The Effect of Short-Term Nutritional Intake on the Reproduction of Male Black-Horned Tree Crickets, *Oecanthus nigricornis*

By
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April 4, 2011



Allegheny College

Department of Biology

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

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Pledge

Approval by the Senior Thesis Committee:

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Dr. Mumme

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Dr. Ostrofsky

Abstract

The fitness and life history of an animal depends on the allocation of resources. Many studies have focused on resource allocation with females but few have examined its effects on reproduction in males. Reproduction can depend on how often an organism takes in food, and whether the organism is a capital breeder that relies on stored reserves or income breeder that relies on continuous intake of resources. Feeding effects on male *Oecanthus nigricornis* reproduction were investigated with the expectations that they are income breeders. This species is of interest due to the male investing nutrients to the female during mating by metanotal gland secretions. Adult male *O. nigricornis* were collected and fed with glucose, a low-quality diet of apples, or a high-quality diet of cricket food for 7 days. Females were collected and fed the high-quality diet for 7 days. Males were mated every other day with a random female for 10 days. Mating behavior was observed by recording courtship song frequency and observing gland feeding and copulation. The high-quality males were shown to have a slightly positive increase in body mass compared to low-quality and glucose males that had a slight negative change in body mass. There was no significant effect found on the average song rate, the total glandular feeding time during copulation, or in the overall mating success found in males despite the decreasing trend found in between mass and diet. My results indicate that black-horned tree crickets are not income breeders and can sustain high mating success even on low-quality diets.

Introduction

Understanding the role of nutrition is essential to understanding life histories of organisms. The allocation of resources is an imperative issue in organisms and may indicate trade-offs in life histories of animals. Absence of a nutrient may lead the organism to reallocate resources to differing functions, leaving other nutrients to make up for the loss (Colasurdo et al. 2009) and may be costly. Different strategies for each organism, such as feeding habits, lead to different end results that may negatively or positively affect life history. The lack of a nutrient may result in an organism allocating its resources to survival rather than to reproduction which would alter the fitness of the individual.

The quality and quantity of nutrients used in egg production by animals can be acquired by juvenile feeding, adult feeding, and nuptial gifts given to the female during mating (Boggs 1997). Allocating nutritional resources can be investigated in the feeding habits of animals based upon capital or income breeding according to their needs. Capital breeders acquire nutrients as larvae and use them throughout life, while income breeders continually acquire nutrients throughout their life to be used towards reproduction. The frog, *Rana temporaria,* is classified as an income breeder. However, due to the possibility that eggs may not be spawned and may turn into energy stores, *R. temporaria* could potentially use the storage at a later time as capital breeders (Lardner and Loman 2003). Many Lepidoptera, like *Malacosoma disstria*, are capital breeders and consistently select the appropriate ratios of food as larvae to be stored for future use (Colasurdo et al. 2009). However, another Lepidoptera*, Biyclus anynana,* is an income breeder. This fruit-feeding

butterfly depends on an adult fruit diet for female egg laying in reproduction. Initially females starved for 1-2 days immediately began laying eggs, indicating adult food is metabolized rapidly into egg production for income breeders (Fischer et al. 2004). Clearly variations occur in whether organisms are income or capital breeders that may make simple classification difficult.

Income versus capital breeding in the black-horned tree cricket *Oecanthus nigricornis* male life history strategies can be examined through reproduction. Black-horned tree crickets are found in southern Canada and the northern USA. Adults emerge mid-August and mate until late October when they die off due to the approaching winter. Males attract females over short distances with the odors from the dorsal metanotal gland, through a calling song lasting about 30 seconds, and through vibrations called tribulations (Brown 1997; Brown 2008). The female mounts the male from the rear after the male backs up into her and then feeds on his nuptial gift of glandular secretions from the metanotal gland. Copulation occurs when both the female and male bend their genitalia to meet while the female continues to feed on his glandular secretions. Metanotal courtship glandular feeding by females is necessary for female reproduction in *O. nigricornis* (Brown 2008). Since females require nutrition for reproduction, it is possible males also require a meal for reproduction, making them income breeders.

Courtship feeding is beneficial to the female by increasing the female egg production and reproductive lifespan of the female which ultimately increases the male fitness (Brown 2010). This continual allocation from the metanotal gland allows for the insemination of the

female as well as ensures adequate time before the female consumes the spermatophore. Due to the costs of reproduction by the male, the condition of the male may affect his success at mating and ensuring fertilization.

Female courtship behavior is rather well-known but few have investigated male mating behavior and nutrition. I examined courtship and mating behavior of male *O. nigricornis* in response to three different nutritional treatments. *O. nigricornis* are an ideal model system for sexual selection studies because males offer a nuptial gift. A short life span, loud acoustic system, and ability to manipulate diet make the cricket a good model system for this study (Brown 2008). The calling song and male body size are both important factors in the mating behavior of black-horned tree crickets. In other species of crickets, *Gryllus capestris* calling song (Scheuber et al. 2003) and *Gryllus veletis* body mass (Burpee and Sakaluk 1993) decreased when food-deprived, indicating both respond to nutritional conditions.

Understanding the relationship between reproduction and nutrition on a finer scale is necessary to identify whether stored or current resources are more important to *O. nigricornis* reproductive patterns. I hypothesized that crickets fed a high-quality diet would retain reproductive capability, while crickets fed a low-quality diet should decrease in mating behavior. The objective of this study is to determine how diet affects male *O. nigricornis* reproductive behaviors and to determine if they are income breeders.

Materials and Methods

Collecting and Feeding Crickets

About 45 adult *O. nigricornis* were collected between 25 August and 27 August 2010 from goldenrod fields around Allegheny College. Male black-horned tree crickets can be identified by a distinct dorsal gland or depression on the metanotum. Males were randomly separated into three groups of 10 to 15 and placed into individual 500 mL plastic containers with netting. Females were housed in a separate 36 L fish tank. They were housed by a window in a room receiving a natural photoperiod at room temperature. Males were treated for 7 days prior to the onset of mating trials. Males in treatment 1 were given no food for 7 days but a 10% solution of glucose in a water vial. Treatment 2 males were fed a low-quality diet of a slice of apple for 7 days. Treatment 3 males were fed a high-quality diet of cricket food, apples, oatmeal and bee pollen for 7 days. Females were fed cricket food, apples, oatmeal, and bee pollen. Water was sprayed on the crickets and food was provided ad libitum.

Matings

Adults were mated in a 500 ml. plastic jar with netting after 7 days of treatment. Male crickets were randomly paired with females 5 times, once every other day for 10 days (Table 1). Each trial lasted a minimum of 20 minutes or until all mating behaviors ceased for 5 minutes. Each female was randomly used once until all the females participated in a trial and then randomly cycled through as needed. Multiple matings took place to determine if the mating success declines over time. For each interaction, 1.) courtship and song rate were recorded by observing to see if the male backs into the female and recording the song

frequency. 2.) Mating success and how long it took to mate were recorded by watching mating behavior and the number of times the female mounted the male. Spermatophore transfer and time was recorded. 3.) Duration of glandular feeding behavior was recorded by timing how long the female mouthparts were feeding from the metanotal gland. When the female dismounted timing would stop until she remounted. The individual mounts in one mating period added together made the total time feeding. Males were weighed before diet treatments began and after the fifth mating.

Table 1: Mating Schedule. Five of the 10 crickets were mated on the first day while the second 5 crickets were mated on the second day. Crickets had the opportunity to mate once every 48 hours for a total of 5 mating chances with the first mating on September 4, 2010 and the last mating on September 14, 2010.

	Cricket Chow	**Apple**	**Glucose**
Day 1-Mating 1	First 5 crickets	First 5 crickets	First 5 crickets
Day 2	Second 5 crickets	Second 5 crickets	Second 5 crickets
Day 3-Mating 2	First 5 crickets	First 5 crickets	First 5 crickets
Day 4	Second 5 crickets	Second 5 crickets	Second 5 crickets
Day 5-Mating 3	First 5 crickets	First 5 crickets	First 5 crickets
Day 6	Second 5 crickets	Second 5 crickets	Second 5 crickets
Day 7-Mating 4	First 5 crickets	First 5 crickets	First 5 crickets
Day 8	Second 5 crickets	Second 5 crickets	Second 5 crickets
Day 9-Mating 5	First 5 crickets	First 5 crickets	First 5 crickets
Day 10	Second 5 crickets	Second 5 crickets	Second 5 crickets

Statistical Analysis

Statistical analysis was performed by an ANOVA test comparing the three diet treatments. Statview 5.0 was used for all statistical procedures.

Results

Change in Body Mass

Although there was no statistically significant effect of diet on change in body mass (P=0.19), there was a general trend that the high-quality crickets increased in weight while the other two treatments decreased in weight (Figure 1).

Song Rate

Diet did not appear to have any effect on the song rate of male crickets. The average song rate was highest among the high-quality diet males but was not significantly higher (P= 0.2290) than the low-quality and glucose diet males (Figure 2).

Glandular Feeding

Diet did not have an effect on the total duration of glandular feeding. Females fed from the high-quality crickets and glucose males for very similar periods of time while (P=0.4763) females fed from males of the low-quality diet for slightly less time (Figure 3). These results are not significant as there was not much difference in the period of feeding between the treatments.

Overall Mating Success

Diet did not have an effect on the overall success of mating. Although there was not a significant difference between treatments (Figure 4), most success was found in the cricket chow treatment and the least was in the apple treatment (P=0.1394).

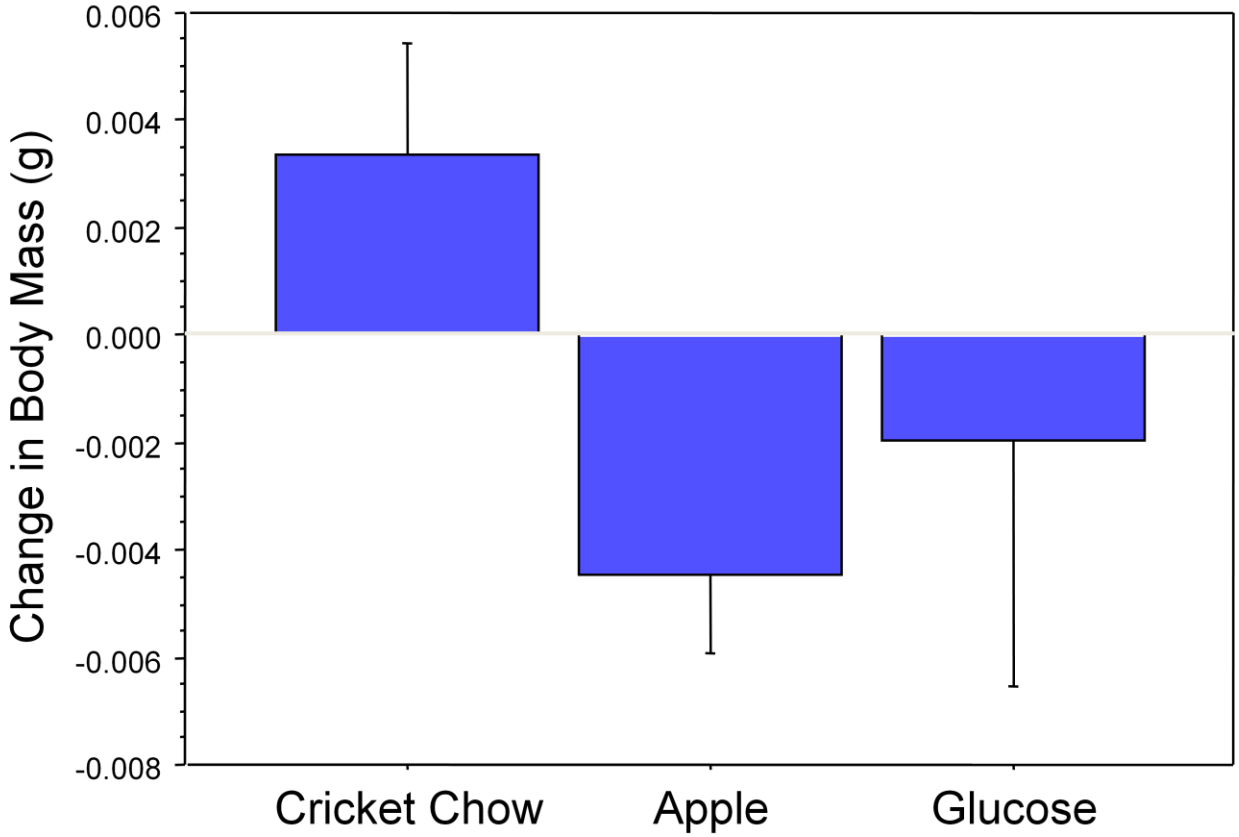

Figure 1: The effect of diet treatments on male mass. Males were massed before treatment and after the last mating. Error bars represent the standard error of the mean.

Table 1: ANOVA table for change in mass

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Diet Treatment	2	0.0003	0.0002	1.7575	0.1916
Residual	27	0.0025	0.0001		

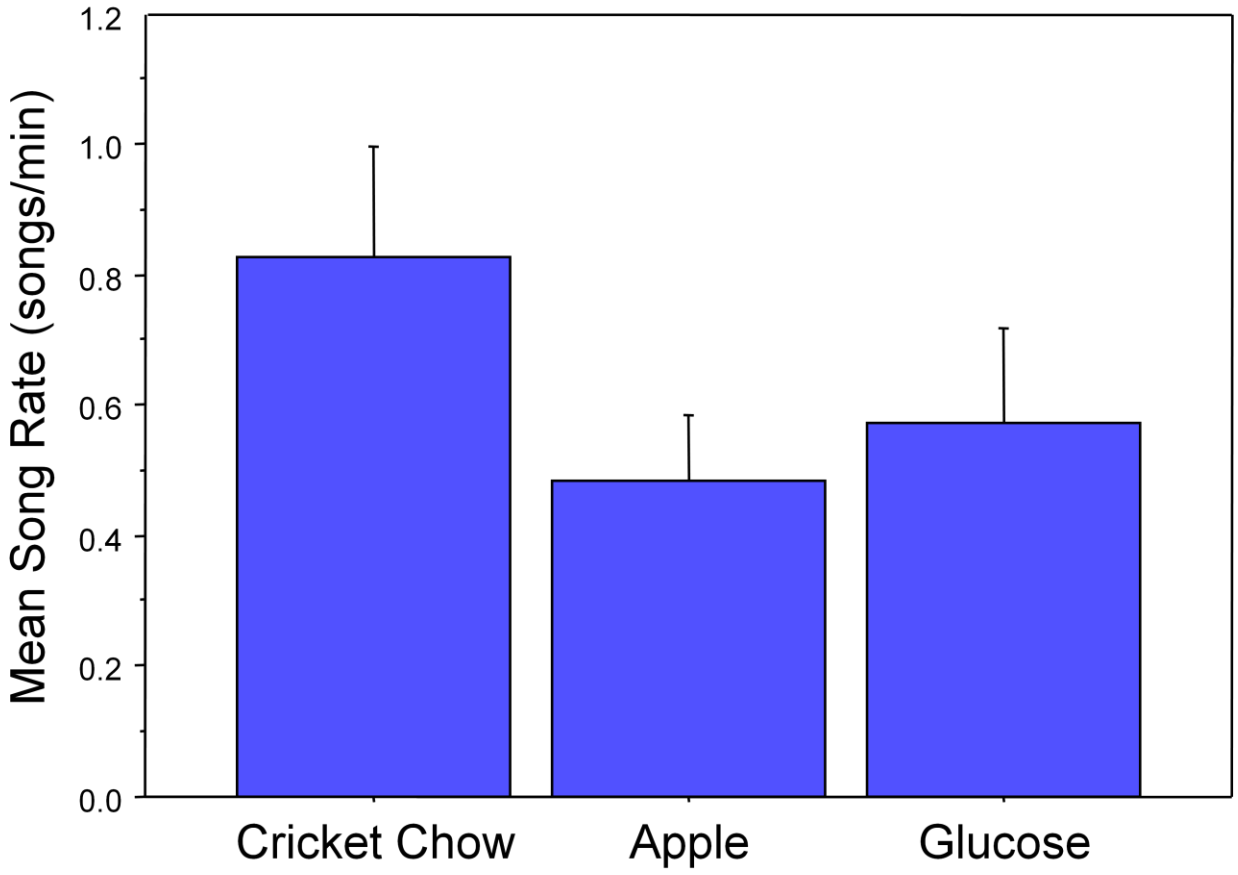

Figure 2: Average song rate of male *Oecanthus nigricornis* treated with a diet of glucose, apple, and cricket chow. Song rate was calculated by counting the number of songs during the mating period. Error bars represent the standard error of the mean.

Table 2: ANOVA table for mean song rate

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Diet Treatment	2	0.6349	0.3174	1.5576	0.2290
Residual	27	5.5027	0.2038		

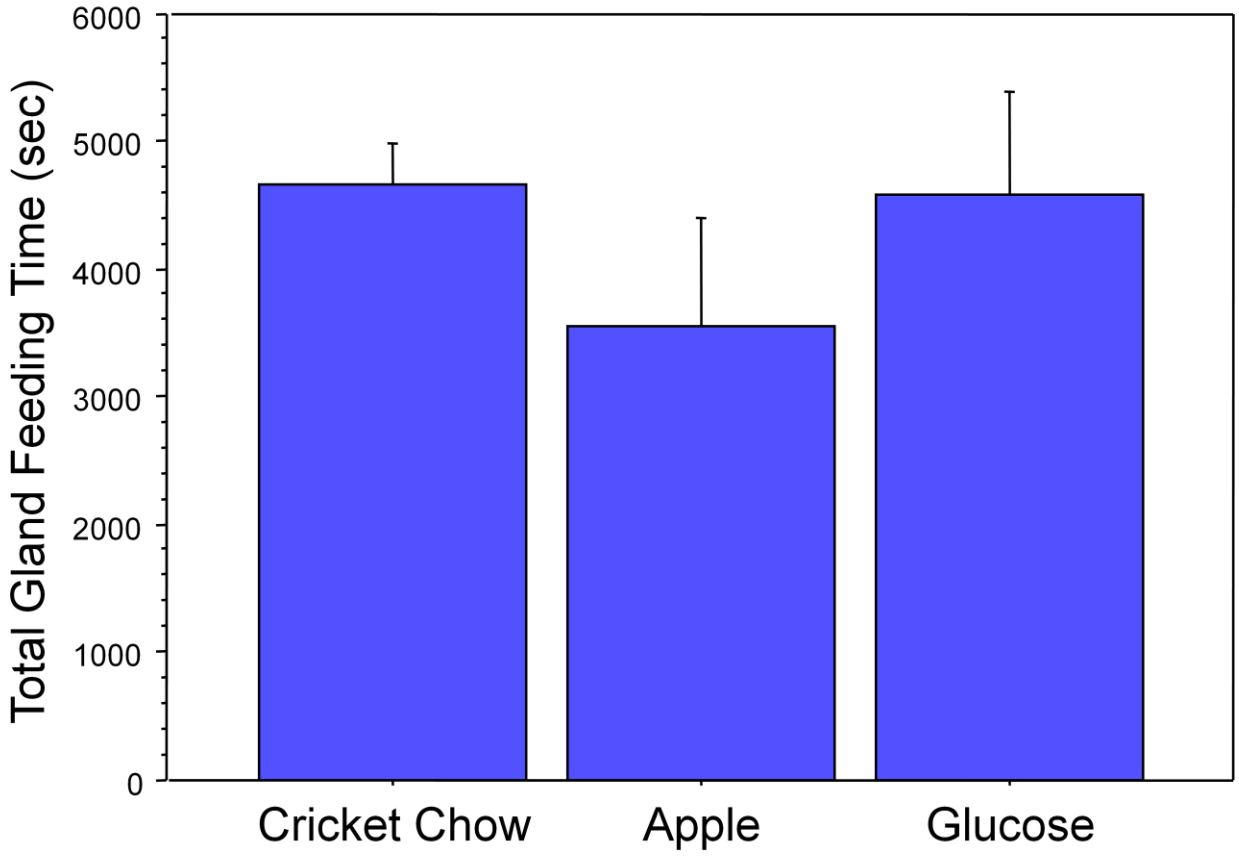

Figure 3: **Total duration of gland feeding** during mating behavior. Males were timed for the how long the female had her mouth parts on the male metanotal gland. Time was stopped when the female dismounted and added for each mount to make total glandular feeding during mating. Error bars represent the standard error of the mean.

Table 3: ANOVA table for total gland feeding

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Diet Treatment	2	7596154.0667	3798077.0333	0.7624	0.4763
Residual	27	134511042.9000	4981890.4778		

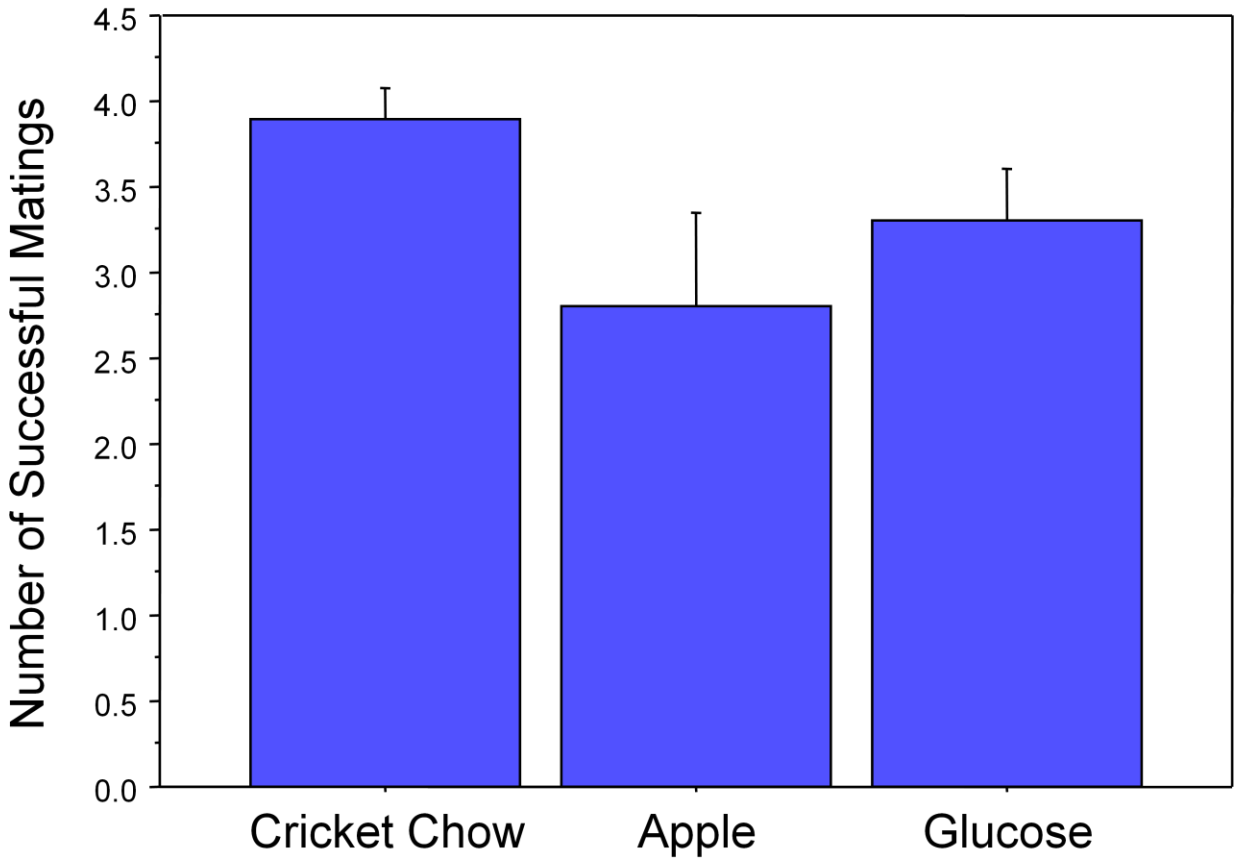

Figure 4: The average number of successful matings. A successful mating was defined as the complete transfer of spermatophore to the female. Error bars represent the standard error of the mean.

Table 4: ANOVA table for N mating success

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Diet Treatment	2	6.0667	3.0333	2.1218	0.1394
Residual	27	38.6000	1.4296		

Discussion

I expected the crickets to show reduced mating behavior in response to the diet of glucose, while the crickets fed a high-quality diet of cricket chow to show increased or stable mating behavior over 5 mating trials. There were no trends found in mating behavior in response to diet. The average song rate (Figure 2), the total glandular feeding (Figure 3), and the total mating rate (Figure 4) remained constant throughout the trials. Although the mating behavior did not decrease as expected in the glucose treatment, there was a trend of decreased mass in the glucose crickets (Figure 1). This study demonstrated that short-term feeding is not necessary for male mating in *O. nigricornis.*

Effects of Diet on Mass

There was a trend in weight loss in the males on the glucose diet (Figure 1). The resource-limited males had a slight decrease in weight while the high-quality diet males were approximately the same mass. Other studies suggest that male weight loss is due to longer periods of postcopulatory feeding by the female (Brown and Kuns 2000). Postcopulatory feeding may have helped in decreasing weight in tree crickets, but the overall trend of increased weight loss in the resource-limited diets suggests this is more likely due to diet.

Effects of Diet on Calling

The effectiveness of male signaling can be dependent on how energetically costly it is to the male. My results suggest that the song rate of male *O.* nigricornis is not condition-dependent (Figure 2). The singing of male *Requena verticalis* was not affected by diet either

(Schatral 1993). However, one study suggested that male calling increased with the amount of protein present in the diet (Hunt et al. 2011). Song rates may be affected during the lifetime due to differing diets but not during mating. This effect of high-quality diet on calling may be considered over the lifetime of the cricket as opposed to during mating behaviors. These differences indicate that some types of calling are more costly to produce than others (Hoback and Wagner 1997). The lack of difference in song rate between males on differing diets in tree crickets indicates that calling to a prospective mate is more important than the cost of calling itself.

Effects of Diet on Glandular Feeding and Mating Success

Although female mating behavior has been studied extensively, little work has been documented on the effects of diet on the male mating behavior of *Oecanthus nigricornis.* My results showed that short-term feeding does not inhibit male mating. In other species of crickets nuptial gifts also did not suffer from dietary constraint, such as the ground cricket *Pteronemobius* sp. (Hall et al. 2008; Brown 2010) and the katydid *Requena verticalis* (Simmons 1993; Brown 2010). The ground crickets were treated with two diets differing in protein content and observed mating behavior for 12 hours. Reproductive investment did not vary between the two diet treatments in *Pteronemobius sp.* (Hall et al. 2008). Despite limited resource availability in *R.* verticalis, males were less attractive but continued to provide spermatophores for 20 days of mating (Simmons 1993). Also, the bushcricket *Decticus verrucivorus* may show that the cost of reproduction is worth the allocation of limited resources. Reproduction was not affected by a protein-limited diet, and the

bushcricket continued to transfer spermatophores with short recovery times (Simmons 1993). The crickets are allocating resources to reproduce and increase fitness rather than to survival.

However, similar studies show that male *O. nigricornis* are limited in their ability to remate. Mating multiple times was found to deplete protein reserves (Bussiere et al. 2005). Courtship-feeding duration also decreased as the number of mates increased (Brown and Kuns 2000), showing that males cannot replenish metanotal protein reserves in order to remate at the frequency of 24-hr mating intervals (Brown 2010). The postcopulatory feeding will be shortened if the male does not maintain enough metanotal protein to keep the female occupied. Longer periods of feeding is highly correlated with insemination from the spermatophore (Brown 2010) indicating shorter postcopulatory feeding decreases the likelihood of sperm transfer to females and lowers fecundity.

The observation that total glandular feeding remained constant (Figure 3) throughout the treatments may be a result of male choice. It has been reported for role reversal between sexes to occur when nutritious foods are rare, making males the choosing sex (Schatral 1993). Males may adjust the rate of secretion of metanotal protein gift depending on female quality (Brown 2010). In the present study, females were fed a high-quality diet for a similar period of time which may have caused the males to adjust the secretion. The males on the glucose diet may have been able to achieve higher levels of reproductive success by secreting smaller amounts of the metanotal gland for a longer period of time to prevent the female from consuming the spermatophore too soon, while

the high-quality males may have secreted more metanotal gland for the same period of time.

Male choice may also have a say in the type of females they mate with in order to achieve the most success. *Requena verticalis* fed a low-quality diet reject the virgin female mating attempts but readily mate with females paired before, while the frequency of male mating attempts with non-virgin females was not affected by male diet (Schatral 1993). Already mated females may be better to mate with since it may appear to the males that they have higher fitness which in turn would increase the male fitness as well. It is also possible that the non-virgin females may be less inclined to feed for as long as virgin females which would be less costly if the males are already depleted of nutrients (Schatral 1993). In my study males that mated multiple times did not differ in the number of matings as well (Figure 4), indicating that the males with limited resources continue to mate with high-quality females of their choosing.

Adaptive plasticity in male choice is known in other courtship-feeding insects, like the male *Gryllodes sigillatus* that can change the size of spermatophylaces to increase sperm transfer in response to male rivals (Bussiere et al. 2005). Increasing the amount of sperm will help the insect achieve a better chance at inseminating the female verse the competition. An example of males adjusting to female quality is *Requena verticalis* that allotted less sperm to older females. Although *R.* verticalis do not invest as heavily in older females, *O. nigricornis* females feed more vigorously from males (Brown and Kuns 2000). Also, males are capable of providing larger meals when fed from often, indicating that

insects can alter their reproduction behavior (Bussiere et al. 2005). This ability to alter reproductive behavior may be why the crickets on the glucose diet were able to feed to high-quality females for the same amount of time as the males on high-quality diets.

It has been suggested that males that mate at 24-hour intervals are depleted of protein reservoirs in the male *O. nigricornis* (Bussiere et al. 2005). This short amount of time may not be adequate to allow the male to replenish glandular stores. However, 48 hours occurred between matings in my study. This extra 24 hours may have allowed the male to rebuild stores even if on the glucose diet. It could be that males only need a bit of energy in them, such as the diet of low glucose, with some extra time to provide enough resources for a female.

It is also possible that the males depleted of protein reserves are allocating the limited energy to other strategies to ensure reproductive success. The resource-limited males may have the intention of ensuring fertilization success by feeding the female for longer periods of time rather than a shorter period of time. Males of the Indian meal moth *Plodia interpunctella* anticipate sperm competition by producing more sperm. However, resource-limited *P. interpunctella* produce as much sperm as the Indian meal moth males on a high-quality diet. The difference lies in the fact that resource-limited males produce more non-fertile sperm, which is less costly to produce than fertile sperm (Lewis et al. 2011). This is a type of male mating strategy that increases their chance of mating while decreasing the success of competition.

In contrast with producing more non-fertile sperm, another possibility is that the black-horned tree cricket produced smaller spermatophores to attain a high mating frequency observed in this study. The adaptive variation in spermatophore size seen in *Requena verticalis* indicates that males are capable of storing energy for future reproduction (Simmons 1995). Rather than allocating resources to spermatophore production, males can increase fitness by mating more frequently. The ability to change the size of spermatophores allows trade-offs to occur in order to achieve the most success in reproduction.

Investigating the reproductive habits of male *O. nigricornis* is beneficial in understanding the consequences of limited resources. Repeating this experiment will help to clarify that the resource-limited males did not suffer from a lack of energy due to a glucose diet. Also repeating this experiment but with 24-hour intervals between matings would clarify if the extra time allotted for recovery had an effect on how the crickets were able to rebuild their resources to continue mating. In addition to this experiment, determining if the lifespan of crickets is affected by diet could play a role in life history. Increased longevity may be a result of diet restriction (Hunt et al. 2004) and may have an effect on male availability for mating which may alter the sex ratio.

My results indicate that the average song rate (Figure 2), total gland feeding (Figure 3), and mating success (Figure 4) are not affected by limited resources, and that black-horned tree crickets are not income breeders. Further research investigating the mating

habits of male *O. nigricornis* may help to uncover discrepancies of how black-horned tree crickets store and use resources for reproduction.

In conclusion, I have shown that resource-limited *O. nigricornis* males allocated enough resources to make trade-offs to provide optimal reproductive investment in females. The ability to continue calling to females and to produce nutrients to feed females even with reduced weight on a low-resource glucose diet indicates that crickets are allocating their nutrients to reproduction. Males may have control over their gift size to females as well as the choice of female. In addition, males may have the ability to recover nutrients after 48 hours as compared to 24 hours. Black-horned tree crickets thus provide an interesting system to interpret the complexity of reproductive behaviors and the allocation of limited resources.

Acknowledgments

Firstly, I would like to thank Dr. Mumme for his advice and much needed knowledge in preparing for and completing this paper, as well as the weekly pizza/calming sessions. I would also like to thank Dr. Ostrofsky for reading this paper.

I thank Julie Laux, Andrew Bean, and Megan Knox, for their help in cricket collecting and moral support, as this project would not have been possible without them.
An extra thanks to Julie and Bean for their motivational signs in my comp cube as well as their support.

Thank you to Ashley Whitehead for helping me to stay calm and down to earth throughout this entire year, as well as attempting to teach me how to interview and speak in front of others. I would be lost without you.

Thank you to Brandon Staley for his fish bowl idea that thoroughly helped me to get through this process, even though he never did his dishes.
I am also in deep thanks to all of the alumni who gave their advice and shared their own personal experiences and encouragement.

Finally, I would like to thank Jody Sullivan and my family for their continual support of my cricket project. All of the questions they asked and Jody's help of relating my comp deadline to her taxes were extremely beneficial.

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