Original Article

The nutritional ecology of maturation in a carnivorous insect

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Herbivores and omnivores, faced with a nutritionally complex diet, have evolved the capacity to balance the intake of specific nutrients. Recent studies have found that carnivores also have this capacity, despite their more nutritionally homogeneous diet. However, unlike herbivores and omnivores who prioritize protein intake when restricted to imbalanced foods, carnivores instead show much stricter regulation of fat intake. These choices to over- or under-consume nutrients when the intake target cannot be achieved are known as *rules of compromise*. To date, studies examining these rules have all been carried out at a single life stage, and it is unclear if these rules regarding the prioritization of nutrients are fixed or labile. We address this question with a carnivorous beetle, *Nicrophorus vespilloides*. We use a combination of dietary restriction and choice tests to determine the intake target and rules of compromise in reproductively mature beetles and in newly emerged adults undergoing a period of maturation feeding. We show that, despite having very similar intake targets, the rules of compromise differ between the 2 life stages. Although mature adults follow the typical carnivore rule of fat prioritization, immature adults behave more like omnivores, showing strict regulation of protein intake, resulting in obesity when restricted to protein-poor diets. These alternate rules suggest different mechanisms or capacities to cope with excess protein across these life stages. Examining how intake targets and rules of compromise change across life stages could be a valuable approach for our understanding of how animals will fare under rapidly changing environmental conditions.

Key words: **carnivore, feeding, geometric framework, insect, intake target, maturation, nutrient regulation, nutritional ecology, predator, rules of compromise**

INTRODUCTION

One of the main challenges faced by organisms during their life is the acquisition of resources, the availability of which is a key factor in determining multiple fitness-related traits, such as body size, condition, immunity, survival, and the number and quality of offspring produced (e.g., Festa-Bianchet 1998; Nager et al. 2000; Kotiaho 2002; Møller and Petrie 2002; Siva-Jothy and Thompson 2002; Senar et al. 2003; Ujvari and Madsen 2006; Smith et al. 2007; Judge et al. 2008; Barrett et al. 2009; Cotter et al. 2011; Graham et al. 2015; Pincheira-Donoso and Hunt 2017). When resources become limited, growth, development, reproduction, and somatic maintenance can be constrained by trade-offs. Nutrient acquirement and allocation are therefore at the heart of our understanding of life history theory (Roff 1992; Stearns 1992).

In ecology, resources were traditionally considered as a black box, or simplified to energy or nitrogen availability, if either

was more likely to be considered limiting (Stephens and Krebs 1986). However, resources are a complex mixture of macro- and micronutrients, indigestible components, and toxins, the maze of which must be navigated to gain the optimum balance for fitness (Raubenheimer et al. 2009). Selection should, therefore, have shaped the ability of organisms to self-select a diet that comprises a specific nutrient composition that maximizes fitness (Simpson and Raubenheimer 2012). The geometric framework for nutrition (GFN) is a state-space modeling approach that can be used to address how animals balance their intake of multiple nutrients across a multidimensional nutritional environment (Simpson and Raubenheimer 1995; Raubenheimer and Simpson 1997; Simpson et al. 2004). The strength of the GFN is that it allows the animal to exhibit their preference to different nutrients, and the fitness outcomes correlating with this preference can be measured. The GFN can be applied to any animal, including humans (Simpson et al. 2003; Simpson and Raubenheimer 2005, 2007). The GFN considers the animal as living within a multidimensional nutrient space, where each functionally relevant nutrient is assigned an axis. The "intake target" can be defined as the optimal mixture and blend of these nutrients (Simpson and Raubenheimer 1995; Raubenheimer and Simpson 1997; Simpson et al. 2004). The location of this

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target is variable, and it can be modified according to the animal's life stage, development status, and its current physiological state (Simpson et al. 2006; Nestel et al. 2016).

Numerous organisms have been shown to defend an intake target when allowed to self-select their diets, including herbivores (e.g., Simpson et al. 2004; Lee et al. 2006; Behmer 2009), omnivores (e.g., Simpson and Raubenheimer 1997; Simpson et al. 2003, 2006; Raubenheimer and Jones 2006) and social insects (e.g., Dussutour and Simpson 2008, 2009; Paoli et al. 2014). Carnivores were expected to maximize energy intake because their food source, meat, was nutritionally homogeneous and closely mirrored their own body composition (Kuipers et al. 2010; Eisert 2011). This view was overturned when the GFN was applied to a range of carnivores, showing that they also had specific nutrient intake targets and were able to self-select a diet that comprised that target from variable sources (Mayntz et al. 2005, 2009; Raubenheimer et al. 2007; Hewson-Hughes et al. 2011, 2013; Jensen et al. 2012).

Optimal foraging requires the regulation of nutrient intake under conditions of both resource scarcity and excess, and indeed, as the availability and composition of resources can vary over both time and space, some nutrients may be abundant in a given environment when others are scarce. Under these conditions, organisms may find themselves temporarily restricted to imbalanced diets, unable to reach their intake target and consequently forced into a trade-off between ingesting an excess of some nutrients against a shortage of others (Simpson and Raubenheimer 1995; Raubenheimer and Simpson 1997, 2018; Simpson et al. 2004; Boggs 2009; Nestel et al. 2016). During dietary restriction, herbivores and omnivores have typically been shown to prioritize protein intake (Simpson and Raubenheimer 2005; Sørensen et al. 2008; Martinez-Cordero et al. 2011, 2012; Jensen et al. 2013), due to the expectation that they are typically protein-limited (but see Rothman et al. 2011). In contrast, carnivores were shown to respond very differently when restricted to imbalanced foods, instead showing stricter regulation of fat intake, which is more likely to be the limiting nutrient (Raubenheimer et al. 2007; Jensen et al. 2011, 2012). These choices to over- or under-consume nutrients when the intake target cannot be achieved are known as *Rules of Compromise* (Simpson and Raubenheimer 1995; Raubenheimer and Simpson 1997; Simpson et al. 2004).

Just as an intake target should be shaped by selection to be optimized for an animal's age or state, so should their rules of compromise. To determine these rules, an animal can be restricted to one of a range of suboptimal foods, or to only noncomplementary foods that do not allow it to reach its intake target, thereby forcing it to overeat some nutrients and undereat others. The nutrient intake points across the range of food rails form a pattern called an intake array that determines how animals prioritize the intake of specific nutrients (Simpson and Raubenheimer 1995). Analysis of the shape of these intake arrays can provide valuable insights into an animal's ecology, behavior, and/or its mechanisms of nutrient regulation (Raubenheimer and Simpson 1993; Simpson et al. 2002, 2006; Lee et al. 2003, 2004, 2006; Martinez-Cordero et al. 2011; Lihoreau et al. 2014) and so are vital for understanding how an animal will cope with changes in its nutritional environment.

Animals typically have a specific intake target, but this can change with age or state (Mayntz et al. 2005; Raubenheimer et al. 2007; Runagall-McNaull et al. 2015). Periods of illness can change nutritional demands, requiring higher protein intake (e.g., Povey et al. 2009, 2014) increased lipids (e.g., Miller and Cotter 2018) or carbohydrates (Graham et al. 2014) relative to the noninfected

state. Nutrient requirements can also shift seasonally, with a greater need for fats in colder months, particularly in preparation for hibernation. For example, when food is abundant, brown bears have been shown to prioritize the fattiest parts of the salmon (eggs and brain) readily discarding the protein-rich flesh as they fatten up during autumn (Gende et al. 2001).

Requirements can be life-stage specific; juveniles, for example, are growing and so often require more protein in the diet than adults, particularly in herbivores/omnivores (e.g., fish: Guy et al. 2018; Sealey et al. 2013; Mammals: FAO Nutrition Meetings Report Series 1973; birds: Oliver 1998). Insects too have distinct life stages during which nutrient requirements could be expected to differ (Scriber and Slansky 1981; Dussutour and Simpson 2009; Runagall-McNaull et al. 2015). This is most extreme in holometabolous insects where larval and adult diets can differ completely. For example, many lepidopteran larvae are leaf feeders while adults solely feed on nectar or do not feed at all (Speight et al. 2009). As for vertebrates, this reflects a higher requirement for protein in juveniles versus adults (Speight et al. 2009).

The requirements for protein in adults are also modified depending on whether the reproductive system is immediately mature or requires further maturation as adults. Mayflies, for example, emerge as adults fully reproductively competent, while other species require a significant period of maturation feeding before reaching this stage (Jervis et al. 2005). Mosquitoes are a prime example, females cannot mature their eggs until they have taken a blood meal, but examples occur across taxa (Jervis and Ferns 2004).

Sex-specific requirements are also common (e.g., Maklakov et al. 2008; Harrison et al. 2014; Jensen et al. 2015), for example, *Drosophila* males maximize their lifetime reproductive success on low-protein diets, whereas female fecundity is optimized at a higher protein intake (Jensen et al. 2015). It is likely that differences between the sexes will occur more frequently where they are under contrasting selective pressures for reproduction, that is, where females invest heavily in offspring and males invest heavily in competition for females (Harrison et al. 2014). However, despite the breadth of data on variation in nutritional requirements, we currently do not know how, or even if, rules of compromise change across different life stages (Raubenheimer and Simpson 2018).

Here, we address this question in a carnivore model, the burying beetle, *Nicrophorus vespilloides*. Adult beetles breed on small vertebrate carcasses. The female lays her eggs in the soil, and after 2–3 days, the larvae hatch and crawl to the carcass, where they take up residence. Parents engage in biparental care of the young; males typically leave the carcass before females but nonetheless invest heavily in brood care, and can rear a brood successfully on their own if widowed (Eggert and Muller 1997). The carcass is typically fully consumed within 5 days, and the larvae move into the soil to pupate. Newly eclosed adult beetles must undergo a period of maturation feeding before attaining reproductive competency, which takes approximately 10 days (Trumbo et al. 1995). Adult beetles are active predators, known to feed on fly larvae and beetle larvae as well as adult beetles (Pukowski 1933), and they will consume meat from carrion sources (Trumbo and Robinson 2004). During this time, beetles will gain weight until they reach a steady state at approximately 5–6 days post-eclosion (Hopwood et al. 2013), which is typically maintained, with minor fluctuations, for the remainder of their lives (Cotter SC, personal observation; Hopwood et al. 2013). During maturation, the ovaries increase in mass and juvenile hormone titers increase in females (Trumbo 1997). Males too must mature their reproductive systems, with growth and

maturation of the testes and accessory glands common across taxa (e.g., Odhiambo 1966; Anciro and Palli 2015).

Here, we measure the intake target and examine the rules of compromise in beetles during 2 life stages. The first upon adult emergence when the beetles must undergo maturation feeding and the second when the beetles are reproductively mature. Burying beetles are not sexually dimorphic, typically share the burden of parental care and as both males and females need to undergo maturation feeding we might expect there to be little variation in the requirements of males and females within each life stage (Ward et al. 2009). We therefore predict:

1) That burying beetles will have specific intake targets for protein and fat.
2) That beetles undergoing maturation feeding will have a more protein-biased intake target than mature beetles, reflecting the requirement for protein to mature the reproductive system
3) Immature beetles will show a stricter prioritization of protein than mature adults due to the fitness consequences of delayed reproductive maturation.
4) We also predict that males and females will respond similarly to dietary restriction within life stages due to similar selective pressures on fitness.

MATERIALS AND METHODS

Nicrophorus vespilloides colony

The colony was established in February 2011 from an outbred colony maintained in the Zoology Department at the University of Cambridge. Maintaining the genetic diversity required adding wild-caught beetles each year. Adult beetles were housed in individual boxes (12 cm × 8 cm × 2 cm) and maintained in a temperature controlled room at 21 °C under a 16:8 light:dark cycle, and fed with small pieces of minced beef twice a week until required for experiments or breeding. During breeding, each male was paired with a nonsibling female and placed together in a plastic breeding container (17 cm × 12 cm × 6 cm), one-third filled with moist, nonsterile soil and provided with a newly defrosted mouse carcass of approximately 20–25 g in weight. Breeding containers were kept in a compartmentalized cupboard to simulate the underground conditions that beetles might experience in nature after finding a small carcass. About 7 days after the parents were paired their larvae began dispersing from the carcass. At this stage, larvae were removed from the soil and placed individually in compartments of 25 cell Petri dishes, one per family, and topped up with moist soil.

Around 20 days after larval dispersal, adult beetles' eclosion began, and then again, beetles were set up in their individual containers.

Diet preparation

Five artificial diets were prepared by mixing minced beef with lard and peptone (Sigma C-5890). The lipid and protein percentage in the minced beef were measured by drying 100 g of mince for 48 h in an incubator at 35 °C to a constant mass, the dry mince was then extracted with chloroform to remove the fat. The amount of lipid was measured as the difference between initial dry mass and final dry mass of mince. The amount of protein approximated to the final dry mass of mince. The minced beef contained approximately 34.5% lipids and 20.5% protein on a dry mass basis and the appropriate amount of peptone or lard was added to the mince to create the desired final percentage protein (experiment 1: 15%, 30%, 45%, 61%, or 77% protein; experiment 2: 22%, 30%, 45%, 61%, or 74% protein as a proportion of the total digestible nutrients; Table 1). The range of protein concentrations was restricted after experiment 1 due to reduced long-term survival on the lowest and highest percentage protein diets. For each of the experiments, adult beetles at either age 0 (immediately after eclosion) or age 21 days were restricted to, or given a choice between, specific diets containing various amounts of protein and fat, hereafter referred to as P and F, respectively. In the no-choice experiments beetles were restricted to a single diet, in the self-selecting experiments, beetles were restricted to 1 of 3 diet pairs varying in their P:F (22% vs. 74% P; 30% vs. 74% P; 45% vs. 74% P). Before and after feeding, food and dishes were weighed, and diet consumption was calculated by subtraction. Nutrient intake was calculated by multiplying the proportion of nutrients in the diet by the dry masses consumed. In each experiment, beetles were provided with blocks weighing c. 1.5 g, the amount consumed every 2 days was measured for 10 days and survival monitored (Cotter et al. 2011).

Experiment 1: the role of diet on maturation feeding and weight gain

To examine the effects of diet on weight gain, 280 beetles, 14 males and 14 females per diet at either age 0 or 21 days post-eclosion, were weighed to the nearest 0.1 mg and each was housed in individual boxes (12 cm × 8 cm × 2 cm). Beetles were assigned to of 1 of the 5 diets varying in their P:F (13%, 30%, 45%, 61%, 77% P) and provided with a preweighed diet block (weighing c. 1.5 g). Beetles were weighed every 2 days for 10 days. Food was replaced every 2 days, and the remaining food was removed and dried in an incubator at 35 °C to a constant mass. The amount consumed was measured as the difference between initial dry mass (estimated from initial wet mass) and the final dry mass

Table 1

Ingredients and nutritional content of the diets used in both experiments

	Diet	Protein % (dry mass)	Beef mince (g)	Peptone (g)	Lard (g)	Water content (g)	Total fat (g)	Total protein (g)
Experiment 1	1	15	50	0	43	22.5	60.25	10.25
	2	30	50	13	37	22.5	54.25	23.25
	3	45	50	25	25	22.5	42.25	35.25
	4	61	50	37	13	22.5	30.25	47.25
	5	77	50	47	0	22.5	17.25	57.25
Experiment 2	1	22	50	7	43	22.5	60.25	17.25
	2	30	50	13	37	22.5	54.25	23.25
	3	45	50	25	25	22.5	42.25	35.25
	4	61	50	37	13	22.5	30.25	47.25
	5	74	50	47	3	22.5	20.25	57.25

of the food. Although some previous studies have not measured individual consumption rates (e.g., Fricke et al. 2008), the link between the amount of food provided and nutrients consumed can change under compensatory feeding conditions (Lee et al. 2004). Therefore, we measured each individual beetle's consumption, every 2 days, so that the amount of protein and fat ingested could be accurately calculated.

Experiment 2: intake target and rules of compromise

For the self-selecting treatments, 168 beetles, 14 males and 14 females at either age 0 or 21 days post-eclosion, were weighed to the nearest 0.1 mg and each was housed in individual boxes (12 cm × 8 cm × 2 cm) with 2 preweighed blocks (weighing c. 1.5 g) of 1 of 3 diet pairs, varying in their P:F (either 22% vs. 74% P; 30% vs. 74% P; 45% vs. 74% P). Beetles were given the opportunity to self-select between the diets to examine to which point they would regulate their intake of protein and fat. Since the paired diets differed in their concentration of protein and fat, beetles in each treatment would have to consume different amounts of food to converge at the same point, their intake target (Simpson and Raubenheimer 1995). Food was replaced every 2 days for 10 days, and consumption measured exactly as described for experiment 1 above. For the no-choice treatments, we used the same procedure as in experiment 1 except beetles were restricted to a different set of 5 diets (22%, 30%, 45%, 61%, or 74% P).

Statistical analyses

All analyses were carried out in the package R version 3.1.2 (R Core Team 2017). All data were analyzed using general linear models or general linear mixed effects models. For experiment 1, a mixed effects model was fitted with beetle ID as a random effect. The explanatory variables for weight change over the 10 days of feeding on the 5 diets were *day*, *day squared* (to account for curvature in the data), *age* (coded as a factor, 0 or 21), *sex*, and *percentage protein* (coded as 15%, 30%, 45%, 61%, and 77%). The effects of percentage protein appeared to be continuous and were included in subsequent analyses as a linear variable, but were kept as a factor in the weight change analysis for ease of visualization.

For experiment 2—diet preference, the explanatory variables for the amount of protein and fat consumed were *age*, *sex*, and *diet pair*. For the nonchoice consumption data, the explanatory variables were *age*, *sex*, *percentage protein* in the diet, and *percentage protein squared* (to account for curvature in the data). In each case, the minimum adequate model was selected via stepwise deletion of nonsignificant terms from the full model containing all possible interactions. Residuals from each model were visually inspected and all conformed to the expectations of normality. To visualize the predicted effects of diet on the measured traits, fitted lines were generated from each minimum adequate model using the *predict* function in R and plotted over the raw data on the figures. To calculate the intake targets, the global mean and standard error was used for protein, as no explanatory variable significantly affected intake. For fat, age-specific means and standard errors were calculated as fat consumption was significantly predicted by beetle age.

RESULTS

Experiment 1: the role of diet on maturation feeding and weight gain

The difference in consumption between the age groups is illustrated by analyzing the weight change over time for young and mature

beetles on the 5 diets (Figure 1). There was a significant interaction between day, diet, and age (Table 2) suggesting that the effects of diet on weight change over time were different for young and mature beetles. The effects can be seen clearly in Figure 1. The most striking difference is between immature and mature beetles. The weight gain of newly eclosed adults undergoing maturation feeding is strongly affected by the diet, with weight increasing across all diets but being most rapid on the highest fat diets and slowest on the highest protein diet. Weight gain tends to level out after 6 days, although in the case of the highest protein diet it actually starts to fall again after this point (Figure 1a,b). In contrast, mature beetles maintained their weight, with a slight increase for those who were fed on rich fat diets (Figure 1c,d). Although males gained slightly more weight than females (estimate 0.0018 [±0.0007 g]), the effects were consistent across age groups and diets (Table 2).

Experiment 2: intake target and rules of compromise

When offered a choice between the pairs of diets, there was no significant difference between young or mature beetles in the amount of protein consumed (Table 3), nor was there a difference between the diet pairs (Table 3), indicating that beetles were actively regulating their protein intake to a target (mean protein consumption ± SE = 0.185 ± 0.006 g; Figure 2a,b). Fat consumption did differ between age groups, with young beetles consuming more fat than old beetles (mean fat consumption ± SE, age 0 = 0.172 ± 0.008 g; age 21 = 0.149 ± 0.007 g) but again, there was no effect of the diet pair the beetles were restricted to (Table 3; Figure 2a,b). There was also no effect of sex on the amount of protein or fat consumed (Table 3). This difference in fat consumption meant the protein:fat intake target of immature and mature beetles was slightly different, with young beetles selecting ~52% and mature beetles ~55% protein. Examination of the cumulative intakes by day shows that the choice was actually very similar for immature and mature beetles (Figure 2a,b). During the first 8 days, beetles in both age groups showed the same pattern in their consumption, broadly following the same intake trajectory, but by day 10 young beetles attempted to increase the amount of fat in their diets, resulting in an intake target that was more fat biased than that chosen by mature beetles. Although the intake trajectory was very similar across age groups, mature beetles' diet choice was more tightly regulated than that of young beetles, as the choice on each of the diet pairs was much more similar across the first 8 days than that shown by young beetles (Figure 2a,b).

The total amount of protein and fat consumed after 10 days, either immediately post-eclosion, or after 21 days (days 0–10 and 21–31) under no-choice conditions, was affected by the beetles' age, sex, and dietary P:F ratio, as well as interaction effects (Table 4). The most striking effect was in the difference in the shape of the intake array between young and mature beetles, despite the similarities in their intake targets (Figure 2c,d). For young beetles (age 0), the intake points approached the vertical position (Figure 2c), indicating that they aimed to prioritize their protein consumption. This is supported by the larger CV for fat than for protein consumption (fat = 51.47, protein = 34.25). Protein regulation on diets with a high protein percentage (45%, 61%, and 74%) was almost complete, as all of the intake points were approximately equal despite the 74% diet having almost double the protein content of the 45% diet (Figures 2c and 3a). In contrast, there was no evidence for fat regulation (Figure 3b), as there was a steep decline in fat consumption as the fat content of the diet dropped. Also, there was no
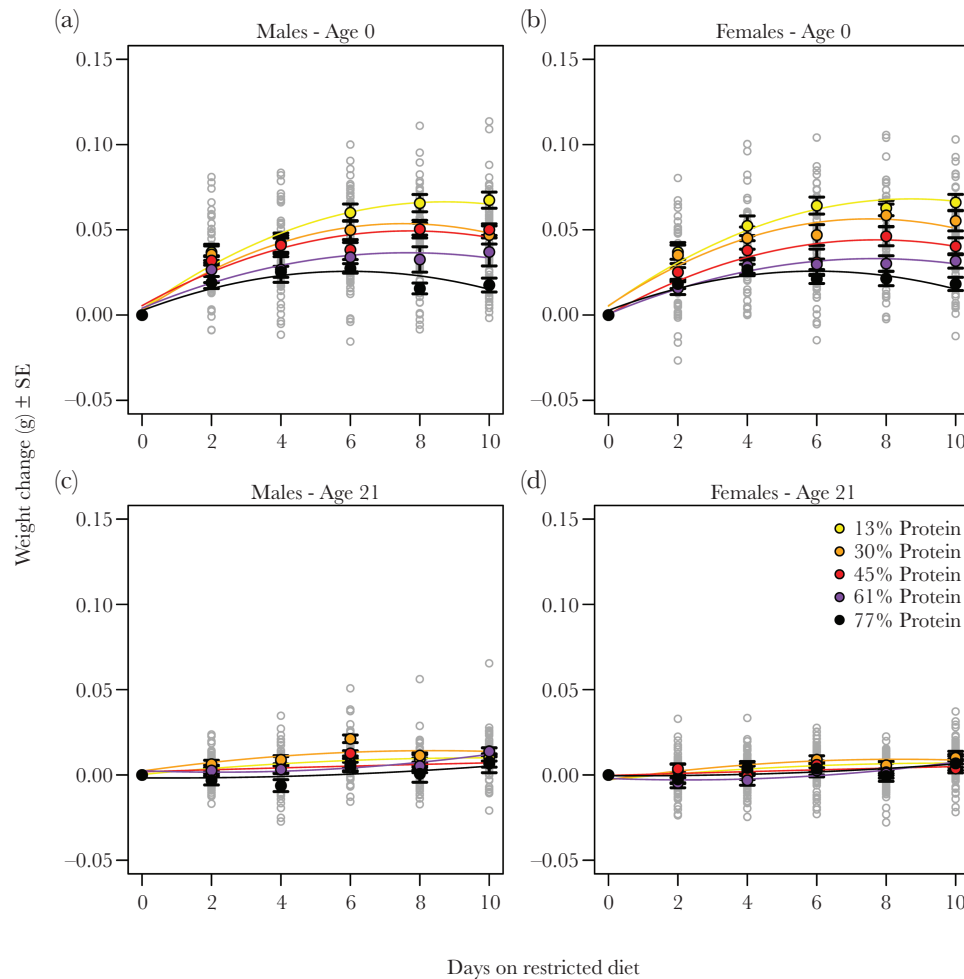
Figure 1

Weight change over 10 days across 5 diets that differ in their percentage protein for young (age 0) males (a) and females (b) and mature (age 21) males (c) and females (d). Open circles are the raw data points, filled circles are the mean and SE of the raw data for each day–diet combination. The colored lines represent the predictions from the minimum adequate model (Table 2).

Table 2

ANOVA for the effects of diet on female and male weight change

Predictor	df	F	P
Day	1,1514	345.27	<0.001
Diet	4,417	0.05	0.994
Age	1,1514	6.81	0.009
Sex	1,130	1.74	0.189
Day^2	1,1514	141.20	<0.001
Day: diet	4,1514	9.12	<0.001
Day: age	1,1514	248.88	<0.001
Diet: age	4,1514	0.39	0.816
Diet: sex	4,130	0.46	0.766
Age: sex	1,1514	2.40	0.121
Diet: day^2	4,1514	3.93	0.003
Age: day^2	1,1514	137.72	<0.001
Day: diet: age	4,1514	18.31	<0.001
Diet: age: sex	4,1514	2.54	0.038

Full model: weight change = day × diet × age × sex + day^2 × diet × age × sex + (1|beetle). All remaining interaction terms were not significant.

evidence for calorie regulation, the calories consumed by immature beetles declined steeply with the increasing protein content of their diets (Figure 3d).

Table 3

Choice: ANOVA for the effects of diet on female and male protein and fat consumption

Response		Protein		Fat	
Predictor	df	F	P	F	P
Age	1	0.08	0.773	4.87	0.029
Diet pair	2	0.39	0.679	1.46	0.236
Sex	1	<0.01	0.987	0.53	0.466

Full model: protein eaten = age × diet pair × sex. All interaction terms were nonsignificant.

For mature beetles (age 21), greater regulation was shown in fat consumption than protein, where the intake points approached the horizontal position (Figure 2d). This is supported by the larger CV for protein than for fat consumption (fat = 36.59, protein = 54.34). Furthermore, the decrease in fat consumption as the fat content of the diet fell was shallower for mature beetles than young beetles, suggesting better regulation (Figure 3b). However, the strongest evidence is for the regulation of calorie intake as mature beetles' performance in calorie consumption was very similar across the diets (Figure 3d).
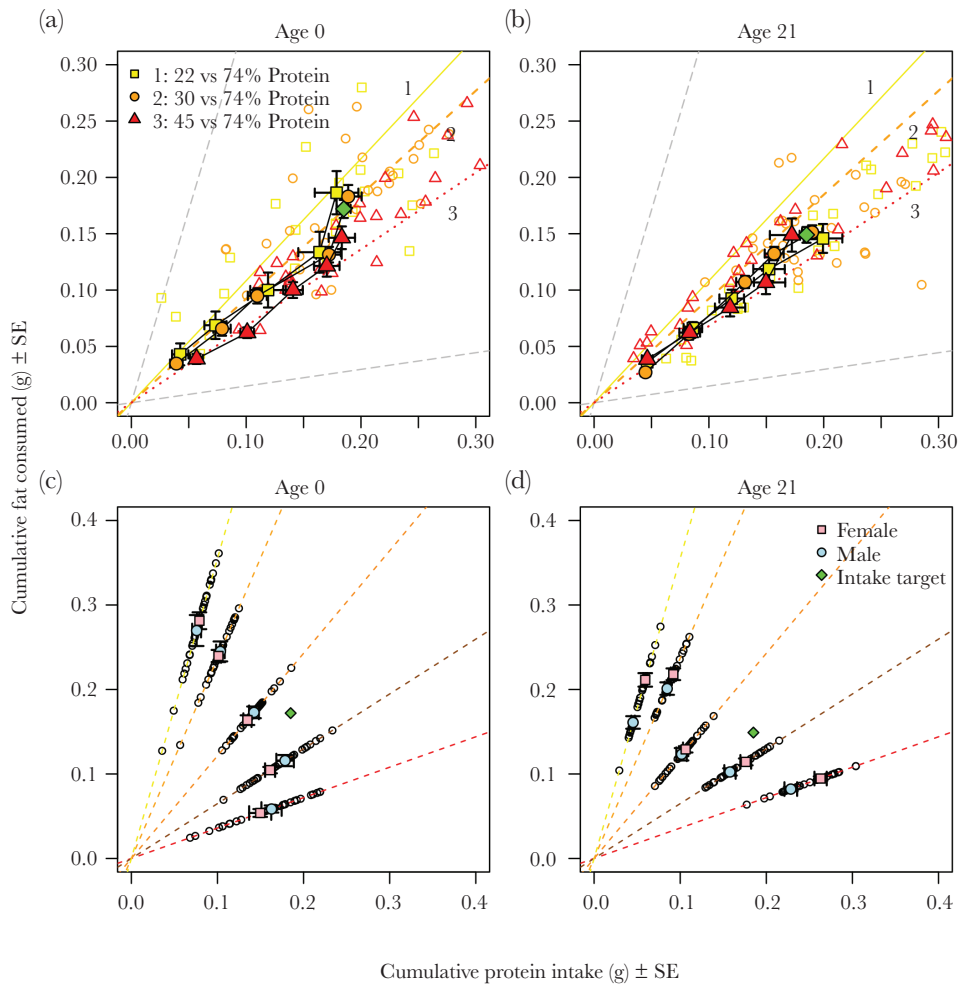
Figure 2
Protein and fat intake targets for a) young (age 0), and b) mature (age 21) beetles, when given a choice between 1 of 3 pairs of diets differing in their protein:fat ratios. Cumulative intakes for both sexes combined are plotted for every 2 days up to 10 days. Choice 1: 22 vs 74% protein (solid line, square symbols), choice 2: 30 vs 74% protein (short dashed line, round symbols) or choice 3: 45 vs 74% protein (dotted line, triangle symbols). Raw data are plotted in open symbols for each choice, means for each choice are plotted in closed symbols. The diamond represents the intake target for each age group. Long dashed lines represent the minimum and maximum ratio of protein to fat that beetles could have chosen to consume. Intake arrays from Experiment 2 are plotted for (c) age 0 and (d) age 21 beetles. Total intake after 10 days feeding is represented for males (round symbols) and females (square symbols) for each diet. Dotted lines represent the ratio of protein to fat for each of the 5 diets. The diamond represents the intake target calculated from the choice data.

Sex was included in the final models for all measures of consumption (Table 4; Figure 3). The effects of sex were less pronounced than the effects of diet or age, but typically, young females consumed less than the males, while mature females consumed much more than the mature males. The total amount eaten was higher in young beetles than mature beetles (Table 4; Figure 3c).

DISCUSSION

A longstanding assumption in nutritional ecology was that carnivores did not require the ability to forage for specific nutrients because the composition of their prey was sufficiently similar to their own body composition, and as such, they were expected to forage quantitatively, rather than qualitatively (Stephens and Krebs 1986; Kohl et al. 2015). Several studies have now shown definitively that this is not the case, and that as the body composition of prey can vary both within and between prey items, so predators have an intake target for specific nutrients,

comparable to that found in herbivores and omnivores (Mayntz et al. 2005, 2009; Hewson-Hughes et al. 2011, 2013; Jensen et al. 2012). However, what is not yet known is how nutrient-specific requirements change during growth and development. In the current study, we provide evidence that both mature and immature beetles had the ability to regulate their nutrient intake to a target, which was broadly similar across age groups and did not differ between the sexes. However, despite this similarity, the shape of the intake array between immature and mature beetles was remarkably different, with young beetles prioritizing protein consumption whereas mature beetles prioritized calorie intake, something that has not yet been shown for any carnivore. As a consequence of these different intake strategies, dietary restriction had different effects on young versus mature beetles. Restriction during maturation feeding resulted in large differences in body weights, with beetles on the low-protein/high-fat diets gaining excessive weight, while mature beetles maintained a consistent weight irrespective of diet.

Table 4

No choice: ANOVA for the effects of diet on female and male protein, fat, total food (g) and calories consumed

Response	Protein			Fat		Total food		Calories	
Predictor	df	F	P	F	P	F	P	F	P
Age	1	0.77	0.380	54.27	<0.001	15.51	<0.001	29.17	<0.001
Protein	1	1083.27	<0.001	1165.29	<0.001	18.47	<0.001	275.46	<0.001
Sex	1	1.98	0.161	4.65	0.032	3.83	0.051	4.27	0.040
Protein^2	1	0.09	0.771	3.20	0.075	1.44	0.231	2.13	0.146
Age: protein	1	135.54	<0.001	108.82	<0.001	139.52	<0.001		
Age: sex	1	15.33	<0.001	10.28	0.002	14.39	<0.001	13.00	<0.001
Protein: sex	1	0.03	0.855	6.08	0.014	4.84	0.029	5.48	0.020
Age: protein^2	1	75.76	<0.001	4.77	0.030	18.83	<0.001	7.34	0.007
Sex: protein^2	1					4.21	0.041	4.56	0.034
Age: protein: sex	1	6.06	0.014						

Full model: response = age × protein × sex + age × protein^2 × sex.
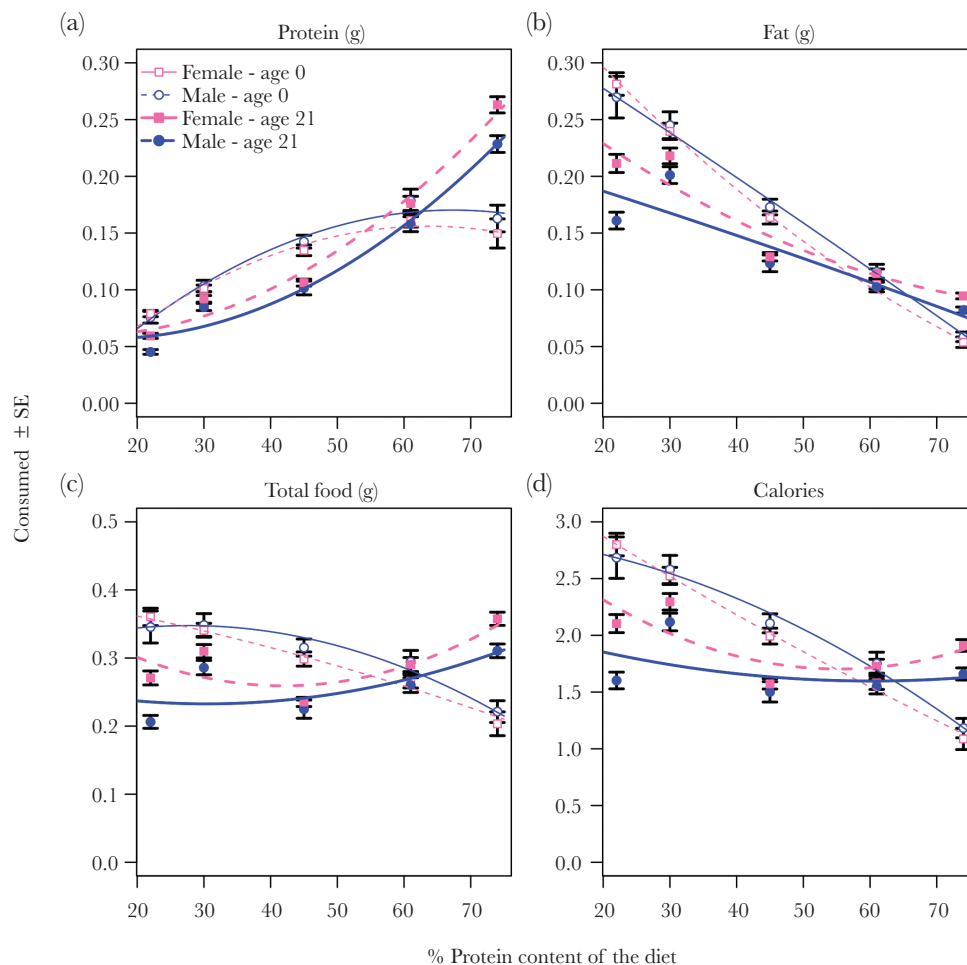
Figure 3
Consumption patterns across 5 diets differing in their protein and fat ratios. Predicted consumption of (a) protein, (b) fat, (c) total amount of food, and (d) calories is plotted against the percent protein content of the diets. Predictions are calculated from the relevant GLM for each variable and are plotted separately for males and females and for young and mature beetles.

As predicted, burying beetles had a defined intake target in terms of protein and fat. Young and mature beetles defended an intake target of 52% and 55% protein, respectively (Figure 2), which was contrary to our second prediction that young beetles would require more protein than mature adults. These estimates fall within those calculated for other carnivores [% protein = P/(P + F)], the domestic cat, *Felis silvestris catus*, 74% (Hewson-Hughes et al. 2011), the predatory ground beetles, *Anchomenus dorsalis*, 74% (Jensen et al. 2012) and *Agonum dorsale*, 61%–67% (Raubenheimer et al. 2007), Mink, *Mustela vison*, 41% (Mayntz et al. 2009), and the

domestic dog, *Canis lupus familiaris*, 32% (Hewson-Hughes et al. 2013). Interestingly, this shift to a more protein-rich diet during maturation feeding mirrors that seen in the study on the predatory ground beetle *A. dorsale*, which has an adult winter diapause (Raubenheimer et al. 2007). In this case, newly emerged beetles preferred 61% protein, but by day 10 their preference had shifted to 67% protein. This initial preference for fat was explained as a need to replenish energy stores depleted during diapause. A similar argument could be used for *N. vespilloides*, as pupation is a nonfeeding stage and could similarly deplete fat reserves. It is also worth noting, that young beetles ate significantly more food than mature beetles, such that their absolute protein intake was higher than that of mature beetles, perhaps accounting for a greater requirement for protein (to mature the reproductive system) and fat.

However, despite the relatively similar intake targets, the rules of compromise differed markedly between the age groups (Figure 2). When immature beetles were restricted to suboptimal diets, they tended to overconsume fat to gain limiting protein, but they would not overeat protein to gain fat. This resulted in strict regulation of protein, resulting in an intake array shifted toward the vertical, which matched our prediction (Figure 2c). A comparison with the figures for the regulation of protein intake and total food intake suggests that young beetles can regulate protein intake tightly on the 3 highest protein diets, but regulation fails on the 2 lowest protein diets (Figure 3a). The total amount of food consumed is at its highest and equal on the 2 lowest protein diets (Figure 3c), suggesting that this failure of protein regulation may be driven by the ability of the beetles to consume more food, they may have reached the physical capacity of their gut. Consequently, the weight gain of newly eclosed adults undergoing maturation feeding was strongly affected by the diet, with weight increasing across all diets but being most rapid on the highest fat diets and slowest on the highest protein diet (Figure 1a,b).

A previous study on this species found that beetles increased their body mass by ~18% over the first 6 days of maturation feeding on a diet of mealworms (Hopwood et al. 2013). This growth rate is comparable to the growth we see on the 45%–61% protein diets, which encompasses the intake targets of both immature (52% protein) and mature beetles (55% protein) suggesting that the weight gain on the high-fat diets is excessive, beyond what would be seen on a typical diet, arguably resulting in "obese" beetles. This pattern of consumption is similar to that seen in the carnivorous, European whitefish, *Coregonus lavaretus* (Ruohonen et al. 2007), which prioritizes the intake of protein over nonprotein energy, similarly resulting in overconsumption on protein-poor diets. This behavior, known as "protein leverage," is more typical of omnivores, including humans, and is thought to be driven by the requirement to gain sufficient protein, without overconsumption, in an environment where the overconsumption of protein was a much greater possibility than the overconsumption of nonprotein energy (Simpson and Raubenheimer 2005). Protein leverage has been argued to play a major role in the worldwide human obesity crisis, as the ease of access to foods with a low-protein to nonprotein energy ratio (P:NPE) has increased (Simpson and Raubenheimer 2005, 2007, 2012).

In contrast to immature beetles, mature beetles maintained their weight over 10 days of dietary restriction, with only a slight increase for those who were fed on the highest fat diet (Figure 1c,d). Mature beetles showed tighter regulation of fat than protein consumption (the average intake points are closer to horizontal than vertical; Figure 2d). These rules of compromise have been examined across herbivores, omnivores, and carnivores (Simpson and Raubenheimer 1995; Lee et al. 2002, 2006; Raubenheimer and Simpson 2003; Mayntz et al. 2005, 2009; Hewson-Hughes et al. 2011, 2013; Jensen et al. 2012, 2013). While herbivores and omnivores tend to prioritize the intake of proteins, all invertebrate predators that have been studied to date have presented the same intake patterns as those shown here for mature beetles, that they more tightly regulate the intake of fat than of protein (Mayntz et al. 2005, 2009; Raubenheimer et al. 2007; Jensen et al. 2011, 2012).

It is well known that, compared with other feeding guilds, predators have the ability to over-ingest protein, while herbivore and omnivore species typically do not overconsume protein to maximize their energy intake, due to the lack of the physiological mechanisms to deal with the surplus amount of consumed protein (Behmer 2009) (but see Rothman et al. 2011), which can result in considerable deleterious effects on their fitness (Lee et al. 2008; Maklakov et al. 2008; South et al. 2011; Dussutour and Simpson 2012). In mammalian carnivores, consumption of protein in high proportions, compared with other macronutrients, reflects the nutritional composition of their prey (Kuipers et al. 2010; Eisert 2011). Consequently, carnivores have evolved adaptations that allow them to cope with excess protein, such as a specialized gut microbiota, whose functions are enriched for amino acid breakdown (Muegge et al. 2011), and the ability to excrete excess nitrogen (Chew et al. 2007). However, this can be a liability on low-protein diets as there is no evidence that predators can downregulate protein catabolism when protein levels are very low (Walton 1986; Mustonen et al. 2005; Green et al. 2008).

So why are the rules of compromise so different for immature versus mature beetles? Unlike vertebrates, beetles gain their nutrients for growth during the larval stage, and these are fixed at pupation, giving discrete phases of growth and maintenance that are clearly separated between juveniles and adults (Browne 1995). Maturation feeding is therefore not designed to fulfill requirements for growth, but to replenish energy stores lost during pupation and to gain nutrients required for the maturation of the reproductive system (Browne 1995). As rules of compromise are shaped by selection pressures, maintaining adequate protein intake must, therefore, be particularly important during maturation feeding, when the reproductive systems of males and females are maturing, such that beetles try to minimize underconsumption. The unwillingness to overconsume protein may be driven by a lack of adequate regulatory mechanisms to cope with excess protein intake. One possibility is that, like vertebrate carnivores (Muegge et al. 2011), carnivorous invertebrates also possess specialized gut microflora for the breakdown of amino acids, but that this takes time after eclosion to develop to its full effect. Further studies on how the microflora develop during maturation feeding would be needed to test this hypothesis.

For carnivores, the expectation is that lipids are more likely to be the limiting nutrient (Wilder et al. 2013), and we provide some evidence for this, as fat intake is more tightly regulated than protein intake in mature beetles. However, our data strongly suggest that it is actually calorie intake that beetles are regulating, as this stays remarkably consistent across the 5 diets, with beetles consuming more on a low-fat diet to maintain the same calorie intake (Figure 3d; Lee et al. 2008; Maklakov et al. 2008; Mayntz et al. 2009; Jensen et al. 2012, 2013; Simpson and Raubenheimer 2012; Simpson et al. 2015a, 2015b,). So why might these beetles be preserving their calorie intake when restricted to imbalanced diets? Despite the assumption that weight indicates condition in many

studies, there is evidence that excess weight gain can result in reduced fitness in wild animals (e.g., Warbrick-Smith et al. 2006; Harrison et al. 2014). The drivers behind the diversity of rules of compromise seen across taxa are largely unknown, but one likely candidate is diet breadth (Raubenheimer and Simpson 2018). Generalist feeders are typically less constrained in their ability to overconsume particular nutrients, because the likelihood that they will find an alternate food source that represents the "nutritional antidote" is much higher than for specialist feeders (Simpson and Raubenheimer 2012). This argument is also likely to hold for active predators, who encounter a range of prey items that can vary in their protein to fat ratio, both between prey items and within individual prey items (Mayntz et al. 2005; Raubenheimer et al. 2007). Indeed, a previous study that examined the nutritional preferences of three types of predator showed that the active hunter selected between prey items to balance its intake, the low mobility predator selected tissues within single prey items, while they sit and wait predator had the capacity to alter their utilization of nutrients postingestion (Mayntz et al. 2005). For an active predator, flexibility in nutritional intake would allow the animal to maintain the ideal weight for fitness by over- or under-consuming nutrients from available prey, followed by compensatory feeding to restore nutritional balance in subsequent meals (Raubenheimer et al. 2007). Furthermore, as restriction to a single, unbalanced food type is significantly less likely in an active predator, mechanisms to cope with chronic overconsumption of a single nutrient are unlikely to have evolved, highlighting the problems of an animal finding itself restricted to a nutritional environment that is mismatched with its evolved behavior and physiology (Simpson and Raubenheimer 2005).

In conclusion, our study confirms that the capacity to forage for specific nutrients is not limited to herbivores and omnivores, but also plays a significant role in the nutritional ecology of carnivores (Mayntz et al. 2005, 2009; Raubenheimer et al. 2007; Hewson-Hughes et al. 2011, 2013; Jensen et al. 2012). However, we show for the first time that how a carnivore prioritizes the intake of those nutrients when restricted to imbalanced diets changes with age. While reproductively mature beetles showed similar intake patterns across varying P:F ratios to those that have been found in other carnivores, young beetles, undergoing maturation feeding behave more like omnivores/herbivores, showing a moderately strict regulation of protein intake. This protein leverage can result in "obesity" in beetles restricted to a diet that is high in fats and low in proteins. This age-related shift in the behavioral regulation of nutrient intake is little studied and potentially widespread and could have implications for understanding how diet influences fitness across different life stages and/or environmental conditions across taxa. It highlights the importance of understanding the fitness consequences of restriction to an evolutionarily mismatched nutritional landscape, which can occur in captivity, or under conditions of habitat loss or degradation (Simpson and Raubenheimer 2005).

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