The effect of temperature and diet on the growth and mortality of *Camnula pellucida* (Orthoptera: Acrididae)

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Abstract

Temperature and diet are important factors that affect the growth and mortality of grasshoppers (Orthoptera: Acrididae). *Camnula pellucida* (Scudder) are commonly found species of grasshoppers and can cause detrimental damage to agricultural crops. This experiment will examine the contribution of heat-exposure (8 hours vs. 16 hours of preferred temperatures) and food quality (low nitrogen vs. high nitrogen) to the growth and mortality of *C. pellucida* nymphs from UNDERC-East in Upper Michigan. I hypothesized that heat and diet would significantly affect growth and mortality, with the highest rates in high heat - high nitrogen conditions. The results showed that heat had a significant effect on nymph growth, but dietary nitrogen did not. Temperature and diet are both important factors that affect growth and mortality when the factors are independent of each other, but when the factors were combined, only temperature showed significant effects. Another experiment also looked at how heat-exposure affected the metabolism of the nymphs by measuring food consumption. I predicted that food consumption would be the highest in high heat conditions because of increased activity in grasshoppers, and the results supported this hypothesis.

Introduction

The primary diet for grasshoppers (Orthoptera: Arcididae) consists of grasses and forbs (Behmer 1994). Grasslands comprise over 40% of the earth's terrestrial surface (Williams et al. 1968), but grasses have very poor nutrition relative to other plant types (Tscharntke and Greiler 1995). Plants are considered to be high quality if it contains high nitrogen levels relative to carbon levels (low C:N ratios; Strengbom, et al. 2008). Forbs, which contain higher concentrations of nitrogen, phosphorus, and sugar, are considered better nutritional foods than grasses depending on levels of defensive chemicals (Randolph et al. 1995). Grasshoppers can change their nutritional intake by altering the amount of food that they eat or by consuming foods with different nutritional values (Waldbauer and Friedman. 1991). Each species has different nutritional needs, and consumes foods with proportions of carbohydrates and proteins best needed to increase overall fitness of the individual (Simpson et al. 2004). Many previous studies indicate that nutritional deficiencies are one of the main causes of reduced body size and prolonged development in grasshoppers (McNeil and Southwood 1978).

Elemental compositions, such as carbon and nitrogen, from the diets are key aspects that affect grasshoppers. Previous studies looked at the relationship between body mass and elemental composition in *Schistocerca americana*, and showed that the amount of carbon, nitrogen, and phosphorus in the grasshopper increased linearly with increase in body mass (Yang 1994). Although nitrogen and phosphorus are both important elements in grasshoppers, they do not have the same effects. In studying spatial patterns, grasshopper behavior was significantly affected by foliar-N in host plants, but not significantly by P-levels (Vonas et al. 2010).

Environmental conditions also affect the behaviors of grasshoppers since they are ectotherms. Ectotherms' physiological processes are heavily influenced by body temperature (Huey 1982), and generally incorporate one of the following two strategies to adapt to its thermal environment. The first is to be a eurythermal (thermal generalist), which means they are not affected as much by temperature change and can tolerate a broader range of temperature. The second is to be a stenothermal (thermal specialist), meaning that they can only tolerate a relatively narrow range of temperatures (Willott and Hassall 1998). Grasshoppers have been shown to be sensitive to temperature in both the laboratory (Whitman 1986) and in the field (Richards and Waloff 1954). Most grasshoppers lie somewhere in between thermal generalists and high-temperature thermal specialists. Their primary way of managing their body temperature is through behavioral thermoregulation (Young 1979). This means grasshoppers will bask in heat to raise body temperatures, or seek shade to avoid extremely high body temperatures. In a study conducted with four different species of grasshoppers (*Chorthippus brunneus, Omocestus viridulus*, *Myrmeleotettix maculatus* and *Stenobothrus lineatus*), each of the species showed significant growth and development rates with increased temperatures (Willott and Hassall 1998).

This study only examined one species of grasshoppers, *Camnula pellucida* (Scudder), to eliminate any possible confound variables between species. *Camnula pellucida,* commonly known as clear-winged or warrior grasshoppers, are economically detrimental species, because they may swarm into cultivated areas and cause serious damage to vegetables and other agricultural crops, especially grain (Ball et al. 1942).

I studied how different levels of heat exposure (low heat vs. high heat) and dietary nitrogen (low nitrogen vs. high nitrogen) affected the growth and survival of *C. pellucida* nymphs. Temperature and diet were shown independently to have influenced grasshopper development (Joern and Behmer 1997), but these two factors were never tested together. I predicted that grasshopper growth would be the largest in high-heat exposures and high-nitrogen diets, because longer hours of heat exposure and high food quality are important factors in the development of grasshoppers (Young 1979, McNeil and Southwood 1978). Oppositely, I predicted that grasshopper mortality rate would be the highest in low-heat and low-nitrogen conditions, because high food quality (high levels of dietary N) and heat are important factors in grasshopper survival rates (Joern and Behmer 1997). Moreover, I predicted there will be a significant interaction between heat and dietary nitrogen levels for both growth rate and mortality rate of grasshoppers, since both factors are important in the development and survival of grasshoppers (Joern and Behmer 1997).

I also tested how *C. pellucida* nymphs' metabolism differed based on heat exposure (low heat vs. high heat) by measuring the food consumed. Food consumption was undoubtedly higher in the high-heat cages, because grasshoppers at higher temperatures were able to have higher body temperatures, which allowed them to be more active and use more energy than in lower temperatures (Willott and Hassall 1998).

Materials and Methods

Grasshopper Collection: I looked at the development of graminivorous grasshopper *C. pellucida*, species commonly found late June to late August in southern Canada, western-half and northeastern parts of the United States (Brooks 1958). I collected the *C. pellucida* nymphs on "Grasshopper Nation," a field located on the west side of University of Notre Dame Environmental Research Center (UNDERC-East) property, which is on the Upper Peninsula of Michigan. The site's elevation was 513 meters according to the Garmin® geographic position satellite.

Cage Set-up: Four treatment combinations of diet and temperature were set up: low-nitrogen diet with low-heat exposure (8 hours of heat), low-nitrogen with high-heat exposure (16 hours of heat), high-nitrogen with low-heat, and high-nitrogen with high-heat. I used a 100-watt light bulb as a source of heat and maintained the temperature in the cage around 35-40°C, since most grasshoppers including *C. pellucida* maintain body temperatures around 36-38°C, if possible (Joern and Behmer 1997). Each cage (34.5 x 21.6 x 10.6 cm) was made of mesh in order to keep the humidity low, which is good for the survival of grasshoppers (Joern and Behmer

1997). Diets were elevated with nitrogen by adding casein to their normal diets of grasses and forbs (Joern and Behmer 1997).

Ten nymphs were randomly distributed into each cage. Once a week, I recorded the number of living and dead grasshoppers. Also I recorded the grasshoppers' sizes, using femur and pronotum length as indices to measure growth for the living grasshoppers. I ran the experiment for three weeks.

In a separate experiment, I looked at the food consumption of five *C. pellucida* nymphs in low and high heat conditions. I recorded the amount of daily food (forbs and grasses) intake for four days by weighing the food. No grasshoppers died during this experiment.

Statistical Analysis: All the statistical tests were run using SYSTAT. I tested all my data for normality using the Kolmogorov–Smirnov test (Lilliefors). I used a two-way ANOVA to test for factors affecting grasshopper growth, with the nitrogen levels and heat exposure as independent factors, and grasshopper growth as the dependent factor. I used femur length and pronotum length as indices to measure grasshopper growth, because these two body parts have been shown to be closely related to grasshopper size (Blackith 1960). To compare how mortality rates differed in each condition, I used a two-way ANOVA with the same independent factors as the previous test but changing the dependent variable to the number of deaths. I compared the grasshoppers' metabolism rate in different levels of heat exposure using a one-way ANOVA. The independent variable was the level of heat exposure, and dependent variable was the amount of food eaten. I used an alpha value of 0.05 to test for significance on all tests.

Results

Grasshopper Growth: I used a two-way ANOVA to compare how heat-exposure and dietary nitrogen affected the growth of *C. pellucida* nymphs, using femur length and pronotum length as indices for grasshopper growth. I tested my data for normality using the Kolmogorov–Smirnov test (Lilliefors), and found femur length and pronotum length to be normal (Femur: Test-stat=0.115, p=0.41; Pronotum: Test-stat=0.119, p=0.356). Both femur growth and pronotum growth were significantly higher in high-heat conditions than low-heat (Femur: $F_{1,25}$=6.18, p=0.020; Pronotum: $F_{1,25}$=5.86, p=0.023; Figure 1), but dietary nitrogen did not have a significant difference (Femur: $F_{1,25}$=1.06, p=0.313; Pronotum: $F_{1,25}$=2.26, p=0.145; Figure 2). The average growth of the femur in high-heat was 5.031 ± 0.289 mm compared to 3.882 ± 0.357 mm in low-heat, and average growth of pronotum in high-heat was 1.964 ± 0.476 mm compared to 1.384 ±0.269 mm in low-heat. The growth of femur in high nitrogen was 4.877 ± 0.378 mm and 4.294 ± 0.316 mm in low nitrogen. The growth of pronotum in high nitrogen was 1.552 ± 0.211 and 1.863 ± 0.179 mm in low nitrogen. The interaction between heat and nitrogen was not significant in both femur growth and pronotum growth (Femur: $F_{1,25}$=0.291, p=0.594; Pronotum: $F_{1,25}$=1.03, p=0.320).

Mortality Rate: I tested how heat-exposure and dietary nitrogen affect mortality rates in *C. pellucida* nymphs. The data for mortality rate was normal according to the Kolmogorov–Smirnov test (Test-stat=0.235, p=0.239). The mortality rate of nymphs in low-heat cages were significantly higher than high-heat ($F_{1,4}$=8.33, p=0.0447, Figure 3), but nitrogen levels did not show a significant difference ($F_{1,4}$=3.00, p=0.158). The mortality rate in low heat was 40.00 ± 8.165%, which was significantly higher than 25.00 ± 9.574% in high heat. Mortality rate in low nitrogen diet was 25.00 ± 9.574% and 30.00 ± 10.00% in high nitrogen. The interaction between heat-exposure and dietary nitrogen was not significant ($F_{1,4}$=0.333, p=0.594).

Consumption Rate: I used a one-way ANOVA to test if the amount of food consumed by *C. pellucida* nymphs depended on the heat conditions. The data for food consumption was normal according to the Kolmogorov–Smirnov test (Test-stat=0.112, p=1.00). Nymphs in high-heat consumed significantly more food than in low-heat ($F_{1,14}$=19.14, p=0.000635, Figure 4), consuming 0.0678 ± 0.007421 g in high heat and 0.0306 ± 0.00417 g in low heat.

Discussion

Temperature and diet quality are both important factors in the development of *C. pellucida* nymphs, but this experiment showed that temperature had a greater effect on their growth. Using femur length and pronotum length as indices for grasshopper growth, I found that nymphs that were exposed to 16 hours of heat grew significantly larger than ones exposed to 8 hours of heat in both femur length and pronotum length. This is consistent with my initial hypothesis that high heat-exposure would show the greatest grasshopper growth, because temperature was shown to be an important factor in grasshopper growth (Joern and Behmer 1997).

The difference in diet quality, however, did not significantly affect grasshopper growth. Femur length was slightly larger with high nitrogen diets than low nitrogen, but the difference was not significant. Pronotum length also did not yield a significant difference. This finding went against my original hypothesis that high quality food (high dietary nitrogen) would show greater grasshopper growth, since dietary intake was shown to be important in grasshopper development

(McNeil and Southwood 1978). Also contrary to my hypothesis, there was no significant interaction between temperature and dietary nitrogen in both femur length and pronotum length.

A possible reason for diet quality not having a significant effect on growth may be that when there are two factors present (temperature and diet quality), temperature may have a greater influence on the growth. When these two factors were tested independently of each other, each factor influenced the grasshoppers' growth (Young 1979, McNeil and Southwood 1978), but when they were combined, my results indicated that only temperature had a significant effect. When I looked at how food consumption rate changed between temperatures, I found that nymphs in high-heat consumed significantly more food than in low-heat. This result shows that heat had a significant influence on the metabolism rates of *C. pellucida* nymphs. Diet quality is important in grasshopper growth in constant temperatures since their metabolisms will be about the same (Joern and Behmer 1997), but when heat exposure differs, body temperatures and metabolism rates change, and the amount of food consumed plays a bigger role than the quality of the food itself.

Similar to grasshopper growth, temperature had a significant effect on the mortality rate of grasshoppers, but dietary nitrogen did not have a significant effect. The mortality rate in low heat was significantly higher in high heat, and the interaction between heat exposure and dietary nitrogen was also not significant. Like grasshopper growth, when these two factors were tested independently of each other, they showed significant effects on mortality (Joern and Behmer 1997), but when these two factors were combined, only temperature had a significant effect. As ectotherms, grasshoppers manage their body temperature through behavioral thermoregulation (Young 1979). Their ideal body temperature is 36-38°C (Joern and Behmer 1997), but they can only reach this temperature if there is an external heat source available. In low heat-exposure cages, they regulated their body temperature for 8 hours a day, but the ones in high heat-exposure cages regulated their temperatures for 16 hours a day. This experiment showed that body temperature regulation was an important influencing factor on the mortality rate of grasshoppers. With the average global temperature increasing rapidly (Root et. al 2002), grasshoppers are expected to grow larger and live longer.

There are many sources of error in this experiment. For one, all the *C. pellucida* nymphs were not in the same instar stages when I captured them. Younger instar nymphs have more potential to grow, which may have affected the nymphs' growth rates, and also the younger ones are more likely to die than older instars, which may have affected mortality rates. Future research can study *C. pellucida* nymph growth and mortality rates with nymphs from all the same instar stages. Another source of error may have come from the gender differences of the nymphs. Previous studies have shown there may be sex-specific differences between feeding patterns in grasshoppers (Behmer and Joern 1994). My study did not take grasshopper gender into account; however, future research can look at if gender is a significant factor by separating the female and male grasshoppers into separate cages. Lastly, humidity is an important factor in the survival of grasshoppers (Joern and Behmer 1997), but I did not take this factor into account. I attempted to keep the humidity low by making the cages out of mesh, but I did not have a way of quantifying the level of humidity. Future research can study exactly how important humidity is to the survival of grasshoppers and how it affects the growth the metabolism of grasshoppers.

I conducted my experiment in a laboratory setting where I was able to control most of the factors. There may be other confounding variables present in the field that I did not consider. Future studies can use the information from laboratory experiments and see how they are affected out in the field. Certain grasshoppers like *C. pellucida* can cause large economic damages to agricultural industries (Ball et al. 1942), so studying the factors that affect the growth, mortality, and metabolism of these species may help prevent outbreaks and reduce economic damages.

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Figures

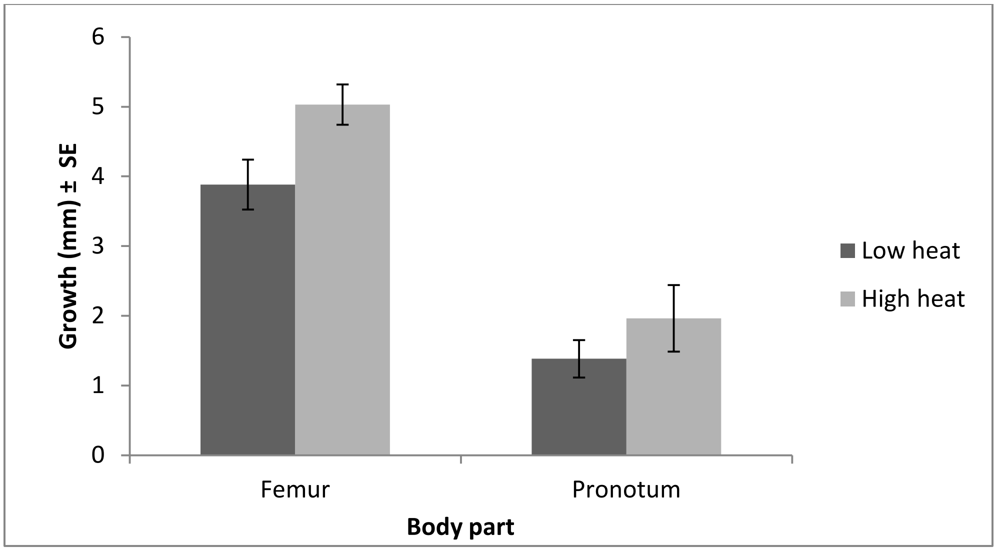

Figure 1: Effect of heat-exposure on the growth (mm) ± SE of *C. pellucida* nymphs
The average growth of *C. pellucida* nymphs in a three-week period was significantly higher in high-heat exposure cages (16 hours of heat) than low-heat (8 hours) for both femur growth ($F_{1,25}$=6.1774, p=0.01998) and pronotum growth ($F_{1,25}$=5.8609, p=0.02307). The average growth of the femur in high-heat was 5.0311 ± 0.2887 mm compared to 3.8817 ± 0.3571 mm in low-heat, and average growth of pronotum in high-heat was 1.9635 ± 0.4762 mm compared to 1.3842 ±0.2689 mm in low-heat.

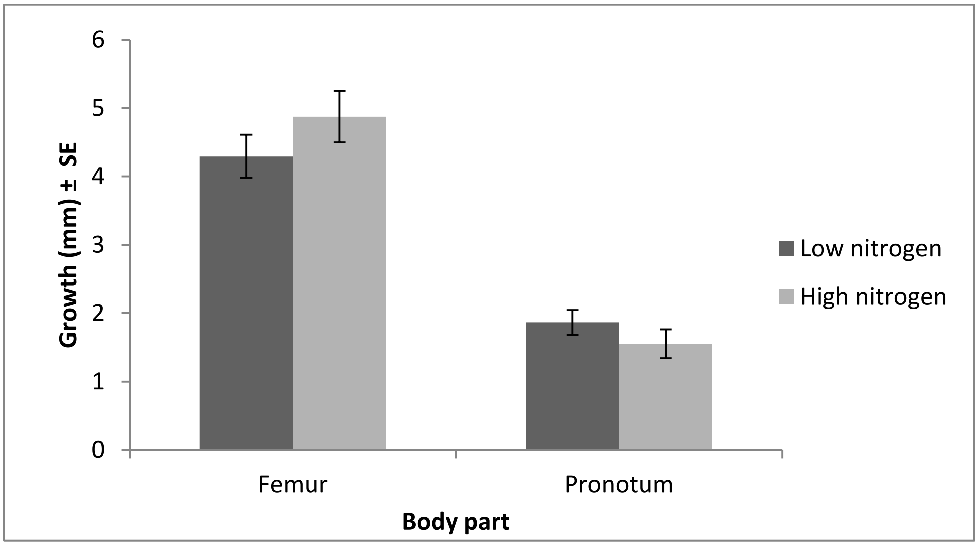

Figure 2: Effect of dietary nitrogen on the growth (mm) ± SE of *C. pellucida* nymphs
The differences in growth rates between low-nitrogen and high-nitrogen diets were not significant in both femur growth ($F_{1,25}$=1.05959, p=0.313164) and pronotum growth ($F_{1,25}$=2.25728, p=0.1455147). The growth of femur in high nitrogen was 4.8769 ± 0.3782 mm and 4.2944 ± 0.3165 mm in low nitrogen. The growth of pronotum in high nitrogen was 1.5523 ± 0.21154 and 1.8631 ± 0.17927 mm in low nitrogen.

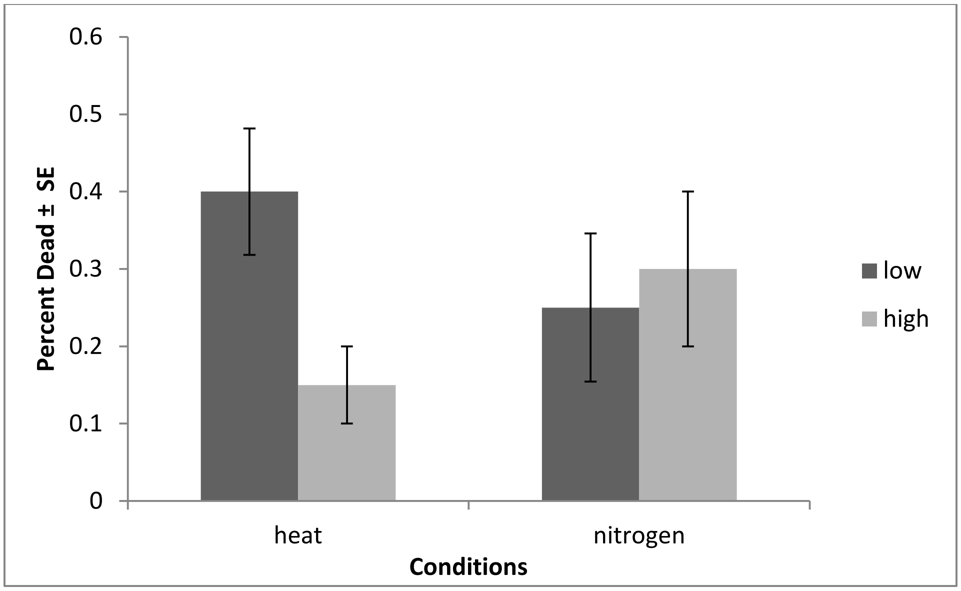

Figure 3: Effect of heat-exposure and dietary nitrogen on the mortality rate (%) ± SE of *C. pellucida* nymphs

The difference in mortality rates between heat exposures was significant ($F_{1,4}$=8.3333, p=0.04471), but not significant between dietary nitrogen ($F_{1,4}$=3.0000, p=0.15830). The mortality rate in low heat was 40.00 ± 8.165%, which was significantly higher than 25.00 ± 9.574% in high heat. Mortality rate in low nitrogen diet was 25.00 ± 9.574% and 30.00 ± 10.00% in high nitrogen.

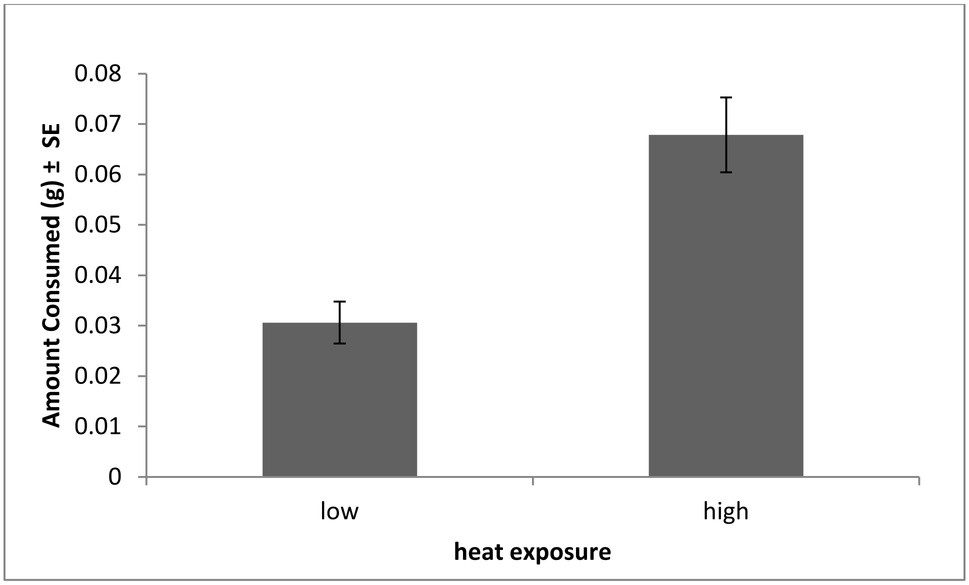

Figure 4: Amount of food consumed (g) ± SE between of heat exposure (low vs. high)

Recorded amount of food consumed by *C. pellucida* nymphs for four days. The nymphs consumed significantly more food in high-heat than in low-heat ($F_{1,14}$=19.1394, p=0.00063482). They consumed 0.067825 ± 0.007421 g in high heat and 0.030588 ± 0.004169 g in low heat.