

Optimal foraging for specific nutrients in predatory beetles

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Evolutionary theory predicts that animals should forage to maximize their fitness, which in predators is traditionally assumed equivalent to maximizing energy intake rather than balancing the intake of specific nutrients. We restricted female predatory ground beetles (*Anchomenus dorsalis*) to one of a range of diets varying in lipid and protein content, and showed that total egg production peaked at a target intake of both nutrients. Other beetles given a choice to feed from two diets differing only in protein and lipid composition selectively ingested nutrient combinations at this target intake. When restricted to nutritionally imbalanced diets, beetles balanced the over- and under-ingestion of lipid and protein around a nutrient composition that maximized egg production under those constrained circumstances. Selective foraging for specific nutrients in this predator thus maximizes its reproductive performance. Our findings have implications for predator foraging behaviour and in the structuring of ecological communities.

Keywords: carnivore; nutrient balancing; lipid : protein ratio; fecundity; fitness; Carabidae

1. INTRODUCTION

Self-selection of diet nutrient compositions that maximize fitness components has been shown experimentally in herbivores [1–3], omnivores [4–6] and social insects [7], but has not been demonstrated in predators. Traditional optimal foraging theory predicts that predators maximize their fitness simply by optimizing their rate of energy intake [8]. It has been argued that predators forage to satisfy their energetic needs, not to balance their intake of specific nutrients, and that prey tissue provides a well-balanced nutrient source [9–12]. However, prey items vary considerably in nutrient composition depending on species [13–16], food sources [17–19], season [20,21], life stage [22] and sex [23]. Moreover, it appears that predator performance components are affected by prey nutrient composition [17,24,25]. It has not, however, been determined whether there is a direct link between specific nutrient intake and reproductive success in predators. Furthermore, it is not known whether predators selectively ingest prey of balanced nutrient composition so that reproductive output is maximized.

In some of the studies which show that the nutritional composition of the prey affects its quality as food for predators [17,25], high-quality prey was rich in protein, which suggests that high growth rates of predators may be limited by protein intake as well as by energy intake *per se*. Others have interpreted the high nitrogen content

of predator bodies compared with herbivores as evidence that predators are typically nitrogen limited [13,26,27]. This hypothesis, however, has been challenged [28–30], with the argument being made that the high nitrogen concentration in the bodies of predators may reflect a limitation of non-protein energy sources such as lipids, rather than a high requirement for nitrogen.

Whereas predator performance may differ depending on the types of prey, performance is often maximal when predators feed from a mix of prey species [31,32]. In addition, recent evidence suggests that invertebrate and mammalian predators regulate their consumption of macronutrients towards an ‘intake target’ [28,33,34] where fitness is maximal. Whereas evidence indicates that predators regulate their intake of multiple nutrients [35], it remains to be shown how this nutritional regulation is linked to fitness (i.e. whether predators adaptively regulate their intake around a target intake where fitness is maximal) [1].

Here, we apply the geometric framework for nutrition [1,4,29] to test the hypothesis that predators will maximize their reproductive output by ingesting optimal amounts of both protein and lipids, thus regulating the ingested amount and ratio of the two nutrients around an intake target. We used female ground beetles, *Anchomenus dorsalis* (Coleoptera: Carabidae), collected in spring as they emerged from hibernation to start foraging and reproducing. These beetles are active hunters and scavengers that are able to cover long distances during their search for prey, and have previously been shown to regulate their nutritional preferences in response to their recent feeding history and immediate requirements [28,35]. They thus

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provide a good model system for examining whether predators may selectively forage for a nutritionally balanced diet that maximizes their reproductive output.

The present study shows that total egg production in *A. dorsalis* is maximized when beetles ingest a diet with a balanced lipid and protein composition. Furthermore, the beetles will self-select this optimal nutrient composition when given free access to two diets differing in nutrient composition. Moreover, beetles that were restricted to a single nutritionally imbalanced diet balanced the costs of over- and under-ingesting the two nutrients and regulated intake to closely follow the ridge of maximal reachable fecundity. Our results thus provide evidence that *A. dorsalis* adaptively regulate their intake of lipids and protein so fitness is maximized.

2. METHODS

(a) *Experimental animals*

We collected *A. dorsalis* under stones and logs bordering an agricultural field near Aarhus, Denmark, during the first warm and sunny days of spring as they were about to emerge from hibernation. The beetles were kept in darkness at 5°C without food for six weeks before the initiation of the experiment.

(b) *Experimental diets and measuring nutrient intake*

Ten semi-artificial foods were prepared by mixing dry pulverized locusts (*Locusta migratoria*) with lard and casein (Sigma C-5890) at different ratios (figure 1a). The locusts were obtained from a gregarious culture, raised on fresh wheat grass and wheat germ, and contained approximately 17.8 per cent lipids and 74.5 per cent protein on a dry mass basis [28]. At the start of the experiment, the female beetles were weighed and transferred to individual 14 cm diameter Petri dishes. The bottoms of the Petri dishes were half-covered in sand that was kept moist during the experiment, and the remaining half was kept dry and cleaned after each feeding period. Each beetle was provided with either one (no-choice trial) or two (choice trial) food types provided on a 19 mm diameter glass dish. Foods were dried for at least 24 h at 60°C prior to being given to beetles and then again after recollection following feeding. Dry food and dishes were weighed to the nearest microgram before and after feeding, and diet ingestion was calculated by subtraction. Nutrient intake in each feeding period was calculated by multiplying the proportion of nutrients in the diet by the dry masses ingested. Beetles were fed seven times over 28 days with each feeding period lasting 2 days (days: 0–2, 3–5, 7–9, 11–13, 16–18, 20–22 and 26–28), and both choice and no-choice trials were performed simultaneously. No food was provided in the periods between feedings and all females were randomly assigned a male to allow mating between the first and the second feeding period. The experiment was performed in a room at 21–25°C with a 16 L : 8 D regime.

(c) *No choice of diet on 10 nutritional rails at four quantities*

To determine the effects of protein and lipid intake on egg production, we confined a total of 505 female beetles to one of 25 different diet treatments varying in lipid-to-protein composition and in the amount of food available to ingest. The beetles in 10 groups were provided one of the 10 semi-artificial diets (figure 1a) in ad libitum amounts. To test whether the beetles would balance lipid and protein intake irrespective of diet micronutrient content when fed

ad libitum, we halved the amount of pulverized locust relative to added nutrients in 5 of these 10 diets (figure 1a). In order to cover the areas of the landscape between ad libitum feeding and the origin, the beetles in the remaining 15 groups were provided with one of the 5 diets with high content of pulverized locusts (L:P = 0.91, 0.64, 0.43, 0.27 or 0.14; figure 1a) in restricted quantities representing approximately one-third, one-half or two-thirds of ad libitum intake. After the seventh feeding period, all beetles were killed by freezing at –18°C and dissected to count the total numbers of eggs in their ovaries. No eggs or larvae were found in the Petri dishes during or after the experiment. We dissected and counted eggs in an additional 26 female beetles at the start of the experiment before any food was ingested.

(d) *Measuring nutrient intake under choice*

To determine if female beetles were capable of selecting an optimal diet with a lipid and protein composition that maximized egg production, we allowed 23 female beetles to self-select their diet from two nutritionally complementary foods that differed only in protein and lipid content: lipid-rich diet (L:P = 0.91) and protein-rich diet (L:P = 0.14; figure 1a). Food dishes were placed in inverted Petri dish lids (5 mm height, 35 mm diameter) to prevent mixing of diets when spilling outside the dish during feeding.

(e) *Statistical analyses*

We used a multivariate response-surface approach [36] to estimate the linear and nonlinear effects of protein, lipid and micronutrient intake on total egg production by the female beetles. As female fecundity did not conform to a normal distribution, we assessed the significance of the linear and nonlinear gradients of the response surface using a randomization procedure implemented in PopTOOLS v. 3.2. We started by running the full parametric model including all linear and nonlinear terms. We then randomly shuffled egg number across females consuming the different diets to obtain an expected distribution under random association and used a Monte Carlo procedure to simulate this 10 000 times. The proportion of times (P) in which the randomized gradients were greater than or equal to the original estimates was calculated and two-tailed significance values (p) were calculated for each term in the model as $2P$ if $P < 0.5$ or as $2(1 - P)$ if $P > 0.5$ [37]. We visualized the nutritional landscape using a nonparametric thin-plate spline in R v. 2.9.12.

The self-selected intake point was calculated as the mean total intake of protein and lipids across the choice females, and was mapped on the nutritional landscape (figure 1b) to test the prediction that females optimize nutrient intake to maximize total egg production. To determine whether beetles preferentially consumed one of the diets over the other over the experiment and within feeding periods, we compared the absolute consumption of each food using a paired t -test. To determine whether the consumption of the protein- and lipid-rich diets significantly changed over the duration of the experiment, as well as across females, we analysed diet consumption across the seven feeding periods using repeated-measures ANOVA.

To test how beetles would balance over- and under-ingestion of lipids and protein, when restricted to feeding on a single nutritionally imbalanced diet, we estimated the slope of the relationship between mean protein and lipid intake across the 10 diets (β_a) using a regression analysis,

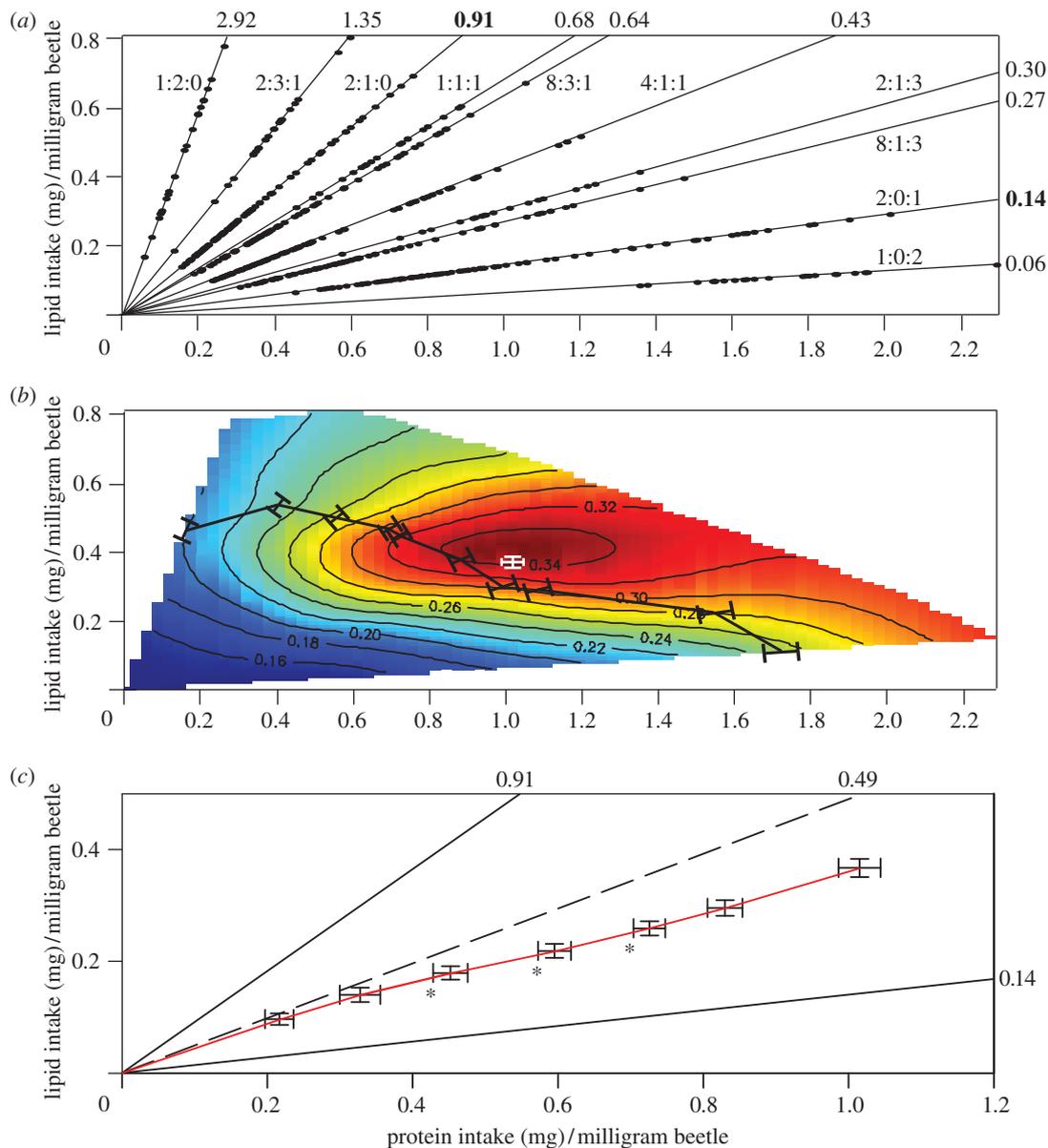


Figure 1. (a) Nutrient rails for the 10 semi-artificial diets and individual nutrient intake points for 505 female *Anchomenus dorsalis* over 28 days of feeding. Each beetle was provided a single diet either in ad libitum amounts or at various levels of limitation (see below). The locust : lard : casein ratio for each diet is indicated by the rails within the figure and the diet lipid : protein (L : P) ratios are provided outside of the figure frame. The L : P ratios are equivalent to the slopes of the rails. All ratios are mass-based. The two L : P ratios marked in bold (L : P = 0.91 and 0.14) represent the two diets provided to beetles in the choice trial. The five diets containing two-thirds pulverized locusts (L : P = 0.91, 0.64, 0.43, 0.27 and 0.14) were provided ad libitum or in quantities representing approximately one-third, one-half or two-thirds of ad libitum intake. The five diets containing one-third pulverized locusts (L : P = 2.92, 1.35, 0.68, 0.30 and 0.06) were provided ad libitum only. (b) Effects of protein and lipid intake on the total number of eggs produced (no. of eggs/milligram beetle) by the 505 female *A. dorsalis* after 28 days of feeding on one of 10 semi-artificial diets. The landscape also includes 26 females that were killed at the start of the experiment prior to ingesting any food. The mean (\pm s.e.) protein and lipid intake of the 23 beetles from the choice test (i.e. the selected intake point) is overlaid (white) on the landscape. The mean (\pm s.e.) protein and lipid intake for 214 beetles that were provided ad libitum amounts of one of the 10 diets are overlaid (black) and the means are connected by a line. This is referred to as the intake array and represents the regulated intake when feeding on single, mainly imbalanced diets. As ingestion of only one nutrient ratio is possible on single diets, the error bars of the intake array follow the rails of the diets. (c) Nutrient intake trajectory (red) of the 23 beetles allowed to select their diet nutrient composition from a food with L : P = 0.91 and a food with L : P = 0.14 (solid black lines). Each intake point (mean \pm s.e.) shows the cumulative lipid and protein intake for the seven feeding periods. The broken line (L : P = 0.49) shows the average nutrient composition of the two foods and is the nutrient ratio the beetles would ingest if they fed indiscriminately. The slopes of the intake trajectory show the selected nutrient ratio within each of the seven feeding periods. Asterisks indicate feeding periods where consumption from the two foods differed significantly (paired *t*-tests, $p < 0.01$). Overall, the beetles selected an average L : P ratio of 0.36 over the 28 days of the experiment.

and tested this against a hypothetical slope (β_h) of -1 and -0.5 using a *t*-test, where $(\beta_a - \beta_h)/(\text{s.e.}_{\beta_a})$ approximates a *t*-distribution with $n - 2$ degrees of freedom [38]. A slope of -1 would be consistent with beetles maintaining a

constant intake of nutrients, whereas a slope of -0.5 would be consistent with beetles maintaining a constant energy intake, as lipids contain twice as much energy as protein per unit ingested (9 and 4 cal mg^{-1} for lipids and

protein, respectively). We used a Levene's test to compare the variation in the amount of lipids and protein ingested by all beetles fed single diets ad libitum. For both nutrients, we expressed this variation in a normalized form using the coefficient of variation (CV). Finally, we compared the dry mass and energy intake of beetles across the 10 diets when feeding ad libitum using ANOVA.

3. RESULTS

(a) *Effects of lipid and protein intake on egg production*

Visualization of the nutritional landscape shows that total egg production by female *A. dorsalis* was maximized on diets that contained L : P ratios close to 1 : 2, when beetles had access to unlimited food (figure 1*b*). Linear analysis of the response surface revealed a significant increase in egg production with both increasing lipid intake and protein intake, although the effect of increasing lipid intake was much stronger than that of protein intake (table 1). There were also significant nonlinear effects of protein and lipid intake on total egg production, and in both instances the quadratic terms describing these relationships were negative, indicating the presence of a fitness peak for both nutrients (table 1). Again, the peak for lipid intake was more pronounced (i.e. had stronger curvature) than that for protein intake (table 1). The nutritional landscape (figure 1*b*) thus indicated a clear intake target, which identifies the diet lipid and protein intake that maximizes female fecundity, and that female beetles should ingest if they regulate their intake of nutrients so that total egg production is maximized [1]. We found no significant effects of varying micronutrient intake on female egg production (table 1).

(b) *Test of optimal foraging to maximize egg production*

After having established a target of optimal lipid and protein intake, we tested whether female beetles would forage in a way that achieved this target intake. Our results show that the selected intake point coincided with the peak of maximal egg production on the nutritional landscape (figure 1*b*), demonstrating that female *A. dorsalis* optimize their intake of nutrients to maximize reproductive output. The beetles reached this intake point by ingesting significantly more of the protein-rich food than the lipid-rich food over the 28 days of the experiment (paired *t*-test: $t_{1,23} = 4.86$, $p = 0.0001$), indicating that the beetles did not feed randomly from the two foods over the experiment. Consistent with previous work on this species [28], we found that the amount of protein-rich and lipid-rich food consumed by the beetles changed significantly over the course of the experiment (repeated-measures ANOVA, within-subjects, protein-rich food: $F_{6,161} = 8.96$, $p = 0.0001$; lipid-rich food: $F_{6,161} = 10.92$, $p = 0.0001$), as beetles recovered from hibernation and commenced egg production (figure 1*c*). Variation in the consumption of the two foods did not, however, differ significantly across females (repeated-measures ANOVA, between-subjects, protein-rich diet: $F_{22,161} = 0.65$, $p = 0.65$; lipid-rich diet: $F_{22,161} = 0.68$, $p = 0.86$), indicating that all females changed their feeding preference in a similar manner.

We next investigated the feeding responses of beetles provided free access to single imbalanced diets. Under these

Table 1. The linear and nonlinear effects of lipid, protein and micronutrient intake on the number of eggs produced by female *Anchomenus dorsalis*. *P* indicates the proportion of times in which the randomized gradients were greater than or equal to the original estimates (see §2), and *p* indicates values of significance.

	slope \pm s.e.	<i>P</i>	<i>p</i>
<i>linear terms</i>			
lipid (L)	0.691 ± 0.153	0/10 000	0.0001
protein (P)	0.108 ± 0.049	216/10 000	0.043
micronutrients (M)	-1.582 ± 1.136	8911/10 000	0.218
<i>nonlinear terms</i>			
L \times L	-0.847 ± 0.156	10 000/10 000	0.0001
P \times P	-0.044 ± 0.018	9859/10 000	0.028
M \times M	9.194 ± 7.796	1498/10 000	0.300
L \times P	0.088 ± 0.099	2061/10 000	0.412
L \times M	0.992 ± 1.671	2965/10 000	0.593
P \times M	0.321 ± 0.544	2999/10 000	0.600

conditions, the beetles faced the challenge of balancing the costs of over-ingesting the surplus nutrient in the food with the costs of under-ingesting the deficient nutrient [1]. When analysing the intake array of lipid and protein across the 10 diets (figure 1*b*), a number of important features are notable. First, the intake array was tilted towards the horizontal (protein) axis with a slope ($\beta \pm$ s.e.: -0.262 ± 0.034 , $t_9 = 7.67$, $p = 0.0001$) differing significantly from -1 ($t_8 = 21.71$, $p = 0.0001$) and -0.5 ($t_8 = 7.00$, $p = 0.0001$). Accordingly, the beetles showed significantly less variation in lipid intake than in protein intake (Levene's test: $F_{1,426} = 164.60$, $p = 0.0001$; $CV_{\text{protein}} = 58.26$, $CV_{\text{lipid}} = 43.47$). These findings indicate that the beetles regulated lipid intake more strongly than they regulated protein intake. The data also show that beetles were not maintaining a constant intake of lipids plus protein, nor of total energy, when feeding on diets of different nutrient composition. Hence, there was highly significant variation across diets in both dry mass eaten (ANOVA: $F_{9,214} = 59.92$, $p = 0.0001$) and total energy intake (ANOVA: $F_{9,214} = 12.51$, $p = 0.0001$). The intake array (figure 1*b*) shows that the beetles did not in general ingest more food when provided with diets containing half the amount of pulverized locusts, which indicates that the beetles regulated their food intake according to lipid and protein intake rather than the intake of micronutrients or other chemical components found in the locusts.

A second key point from the single-diet trial is that the intake array across the 10 diets traced a path along the ridge of maximal fecundity on the egg production landscape. Nutrient intake points fell on the contour of maximal total fecundity possible on the allocated diet (figure 1*b*). This pattern demonstrates that, even when beetles are restricted to a single imbalanced food source, and hence unable to reach the global optimum on the egg production landscape, they are able to balance the over- and under-consumption of protein and lipids to achieve the maximum reproductive output that is locally accessible. Only the beetles allocated to the two diets with the highest ratio of protein to lipids (L : P = 0.06 and 0.14) did not, on average, meet the very high intake that was necessary to reach the estimated maximal egg production on these diets (figure 1*b*).

4. DISCUSSION

Our results show that both lipid and protein limit egg production in predatory beetles when either under- or over-consumed (figure 1*b*). Furthermore, when offered a choice of complementary foods, the beetles selected protein and lipids in proportions and amounts that maximized egg production (figure 1*b*). Thus, our study shows that the ability to balance the intake of nutrients to maximize fitness is not only restricted to herbivores and omnivores, but is also likely to play an important role in the feeding ecology of predators.

When beetles were restricted to a single imbalanced diet, the intake array (figure 1*b*) aligned with the shape of the egg production surface, indicating an underlying fitness cost structure in which the costs of ingesting excesses and deficits of protein and lipid was asymmetrical around the intake target [1,39]. This pattern of intake required that beetles had a large capacity to over-consume protein to gain limiting lipids, relative to the intake target (figure 1*b*); in contrast, they tended to avoid over-eating lipids to gain protein when this was the limiting nutrient (figure 1*b*). Such a pattern has been recorded in a number of other predator species [17,33,34], and here we have shown that the stronger regulation of lipids than of protein corresponds with the steeper fitness peak found for lipids than for protein. Although the beetles were able to over-ingest substantial amounts of protein relative to the intake target to increase fitness, constraints such as gut capacity and the energetic costs associated with voiding excessive amino acids probably limited the majority of beetles fed the most protein-rich diets from ingesting enough food to gain sufficient lipid to maximize egg production.

The greater capacity of *A. dorsalis* and other predators to over-consume protein when compared with animals from other feeding guilds is likely to reflect the fact that they are adapted to protein-rich diets [40], as well as to feeding on prey that is frequently lipid-deficient compared with their optimal requirement. Herbivore and omnivore species typically do not over-ingest protein to increase energy intake because they lack the physiological mechanisms to handle large excesses of ingested protein [2], which may have significant deleterious effects on important fitness components [41,42] (but see [43]).

Having established the shape of the egg production landscape (figure 1*b*), we can use this surface to infer the reproductive consequences of food limitation in the wild, using the following logical argument. A useful surrogate for food availability is provided by the sum of protein and lipid eaten, which together constitute the majority of the usable dry mass in natural foods for these predators. Hence, the effects of progressively limited food intake (and availability) can be visualized by moving a line with a slope of -1 (i.e. a line along which all points sum to the same total mass of protein plus lipid eaten) from the intake target back towards the origin. It can then be seen how the nutrient ratio supporting maximal egg production changes with increasingly limited food intake by noting at which nutrient ratios the line tangents with the contour lines on the fecundity landscape. Using this logic, it is evident that as food intake becomes progressively limited, total egg production is maximized on increasingly lipid-rich diets (figure 1*b*).

Predators are often reported to be food-deprived in nature [44,45] and a shift towards a higher optimal

lipid-to-protein ratio under conditions of food deprivation might explain the traditional assumption that predators forage to maximize energy intake [8,12], given that lipids have twice the caloric content of protein per gram ingested. A recent study, however, suggests that the assumption that predators are normally energy-limited is likely to be an over-simplification [46]. In almost 90 per cent of the vertebrate species examined, the kill rates of prey exceeded the energetic demands of the predators, suggesting that energy intake in predators is unlikely to be as limiting as once believed. More work is needed, however, to determine whether this is also true for invertebrate predators.

If predators in general have evolved mechanisms that balance their intake of nutrients, then it is likely to have important implications for the role that predators play in community and food web ecology [29,47,48], and for modelling predator–prey interactions and nutrient flow across trophic levels in ecosystems [49]. For instance, if most prey contain less lipid than is optimal, then our nutritional landscape suggests that predators would be likely to kill and eat more prey than if the prey were rich in lipid. A situation such as this might occur, for example, immediately after winter hibernation, where both predators and prey are likely to have depleted lipid stores [28,50]. Conversely, if some prey species contain a higher concentration of lipid than is optimal, then predators should switch their focus towards more protein-rich prey species. A functional response matching this scenario was recently documented in wolf spiders (*Pardosa amentata*), which increased capture rate at increasing prey density when prey was protein-rich but reduced capture rate above a certain prey density when the prey was lipid-rich [51].

In conclusion, our experiment demonstrates for the first time that predators are able to forage optimally for multiple nutrients by self-selecting a diet with a protein and lipid content that maximizes total egg production. These findings indicate that predators are more discriminative foragers than previously thought, which is likely to have widespread and important implications for prey selection and the shaping of animal communities.

This study was supported by a grant from the UK Biotechnology and Biological Sciences Research Council. D.M. and S.T. were in receipt of grants from the Danish Research Council. J.H. was funded by NERC and a Royal Society Fellowship. D.R. is part-funded by the National Research Centre for Growth and Development, New Zealand and the Massey University Research Fund. S.J.S. was in receipt of a Federation Fellowship and currently a Laureate Fellowship from the Australian Research Council.

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