see Table 1. Same letters at the top of each plot indicate no significant difference between groups (least square means contrasts, $\alpha = 0.05$). *HP* fruit flies (*Drosophila melanogaster*) of high protein content, *HL* fruit flies of high lipid content

The effect of treatment prey was significant for predation on HP-flies throughout the first three days but disappeared over the course of the experiment. In contrast, the effect of treatment prey for predation on HL-flies was non-significant on day 1 but significant over days 1–3 and days 1–15. The result supports hypothesis 2, claiming that predation would be enhanced if the target prey prevents predators from regaining their nutritional balance.

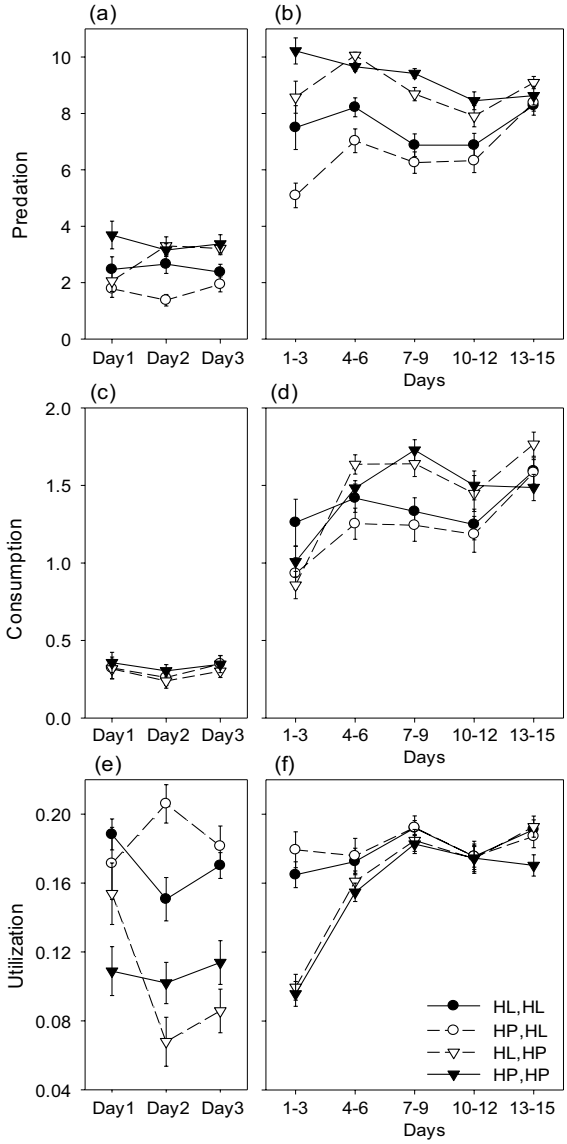

Fig. 3 Course of predation, consumption and utilization of test prey (HP-flies or HL-flies) by wolf spiders *Pardosa amentata*, dependent on treatment prey (HP-flies or HL-flies) during days 1–3 (**a**, **c**, **e**; units: predation, consumption, utilization per day), and during the five three-day periods (**b**, **d**, **f**; units: predation, consumption, utilization per three days) of the experiment. Values shown are least squares means (mean ± SE) from full factorial three-way repeated-measures MANOVA. For statistics, see Table S1. Treatment groups are treatment prey-test prey combinations. *HP* fruit flies (*Drosophila melanogaster*) of high protein content, *HL* fruit flies of high lipid content

This result is significant though the main effect of test prey is even stronger than the treatment prey × test prey interaction (Table 1). Thus, predation and consumption of HP-flies were overall larger than those of HL-flies (Figs. 2–3). This result was predicted based on previous findings that predators easily overconsume protein but not lipid relative to their protein/lipid-target (Jensen et al. 2011). A mechanistic explanation may be that wolf spiders show increased locomotor and predatory activity when fed protein-rich compared with lipid-rich food (Wilder and Rypstra 2008; Koemel et al. 2019).

We had expected that a longer (six days) treatment duration affected predation more than a short treatment duration (two days). Our results showed the opposite, as a two-day treatment with HP-flies increased predation on HP-flies more than a six-day treatment on day 1 (Fig. S1). Otherwise, treatment duration had no significant effects on the level of predation, except that because of the effect on day 1, it affected the way predation rate changed throughout the experiment (Table S1).

Consumption of both fly types was unaffected by the treatment prey (Table 1). We had expected the two groups in which treatment prey differed from the test prey to consume more than the two groups whose treatment prey and test prey were the same, but this was not confirmed. Consumption increased during the course of the experiment in all treatment groups (Fig. 3d), and this increase was influenced by weak effects of test prey and treatment duration. In fact, the overall higher consumption of HP- than HL-flies was due to a very strong increase in HP-fly consumption during the experiment (Fig. 3d), though consumption of HL-flies also increased. Figure 3d indicates that the consumption increase is higher in the two groups in which test prey differed from treatment prey, as would be expected and was supported by a marginally significant time × treatment prey × test prey interaction (Table S1).

The results of Schmidt et al. (2012) are qualitatively similar to ours. These authors used "low-quality" and "high-quality" fruit flies, which nutritionally should be equivalent to our HL- and HP-flies, respectively. They found that wolf spiders treated with high-quality flies killed more high-quality flies than spiders treated with low-quality flies. In spite of this, consumption of high-quality flies was independent of treatments. Unfortunately, they did not test predation and consumption of low-quality flies.

Prey utilization was influenced by treatment prey, test prey and treatment duration in

various combinations of main effects and interactions throughout the experiment (Table 1). The strongest effect was that of test prey, reflecting that HL-flies were utilized much more efficiently than HP-flies (Fig. 3e-f). Due to this high efficiency, the curves for predation and consumption of HL-flies follow each other closely (Fig. 3b,d).

As we could not identify the cause of death of flies that had not been sucked out by the spiders, the low utilization of HP-flies might in part be due to fly mortality not directly caused by the spiders. However, as the spiders do not normally consume already dead prey, in particular when live prey is still available, the high consumption rate of HP-flies shows that spider-independent mortality cannot be a main cause of the high predation rate on HP-flies. Predators often kill prey without consuming them (wasteful killing), a behaviour that may be associated with suboptimal prey (Lang and Gsödl 2003; Fantinou et al. 2008). In our experiment, it was most prominent with HP-flies during the early three-day periods (Fig. 3b,d,f), when the spiders presumably were least hungry.

Partial consumption of prey has been related to factors like satiation and prey density (Sih 1980; Samu 1993). In arthropods with extra-oral digestion (like spiders), protein and glycogen are extracted from the prey in the early phase of the feeding process, whereas lipid is extracted later (Cohen 1995). Our results showed that high-protein flies were less efficiently utilized than high-lipid flies. Thus, macronutrient composition may be added to the list of factors responsible for partial consumption. The changing utilization of HP-flies during the course of the experiment (Fig. 2f) indicates that other factors, perhaps satiation/hunger, interacted with macronutrient composition to make partial consumption a dynamic variable.

Over the whole experiment, predation on HL-flies was lower than that of HP-flies. This was because the spiders consumed less and utilized each fly very efficiently. Utilization of HP-flies increased during the experiment, while utilization of HL-flies remained at the same high level (Fig. 3f). As predation on HP-flies was constant or declining (Fig. 3b), the increased consumption of HP-flies (Fig. 3d) must have been due to the increasing utilization efficiency. The reason for this increased efficiency of utilization of HP-flies along the experiment is unclear. The increasing consumption might indicate an increasing level of hunger, which might be met only by increasing utilization efficiency as fly availability was constant.

The high utilization of HL-flies is in line with the findings of Wilder et al. (2010) that spiders extract nearly all of available lipid in the prey. This may reflect a deeply rooted ability of predators to prioritize extraction of lipid. In their natural habitat, most arthropod predators are not only limited by food but additionally, they are specifically limited by lipid (Wilder et al. 2013; Toft et al. 2019). This may have constituted a selection pressure for unconditional high extraction efficiency of lipid, i.e., lipid may be extracted at the maximal possible rate. In contrast, protein limitation is rare among arthropod predators (Toft et al. 2019). Their available prey tend to be protein biased compared to predators' demands (Wiggins and Wilder 2018). It therefore seems that extraction of protein has evolved to be flexible dependent on factors like prey availability, prey nutrient content, and the level of imbalance of the predator itself (Wilder et al. 2010). Alternatively, or additionally, the high extraction efficiency of HL-flies may be a seasonal effect (Raubenheimer et al. 2007; Bressendorff and Toft 2011). The experiment was performed during the autumn when the spiders were preparing for the winter, i.e., were building up lipid stores for at least six months of hibernation (Arrese and Soulages 2010). Whatever the explanation, the results suggest that the widespread mismatch between predators' nutritional demands and the nutritional composition of their prey in nature may serve to enhance predation rates and thus the overall impact of predators on their prey populations.

We suggest that macronutritional manipulation of predators may be a possible additional strategy to enhance biocontrol efficiency. Generalist predators use dietary mixing in order to reach the intake target that gives them an overall balanced diet (Lefcheck et al. 2013; Marques et al. 2015). If an agricultural pest has a nutritional composition that differs from the demands of its predators, it will be selected only if alternative prey have an even more biased composition, or if the pest is the most abundant prey available. In both cases, the predator may be forced to accept it even if it does not re-establish the nutritional balance. From the results of this study it seems to be possible to increase predation on an abundant non-optimal (but palatable) prey by feeding the predator with prey of the same nutrient composition as the pest prior to

release. This means that the pest species itself may be used for predator pre-treatment, which will make the practical application easier, since no special search for an optimal pre-treatment prey is needed.

Against our conclusions, Schuldiner-Harpaz et al. (2022) predicted that a nutritionally non-optimal pest would be best controlled by a nutritionally balanced predator because of its lower choosiness in prey selection. The difference between their conclusion and ours may lie in different assumptions about the environment. Our experiment was a simple system composed of only a single predator and a single prey, mimicking a pest outbreak in a crop with few other resources for the predator. In contrast, Schuldiner-Harpaz et al. (2022) modelled a richer system with alternative resources for the predator. Thus, the optimal pre-treatment may depend on the complexity of the habitat.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

Research involving human/animal participants The work complies with Danish legislation on animal welfare.

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