

ABSTRACT

Title of Document: NUTRIENT REGULATION BY AN
OMNIVORE AND THE EFFECTS ON
PERFORMANCE AND DISTRIBUTION

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Omnivores have a unique interaction with their nutritional environment because they have adapted to consume food from different trophic levels. To successfully navigate their variable resources, omnivores must maintain some level of nutrient regulation. To explore the effects of nutrient regulation by an omnivore, I used a salt marsh katydid, *Conocephalus spartinae*.

To first address the ability of *Conocephalus* to perform on a wide range of diets and to regulate their nutritional intake, I used artificial diets that differed in relative amounts of protein and carbohydrate (Chapter 1). I found that *Conocephalus* survival decreased on a high protein diet due in part to a decrease in lipid stores but growth was not affected by diet. In a second experiment *Conocephalus* showed a degree of nutrient regulation as evidenced by the difference in what they actually ate and the predicted consumption if they had been feeding equally on the diets presented

in each treatment. However, I did not find evidence for tight macronutrient regulation.

Next I explored capacity of *Conocephalus* to regulate their nutrient intake (nitrogen and lipid) when fed naturally co-occurring prey (Chapter 2). I first established that the prey differed in their protein and lipid content and that these differences were related to the size of the prey species. When *Conocephalus* were fed different prey species individuals showed no differences in either growth or survival. In the final experiment, I found that *Conocephalus* did show evidence of a degree of nitrogen and lipid regulation because they did not feed equally on all of the prey species offered.

Lastly, I documented the relationship between the ability of *Conocephalus* to locate plant and prey resources and the effect that these resources have on omnivore performance (Chapter 3). I found that *Conocephalus* aggregates in areas of high plant quality but that their numbers do not correspond to areas of high prey density. However, I found that katydid growth and survival was enhanced by prey availability but not plant quality.

Overall, I documented how an organism like an omnivore relates to its nutritional environment and how nutrient regulation might affect performance and distribution. Last, I documented the relationship between the ability of katydids to locate plant and prey resources and the effect that these resources have on omnivore performance (Chapter 3). I found that katydids aggregate in areas of high plant quality but that their numbers do not correspond to areas of high prey density.

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PERFORMANCE AND DISTRIBUTION

By

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Dedication

I would like to dedicate this dissertation to my amazingly supportive “dirt-bag” of an advisor, Bob Denno. He is the reason that I came to Maryland. If it wasn't for him I would probably have a completely different life. Bob's curiosity and enthusiasm were inspiring to everyone around him. He expected a lot from his students, but in the end we all learned a great deal from being mentored by him. He is and will always be sorely missed.

I would also like to dedicate this to my uncle Sam Greenfield who told me many times that he wanted to see me become a doctor. I really wish that I could call him and tell him that I finally made it and that I love him very much.

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Chapter 1: Effects of diet quality on performance and nutrient regulation in an omnivorous katydid

Abstract

Omnivores by definition feed on plants and animals. However, little is known about how diet macronutrient quality affects omnivore performance, or the extent to which omnivores can regulate their macronutrient intake. We assessed these questions using the omnivorous salt marsh katydid, *Conocephalus spartinae* (Tettigoniidae). In our first experiment artificial diets with different macronutrient content were used to assess performance. We found that a high protein diet negatively affected *Conocephalus* survival. Growth in surviving individuals was not significantly different across the treatments, but lipid content decreased significantly as the protein-carbohydrate ratio of diets increased. In a second experiment we explored the ability of *Conocephalus* to regulate their protein-carbohydrate intake. The results suggested that *Conocephalus* can partially regulate their nutrient intake, particularly when presented with foods that are both nutritionally suboptimal, but complementary, and that carbohydrate intake is more tightly regulated than is protein intake. However, the results also suggest that katydids do not show tight homeostatic regulation. We discuss evidence for greater carbohydrate regulation compared to protein regulation within the context of a recent suggestion in the literature that predators (or animals feeding predominately on prey items) are energy-limited, rather than protein-limited.

Introduction

By eating both plants and animals, omnivores play important roles in a number of different terrestrial systems (Agrawal & Klein, 2000; Eubanks & Denno, 2000b; Ho & Pennings, 2008; Zhi et al., 2006), but as is the case for strict herbivores and predators, resource limitations, including food resources, mates, or shelter, can potentially have large impacts on their fitness (Jacot et al., 2009; Kaspari et al., 2001; Sasakawa, 2009). However, an omnivore's ability to include both plant and animal material in its diet implies that they are not affected by limitations in food resources in the same manner as strict herbivores or carnivores. Instead, a key issue for an omnivore concerns how long it can tolerate feeding exclusively on plant material in the absence of prey, or alternatively, feeding only on prey. This is an important issue because the nutritional composition of plants and prey are very different, particularly with respect to their elemental and macronutrient profiles (e.g. Raubenheimer et al., 2009; Sterner & Elser, 2002).

Plants and prey differ in the makeup of their energy-containing components. Plants generally contain digestible carbohydrates (e.g. simple sugars and starch), and only small amounts of lipids (primarily fatty acids, e.g. linoleic and linolenic acid) in addition to protein (Taiz & Zeiger, 2006). In contrast, prey typically contain protein and lipids (e.g. triacylglycerids), and only small quantities of simple sugars (e.g. glucose) (Chapman, 1998). From an omnivore's perspective, digestible carbohydrates and lipids are functionally similar in that they provide a source of energy, so another key nutritional difference between plants and prey is the ratio of protein to energy. Generally plants contain protein-energy ratios that range from equal protein-energy ratios to energy-bias (rich in digestible carbohydrates relative to protein), while prey items tend to be

extremely protein-biased (Pearson et al. unpublished, Chapman, 1998; Raubenheimer et al., 2009) (see Figure 1). However, apart from a single study on an extreme generalist cockroach, *Blattella germanica* L. (Blattellidae) (Raubenheimer & Jones, 2006), we know very little about how the nutritional composition of an insect omnivore's food influences its fitness.

When food resources are not limiting, omnivores will have the opportunity to mix their diet, and in doing so can more closely match their nutritional needs. Numerous studies have shown that insect herbivores (reviewed by Behmer, 2009), and more recently arthropod predators, actively regulate their nutrient intake in such a way that it redresses recent nutritional imbalances, via both behavioral and physiological mechanisms (Mayntz et al., 2005). In the case of both insect herbivores and arthropod predators, nutrient regulation directly optimizes performance and fitness (e.g. Behmer & Joern, 2008; Mayntz & Toft, 2001; Simpson et al., 2004). However, we currently know very little about the ability and extent to which insect omnivores regulate their intake. For example, are omnivores more protein limited, more carbohydrate limited, or do they require a balanced intake of protein and carbohydrate? The nutritional landscape that an omnivore occupies is much broader than that of a herbivore, and extremely broad compared to that of an arthropod predator (Raubenheimer et al., 2009). Therefore, when resources are in abundant supply, omnivores will have ample opportunity to optimize their nutritional intake by mixing among the available food items.

Due to the widespread nature of omnivory (Coll & Guershon, 2002), understanding the factors that underlie feeding choices in omnivores is fundamental to the study of population ecology and food-web dynamics (Eubanks & Denno, 2000a,

2000b; Fagan, 1997; McCann et al., 1998; Menge & Sutherland, 1987; Polis et al., 1997; Rosenheim, 1998), as well as to biological control (Hodge, 1999; Rosenheim et al., 1993). Omnivores may have either stabilizing or destabilizing effects on food web dynamics, depending on the relative strength of their effect on plants and prey (Eubanks & Denno, 2000a, 2000b; Fagan, 1997). Understanding the relative importance and interactive effect of the various resources that influence omnivore performance helps to elucidate their effects on food web stability. In this study, we explore the ability of an omnivorous salt marsh arthropod, the katydid *Conocephalus spartinae* Fox (Tettigoniidae, hereafter *Conocephalus*), to maintain survival and growth on foods that differ in their macronutrient content, and to regulate its macronutrient intake when given a choice between foods that differ in their protein-carbohydrate composition. If omnivores are nitrogen-, or protein-, limited as suggested by Denno and Fagan (2003), we would predict improved *Conocephalus* survival and growth on high protein diets as compared to high carbohydrate diets. However, our results suggest that *Conocephalus* performs poorly on diets that are extremely protein-biased. This finding accords with a recent paper by Raubenheimer et al. (2009) suggesting that predators, compared to herbivores, are actually carbohydrate- or lipid-limited. Our data also indicate that the omnivorous *Conocephalus* regulates its protein-carbohydrate intake, although not as tightly as has been found for most insect herbivores.

Methods

Study system

North American Mid-Atlantic coast intertidal marshes are dominated by extensive pure stands of the salt marsh cordgrass, *Spartina alterniflora* Loisel (Poaceae, hereafter *Spartina*) (Denno, 1983; Gallagher et al., 1988). *Spartina* varies extensively in its nitrogen content (1-5%N) across marsh habitats (Denno, 1983; Denno et al., 2000; Ornes & Kaplan, 1989; Squiers & Good, 1974). In general, low-marsh *Spartina* plants (those growing along tidal creeks) are more robust, have higher nitrogen content, and set more seed due to the higher availability of nutrients associated with tidal flushing. High-marsh *Spartina* tends to be more nitrogen-limited and therefore has a decreased biomass and nitrogen content (Denno et al., 2000; Ornes & Kaplan, 1989). The greatest disparity in plant nutritional content between the high and low marsh typically occurs early in the growing season (Ornes & Kaplan, 1989).

Spartina typically has a low toxin load and instead use a combination of chemical and physical defenses like silica (Pennings et al., 1998; Salgado & Pennings, 2005). However, in its southern range in Georgia *Spartina* plants do contain a higher phenolic concentration acting as a deterrent to herbivores whereas in the Mid-Atlantic, phenolic concentrations are lower and do not deter orthopteran herbivores (Salgado & Pennings, 2005; Siska et al., 2002). Thus there is a decreased probability that consumers in our study mix their diet as a consequence of plant toxicity (Singer et al., 2004). Therefore, the potential effects of plant nutrient-allelochemical interactions are less confounded in

Spartina's northern range allowing for a focus on the singular effect of plant nutrition on consumer performance and nutrient regulation.

Conocephalus is the most common omnivore at the study site located on the Eastern shore of Maryland on Chincoteague Bay (38.13°N, 75.30°W) where it feeds on resources from three different trophic levels (plant leaves and seeds, herbivores and predators) that differ in their macronutrient content here defined as proteins, carbohydrates and lipids. *Spartina* differs seasonally in its N content and C:N ratios while herbivores and predators differ seasonally in abundance (Denno, 1983; Denno et al., 2000; Denno et al., 1980; Matsumura et al., 2004). The N content (%) and C:N ratio of *Conocephalus* is intermediate between marsh herbivores and predators (Matsumura et al., 2004). On the mid-Atlantic marsh, *Conocephalus* has only one generation per year, with eggs hatching in May or June depending on weather conditions (Pearson personal observation). The juveniles then mature on the marsh grass where they molt into adults in August. Females lay their eggs at the base of the grass culms in between the furled leaves. The last adults are seen in August or September depending on the year (Pearson personal observation).

Performance on different diets

We determined the effect of variation in diet macronutrient content on *Conocephalus* survival and growth by feeding them one of four artificial diets that differed in the amounts of protein and energy, using only digestible carbohydrates (henceforth simply carbohydrate) as our source of energy. We formulated diets following the published

protocols as outlined in Dadd (1961) and Simpson and Abisgold (1985), and presented the diets suspended in a 1% agar solution, at a 1:6 dry diet to agar ratio (Lee et al., 2004).

In total, four diets, ten replicates of each (5 males and 5 females), with different protein-carbohydrate ratios (p:c) by mass were made: (1) a highly protein biased diet (p75:c5), (2) a slightly protein biased diet (p50:c30), (3) an equal diet (p40:c40), and (4) a highly carbohydrate biased diet (p30:c50). The combination of protein and carbohydrates in the diets were chosen because they bracket the possible protein-energy ratios *Conocephalus* would encounter in the field (Figure 1). For instance, the average protein-carbohydrate ratio for *Spartina* is approximately 1:2 by mass (Matsumura et al., 2004; Seliskar et al., 2002). In contrast, the protein-lipid ratio for the herbivorous planthoppers (*Prokelisia*), which are often prey items for *Conocephalus*, is 3:1 (Chapter 2). Finally, *Conocephalus* also occasionally feeds on the predaceous spider *Pardosa* (Pearson unpublished data), which has a protein-lipid ratio of approximately 7:1 (Chapter 2). With all of these artificial diets, the total macronutrient composition (proteins and carbohydrates) by mass was 80%. The remaining 20% of each diet had identical proportions of other ingredients, including vitamins, cholesterol, salts, fatty acids and cellulose (Table 1).

We collected early-instar *Conocephalus* in June 2007 using a sweep net and kept them for 48 hours with only a water source prior to the experiment. Immediately preceding the start of the experiment, the starting wet-weight mass of each *Conocephalus* was measured and individuals were assigned randomly to circular feeding arenas (15cm diameter; 6cm height) that housed four evenly spaced feeding dishes each containing the same diet and a water source in the center. All of the cages were maintained in a growth

chamber at a temperature of 27°C under a 13:11 light:dark cycle to approximate summer conditions. We separated the cages with partitions so that *Conocephalus* could not see each other. The experiment was conducted over the course of 12 days. Every two days the *Conocephalus* were monitored for survival, and their food and water were replaced.

Following the completion of the experiment we weighed the surviving *Conocephalus* and then stored them in a -20°C freezer for further processing. Lipid content of the individuals was determined by first drying the individuals in a 60°C oven and then weighing them to the nearest 0.001mg. We extracted the lipids from the dried *Conocephalus* using a chloroform wash (Loveridge, 1973). Here *Conocephalus* were suspended three times in succession in a chloroform bath for 24 hours. Following this procedure they were dried at 60°C in a drying oven to a constant mass and then reweighed. Lipid mass was calculated as the difference in the two mass measurements.

Analyses for the performance measures of survival and growth proceeded first by confirming that there was no significant treatment effect at the outset of the experiment for initial mass. Likewise, there was no effect of gender on any of the performance variables tested so it was removed from all of the models. *Conocephalus* survival on the different treatments was analyzed using the non-parametric log-rank test (SAS: Proc Lifetest). Data were right-censored to account for the individuals that did not die by the time the experiment ended. To determine if *Conocephalus* survival was based on initial mass we performed a post-hoc test using ANOVA (SAS: Proc Mixed).

All of the analyses for growth used *Conocephalus* still alive at the end of the experiment. The models with the fewest numbers of variables for mass gained and lipid mass were chosen using Akaike Information Criterion. To examine the effect of diet

composition on katydid growth we analyzed total mass gained using ANCOVA (SAS: Proc GLM) with diet type as the explanatory variable and initial mass as a covariate. We analyzed the data for differences in lipid mass of the *Conocephalus* on the different diets using ANOVA (SAS: Proc Mixed) with multiple comparisons using diet type and initial mass as the explanatory variables.

Regulation of dietary intake

To determine whether omnivorous early-instar *Conocephalus* nymphs regulate their diets, we performed a choice experiment in which individuals were offered two complementary foods from a possible four total artificial diets. In this way, *Conocephalus* could self-compose their preferred nutritional intake. The diets used were the same as the previous experiment, but there were three treatments: (1) p30:c50 paired with p75:c5, (2) p40:c40 paired with p75:c5, and (3) p50:c30 paired with p75:c5. Each treatment had ten replicates (5 males and 5 females) for a total of 30 experimental units. *Conocephalus* were collected in the field in June 2007 and maintained before and during the experiment in the same manner as above. These experiments were completed over a 6 day period, and the diet cubes in each arena were replaced every 48 hours with a fresh cube.

Two approaches were employed to determine if the *Conocephalus* regulated their dietary intake of proteins and carbohydrates. First, a two-tailed, one sample t-test was used to compare the observed ratio of protein to carbohydrate ingested with the expected ratio of protein to carbohydrate ingested if the *Conocephalus* were feeding randomly on the different diets. Second, MANOVA was used with protein and carbohydrate consumed

as the multiple response variables, and diet type, *Conocephalus* sex and initial mass as the explanatory variables (SAS 9.1.2). The amount of water in each of the diets (75%) was subtracted before analysis was completed. Data were analyzed for three time periods (days 0-2; days 0-4; days 0-6). The protein and carbohydrate consumed for all three time periods met the assumptions of homogeneity of variance and normality required to perform ANOVA.

Results

Performance on different diets

Survival curves for each of the four treatments are shown in Figure 2. The log-rank test indicated that survival was significantly poorer on the high protein diets (p75:c5) and there was an overall difference in survival between the treatments over the 12-days of this experiment ($\chi^2=12.80$, $P=0.005$). There was a slight decrease in survival on the p50:c30 diet, but this difference was not significant compared to survival on the p40:c40 and p30:c50 diets. Initial mass at the start of the experiment did not affect survival ($F_{1,37}=1.23$, $P=0.274$).

There was a main effect of initial mass on mass gained ($F_{1,24}=10.07$, $P=0.004$) but there was no significant effect of treatment ($F_{3,24}=1.54$, $P=0.231$) or the interaction of treatment and initial mass ($F_{3,24}=1.27$, $P=0.306$). Paired contrasts of all of the slopes of the relationship between initial mass and mass gained showed no significant differences between them. Analysis of lipids in the *Conocephalus* carcasses showed a significant treatment effect ($F_{3,31}=14.56$, $P<0.0001$; Figure 4) and a significant effect of initial

Conocephalus mass ($F_{1,31}=22.72$, $P<0.0001$). In general, lipid body content decreased as the protein-carbohydrate ratio decreased. Lipid contents were highest on the p30:c50 and p40:c40 diets, intermediate on the p50:c30 diet, and lowest on the p75:c5 diet (Figure 4).

Regulation of dietary intake

Macronutrient regulation was analyzed using two different approaches. First we asked whether *Conocephalus* fed randomly from the two food types presented in their arenas. Here we employed a t-test and compared the observed ratio of protein to carbohydrate ingested against the expected ingested protein:carbohydrate ratio (calculated based on the null expectation when individuals were given the choice between the food dishes in each treatment). Of the three comparisons, we observed regulated feeding – i.e. consumption that differed from the null expectation – on two of these. *Conocephalus* on the treatments pairing p30:c50 food with p75:c5 foods had a significantly higher p:c ratio (indicating a preference for protein over carbohydrate) for each of the three periods than would have been expected if consumption was not selective ($t_{11}=6.17$, $P<0.0001$; $t_{11}=7.65$, $P<0.0001$; $t_{11}=6.91$, $P<0.0001$; Figure 5). Likewise, individuals on the treatment pairing the p40:c40 food with the p75:c5 food also showed active regulation by consuming protein and carbohydrate in a ratio different from random for all three time periods ($t_9=4.43$, $P=0.002$; $t_8=4.61$, $P=0.002$; $t_8=4.55$, $P=0.002$; Figure 5). In contrast, the protein-carbohydrate ratio for *Conocephalus* that were given the choice between the more moderate protein diet, p50:c30 and the high protein diet, p75:c5, did not differ from the null expectation (Figure 5).

Second, we tested whether *Conocephalus* tightly regulated their protein-carbohydrate intake by performing a MANOVA to explore the extent to which the intake targets on the three different treatments overlapped. If the intake points for each diet pairing overlapped, then we would conclude that *Conocephalus* is capable of tight nutrient regulation. We observed a significant treatment effect for each of the 2-day periods (Day 2: $F_{4,54}=10.03$, $P<0.0001$; Day 4: $F_{4,54}=11.59$, $P<0.0001$; Day 6: $F_{4,54}=13.24$, $P<0.0001$; Figure 5), indicating a lack of overlap in protein-carbohydrate intake. However, there was no significant effect of gender, initial mass or an interactive effect of treatment and initial mass on the amount of protein and carbohydrates eaten. Paired contrasts showed a significant difference in the total amount of proteins and carbohydrates eaten for each of the three pairs (p30:c50 vs. p40:c40, p30:c50 vs. p50:c30 and p40:c40 vs. p50:c30) at each of the three time periods (day 2, day 4, day 6).

Discussion

In contrast with most nutritional studies of herbivores (Behmer, 2009) and predators (e.g. Mayntz et al., 2009), *Conocephalus* did not tightly regulate their macronutrient intake. For omnivores, meeting a protein and carbohydrate intake target may not always be possible. This may be because feeding on plants or prey may not represent an “either / or” situation, but rather a nutritional continuum where they benefit by constantly sampling their surroundings or complementary feeding to meet their nutritional requirements. The degree to which an omnivore mixes its diet may depend not on whether a plant or an animal itself is more nutritious, but rather how each, when

combined, fulfill an omnivore's nutritional needs at that particular time. These needs, of course, can change over time depending on both the state of the omnivore (age, sex etc.) and the state of its environment (resource availability, abiotic conditions, toxins etc.) (Simpson & Raubenheimer, 1995). The subsequent effect of eating diets differing in their macronutrient composition on fitness measurements of insect omnivores is poorly known (but see Raubenheimer & Jones, 2006).

That *Conocephalus* survival was strongly reduced on the high protein diets was somewhat surprising because omnivores and predators, are generally considered protein- or nitrogen-limited (Denno & Fagan, 2003). Our result does, however, support the recent suggestion that animals that feed predominantly on other animals, and thus have diets deficient in carbohydrates or lipids, are more likely to be carbohydrate- or lipid-limited (Raubenheimer et al., 2009). Similar manipulative studies of an orthopteran herbivore, *Locusta migratoria* L. (Acrididae), showed a decrease in percent survival on both high protein and a high carbohydrate diets (Raubenheimer & Simpson, 1999) but studies of another omnivore, the German cockroach (*Blattella germanica*), showed no difference in survival across a broad range of protein-carbohydrate ratios (Raubenheimer & Jones, 2006). Here the authors postulated that *B. germanica* adjusted to variations in the balance of ingested nutrients because they are physiologically able to take advantage of periods of excess while also surviving periods of famine. In particular, cockroaches are opportunistic scavengers and extreme generalists that have the ability to store nitrogen, in the form of uric acid, and carbohydrates, in the form of lipids, in their fat body. In doing so they can consume nutrients in excess with no performance penalty (Douglas, 1989). Cockroaches also have a number of paunches in the hindgut, that house bacteria and

provide them with essential nutrients thus aiding their digestion (Bourtzis & Miller, 2003). In contrast, *Conocephalus* have a less well developed fat body system, are much leaner compared to cockroaches, and do not harbor endosymbionts within their alimentary canals (Nation, 2001). That *Conocephalus* do not generate large lipid stores may not be surprising, though, as they generally have access to sufficient quantities of plant vegetative tissue that compared to prey is relatively rich in digestible carbohydrates.

Despite survival differences among treatments, no differences in growth were observed for those *Conocephalus* that survived to the end of the 12-day experiment. However, there was a significant drop in the lipid content of individuals fed the high protein diets compared to the high carbohydrate diet. Although both *B. germanica* and generalist herbivores also show a significant decrease in their lipid stores on lower carbohydrate diets, counter to *Conocephalus*, *B. germanica* and generalist herbivores grew more slowly on unbalanced foods (Joern & Behmer, 1997; Raubenheimer & Jones, 2006; Raubenheimer & Simpson, 1997). If *Conocephalus* have sufficient nitrogen stores, the key limiting factor would be to have sufficient energy to maintain growth, and this is where lipid stores potentially become important. Although we did not make measurements of post-ingestive processes such as excretion, the *Conocephalus* that died on the high protein diets would have been able to excrete excess amino acids as has been shown for *L. migratoria* (Zanotto et al., 1993), but they would have had no means of replenishing their energy stores.

Conocephalus in the field will rarely be restricted to a narrow diet, and our choice experiments demonstrate that when given the opportunity to self-select they ingest more protein than carbohydrate. However, our results also suggest that *Conocephalus* may

more tightly regulate their carbohydrate consumption. Variation in the mass of carbohydrates eaten by the *Conocephalus* was generally lower than the variation in the mass of consumed protein (Figure 5). At least in the short term, *Conocephalus* may over-ingest proteins to ameliorate a carbohydrate deficit. This apparent regulation of carbohydrates by *Conocephalus* provides additional support for the hypothesis that higher trophic level organisms such as omnivores are limited more by carbohydrates and lipids, in contradiction to predictions that high level consumers are nitrogen- or protein-limited (Denno & Fagan, 2003). This limitation would motivate consumers to feed on energy rich resources at lower levels on the food chain (Raubenheimer et al., 2009) in preference to feeding up the food chain (Denno & Fagan, 2003).

Overall, though, it appears that omnivorous *Conocephalus* are not strong macronutrient regulators since instead of finding convergence of the intake points for each diet pairing, we found that the protein-carbohydrate intake points across the three choice treatments were significantly different from one another at the end of the 6-day experiment, and for each of the three 2-day intervals. Nutritional regulation occurs on a continuum from very tight homeostasis to the absence of dietary regulation. Tight regulation has been shown for numerous herbivores (see Behmer, 2009), predators (e.g. Mayntz et al., 2009), and one omnivore (Raubenheimer & Jones, 2006). However, the regulation demonstrated by *Conocephalus* falls somewhere in the middle of the extremes on the continuum. While they do not show tight regulation, they show evidence for a degree of self-selection (Figure 5); however, the intake of macronutrients by *Conocephalus* depended on the pairing of diets that they were presented with. Similar results were reported for the generalist herbivore *Melanoplus sanguinipes* Fabricius

(Acrididae) (Fielding & Defoliart, 2008), which showed partial regulation by consuming protein and carbohydrate at a ratio different from the null, but not overlapping in their intake when fed different diet pairings.

Tight regulation might not be essential if *Conocephalus* possess efficient post-ingestive physiological processes that allowing compensation for differences in nutrient intake. The use of post-ingestive processes as a mechanism for increasingly efficient use of macronutrients has been suggested for populations of *M.sanguinipes* that demonstrated imprecise regulation of protein and carbohydrate intake (Fielding & Defoliart, 2008). In cases of excessive carbohydrate consumption, post-ingestive processes may increase fat storage (Simpson et al., 2002) or respiration rates (Zanotto et al., 1997; Zanotto et al., 1993). Some insects can process excess nitrogen, for example, some orthopterans (e.g. *Locusta migratoria*), can metabolize excess protein and use amino acids as a source of energy via deamination (Raubenheimer & Simpson, 2003). We do not know if *Conocephalus* has this ability, or the extent to which other predators or omnivores can produce energy via deamination. Alternatively, if excess protein cannot be used to generate energy, protein can be metabolized during digestion, and amino acids in excess of requirements can be voided during excretion (Zanotto et al., 1993).

As an omnivore, *Conocephalus* includes both plant and animal material in its diet despite large differences among which are differences in macronutrient composition. In our short-term feeding experiments, *Conocephalus* was flexible in its acceptance of a wide range of artificial diets and its ability to maintain significant growth. However, their survival negatively impacted by a high protein diet suggesting that they cannot rely on a diet of prey but need an additional source of carbohydrates provided by plants. Resources

rich in carbohydrates may be more important than previously realized for omnivores and predators that mainly feed on diets of protein-rich prey. The apparent short term nutritional flexibility of *Conocephalus* makes sense when consideration is given to the resource landscape of the salt marsh where planthopper prey can reach outbreak numbers and plant quality is variable over space and time. *Conocephalus* can utilize these variable conditions by consuming available resources and, like generalist herbivores, consuming unbalanced foods when they are encountered because the probability of encountering a complementary food will be high (Behmer, 2009).

Figures and Tables

Figure 1: The ratio of protein to carbohydrate/lipid for marsh representatives of the three trophic levels: plant (*Spartina*), herbivore (*Prokelisia*) and predator (*Pardosa*).

Figure 2: Percent survival for each of the diet treatments. Pairwise comparisons showed a significant difference in the percent survival of *Conocephalus* in the 75:5 treatment group compared to 30:50 group ($p=0.0417$), the 40:40 group ($p=0.0315$) and the 50:30 group ($p=0.0315$).

Figure 3: Mean proportional mass gained (\pm SE) of the *Conocephalus* in the four diet treatments. Proportional mass gained was calculated as the final *Conocephalus* mass/initial *Conocephalus* mass.

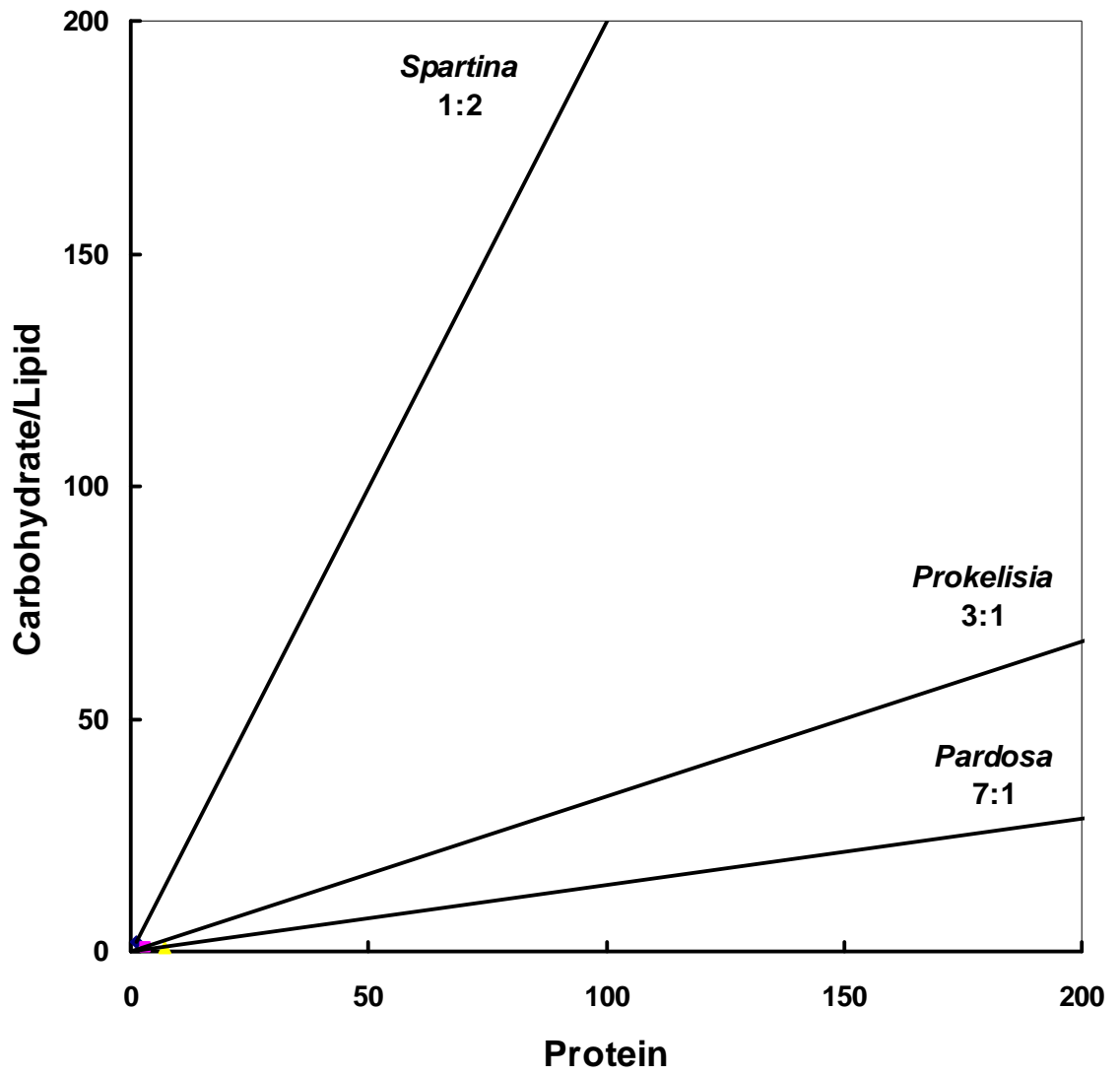
Figure 4: Comparison of the mean percent body lipids (\pm SE) of the *Conocephalus* carcasses fed one of four diets differing in protein:carbohydrate (P:C) diet treatment for twelve days. Means with different letters are significantly different ($P<0.05$).

Figure 5: Bivariate means of protein and carbohydrates consumed (\pm 95% CL) by *Conocephalus spartinae* when given one of three paired diets: (\blacklozenge) 30:50 and 75:5, (\blacksquare) 40:40 and 75:5 and (\blacktriangle) 50:30 and 75:5. The first set of points in the series represents the amount consumed after the first 2 days. The second set of points is the total amount consumed after 4 days and the final set of points is the total amount consumed after 6

days. The dark lines represent the nutritional rails of the four diets as listed in the margin. The fine lines () corresponds to the 30:50 and 75:5 pairing, (---) corresponds to the 40:40 and 75:5 pairing, and () corresponds to the 50:30 and 75:5 pairing) represent the predicted ratios of protein:carbohydrate if the two diets offered were being eaten in equal proportions so that the *Conocephalus* were not self selecting their intake.

Table 1: Constituents of 4 artificial diets fed to *Conocephalus*. Quantities are shown in grams unless otherwise noted.

Figure 1



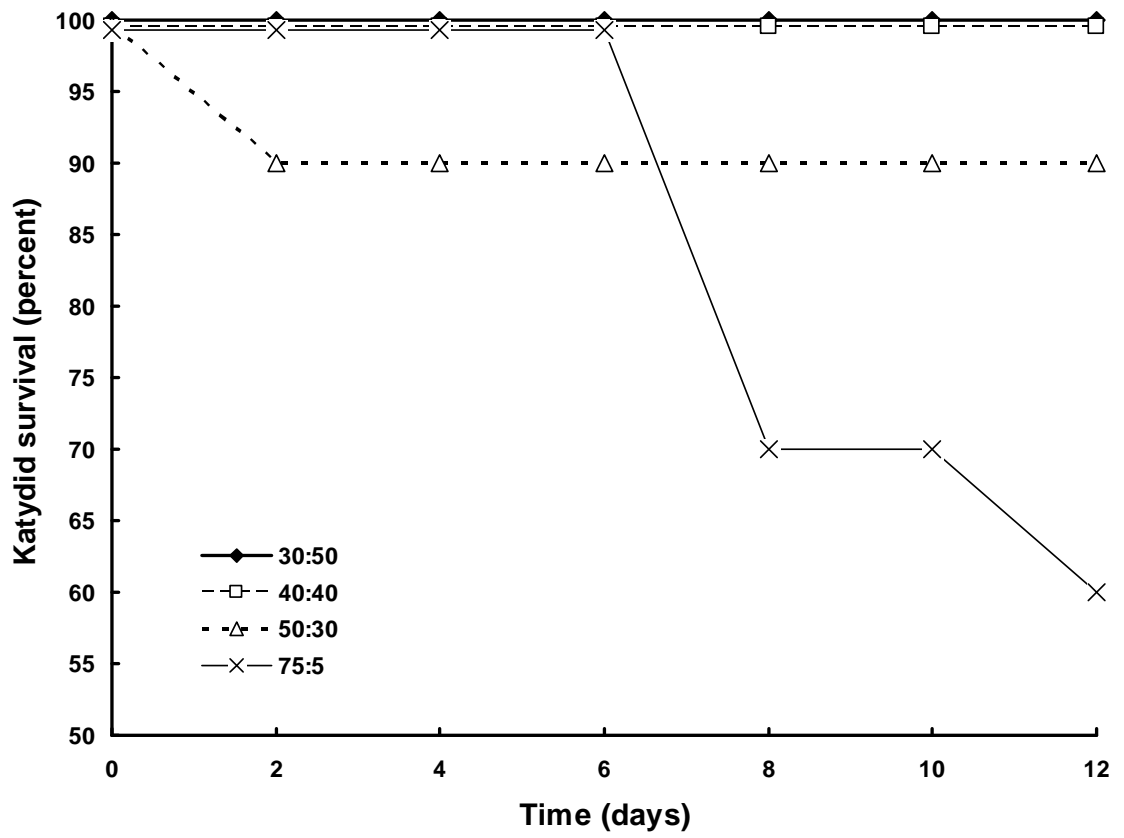
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Figure 2



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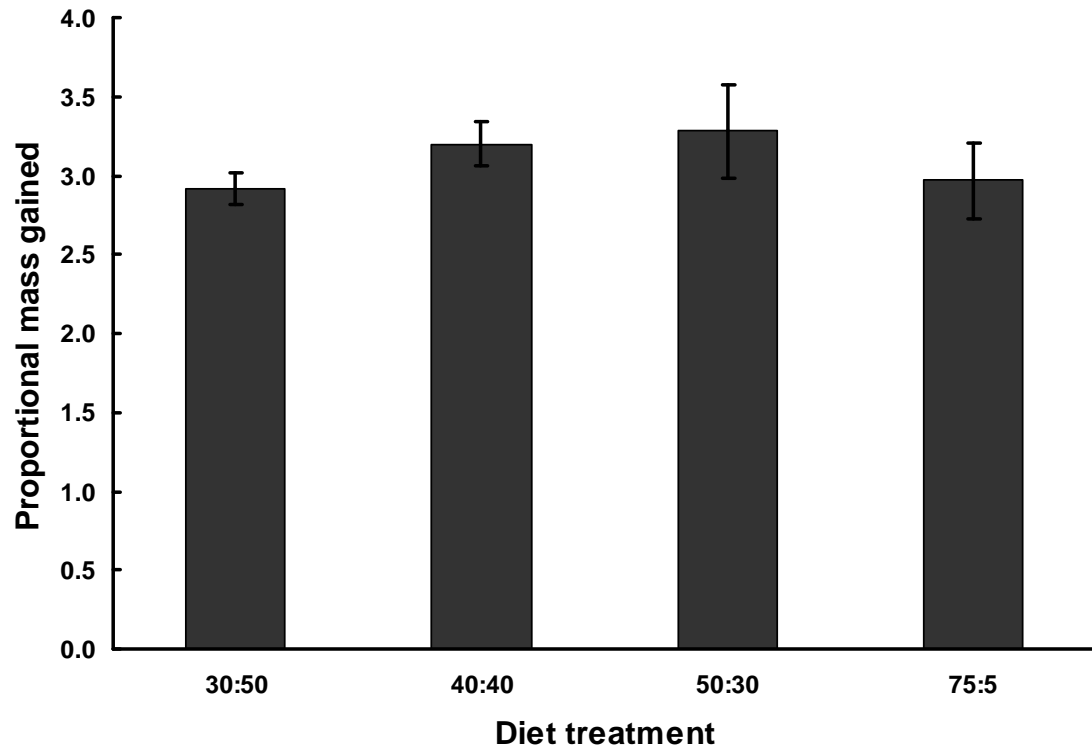
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Figure 3



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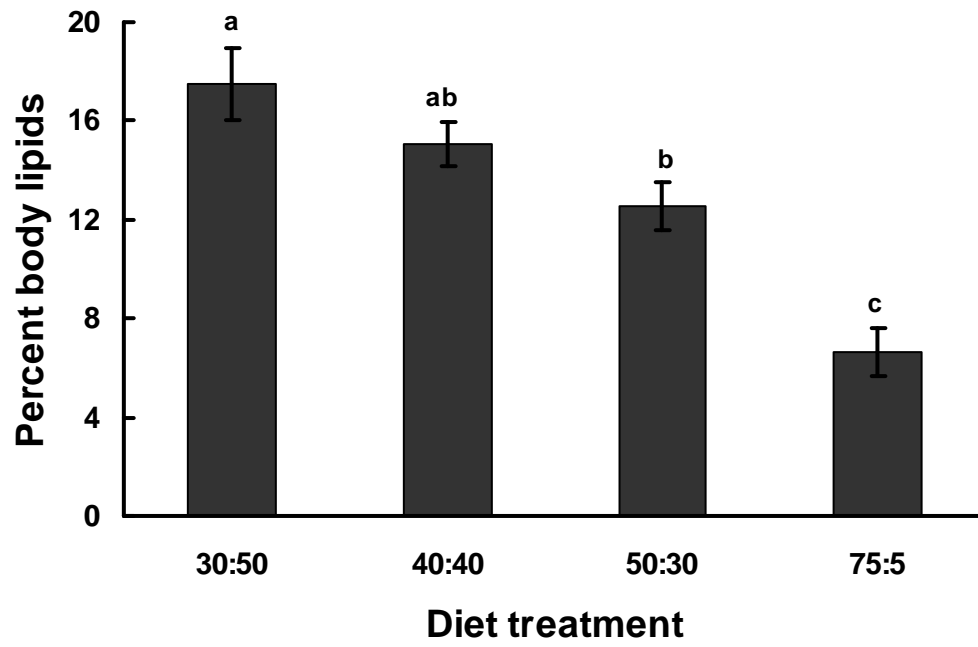
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Figure 4



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Figure 5

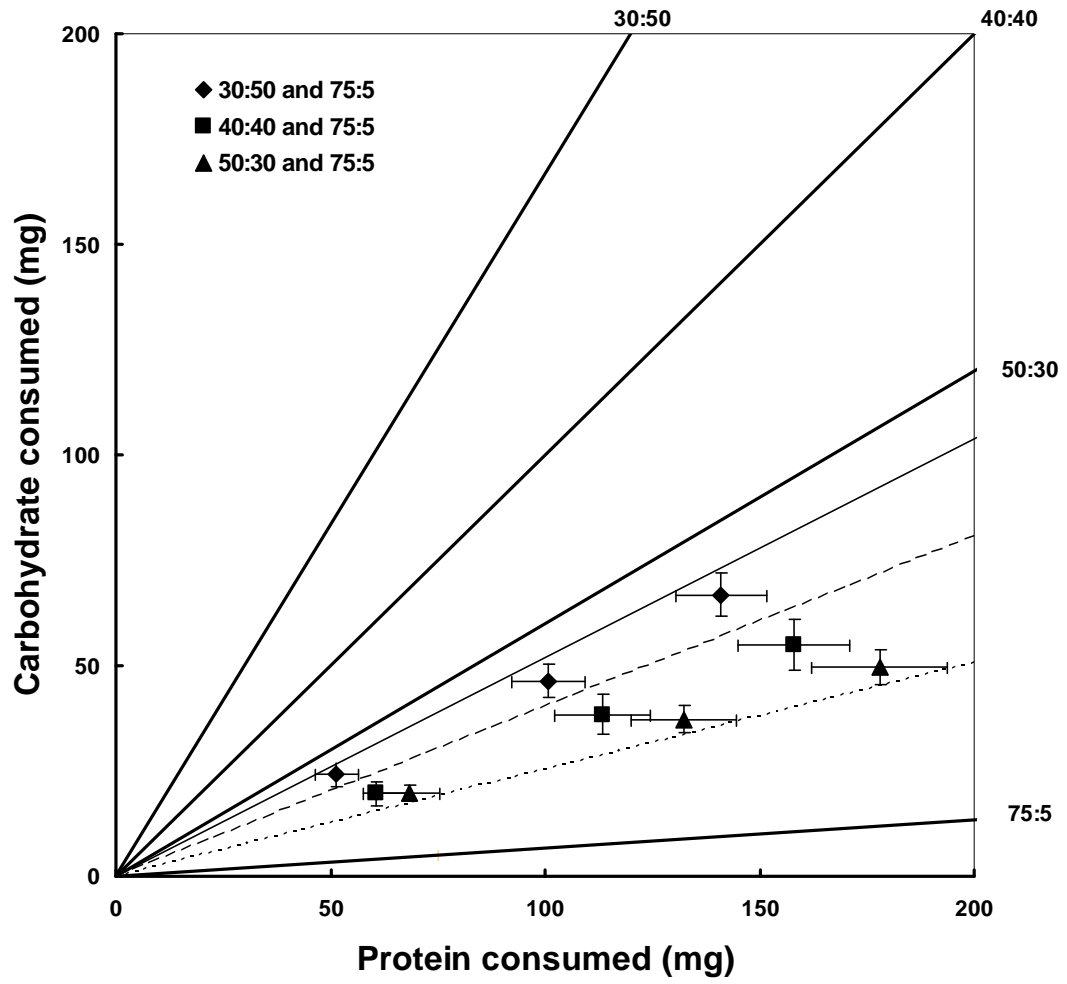


Table 1

Nutrient	Diet			
	<i>High carbs</i>	<i>Equal</i>	<i>Moderate protein</i>	<i>High protein</i>
P:C ratio	30:50	40:40	50:30	75:5
Casein	18.0	24.0	30.0	45.0
Peptone	6.0	8.0	10.0	15.0
Albumin	6.0	8.0	10.0	15.0
Sucrose	25.0	20.0	15.0	2.5
Dextrin	25.0	20.0	15.0	2.5
Cellulose	16.0	16.0	16.0	16.0
Linoleic Acid	0.55ml	0.55ml	0.55ml	0.55ml
Ascorbate	275mg	275mg	275mg	275mg
Cholesterol	550mg	550mg	550mg	550mg
Vitamin mix	180mg	180mg	180mg	180mg
Wesson's salts	2.5	2.5	2.5	2.5

Chapter 2: The performance and nutrient regulation of an omnivore feeding on naturally co-occurring prey

Abstract

Omnivores must contend with relative variation in availability and nutritional content of diverse food resources. Current research has begun to recognize substantial inter- and intraspecific differences in prey nutritional quality as they pertain to consumer performance and nutrient regulation. In our study we examined the nutritional differences of six co-occurring potential prey species of an omnivorous katydid, *Conocephalus spartinae*. We found that these species differed in their nitrogen and lipid content, which were in part a function of body size: a characteristic used by consumers when selecting prey. Despite these differences, we did not detect differences in *Conocephalus* performance. However, individuals showed a degree of regulation in their consumption of nitrogen and lipids by selecting prey unequally when given the opportunity to choose between the six species. We discuss our results in the context of a combined biomolecular and elemental approach to nutrition.

Introduction

Omnivores, consumers that feed on both plants and prey, require traits that allow them to exploit nutritionally dissimilar resources. For instance, plant nutritional content, particularly macronutrient profile and elemental analysis, can vary over both space and time (Denno 1983; Joern and Mole 2005). Despite previous suggestions that prey nutrient content is relatively homogeneous (1996; Slansky and Scriber 1985; Stephens and Krebs 1986), more recent data suggest that there is variation in both prey stoichiometry (C:N) and macronutrient content (protein and lipids) (Elser et al. 2000; Fagan et al. 2002; Matsumura et al. 2004; Mayntz et al. 2005). Furthermore, body composition of prey does not necessarily provide nutritional balance for consumers, requiring omnivores, like predators, to regulate their nutritional intake through active food selection to maintain performance (Mayntz et al. 2009; Mayntz et al. 2005; Mayntz and Toft 2001; Toft 1999). Therefore, in this heterogeneous nutritional landscape, omnivores are likely to increase their fitness if they can compose their diet from a range of options.

Diet choice in omnivores can be influenced by both nutritional and non-nutritional factors such as prey abundance, mobility, body size, risk of predation, and toxin load (Eubanks 2005; Singer and Bernays 2003). Indeed, prey nutrient composition and body size have been used independently to explain food selection and prey choice in omnivores (Coll and Hughes 2008; Denno and Fagan 2003; Diehl 1993; Elser et al. 2000; Raubenheimer and Jones 2006; Sterner and Elser 2002) For example, Denno and Fagan (2003) postulated that the mismatch between plant nitrogen content and omnivore C:N stoichiometry leads omnivores to supplement their diets with prey from higher trophic levels. Predators that feed on herbivores may also compensate for nutrient limitation by

supplementing their diet with other predators, a phenomenon known as intraguild predation (Denno and Fagan 2003). The use of elemental analysis is important for ecological studies because elements are relatively easy to measure and using stoichiometry, they are the common thread that allow for connections to be made between disparate organisms, ecological communities and organic and inorganic constituents (Sterner and Elser 2002).

Elements are nutrients at a fundamental level. However, there can be problems associated with considering only elements when questions are behavioral and physiological in nature because organisms do not relate to their environment through elements. The nitrogen content of food has been used as a proxy for protein (Raubenheimer and Jones 2006) however, carbon occurs readily in both digestible and indigestible forms which cannot be distinguished with carbon analysis alone (Andersen et al. 2004; Raubenheimer et al. 2009). In this situation, a macronutrient, or molecule such as lipids that are used in large quantities, may be a more appropriate unit of measure (Raubenheimer and Simpson 2004; Raubenheimer et al. 2009). Using data from studies that employ a biomolecular approach, Raubenheimer et al. (2009) proposed that predators may be carbohydrate- or lipid-limited, rather than nitrogen-limited, as is generally argued to be the case for herbivores. They contend that predators feed on higher trophic levels that are rich in protein, but generally are low with regard to energy containing biomolecules, particularly carbohydrates. Therefore predators, to redress the lack of energy-rich nutrients, have a greater incentive to consume resources from lower trophic levels (Raubenheimer et al. 2009).

Prey characteristics such as element and macronutrient composition (nitrogen and lipid) may play an important role in omnivore prey selection, but currently we know very little about how prey characteristics influence the nutritional decisions of omnivores and their performance. In this study we tested the effect of variable prey nutrient content and associated characteristics on the performance of an omnivorous salt marsh arthropod and its ability to regulate consumption of nitrogen and lipids. We addressed three specific objectives: (1) to establish the difference in the nutritional content (nitrogen and lipids) of different potential prey; (2) to compare the effect of prey identity on survival, and growth of an omnivore fed one of six different prey; (3) to determine if this omnivore regulates its dietary intake. Contrary to the assumption that prey nutrient content is relatively homogeneous, our results suggest that prey are heterogeneous in nitrogen and lipid content as well as size, however, these differences did not affect performance. Our results also indicated that katydids demonstrate a degree of regulation when given a mixture of prey items.

Methods

Study system

Salt marshes along the Atlantic coast of North America are well-studied systems that offer many advantages for the investigation of food-resource characteristics and diet choice. Atlantic coast intertidal marshes are dominated by the perennial cordgrass, *Spartina alterniflora* (Poaceae) (hereafter *Spartina*). From the high marsh to the low marsh, *Spartina* differs in its growth forms and nitrogen content influenced by soil

conditions, litter decay, salinity, tidal inundation and nutrient influxes (Denno 1983; Gallagher et al. 1988; Teal 1962) Low marsh *Spartina* tends to be higher in nitrogen and more vigorous whereas high marsh *Spartina* is much shorter in stature, with a lower nitrogen content (Denno 1983; Gallagher et al. 1988). These contrasts in plant characteristics affect arthropod distributions across the marsh (Denno 1983), and we know that numerous common arthropod species differ consistently in their nitrogen and carbon contents within the marsh (Matsumura et al. 2004). Our study focused on the nutritional ecology of a common and abundant omnivore, the katydid *Conocephalus spartinae* (Orthoptera: Tettigoniidae) (hereafter *Conocephalus*). Evidence from gut contents of field caught *Conocephalus* revealed identifiable parts from plants, planthoppers, leafhoppers, spiders, flies and beetles (Chapter 3). Because *Conocephalus* appears highly catholic in food selection habitats and because of its high mobility, *Conocephalus* individuals can encounter numerous different prey items in the salt marsh habitat.

For this study we selected six common potential prey species to represent variation in phylogeny, trophic level (3 herbivores and 3 predators) and body size. Members of the sap-feeding guild (planthoppers, leafhoppers and mirid bugs) are the most abundant herbivores on the marsh (Denno 1983; Denno et al. 2000; Denno et al. 1980) and are known prey for *Conocephalus*. Making up the largest proportion of this guild are specialist *Spartina*-feeding planthoppers from the genus *Prokelisia* (Hemiptera: Delphacidae). We used *Prokelisia dolus*, one of the two most common species that typically lacks flight wings in the adult stage (Denno et al. 2000; Denno et al. 1996). We included two other locally abundant herbivorous insects as prey for *Conocephalus*: the

slender-bodied mesophyll-feeding mirid bug *Trigonotylus uhleri* (Hemiptera: Miridae), and adults of the larval stem-boring fly *Chaetopsis aenea* (Diptera: Ulidiidae). We also included three common predator species as potential *Coenocephalus* prey: a large, actively-hunting wolf spider, *Pardosa littoralis* (Araneae: Lycosidae); a small sit-and-wait, web-spinning spider, *Grammonota trivittata* (Araneae: Linyphiidae), and an actively foraging hemipteran, *Pentacora hirta* (Hemiptera: Saldidae). Each of these three predators hunt and feed throughout the marsh canopy and across an elevational gradient.

Macronutrient content of prey

Individuals of the six prey species were collected during July 2007 at two marsh locations (Tuckerton, Ocean County, New Jersey: 39°31'N, 74°19'W and Public Landing, Worcester County, Maryland: 38°13'N, 75°30'W) using sweep net and D-Vac® (Rincon-Vitova, Ventura, California) suction sampling techniques. Arthropods were kept on dry ice for transport from the field, and then moved to a -20°C freezer in the laboratory. All arthropods were oven-dried for approximately 72 hours at 60°C and then weighed to the nearest 0.001 mg.

Mean nitrogen and lipid content was determined for the six potential prey species. A mass of 0.9-1.1mg was required for analysis so, small-bodied arthropods were pooled into samples of 2-11 individuals and packed into a tin capsule. Each tin capsule represented one replicate. Larger arthropods were ground to a powder after drying. One subsample of powder per individual was then packed into a tin capsule for analysis. For each of the six potential prey species there were at least five replicates. We also analyzed

the nitrogen content of *Conocephalus* (10 replicates). The samples were sent to the Cornell University Stable Isotope Laboratory (Cornell University, Ithaca, New York, USA) for analysis. Each sample was analyzed for percentage N using an isotope-ratio mass spectrometer (Finnigan MAT Delta Plus, San Jose, California, USA) connected to a Carlo Erba NC2500 (Milan, Italy) elemental analyzer through a Conflo II (Thermo Finnigan LLC, San Jose, California) open split interface. To date, although there is ongoing work to determine a more precise conversion factor for nitrogen to protein (Lourenco et al. 2004; Mariotti et al. 2008; SaloVaananen and Koivistoinen 1996; Sosulski and Imafidon 1990), the typically used conversion factor of 6.25 to determine the protein content of arthropods represents only a linear re-scaling constant. Therefore we used simple nitrogen content as a proxy for protein.

We couldn't use the same individuals for the lipid analysis because of the destructive nature of the nitrogen analysis, so we used comparably sized individuals from the same collection. Species lipid content was estimated through lipid removal in a series of 24-hr chloroform washes (Lee et al. 2003). Arthropods were dried in a 60°C oven to a constant mass (weighed to the nearest 0.001mg), then placed in a chloroform bath, completely submerged, for 3, 24 hr cycles. Following the final chloroform wash the arthropods were dried again in a 60°C oven and then reweighed. Lipid content was calculated as the difference in the masses before and after the chloroform bath.

We used a series of analysis to determine if prey species differed significantly in their compositions. Analysis across the six prey species for differences in nitrogen and lipids was conducted using separate ANOVA tests (SAS: Proc Mixed). For percent nitrogen and percent lipids we used species and species mass as the explanatory

variables. We conducted a series of five independent contrasts to compare percent nitrogen and percent lipids between species, orders, classes and trophic groups. The relationship between the mean absolute amount of lipids and mean species mass as well as the mean absolute amount of nitrogen and mean species mass was investigated using regression procedures (SAS: Proc Reg). Similarly, regression procedures were used to analyze the relationships between percent lipid and percent nitrogen and body mass (SAS: Proc Reg). For all analyses, we verified model assumptions of normality and homogeneity of variance. No transformations were necessary.

Performance of *Conocephalus* reared on different prey

To determine the effect of prey identity and nutritional content on katydid performance (growth and survivorship) a no-choice rearing experiment was conducted, using the six prey listed above. All prey were field collected in New Jersey and Maryland in July just prior to the start of the experiment, maintained alive until they were needed, and then killed by freezing in a -20°C freezer for 10 minutes. By killing prey we removed prey behavior so that katydid foraging decisions were based predominantly on prey identity and nutrient content.

The *Conocephalus* individuals used in this laboratory experiment were collected with a sweep net in July 2007 from the Maryland salt marsh. Individuals were collected as mid-instar nymphs, just prior to the start of the experiment and maintained in the lab with a water source for 48 hours prior to the start of the experiment. Upon commencement of the experiment, sixty *Conocephalus* (30 of each gender) were weighed

and randomly assigned to one of the six prey treatments. Individuals were housed in closed, cylindrical feeding arenas (16cm diameter; 4cm height) with a water source in the center and were offered a continuous supply of a single prey species over an 8-day period. Whole prey (10mg of each) were offered in feeding dishes (2.5cm diameter), and all of the cages were kept in a growth chamber under a 13:11 hour light:dark cycle at 27° C. Individuals were monitored every two days to determine survival and to restock prey. The experiment was terminated after eight days and the mass of each survivor was recorded.

To evaluate our expectation that initial *Conocephalus* mass did not differ systematically, we conducted an ANOVA (SAS: Proc Mixed) and found that there was no significant effect of initial mass across treatments ($F_{5,46}=0.12$, $P=0.987$). However, there was a significant and unavoidable sex effect ($F_{1,46}=11.52$, $P=0.001$) with females initially larger than males. *Conocephalus* survival was analyzed using the non-parametric log-rank test (SAS: Proc Lifetest) in which those individuals that did not die by the end of the experiment could be right censored. To determine if there was an effect of prey treatment on growth, total relative growth ($(\text{final}-\text{initial}*\text{initial}^{-1})$) during the course of the experiment was analyzed using ANOVA (SAS: Proc Mixed). The analyses of mass gained included prey treatment, sex and the interaction of the two as explanatory variables.

Nutrient regulation on multiple prey

To determine if *Conocephalus* regulates their nitrogen-lipid intake when feeding on prey, an experiment was performed in which individuals were offered a choice of all six prey

species used in the previous experiment. Individual *Conocephalus* were treated in the same manner as above, and prey were offered freshly killed in order to remove prey behavior. Individuals were given free access to all six different prey species (10 mg each) simultaneously, and this treatment was replicated 16 times, with 8 of each gender.

Prey species were offered in equally spaced feeding dishes (2.5cm diameter) within an arena (16cm diameter; 4cm height). Each individual received 10mg of each prey species in an amount that exceeded what they could consume during the 48-hour experiment. A water source was included to reduce the importance of prey water content. All of the arenas were kept in a growth chamber under a 13:11 hour light:dark cycle at 27° C. Following 48-hours in the arenas, each prey species was weighed and mass consumed in the choice experiment was calculated.

A two-tailed, one sample t-test was used to compare the observed ratio of nitrogen to lipid ingested with the expected ratio of nitrogen to lipid ingested if the *Conocephalus* were feeding equally on the different prey species.

Results

There were significant differences in percent nitrogen between the prey ($F_{5,11.5}=14.08$, $P<0.0001$; Figure 1). Similarly, for percent lipids, there was a significant species effect ($F_{5,26.3}=12.07$, $P<0.0001$), but no size effect ($F_{1,30.4}=0.91$, $P=0.347$; Figure 1). There were also some notable differences in percent nitrogen and percent lipids between species, orders, classes and trophic groups within an order (Table 1). For instance *Chaetopsis* had a lower nitrogen (%) content than both *Trigonotylus* and *Prokelisia* and the spiders (*Grammonota* and *Pardosa*) have a higher nitrogen (%) content than the insect predator,

Pentacora. However, when all six species were analyzed, there was no effect of trophic group (%Nitrogen: $F_{1,29,2}=1.05$, $P=0.313$; %Lipid: $F_{1,49,3}=2.32$, $P=0.134$). Regression analysis of nitrogen mass and species body mass yielded a positive slope (0.099) that was significantly different from zero ($P_{1,39}<0.0001$, $\text{AdjR}^2=0.947$; Figure 2A). Likewise, there was also a positive relationship between lipid mass and prey size ($b_1=0.163$, $P_{1,83}<0.0001$, $\text{AdjR}^2=0.720$; Figure 2B). Percent nitrogen was significantly, negatively related to mean prey species mass, ($b_1=-0.785$, $P_{1,39}=0.005$, $\text{AdjR}^2=0.163$; Figure 2C). However, the regression of percent lipids and species size was not significant ($P_{1,83}=0.287$, $\text{AdjR}^2=0.002$; Figure 2D).

An initial comparison of survival curves, after controlling for the effects of gender, revealed no difference between no-choice feeding treatments of the six prey species ($P=0.688$ for the log-rank test; Figure 3). Following the 8 day experiment, analysis of total relative growth of *Conocephalus* across the treatments (Figure 4) yielded non significant effects of sex ($F_{1,39}=2.08$, $P=0.157$), prey treatment ($F_{5,39}=1.76$, $P=0.144$) and the interaction of sex and prey treatment ($F_{5,39}=0.37$, $P=0.867$).

The prey choice experiment suggested circumstantial evidence for *Conocephalus* regulation of their nutrient intake. Individuals consumed nitrogen and lipid in a 1:2.89 mean ratio, which was lower in protein than the expected 1:1.28 ratio that would have been observed if all species were consumed with proportional intensity ($t_{14}=6.03$, $P<0.0001$, Figure 5).

Discussion

Effects of the differential macronutrient content of different diets on fitness and dietary regulation has been explored for herbivores (reviewed in Behmer 2009), predators (Mayntz et al. 2009; Mayntz et al. 2005), and a single omnivore species (Jones and Raubenheimer 2001; Raubenheimer and Jones 2006). However, little is known about how the differences in nutritional composition of the prey themselves, and associated characteristics (such as size), affect the extent to which omnivores survive, grow and regulate their nutrient intake. To our knowledge, few studies have analyzed the nitrogen-lipid content of an omnivore's potential prey, and how prey nutritional content relates to prey body size (another prey characteristic that may influence consumer choice). Our analysis of the nitrogen and lipid content of potential prey of the omnivorous katydid, *Conocephalus*, revealed significant differences among species. In addition, there was a significantly positive relationship between the size and both nitrogen and lipid content (by mass) and mean body mass among species. Interestingly, there was a significant, and negative, relationship between size and the percentage of nitrogen in the prey species, suggesting that proportional allocation to nitrogen decreases with size. However, this pattern was not observed when percent lipid content was regressed against body size.

The nitrogen-lipid values for the six prey items studied support the notion that prey items, in terms of their nutritional content, are not created equal, although the range of food composition available to a carnivore is more restricted compared to herbivores (Raubenheimer and Simpson 1999; Raubenheimer et al. 2009). The observed differences between prey can at least partially be explained by biological and phylogenetic differences of the species. The nitrogen content (as a %) of the two hemipteran

herbivores was not significantly different. However, the %lipid content of *Trigonotylus* (~7%) was significantly lower than *Prokelisia* (~21%). The high %lipid content of *Prokelisia* may be explained in part by their diet of carbohydrate-rich phloem. The finding of differences between both %nitrogen and %lipid content between *Chaetopsis* and the combination of *Prokelisia* and *Trigonotylus* support analyses conducted by Fagan et al. (2002) that found that there were significant differences in protein content among lineages of herbivores. More derived groups, such as Diptera (e.g. *Chaetopsis*), have lower protein content than older lineages like Hemiptera (e.g. *Prokelisia* and *Trigonotylus*) perhaps because of evolutionary differences in the time since the adoption of phytophagy, nitrogen stress at the time of species origin and the feeding habit that preceded phytophagy. The significantly lower %nitrogen and %lipid content of *Pentacora* compared to the spiders may be due to a taxonomic difference between the classes Insecta and Arachnida. In a stoichiometric study of salt marsh taxa, Matsumura et al. (2004) found that spider predators (Arachnida) had a higher nitrogen content than insect predators. The higher nitrogen signal in spiders may be due to the incorporation of nitrogen into non-muscular structures like silk and peptide rich toxins (Grip et al. 2009; Ueberheide et al. 2009).

Contrary to stoichiometric analyses that have shown predators have a higher nitrogen content and lower carbon to nitrogen ratio than herbivores (Fagan et al. 2002; Matsumura et al. 2004), we did not find a clear effect of trophic level when nitrogen and lipid content were analyzed for the six marsh species. The discrepancies between our findings and that of previous studies may be because we analyzed only a small subset of herbivores and predators. Another explanation derives from our analysis of biomolecules

in addition to elemental nutrients. Insects interact with their nutritional environment using both pre- and post-ingestive processes that are sensitive to molecular complexes such as amino acids and carbohydrates, and not elements (Gwynne 2001; Raubenheimer and Simpson 2004; Raubenheimer et al. 2009). It is to these molecules that insects have evolved regulatory mechanisms (Raubenheimer and Simpson 2004). Thus biomolecules are a more appropriate unit of measure considering that we were interested in the behavior and physiological regulation of *Conocephalus* (Raubenheimer and Simpson 2004; Raubenheimer et al. 2009). The correlation between nitrogen and protein has been used successfully in many studies (Raubenheimer et al. 2009) but the widely used conversion factor of 6.25 is an approximation and is not perfect. There may be other nitrogenous compounds that inflate the amount of nitrogen found in an organism and thus the amount of protein. There is also variation in the amount of nitrogen found in different proteins (e.g. Lourenco et al. 2002). Therefore we chose to use nitrogen as a proxy for protein. However, the elemental analysis of both plants and animals for carbon does confound both indigestible carbon, such as that found in cellulose and chitin, with digestible carbon found in carbohydrates and lipids. Therefore by using elemental analysis when measuring the consumption of food nitrogen may be a good estimation, but by measuring carbon, the outcome may be the failure to accurately detect regulation of digestible carbon thereby missing information on a large component of food choice and behavior (Raubenheimer et al. 2009).

Body size may influence the nutritional content of prey and thus food choice. We found a significant relationship between both the mass of the nitrogen content of the prey (Figure 2A) and the mass of the lipid content of the prey (Figure 2B), and prey size. The

slopes of these two relationships were positive. For both nitrogen and lipids, prey size constrains the absolute amount of each that a consumer can eat, so that larger prey contain more absolute nitrogen and lipids. However, there was a negative relationship between %nitrogen and size indicating that larger prey have a lower %nitrogen, while there was no relationship between %lipids and size. Therefore, smaller prey may actually be relatively richer in their nitrogen to lipid ratio and they are easier to capture and consume. For example, size played a role in the enhanced performance of the omnivorous pentatomid bug, *Oeochalia schellenbergii* when they were allowed to consume smaller rather than larger noctuid larvae (Coll and Hughes 2008). Their enhanced performance was hypothesized to be the result of a lower energetic cost associated with subduing smaller compared to larger prey. Other studies have also shown evidence of greater capture success being related to smaller prey size (e.g. Cogni et al. 2002). However, there may also be costs associated with small prey due to the higher surface area to volume ratio and thus a greater amount of indigestible chitin (Molles and Pietruszka 1987). Therefore smaller prey may be more costly to consume because of the increased time spent eating a greater quantity of smaller prey to get the same absolute amount of nutrition from a lower number of larger prey.

The differential macronutrient content and identity (including body size) of the six species used in this study did not have an effect on growth or survival over the 8 day time period, despite differences in prey nitrogen from 9% to 13% and in lipids from 7% to 21%. These results are similar to results found for a predator that showed no change in body mass when fed different artificial diets (Mayntz et al. 2009). As an omnivore, *Conocephalus* is flexible in its acceptance of a wide range of artificial diets and its ability

to maintain significant growth, although survival on very protein-rich artificial diets (a protein-digestible carbohydrate ratio of 75:5) was very low (Chapter 1). In the current study the *Trigonotylus* prey treatment had a protein-lipid ratio of 79:7 (when converted using 6.25), which is not too dissimilar to the previously mentioned artificial diet. There are two possible explanations for why survival on the *Trigonotylus* treatment was not different compared to the other treatments, and compared to results with the protein-rich artificial diet. First, we may have failed to observe an effect of prey because of the length of the study – it was run for 8 days, as compared to 12 days for the artificial diet study where we saw a majority of the mortality between day 6 and 10. Second, lipids, compared to carbohydrates, yield more energy per unit mass (9 kcal/g compared to 4 kcal/g, respectively).

Our choice experiment suggests that when *Conocephalus* is given the opportunity to self-select from among a range of prey items, they consume prey in proportions such that their nitrogen-lipid intake ratio is less than would be expected if they were feeding equally on all species offered. Choice experiments using artificial diets varying in protein to carbohydrate ratios also suggested energy needs are a key factor driving foraging behavior for this omnivorous katydid (Chapter 1). Here *Conocephalus* more tightly regulated their lipid intake than they did their nitrogen intake. These data suggest that when omnivores are confined to a prey-only diet, like strict predators, they may be carbohydrate and lipid limited (Raubenheimer et al. 2009). Therefore, omnivores may ameliorate this limitation by feeding on plants in addition to prey (Eubanks and Denno 1999).

We have shown that there is natural variability in prey nutrient composition and that this variability is associated with the biology and phylogeny of prey species as well as mean prey size. The omnivorous *Conocephalus* is capable of distinguishing between the body composition of different prey despite previous suggestions that prey availability and not prey nutrient composition determines patterns of consumer foraging (Stephens and Krebs 1986). Evidence for this comes from the ability of mid-instar *Conocephalus* to regulate intake when offered a complex of prey that are abundant on the marsh. Omnivorous consumers may therefore regulate their dietary intake by eating prey in the short term without negative impacts on performance. Maintaining a diet that is this rich in nitrogen may be difficult for *Conocephalus*, but as omnivores, they are able to mix their nitrogen-rich diet of prey with a carbohydrate-rich diet of plants. This flexibility in acceptance of vastly different food resources requires both physiological and behavioral adaptations and is one mechanism by which organisms may solve the dilemma of nutrient balancing.

Figures and Tables

Figure 1: Comparison of the mean percent nitrogen and mean percent lipids (\pm SE) of the six different prey offered to *Conocephalus*. The black symbols followed by the genera names represent herbivores (H) and the grey symbols followed by the genera names represent predators (P).

Figure 2: Contrasting A) the relationship between mean mass of nitrogen (mg) (\pm SE) and B) the mean mass of lipids (mg) (\pm SE) with the size (mg) of six potential prey species. Comparison of C) the relationship between mean %nitrogen (\pm SE) and D) mean %lipid (\pm SE) and size (mg) of six potential prey species. The black symbols represent herbivores (H) and the grey symbols represent predators (P).

Figure 3: Percent survival of *Conocephalus* after eight days on the six prey treatments.

Figure 4: Mean relative growth (\pm SE) of *Conocephalus* after eight days on one of the six prey treatments.

Figure 5: Nitrogen and carbohydrates consumed by *Conocephalus* when given six prey that differed in their protein and lipid content. Clear diamonds represent the individual katydids and the dark triangle represents the mean (\pm 95% CL). The fine line represents the predicted ratio (N1:L1.28) of nitrogen:lipids if the six prey were being eaten in equal proportions so that the katydids were not self selecting their intake. The dark line

represents the ratio (N1:L2.89) of actual amount of nitrogen to lipids eaten by *Conocephalus* in the experiment.

Table 1: Comparisons of percent nitrogen and percent lipids between groups of herbivores, predators and trophic levels.

Figure 1

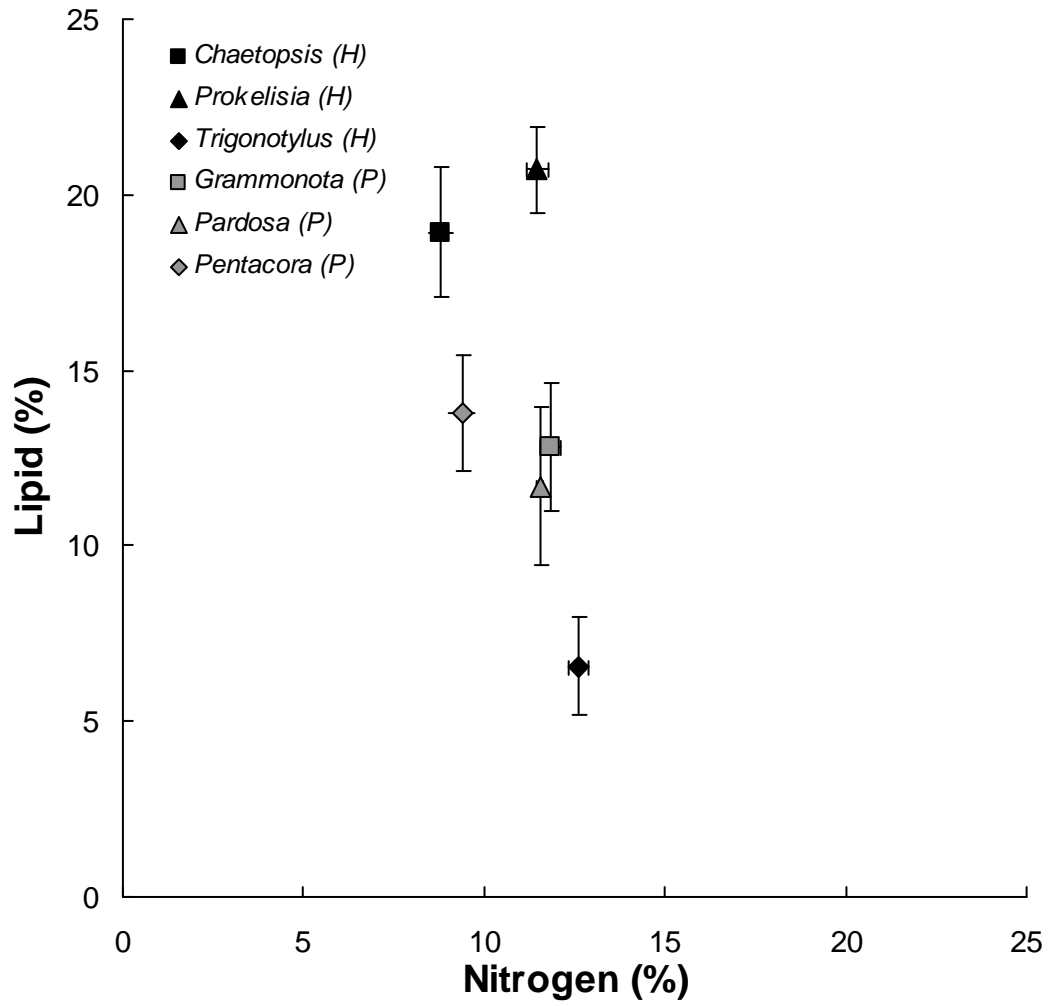


Figure 2

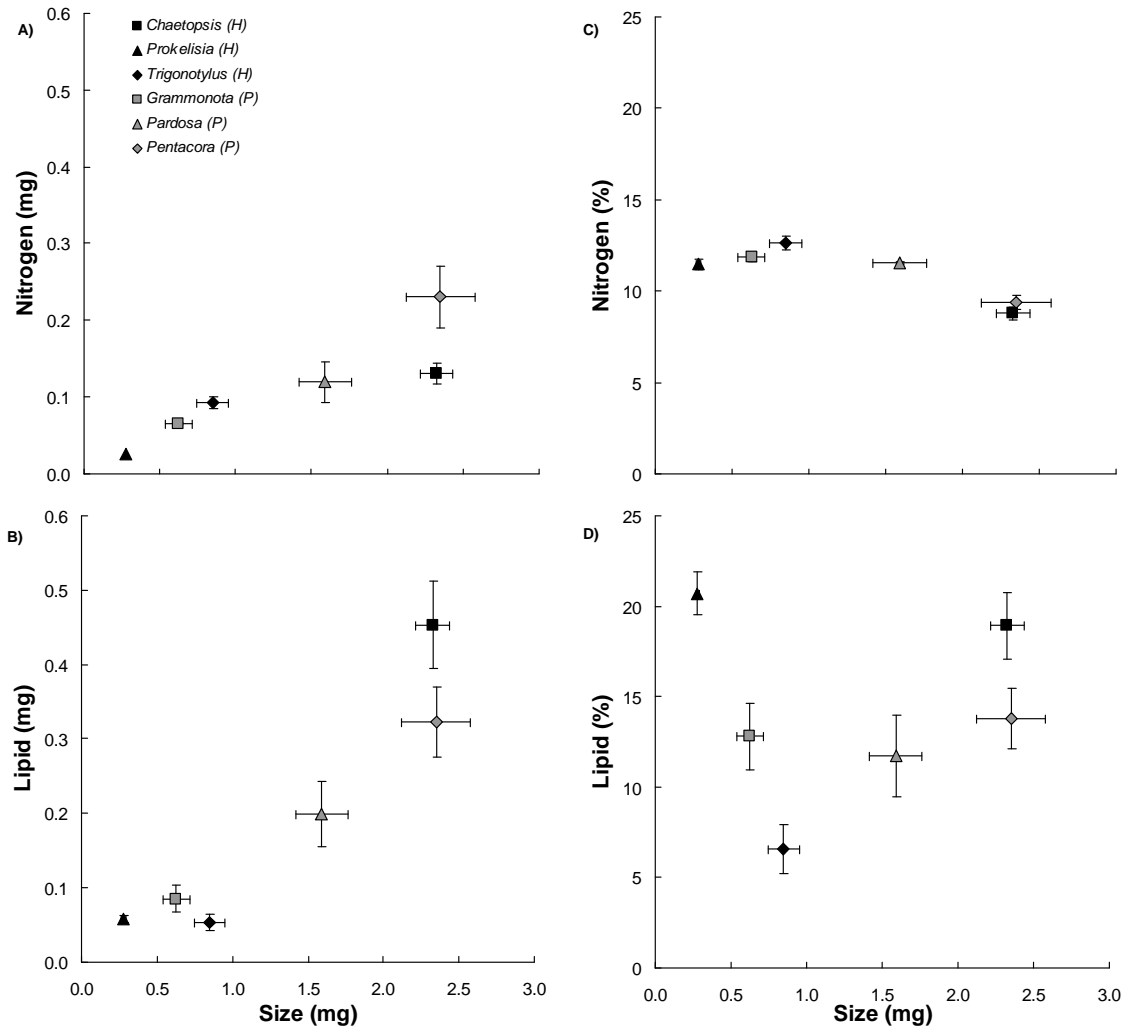


Figure 3

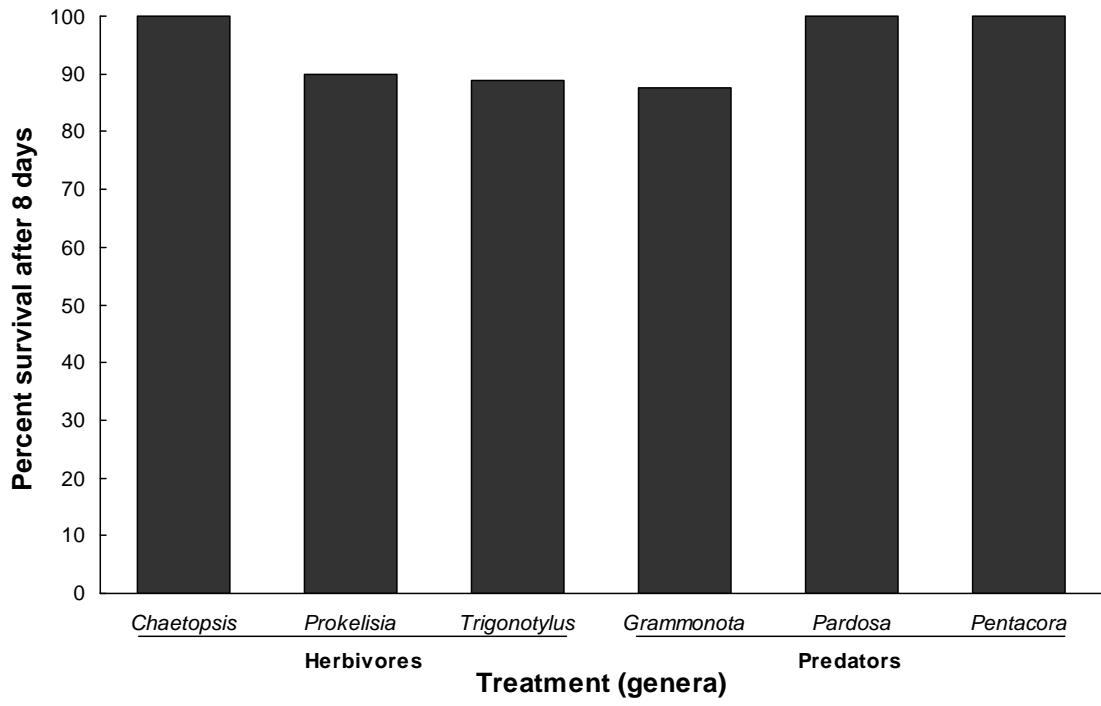


Figure 4

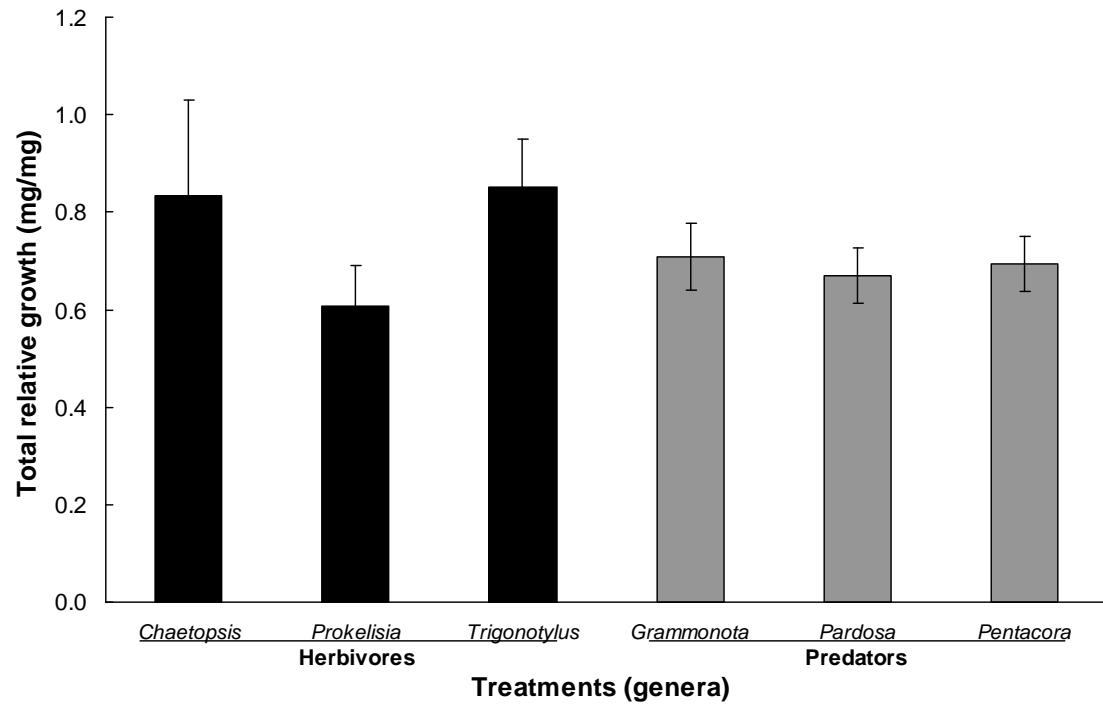


Figure 5

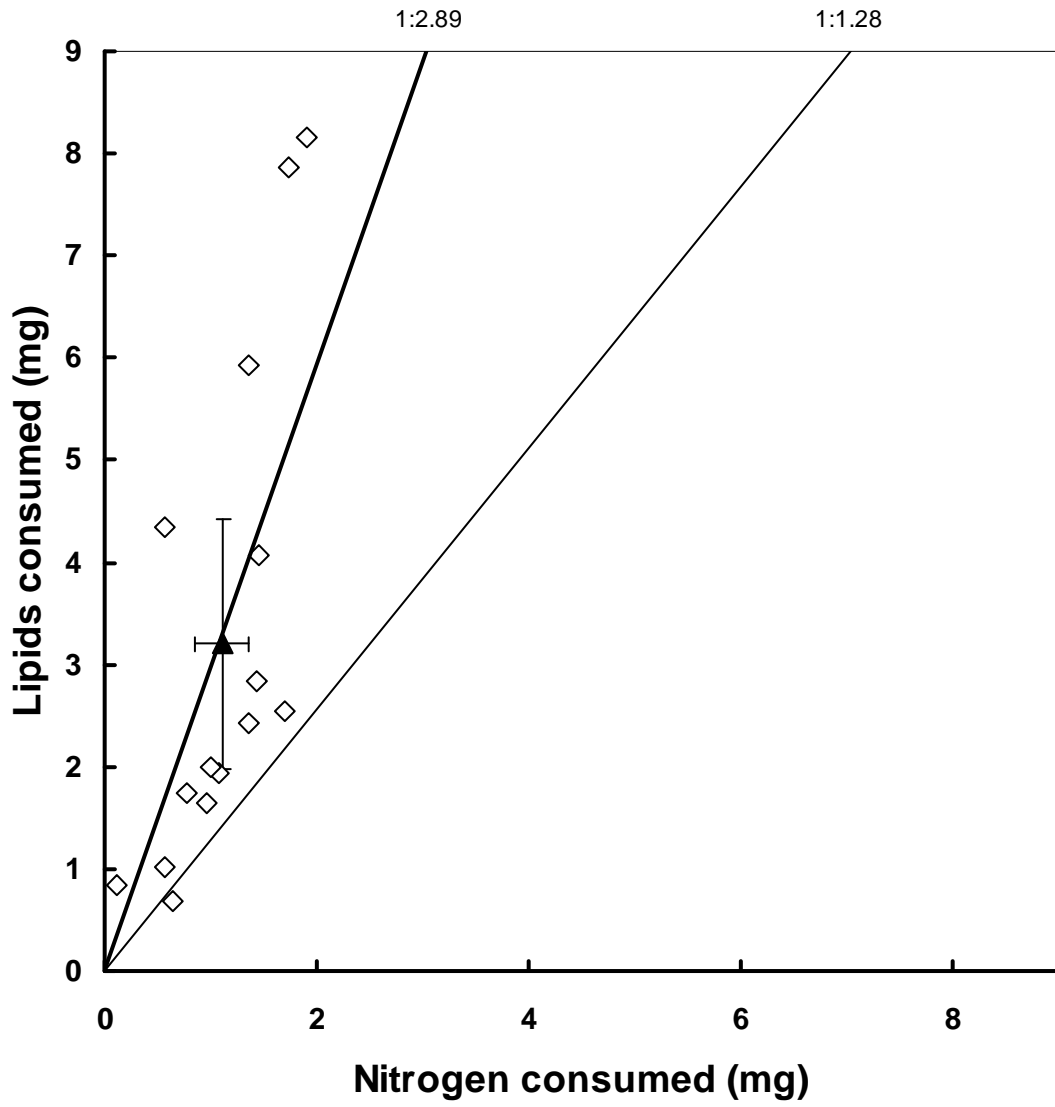


Table 1

Contrasts	% Nitrogen			% Lipid		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Herbivores						
<i>Trigonotylus</i> vs. <i>Prokelisia</i>	1, 10.6	0.41	0.537	1, 13.3	11.13	0.005
<i>Chaetopsis</i> vs. <i>Prokelisia</i> & <i>Trigonotylus</i>	1, 11.5	50.65	<0.0001	1, 15.0	16.76	0.001
Predators						
<i>Grammonota</i> vs. <i>Pardosa</i>	1, 10.8	0.57	0.467	1, 20.2	0.03	0.867
<i>Pentacora</i> vs. <i>Grammonota</i> & <i>Pardosa</i>	1, 6.6	31.47	0.001	1, 44	17.87	0.0001
Trophic level						
<i>Pentacora</i> vs. <i>Prokelisia</i> & <i>Trigonotylus</i>	1, 6.6	29.31	0.001	1, 4.5	41.98	<0.0001

Chapter 3: Plants drive patterns of distribution but prey enhance performance of an omnivorous katydid

Abstract

Understanding the ecology of omnivores is central to understanding their distribution and their subsequent effects on the structure and dynamics of plant and prey communities. However, there is still much to learn about the complex relationship between the ability to locate plant and prey resources and the differential effect that these resources have on omnivore performance. Here we examine the distribution and performance of an omnivorous katydid, *Conocephalus spartinae*, as it relates to variation in the quality of salt marsh cordgrass, *Spartina alterniflora* and the abundance of their herbivorous prey, *Prokelisia* planthoppers, using a combination of survey techniques, laboratory and field experiments. In the survey of natural populations, we found that *C. spartinae* tracks plant quality but not prey availability, although gut content analysis shows evidence of the inclusion of both plants and prey in their diet. In a manipulative 2X2 factorial field experiment with two levels of plant quality and two levels of prey abundance, *C. spartinae* were more abundant in the high plant quality treatment, but did not respond to variation in prey availability. Their response lead to six times more damage on fertilized compared to unfertilized plots. In a second experiment conducted in the laboratory, *C. spartinae* performed best when they were given the opportunity to include prey in their diet, and their survival and growth decreased when they were confined to plants only. We

discuss the possible explanations for the ability of omnivorous katydids to track plant quality despite the obvious performance benefits of supplementing a plant diet with prey.

Introduction

Omnivores, consumers that eat a mixture of plants and prey, require morphological, physiological and behavioral adaptations to be able to locate and consume resources that differ in abundance, and nutrition (Coll and Guershon 2002, Eubanks et al. 2003). These adaptations have given some omnivores the ability to suppress herbivores to a greater extent than strict predators. This suppression occurs directly through decreasing herbivore numbers through consumption, and indirectly by competing for food plants that the omnivores share with their herbivore prey (Holt and Polis 1997, Diehl and Feissel 2000). Support for this comes from both mathematical models (e.g. Pimm and Lawton 1977, 1978, e.g. 1997, McCann et al. 1998) and experimental studies (McMurtry and Scriven 1966b, 1966a, Eubanks and Denno 1999, 2000). Due to the prevalence of omnivory among insects, with at least one omnivorous species present in 40 insect families belonging to 12 orders (Coll and Guershon 2002), and the marked effects that omnivores can potentially have on population dynamics, it is important to understand the behavioral adaptations required for an omnivore to locate its resources.

To answer questions about the ecological consequences of omnivory, the ability of omnivores to respond to variation in plants and prey must be explored. The variation in these resources has been well established (Kiman and Yeargan 1985, Bjorndal 1991, Milne and Walter 1997), but if omnivores are able to substitute plants for prey and vice versa, then they would be afforded a great deal of ecological flexibility by consuming the

most readily available food at a particular point in time. However, if plants and prey are not substitutable, then these resources may complement each other and be necessary in combination for an omnivore to maintain performance. Therefore they would require the ability to track or find both resources (Eubanks and Denno 1999).

The nutritional necessity of both plants and prey has ramifications for the distribution of the omnivore and for the structure and dynamics of plant and prey communities (Eubanks and Denno 1999). Salt marshes along the Atlantic coast are an ideal system to investigate the differences in food-resource quality and the resulting effects on the distribution and performance of omnivores like *Conocephalus spartinae* (Orthoptera: Tettigoniidae) because host plants in the genus *Spartina* (Poaceae) vary in their nutritional quality over space and time. In addition, herbivores in the genus, *Prokelisia* (Hemiptera: Cicadellidae) are known to outbreak in association with nitrogen-rich plants (Denno and Peterson 2000) creating both spatial and temporal variability in prey abundance.

In this study, we examined the possible effects of variation in plant nutrient levels and prey density on an insect omnivore in a mid-Atlantic salt marsh. To our knowledge this is the first study to explore reasons for the apparent conflict in the variables that drive distribution and those that lead to enhanced performance of omnivores. Our specific objectives were to: (1) Establish patterns of omnivore abundance relative to habitat type, plant nutritional value (nitrogen) and prey abundance; (2) examine the separate and combined effects of plant nutritional content and prey abundance on omnivores, and; (3) determine how differences in both plant quality and prey presence affect omnivore performance.

Methods

Study site and system

Field work was conducted on an intertidal salt marsh on the Eastern shore of Maryland on Chincoteague Bay at the end of Tanhouse Road, Public Landing (38.13°N, 75.30°W). The intertidal marsh in the mid-Atlantic is dominated by perennial cordgrass in the genus *Spartina*. The low and mid-marsh and upland marsh are dominated by *Spartina alterniflora*, but the upland marsh is a mixture of *Spartina alterniflora*, *Spartina patens* and *Distichlis spicata*. Variation in the growth forms of *S. alterniflora* from low to high marsh is due to differences in the physical conditions of the soil, tidal disturbance, nutrient subsidy and litter decay (Teal 1962, Denno 1983, Gallagher et al. 1988). Low marsh *S. alterniflora* tends to be more robust, taller, set more seed, and has a higher nitrogen content than high marsh *S. alterniflora* (Denno 1983, Gallagher et al. 1988).

Conocephalus spartinae (Orthoptera: Tettigoniidae) is the most common omnivore found on mid-Atlantic salt marshes (Vince et al. 1981). In New England, populations of this katydid have large impacts on *Spartina* plant biomass by eating leaves as well as pollen and seeds (Bertness et al. 1987, Bertness and Shumway 1992, Sala et al. 2008). However, Vince et al. (1981) found insect remains in their frass and a carbon isotopic signature that suggested katydids were eating food other than plants. More recently, stoichiometric analysis of *C. spartinae* showed a signal that was intermediate between predators and herbivores (Matsumura et al. 2004).

Planthoppers, *Prokelisia dolus* and *Prokelisia marginata*, are the most abundant and one of the most vulnerable (Matsumura et al. 2004) prey present on *Spartina* along the Atlantic Coast of North America (Denno et al. 1987). Both species are wing dimorphic with macropterous, migratory adults as well as brachypterous, flightless adults (Denno and Peterson 2000). However, a majority of *P. dolus* adults are flightless and thus more sedentary than the highly mobile *P. marginata*, a large proportion of which are macropterous and thus more adept at tracking plant quality (Denno et al. 1996). Outbreaks of both species occur in association with nitrogen-rich plants (Denno and Peterson 2000). Due to the abundance and vulnerability of planthoppers, it is likely that they are a primary prey item of *C. spartinae*. Evidence for this was found both in gut content analysis of field-collected katydids (Pearson unpublished data) and the ability of katydids to readily eat planthoppers when presented with them.

Field survey

A field survey was conducted to determine the distribution of katydids in response to plant quality and prey abundance across a diversity of marsh habitats. Replicated transects (n=3) were established that traversed three habitats (low marsh, mid marsh and high marsh); these habitats vary in plant quality and prey abundance. Three sample sites (each separated by at least 10 m) were established along each transect with one site in each of the three habitats, and sampled in August 2005 when prey (e.g., planthoppers) were abundant. Each site was sampled twice in the opposite direction using two different sampling techniques to measure the abundance of both *Prokelisia* and *C. spartinae*. All vacuum samples were taken in the southerly direction from a pre-determined sample

point by setting the head of a D-Vac® suction sampler (Rincon-Vitova, Ventura, California, USA) down on the marsh surface 10 times for 5 seconds each time; this method has been successfully used in previous studies to estimate small arthropod densities on the marsh (Denno et al. 2002). Sweep samples (10 sweeps with a 38cm diameter net) were also taken from the same pre-determined sample point in the northerly direction to determine the number of larger katydids. The arthropods found in both sampling techniques were totaled to determine a composite estimate.

Plant quality (%N) was determined from samples taken at each sample site (see Denno et al. 2002). In addition, six katydids from each sample site were dissected and scored for the presence of plant and arthropod parts in their gut to confirm that *C. spartinae* are consuming both plants and arthropods in the field. To test whether omnivore densities differed among habitats in response to planthopper density and plant quality, a general linear model (SAS: Proc GLM) was conducted with *C. spartinae* density as the response variable and planthopper density, *Spartina* %N, habitat (low marsh, mid marsh, or high marsh) and %N by location interaction as the predictor variables. The best model with the lowest number of predictor variables was chosen using the Akaike Information Criterion.

Field experiment

A field experiment was conducted to determine the numerical response of katydids to plant quality (%N and C:N) and planthopper abundance. The effects of plant quality and planthopper abundance on habitat selection by katydids was determined using a 2x2

factorial design with plant quality (high vs. low) and planthopper abundance (supplemented vs. removed) as factors in a randomized complete block design that was replicated ten times (n=40 total plots). Each plot was 2m² and was located 1.5m from the other 3 plots in the block; different blocks were located at least 5m apart. It was assumed that katydid densities were the same in all of the plots at the start of the experiment.

High and low plant quality treatments were achieved by differential fertilization. High quality plants were fertilized with 45gN/m² in the form of ammonium nitrate, 15gP/m² in the form of triple super phosphate and low quality plants were left unfertilized (see Denno et al. 2002). Fertilizer was added to the high-quality plots once at the end of May and a second time at the end of June. Prey treatments (supplemented vs. removed) were imposed at the beginning of July when the majority of the planthoppers were in the nymphal stage, thus minimizing planthopper dispersal among plots. Planthoppers were collected from *Spartina alterniflora* using a D-Vac and added to prey-supplemented plots twice. To mimic possible disturbance from the D-Vac heads, plots with planthopper addition treatments were agitated using the D-Vac hose head without the vacuum. The prey removal treatment was applied on the other half of the plots by repeatedly setting the head of a D-Vac suction sampler onto the marsh surface within the entire plot. In this way we did not expect to remove all of the planthoppers, but instead we aimed to create a density difference between the removal and the supplemented plots. In the prey removal treatments, all katydids collected with the D-Vac were returned to the plots.

To verify that the fertilizer treatment enhanced plant quality, plants were sampled at the end of the experiment with quadrats to determine nitrogen content (see Denno et al. 2002) and percent katydid damage ($100 \times \text{damaged \#culms} / \text{total \#culms}$). All plots were

defaunated on 20 July 2006 first with a sweep net and then by D-Vac, to assess the density of katydids and arthropod prey.

To assess the direct and interactive effects of plant quality and prey density on katydid abundance and to verify the effectiveness of fertilization on grass nitrogen and the prey enhancement and removal treatments on planthopper density, data were analyzed using ANOVA (SAS: Proc Mixed). To determine the extent to which treatment had an effect on percent katydid damage to *Spartina*, data were first square root transformed to meet the assumptions of homogeneity of variance for ANOVA (SAS: Proc Mixed).

Laboratory experiment

To determine the effects of host plant quality and prey presence on the survival and performance of an omnivorous katydid, a manipulative laboratory experiment (Randomized complete block, 2x2 factorial design) was conducted. Two levels of *Spartina* quality (high or low) were crossed with prey (*Prokelisia dolus* late instar nymphs) presence or absence. Each treatment combination was replicated 19 times. *Spartina* plants were started from seed (Environmental Concern, Inc., St. Michaels, MD) in sterilized sand in the greenhouse during April of 2003. Potted *Spartina* plants were grown in flats (80 pots per flat) and fertilized every three weeks from May through July. Fertilizer was applied as a combination of ammonium nitrate and triple super phosphate at two levels (3g nitrogen, 1g phosphorus/ flat/ application or 12g nitrogen, 5g phosphorus/ flat/ application). At the start of the experiment, the potted *Spartina* were transferred to the laboratory and placed into water-filled tubs under greenhouse grow-lights. Plants were thinned to three culms per pot which constituted one experimental

unit. A clear plastic tube cage (diameter 8cm and approximate height 12.5cm) with four mesh-covered holes and a mesh top was placed over each pot and used to contain the katydids and prey. *P. dolus* nymphs were collected in the field with a D-Vac® insect suction sampler and then transported on plants back to the lab. To ensure that katydids had a continuous source of prey, 25 late-instar planthopper nymphs were added to the prey-supplemented treatment cages twice weekly, using an aspirator. Katydids were collected in the field with a sweep net and transported back to the laboratory in individual containers with a water source. Each experimental cage contained a single katydid nymph that was randomly assigned to one of the four treatments and monitored every 3-4 days throughout the 35 day experiment to determine the effects of the diet treatments on survivorship and growth.

The effect of host plant quality and prey presence on katydid survival was analyzed using the non-parametric log-rank test (SAS: Proc Lifetest) in which the data could be right-censored to account for the individuals that did not die by the time the experiment ended. Relative growth rate $(\text{mass}_{\text{final}} - \text{mass}_{\text{initial}}) / (\text{mass}_{\text{initial}} * \text{time}^{-1})$ was calculated to correct for differences in starting mass as well as differences in the amount of time each individual was in the experiment. ANOVA (multi-factorial general linear model incorporating Tukey-Kramer's pairwise mean comparison) was used to determine the direct and interactive effects of host plant quality and prey abundance on katydid total relative growth rate.

Results

Field survey

When katydids were surveyed in the field across three different marsh habitats from low marsh to high marsh, there was a significant effect of %N of *Spartina* ($F=69.68$, $P=0.004$), marsh location ($F=17.37$, $P=0.022$), and the interaction of marsh location and %N ($F=20.60$, $P=0.018$; Figure 1), but there was not a significant effect of planthopper density ($F=3.27$, $P=0.168$) on katydid numbers. Gut content analysis showed that a higher percentage of katydids collected across the marsh had arthropod parts in their guts ($88.46\% \pm 0.05$ SE) as opposed to plant parts ($53.846\% \pm 0.069$ SE).

Field experiment

Planthopper density was greater in plots augmented with planthoppers than it was in plots where planthoppers were removed ($F_{1,36}=41.27$, $p<0.0001$; Figure 2A) and did not differ between fertilizer treatments ($F_{1,36}=0.40$, $P=0.532$). Fertilization increased the nitrogen content (%) of *Spartina* ($F_{1,36}=98.48$, $P<0.0001$; Figure 2B). Although increasing plant quality had a positive effect on katydid abundance ($F_{1,36}=4.76$, $P=0.036$, Figure 3), enhancing the density of planthoppers did not change katydid abundance ($F_{1,36}=1.84$, $P=0.184$), and there was no interactive effect of plant quality and planthopper abundance on katydid abundance ($F_{1,36}=0.90$, $P=0.768$; Figure 3). Pairwise mean comparisons showed a difference between densities of katydids in the plots with a combination of high planthopper density and high plant quality compared to plots with low planthopper densities and low plant quality ($t=2.50$, $P=0.017$). In addition, *C. spartinae* inflicted

more damage to plants in fertilized plots ($37.57\% \pm 4.83$ SE) compared to the unfertilized plots ($6.87\% \pm 2.18$ SE; $F_{1,37}=49.05$, $P<0.0001$) whereas planthopper density had no effect on katydid damage ($F_{1,37}=0.11$, $P=0.740$, Figure 4).

Laboratory experiment

The nitrogen content (%) of the *Spartina* differed for the plants grown with low levels of fertilizer compared to those that received higher quantities ($F_{1,13}=18.19$ $P=0.0009$; Figure 5). The rank tests indicate that there was a positive effect of prey presence ($P<0.0001$ for the log-rank test) and no effect of plant quality (fertilization) on katydid survivorship ($P=0.385$; Figure 6). Similarly, prey supplementation enhanced the relative growth rate of katydids ($F_{1,34}=16.17$, $P=0.0003$; Figure 7), but there was no effect of plant quality ($F_{1,34}=2.55$, $P=0.120$) nor an interactive effect between prey presence and plant quality ($F_{1,34}=0.35$, $P=0.552$). Although there was no main effect of plant quality on katydid growth rate, pairwise means comparisons show a reduction in katydid growth in both treatment levels where prey were absent, regardless of plant quality, compared to the high fertilizer treatment where prey were present (high fertilizer: $t=3.97$, $P=0.0004$; low fertilizer $t=3.26$, $P=0.003$; Figure 7).

Discussion

For omnivores, such as katydids, variation in the quality and availability of both plant and prey resources affect their distribution and their performance. We found that the natural distribution of an omnivore, *C. spartinae*, is affected by the quality of its host plant,

different marsh habitats (low, mid, and high marsh) and the interaction of plant quality and habitat (Figure 1). However, the effects of plant and prey resources are difficult to decouple because high quality plants are often correlated with greater herbivore densities. Thus in a manipulative field experiment where we were able to separate the effects of plant quality and prey abundance (Figure 2) we found that *C. spartinae* aggregate in areas of high plant quality irrespective of prey abundance (Figure 3). As a result of this aggregation, *Spartina* incurred substantial damage in the high quality plant plots (Figure 4). Despite the lack of an effect of prey abundance on katydid distribution in the field, we found that lab reared katydids required planthoppers to maintain survival (Figure 6) showing an average increase in survival of 74% when planthoppers were available. Katydid growth was also significantly enhanced by prey presence with individuals in the high plant quality, prey present treatment having a significantly higher growth rate than those in the prey absent treatments (Figure 7).

The pattern of *C. spartinae* abundance observed in the field relative to high quality plants concurs with previous studies. For example, the distribution pattern of omnivorous mites in apple orchards is influenced by the distribution of pollen (Addison et al. 2000) and minute pirate bugs are attracted to flowering plants and artificial nectar (Reid and Lampman 1989, Evans and Swallow 1993, Coll 1996). Similarly, the population dynamics of big-eyed bugs is tied to the presence of fruit on plants (Eubanks and Denno 1999) leading to persistence in fields with high quality resources in the absence of prey. There are multiple explanations for these patterns of omnivore, and more specifically, katydid distribution relative to plants. The first explanation could be that katydids are found in greater numbers in areas of high plant quality because they are

only eating plants and not taking advantage of the prey resource. However, the gut contents of field caught katydids reveal that they eat both plants and prey, so we can dismiss this argument. A second reason for uneven katydid distribution could be that they track prey by tracking plant quality and finally, they may not track prey because they can readily eat a mixed plant and prey diet irrespective of prey density.

The second possible explanation for the distribution of katydids relative to high quality plants may be that by tracking the more predictable plant resource, katydids are able to acquire prey (Coll and Guershon 2002). By finding high quality plants, omnivores can take advantage of a nutritious resource thus fulfilling some of their nutritional requirements (Coll 1996, 1998, Eubanks and Denno 1999, Coll and Guershon 2002). For katydids, evidence suggests that they are consuming the fertilized plants due to the high incidence of damage in the fertilized plots compared to the unfertilized plots. Notably, not only are omnivores like katydids attracted to high quality plants, but insect herbivores also respond positively to plant variation by aggregating in areas of high host-plant quality (Scriber and Slansky 1981, Slansky and Rodriguez 1987, Bernays and Chapman 1994, Raubenheimer and Tucker 1997, Schoonhoven et al. 1998, Denno et al. 2002). When we separated the effects of plant quality, prey abundance, and habitat, we found that katydid numbers were highest in plots that had been fertilized irrespective of prey density. Therefore, it is highly likely that katydids are actively choosing areas of high plant quality and they are not attracted to high prey density.

We have not ruled out the possibility that katydids track prey abundance only on small spatial scales within high quality plant habitats. Examples of consumers using the occurrence of high value plant parts to find prey can be found amongst predators. Both

spider and assassin bug abundance could be predicted by the number of flowers per bean plant in a managed habitat due to the higher densities of prey attracted to the flowers (Eubanks and Denno 1999). Similarly, crab spiders were positively associated with the high nectar producing inflorescences of milkweed that attracted greater numbers of prey compared to low quality, low nectar producing inflorescences (Morse and Fritz 1982). Thus like predators, through their ability to track plant quality, *C. spartinae* may be able to simultaneously take advantage of planthoppers that also occur at high densities in areas of high plant quality (Denno 1983).

The final, and most likely, explanation for the greater numbers of katydids associated with higher plant quality may be that katydids can alternate between plant and prey feeding irrespective of prey density. For intraguild omnivores that feed on several different prey, relative consumption is thought to be based on relative size and abundance of the prey (Warren and Lawton 1987, Diehl 1993). However, in a system like the salt marsh where omnivorous katydids feed on plants and prey, there is a large biomass difference in the plants relative to the prey so while planthoppers are the most abundant herbivore on the marsh, their relative biomass compared to *Spartina* is low. Thus, because high quality plants are not in short supply, it is not likely that plant abundance will affect katydid switching between plants and prey (Coll 1996, Coll and Guershon 2002). Furthermore, katydids had a greater incidence of prey parts in their gut relative to plant parts irrespective of plant quality and prey abundance, suggesting that although katydids are more abundant in areas of high plant quality and are not tracking planthopper prey, they are managing to obtain nutritional resources from both.

The necessity for *C. spartinae* to include prey resources in addition to plants in their diet was demonstrated by their enhanced survival and growth when they were offered both. Thus performance results suggest that although katydids are seemingly unable to track prey abundance, they do require prey. Furthermore we know from nutritional studies on *C. spartinae* (Chapter 1) that katydids are unable to survive on high protein diets, requiring sources of energy rich nutrients (carbohydrates or lipids) in addition to protein. In a review of omnivorous heteropterans, it was found that by supplementing a prey diet with plants their development was accelerated, longevity of both nymphs and adults increased and there was enhanced fecundity (Coll 1998). A subsequent review of insects by Eubanks and Styrsky (2005) also found that in 36 out of 50 cases, supplementing prey with plant food resulted in faster development of immatures. Therefore, evidence points to the nutritional complementarity of plants (which are more carbohydrate-rich) and prey (which are more protein-rich) as resources for *C. spartinae* and omnivores in general.

Here we aimed to elucidate the effects of plants and prey on the distribution and performance of the omnivorous katydid *C. spartinae* in a variable landscape. Determining how food resources interact to affect performance is critical to predicting the impact of omnivores on plants and prey, their spatial distribution in the field, and ultimately their role in food-web dynamics. Over time plants change in quality and prey change in abundance. However the changes in prey abundance are irrelevant if katydids are able to track plant quality irrespective of prey density. Thus katydids are able to maintain a high level of performance by eating a complementary diet of both plants and prey, thereby fulfilling their nutritional requirements

Figures

Figure 1: The interactive effect of plant quality and marsh habitat on *C. spartinae* number. Mean percent nitrogen (\pm SE) is noted above the bars. Means (\pm SE) with different letters are significantly different ($P < 0.05$).

Figure 2: The effect of prey and fertilizer regime on A) planthopper density and B) %N of *S. alterniflora* in the field experiment. Means (\pm SE) with different letters are significantly different ($P < 0.05$).

Figure 3: Field population densities of *C. spartinae* as a result of differential prey densities (planthoppers added or removed) and plant quality (fertilized or control). Letters above the means (\pm SE) indicate significant differences ($P < 0.05$).

Figure 4: The percent culms per quadrat that were damaged by *C. spartinae* in the different treatments. Bars represent means (\pm SE) and those with different letters are significantly different ($P < 0.05$).

Figure 5: The effect of the two different fertilizer regimes on *S. alterniflora* percent nitrogen in the laboratory. Means (\pm SE) with different letters are significantly different ($P < 0.05$).

Figure 6: The effect of plant quality (high vs. low nitrogen fertilization) and prey availability (planthoppers present or absent) on *C. spartinae* survivorship in the laboratory.

Figure 7: The effect of plant quality (high vs. low nitrogen fertilization) and prey availability (planthoppers present or absent) on *C. spartinae* total relative growth rate in the laboratory experiment. Means (\pm SE) with different letters are significantly different ($P < 0.05$).

Figure 1

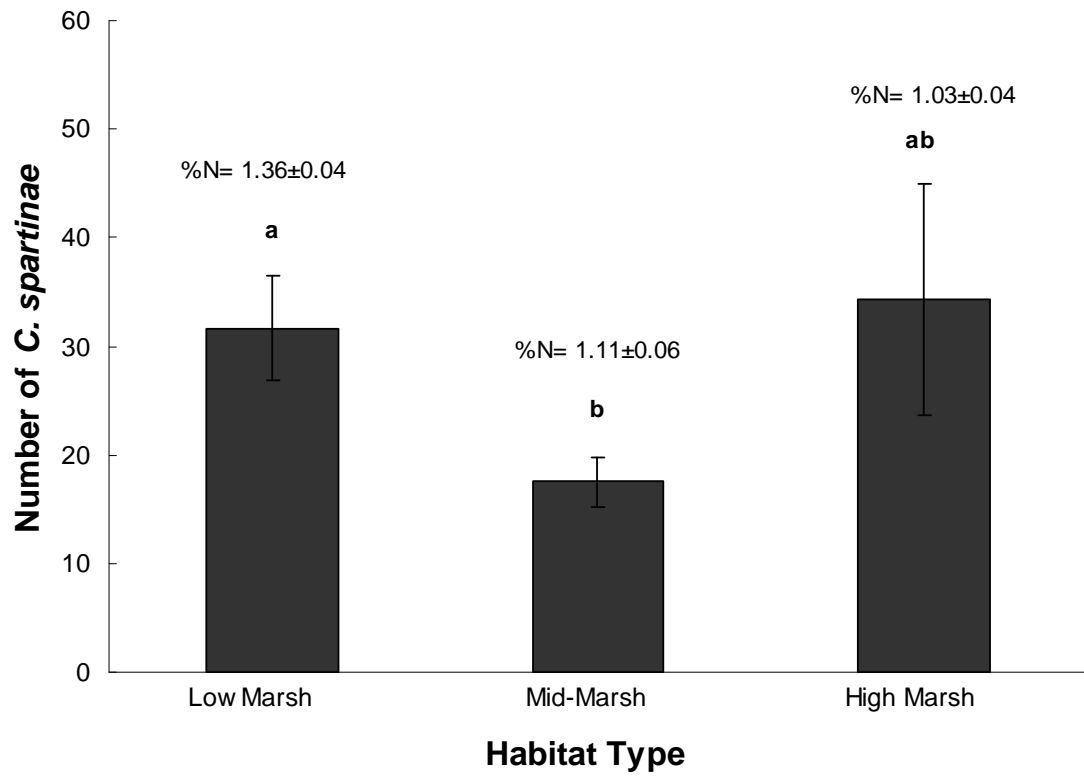


Figure 2

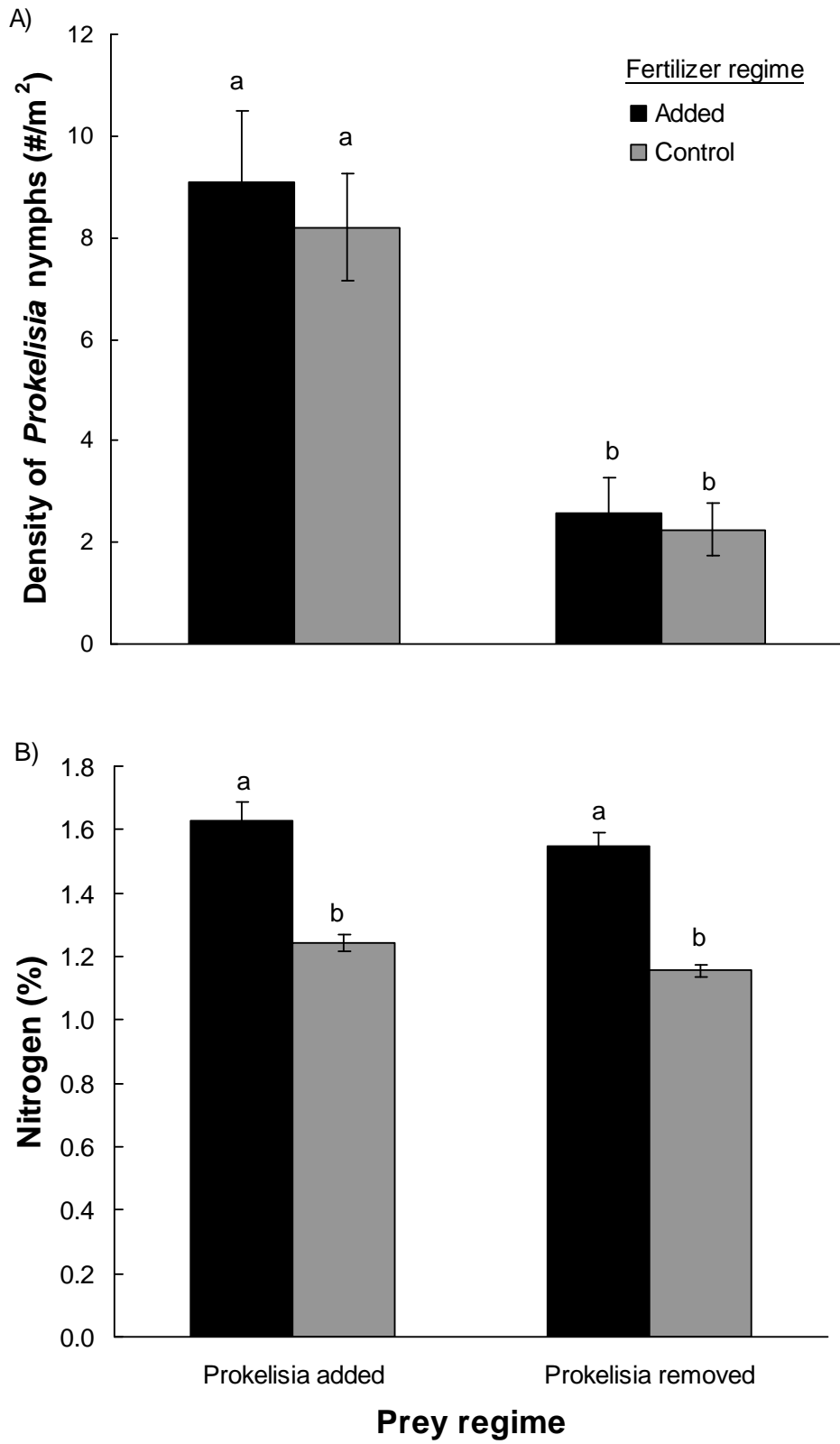


Figure 3

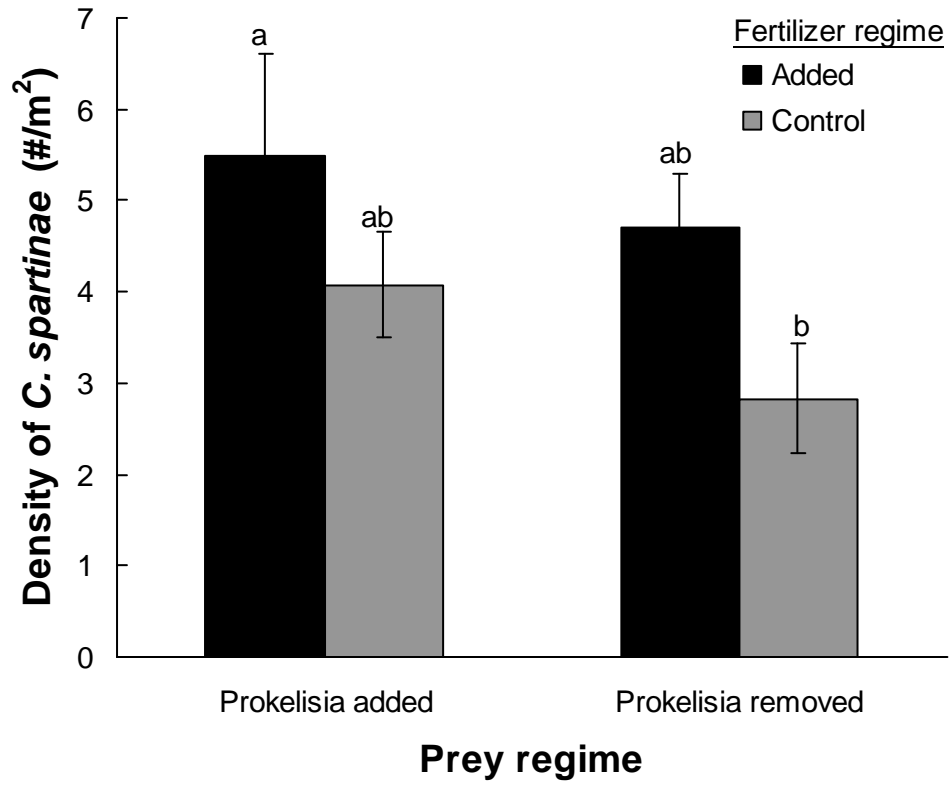


Figure 4

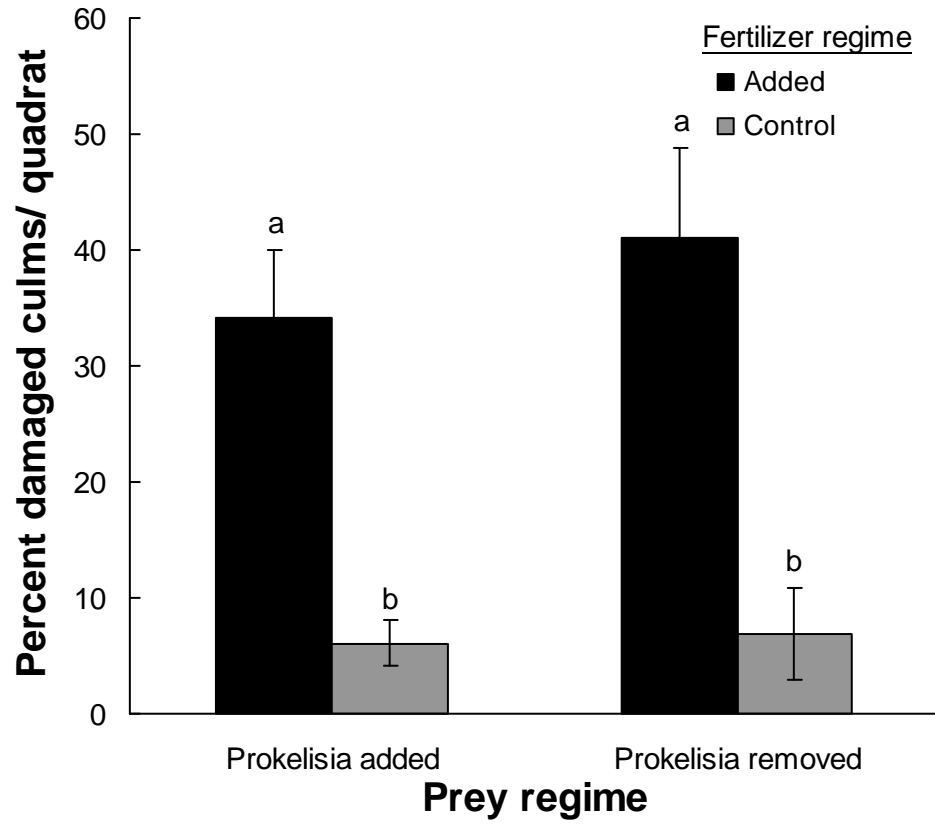


Figure 5

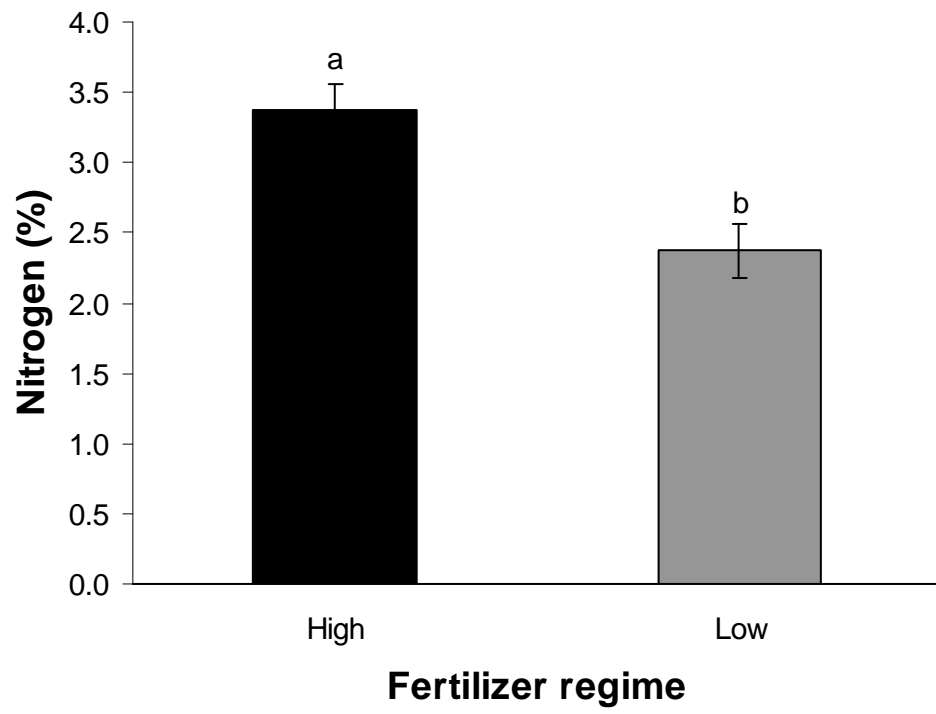


Figure 6

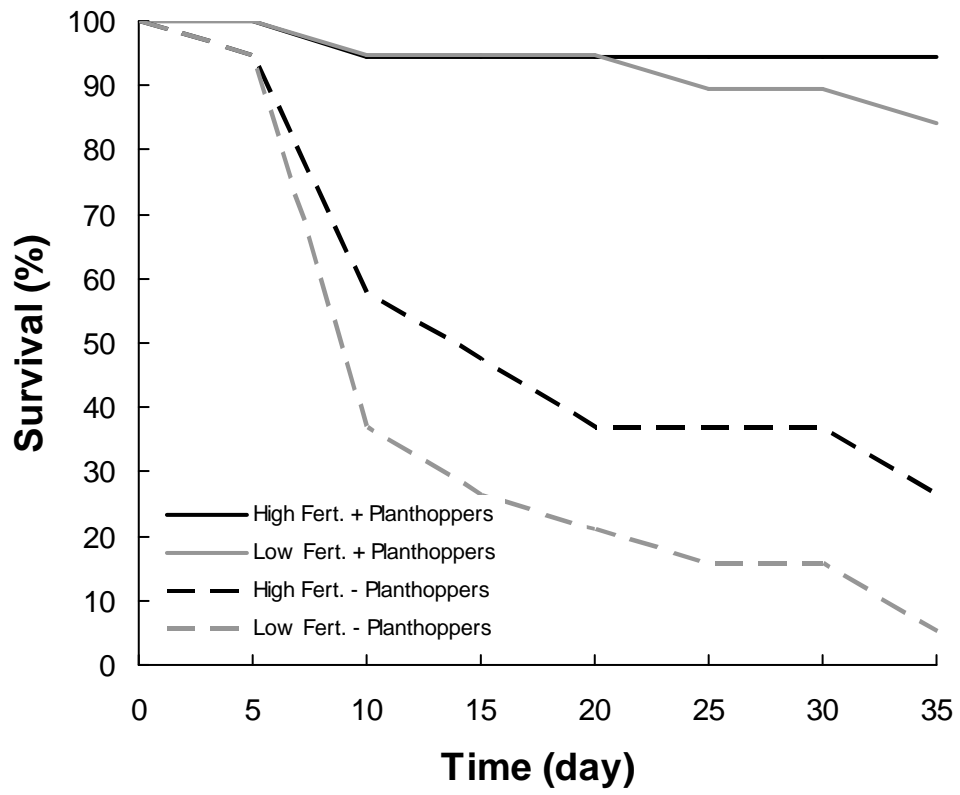
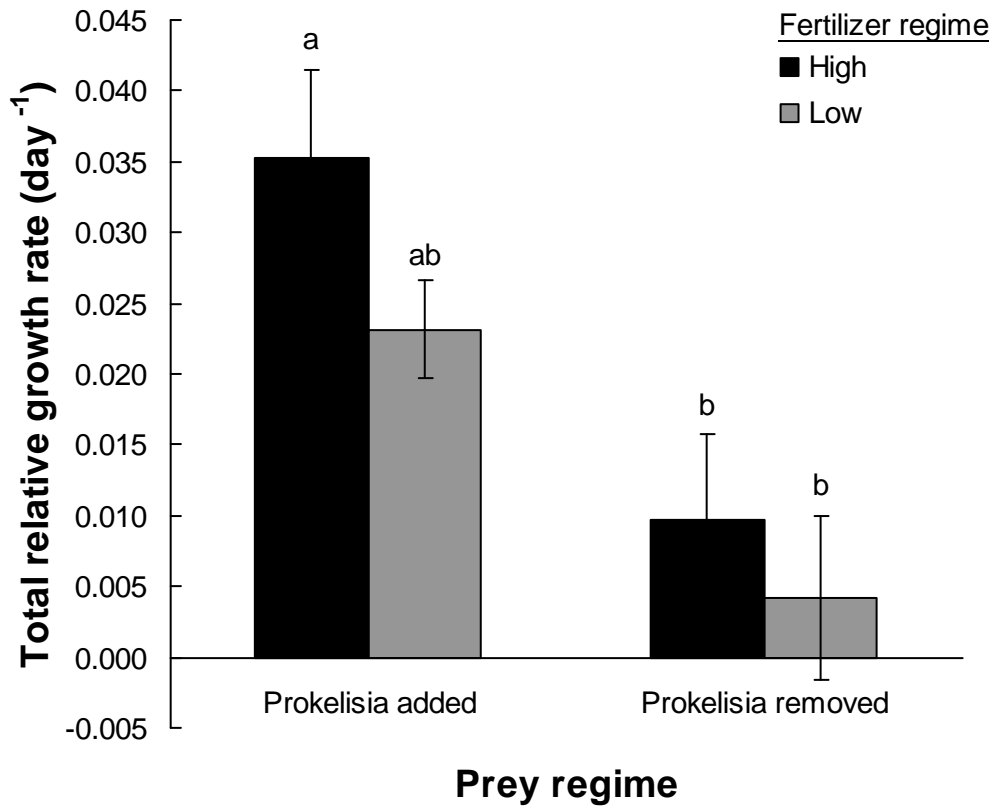


Figure 7



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