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Accepted Article

Food and specific macronutrient limitation in an assemblage of predatory beetles

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Abstract

Habitats vary in food resources with carnivores often being prey limited, but it is unclear whether habitats facilitate a nutritionally balanced diet. Two paradigms in nutritional ecology, ecological stoichiometry and nutritional geometry, predict that carnivores are limited mainly by protein or lipid, respectively. Using the carabid beetle *Anchomenus dorsalis* and 10 other predatory beetles from agricultural fields, we developed and tested two simple procedures for quantifying macronutrient-specific habitat conditions without requiring information about the natural prey. Both procedures assume that predators forage for nutrients rather than specific prey. Our results show that 10 of 11 species were food limited. Five species were lipid limited and one species was protein limited in the field. Co-existing predator species showed considerable segregation of fundamental macronutritional niches. A linear relationship between specific nutrient limitation and the target lipid:protein (L:P) intake ratio indicates that species with high L:P target are more protein limited while species with low L:P target are more lipid limited. The study illustrates how species within a natural assemblage vary in nutritional niche and in specific nutrient limitation.

Keywords: Carabidae, field conditions, habitat quality, nutritional ecology, nutritional niche, nutrient limitation, optimal foraging, Staphylinidae

Introduction

Nutrient limitation in the field occurs if the food available prevents animals from composing a diet of optimal nutrient composition, i.e. the composition that maximizes their fitness. Based on the theory of ecological stoichiometry (Sterner & Elser 2002), it has been argued that carnivorous arthropods should tend to be nitrogen/protein limited (e.g. Fagan et al. 2002; Fagan & Denno 2004) because the ratio of carbon to nitrogen (C:N) in herbivorous insects is higher than in predators. Assuming that the demand for C and N is reflected by the body content, N-rich predators will have difficulties getting their N-demand satisfied by preying on N-poor herbivorous insects. Against this hypothesis stands the argument of Raubenheimer et al. (2007, 2009; see also Wilder & Eubanks 2010; Wilder et al. 2013), based on nutritional geometry (Simpson & Raubenheimer 2012), claiming that an organism's nutrient demand is not dictated by its body load but by the turnover rate in the body, which is highest for the nutrients involved in metabolic maintenance, i.e. non-protein energy sources. Very few direct measurements of specific nutrient limitation in the field exist to evaluate these opposing hypotheses. Thus, the controversy is unsettled due to lack of empirical evidence.

In contrast, studies of predator food limitation in the field have a long history (White 1978, 1993; Wise 1993). Food limitation has been documented in predators at both the population and the individual level, and there is general agreement that most arthropod predators are food limited at least part of the time (Lenski 1984; Juliano 1986; Wise 1993; but see Jeschke 2007). The most direct evidence of general food limitation in the wild comes from experiments involving food supplementation, where food limitation is confirmed if supplementation increases individual growth rates, survival, fecundity or population density (Wise 1993). In an alternative approach, Bilde & Toft (1998) devised a method in which a reference curve was used to quantify the level of hunger experienced by arthropod predators in the field.

The typical approach to study animals' nutrient balance in nature has aimed at analyzing the predator's diet composition, from this to calculate its nutrient budget, and then test if individuals select a diet with different nutrient composition if presented with food items with a wider than the natural range of nutrient compositions (Raubenheimer et al. 2015, Machovsky-Capuska et al. 2016a,b,d). However, the analysis of diets and macronutrient budgets of animals in the field is fraught with methodological challenges (Raubenheimer et al. 2015). This is not least true for small invertebrate predators (Birkhofer et al. 2017). Therefore, most existing studies of nutritional balancing in the field deal with vertebrates, e.g. birds whose feeding behavior can be readily observed (Tait et al. 2014; Machovsky-Capuska et al. 2016b,c; Coogan et al. 2017), or herbivorous or omnivorous mammals (Felton et al. 2009; Rothman et al.

2011; Johnson et al. 2013; Coogan et al. 2014; Raubenheimer et al. 2015) that can be followed continuously or from which extensive quantitative studies of the natural diet exist. The most direct evidence for macronutrient limitation in nature comes from Coogan et al. (2014), who found that the food available to a population of grizzly bears during spring and early summer did not embrace the intake target, i.e. the optimal macronutrient combination. These animals were limited in non-protein energy, i.e. lipid and carbohydrate. In a food supplementation experiment, Australian white ibis selected a diet with a higher carbohydrate content than was available in their natural food. Likewise, there is indirect evidence for lipid limitation in marine mammals and birds, e.g. Stellar sea lion *Eumetopias jubatus* (Trites et al. 2003), kittiwakes *Rissa tridactyla* and puffins *Fratercula cirrhata* (Romano et al. 2006). Evidence for protein limitation comes from urban populations of myna *Sturnus tristis* birds (Machovsky-Capuska et al. 2016c), western jackdaws *Corvus monedula* (Meirier et al. 2017) and pine martens *Martes martes* (Remonti et al. 2016). In contrast, chacma baboons *Papio hamadryas ursinus* showed no indication of nutrient limitation (Johnson et al. 2013).

Data are scarce on invertebrates. Simpson et al. (2006) found that mass migrations and cannibalism in a cricket was induced by shortage of protein and salt. German cockroaches had less protein and carbohydrates available in the “field” (human apartments) than is present in the assumed optimal laboratory diet (Kells et al. 1999). Regarding true predators, Salomon et al. (2008) supplemented colonies of a social spider with protein- or lipid-rich prey and found that the latter increased the proportion of reproductive females. Wiggins & Wilder (2018) found that a jumping spider grew best on lipid-rich prey but that protein-rich prey prevailed in the habitat. These authors suggested that the spider might solve this problem through selective feeding on lipid-rich prey.

Thus, from the available evidence nutritional imbalances may be in both directions of relative shortage or surplus of any macronutrient also among predators. If there is any dominating pattern of nutrient limitation, as the two opposing paradigms postulate, information from many more species must be attained. To achieve this, more direct and more cost-efficient methods for measuring nutrient limitation in the field are required.

We here propose two simple procedures to quantify the degree of macronutrient imbalance experienced by animals in the field at a particular time. Both are based on the assumption that predators, if given the opportunity in the form of nutritionally complementary diets, will seek to select a combination of nutrients that redresses any existing imbalance in previous nutrient intake and/or maintain an obtained nutritional balance by composing a diet with the optimal nutrient composition (= the intake target (Simpson & Raubenheimer 2012 p. 13)). Laboratory evidence from several groups of generalist

predatory arthropods indicate that this can be accomplished (Mayntz et al. 2005; Jensen et al. 2012; Wilder et al. 2013). The ability to defend a nutritional balance is no guarantee, however, that this target can also be reached under varying prey availabilities in the field. In the first procedure (the “double-test procedure”), we measure the self-selected diet of predators immediately after collection in the field and then feed the same animals to satiation and nutrient balance during one week before measuring the self-selected diet a second time. The second self-selected diet is assumed to reveal the animals’ “fundamental intake target” or “fundamental nutritional niche position” (Machovsky-Capuska et al. 2016d, 2018), because it is uninfluenced by the habitat. The nutritional conditions in the field can be evaluated by comparison of the first (habitat-dependent) and second (habitat-independent) self-selected diet. This procedure additionally allows us to distinguish between general food limitation by comparison of the amounts of food eaten in the two tests, and specific nutrient limitation by comparison of the two self-selected lipid:protein (L:P) ratios. We can thus identify whether predators are both food and nutrient limited, only food limited, only nutrient limited, or unlimited in both respects (illustrated in Fig. 1).

In the second procedure (the “reference curve procedure”), we first expose groups of animals to fixed diets varying in macronutrient composition (no-choice pre-treatment). After a predetermined length of time, we let the animals self-select for 24 hours among two complementary diets varying widely to both sides of the optimal composition. Their choice will form a reference curve relating the self-selected nutrient composition to that of the pre-treatment. One point of this curve is of particular interest: its intersection with the $y = x$ line. At this point the self-selected L:P ratio is identical with the pre-treatment L:P ratio, and thus it represents the animals’ intake target. After establishing the reference curve, we collect animals from the field and measure the nutrient composition of their self-selected diet during 24 hours. From the reference curve, we can then infer the L:P ratio experienced by the animals in the field prior to collection. In particular, we want to test whether the self-selected L:P ratio differs from the intake target, and if it does, to which side it deviates, i.e. whether the animals were lipid or protein limited in the field.

We use extensive data from the carabid beetle *Anchomenus dorsalis* from agricultural fields to demonstrate the two procedures. This species is already used in several studies on predator nutrient balancing. Thus, we know that these beetles can redress an induced macronutrient imbalance (Mayntz et al. 2005), select a nutrient composition that maximizes its fecundity (Jensen et al. 2012), and they probably use sugar and lipid as substitutable non-protein energy sources (Noreika et al. 2016). They are also known to have very high starvation tolerance (Knapp 2016). Concurrently collected results on other predatory beetles (Carabidae and Staphylinidae) from the same fields are also presented in order to

answer the following questions: Are predatory beetles from agricultural fields food and/or macronutrient limited in their habitat? If macronutrient limited, are they mostly lipid or protein limited? Are females and males equally food and/or macronutrient limited? The results from multiple species allowed us to analyze a relationship between species' intake target and degree and direction of macronutrient limitation. We expected that species with a high (i.e. lipid biased) L:P target would tend to be lipid limited, and species with a low (i.e. protein biased) L:P target would tend to be protein limited. This is because the more biased a predator's demand is, the more difficult it might be to find a sufficient number of prey that satisfies this demand. The results support the idea that predators are mainly lipid limited. We found, however, a relationship between the species' fundamental intake targets and the direction and degree of macronutrient limitation not predicted by the nutritional ecology paradigms but which may provide an alternative explanation for why some species are lipid and protein limited, respectively.

Materials and methods

Animals

The animals used for the present study were of the same population as those of our previous studies (Mayntz et al. 2005; Raubenheimer et al. 2007; Jensen et al. 2012; Noreika et al. 2016; Toft & Nielsen 2017), all collected near Stjær, Denmark (56°7'N, 9°57'E). The animals hibernate as adults in the soil at the margins of agricultural fields. On emergence from hibernation, they aggregate under stones for some weeks before dispersing into the fields where they reproduce during May to June. We collected *A. dorsalis* in 2013 at the hibernation sites by turning stones. These beetles were used for producing the reference curve. In 2013 and 2018 we also collected beetles within fields during the reproductive season. These beetles were used for performing the double test and as population estimates on the reference curve. One sample was collected from a rape-seed field in June 2013, and another five samples were collected in 2018: two from rape-seed fields, two from winter wheat fields, and one from a winter barley field. Approximately 20 *A. dorsalis* were collected in each field (for final sample sizes, see Table 1). Concurrent with the studies of *A. dorsalis* in 2018, we collected and tested other species of the predatory beetle assemblage from the same fields. These included the carabid species *Agonum muelleri* (body length 9 mm), *Amara similata* (9 mm), *Bembidion lampros* (3 mm), *Bembidion quadrimaculatum* (5 mm), *Demetrias atricapillus* (5 mm), *Loricera pilicornis* (8 mm), *Nebria brevicollis* (12 mm), *Pterostichus melanarius* (16 mm) (identified using Lindroth 1985, 1986), and the staphylinid species *Philonthus fuscipennis* (10 mm) and *Tachinus rufipes* (6 mm) (identified using Hansen 1952). We aimed to collect 20 individuals of each species and combined individuals from across the different fields to get this number (see sample sizes in Table 2).

Housing and measuring consumption

Following procedures in Jensen et al. (2012) and Noreika et al. (2016), beetles were sexed, weighed and placed individually in 9 cm Petri dishes with a small tube of drinking water plugged with cotton and one or two small glass dishes (diameter 1.9 cm) on which the semi-artificial foods were served. Each dish was placed in an inverted 2.5 cm diameter plastic lid with a 2 mm high edge. This arrangement allowed easy recollection of spilled food and prevented mixing of food from the two dishes. Dishes and food were weighed before feeding. Afterwards, dishes with remaining food were dried in a vacuum oven (VacuTherm VT6060M, Thermo Scientific, Langenselbold, Germany) at 60 °C for 24 h, and reweighed. Consumption from each food was calculated as the difference in food mass before and after feeding. Controls without beetles were run concurrently to correct for any changes in food mass not caused by consumption. Lipid and protein consumption were calculated by multiplying dry mass consumption with the proportion of each nutrient in the two foods and summing from both foods.

Semi-synthetic foods

We used the recipes of Jensen et al. (2012) for producing five semi-synthetic foods with different composition of protein and lipid. Half of the dry mass of each food consisted of dried and powdered grasshopper (*Locusta migratoria*), while the other half was composed of lard and/or casein in different proportions, creating foods with L:P ratios of 1.58, 0.93, 0.54, 0.29 and 0.1, respectively. The two extreme foods (L:P ratios 1.58 and 0.1) are known to be complementary; thus, with both foods available the beetles can compose a diet with an optimal L:P ratio (Jensen et al. 2012). The two complementary foods contained 87.25% protein and 8.9% lipid versus 37.25% protein and 58.9% lipid, respectively (Noreika et al. 2014). All food components were pulverized, dried at 60 °C for 24 h in the vacuum oven, and thoroughly mixed. The foods were kept dry in a desiccation chamber with dry silica gel before weighing and serving to the beetles. The experiments were performed at 21-23 °C and natural light following the season.

Double-test procedure

After collection, beetles were transported in a cooled container and immediately set up in experiment upon arrival to the laboratory. Here, individual consumption was recorded during 24 h of self-selection between the two most extreme semi-synthetic foods (test 1). The beetles were then offered chopped house fly (*Musca domestica*) pupae and mealworm (*Tenebrio molitor*) larvae plus the two extreme semi-synthetic foods ad libitum for seven days with a replacement of food and water at day four. Dead insects were included as they are probably more palatable than the semi-synthetic foods to secure that the beetles

would reach satiation. We then moved the beetles to clean Petri dishes and again recorded consumption during 24 h of self-selection between the two most extreme semi-synthetic foods (test 2).

Reference curve procedure

To produce the reference curve, we first standardized the beetles by providing all five semi-synthetic foods for six weeks. This allowed the beetles to feed to satiation and reach their preferred nutritional balance while developing into the reproductive phase. The beetles were then separated into five groups of 10 females and 10 males, and beetles in each group were given ad libitum access to one of the five semi-artificial foods over seven days. Following this pre-treatment, the beetles were transferred to individual petri dishes, and consumption was recorded during 24 h of self-selection from the two most extreme semi-synthetic foods. We could then model the relationship between dietary L:P ratios in the restricted no-choice period with the dietary L:P choice in the subsequent self-selection period.

Data analysis

Double-test procedure. We analyzed the amounts of food eaten and the self-selected L:P ratios separately, as the former gives information about general food limitation while the latter provides information about specific nutrient limitation and nutritional imbalances. Amounts of food eaten were analyzed in three ways, i.e. as the sum of dry mass consumption from the two foods; as the sum of protein and lipid consumption; and as the sum of protein and lipid energy consumption (using 17 Joule/mg for protein and 37 Joule/mg for lipid; N.R.C. 1989). Since all three analyses gave similar results with respect to the factors “field” and “sex”, only the results of food dry mass consumption are presented. We applied the multivariate repeated-measures MANOVA to compare consumption and L:P ratios in test 1 and test 2 (referred to as Consumption1, Consumption2, L:P1, and L:P2), using the within-subjects factor “time” and its interactions with “field” and “sex” as the critical statistics.

Reference curve procedure. Appendix S1 in Supporting Information details the construction of the reference curve (shown in Fig. 3) and calculation of confidence limits (results in Table 1) by which the data from field-collected animals were evaluated against the reference curve (cf. Bilde & Toft 1998). The estimated field-L:P values ($L:P_{\text{field}}$) can be considered the “realized intake targets” or “realized nutritional niche positions”. We consider them significantly different from the estimated intake target if the target lies outside the confidence limits.

Data for calculating the realized nutritional niche width of the population are available only for *A. dorsalis* by means of the reference curve ($L:P_{\text{field}}$). Thus, we cannot directly compare the realized nutritional niche widths of all species in the assemblage. However, assuming that the species order of niche positions ($L:P_{\text{field}}$) will be approximately the same (though opposite because the reference curve has

a negative slope) as the species order in the L:P1 data, and because these data were not normally distributed, we used a non-parametric analysis (Kruskal-Wallis test) of the L:P1 data to compare the niches of the 11 species. Thus, we used the medians as expression for niche positions and the 25/75% quartiles for niche widths. Tests for the two quartiles gave similar results; therefore we report only those for the 25% quartiles. We used the same approach to analyze the fundamental niche positions and widths from L:P2 data. It should be remembered, however, that the L:P2 data were created as optimal individual self-selection points and thus may represent only the plateau of the fundamental niche curve where fitness is maximized (Behmer & Joern 2008). All statistical analyses were made in JMP 13.0.0 (SAS Institute Inc., Cary, NC, USA).

“Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.XXXXXX>”.

Results

Double-test procedure using A. dorsalis from different field habitats

Consumption by *A. dorsalis* from across fields differed between the first and the second self-selection test, between the sexes, and between beetles from the five fields (Appendix S2 in Supporting information). Beetles of both sexes consumed more food in the first than in the second test (Fig. 2A-B), with significantly higher consumption in the first test by females than by males (log-transformed data, ANOVA $F = 23.6$, $P < 0.0001$). There were significant differences in consumption by females from the different fields (log-transformed data, ANOVA $F_{4,51} = 3.53$, $P = 0.0128$), which was not the case in the males ($F_{4,50} = 2.07$, $P = 0.0992$). Females furthermore showed the largest difference in consumption between the two tests; overall, they consumed 6.9 times more food in the first than in the second test, while males only consumed 4.7 times more food in the first test. There was no significant difference in consumption between the sexes in the second self-selection test ($F_{1,101} = 2.5$, $P = 0.12$), with marginal variation between beetles from different fields (females $F_{4,51} = 2.25$, $P = 0.0767$; males $F_{4,50} = 2.55$, $P = 0.0504$) (Fig. 2A-B). These results demonstrate that both sexes of *A. dorsalis* were food limited in the field, that females were more food limited than males, and that females were more food limited in some fields than in others.

The consumption ratio of lipid to protein in the first test (L:P1) was significantly higher than in the second test (L:P2), but did not vary depending on sex or field of collection (Table S1, Fig. 2C). L:P1 was higher than L:P2 in all five fields (binomial test $P = 0.031$). Thus, *A. dorsalis* was lipid-limited in the field with no difference between sexes and fields.

Reference curve procedure using *A. dorsalis*

Using the fitted curve (see Appendix 1 in Supporting Information), we estimated an intake L:P target of 0.378 under nutritional balance, given as the intersection point between the reference curve and the $y = x$ line (Fig. 3). This value is slightly more lipid-biased than the equivalent estimate for the beetles collected in 2018 (overall L:P2 = 0.334), but within the range of field means (0.263-0.382), and not significantly different from any of these (all $P > 0.05$).

The L:P1 values selected by animals from the field were all higher than the intake target L:P estimated from the reference curve (binomial test $P = 0.016$; Fig. 3). The upper 95% confidence limits of the L:P_{field} ratios lie below the calculated intake target for the complete 2018 data set (i.e. beetles from all five fields merged) and for one field separately (Rape2; Table 2). This shows that the beetles' food in the field was protein biased relative to the intake target and thus that the beetles were lipid limited; in one field, the beetles were strongly lipid limited. The *A. dorsalis* collected post-hibernation in 2013 selected an extremely lipid-biased diet (L:P = 1.142), which indicates severe exhaustion of lipid reserves following hibernation in these beetles (Fig. 3).

Double-test procedure across the predatory beetle assemblage

For the 10 additional species of the predatory beetle assemblage, total food consumption was higher in the first than in the second test for all species, although in one species (*L. pilicornis*) the difference was not significant (Table 2). Thus, 9 of 10 species were significantly food limited in the field. In three species (*D. atricapillus*, *B. quadrimaculatum*, *Pt. melanarius*), the level of food limitation differed between the sexes; in all these cases females were more food limited than males. Four of the species were significantly lipid limited (*Pt. melanarius*, *N. brevicollis*, *A. muelleri*, *Ph. fuscipennis*; Fig. 1E). One species was significantly protein limited although not significantly food limited (*L. pilicornis*; Fig. 1C). Five species were food limited but not macronutrient limited, i.e. they had eaten a restricted but nutritionally balanced diet before capture (*B. lampros*, *B. quadrimaculatum*, *A. similata*, *D. atricapillus*, *T. rufipes*; Fig. 1D).

The realized nutritional niches (calculated from L:P1 data) differed significantly across the species (Kruskall-Wallis test $\chi^2_{10} = 70.5$, $P < 0.0001$; Fig. 4a). The species formed two groups, one with realized niches concentrated in the protein-biased end, the other with higher L:P ratio targets, and with high niche overlap within each group. Species' realized nutritional niche widths were significantly and positively correlated with the realized niche positions ($t_9 = 6.05$, $P = 0.0002$ (Fig. 4b)). The fundamental nutritional niches (calculated from L:P2 data) also differed significantly across species (Kruskall-Wallis test $\chi^2_{10} = 53.7$, $P < 0.0001$; Fig. 5a). The fundamental niche positions are more evenly spaced than the realized

niche positions, indicated by the coefficient of variation of nearest-neighbour values (0.44 for L:P2 compared with 1.02 for L:P1). The fundamental nutritional niche widths correlated significantly and positively with the corresponding niche positions ($t_9 = 2.39$, $P = 0.041$; Fig. 5b), i.e. species with protein-biased target intake had smaller niche widths than species with more lipid-biased targets. The realized niche positions (median L:P1) were independent of the fundamental niche positions (median L:P2) ($t_9 = -0.99$, $P = 0.35$), and the realized niche widths (25% quartile L:P1) were independent of the fundamental niche widths (25% quartile L:P2) ($t_9 = 0.38$, $P = 0.72$). Across all 11 beetle species, there was a significant linear relationship between the species' fundamental intake target ratio (median L:P2) and the deviation from this target ratio in the self-selected L:P ratio following collection in the field (median L:P2 minus median L:P1) ($t_9 = 3.32$, $P = 0.0090$; Fig. 6). The figure shows that species with a fundamental intake target in the intermediate range tend to be in macronutrient balance, while species with a protein-biased intake target tend to be lipid limited and species with a lipid-biased intake target tend to be protein limited in the field (Fig. 6).

Discussion

Food and macronutrient limitation

The two experimental procedures (the double-test procedure in particular) provide information on macronutrient limitation in the field at low costs in money and time. Importantly, the simplicity of the procedures allows the concurrent analysis of multiple species even if little is known about their biology. Thus, we were able to analyze all the most abundant species of the predatory beetle assemblage in agricultural fields within a single reproductive season. We confirm that insect predators in agricultural fields are food and energy limited, and show that macronutrient limitation is widespread among these predators. We also found substantial segregation in both the realized and the fundamental nutritional niche positions of the species. Because of niche segregation, prey availability differs between co-occurring species, i.e. the nutritional conditions are experienced differently for each species (or even individual) in the same field. Therefore, the traditional approach for measuring prey abundance and species composition by standard entomological sampling methods is insufficient, because these techniques do not provide information about the availability for each predator species or individual. In order to understand how each animal experiences the nutritional conditions in its habitat, we must “ask the animal” by testing the animal itself. This is what our procedures were designed for (cf. Raubenheimer et al. 2009).

Both procedures lead to similar results regarding the nutritional conditions in the field of *A. dorsalis*, although they were founded on independent data (test2 results and reference curve, respectively). Thus, both procedures show that *A. dorsalis* was lipid limited in both sexes and that lipid limitation was most severe in the Rape2 field. Our survey of the whole predatory beetle assemblage revealed that 10 out of 11 species were food limited and 6 were macronutrient limited with lipid limitation being predominant (5 out of 6 species). These results can be seen as numerical support of the general prediction that predators are mainly lipid limited (Raubenheimer et al. 2007, 2009). While our study is conclusive for the investigated field sites, we see it only as a preliminary confirmation. Agricultural fields are inhabited by a relatively species-poor invertebrate fauna, especially under intensive conventional crop production (Tsiafouli et al. 2015) like the fields of the current study. Similarly collected data from a range of habitat types and other predator groups are needed for a final conclusion. Results on two species of harvestmen (Opiliones) indicate that non-protein limitation is also prevailing among these predators in urban as well as in forest habitats (S. Toft, unpublished).

The results show that a population can be food limited without macronutrient limitation, and that macronutrient limitation may exist without food limitation. Thus, all six scenarios depicted in Fig. 1 seem to be realistic (though not all are represented in this study). Fig. 4 indicates that species with fundamental intake targets (L:P2) in the intermediate range tend not to be macronutrient limited, while species with higher or lower intake targets are protein or lipid limited, respectively. What exactly lies behind this relationship is unclear. The differences in size and the spread of fundamental intake target (L:P2) values between species (Fig. 4) would indicate that nutritional demands may secure segregation in prey types between species (cf. Behmer & Joern 2008). This view must be modified, since we found no relationship between realized and fundamental niches. This was true for both the niche positions and the niche widths. Further, the regular spacing of fundamental niches was transformed into a more clumped distribution of realized nutritional niches. These results indicate that realized niches were determined more by the actual prey availability, i.e. the macronutrient composition of available prey, than by the species' fundamental niches.

With the exception of *L. pilicornis*, which is a specialized Collembola hunter (Hintzpeter & Bauer 1986), all species studied are generalist predators (Toft & Bilde 2002), and one species (*A. similata*) is additionally a granivore (Jørgensen & Toft 1997). We expected *A. similata* to be less macronutrient limited than pure insectivores, because this species not only has access to protein and lipid from insect prey but also to carbohydrate and lipid (oils) from seeds. This prediction was confirmed, as *A. similata* was among the three species whose L:P1 was very close to its intake target (L:P2) (Table 2). The two

others (*B. lampros* and *B. quadrimaculatum*) are strict insectivores, and we cannot conclude that the ability of *A. similata* to reach its fundamental target intake ratio in nature is caused by its broad diet.

As indicated, macronutrient imbalance is not necessarily implied when food is scarce. In this case, food and energy are scarce but without causing imbalance, such that protein and lipid are equally limiting. This may be the case if prey populations are low but the optimal prey diversity maintained, or if available prey are of optimal macronutrient composition (Fig. 1D). This situation is exemplified by *A. similata*, *B. lampros*, *B. quadrimaculatum*. Likewise, macronutrient limitation without food limitation is possible as in the case of *L. pilicornis* (Fig. 2C). We did not find lipid limitation without overall food limitation (Fig. 2B), but examples from other ecosystems have been described. Trites & Donnelly (2003) argue that the decline of Stellar's sea lions in the Northern Pacific may be due to a change in the populations of fishes to species of lower oil content rather than to a decrease in the abundance of fish.

The relationship between the species' fundamental intake targets (L:P2) and the difference between the fundamental intake target and the realized intake target for each species in the field (L:P1 – L:P2) was opposite to our prediction. An alternative hypothesis would be that a species with a very lipid biased intake target is primarily adapted to seek lipid-rich prey, i.e. be a macronutritional specialist (sensu Machovsky-Capuska et al. 2016d). If this nutritional specialization is coupled with a behavioural trade-off that reduces the ability to identify protein-rich prey or reduces the ability to switch to preference for protein-rich prey under nutritional imbalance - and vice versa for species with protein biased intake target - the relationship can be explained. In the same vein, species with intermediate intake L:P targets may be considered generalists and possibly have less selective prey preference (a wider prey niche), and in this way they can more easily defend their intermediate L:P target. This suggestion was partly confirmed by the positive relationship between niche width and niche position (Fig. S1-2 in e-supplement S3) showing that species with protein-biased intake targets have narrower niches than species with higher (more lipid-biased) targets.

Procedures

Our procedures are based on the assumption that predators forage primarily for nutrients rather than for specific prey (Kohl et al. 2015; Machovsky-Capuska et al. 2016a). Thus, they circumvent the problems associated with identifying and quantifying the natural diet of our subject species, and further restrict the field part of the study to collection of the animals. Our procedures only require nutritionally complementary foods with known macronutrient content that are acceptable to the animals. As such, these procedures may not be restricted to predators, but can be applied to species of all feeding guilds for which suitable complementary food combinations are attainable. Some major drawbacks are the need to

take the animals out of their habitat and that only point estimates of their nutritional condition in the field are possible.

Both procedures provide information on the species' fundamental nutritional niche positions (L:P2 or cross point of reference curve with $y=x$). The reference curve method also reveals the realized individual nutritional niche positions, from which the realized population niche width can be calculated. We cannot interpret the scatter of L:P2 data as niche width, because these points are measured as optimal individual niche positions. The species that were macronutrient limited in the field show that the beetles may live at and tolerate a large scatter in L:P intake and considerable food limitation. Results from the Rape2 field, whose beetles stood out as more food and lipid limited than those from other fields, indicate that *A. dorsalis* females will tolerate a situation with strong food and lipid limitation. Interestingly, the Rape2 field had been insecticide sprayed only 10 days prior to collection of the animals. This indicates that our procedures may be useful for monitoring how the nutritional conditions of arthropod predators of insect pests are influenced by agricultural treatments, and how they vary between habitats or between seasons within habitats.

Both the reference curve procedure and the double-test procedure have their strengths and weaknesses. An important strength of the double-test procedure is that it allows us to determine limitation of food/energy as well as limitation of specific macronutrients concurrently. By the reference curve method we can determine nutrient limitation but not food limitation. A benefit of the reference curve procedure is that it allows estimation of the L:P ratio of the beetles' field diet and the realized population niche width. The double-test procedure is the simplest both practically and analytically. It can provide important information about the living conditions of a population with very low costs in money and time, because significant results can be obtained from simple feeding tests with few individuals. As with other approaches in nutritional ecology (Machovsky-Capuska et al. 2016a,d), it may be useful in several applied contexts. For example, it might be used for testing the nutritional conditions experienced by predators released in biocontrol programs or in interactions between invasive species and their native relatives that potentially suffer from the competition. In conservation biology it can be used to follow the health of populations of animals that are released to establish in the wild. Because the method is non-destructive, it might even be used on endangered species, and the animals can be returned a few days later in a better nutritional condition than when they were caught.

Declarations

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Figure legends

Fig. 1. Schematic presentation of possible outcomes of the double-test procedure. The procedure consists of two self-selection experiments, the first performed immediately after the animals have been collected in the field; this produces Target 1 (T1; black dot). The second is performed after the same animals have been fed to satiation and nutrient balance during one week; this produces Target 2 (T2; large red dot). Target 2 is equal to the Intake target. If the two targets differ, the animals were food and/or macronutrient limited when collected in the field. (A) The selected intake points are identical: the animals were not food limited and they were in nutritional balance in the field. (B) Target 1 lies on the line through the Intake target (T2) with slope -1 and to the left and above T2, i.e. the animals consumed the same amount of food in both tests, but with a higher lipid content in the first test; the animals were lipid limited but not food limited in the field. (C) Target 1 lies on the line through the Intake target (T2) with slope -1 and to the right and below T2, i.e. the animals consumed the same amount of food in both tests, but with a higher protein content in the first test; the animals were protein limited but not food limited in the field. (D) The animals from the field consumed more food in the first test but maintained the same L:P ratio in both tests, i.e. they were food limited but in nutritional balance in the field. (E) The animals from the field consumed more food and the food had a higher lipid content, i.e. they were both food limited and lipid limited in the field. (F) The animals from the field consumed more food and the food had a higher protein content, i.e. they were both food limited and protein limited in the field.

Fig. 2. Total consumption (A: females, B: males) and lipid:protein (L:P) ratio (C: both sexes) (mean + SE) of food consumed by *Anchomenus dorsalis* in two self-selection tests. Test 1 was carried out immediately after the beetles had been collected in the fields. Test 2 was carried out with the same beetles after they had been fed to satiation and nutrient balance during one week in the laboratory. X-axes show separate fields (names indicate crop type).

Fig. 3. Reference curve procedure. Black dots and red curve show the relationship between pre-treatment L:P ratio and the mean L:P ratio subsequently selected by *A. dorsalis* beetles. The intersection point between the reference curve and the $y = x$ line indicates the species' intake target (self-selected L:P equal to pre-treatment L:P). Green lines combine the self-selected L:P ratio of beetles collected from 6 fields via the reference curve to the "pre-treatment L:P", i.e. the predicted L:P ratio of the beetles' field diet. The beetles from the field selected a diet with higher lipid content than the intake target, i.e. their field diet was protein biased. Thus, they were lipid limited in the fields. The purple point shows the L:P ratio selected by *A. dorsalis* after being collected in early spring at the hibernation sites, indicating complete exhaustion of lipid reserves after the winter.

Fig. 4. A. Realized macronutritional niches (measured as L:P1) of predatory beetles from agricultural fields (in ascending order). Box plots show the median (horizontal full line), 25 and 75% quartiles (box), 10 and 90% quantiles (bars) and outliers (dots). B. Relationship between realized macronutritional niche position (median L:P1) and realized niche width (25% quartile L:P1) of the same 11 species.

Fig. 5. A. Fundamental macronutritional niches of predatory beetles from agricultural fields (in ascending order). Box plots show the median (horizontal full line), 25 and 75% quartiles (box), 10 and 90% quartiles (bars) and outliers (dots). B. Relationship between macronutritional niche position (median L:P2) and niche width (25% quartile L:P2) of the same 11 species.

Fig. 6. Relationship between the fundamental macronutritional intake target (median L:P2) and the difference between median L:P2 and median L:P1 (i.e. self-selected deviation from the intake target) in 11 species of carabid and staphylinid beetles just after collection from agricultural fields. Horizontal dotted line indicates complete nutrient balance in the field; species below this line were lipid limited and species above the line were protein limited in the field. Abbreviation of species names: Ad: *Anchomenus dorsalis*; As: *Amara similata*; Am: *Agonum muelleri*; Bl: *Bembidion lampros*; Bq: *Bembidion quadrimaculatum*; Da: *Demetrias atricapillus*; Lp: *Loricera pilicornis*; Nb: *Nebria brevicornis*; Pf: *Philonthus fuscipennis*; Pm: *Pterostichus melanarius*; Tr: *Tachinus rufipes*. * following species abbreviation indicates that L:P1 and L:P2 are significantly different (Table 2).

Table Legends

Table 1. Reference curve procedure (cf. Fig. 3): Calculation of the L:P ratio experienced by the beetles in the field prior to collection ($L:P_{\text{field}}$) and its 95% confidence limits. L:P1 is the L:P ratio selected by the beetles in self-selection tests carried out immediately after the animals were collected in the field. All L:P1 values lie above the intake target defined by the reference curve (0.378). Upper confidence limits in bold indicate that the estimated confidence limits do not embrace the intake target.

	N	L:P1	$L:P_{\text{field}}$	95% c.l.	Upper c.l.
Total 2018 data set	111	0.469	0.251	0.117	0.368
Rape 1	20	0.447	0.278	0.121	0.399
Rape 2	23	0.539	0.182	0.130	0.312
Barley	19	0.451	0.273	0.122	0.394
Wheat 1	31	0.458	0.264	0.119	0.383
Wheat 2	18	0.442	0.283	0.121	0.404
Rape 2013	17	0.439	0.288	0.121	0.409

Table 2. Total consumption (mg) and L:P ratio of self-selected diet in test 1 and test2 by 10 species of carabid and staphylinid beetles from agricultural fields. For species in which sex was a significant factor, results are given for both sexes. F: females; M: males. * indicate significant difference between test 1 and test 2 (repeated-measures MANOVA); the higher value indicated in bold. For all species except *L. pilicornis*, Consumption1 > Consumption2, i.e. the species were food limited in the field. In four species, L:P1 > L:P2, i.e. they were lipid limited; in one species, L:P2 > L:P1, i.e. it was protein limited.

	<i>n</i>	Test 1	Test 2	
Total consumption (mg)		Consumption1	Consumption2	
<i>Agonum muelleri</i>	6	6.39 ± 1.07	1.36 ± 0.58	*
<i>Amara similata</i>	24	8.02 ± 0.76	2.92 ± 0.32	*
<i>Bembidion lampros</i>	23	0.47 ± 0.05	0.28 ± 0.02	*
<i>Bembidion quadrimaculatum F</i>	6	2.27 ± 0.19	0.53 ± 0.03	*
<i>B. quadrimaculatum M</i>	13	1.64 ± 0.09	0.50 ± 0.03	*
<i>Demetrias atricapillus</i>	5	0.31 ± 0.04	0.05 ± 0.02	*
<i>Loricera pilicornis</i>	25	1.37 ± 0.41	0.85 ± 0.14	
<i>Nebria brevicollis</i>	23	7.24 ± 0.89	1.31 ± 0.19	*
<i>Philonthus fuscipennis</i>	19	3.91 ± 0.34	2.14 ± 0.33	*
<i>Pterostichus melanarius F</i>	10	47.09 ± 3.36	8.49 ± 3.80	*
<i>Pt. melanarius M</i>	16	30.90 ± 2.69	4.85 ± 1.28	*
<i>Tachinus rufipes</i>	17	1.51 ± 0.12	0.45 ± 0.11	*
L:P ratio		L:P1	L:P2	
<i>Agonum muelleri</i>	6	0.916 ± 0.127	0.317 ± 0.115	*
<i>Amara similata</i>	24	0.369 ± 0.042	0.422 ± 0.066	
<i>Bembidion lampros</i>	23	0.461 ± 0.048	0.508 ± 0.042	
<i>Bembidion quadrimaculatum</i>	19	0.669 ± 0.045	0.597 ± 0.031	
<i>Demetrias atricapillus</i>	7	0.395 ± 0.064	0.844 ± 0.268	
<i>Loricera pilicornis</i>	25	0.394 ± 0.040	0.539 ± 0.080	*
<i>Nebria brevicollis</i>	23	0.680 ± 0.073	0.246 ± 0.035	*
<i>Philonthus fuscipennis</i>	19	0.655 ± 0.045	0.478 ± 0.065	*
<i>Pterostichus melanarius</i>	26	0.839 ± 0.050	0.380 ± 0.049	*
<i>Tachinus rufipes</i>	17	0.638 ± 0.102	0.426 ± 0.063	