EFFECTS OF WEATHER AND BEHAVIOR ON BODY TEMPERATURE AND THE CONSEQUENCES OF TEMPERATURE FLUCTUATIONS ON DEVELOPMENT AND REPRODUCTION IN *Schistocerca americana*: IMPLICATIONS FOR PHENOLOGY AND POPULATION MODELING

BY

JASON G. FROEBA

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2007

To my Aunt Linda, who always nurtured my scientific interests, and to my wonderful wife Emily, without whom this accomplishment would not have been possible

ACKNOWLEDGMENTS

I thank my supervisory committee chair, Dr. John L. Capinera, for his advice and support, his firm motivational style, and for always keeping my best interest in mind. I thank my other committee members, Dr. Daniel Hahn and Dr. Heather McAuslane, for their mentoring and constructive criticism, which greatly improved this work. I would like to thank Seth Bybee and Jennifer Zaspel for their help and anyone else who provided input, support, or materials. Finally, I would like to thank my wife Emily for her support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

EFFECTS OF WEATHER AND BEHAVIOR ON BODY TEMPERATURE AND THE CONSEQUENCES OF TEMPERATURE FLUCTUATIONS ON DEVELOPMENT AND REPRODUCTION IN *Schistocerca americana*: IMPLICATIONS FOR PHENOLOGY AND POPULATION MODELING

By

Jason Froeba

May 2007

Chair: John Capinera
Major: Entomology and Nematology

Phenology modeling is central to many pest management strategies and often consists of modeling temperatures by using ambient temperatures and insect developmental data derived from constant temperature studies. Not surprisingly, discrepancies between predictions and the actual timing and size of insect populations can often be attributed to differences between actual body temperature and the temperature used in the model. In addition, temperature fluctuations are a potentially important, yet largely ignored area in insect thermal biology and modeling, as most models are based on data collected at constant temperature. Grasshoppers such as *Schistocerca americana* are well known for maintaining body temperatures different from ambient temperature. This, plus the fact that it is one of the few grasshoppers in the eastern USA that reach damaging levels, make *S. americana* a relevant subject on which to assess the effects of thermoregulation on development and reproduction.

Body temperatures of grasshoppers, along with several environmental parameters, were monitored continuously to ascertain the effects of weather and behavior, and to determine what body temperatures grasshoppers could attain and would prefer in the field. Body temperatures averaged about 30 and 38°C during the daylight hours for grasshoppers on overcast and sunny

summer days, respectively, compared to average ambient temperatures of 28 and 32° for overcast and sunny conditions. Daytime temperatures fluctuated frequently, especially on overcast days. There were significant positive relationships between body temperature in *S. americana* and sunlight intensity, ambient temperature and behavior. The rate of increase in body temperature due to direct solar radiation was as high as 2.5°C /min, which greatly elevated body temperatures, up to 19.6°C above ambient. Adverse weather conditions (cloud cover and rainfall) reduced mean daily body temperatures by up to 8°C and caused up to a 38% decrease in body temperature at a mean rate of 0.978ºC/minute and 0.552ºC/minute for cloud cover and rainfall, respectively.

Laboratory studies were conducted at constant temperatures and fluctuating temperatures of two amplitudes and frequencies for two mean temperatures of 29.5°C and 31.5°C for a total of ten treatments. A mean daily temperature increase of 2°C caused a decrease in mean development time (up to about 6 d) and increase in mean reproductive output (up to 175 eggs / female increase). Amplitude of temperature fluctuation was also shown to affect development rates and fecundity, but more so in the 29.5°C treatments than in the 31.5°C treatments. The effects of frequency of temperature change were significant for all parameters tested except days to oviposition, but could not be separated from effects which may have been caused by the separation of treatments into two time periods. Overall, temperature fluctuations significantly affect development and reproduction in *S. americana*. The results suggest that grasshopper body temperatures are often very different from ambient temperatures, that phenology models based on ambient temperatures can be inaccurate, and that use of more relevant mean body temperatures should be considered for species that thermoregulate or attain body temperatures different than ambient.

CHAPTER 1
INTRODUCTION

Temperature plays an important role in the life processes of many organisms. This is especially true in poikilothermic ectotherms, which rely on outside sources of heat to maintain internal body temperature. Temperature affects the rate at which biochemical processes take place, and can affect metabolism, developmental rate, reproduction, and body size. The developmental stage of Atlantic cod, *Gadus morhua*, at hatch is negatively affected by suboptimal temperatures, with development being incomplete at both low and high temperatures (Jordaan et al. 2006). Incubation time in northern shrimp, *Pandalus borealis*, was reduced from 214 days at 2°C to 123 days at 8°C (Brillon et al. 2005). Insects are particularly sensitive to changes in temperature. *Listronotus texanus* Stockton (Coleoptera: Curculionidae) exhibited a decrease in total development time of 35 days from 65 to 30 days when the temperature was raised from 20°C to 27.5°C (Woodson and Edelson 1988). *Euzopherodes vapidella* Mann (Lepidoptera: Pyralidae) showed an increase in mean fecundity from 51.8 eggs per female at 20°C to 124.4 eggs per female at 33°C. However, wing span and body length was significantly less at 33°C than at lower temperatures tested (Ashamo and Odeyemi 2000). As advantageous as temperature increases seem to be, there is a limit to the extent temperature increases can be beneficial.

Extremes in temperature can have severe negative effects on an organism (e.g. desiccation and deactivation of enzymes) causing thermal death. The temperatures at which severe negative effects occur define the tolerance range of an organism, which estimates the range of temperatures over which survival is possible (Huey and Stevenson 1979). While temperatures outside of the tolerance range lead to death, the effects of less extreme suboptimal temperatures are usually more subtle (e.q., prolonged developmental time and diapause, and

decreased fecundity and body size). In *Mallada desjardinsi* (Navas) and *Chrysoperla nipponensis* (Okamoto) (Neuroptera: Chrysopidae) body size was reduced at both low and high suboptimal temperatures (Nakahira et al. 2005). Castillo et al. (2006) showed that developmental time in *Quadrastichus haitiensis* (Gahan) (Hymenoptera: Eulophidae), decreased with increasing temperature up to a certain point, after which increases in temperature caused increases in developmental time. This increase in developmental time at suboptimal temperatures is very common in insects, and the temperatures at which the decrease occurs are often called critical thermal temperatures.

There are both critical thermal maximum and critical thermal minimum temperatures, and they are often used to define ecological and behavioral thermal limits (Hu and Appel 2004). The critical thermal minimum and maximum temperatures help define what is known as the thermal performance breadth of an organism, which estimates the range of temperatures over which an organism performs well (Huey and Stevenson 1979). When compared to larger organisms, insects exhibit a broad thermal performance breadth. This may be because insects will encounter the same thermal environments as many other organisms but, due to their small size, body temperature will be more sensitive to small changes in the thermal environment (Heinrich 1993). In addition to having a broad thermal performance breadth, some insects (e.g., termites) have been shown to have critical temperatures that fluctuate seasonally (Hu and Appel 2004). Of even more importance than the thermal performance breadth is the thermal optimum or optimal temperature at which an insect will experience the highest fitness level possible (Huey and Stevenson 1979), usually by a combination of increased developmental and reproductive rates. However, environmental temperatures are often not those that would be considered optimal for insects, and so body temperature must be controlled in order to optimize fitness.

All insects are poikilothermic and can be ectothermic (relying on external sources of heat), endothermic (able to use heat generated by muscle activity), or both. In either case, insects must thermoregulate to maintain functional body temperatures when in a suboptimal thermal environment. Thermoregulation is the maintenance of an optimal internal body temperature different from environmental temperatures. Thermoregulation not only includes raising body temperature above ambient, but also systematically decreasing body temperature in cases where ambient temperatures become too high. While almost all insects possess some ability to avoid undesired temperatures (Casey 1981), many insects can maintain body temperatures within an optimal range by actively altering specific behaviors.

Insect orders in which behavioral thermoregulation has been observed include Lepidoptera, Odonata, Orthoptera, Coleoptera, Hemiptera, Hymenoptera, and Diptera (Heinrich 1993). Methods of thermoregulation in insects are variable and differ significantly between orders. Hymenoptera make use of heat produced by the shivering of flight muscles. Honeybees and bumblebees have been known to use this method to keep nests warm (Heinrich 1996). Among Lepidoptera, *Hemileuca oliviae* Cockerell (Lepidoptera: Saturniidae) larvae ascend vegetation and rest during the heat of the day to stay cool. They shift position so that the ventral surface of the body is always facing the sun and covered by vegetation (Capinera et al. 1980). Codling moth larvae, *Cydia pomonella* (L.) (Lepidoptera: Torticidae), exhibit thermoregulatory behavior by selecting favorable microhabitats within apples (Kührt et al. 2005). Kührt showed that 74% of larvae built larger cavities in the warmer hemispheres of apples closest to the heat source. Kührt also found that, unlike the previous larval stages, when larvae sought to cocoon there was no difference in distribution of the mature larvae between the temperature gradient and the control, suggesting little if any preference for temperature at this stage. He concluded that at

least in the codling moth, behavioral thermoregulation is life stage dependent, and that larvae change their thermoregulation behavior during development based on benefits, needs, and constraints (Kührt et al.2005).

One group in which behavioral thermoregulation has been extensively studied is Orthoptera, specifically Acrididae, due to their great economic importance. Some Orthoptera, such as katydids, have been observed shivering flight muscles to raise muscle temperature before singing, similar to muscle twitching in Hymenoptera (Heinrich 1993). However, Acrididae do not use their wing muscles to sing, and so far no such warming mechanism has been observed in the group. Consequently, most Acrididae rely solely on external sources of heat obtained behaviorally (Casey 1981), primarily radiant heat from the sun, also known as insolation. The movement into or out of sunlight is the most common form of behavioral thermoregulation in Acrididae (Lactin & Johnson 1998b). Moving into and remaining in a radiant heat source, usually direct sunlight, as a way to increase body temperature is known as basking. While there are other sources of heat, such as radiant heat from the ground and small amounts of metabolic heat, radiant energy acquired by basking is the most important heat source for any acridid (Uvarov 1966a). In one study conducted on the genus *Locusta*, body temperatures rose from 27.7ºC to 36ºC at a rate of 0.83ºC per minute for ten minutes, thereafter rising at a rate of 0.104ºC per minute until reaching 42.7ºC, when exposed to radiant heat (Uvarov 1966a). While metabolic heat may have played a role in causing basal body temperature to be different from ambient temperature, it is very unlikely to raise body temperature by 0.83°C per minute, especially in a resting grasshopper. Lactin and Johnson (1996) found that nymphs of *Melanoplus sanguinipes* (Fabricius) oriented themselves around artificial heat sources and reached average body temperatures (near 40°C) that were different than those expected if the grasshoppers were

randomly dispersed. They considered this conclusive evidence that *M. sanguinipes* was actively thermoregulating. In a later study, Lactin and Johnson (1998b), showed grasshoppers thermoregulate not only in the laboratory, but also in the field. They estimated the body temperature of free ranging grasshoppers using a series of complex equations that accounted for different environmental factors affecting body temperature. They also placed objects with thermodynamic properties similar to those of grasshoppers at random in the field and recorded the temperature of these objects. The estimated body temperatures of the free ranging grasshoppers differed significantly from those of the objects that were placed at random. This was one of the first field studies that provided strong evidence that grasshoppers actively thermoregulate in the field by choosing warmer locations and altering body posture.

When trying to understand the thermal biology of an organism it is not only essential to know if the organism possesses the ability to thermoregulate, but also what other factors might affect that ability and body temperature. Fortunately, an extensive amount of work has been conducted for the purpose of understanding the individual mechanisms of heat transfer that affect a grasshopper's body temperature and ability to thermoregulate. However, pathways of heat transfer are very complex and largely independent of each other, making them very hard to quantify. Some factors affecting these pathways include insect orientation, body texture, color, and size, radiant intensity, substrate conductivity, wind speed, and turbulence (Chappell and Whitman 1990).

While basking, acridids will adopt different orientations with relation to the sun's rays (Uvarov1966b). There are two basic orientations that a grasshopper will adopt, perpendicular or parallel. In perpendicular basking, the long axis of the body is oriented perpendicular to the sun's rays (Uvarov 1966b). While in this position, grasshoppers will often assume a flanking position,

in which the hind leg shaded by the abdomen is raised, and the other is lowered to prevent shading of the abdomen (Heinrich 1993). This exposes the largest amount of surface area possible for heating. Parallel posture is one in which the long axis of the body is parallel to the sun's rays. In most acridid species the parallel posture only exposes about one-fifth the surface area that perpendicular flanking basking does (Uvarov 1966b).

Body size and color are two other factors that have the potential to affect body temperature. Rates of temperature increase and final equilibrium temperature (the point at which temperature no longer increases as a result of a fixed amount of radiant energy) were examined in 1st and 5th instar stages of *Schistocerca gregaria* (Forskal) (Uvarov 1966a). First instars had a higher rate of temperature increase but lower equilibrium temperatures than 5th instars. In contrast, Willot (1997), while investigating thermoregulation in four species of grasshoppers, found there to be no significant temperature differences between males and females within species. Together, the two studies suggest that while large size differences between 1st and 5th instar nymphs and possibly species affect temperature, the smaller size differences in adults between males and females has little if any effect on body temperature. Indeed, Lactin and Johnson (1997) found only a 2°C difference in body temperature between a body mass of 0.03g and 0.30g, a ten-fold difference in weight. Forsman (1997) conducted a study to determine if differences in color and size would affect body temperature in *Tetrix subulata* (Linnaeus) (Orthoptera: Tetrigidae). He found that body size had no effect on body temperature. However, black individuals achieved higher body temperatures than brown or white individuals. He then conducted a study to see if these differences in body temperature affected reproductive performance in females of the *T. subulata*. Forsman (2000) again found significant variation in body temperature due to color morphs in females, but no difference in overall performance. He

concluded that even though darker color morphs achieved higher temperatures, there were possible physiological differences between morphs that caused them to required higher optimum temperatures. Similarly, in their study on *H. oliviae*, Capinera et al. (1980) found that artificially blackened larvae attained higher body temperature than normal larvae, however, performance implications were not evaluated.

There are several factors that can potentially lower a grasshopper's body temperature, including convective and evaporative cooling, and long wave radiation, which refers to the fact that all objects lose heat by emission of energy in the form of infrared radiation (Uvarov 1966a). *S. gregaria* will climb onto vegetation, increasing convective cooling when ambient temperature rises to 40ºC (Heinrich 1993). Some grasshoppers can extend their legs to raise the body above the ground, a behavioral mechanism called stilting, which helps the grasshopper to escape high surface temperatures while increasing convective cooling (Heinrich 1993). There have been several studies concerned with the effects of convection and evaporative cooling on body temperature in grasshoppers. Lactin and Johnson (1998a) showed that orientation to the wind had no effect on body temperature in *M. sanguinipes*; however, wind speed did, and as wind speed increased, body temperature decreased. In a study on the caterpillar *H. oliviae*, body temperature was shown to be inversely related to air speed, but body temperature was always above ambient temperature, even at air speeds of 4m/s (Capinera et al. 1980). This suggests that while wind cannot prevent temperature increase, it can reduce the maximum attainable temperature and possibly the rate of increase. Evaporative cooling can become a factor in low humidity environments, where evaporation rates are high. This can be seen in an experiment in which nymphs of *S. gregaria* were held at different humidity levels, and the rate at which thermal equilibrium occurred was measured (Uvarov 1966a). Nymphs held in high humidity

environments reached equilibrium more quickly than nymphs held in low humidity environments (Uvarov 1966a). The higher humidity reduced heat loss through evaporation allowing the nymph to retain more heat and attain the equilibrium temperature faster. Grasshoppers have also been known to "pant," increasing breathing rate and increasing evaporative cooling, and therefore lowering body temperature (Casey 1981, Heinrich 1993). Prange (1990) conducted a study on the effect of respiratory rate on evaporative cooling in three species of grasshoppers *Schistocerca nitens* (Thünberg), *Locusta migratoria* (L.), and *Tmethis pulchripennis* (Bolivar). He showed that rate of evaporation and ventilation frequency remained relatively constant up to about 45°C, after which both rates increased significantly. Grasshoppers were able to maintain temperatures below the lethal limit of 48°C in air temperature as high as 52-53°C. The maintenance of such temperatures caused water loss in the grasshoppers to reach 8% of their body mass per hour. Grasshoppers could tolerate a water loss of 33% of their mass, meaning they could maintain sub-lethal temperatures for about 4 hours. With the exception of deserts, there are few places where ambient temperature can rise to such lethal levels requiring such an extreme form of behavioral thermoregulation.

By far the most determining factor of body temperature in grasshoppers is radiant energy from the sun and is the main reason body color and orientation can affect body temperature. It is common knowledge that objects heat up when exposed to direct sunlight, but more specific parameters such as maximum attainable temperature and rate of temperature increase differ depending on the nature of the object. There is also diffuse solar radiation from the sun that reflects off surrounding objects which could possibly affect temperature. Lactin and Johnson (1997) conducted a study on *M. sanguinipes* to determine the effects of direct and diffuse solar radiation on body temperature. They found that direct solar radiation had a highly significant

effect on body temperature while diffuse solar radiation did not. It is possible that diffuse solar radiation does not have enough energy to heat objects, or perhaps body temperature did not differ from ambient because diffuse solar radiation was also warming the surrounding environment. Either way, diffuse solar radiation likely plays little if any part in maintaining body temperatures different from ambient. In simulations based on this work, Lactin and Johnson found that direct solar radiation can cause a temperature increase of 0.008°C / W/m^2. In another study, Fielding (2004) placed freshly killed *M. sanguinipes* grasshoppers perpendicular to the sun at ground level. A thermocouple was inserted into the sternum to obtain temperatures under direct insolation. Fielding found that body temperature could be raised 15-20°C above ambient temperature near the ground when exposed to direct insolation. He also compared predicted developmental times using ambient temperature and solar adjusted temperatures and showed the time spent as nymph could be cut in half. While sunlight is the most significant source of temperature elevation in grasshoppers, it is not always available due to adverse weather conditions. Because the sun is the most important factor for raising body temperature, adverse weather conditions or the absence of the sun, is the most important factor hindering the ability to raise body temperature. In environments such as mountain ranges where ambient temperatures are low and the warmer seasons are short, cloudy days can prevent grasshoppers from obtaining the necessary amount of heat to complete development (Berner et al. 2004).

Aside from behavioral responses and abiotic environmental factors, there are ecological factors which can also play a role in a grasshopper's ability to thermoregulate. When considering an insect's ability to thermoregulate, one must also consider the biotic environment in which the insect lives. The vegetation surrounding the grasshopper can dramatically change how each of the abiotic factors mentioned earlier will affect the grasshopper. Vegetation can provide shelter

from sunlight and wind, while at the same time providing a perch that a grasshopper may use to increase exposure to either element. Vegetation can also produce different thermal environments with varying temperature and humidity levels. Willot (1997) studied the temperature differences between swards (large expanses of grass covered soil) of different height. His results showed that shorter swards reach higher temperatures during the day than taller swards. Differences in food quality of vegetation in the environment will also play a role in where a grasshopper will spend its time (Pitt 1999). Along with the surrounding vegetation, other animals can also influence a grasshopper's ability to thermoregulate. Predators can drive grasshoppers away from optimal conditions and into suboptimal temperatures and areas of reduced food quality. In a study by Pitt (1999) on *M. femurrubrum* (DeGeer), avian predators were shown to drive grasshoppers down into vegetation away from high quality food and sunlight into cooler temperatures.

It may seem like grasshoppers go through a large amount of trouble to maintain optimal temperatures, but there is merit in their effort. Temperature can have profound effects on physiological processes in acridids and in insects as a whole. Most chemical reactions occur at faster rates when subjected to higher temperatures. This generalization can be applied to most processes in the biological world, and is especially important to poikilothermic organisms and specifically in grasshoppers. Some processes that benefit from higher temperatures include speed of muscle contraction, food consumption, metabolism, immune response, development, and reproduction. Grasshoppers usually need to maintain body temperatures above ambient in order to optimize these processes.

The speed at which muscle contractions occur is highly dependent on temperature. This has profound effects on minimum temperatures at which any insect moves, and more importantly, flies. This dependence on a minimum temperature is so important that some insects

can even shiver their flight muscles to warm up to minimum flight temperatures (Heinrich 1996). In *S. gregaria*, the minimum temperature required for flight is around 20ºC, while optimum is around 35ºC (Uvarov 1966b). Feeding rates are also positively correlated with temperature; both chemical digestion and the muscle contractions of the digestive tract are temperature dependent. Whitman (1988) conducted a study on thermoregulation in *Taeniopoda eques* (Burmeister), part of which was measuring feeding rates at different temperatures. The study showed that as temperature increased, rates of feeding and defecation increased. Harrison and Fewell (1995), in a similar study on *M. bivittatus* (Say), showed that there were minimum thresholds for feeding, 10ºC for lab studies and 25ºC for field studies. They also showed there to be a maximum rate of consumption in the laboratory beyond which feeding does not increase even with an increase in temperature. This suggests an upper feeding threshold at which rate of consumption would exceed the maximum rate of digestion. In addition, Harrison and Fewell (1995) also showed that net energy intake increased dramatically from 0.008W at 15°C to 0.38W at 35°C. This was also accompanied by an increase in metabolic rate.

Gündüz and Gülel (2002) conducted a study on *S. gregaria*, investigating food consumption and body weight in relation to two different temperatures, 25°C and 30°C. They found that food consumption increases with each stage and then begins to decline after the end of the first week of adult life, and that during this time consumption increased with an increase in temperature. After the first week of adult life, temperature increases resulted in decreases in food consumption. Gündüz and Gülel reported high weight gain from first instar to the first week of adult life in high temperature treatments relative to low temperature treatments. However, average weight at the end of the experiment was not significantly different between treatments. They concluded that the high weight gain was due to increased food consumption rates, that

there was some critical weight which was needed for reproduction to begin, and that temperature does not affect the critical weight needed but how fast it is attained. In *M. sanguinipes,* body weight was lower at the low (21°C and 24°C) and high (39°C and 42°C) extremes of the temperatures tested relative to intermediate temperatures (Fielding 2004). It is possible that body weight was affected in this study and not the other because this study used sub-optimal temperatures.

Temperature also plays an important role in a grasshopper's ability to fight infection. Several studies have investigated levels of mycosis at different temperatures in grasshoppers. In *M. sanguinipes*, continuous exposure to high temperatures was detrimental to the mycosis of *Beauveria bassiana*. Nymphs that were inoculated with *B. bassiana* and then placed on a heat gradient remained in warmer areas of the gradient (Inglis et al. 1996). Carruthers (1992) performed a study on the mycosis of *Entomophaga grylli* in the clear winged grasshopper, *Camnula pellucida* (Scudder). The study showed that exposure to temperatures of 38ºC-40ºC for more than four hours each day was detrimental to survival of *E. grylli*. The ability of high temperatures to fight infection gives grasshoppers one more reason to maintain body temperatures well above ambient.

The effects of temperature on development and reproduction in grasshoppers can be substantial and have been well documented. A study by Begon (1983) showed that when 4th instars of *Chorthippus brunneus* (Thunberg) were exposed to a radiant heat source, developmental rate could be 5.6 times greater than when the grasshoppers were not exposed. The same study also showed that adult females held in cages with longer periods of radiant heat laid more egg pods than females in cages with shorter periods of radiant heat. Putnam (1963) conducted a study that shows development time in three species of grasshopper ranging from 53

days at 24ºC, to 17 days at 38ºC. In another study by Willot (1992) on four species of Acrididae, development and reproduction were either zero or very low at temperatures below 25ºC, while the optimum temperature for growth and development was between 35ºC and 40ºC. In *S. gregaria*, nymphal development was 10 days faster and sexual maturation 19 days earlier at 30°C when compared to 25°C (Gündüz and Gülel 2002). *Taeniopoda eques* required 60 days from nymph to adult at 25°C and only 35 days at 30°C (Whitman 1986). Parker (1930) showed an increase from 27°C to 32°C reduced length of larval development by 27 days in *M. mexicanus* Saussure.

While these studies show that temperature increases as small as 4°C can cut development time in half, it is likely that the more important parameter is how close the temperature approximates the optimal temperature. An Arizona population of *Hesperotettix viridis* (Thomas) did not develop at 15°C and 20°C and developed slowly at 23°C. At higher temperatures of 30°C, 35°C, and 40°C, development was the same, approximately 40 days (Gardner and Thompson 2001). Here, small increases of 5°C did not cause an increase in developmental time. Almost all studies show that increases in temperature can increase development, but the amount of increase and actual benefits associated with these increases depend highly on the thermal biology of the organism in question.

Temperature increases development by increasing biological processes in general, but this can also have a negative side effect. Increased metabolic rates tend to shorten an organism's life span. In one study conducted on *C. pellucida*, adults survived for 16 days at 37ºC and 32.6 days at 27ºC (Uvarov 1966a). At first glance one might conclude that those grasshoppers living at 27ºC have a higher fitness. However, at 27ºC females laid an average of only one egg pod, as opposed to the average of four egg pods laid at 37ºC (Uvarov 1966a). Even though the longevity

of the grasshopper had been reduced, its fecundity had quadrupled. These results were similar to those of Begon (1983).

Overall, basking can help a grasshopper maintain an optimal body temperature and increase development rate and egg production. Increases in development rate are beneficial to most grasshoppers, as adult grasshoppers have fewer natural enemies than nymphs, and the faster one reaches adulthood, the sooner one can leave behind certain risk factors (Kemp 1986). Increases in developmental rate due to temperature are also essential to the survival of some species of grasshoppers that live in cooler climates which could not complete their life cycle in a single season if it were not for the ability to bask and increase their developmental rate (Heinrich 1996). In a study on *T. eques*, Whitman (1988) found that this species required 850 degree-days to complete its life cycle, while the air temperature of the environment only provided 692 degree-days. The deficit in the supply of heat was made up by the grasshopper's ability to thermoregulate.

Most of the studies mentioned above were conducted at constant temperatures. While this simplifies the execution and analysis of experiments, it fails to mimic natural conditions. In nature, terrestrial organisms are submitted to daily fluctuations in temperature, often exceeding a 10°C difference (Petavy et al. 2001). Daily temperature fluctuation, which is most commonly thought of as a day / night cycle, is referred to as thermoperiod. Beck (1983) conducted a review on thermoperiod in insects and found that thermoperiod is known to affect development, fecundity, circadian rhythms, diapause, and biological clocks in insects. The effects of thermoperiod can differ between different insects; under cyclic temperatures some species will develop more rapidly, others will show no difference, and even a few will develop more slowly (Beck 1983). For example, the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera,

Pyralidae), exhibited no difference in development time under thermoperiodic conditions when compared to constant temperatures, but did exhibit larger late instar larvae under thermoperiodic conditions (Beck 1983). On the other hand the pitch plant mosquito, *Wyeomyia smithii* (Coquillett), developed more slowly under thermoperiodic conditions. However, larvae produced larger, heavier, and more fecund mosquitoes when compared to larvae reared under constant conditions (Beck 1983). While the benefits of thermoperiodic conditions in these two species may not be readily apparent in developmental data, their increased fitness becomes apparent in reproductive aspects of their life history. On the other hand, the benefits of thermoperiodic conditions may be blatantly obvious. Satar et al. (2005) tested the effects of thermoperiod on the aphid *Brevicoryne brassicae* (L.). At alternating temperatures of 25°C and 30°C, developmental time decreased, mortality decreased, longevity increased, and reproduction increased when compared to those reared at a constant 30°C even though they had received less overall heat.

Many studies regarding thermoperiod in insects have yielded ambiguous results and those that have produced clear results often conflict with results from other studies (Beck 1983). Studies dealing with thermoperiod often compare fluctuating treatments against a constant temperature at the average or midpoint temperature of the alternating treatment. The premise for this is that if development is the same under both alternating temperature and the midpoint temperature, then the relationship between development time and temperature can be assumed to be linear. However, the relationship is usually not linear and there is often a deceleration or acceleration of development rate which is called the Kaufmann effect (Petavy et al. 2001). In such situations, midpoint temperatures are not suitable for predicting development times under alternating conditions. An alternative must be used and is called the equivalent development temperature (EDT), which is the constant temperature that provides the same developmental time

as that observed under a given alternating temperature (Petavy et al. 2001). Petavy et al. (2001) conducted a study on relatively simple thermoperiods in *Drosophila*. They consisted of two 12 hr phases (day and night) and total of 14 treatments with mid range temperatures from 10°C to 27°C and amplitudes of 6°C to 22°C. Overall, there was a decrease in development time with increasing temperature. Mortality was 100% at alternating temperatures of 4°C/26°C, 9°C/33°C, and 21°C/34°C, and temperatures above 28°C caused increases in developmental time. As expected, mortality was caused by extremes in temperature, while suboptimal temperatures caused less severe deleterious effects. They determined that the relationship between development time and temperature is a positive function of amplitude and a negative function of midpoint. The study showed that development time under alternating temperatures can be up to 20% superior or inferior to the expected development time at the midpoint. Depending on the species, a given amount of heat from alternating temperature could provide some developmental advantage over the same amount of heat provided at constant temperature.

This poses a problem for the common practice of using degree-day accumulation based on average ambient temperature, or the midpoint, and known developmental rates obtained at constant temperatures to develop phenology models for pest management purposes. This method assumes there to be a linear relationship between life history traits and accumulated degree days. For many insects the relationship is not linear and helps explain why Kührt et al. (2005) report that in the codling moth and other insects there are time discrepancies between predicted population numbers and actual numbers observed in the field. Grasshopper phenology is a foundation of grasshopper management (Gardner and Thompson 2001) and understanding the effects of temperature on phenology is essential to developing reliable pest management techniques.

It is known that grasshoppers develop faster and in general are healthier when reared under day / night temperature cycles (Uvarov 1966b). More recently, in his study on *M. sanguinipes*, Fielding (2004) found that grasshoppers reared at alternating temperatures developed faster than those reared at the midpoint temperature. As with any terrestrial organism, grasshoppers are affected by daily temperature cycles. However, grasshoppers behaviorally thermoregulate by exposing themselves to sunlight, and changes in this behavior or availability of sunlight will affect the cycle of temperatures they experience, specifically daily fluctuations in temperature other than the day / night cycle most commonly investigated. There has been little field work conducted to see what types of temperature fluctuations grasshoppers experience under natural conditions, and investigated even less are the effects these fluctuations may have on life history traits. If development time is affected by the way in which heat is obtained and not just the absolute amount of heat obtained, then other factors such as fecundity and body size might also be affected.

The grasshopper *S. americana* (Drury) is commonly the most economically important grasshopper in Florida and has been known to cause severe damage to citrus and ornamental crops (Capinera 1993). In Florida it is known to have a spring or early summer generation followed by an autumn generation (Kuitert and Connin 1952). This coincides with the report that the numbers of nymphs increase in early summer and late September (Squitier and Capinera 2002a). The eggs of overwintering adults lack a prolonged diapause and hatch in the year they were deposited. Consequently, in Florida, *S. americana* overwinters as an adult (Squitier and Capinera 2002a). *S. americana* has adapted to nearly every Florida habitat (Squitier and Capinera 2002b). Like many *Schistocerca* species, it is a tree and shrub dweller and can usually be found in abundance in areas where both food and perches are available. Disturbed habitats

near the edges of crop fields and roads often contain large numbers of *S. americana*. Squitier and Capinera (2002b) report that abundance in pine plantations is about 20 times greater when pine trees are young and small than in mature pine stands. This may be due to several factors. One, a mature pine stand usually has a less diverse plant community as compared to younger stands (Squitier and Capinera 2002b). Second, fully grown pine trees may provide more shade than *S. americana* prefers. When Miles (1985) placed adults of *S. americana* in thermally heterogeneous environments, he found that on average they spent about 90% of their time in locations where thoracic temperature was 32°C – 44°C. Like many of the grasshoppers mentioned earlier, *S. americana* prefers to maintain high internal body temperatures and does so by basking. Hence, shaded habitats, such as mature pine stands, would likely not be preferred.

In 1991 there was an outbreak of *S. americana* in Florida. Considerable damage occurred in Pasco, Polk, Sumter, and Hernando counties (Capinera 1993b). According to Capinera (1993a), the outbreak was likely due to a 5-year drought which provided an abundance of sunshine and increased the ambient temperature. This was coupled with a mild winter in 1990-91 which allowed for high overwintering adult survival rates and abundance of suitable habitat. All of these combined factors may have induced a gradual population increase over several years to produce an outbreak population. Most grasshopper research is conducted on species that occur in the western United States where outbreak populations frequently occur. The concentration of work on other species and lack of frequent outbreaks of *S. americana* has caused this species to have not been well studied (Capinera 1993a). However, its close relation to two species well known for outbreaks, *S. gregaria* in Africa and *S. piceifrons* in Mexico, along with the 1991 outbreak underscores their potential to reach outbreak populations and warrants further study.

In preliminary laboratory testing, *S. americana* adults oriented themselves around a radiant heat source and reached temperatures of over 40ºC for short periods of time when allowed to position themselves in front of 100w incandescent light bulbs. Achieving temperatures this high in the field is very unlikely without the presence of direct sunlight. During hours when a heat source (in this case an incandescent light bulb) was provided, *S. americana* adults often did not maintain a constant temperature, but allowed their body temperature to fluctuate between 30ºC and 41ºC. This corresponds with the idea suggested by Kemp (1989), that grasshoppers will only use a portion of the heat available to them on a given day. These preliminary tests, along with observations that *S. americana* adults do not bask all hours of the day, suggest that sunlight is only required for a portion of the day. This is relevant in Florida, where during the summer there are very few days with no cloud cover, allowing for a full day of sunlight. Also, there are times when cloud cover can remain for several days, which could be detrimental to grasshopper development and fecundity.

Our first objective was to assess the effects of sunlight on the body temperature of *S. americana* in the field, to determine how adverse weather conditions such as rain and cloud cover may affect body temperature, and to ascertain what sorts of temperature fluctuations a grasshopper might experience throughout the course of a day. The second objective of this study was to determine the effects ecologically relevant cycles of frequency and amplitude of temperature change on development and fecundity by developing standardized alternating temperature regimes based on what grasshoppers experience in the field. Such information will allow for development of more accurate phenological models that are not based on ambient temperature, but more accurate body temperatures, not only for this species but for others as well.

CHAPTER 2
EFFECTS OF WEATHER AND BEHAVIOR ON BODY TEMPERATURE

Methods and Materials

The purpose of this portion of the study was to assess the effects of sunlight, adverse weather, and behavior on the body temperature of *S. americana* in the field, and more specifically, tries to determine what kinds of temperature fluctuations a grasshopper might experience due to these factors. To accomplish this, grasshoppers were wired to thermocouples and placed in the field where observations of body temperature, behavior and environmental conditions were recorded.

Study Site

Members of the genus *Schistocerca* are shrub and tree dwellers. Many of the shrubs and trees used as perches are also host plants, such as citrus. In an effort to promote natural behavior, citrus trees were used as the site of the field study. Three potted grapefruit trees (1.5 – 2m high) were placed on a light gray gravel surface which was assumed to have similar thermal and reflective properties as sandy soil, the normal substrate for citrus. The trees were arranged as if they were a single tree with a dense canopy and several protruding branches. Trees were watered as needed and occasionally sprayed with a copper solution to control scale insects (Hemiptera : Margarodidae). Trees were also fitted with ant barriers (Line Guard Inc., Elyria, OH) to deter predation by ants. Observations were made irregularly from mid-May to mid-November of 2005.

Measurement of Body Temperature

Grasshoppers used in the field study were pre-reproductive adult females of *S. americana* taken from a lab colony at the University of Florida. Only pre-reproductive females were used to prevent any differences in behavior that may be due to necessity of heat intake, which could differ between stage and sex. In order for continuous body temperatures to be taken,

grasshoppers were fitted with a permanent thermocouple (36 gauge, Teflon insulated, thermocouple wire, part# TT-T-36-SLE, Omega Engineering, Inc., Stamford, CT) (Fig. 2-1). The thermocouple consists of a separate copper and constantan wire, with a Teflon coating. One end of the pair was connected to a male terminal, copper to positive and constantan to negative (Sub Mini T/C Connector, Part # SMP-T-M, Omega Engineering, Inc., Stamford, CT). The copper side of the other end was threaded through a sewing needle (size 28), which was then inserted laterally into the central mesothorax, and pulled through so that the end of the wire runs completely through the thorax. The copper wire was then soldered to the constantan wire forming the site of temperature measurement, which was then pulled back into the center of the thorax. Hot glue (SuperPower Slow Setting Hot Melt Glue, Model No. BSS6-4, Arrow Fastener Co. Inc., Saddle Brook, NJ) was used to seal off the wounds and to hold the wire in place. There seemed to be no behavioral effects due to the process, and grasshoppers lived for more than a week in this state. Similar results have also been noted in other studies where thermocouple wires were inserted into the thorax of grasshoppers (Carruthers et al. 1992, Lactin and Johnson 1996). Later dissection of the wired grasshoppers showed there to be no damage to internal organs. This method allows for continuous temperature readings to be taken and is much more accurate than the common “grab and stab” method used in many other studies (Beck 1983, Begon 1983, Kemp 1986, Willot 1997, Blanford and Thomas 2000,).

Four grasshoppers were wired with thermocouples each morning the tests were to take place. One grasshopper was hot-glued to a branch near the center of the canopy to provide continuous shade (shade constrained grasshoppers), allowing the measurement of minimum temperatures that could be encountered by a grasshopper on that particular day. This could then be used as a relative ambient temperature measurement of the grasshopper and be compared with

readings for actual ambient air temperature. A second grasshopper was hot-glued to a green pipe cleaner which was attached to a branch fully exposed to sunlight and positioned so that the long axis of the body was perpendicular to the sun's rays (sun constrained grasshoppers), theoretically allowing the measurement of maximum temperatures that may be encountered. After a few minutes, both stationary grasshoppers quit struggling to get free, therefore minimizing any effects of metabolic heat. The fully exposed grasshopper only began struggling again when it began reaching lethal temperatures. The remaining two grasshoppers were marked with either red or blue lettering enamel (Sign Painters' 1 Shot Paint Peinture Lettering Enamel, 153-L Process Blue and 165-L Rubine Red, Consumers Paint Factory, Inc., Gary, IN) on the hind tip of the forewings. This allowed them to be easily distinguished when behavioral observations were being recorded. These two grasshoppers were placed on the grapefruit trees to roam freely, allowing them to behaviorally thermoregulate and to maintain desired body temperatures. Free roaming grasshoppers were provided with enough thermocouple wire to allow unlimited movement within the trees. This provided measurements of preferred temperatures in contrast to the minimum and maximum measurements provided by the two stationary grasshoppers.

Once all four grasshoppers had been placed on the trees, they were connected to data loggers (Easy View Dual Input Thermometer, model# EA15, Extech Instruments, Waltham, MA). Each data logger had two terminal inputs; hence, one logger was used for the shaded and exposed grasshoppers and one for the two free roaming grasshoppers. The data loggers were housed in plastic containers with a watertight lid. Temperature readings were logged every ten seconds throughout the duration of the tests to provide a more accurate view of how quickly internal body temperature responded to weather changes.

Behavioral Observations

After the data loggers had been started and it had been determined that all were working properly, grasshoppers were allowed to settle for a short period before behavioral monitoring began. Visual monitoring took place every 15 min. during the time when grasshoppers were most actively thermoregulating, usually from the start of the experiment (9:00 a.m.) until about 4:00 p.m. Behavioral observations for the free-roaming grasshoppers were recorded as five different categories: completely shaded, partial sun exposure (about 25-75% of body surface) with a parallel orientation, partial sun exposure with a perpendicular orientation, complete sun exposure with a parallel orientation, and complete sun exposure with a perpendicular orientation. These behavioral orientation responses were then numerically rated as 0, 0.375, 0.50, 0.75, and 1.00, respectively. Previous research (Uvarov 1966b) has shown that orientation of the long axis of the body plays an important role in temperature regulation and that a perpendicular orientation provides for higher attainable temperatures. Therefore, a rating of 1.00 represents a grasshopper receiving the most solar radiation possible. The 0.75 rating for the parallel orientation was derived from measurements taken that showed a 25% reduction in body surface area exposed to sunlight when moving from perpendicular to parallel orientation. Each rating was then arbitrarily halved for partial exposures. After behavioral observations were made, any tangles that may have developed in the wires since the last observation were removed. Also, during this time the two stationary grasshoppers were checked to make sure they remained in position, and that the exposed grasshopper's orientation remained perpendicular. Adjustments needed to maintain a perpendicular orientation were accomplished by repositioning the pipe cleaner. A total of 6 d (2 grasshoppers on each day) yielded behavioral data suitable for statistical analysis.

Environmental Conditions

Measurements taken throughout the test period included sunlight intensity, ambient temperature, and humidity. Sunlight intensity was taken every second and the average recorded every 10 sec as W/m^2 with a Silicon Pyranometer smart sensor (Part # S-LIB-M003, Onset Computer Corp., Bourne, MA) attached to a Hobo Micro Station (Part # DOC-H21-002, Onset Computer Corp., Bourne, MA). The light sensor was placed as close to the test trees as possible without becoming shaded. Temperature and humidity readings were recorded every 10 sec with a Hobo data logger (Hobo U12 Temp/RH/Light/External Data Logger, Part # U12-012, Onset Computer Corp., Bourne, MA) placed on top of a plastic plate supported by cork legs, which was then placed in the shade of a building away from any vegetation.

Statistical Analysis

Descriptive statistics for field data were calculated using Microsoft Excel (Microsoft Corporation, Redmond, WA). A simple linear regression was performed on sunlight intensity and percent temperature difference between sun constrained grasshoppers and ambient temperature. Simple linear regressions were also performed on mean behavior and ambient temperature, and temperature differences between constrained grasshoppers and free roaming grasshoppers and behavior. Body temperatures, ambient temperature, % RH, and sunlight intensity for each day were plotted. Rates of temperature increase and decrease were calculated using the linear portions of body temperature plots. SAS Analyst 9.0 (SAS Institute Inc., Cary, NC) was used to perform multiple linear regression analysis and plot partial regressions on the combined data of body temperatures and behavior, ambient temperature, and sunlight intensity from June 29, July 5, and July 8, 2005.

Results

Measurement of Body Temperature

Body temperature, ambient temperature, % RH, and sunlight intensity were plotted for each of the 7 d tested (Fig. 2-2 – 2-9). For each of the 7 d tested, mean values of body temperature were calculated (Table 2-1). Because of the very large sample sizes (>2500), even extremely small differences between body temperatures (such as 0.05°C) were considered significant by ANOVA, causing every temperature to be considered different. Therefore, this information was omitted from the table.

As expected, sun constrained grasshoppers attained the highest maximum body temperature of all the grasshoppers tested, with the exception of one day tested in November (Table 2-1). What was not expected was how high maximum body temperature reached. On three of the 7 d tested, internal body temperatures rose above 50°C, causing thermal death. Increased respiration rates were observed in sun constrained grasshoppers at such high temperatures, possibly as a cooling mechanism (Heinrich 1993, Casey 1981). Unfortunately, this behavior could not be maintained indefinitely and eventually some of the grasshoppers reached lethal temperatures. Body temperatures at their maximum were 12.4-19.6°C above ambient temperature, often around 75% above ambient temperature. Sun constrained grasshoppers were meant to represent the highest attainable temperature for that day. For the most part this was true, but there were factors acting on sun constrained grasshoppers, such as wind and rain, that the free roaming grasshoppers could avoid. Consequently, mean body temperatures of sun constrained grasshoppers were not always the highest throughout the day.

Shade constrained grasshoppers tended to have the lowest average body temperatures for each day, with the exception of the two hottest and sunniest days, July 5 and July 8, where the

body temperature of the shade constrained grasshopper was very similar to those of the free roaming grasshoppers (Table 2-1). On these days, free roaming grasshoppers spent most of their time shaded from direct sunlight and consequently had body temperatures similar to those of the shade constrained grasshopper. On five of the seven days tested, shade constrained grasshoppers had a mean body temperature greater than ambient temperature, with one day having a maximum body temperature that was 26.9% above ambient temperature. The highest body temperature reported in a shade constrained grasshopper was on the hottest day tested, July 8, where body temperature reached 41.3°C.

Mean body temperatures for free roaming grasshoppers were somewhat harder to generalize, but for the most part they were between those for shade and sun constrained grasshoppers (Table 2-1). With the exception of November 17, maximum body temperature of free roaming grasshoppers never surpassed the maximum for sun constrained grasshoppers. On all days but November 17, free roaming grasshoppers raised their body temperature above 40ºC for short periods of time, which corresponds well with what was found in preliminary laboratory tests.

On several occasions, night-time body temperatures were also recorded for all four grasshoppers. For the most part, all grasshoppers maintained body temperatures very similar to that of ambient, usually within 1ºC above or below ambient.

Environmental and Behavioral Observations

For each of the days where data were available, mean values of sunlight intensity, ambient temperature, relative humidity, behavior, as well as differences between constrained grasshopper body temperatures and ambient temperature were calculated (Table 2-1). Measurements of sunlight intensity were available for four of the days tested. The higher the

mean sunlight intensity the higher the mean percent difference between body temperatures of sun constrained grasshoppers and ambient temperature. Simple linear regression analysis between percent difference in temperature and sunlight intensity produced an R^2 value of 0.99 (Fig. 2-10). A multiple linear regression analysis with sun constrained body temperature and ambient temperature and sunlight intensity revealed significant positive linear relationships between body temperature and both ambient temperature and sunlight intensity (Table 2-2, Fig. 2-11). Not surprisingly, sunlight was the most significant factor, accounting for 0.8664 of the total r^2. The data tend to be clustered in the partial regression plots because most body temperatures of sun constrained grasshoppers were on the higher end and at high levels of sunlight. A multiple linear regression analysis with the body temperature of shade constrained grasshoppers and ambient temperature and sunlight intensity also revealed significant positive linear relationships between body temperature and both ambient temperature and sunlight intensity (Table 2-2, Fig. 2-12). In contrast to the regression analysis for the sun constrained grasshopper, ambient temperature was the most significant factor, accounting for 0.9539 of the total r^2. The models given for predicting body temperature of sun and shade constrained grasshoppers (Figs. 2-11c, 2-12c) were found to be reliable.

Unlike the constrained grasshoppers, free roaming grasshoppers were allowed to thermoregulate behaviorally. Both parallel and perpendicular orientations to the sun were observed, but no grasshopper was ever observed in the flanking position. As a general rule, grasshoppers tended to begin basking immediately in the morning sun after being placed on the test trees. In all but one instance, the grasshopper with the higher mean behavioral orientation rating (the one that spent more time basking) of the two free roaming grasshoppers had the higher mean daily body temperature (Table 2-1). Increases in mean behavioral orientation rating

for different days reflected increases in the mean difference between the body temperature of free roaming grasshoppers and those of shade constrained grasshoppers (Fig. 2-13a). Conversely, increases in mean orientation behavior caused decreases in the mean difference between body temperature of free roaming grasshoppers and those of sun constrained grasshoppers (Fig. 2-13b). Mean daily behavioral orientation rating decreases with increases in mean daily ambient temperature (Fig. 2-14).

A multiple linear regression analysis between the body temperatures of free roaming grasshoppers and ambient temperature, sunlight intensity, and behavioral orientation rating showed significant positive linear relationships between body temperature and all three parameters (Table 2-2, Fig. 2-15). All three parameters were found to be significant contributors to the variation in body temperature, with ambient temperature being the most significant factor, accounting for 0.7819 of the total r^2 while behavior only accounted for 0.0063 of the total r^2. The relationship between body temperature and ambient temperature and sunlight intensity for free roaming grasshoppers follows somewhat similar patterns to those of the constrained grasshoppers (Figs. 2-15a, 2-15b). Unlike the data for constrained grasshoppers, the data for free roaming grasshoppers are much more dispersed along the y-axis, indicating some effect of behavior. The partial regression plot for body temperature and behavior (Fig. 2-15c) takes on a columnar appearance. However, a pattern still emerges; as behavioral orientation rating increases there is a slight increase in body temperature. The model for free roaming grasshoppers (Fig. 2-15d) is not as accurate as those for constrained grasshoppers.

Temperature Fluctuations and Adverse Weather Conditions

Rates of body temperature increase in sun constrained grasshoppers were calculated from linear portions of body temperature plots where sunlight intensity was 800 W/m^2 or greater

(sunny conditions). Sunlight intensity fluctuated too rapidly to allow for rates of temperature increase to be measured at a constant intensity. The mean (± SD) rate of body temperature increase for sun constrained grasshoppers was 1.24°C/min ± 0.009, with a maximum of 2.5°C/min, and a minimum of 0.71°C/min.

Large fluctuations in body temperature (> 15% change in temperature) occurred several times a day, averaging 3.67 ± 1.50 (Mean ± SD) changes per day, and were often the direct consequence of changes in weather or sunlight intensity (Figs. 2-2 -2-9), with a few due to behavioral thermoregulation. We used a 15% change in temperature to define a large fluctuation. This eliminated from the calculations, any small changes that might have been caused by very brief changes in sunlight intensity, where the full effect of the change could not have been observed. Additionally, body temperatures of sun and shade constrained grasshoppers show a high frequency of lower amplitude temperature changes not caused by cloud cover or rain which were also eliminated by the use of the > 15% rule. The rates of change and amplitude (% temperature change) were calculated for large temperature fluctuations caused by cloud cover or rainfall (Table 2-3). Cloud cover caused a higher mean rate of temperature decrease than did rainfall, but had a lower mean % decrease. The frequency and amplitude of temperature fluctuations for the laboratory portion of this study were based on these observations.

The lowest temperature recorded as a result of adverse weather conditions, 20.1°C, was due to rain on June 22 and was 7.6% below ambient temperature. On June 22 and June 29, when frequent cloud cover and rainfall were recorded, mean body temperatures were up to 8ºC lower for all grasshoppers than on predominately sunny days (Table 2-1).

Discussion

Measurement of Body Temperature

Sun constrained grasshoppers achieved body temperatures that were 12.4-19.6°C above ambient. Fielding (2004) reported similar results of 15-20°C above ambient temperature. However, Fielding's grasshoppers were at ground level and dead, reducing both convective and evaporative cooling. The grasshoppers in the current study may have reached even higher temperatures had they been treated in a similar manner. Uvarov (1966a) reports that in *L. migratoria* maximum temperatures reach 42.7ºC, but does not specify the source of radiant heat. *S. americana* exhibited maximum temperatures several degrees higher (Table 2-1), even in free roaming grasshoppers. Though sun constrained grasshoppers did not always attain the highest body temperatures, they provided a reasonable representation of maximum body temperatures that may be attainable by a grasshopper on a summer day in Florida. The body temperatures of sun constrained grasshoppers reported in this study were often around 75% above ambient temperature. Theoretically, grasshoppers with the ability to thermoregulate could also reach such temperatures and, in fact, they do. The fact that grasshoppers possess the ability to raise their body temperature so high above ambient, puts into question the method of using mean ambient temperature to model phenology and population sizes. Surprisingly, body temperatures of sun constrained grasshoppers attained lethal levels (> 50°C) when exposed to direct sunlight, emphasizing the need for cooling. To the author's knowledge there is no other study that documents thermal death in *Schistocerca* species from exposure to direct sunlight.

Shade constrained grasshoppers, for the most part, had the lowest mean daily body temperature. However, there were 5 d when mean daily body temperatures were above ambient, sometimes as much as 26%. The likely explanation for shade constrained grasshoppers having a

higher body temperature than ambient is that the grasshoppers were located within the grapefruit trees and surrounded by vegetation. Sheltering vegetation can provide some amount of insulation by reducing air flow and preventing heat escape, causing air temperature within the trees to be different from that measured out in the open air. The surface of the vegetation can also reflect light back into the canopy and possibly increase temperature even more. The thermal and reflective properties of the gravel also likely added to the increased temperature. In addition, when night time temperatures were recorded they were shown to be very similar to ambient, suggesting that temperature differences between ambient and shade constrained grasshoppers were caused by radiant energy from the sun being trapped within the tree canopy. These data show that even when completely shaded, grasshoppers will experience body temperatures much higher than that of ambient temperature (up to 26%) due to differences in microclimate, and that measurement of microclimatic conditions would likely be more appropriate for modeling purposes.

As expected, the mean daily body temperatures of free roaming grasshoppers were usually between the body temperatures of the constrained grasshoppers, which represent the extremes in temperature that could be experienced. Kemp (1989) suggested that grasshoppers do not utilize all of the available heat in a day and that sunlight is not required all hours of the day for optimal body temperature to be maintained. The data from this study correspond with this on days when ambient temperature is high and sunlight is available for most of the day. Data from July 5 and 8 shows that mean body temperatures of free roaming grasshoppers were well below those of sun constrained grasshoppers, the theoretical maximum (Table 2-1). On days when ambient temperature was cooler or sunlight was limited, grasshoppers seemed to be attempting to utilize most of the heat available to them. Data from June 14, 17, 22, and 29 shows increased

basking behavior and body temperatures of free roaming grasshoppers very close to, and in two instances higher than, those of sun constrained grasshoppers (Table 2-1). However, free roaming grasshoppers did not bask every moment of the day even on cooler days. All of this suggests that there is a certain amount of heat a grasshopper needs obtain to achieve optimal fitness and that they will not utilize more heat than is necessary to do so. High body temperatures do have negative side effects and would be avoided when there is no longer a benefit provided by increasing body temperature. July 5 and 8 would represent days when more than enough heat was available, while June 14, 17, 22, and 29 were days where heat was more limited and grasshoppers took advantage of most of the heat that was available.

November 17, 2005 was the only day tested during the winter months. November 17 provided some interesting data. As mentioned earlier, sun constrained grasshoppers attained the highest maximum body temperature of all the grasshoppers tested, with the exception of this day, when free roaming grasshoppers attained the highest maximum and mean temperatures. November 17 was the coolest day tested, and was characterized by a stiff breeze and low sunlight intensity. Because there were no rainfall events, the most likely explanation for lower sun constrained body temperatures is that the brisk breeze prevented the exposed sun constrained grasshopper from reaching higher temperatures, while free roaming grasshoppers might have been able to position themselves to bask while avoiding exposure to the wind. What is also interesting about November 17 is that the mean body temperature of the shade constrained grasshopper was below mean ambient temperature, and is probably a result of the high wind speed and low sunlight intensity. This helps support the idea that, on dates other than November 17, radiant energy from sunlight trapped by the vegetation was causing shade constrained grasshopper temperatures to rise above ambient.

Environmental and Behavioral Observations

A simple linear regression revealed a very significant positive relationship (R^2=0.99) between mean sunlight intensity and the mean difference between body temperatures of sun constrained individuals and ambient temperature. As expected, the higher the mean sunlight intensity, the higher the body temperature rose above ambient temperature. When a multiple regression analysis was conducted on the raw data, accounting for the effects of both sunlight and ambient temperature on body temperature, sunlight was the most significant factor, accounting for 0.8664 of the total r^2 (Table 2-2). Such a dependency of body temperature on sunlight was expected in sun constrained grasshoppers, and shows how influential sunlight can be on body temperature. These results are in agreement with many other studies. Lactin and Johnson (1997) found direct sunlight to significantly affect grasshopper body temperature. Fielding (2004) also reported a very strong relationship between grasshopper body temperature and sunlight. The majority of data points shown in the plot of predicted body temperatures vs. actual body temperatures given by the multiple regression analysis (Fig. 2-11c) follow the general linear pattern predicted by the model. A model predicting the body temperature of constrained grasshoppers is not likely to prove useful in trying to model the phenology and population of free moving grasshoppers; however, the model does provide some interesting information. It provides a method for calculating a rough estimate of maximum grasshopper body temperature when the ambient temperature and sunlight intensity are known. Additionally, there are some outlying data points which seem to reduce the model's predictive power. These points are artifacts of the delayed response of body temperature to changes in sunlight intensity. Changes in sunlight intensity were recorded immediately, while the effects these changes had on body temperature took some time to manifest themselves in the body temperature recordings.

In contrast to sun constrained grasshoppers, the body temperature of shade constrained grasshoppers was much less dependent on sunlight intensity and more dependent on ambient temperature. The multiple regression analysis showed ambient temperature having a linear relationship with body temperature and accounting for 0.9539 of the total r^2 (Table 2-2). This result was expected, because shade constrained grasshoppers were denied access to direct sunlight. Therefore, the body temperature of shade constrained grasshoppers should be very dependent on ambient temperature. This is partially supported by the fact that the data for shade constrained grasshoppers in the partial regression plot between body temperature and sunlight intensity (Fig. 2-12b) are much more dispersed along the y-axis than the data for the equivalent sun constrained plot (Fig. 2-11b). In addition, the plot of predicted vs. actual body temperatures (Fig. 2-12c) does not exhibit nearly as many outlying values as that of the plot for sun constrained grasshoppers (Fig. 2-11c). This further shows the reduced effect of sunlight on body temperature in shade constrained grasshoppers. It is suspected that the effect of sunlight on body temperature and the positive relationship between the two (Fig. 2-12b) are due to the partial dependency of ambient temperature on sunlight intensity (even though tests for co-linearity were negative) and differences in microclimatic conditions which would also be partially dependent on sunlight intensity.

Blanford and Thomas (2000), among many others, report a non-linear relationship between ambient temperature and body temperature in grasshoppers. They report that body temperatures reach equilibrium at higher ambient temperatures. There is a simple explanation for the discrepancy between their study and this study. They were trying to show behavioral thermoregulation and at very high temperatures grasshoppers were actively cooling their bodies, while at cooler temperatures grasshoppers were basking to raise their body temperature. In the

current portion of this study, behavior has been removed from the equation for constrained grasshoppers and body temperature is solely dependent on environmental conditions. For this reason there is a linear relationship between body temperature of shaded grasshoppers and ambient temperature.

Free roaming grasshoppers adopted both parallel and perpendicular orientations. The fact that grasshoppers were never observed in a flanking position (when a grasshopper raises one hind leg and lowers the other while in a perpendicular orientation to maximize exposure) is not entirely surprising. Flanking has never been reported in *S. americana*. Unfortunately, the experiment was not designed to investigate how body orientation and body temperature interact, and the duration of orientation was never recorded. Such information is necessary to determine how orientation directly affects body temperature in real time and vice versa. However, mean behavioral ratings were used to determine the effects of behavior on mean daily body temperature. Increased mean behavioral orientation rating reflected increases in the mean difference between the body temperature of free roaming grasshoppers and those of shade constrained grasshoppers (Fig. 2-13a) and decreases in the mean difference with sun constrained grasshoppers (Fig. 2-13b). As grasshoppers spend more time basking, their mean body temperatures rise above those of shaded grasshoppers and toward those of sun constrained grasshoppers which proves behavior has an effect on body temperature in *S. americana*. Additionally, of the two grasshoppers tested each day, the one with the higher mean behavioral orientation rating always had a higher mean body temperature for that day. This shows that the behavioral orientation ratings used in this study were suitable for describing grasshopper behavior with respect to sunlight absorption. The fact that mean daily behavioral orientation rating decreases with increases in mean daily ambient temperature (Fig. 2-14) corresponds with

the previous idea that grasshoppers do not use all the heat available to them on very warm days. It also shows that grasshopper behavior is dependent on environmental conditions. The more ambient heat that is available to a grasshopper, the less heat it must obtain through basking. Behavior obviously has an effect on body temperature, and the linear plots of body temperature (Figs. 2-2 - 2-9) reinforce this fact even further by showing differences in body temperature between the two different free roaming grasshoppers for each day which experienced the same exact environmental conditions. The differences alone do not provide enough evidence for behavioral thermoregulation, but help make a strong case in conjunction with the other behavioral data, especially the comparison between mean behavioral rating and mean ambient temperature (Fig. 2-14).

The multiple regression analysis for free roaming grasshoppers revealed results similar to those for constrained grasshoppers. Both sunlight and ambient temperature had significant positive linear relationships. Again, the relationship between body temperature and ambient temperature remained linear. Behavior was also found to be a significant factor. However, behavior only accounted for a small portion of the total r^2 (Table 2-2). The data plotted for the partial regression plot between behavioral orientation and body temperature (Fig. 2-15c) tries to show the direct effects of behavior on body temperature. However, this data takes on a columnar appearance because the times of data collection for a continuous variable, body temperature, do not perfectly coincide the times of data collection for a discontinuous variable, behavior. As mentioned before, the duration of behavioral orientations were not recorded and behavior was only recorded every 15 min, then paired with the corresponding 15 min of temperature data. This data cannot account for any changes in behavior between the 15 min intervals. Even so, a general trend can be seen, that as behavioral orientation rating increases so does body temperature (Fig.

2-15c). Unlike the data for constrained grasshoppers, the data for free roaming grasshoppers are much more dispersed along the y-axis (Figs. 2-15a and 2-15b). This is most likely because behavior allowed grasshoppers to maintain similar body temperatures over a broader range of environmental conditions and shows how behavior can affect body temperature by controlling for environmental conditions.

Why ambient temperature is the most significant factor contributing to the variation in body temperature in free roaming grasshoppers is uncertain. It could be that the behavioral data are discontinuous and do not accurately represent how behavior affects body temperature. It is more likely, though, that the effects of ambient temperature on body temperature are not affected by grasshopper behavior (unlike the effects of sunlight which can be avoided by seeking shade) and, therefore, ambient temperature is the most consistent factor affecting body temperature. The fact that the model for free roaming grasshoppers (Fig. 2-15d) is not as accurate as those for constrained grasshoppers is likely due to the time discrepancies and discontinuous nature of the behavioral observations.

Temperature Fluctuations and Adverse Weather Conditions

Rates of body temperature increase in sun constrained grasshoppers averaged 1.24°C/min ± 0.009 (mean ± SD). These results are somewhat similar to the rate of increase of 0.828°C/min in *Locusta* reported by Uvarov (1966a). Lactin and Johnson (1997) provide a measurement of overall temperature increase per W/m^2, but give no measurement of rate. The maximum rate recorded, 2.5°C/min, is very high and at such rates grasshoppers could reach optimal body temperature in a very short period of time. Rates of decrease and % decrease caused by adverse weather conditions (Table 2-3) show just how significant the impact of cloud cover or rain can be on grasshopper body temperature. Unexpectedly, cloud cover caused a faster rate of decrease

than did rainfall. However, rates were calculated using the temperature when the event started and the lowest temperature recorded within the decrease. Rainfall caused much larger percentage decreases in temperature than did cloud cover, and at lower temperatures the rate of body temperature decrease tapered off, therefore causing decreases due to rainfall to have a reduced rate. There were many small fluctuations in grasshopper body temperature throughout the day. These changes are possibly due to several factors, including small changes in sunlight intensity, changes in evaporative cooling, and changes in wind speed. The timing of these small changes in body temperature is similar between sun and shade constrained grasshoppers, but the amplitude of change is often larger in sun constrained grasshoppers than in shade constrained grasshoppers. Sun constrained grasshoppers were more exposed to small changes in both wind speed and sunlight intensity, whereas shade constrained grasshoppers were sheltered from such changes by vegetation. All of these observations suggest that these small temperature changes are due mainly to small changes in abiotic factors.

Aside from limiting a grasshoppers ability to achieve optimal body temperature, adverse weather conditions caused grasshoppers to experience an average of 3.67 large fluctuations in body temperature (> 15%) during daylight hours. When trying to model insect populations, it is common to use data collected from subjects reared under constant conditions. Whether or not it is proper to use such data should be investigated on an individual basis. Depending on the answer, it might be possible to use daily averages for environmental parameters to model insect populations, or it may be necessary to record and include fluctuations that occur in environmental parameters. The next part of this study attempts to determine if there are developmental and reproductive consequences associated with daily fluctuations in temperature, such as those observed in the field portion of this study

Table 2-1. Grasshopper body temperatures and behavioral orientation ratings, and environmental data (mean ± SD) obtained from field studies. Differences between constrained grasshoppers and ambient temperature are given in percent difference. See text for explanation of treatments and environmental parameters.

	June 14 2005					June 17 2005					June 22 2005				
Parameter	Mean			Min	Max	Mean			Min	Max	Mean			Min	Max
Free Roaming Blue (°C)	37.03	±	2.99	29.80	44.40	37.37	±	2.16	32.90	43.40	28.19	±	7.42	20.90	47.20
Blue Behavior	0.56	±	0.43			0.38	±	0.41			0.73	±	0.40		
Free Roaming Red (°C)	35.61	±	2.61	30.60	42.70	41.71	±	1.84	34.70	44.90	27.45	±	6.25	20.80	43.80
Red Behavior	0.36	±	0.35			0.78	±	0.23			0.46	±	0.45		
Shade Constrained (°C)	33.61	±	2.52	28.20	38.80	33.82	±	1.87	28.10	36.10	26.20	±	4.27	21.10	37.10
Sun Constrained (°C)	39.31	±	3.11	33.20	45.80	40.36	±	3.30	32.60	45.70	28.13	±	7.77	20.20	50.40
Ambient Temperature (°C)	30.41	±	1.77	27.80	33.40	28.95	±	1.45	26.60	31.40	27.68	±	1.37	25.10	30.80
Shade Const. - Ambient (%)	10.44	±	2.91	1.40	18.30	16.86	±	4.67	-0.60	26.90	8.76	±	6.26	-7.90	22.10
Sun Const. - Ambient (%)	29.64	±	12.44	2.69	62.60	39.35	±	8.58	14.94	55.90	30.26	±	18.22	-7.60	76.40
% RH						58.43	±	8.66	36.80	76.70	68.19	±	5.91	54.30	79.90
Sunlight Intensity (w / m^2)															

Table 2-1. Continued.

	July 5 2005					July 8 2005				
Parameter	Mean			Min	Max	Mean			Min	Max
Free Roaming Blue (°C)	38.17	±	1.45	33.80	41.60	37.40	±	1.85	32.50	41.30
Blue Behavior	0.12	±	0.24			0.06	±	0.13		
Free Roaming Red (°C)	38.32	±	1.87	32.10	42.90	37.35	±	2.09	31.60	42.20
Red Behavior	0.13	±	0.25			0.07	±	0.15		
Shade Constrained (°C)	37.68	±	1.68	32.80	40.10	37.76	±	2.27	32.10	41.30
Sun Constrained (°C)	45.90	±	2.96	33.20	52.10	46.13	±	3.42	33.50	51.60
Ambient Temperature (°C)	32.20	±	1.04	28.50	33.30	32.58	±	1.25	28.70	34.50
Shade Const. - Ambient (%)	17.00	±	2.29	9.56	21.35	15.83	±	3.55	2.90	22.90
Sun Const. - Ambient (%)	42.47	±	6.08	16.60	57.70	41.66	±	9.90	2.40	56.20
% RH	51.07	±	3.28	46.50	63.70	56.19	±	6.52	47.80	74.10
Sunlight Intensity (w / m^2)	801.40	±	99.50	420.60	979.40	805.70	±	204.80	0.60	1014.40

Table 2-1. Continued.

	June 29 2005					November 17 2005				
Parameter	Mean			Min	Max	Mean			Min	Max
Free Roaming Blue (°C)	30.30	±	3.62	25.30	40.40	27.21	±	4.28	17.30	38.40
Blue Behavior	0.60	±	0.40				±			
Free Roaming Red (°C)	30.88	±	3.92	25.10	41.40	27.63	±	5.12	18.70	37.60
Red Behavior	0.60	±	0.37				±			
Shade Constrained (°C)	28.46	±	2.29	25.40	33.60	21.47	±	2.60	13.80	24.80
Sun Constrained (°C)	31.84	±	4.57	25.30	47.80	26.35	±	3.94	15.80	33.10
Ambient Temperature (°C)	27.29	±	1.09	25.00	29.10	21.84	±	1.43	20.70	27.40
Shade Const. - Ambient (%)	4.13	±	4.79	-5.70	17.20	-0.90	±	15.19	-40.90	16.33
Sun Const. - Ambient (%)	16.41	±	13.55	-6.60	66.60	21.58	±	21.79	-29.70	55.90
% RH	81.18	±	4.32	71.50	87.20	29.29	±	2.18	24.60	33.90
Sunlight Intensity (w / m^2)	316.80	±	223.40	39.40	1164.40	460.20	±	196.80	51.90	731.90

Table 2-2. Regression statistics for multiple regressions between grasshopper body temperatures and environmental parameters from June 29, July 5, and July 8 2005.

		SS	F-value	P	r^2	Variable	Parameter Estimate	t value	P	Type II SS	Partial r^2
Sun Constrained	Model	489263	50939	<0.0001	0.93	Intercept	-6.6499	-17.12	<0.0001	1408	
	Error	37531				Ambient Temperature	1.2070	84.03	<0.0001	33910	0.0644
						Sunlight Intensity	0.0165	138.46	<0.0001	92073	0.8644
Shade Constrained	Model	180960	141308	<0.0001	0.97	Intercept	-13.9944	-98.69	<0.0001	6236	
	Error	5003				Ambient Temperature	1.5149	288.82	<0.0001	53412	0.9539
						Sunlight Intensity	0.0033	74.59	<0.0001	3562	0.0192
Free Roaming	Model	192781	18664	<0.0001	0.82	Intercept	-1.4235	-4.14	<0.0001	59	
	Error	42494				Ambient Temperature	1.0921	86.68	<0.0001	25867	0.7819
						Sunlight Intensity	0.0049	46.30	<0.0001	7382	0.0312
						Behavior	1.2106	20.68	<0.0001	1472	0.0063

Table 2-3. Rate of temperature decrease and percent temperature decrease (mean ± SD) of body temperature in sun constrained grasshoppers under cloudy or rainy weather conditions.

Condition	Rate of Decrease	Min	Max	% Decrease	Min	Max
Cloud Cover	0.0163 °C/sec ± 0.0063	0.0074 °C/sec	0.0269 °C/sec	22.34 ± 3.98	17.60	31.20
Rain Fall	0.0087 °C/sec ± 0.0058	0.0031 °C/sec	0.0146 °C/sec	38.83 ± 9.49	30.20	49.00

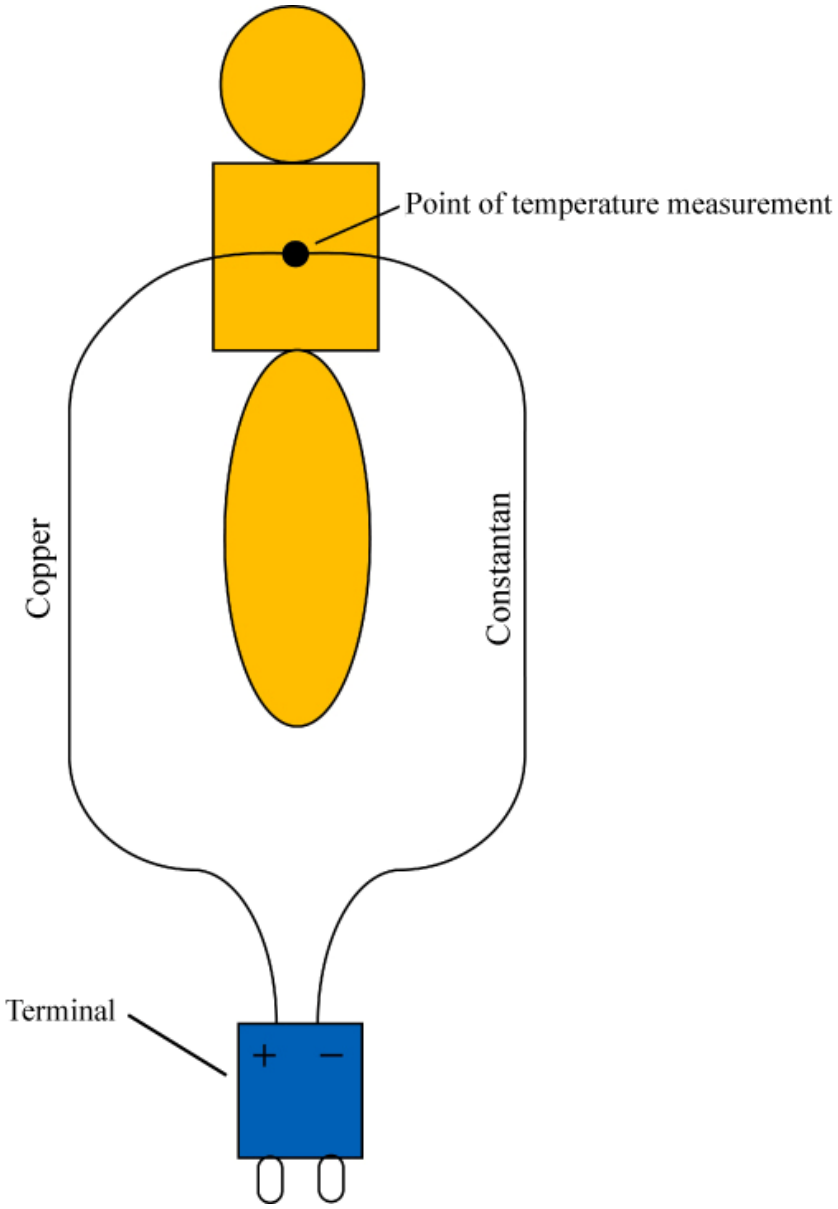

Figure 2-1. Diagram of wiring method for recording internal body temperature of grasshoppers showing how to connect copper and constantan wires.

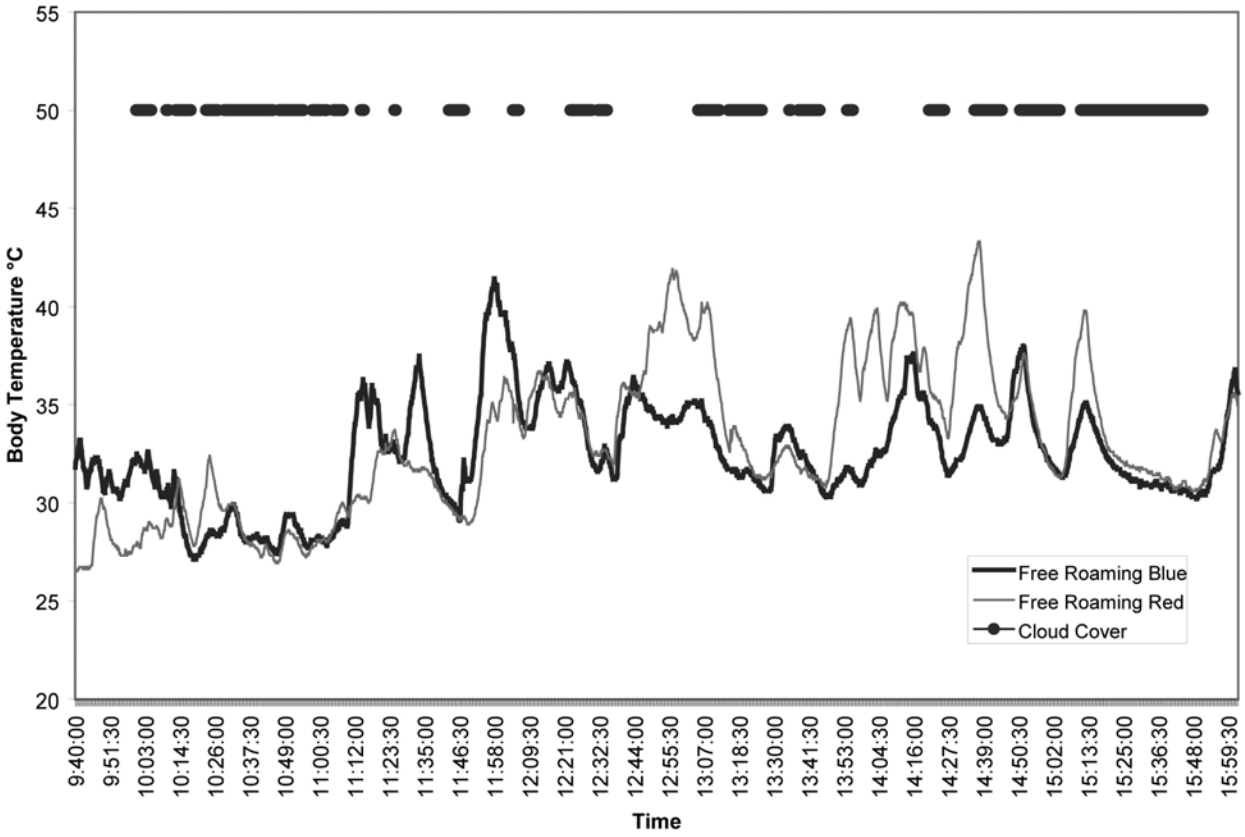

Figure 2-2. Linear plot of continuous body temperatures of free roaming grasshoppers and cloud cover from May 19 2005. Cloud cover is represented by the discontinuous bars in the upper portion of the plot.

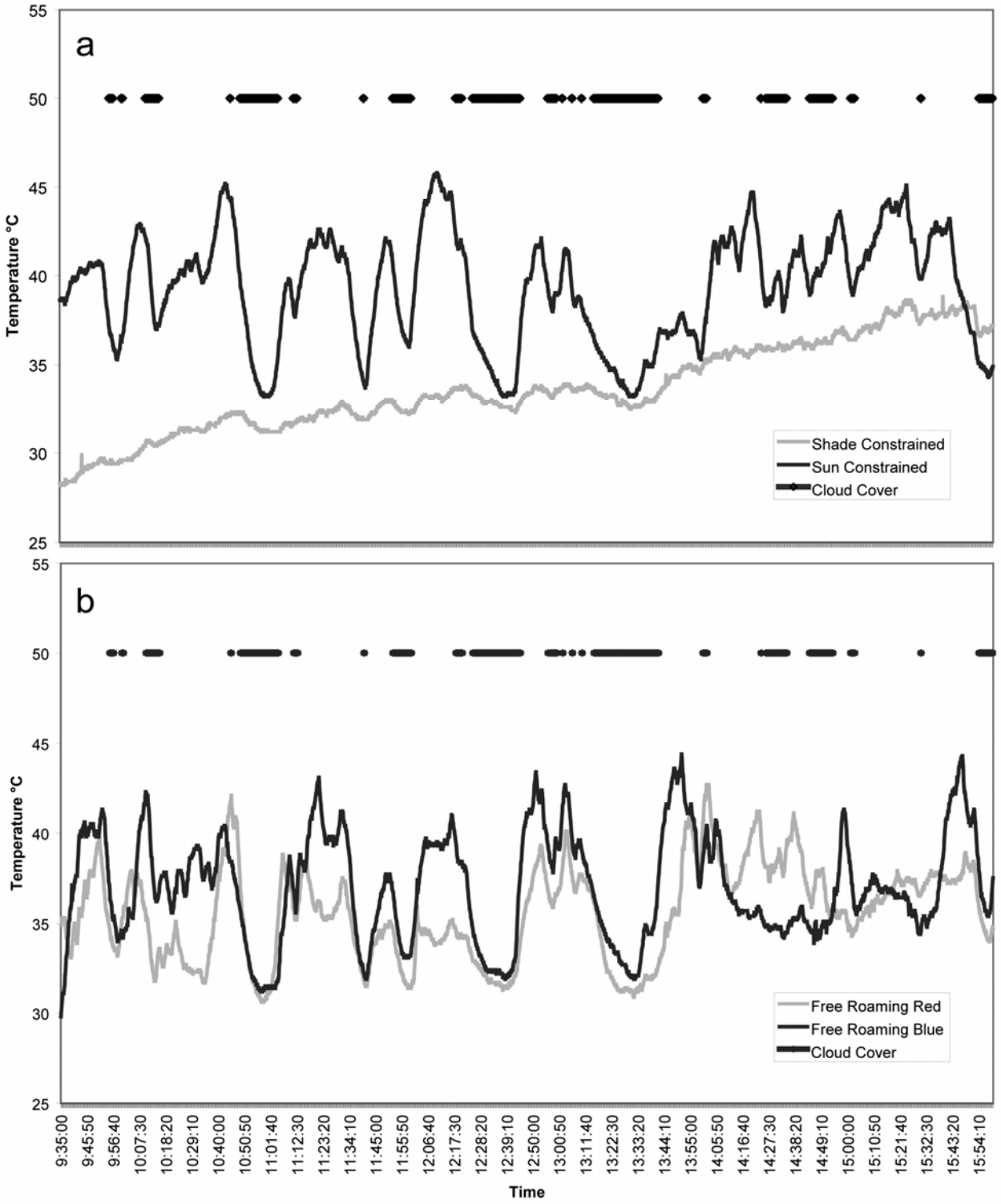

Figure 2-3. Linear plot of continuous body temperatures of (a) constrained grasshoppers and cloud cover and (b) free roaming grasshoppers and cloud cover from June 14 2005. Cloud cover is represented by the discontinuous bars in the upper portion of the plot.

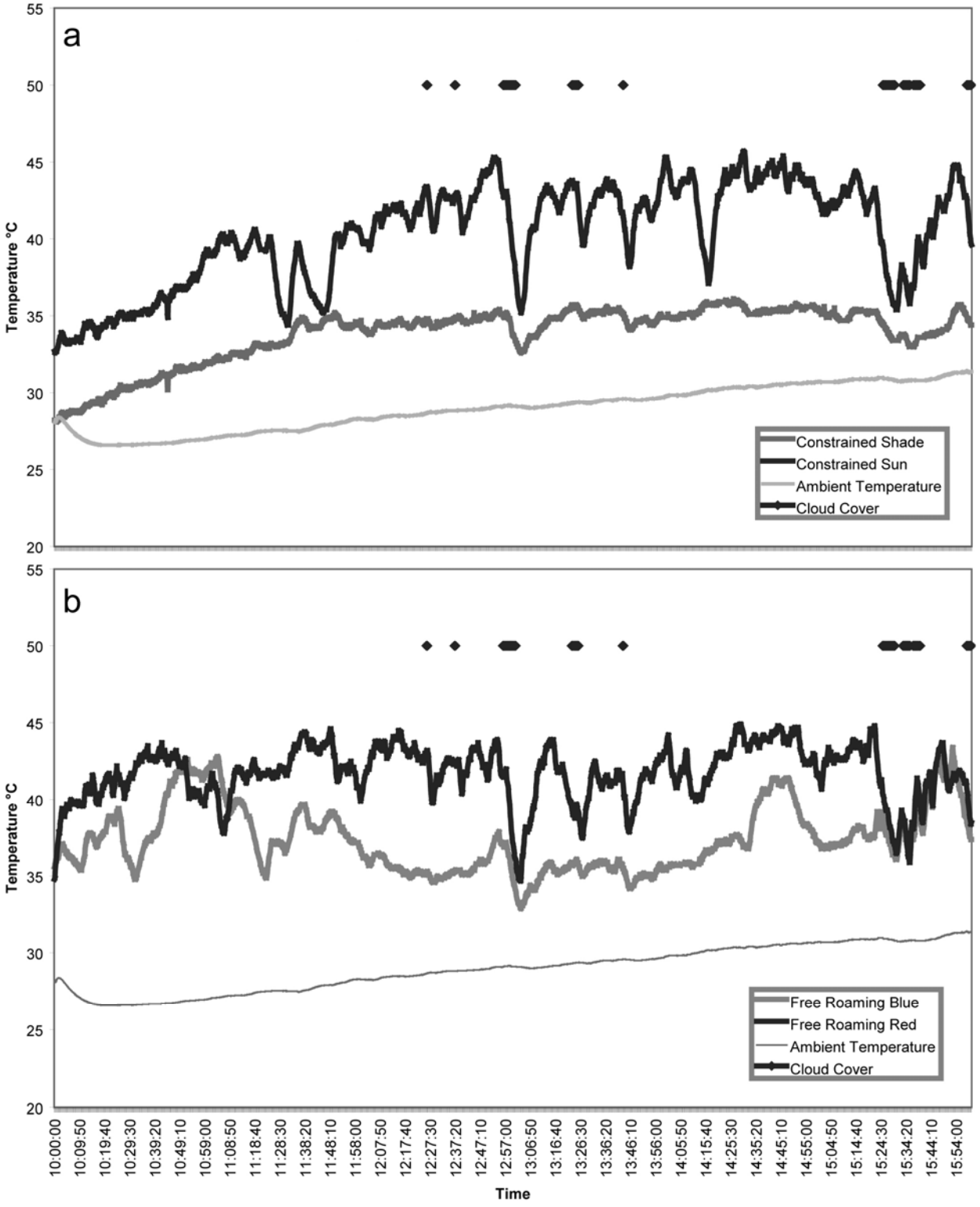

Figure 2-4. Linear plot of continuous body temperatures of (a) constrained grasshoppers with ambient temperature and cloud cover and (b) free roaming grasshoppers with ambient temperature and cloud cover from June 17 2005. Cloud cover is represented by the discontinuous bars in the upper portion of the plot.

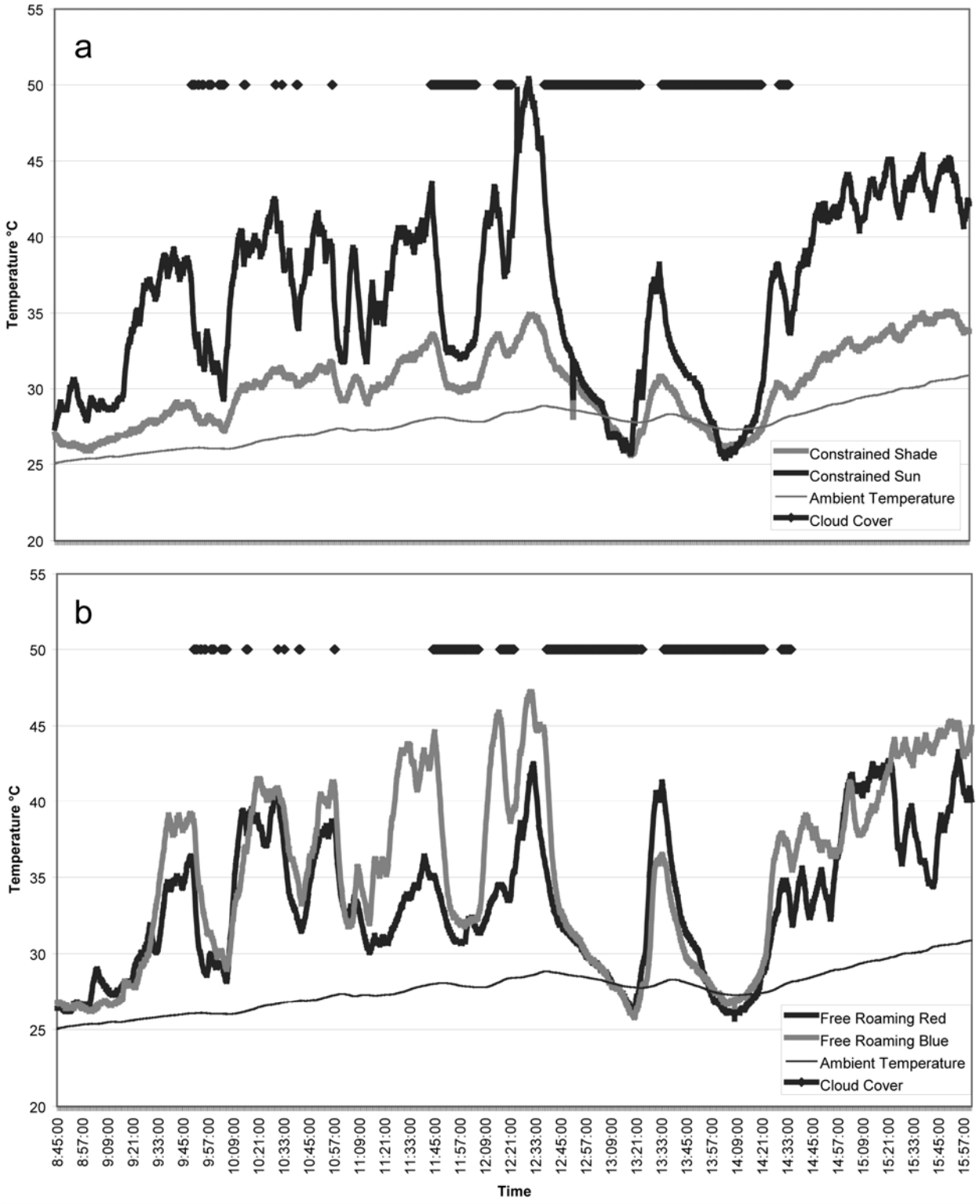

Figure 2-5. Linear plot of continuous body temperatures of (a) constrained grasshoppers with ambient temperature and cloud cover and (b) free roaming grasshoppers with ambient temperature and cloud cover from June 22 2005. Cloud cover is represented by the discontinuous bars in the upper portion of the plot.

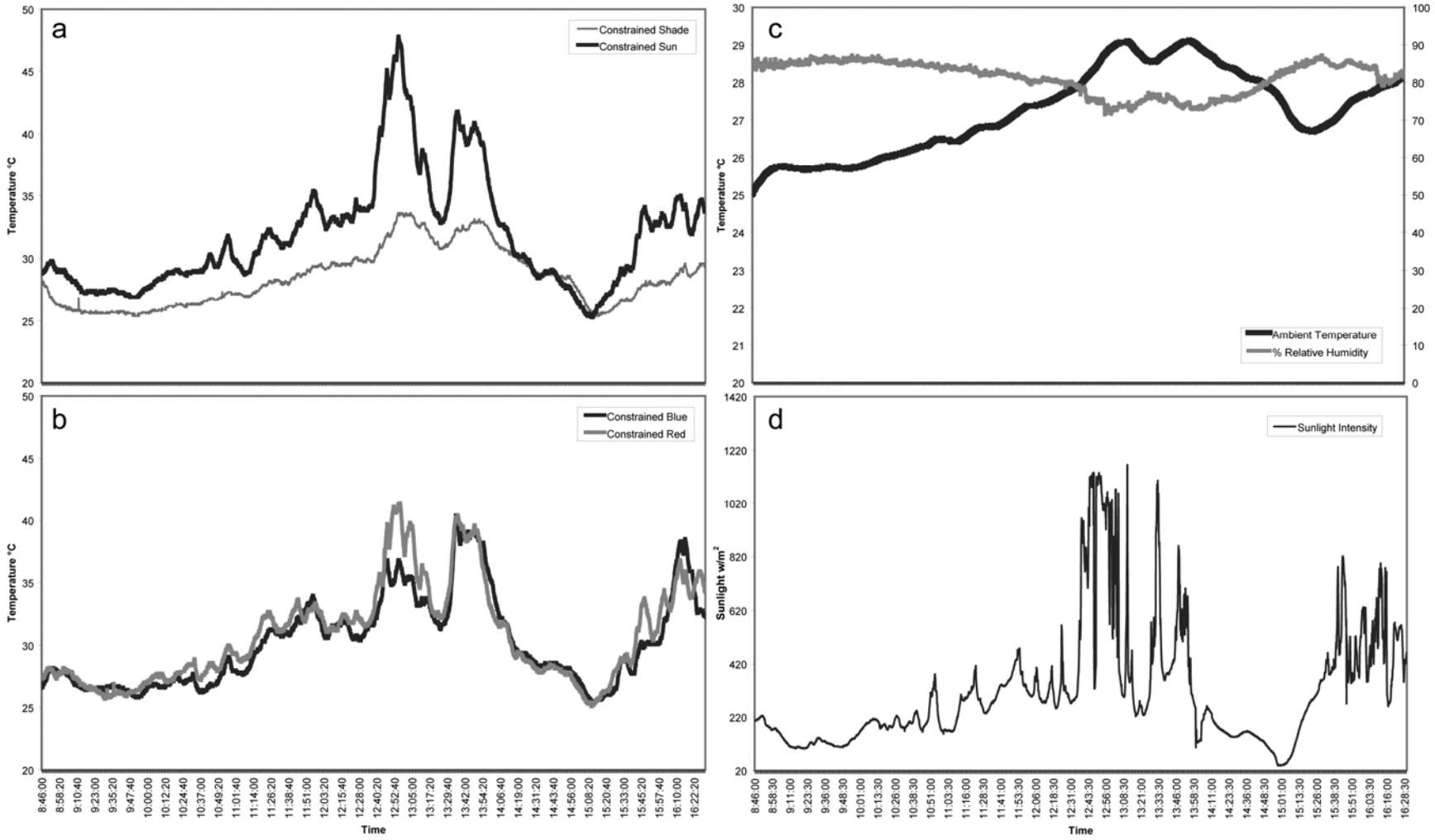

Figure 2-6. Linear plots of continuous body temperatures and environmental parameters from June 29 2005. a) Constrained grasshoppers. b) Free roaming grasshoppers. c) Ambient temperature and relative humidity. d) Sunlight intensity. Secondary axis of c given in %RH.

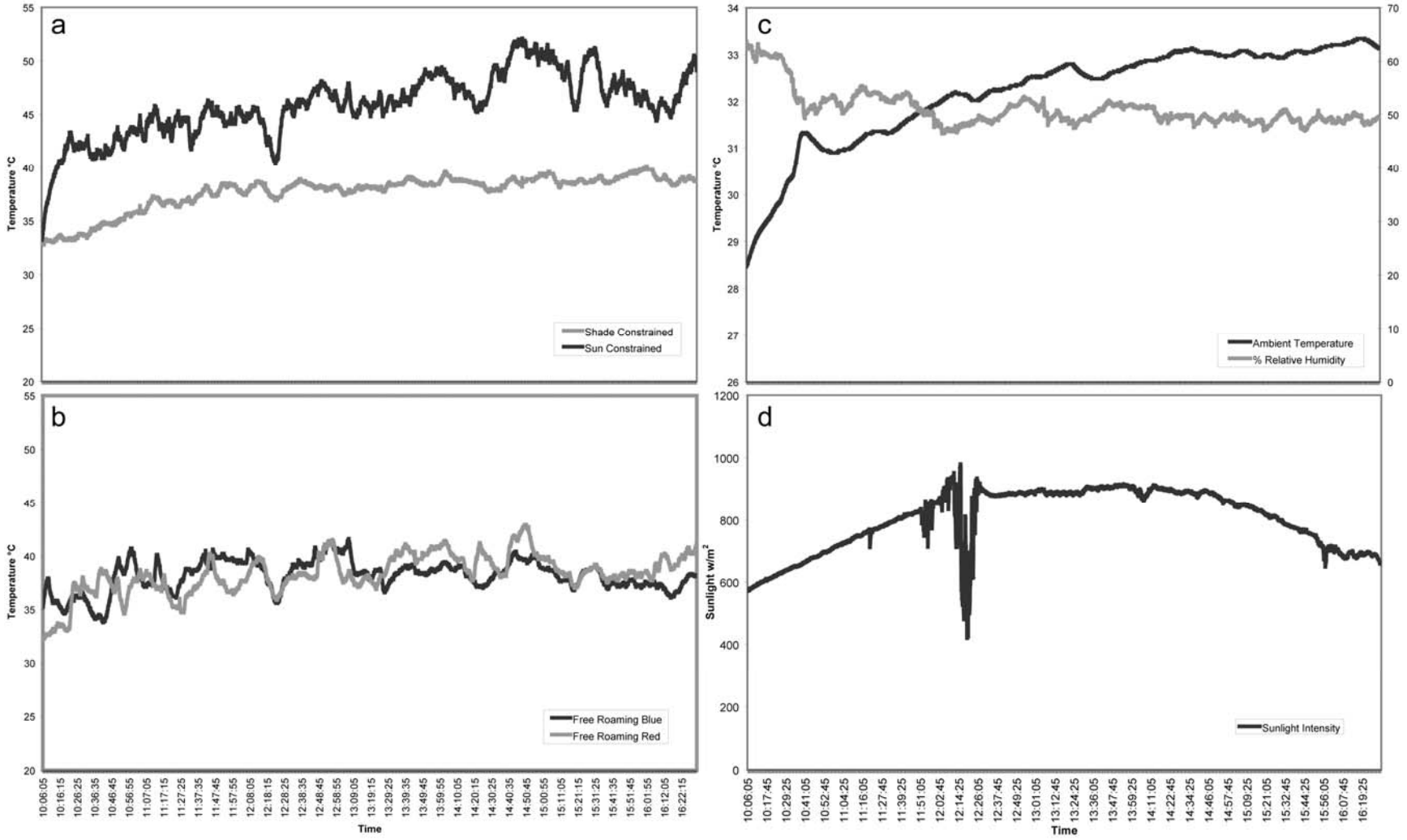

Figure 2-7. Linear plots of continuous body temperatures and environmental parameters from July 5 2005. a) Constrained grasshoppers. b) Free roaming grasshoppers. c) Ambient temperature and relative humidity. d) Sunlight intensity. Secondary axis of c given in %RH.

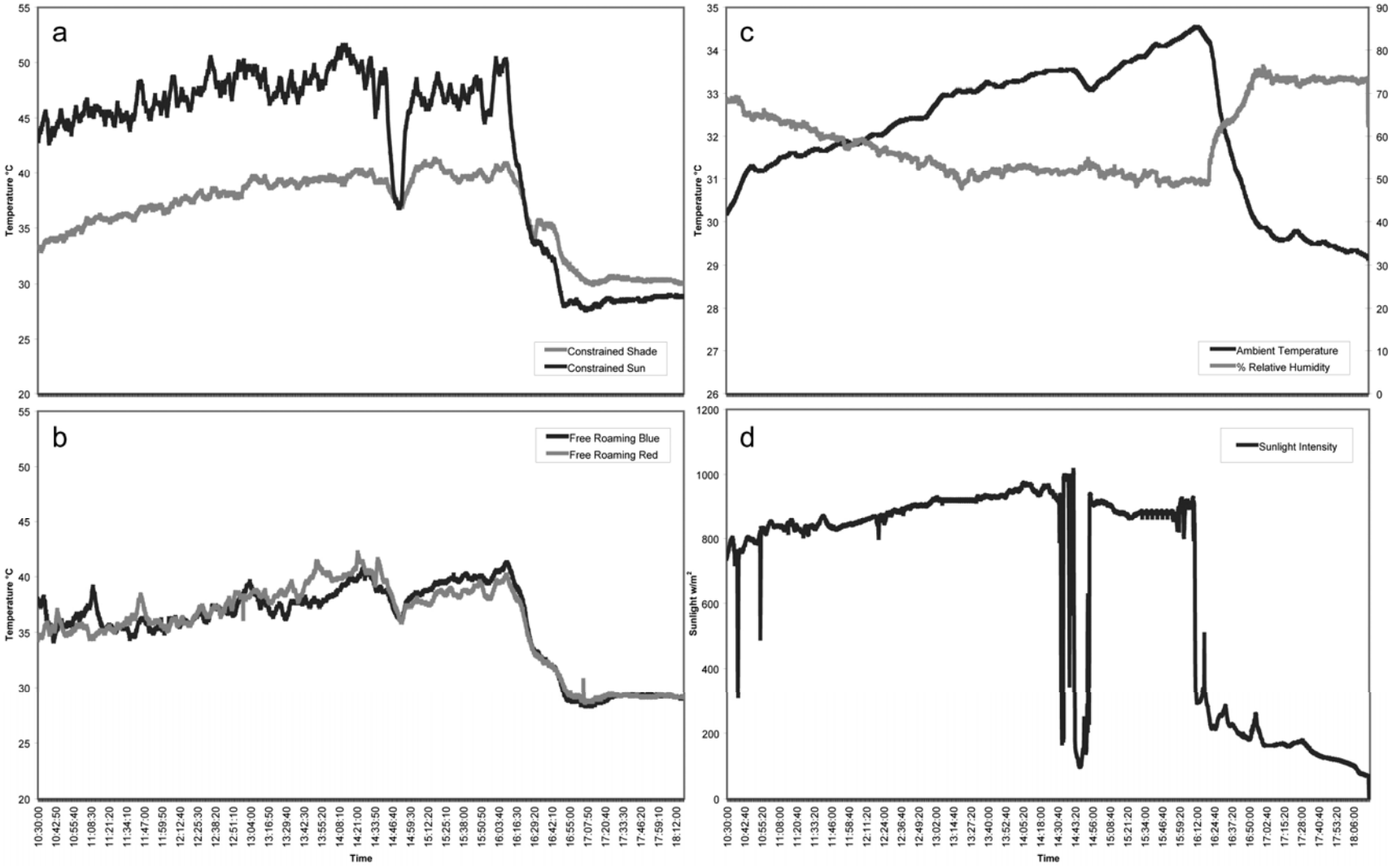

Figure 2-8. Linear plots of continuous body temperatures and environmental parameters from July 8 2005. a) Constrained grasshoppers. b) Free roaming grasshoppers. c) Ambient temperature and relative humidity. d) Sunlight intensity. Secondary axis of c given in %RH.

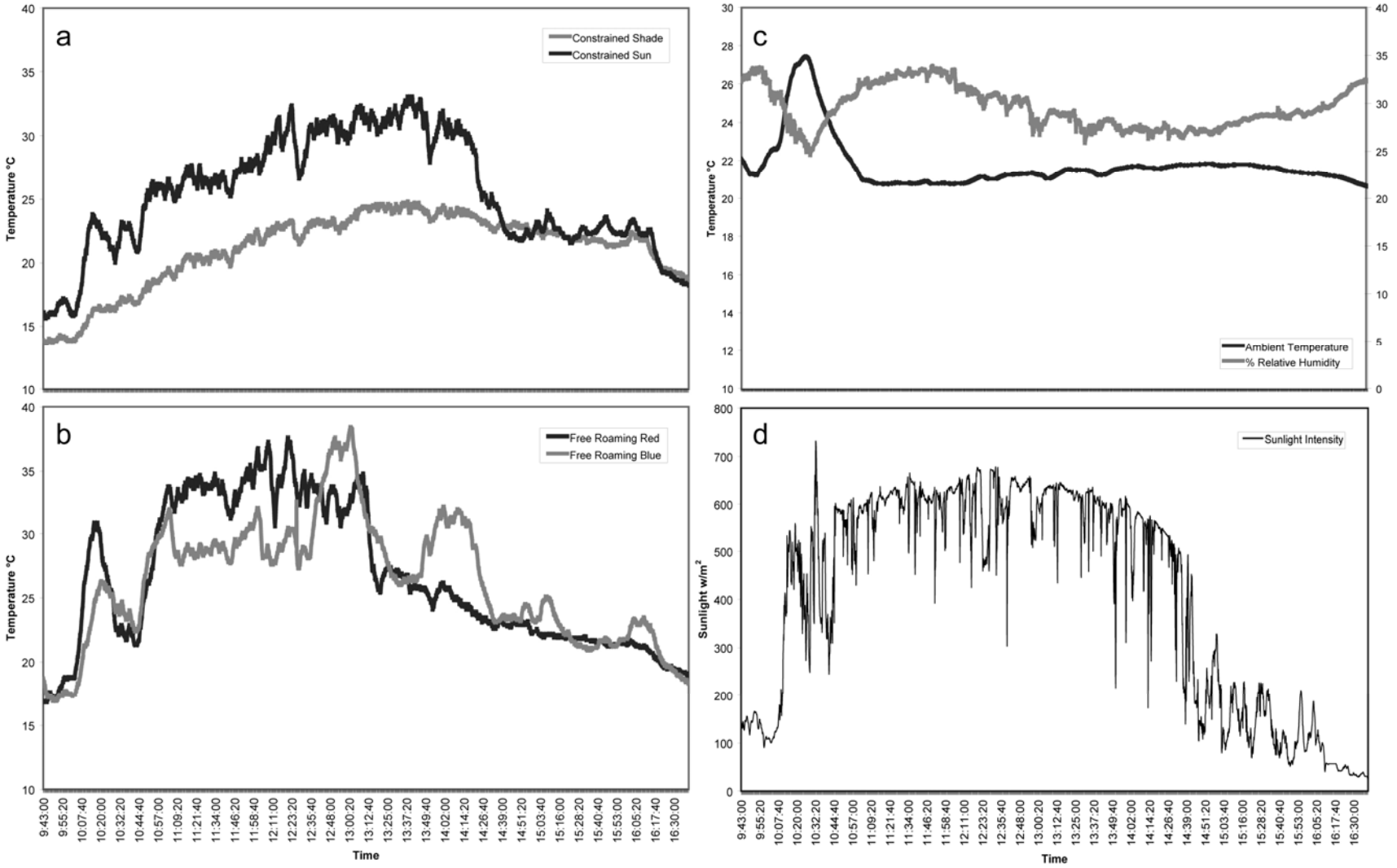

Figure 2-9. Linear plots of continuous body temperatures and environmental parameters from November 17 2005. a) Constrained grasshoppers. b) Free roaming grasshoppers. c) Ambient temperature and relative humidity. d) Sunlight intensity. Secondary axis of c given in %RH.

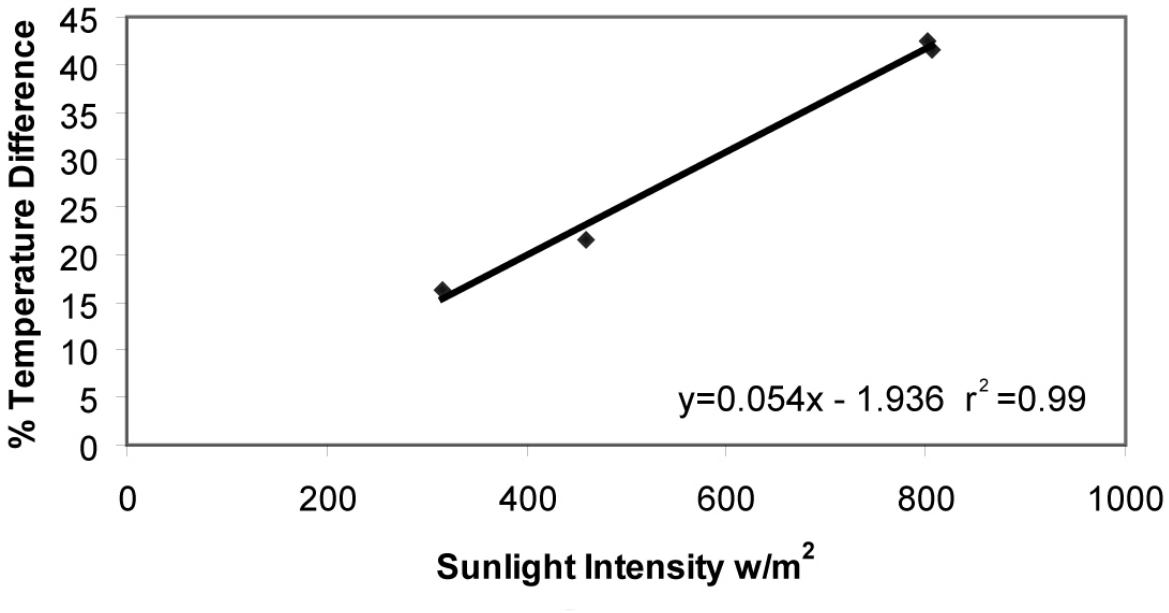

Figure 2-10. Variation in the percent temperature difference between the body temperature of sun constrained grasshoppers and ambient temperature in relation to mean sunlight intensity from June 29, July 5, July 8, and November, 17 2005 ($F=255.13$, $P=0.004$ df = 1, 2).

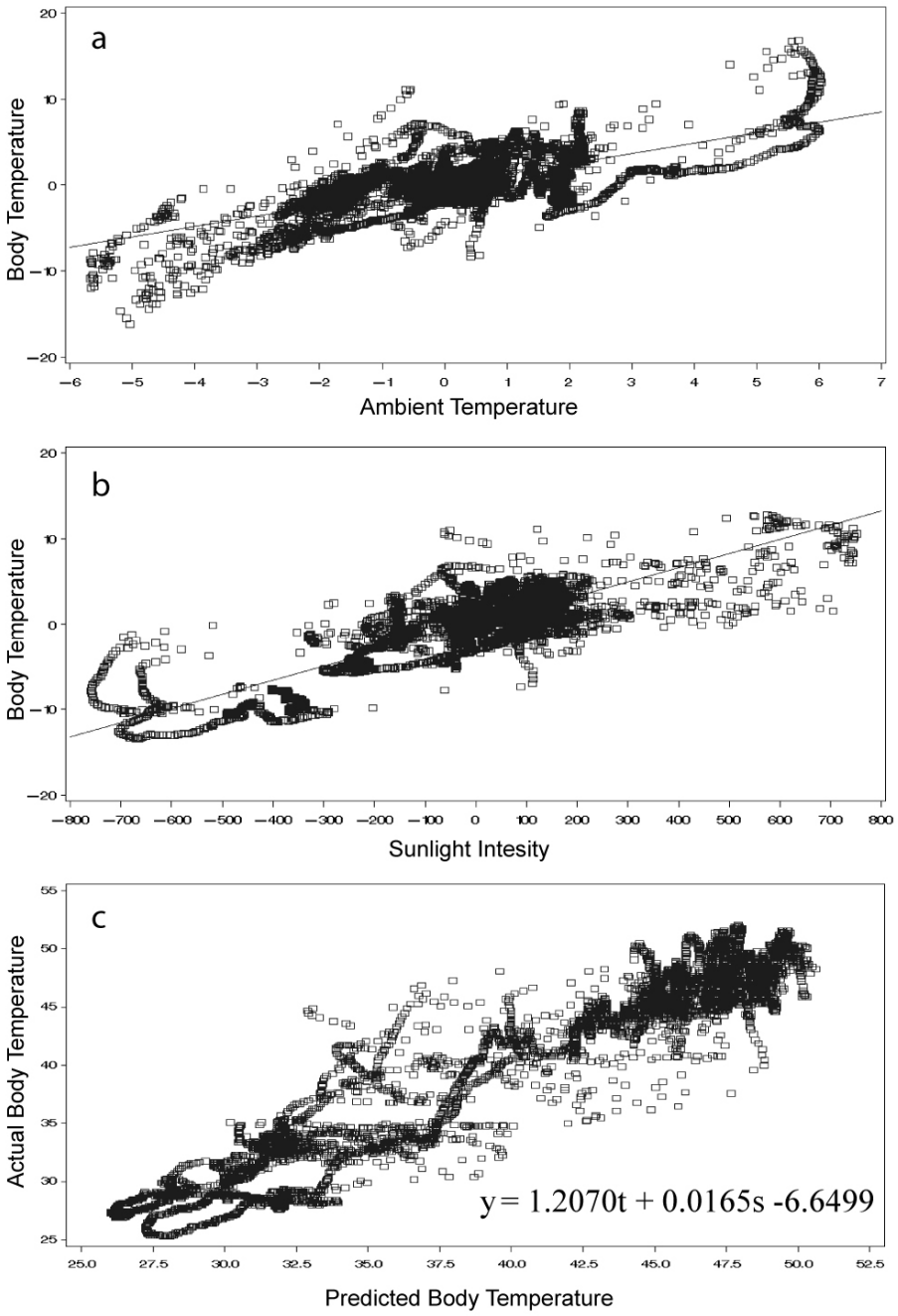

Figure 2-11. Regression analysis of sun constrained grasshoppers from June 29, July 5, and July 8 2005. a) Partial regression plot of body temperature and ambient temperature. Y axis represents residuals of regression between body temperature and sunlight intensity. X axis represents residuals of regression between ambient temperature and sunlight intensity. b) Partial regression plot of body temperature and sunlight intensity. Y axis represents residuals of regression between body temperature and ambient temperature. X axis represents residuals of regression between sunlight intensity and ambient temperature. c) Plot of actual vs. predicted body temperature (t = ambient temperature, s = sunlight intensity).

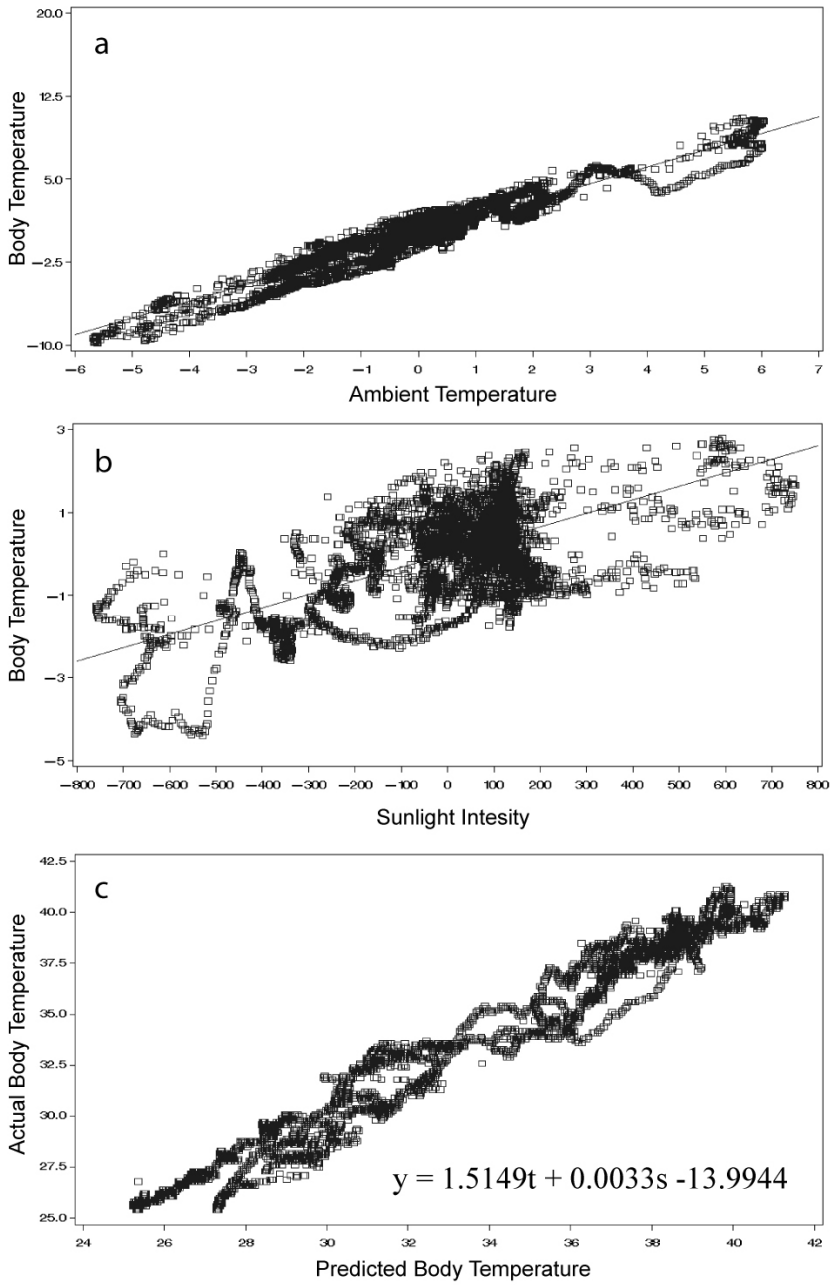

Figure 2-12. Regression analysis of shade constrained grasshoppers from June 29, July 5, and July 8 2005. a) Partial regression plot of body temperature and ambient temperature. Y axis represents residuals of regression between body temperature and sunlight intensity. X axis represents residuals of regression between ambient temperature and sunlight intensity. b) Partial regression plot of body temperature and sunlight intensity. Y axis represents residuals of regression between body temperature and ambient temperature. X axis represents residuals of regression between sunlight intensity and ambient temperature. c) Plot of actual vs. predicted body temperature (t = ambient temperature, s = sunlight intensity).

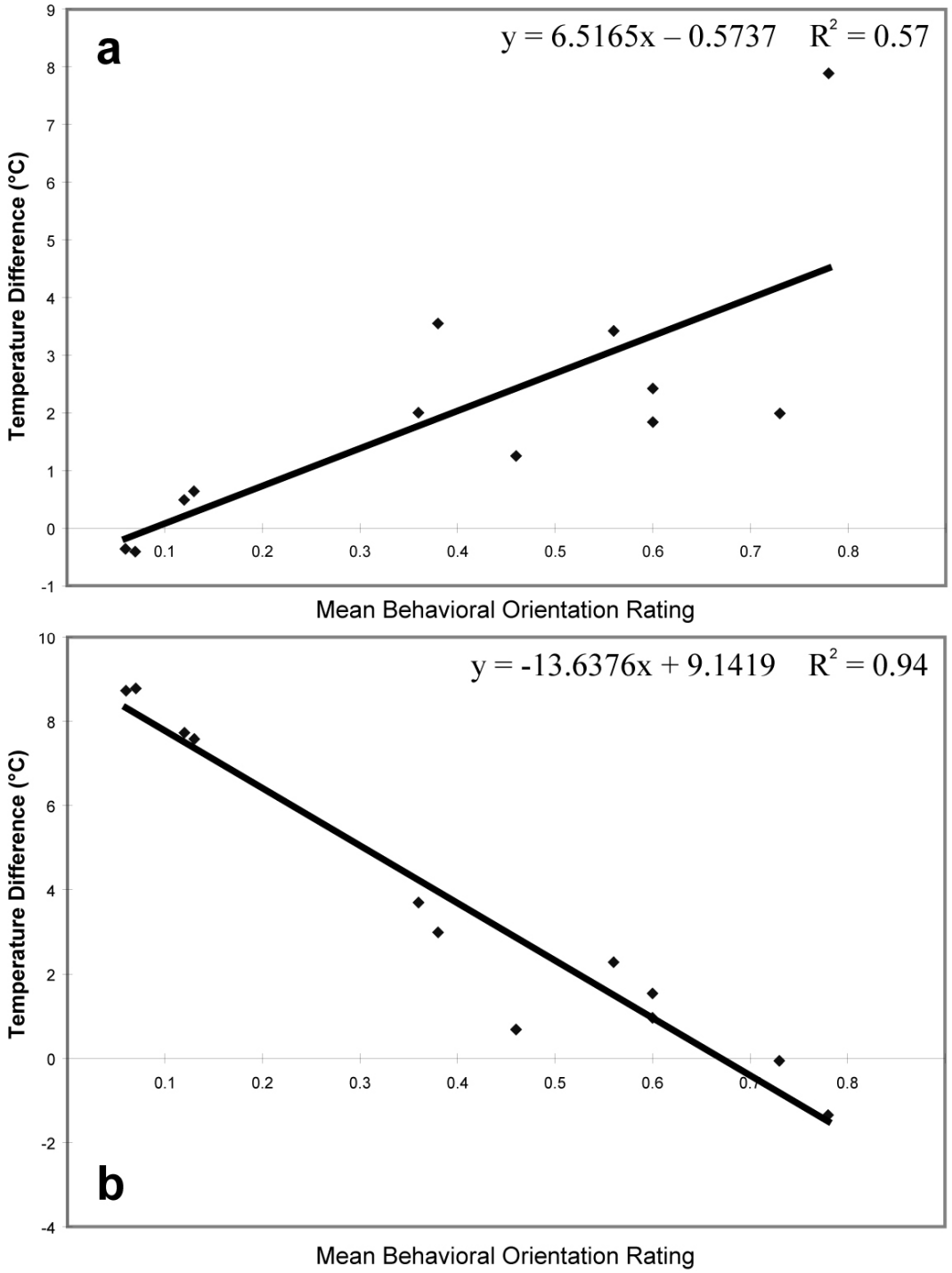

Figure 2-13. Regression analysis of behavioral data from June 14, June 17, June 22, June 29, July 5, and July 8 2005. a) Mean behavioral orientation rating plotted against the difference between free roaming body temperature and shade constrained body temperature in ($F=13.14$, $P=0.005$, $df =1, 10$). b) Mean behavioral orientation rating plotted against the difference between free roaming body temperature and sun constrained body temperature ($F=168.85$, $P=<0.0001$, $df =1, 10$).

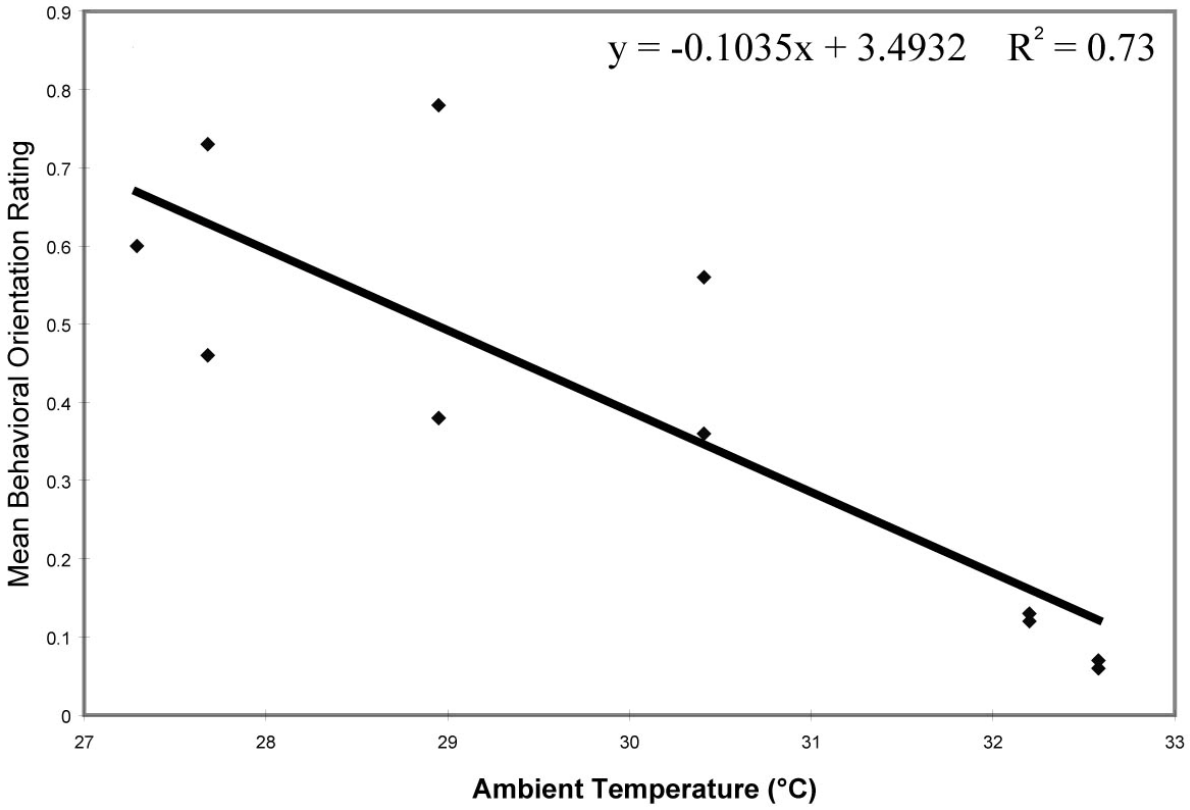

Figure 2-14. Regression analysis for mean behavioral orientation rating plotted against ambient temperature (F=27.58, P=0.0004, df =1, 10).

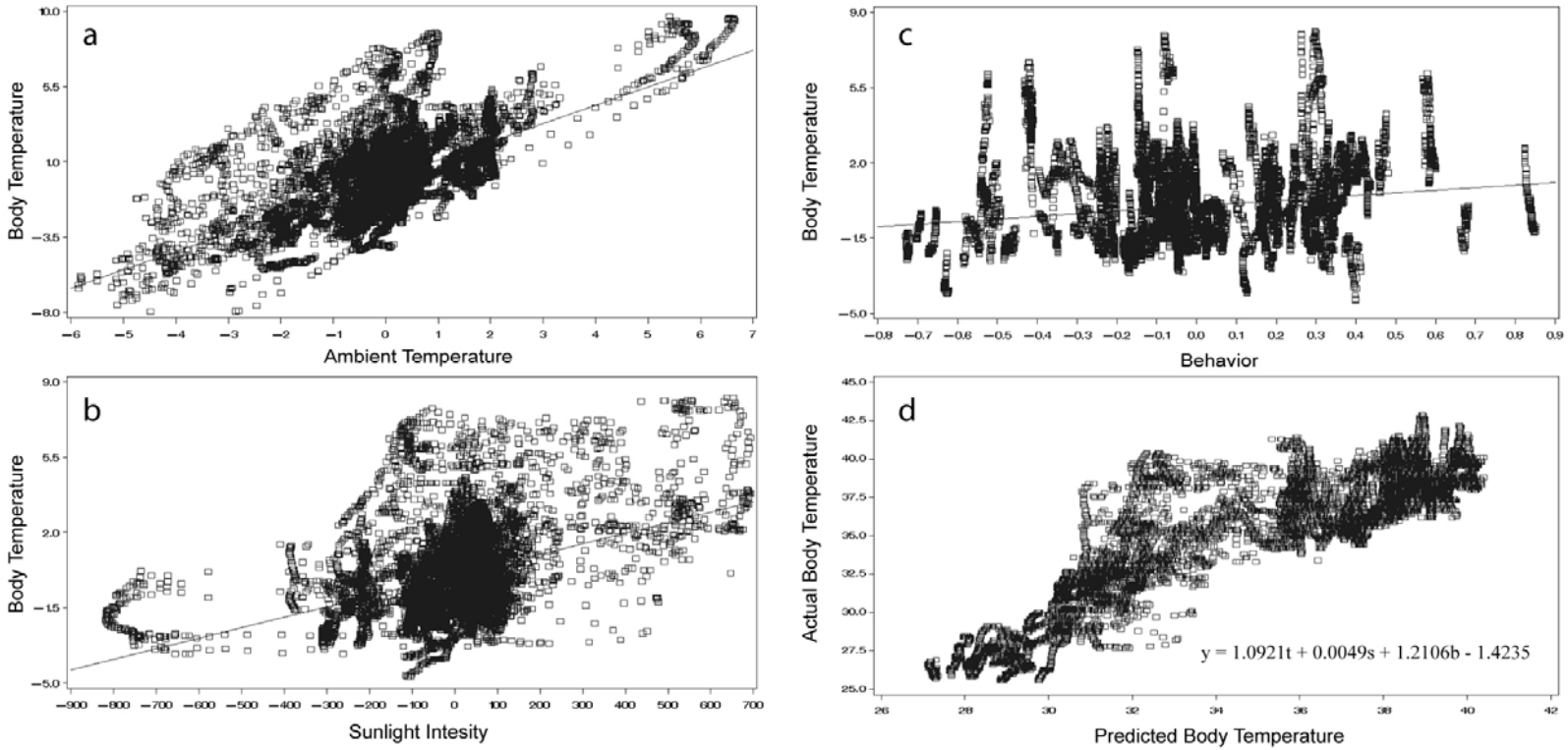

Figure 2-15. Regression analysis of free roaming grasshoppers from June 29, July 5, and July 8 2005. a) Partial regression plot of body temperature and ambient temperature. Y axis represents residuals of regression between body temperature and sunlight intensity and behavioral orientation rating. X axis represents residuals of regression between ambient temperature and sunlight intensity and behavioral orientation rating. b) Partial regression plot of body temperature and sunlight intensity. Y axis represents residuals of regression between body temperature and ambient temperature and behavioral orientation rating. X axis represents residuals of regression between sunlight intensity and ambient temperature and behavioral orientation rating. c) Partial regression plot of body temperature and behavioral orientation rating. Y axis represents residuals of regression between body temperature and ambient temperature and sunlight intensity. X axis represents residuals of regression between behavioral orientation rating and sunlight intensity and ambient temperature. d) Plot of actual vs. predicted values for body temperature (t = ambient temperature, s = sunlight intensity, b = behavioral orientation rating).

CHAPTER 3
EFFECTS OF TEMPERATURE FLUCTUATIONS ON DEVELOPMENT AND REPRODUCTION

Methods and Materials

This portion of the study was conducted in the laboratory in rearing chambers to determine how development time, adult body size, and female fecundity is affected by ecologically relevant temperature fluctuations.

Temperature Treatments

Laboratory experiments took place in environmental control chambers. The temperature fluctuation data obtained from the field was used as a template to construct temperature regimes. Average daytime temperatures of 33˚C and 38˚C were chosen to represent overcast and sunny days. The chambers were placed on a 14/10 photoperiod and a 16/8 thermoperiod with 16 h of respective daytime temperature with the last 2 h hours slowly falling to the 8 h of nighttime temperature at 25˚C. After averaging in nighttime temperatures, the two mean temperatures were 29.5˚C and 31.5˚C, respectively. The mean temperatures of 29.5˚C and 31.5˚C were assigned different frequencies and amplitudes of temperature change. Frequency of temperature changes were 1 and 5 changes per daytime period, while the amplitude of change was 4˚C and 8˚C. The fifth temperature regime was that of constant temperature at 29.5˚C or 31.5˚C. This provided each mean temperature with four fluctuating treatments and one constant temperature treatment for a total of 10 treatments (Fig. 3-1). Unfortunately, because of two failed attempts and time and space restrictions, the high frequency treatments were conducted from October 2005 to February 2006, while low frequency and constant temperature treatments were conducted from February 2006 to June 2006. During this second time period, the 31.5˚C high frequency low amplitude treatment was repeated as a control between the two time periods.

Cages

Four replicates were performed per treatment. Each replicate consisted of an aluminum cage with dimensions of 30 cm X 30 cm X 30 cm, with a solid aluminum bottom, and screen sides and top, stocked with 15 1st instars hatched within the last 24 hours. Each cage was provided with a petri dish of dry food (wheat flour, wheat bran, soy flour, and tropical fish flakes) and a water supply. Fresh romaine lettuce was provided daily until reproduction began, at which point lettuce was provided tri-weekly. Cages were rotated daily to compensate for any temperature stratification that might occur within the chambers. Grasshopper frass was removed daily and cages were cleaned as necessary.

Nymphal Developmental Time

Grasshoppers were monitored daily for molting. Each day, grasshoppers were counted and the number surviving and the number at each stage were recorded. The stage of any dead grasshoppers was also recorded. Data were entered into a Microsoft Excel spreadsheet. Average nymphal development time for each cage was calculated by taking the number of grasshoppers molting to the next stage each day and dividing it by the number surviving, and then adding the products from each day until all grasshoppers had reached adulthood.

Body Size and Reproduction

Once all the grasshoppers in a treatment reached adulthood, the grasshoppers were removed and the sex, femur length, and overall length of each grasshopper were recorded. Even numbers of females and males were then placed back into the cages. Grasshoppers were then monitored for mating behavior, at which point 32 oz. deli cups (approximately 14cm high, 8.9cm in diameter at base, and 11.4cm in diameter at top) filled with moist vermiculite were placed inside the cages as oviposition medium. Each cup was then monitored daily until the first

occurrence of oviposition was recorded. Cages were then monitored tri-weekly and cups were changed weekly. During this time, any egg pods that had been laid outside the cups were recorded. Cups were then stored at 5-10˚C until they could be processed, at which time the number of egg pods per cup was recorded, along with the number of eggs in each pod. The number of egg pods laid per cage was divided by the number of females per cage to arrive at egg pods / female. Those egg pods laid outside of the cups were included in the egg pod mean but not the eggs/pod mean. Unfortunately, total reproduction could not be measured due to pesticide residues detected on lettuce fed to the general colony during the high frequency treatments. Experimental grasshoppers received the inner portions of this lettuce and consequently, all treatments had to be terminated for fear of contamination. Thus, the collection of egg pods was discontinued 115 d after hatch. The same was done for the second set of treatments conducted from February to June.

Statistical Analysis

Differences in total nymphal development time, body size, egg pods per female, eggs per pod, and days to first oviposition were analyzed using ANOVA and the Least Squares Means (LSM) procedure using a Tukey's adjustment in SAS Analyst 9.0. High frequency treatments conducted from October to February were treated as a separate study and analyzed in a separate ANOVA from low frequency and constant treatments conducted from February to June. An ANOVA was conducted on all fluctuating treatments as one group, combining differing frequencies and time periods to test for any frequency or trial effects such as, difference in food quality or change in biology due to seasonal differences. Separate histograms for high and low frequency treatments as well as one for combined treatments of nymphal developmental data

were produced using SAS Analyst 9.0. Bar graphs of body size, egg pods per female, eggs per pod, and days to first oviposition were produced using Microsoft Excel.

Results

To see if the two laboratory studies could be combined, an ANOVA was performed for each parameter on the repeated and original 31.5°C high frequency low amplitude treatments. The tests revealed significant differences between the two in all parameters. Due to these differences, data were analyzed as two separate studies. Data were also analyzed together to note any effects, regardless of whether they were frequency or trial related.

Nymphal development time

Amplitude and temperature effects were found to be significant in high frequency treatments (Table 3-1). On average, grasshoppers reared at 31.5°C reached adulthood 5.2 d faster than those reared in 29.5°C treatments, while those reared in high amplitude treatments reached adulthood 2.1 d faster than grasshoppers reared in low amplitude treatments (Table 3-2). Mean comparisons revealed that the 29.5ºC high frequency low amplitude treatment is the only significantly different treatment within high frequency treatments (Table 3-2). Although both 29.5ºC treatments had longer average nymphal development times, only the low amplitude treatment was found to be significantly different from the 31.5ºC treatments. This treatment likely caused all of the amplitude and the majority of temperature effects. The distribution of data for low amplitude treatments shows a distinct separation (Fig. 3-2), which is caused by the 29.5ºC low amplitude treatment. Grasshoppers in both 31.5ºC treatments took about 38 d to complete nymphal development, showing no effects of amplitude at this temperature (Table 3-2).

Amplitude, temperature, and amplitude x temperature effects were found to be significant in low frequency and constant treatments (Table 3-1). On average, grasshoppers reared at 31.5°C

reached adulthood 1.6 d faster than those reared in 29.5°C treatments (Table 3-2). There was no difference in nymphal development time between grasshoppers reared at high and low amplitude in low frequency treatments. However, those reared at alternating temperature reached adulthood about 2 d faster than grasshoppers reared in constant treatments (Table 3-2). As in high frequency treatments, mean comparisons revealed only one treatment to be significantly different from the others. The 29.5°C constant temperature treatment was significantly different from all treatments except for the 29.5°C low frequency low amplitude treatment and is the likely the reason there are significant amplitude, temperature, and interaction effects (Table 3-2). The distribution of data reveals how similar nymphal development times for low frequency treatments are (Fig. 3-3). The data for constant temperature treatments is very spread out and is likely due to the 29.5°C and 31.5°C constant temperature treatments being so different (Fig. 3-3). Grasshoppers reared in the 31.5ºC constant treatment showed no delay in nymphal development and even developed faster than one of the alternating 31.5ºC treatments.

When high and low frequency treatments were combined (minus constant treatments) there were significant frequency or/and trial effects (Table 4). In general, low frequency treatments tended to have shorter mean total nymphal development times than high frequency treatments, 3.9 d shorter (Table 3-3). This can also be seen in the distribution of the data (Fig. 3-4). However, this difference is likely caused by the 29.5°C high frequency treatments. All other treatments (29.5°C low frequency, and both 31.5°C high and low frequency treatments) were not significantly different (Table 3-3). When all treatments were combined there were no amplitude effects (Table 3-3).

Body size

Sex was the only significant main effect for both femur length and overall length (Table 3-4). In high frequency treatments females had an overall length (in mm, mean ± SD) of 59.81 ± 1.84 and a femur length (in mm, mean ± SD) of 26.45 ± 1.13, while males had an overall length of 51.91 ± 1.94 and a femur length of 23.04 ± 1.17. In low frequency and constant treatments females had an overall length of 61.91 ± 1.84 and a femur length of 27.82 ± 2.59, while males had an overall length of 53.97 ± 1.75 and a femur length of 24.29 ± 0.97. Differences in size between male and female grasshoppers were expected. There were also several significant interactions involving the sex factor, but a closer look at multiple comparisons revealed no real differences other than sex. When low frequency and high frequency treatments were combined, there were both significant sex and frequency or/and trial effects for femur length and overall length (Table 3-4). High frequency treatments tended to produce smaller individuals than did low frequency treatments (Fig. 3-5). The only factor to affect grasshopper body size other than sex was frequency or/and trial effects.

Days to oviposition

Grasshoppers reared in high frequency treatments, on average, took between 56 and 70 d from hatch to egg deposition. Temperature was the only significant factor affecting the number of days to oviposition in high frequency treatments (Table 3-5). Grasshoppers reared in 29.5°C high frequency treatments took, on average, about 9 d longer to the first occurrence of oviposition (Table 3-6). Mean comparisons revealed differences similar to those for nymphal development time. The 29.5°C high frequency low amplitude treatment was shown to be the only significantly different treatment (Table 3-6) and is likely the sole cause for the significant temperature effect. Grasshoppers reared in low frequency and constant treatments, on average,

took between 56 and 93 d from hatch to egg deposition (Table 3-6). The results of the ANOVA (Table 3-5) and mean comparisons (Table 3-6) revealed only the 29.5°C constant temperature treatment to be significantly different. This treatment was so different from the others that it likely the reason for both significant temperature and amplitude effects. There were no differences between high and low amplitudes of low frequency treatments; however, there were differences between constant treatments and fluctuating treatments (Table 3-6). Therefore, the "amplitude" effect was caused by constant temperature treatment and is not a true effect of differences in amplitude of temperature change but more of an effect of frequency or the presence or absence of temperature change(disc). The ANOVA conducted on the combination of high and low frequency treatments (Table 3-5 and Fig. 3-6) revealed there to be no frequency or trial effect on the number of days from hatch to first oviposition, with high frequency treatments averaging 61.8 d ± 7.3 (mean ± SD) and low frequency treatments averaging 63.1 ± 5.6.

Egg Pods / Female

The results of the ANOVA (Table 3-5) and mean comparisons (Table 3-6) performed on high frequency treatments for egg pods / female revealed both significant temperature and amplitude effects, and showed that only one treatment was significantly different from the others, the 29.5°C high frequency low amplitude treatment. Grasshoppers reared under this treatment produced an average 2 egg pods / female compared to the average of about 6 egg pods / female for all other treatments (Table 3-6). It is likely that both the amplitude and temperature effects were caused by this treatment.

Just as in high frequency treatments, the ANOVA for low frequency treatments (Table 3-5) revealed significant temperature and amplitude effects. However, mean comparisons (Table 3-6) revealed more than one significantly different treatment within the low frequency treatments.

Grasshoppers reared in 29.5°C treatments produced on average 2.25 fewer egg pods / female than those reared in 31.5°C treatments. This difference was caused by more than one treatment with all 29.5°C treatments being significantly different from all 31.5°C treatments but the constant treatment (Table 3-6). The treatment with the fewest egg pods / female produced was the 29.5°C constant treatment with only 1.27 egg pods / female (Table 3-6). The amplitude effect seen in low frequency treatments is essentially a frequency effect, as there were no differences between low and high amplitude (Table 3-6). The only differences were between constant and fluctuating treatments. Females in both constant temperature treatments produced significantly fewer egg pods / females than many of their respective alternating treatments (Table 3-6). Unlike high frequency treatments, there is more than one treatment causing differences between temperature and amplitude. When both high and low frequency treatments were analyzed together there were significant differences between the two frequencies (Table 3-5, Fig. 3-6) with females reared in high frequency treatments producing 5.10 ± 1.93 (mean ± SD) egg pods / female and those reared in low frequency treatments producing 4.00 ± 1.48 (mean ± SD) egg pods / female.

Eggs / Pod

Both temperature and amplitude were found to be significant factors affecting the number of eggs per pod in high frequency treatments (Table 3-5). Yet again, the only significant treatment of the high frequency treatments was the 29.5ºC low amplitude treatment with an average of 82.46 eggs / pod compared to the average of near 60 eggs / pod for all other treatments (Table 3-6). This treatments was responsible for both temperature and amplitude effects by causing the eggs / pod average to be greater for 29.5ºC treatments than 31.5ºC

treatments and low amplitude treatments to average more eggs / pod than high amplitude treatments (Table 3-6).

This is in contrast to low frequency treatments, where grasshoppers reared in 29.5ºC treatments produced fewer eggs per pod than 31.5ºC treatments (Table 3-5 and 3-6). The ANOVA conducted on low frequency treatments shows a significant amplitude effect (Table 3-5). However, after examining the mean comparisons analysis (Table 3-6), it is clear that there are no real differences between high and low amplitudes and that, just as before, the amplitude effect is caused by differences between the constant and alternating temperature treatments, which is more of a frequency effect. Females in both constant temperature treatments produced significantly fewer eggs / pod than did their respective alternating treatments. Interestingly, the 29.5°C low frequency high amplitude treatment had a higher mean number of eggs / pod value than the 31.5°C constant treatment and was not considered significantly different from any of the 31.5°C treatments. When both high and low frequency treatments were combined, the ANOVA revealed no differences between high and low treatments in the number of eggs / pod. Females reared in high frequency treatments produced an average of 62.93 ± 16.71 (Mean ± SD) eggs / pod and those reared in low frequency treatments produced 63.17 ± 19.55 (Mean ± SD).

Discussion

Conformity of laboratory treatments to field study results

Mean daily temperatures for laboratory treatments were based on mean daily body temperatures obtained from the field. When able, the majority of free roaming grasshoppers maintained mean body temperatures near 38°C (Table 2-1). Thus, the initial temperature used to simulate mean daily temperature to represent ideal or sunny weather conditions was 38°C. Free roaming grasshoppers on cloudy or rainy days often had body temperature near 30°C (Table 2-

1). However, environmental chamber limitations necessitated the use of a slightly higher temperature and so 33°C was chosen to represent the mean daily temperature for days with adverse weather conditions. In addition to the mean daily temperatures, frequency and amplitude of temperature change were also modeled after data obtained from the field. During ideal conditions on predominately sunny days, grasshoppers often experienced 0-1 large fluctuations (>15% change) in body temperature. The low frequency treatments corresponded well with this and had only 1 fluctuation in temperature per daytime period (Fig. 3-1). The fluctuation was placed near the end of the day because more often then not, cloud cover or rain on such days occurred during the afternoon hours. During predominately cloudy and rainy days, grasshoppers experienced a mean of 3.67 large fluctuations per day. In laboratory treatments 5 fluctuations per day were chosen which is not far from the average and likely provided more opportunity for differences between different frequency treatments than 3 or 4 fluctuations might have. The mean percent change in body temperature of grasshoppers for cloudy and rainy days was 22.34% and 38.83% respectively. The initial amplitude of 8°C corresponds well with those percentages for cloudy days. In 31.5°C high amplitude treatments, an 8°C drop in temperature equals a 19% change in temperature. In 29.5°C high amplitude treatments the 8°C drop equals a 21.6% change in temperature. Low amplitude treatments were included to complete the study design and provide a measurement of the effect of amplitude on the various parameters being investigated. The low amplitude of 4°C was chosen by arbitrarily halving the 8°C amplitude.

Nymphal development time

Total nymphal development time in this study ranged from 35.4 – 45.0 days, with an average of 38.4 days. The difference between average temperatures (29.5°C and 31.5°C) for nymphal development time was 5.2 days for high frequency treatments and 1.6 days for low

frequency treatments. These differences are far less than many differences found in other studies (Parker 1930, Whitman 1986, Gündüz and Gülel 2002). Gündüz and Gülel (2002) reported a difference in development time of 10 d between 30°C and 25°C in *S. gregaria*. One reason these results are different may be that the temperature difference between treatments was only 2ºC compared to the 5ºC or higher difference used in many other studies. Another possible reason is that much lower temperatures, such as 20ºC and 25ºC, were used in these other studies. These low temperatures may have been well below optimal for the grasshoppers being studied, while the higher temperatures tested would have been within their optimal range. As mentioned before, it may not be the temperature difference but where the temperatures fall with respect to optimal temperatures for that species, that matters most. If an increase in 5°C caused the new temperature to cross from the suboptimal range into the optimal range, a greater effect would likely be seen than if the 5°C change occurred within the same range. This can be seen in a study by Gardner and Thompson (2001) on development time in *H. viridis* where 5°C increases in temperature above 30°C did not decrease development time. The lower temperatures tested in the current study likely fell closer to optimal for this species than those of other studies and their respective species, resulting in diminished responses.

Analysis of nymphal development time in high frequency treatments shows the only significantly different treatment as being the 29.5ºC low amplitude treatment. Willot (1992) reports that for four species of Acrididae, optimum temperature for growth and development is between 35ºC and 40ºC. Daytime temperatures within the 29.5ºC treatments averaged 33ºC, which is just below the optimal range reported by Willot. Temperatures in the low amplitude treatment ranged from 31ºC to 35ºC, while those in the high amplitude treatment ranged from 29ºC to 37ºC. The body temperatures of grasshoppers reared in the low amplitude treatment

never reached above 35°C, while body temperatures of those reared in high amplitude treatments reached levels near the middle of the optimal range reported by Willot (1992). While Willot (1992) does not report optimal body temperatures for *S. americana*, they are likely to at least be within the 35-40°C range, if not higher. In contrast to the 29.5°C treatments, 31.5°C treatments had mean daily temperatures of 38°C with low amplitude temperatures ranging between 36°C and 40°C and high amplitude temperatures ranging between 34°C and 42°C, all of which fall very near or within the theoretical optimal range. The fact that only the grasshoppers in the 29.5°C low amplitude treatment had a slower development rate, suggests that amplitude of temperature change may only be important for development time at suboptimal temperatures and that it may be possible that just attaining 37ºC for some portion of the day is beneficial for the development of *S. americana*. Reporting that amplitude only effects nymphal development at suboptimal temperatures entails that there should be a significant interaction between amplitude and temperature. While the interaction was not considered significant, it was very close with a p-value equal to 0.06.

A situation similar to that of high frequency treatments occurred in low frequency treatments, with only one treatment being significantly different. However, instead of the 29.5°C low amplitude treatment being significantly different, it was the 29.5°C constant temperature treatment. Development time in the 29.5°C low amplitude treatment falls between the 29.5°C constant treatment and the rest of the treatments, and is not statistically different from any of the treatments. While every treatment received exactly the same number of degree days with respect to their mean temperatures (calculated using data from environmental chambers), grasshoppers reared in low frequency treatments experienced the high range of their temperature range for longer uninterrupted periods of time than grasshoppers of high frequency treatments. This could

be the reason that nymphal development time in the 29.5°C low amplitude treatment was significantly different in high frequency treatments but not in low frequency treatments. The exposure to uninterrupted periods of 35°C likely provided a greater benefit than several shorter periods of exposure. If this is true, it suggests that the duration (frequency) of uninterrupted high temperatures is important for development.

The significant difference of the 29.5°C constant treatment causes there to be both significant amplitude and temperature effects. However, there are no differences between high and low amplitude treatments. Therefore, the significant amplitude effect is an artifact of the constant temperature treatments and is essentially a frequency effect manifested as a difference between low frequency and no frequency or the presence or absence of temperature change. Data was analyzed this way to preserve the complete block design. Had frequency been analyzed as low frequency and no frequency the model would have lacked high and low amplitudes for the constant treatments since there cannot be amplitude of change if there is no temperature change. As in high frequency treatments, the amplitude factor (or in this case frequency) only had an effect at the lower temperature (Table 3-2), suggesting an interaction between amplitude and temperature. However, in contrast to the high frequency treatments, the amplitude X temperature interaction is significant (Table 3-1). Again, the data suggest that nymphal development is affected by fluctuating temperature only at suboptimal temperatures.

Because different frequency treatments were conducted as different trials, and the treatment repeated between the two trials was found to be significantly different, any significant frequency effects between high and low frequency treatments are confounded with any trial effects. However, the data can still be analyzed if any effects of frequency between the two are also attributed to differences between the trials. It was expected that low frequency might

develop faster because they spent a longer continuous duration of time at high temperatures, even though they received the same number of degree days as that of high frequency treatments. While nymphal development time of grasshoppers experiencing 31.5ºC treatments remain relatively the same between high and low frequency treatments, development time of 29.5ºC treatments is much shorter, 6.5 d shorter (Table 3-3), in low frequency treatments when compared to high frequency treatments, suggesting again that temperature change only affects nymphal development time at suboptimal temperatures. However, development times of grasshoppers reared at a constant 29.5ºC were shorter than that of grasshoppers reared in either 29.5ºC high frequency treatment (Table 3-2). However, it was expected that constant temperature treatments would exhibit the slowest development times. This, along with the idea that fluctuations in temperature that allow the attainment of higher temperatures are more beneficial for nymhpal development at lower mean temperatures, leads to the explanation that trial effects, such as seasonal differences, are likely the cause for the faster development times seen in constant temperature treatments when compared to high frequency treatments. However, if the differences between high and low frequency treatments are due to trial affects, then another question arises. Why is there no acceleration of nymphal development time in the 31.5ºC treatments between high and low frequency treatments? There are two possible explanations assuming experimental error is not the cause. Trial effects are more prominent at suboptimal temperatures or frequency effects are more prominent at suboptimal temperatures. The differences due to a trial effect such as seasonality would not likely be affected by suboptimal temperatures; therefore, the more plausible explanation is that differences in nymphal development time between high and low frequency treatments are due to frequency, whose effects are temperature dependent. This idea is supported by the fact that the same patterns are

seen when frequency treatments are analyzed separately, as discussed earlier. Still, whether or not differences in nymphal development time between low and high frequency treatments should be attributed to frequency or trial effects remains somewhat unclear.

Body size

Studies in the past have shown that thermoperiod can affect weight and size in insects (Beck 1983). However, no such effect was observed in this study. Neither temperature fluctuations nor differences in mean temperature affected body size in *S. americana*. Fielding (2004) reported that body weight of *M. sanguinipes* was lower at the low (21°C and 24°C) and high (39°C and 42°C) extremes (with respect to optimal for *M. sanguinipes*) of the temperatures tested, relative to intermediate temperatures. In the current study, body weight was not recorded and a lack of difference in size does not necessarily equate to a lack of difference in weight. There may have been a possibility that there were differences in weight. However, temperatures tested in the current study would not be considered extreme, and it is likely that there were no differences in weight, just as there were no differences in size. As expected, there were differences between the sexes, but when high and low frequency treatments were combined there were significant differences between the two frequencies. No other facet of temperature affected body size when the treatments were analyzed separately and, therefore, it is unlikely frequency would have an effect and the differences between high and low frequency treatments were most likely caused by trial effects. If trial effects affected a parameter not affected by temperature, it may be that the differences between trials also affected other parameters more so than frequency. This is in contrast to the nymphal developmental data, which suggest that differences between high and low frequency treatments might actually be due to frequency.

Days to oviposition

In high frequency treatments the 29.5°C low amplitude treatment was the only significantly different treatment and there were no differences in sexual maturation between the 29.5°C high amplitude treatment and the 31.5°C treatments for the number of days to first oviposition. The results suggest that amplitude only has an effect at suboptimal temperatures and that mean body temperatures are likely not as important as maximum body temperatures experienced. Just as in nymphal development time, reaching 37°C when in the 29.5°C high amplitude treatment provided some benefit. The analysis conducted on low frequency treatments for days to oviposition also revealed only one significantly different treatment. However, just as for nymphal development time, the treatment was the 29.5°C constant treatment and not the 29.5°C low amplitude treatment. The reasons for this are likely the same as those given for development time, having to do with the duration of continuous time spent in the high temperature range of the treatments, suggesting a frequency effect. Together both sets of results suggest that the number of days to oviposition is affected by both amplitude and frequency of temperature change. Because the differences were only seen in the 29.5°C treatments, it is strongly suggested that these differences only occur at suboptimal temperatures.

The separate analyses conducted on both high and lower frequency treatments for days to oviposition revealed results very similar to those for nymphal development time. This is not surprising as both nymphal development time and sexual maturation are both developmental parameters (together equaling total development time) and should be affected in a similar manner. However, when both high and low frequency treatments were combined, the analysis revealed the effect of frequency to be non-significant, contrary to what was found for nymphal development time. Why this occurs is uncertain, but if the difference between nymphal

development times of both high and low frequency treatments is due to trial effects, it would be very unusual for the frequency or trial effect to be absent in sexual maturation rate considering the two parameters are closely related.

Gündüz and Gülel (2002) reported that *S. gregaria* needed to reach a critical weight before reproduction began, and that temperature did not affect this critical weight but only how fast it was attained. It may be that 29.5°C high frequency and constant treatments did not maintain high enough temperatures for long enough periods of time for grasshoppers to quickly reach this critical weight. One explanation for why differences were observed between 31.5°C treatments and 29.5°C high frequency low amplitude and constant treatments and not the low frequency 29.5°C treatments is that the low frequency 29.5°C treatments may have maintained the high limit of the temperature range long enough to increase rate of weight gain. In contrast, the less optimal treatments (29.5°C high frequency low amplitude and 29.5°C constant) would have decreased rates of weight gain and delayed sexual maturation. Delayed sexual maturation could have several negative effects on a grasshopper population. Grasshoppers with delayed sexual maturation are more likely to die of disease or predation before being able to reproduce. In colder environments, those that survive will have less time to lay eggs, and those eggs that are laid will hatch later and be at a disadvantage compared to eggs which were laid earlier in the season.

Measuring how many days it took female grasshoppers to reach sexual maturity posed a problem in this study and for that reason the accuracy of its measurement may have suffered. All other parameters were taken as cage averages (nymphal development time and pods / female) or as individual measurements of every available grasshopper or egg pod (body size and eggs / pod). The number of days it took to first oviposition was recorded for only one individual per

cage, the first female to lay an egg pod. While it was rather simple to determine total nymphal development time by recording when each grasshopper reached adult hood, it was impossible to record the first oviposition date of every grasshopper in the cage. There was no way to determine which female the egg pod came from. Therefore, only 40 of the 600 grasshoppers used in this study were used for recording the number of days from hatch to oviposition.

Egg pods / female

Mean daily temperature had a significant effect on the number of egg pods laid per female, with 31.5°C treatments laying, on average, 2 more egg pods / female than in 29.5°C treatments for both high frequency and low frequency and constant temperature treatments. Females in 29.5°C constant treatment laid the lowest average with only 1.27 egg pods / female, while 31.5°C alternating treatments laid 5-6 egg pods / female. This is a huge difference with respect to overall fecundity. These results are similar to those reported by Uvarov (1966a) on *C. pellucida*, where females reared at 27ºC laid an average of only one egg pod, as opposed to the average of four egg pods laid by females reared at 37ºC. While the results of the analysis clearly show that temperature has a significant effect on egg pods / female, the effects of amplitude and frequency of temperature change are much harder to identify. The results from high frequency treatments are similar to those for developmental parameters with only the 29.5°C low amplitude treatment being significantly different from all others, suggesting the same inferences made about nymphal development time might also be able to be made about fecundity. However, the results from low frequency and constant treatments are very different from those for developmental data with many treatments being different from each other.

The benefits on nymphal development time provided by the longer periods of continuous high temperatures in low frequency treatments do not seem to apply to reproductive parameters.

The 29.5°C low frequency high amplitude treatment has a much lower average than its high frequency counterpart (Table 3-6). Additionally, the 29.5°C low frequency treatments are statistically different from the 31.5°C treatments, something not seen in developmental data. This suggests that continuous periods of the high range of temperatures have little if any effect on the number of egg pods / female. The notion that temperature fluctuations have more of an effect at suboptimal temperatures is also harder to see because most of the significant differences are between mean temperatures. While the 29.5°C constant treatment seems to have produced the fewest egg pods / female, it is not considered statistically different from the other 29.5°C treatments or even the 31.5°C constant treatment (Table 3-6). On the other hand, the 31.5°C constant treatment is significantly different from one of the 31.5°C treatments. This is in contrast to what has been suggested earlier, that temperature fluctuations are more influential at suboptimal temperatures. These results have made it difficult to decipher the effects of temperature, amplitude and frequency on the number of egg pods laid per female, especially when one considers that high frequency treatments produced, on average, one more egg pod / female than low frequency treatments and were considered statistically different from low frequency treatments.

Eggs / pod

Only the higher values of raw data for the number of eggs / pod were within the range of 76-100 eggs per pod reported by Kuitert and Connin (1952). The means for each treatment, with the exception of one, were never within this range. Why this is remains uncertain, but could be due to a couple of factors. It may be that conditions in this study were not always optimal and could have caused a reduction in the number of eggs per pod. Additionally, the reduced number

of eggs / pod might be an artifact of prolonged laboratory colonization. Regardless, there were significant effects of temperature change on the number of eggs laid per pod.

The effects of mean temperature on the number eggs / pod are less obvious than those for egg pods / female. In high frequency treatments the females reared in 29.5°C treatments produced more eggs / pod than those reared in 31.5°C treatments, while the exact opposite occurred in low frequency treatments (Table 3-6). The differences in amplitude and temperature in high frequency treatments are due to the 29.5ºC high frequency low amplitude treatments, which is the only significantly different treatment. Females from the 29.5°C high frequency low amplitude treatment produced an abnormally large number of eggs / pod when compared to females from the rest of the treatments. This suggests that suboptimal temperatures might cause an increase in the number of eggs / pod. It was originally hypothesized that this increase in number of eggs / pod was possibly a stress induced response to compensate for prolonged development and the reduced number of egg pods laid, as this treatment was always the only significantly different treatment of the high frequency treatments. However, such compensation is not seen in equivalently affected low frequency treatments. This leads to the notion that something may have been wrong with one or more elements of the experiment involving the 29.5°C high frequency low amplitude treatment.

The results for eggs / pod for low frequency treatments were very similar to those for egg pods / female, and many of the same conclusions can be drawn from the results. However, unlike egg pods / female, the 29.5°C constant treatment was considered statistically different from other 29.5°C treatments and the 31.5°C constant treatment (Table 3-6). This exemplifies the negative effects that suboptimal temperatures can have on reproductive parameters. Again, because there are no real differences between high and low amplitude treatments, the amplitude effect seen in

low frequency treatments is actually caused by the presence or absence of temperature change. Unexpectedly, there was no frequency or trial effect on the number of eggs / pod when both high and low frequency treatments were combined, even though there were differences in frequency between constant and low frequency treatments. This was puzzling because there was some amount of frequency or trial effects in every other parameter tested but days to first oviposition.

Table 3-1. ANOVA results for nymphal development time at different treatment combinations.

Treatment	Source	SS	df	MS	F	*P*
High Frequency	Amplitude	17.65	1	17.65	5.34	0.0394
	Temperature	109.97	1	109.97	33.27	<0.0001
	Amp x Temp	13.99	1	13.99	4.24	0.0620
	Error	39.66	12	3.30		
Low Frequency + Constant	Amplitude	19.90	2	9.95	5.59	0.0129
	Temperature	15.60	1	15.60	8.99	0.0077
	Amp x Temp	39.47	2	19.73	11.09	0.0007
	Error	32.03	18	1.78		
High Frequency + Low Frequency	Amplitude	11.39	1	11.39	4.56	0.0431
	Frequency	123.87	1	123.87	49.58	<0.0001
	Temperature	61.70	1	61.70	24.70	<0.0001
	Freq x Amp	6.59	1	6.59	2.64	0.1175
	Temp x Amp	32.33	1	32.33	12.94	0.0014
	Temp x Freq	48.66	1	48.66	19.48	0.0002
	Temp x Freq x Amp	0.16	1	0.16	0.06	0.8049
	Error	59.96	24	2.50		

Table 3-2. Mean comparisons of nymphal development time (in days) in high and low treatments (HF = high frequency, LF = low frequency, HA = high amplitude, LA = low amplitude). Means followed by different letters are significantly different at the 0.05 level (LSM, Tukey's adjustment).

Treatment	Mean ± SD		Amplitude	Mean ± SD		Temperature	Mean ± SD	
31.5ºC HF HA	37.68 ± 2.45	A	HA	39.36 ± 2.50	A	31.5ºC	37.80 ± 1.76	A
31.5ºC HF LA	37.91 ± 1.09	A	LA	41.47 ± 4.14	B	29.5ºC	43.04 ± 2.66	B
29.5ºC HF HA	41.05 ± 0.98	A						
29.5ºC HF LA	45.02 ± 2.25	B						
31.5ºC LF HA	37.26 ± 1.45	A	HA	36.34 ± 1.43	A	31.5ºC	36.30 ± 1.51	A
31.5ºC LF LA	35.40 ± 1.34	A	LA	36.63 ± 1.88	A	29.5ºC	37.94 ± 2.45	B
31.5ºC Constant	36.26 ± 1.48	A	Constant	38.40 ± 2.63	B			
29.5ºC LF HA	35.42 ± 0.68	A						
29.5ºC LF LA	37.86 ± 1.56	AB						
29.5ºC Constant	40.54 ± 1.31	B						

Table 3-3. Mean comparisons of nymphal development time (in days) in high (HF) and low (LF) frequency treatments at fixed levels of amplitude and temperature (HA = high amplitude, LA = low amplitude). Means followed by different letters are significantly different at the 0.05 level (LSM, Tukey's adjustment).

Fixed Parameter	Frequency	Mean ± SD	
HA	HF	39.37 ± 2.50	A
	LF	36.34 ± 1.43	B
LA	HF	41.47 ± 4.14	A
	LF	36.63 ± 1.88	B
29.5ºC	HF	43.04 ± 2.66	A
	LF	36.64 ± 1.71	B
31.5ºC	HF	37.79 ± 1.76	B
	LF	36.33 ± 1.63	B
All	HF	40.42 ± 3.48	A
All	LF	36.48 ± 1.62	B

Table 3-4. ANOVA results for body size at different treatment combinations.

		Femur Length					Overall Length				
Treatment	Source	SS	df	MS	F	*P*	SS	df	MS	F	*P*
	Amplitude	4.18	1	4.18	3.26	0.0700	0.46	1	0.46	0.13	0.7200
High Frequency	Sex	286.34	1	286.34	223.40	<0.0001	1497.47	1	1497.47	414.98	<0.0001
	Temperature	0.00	1	0.00	0.00	0.9600	13.79	1	13.79	3.82	0.0540
	Temp x Amp x Sex	6.94	1	6.94	5.42	0.0200					
	Error	119.20	93	1.28			335.59	93	3.61		
	Amplitude	2.81	2	1.41	0.36	0.7000	8.97	2	4.48	1.95	0.1400
Low Frequency +	Sex	817.03	1	817.03	207.64	<0.0001	4102.27	1	4102.27	1786.85	<0.0001
Constant	Temperature	1.71	1	1.71	0.43	0.5100	3.01	1	3.01	1.31	0.2500
	Temp x Sex						21.47	1	21.47	9.35	0.0030
	Error	991.57	252	3.93			578.54	252	2.30		
High Frequency +	Frequency	97.88	1	97.88	23.48	<0.0001	216.38	1	216.38	11.56	0.0008
Low Frequency	Error	1146.62	275	4.17			5145.32	275	18.71		

Table 3-5. ANOVA results for reproduction at different treatment combinations.

		Days to Oviposition					Egg Pods / Female					Eggs / Pod				
Treatment	Source	SS	df	MS	F	*P*	SS	df	MS	F	*P*	SS	df	MS	F	*P*
	Amplitude	27.56	1	27.56	1.03	0.3300	10.78	1	10.78	24.47	0.0003	3767.46	1	3767.46	16.78	<0.0001
High Frequency	Temperature	351.56	1	351.56	13.17	0.0040	19.95	1	19.95	45.30	<0.0001	6914.56	1	6914.56	30.80	<0.0001
	Amp x Temp	95.06	1	95.06	3.56	0.0800	19.95	1	19.95	45.30	<0.0001	3652.88	1	3652.88	16.27	<0.0001
	Error	320.25	12	26.69			5.29	12	0.44			41089.37	183	224.53		
	Amplitude	1705.75	2	852.88	12.12	0.0005	17.32	2	8.66	12.09	0.0005	8441.19	2	4220.59	11.96	<0.0001
Low Frequency +	Temperature	988.17	1	988.17	14.04	0.0020	32.03	1	32.03	44.71	<0.0001	9780.86	1	9780.86	27.75	<0.0001
Constant	Amp x Temp	523.58	2	261.79	3.72	0.0400	1.59	2	0.80	1.11	0.3500	455.09	2	227.54	0.64	0.5200
	Error	1266.50	18	70.36			12.89	18	0.72			107602.00	305	352.79		
	Amplitude	75.03	1	75.03	3.40	0.0800	4.57	1	4.57	9.57	0.0050					
	Frequency	13.78	1	13.78	0.62	0.4300	9.57	1	9.57	20.01	0.0002	5.68	1	5.68	0.02	0.8966
High Frequency +	Temperature	504.03	1	504.03	22.83	<0.0001	45.36	1	45.36	94.87	<0.0001					
Low Frequency	Freq x Amp	1.53	1	1.53	0.07	0.7900	6.27	1	6.27	13.12	0.0014					
	Temp x Amp	5.28	1	5.28	0.24	0.6300	14.99	1	14.99	31.34	<0.0001					
	Temp x Freq	16.53	1	16.53	0.75	0.4000	0.18	1	0.18	0.31	0.5508					
	Temp x Freq x Amp	132.03	1	132.03	5.98	0.0200	5.98	1	5.98	12.51	0.0017					
	Error	529.75	24	22.07			11.48	24	0.48			139459.82	415	336.05		

Table 3-6. Mean comparisons of reproductive data in high and low frequency treatments (HF = High Frequency, LF = low frequency, HA = high amplitude, LA = low amplitude). Means followed by different letters are significantly different at the 0.05 level (LSM, Tukey's adjustment).

	Treatment	Mean ± SD				Amplitude	Mean ± SD				Temperature	Mean ± SD			
	31.5ºC HF HA	58.25	±	2.63	A	HA	60.50	±	3.02	A	31.5ºC	57.13	±	2.10	A
	31.5ºC HF LA	56.00	±	0.00	A	LA	63.13	±	10.02	A	29.5ºC	66.50	±	7.67	B
	29.5ºC HF HA	62.75	±	0.96	AB										
Days to	29.5ºC HF LA	70.25	±	9.95	B										
Oviposition	31.5ºC LF HA	56.50	±	1.00	A	HA	61.38	±	7.25	A	31.5ºC	62.58	±	7.88	A
	31.5ºC LF LA	63.25	±	2.06	A	LA	64.88	±	2.75	A	29.5ºC	75.42	±	15.99	B
	31.5ºC Constant	68.00	±	11.55	A	Constant	80.75	±	18.35	B					
	29.5ºC LF HA	66.25	±	7.63	A										
	29.5ºC LF LA	66.50	±	2.52	A										
	29.5ºC Constant	93.50	±	14.80	B										
	31.5ºC HF HA	5.92	±	0.57	A	HA	5.92	±	0.68	A	31.5ºC	6.21	±	0.71	A
	31.5ºC HF LA	6.51	±	0.78	A	LA	4.28	±	2.45	B	29.5ºC	3.98	±	2.16	B
	29.5ºC HF HA	5.92	±	0.87	A										
Egg Pods	29.5ºC HF LA	2.04	±	0.28	B										
/ Female	31.5ºC LF HA	4.95	±	0.97	AB	HA	3.94	±	1.37	A	31.5ºC	4.56	±	1.28	A
	31.5ºC LF LA	5.58	±	0.39	A	LA	4.07	±	1.68	A	29.5ºC	2.25	±	1.12	B
	31.5ºC Constant	3.14	±	0.77	BC	Constant	2.20	±	1.40	B					
	29.5ºC LF HA	2.93	±	0.82	C										
	29.5ºC LF LA	2.55	±	0.54	C										
	29.5ºC Constant	1.27	±	1.28	C										
	31.5ºC HF HA	59.12	±	11.09	A	HA	60.88	±	14.37	A	31.5ºC	59.22	±	13.01	A
	31.5ºC HF LA	59.27	±	13.92	A	LA	64.43	±	18.16	B	29.5ºC	70.40	±	20.56	B
	29.5ºC HF HA	62.79	±	17.17	A										
Eggs / Pod	29.5ºC HF LA	82.46	±	19.98	B										
	31.5ºC LF HA	68.11	±	19.89	A	HA	63.73	±	20.22	A	31.5ºC	64.13	±	19.20	A
	31.5ºC LF LA	66.68	±	18.54	A	LA	62.63	±	18.97	A	29.5ºC	54.35	±	19.80	B
	31.5ºC Constant	57.11	±	17.59	B	Constant	53.23	±	19.38	B					
	29.5ºC LF HA	58.20	±	19.43	AB										
	29.5ºC LF LA	55.98	±	17.94	B										
	29.5ºC Constant	40.58	±	19.97	C										

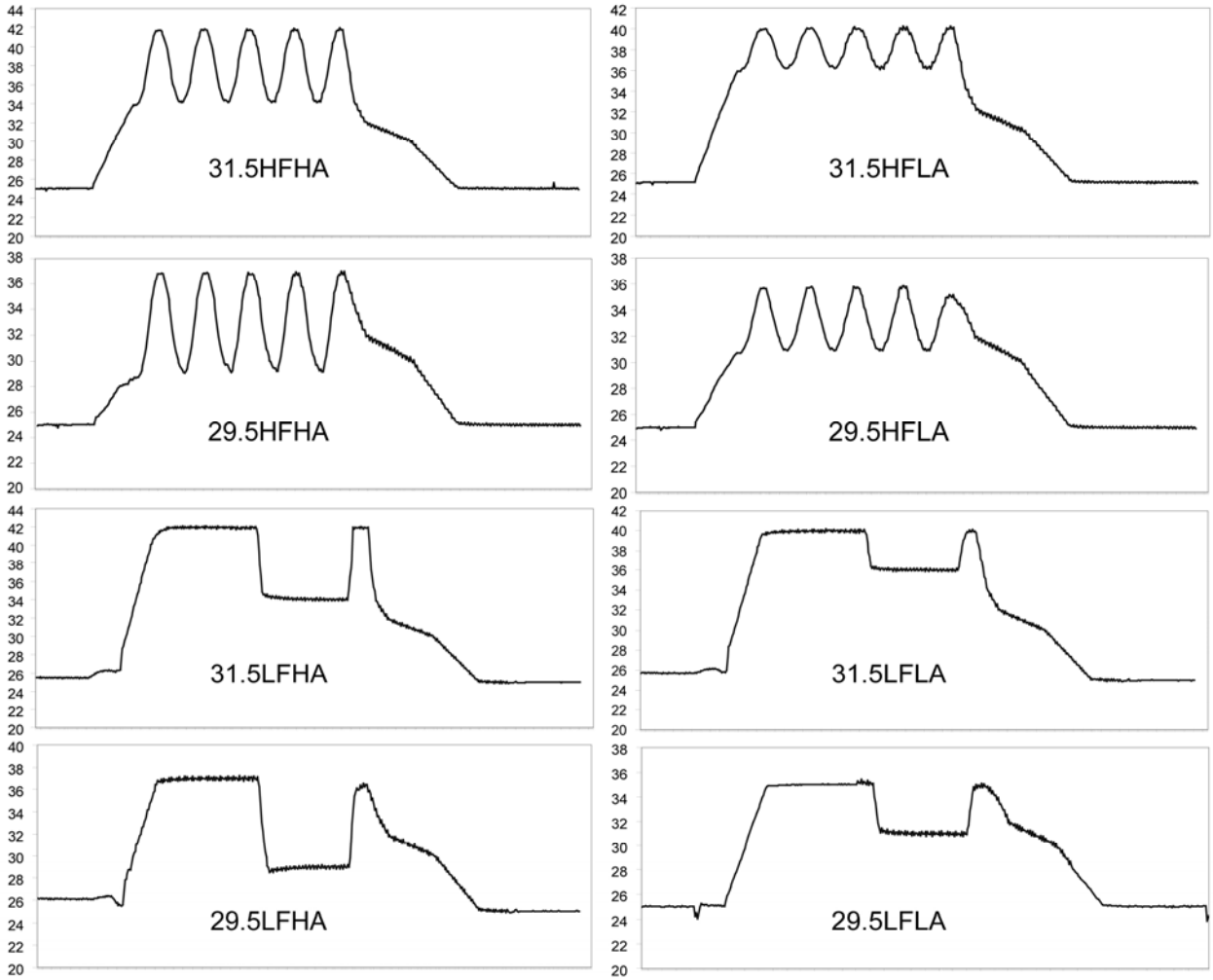

Figure 3-1. Fluctuating laboratory temperature treatments for each mean temperature taken from environmental chamber data loggers over a 24hr time period (resolution = 3min). Y axis is given in °C. (HF = high frequency, LF = low frequency, HA = high amplitude, LA = low amplitude).

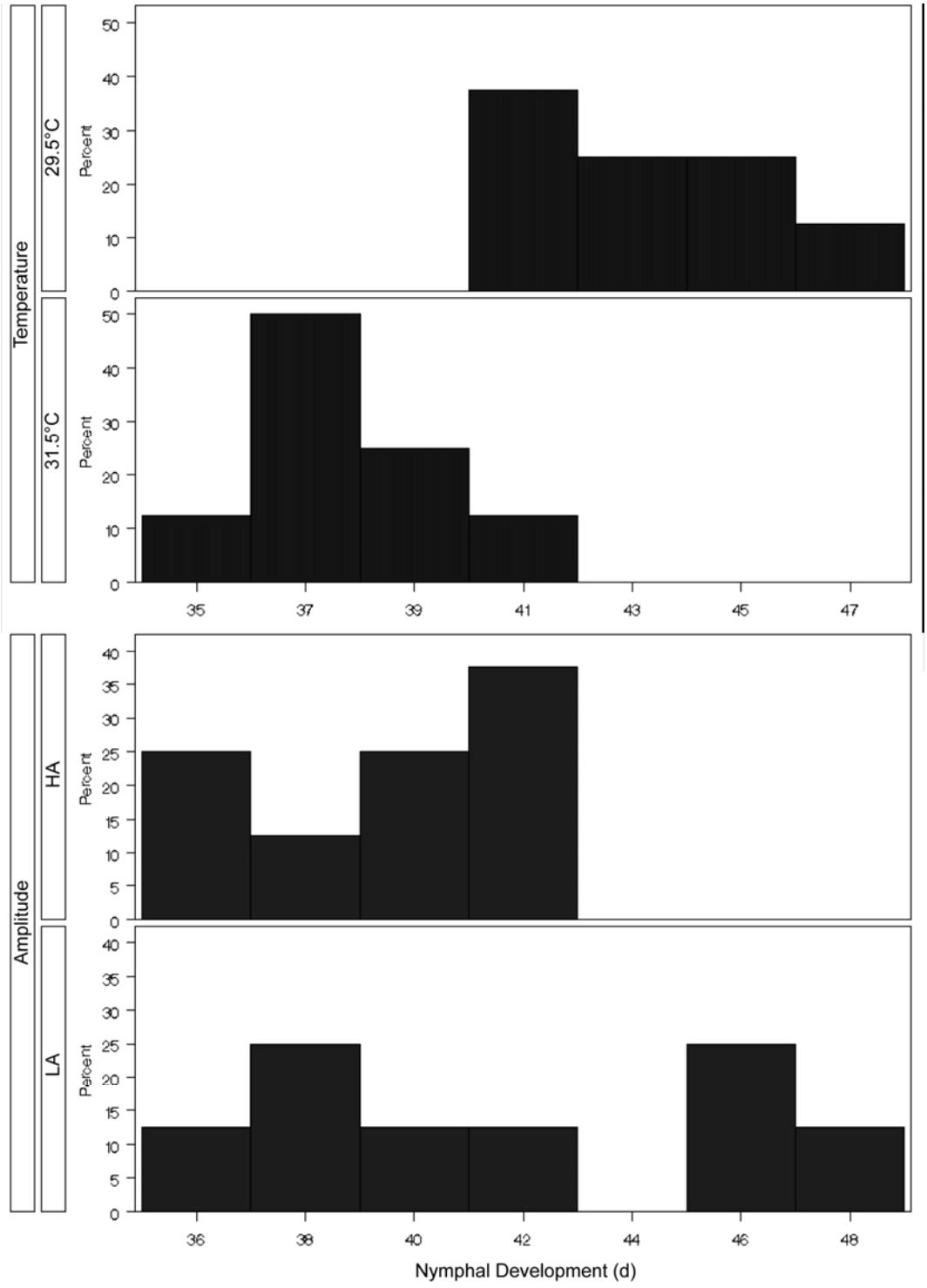

Figure 3-2. Nymphal development time (d) of high frequency treatments at fixed levels of temperature and amplitude.

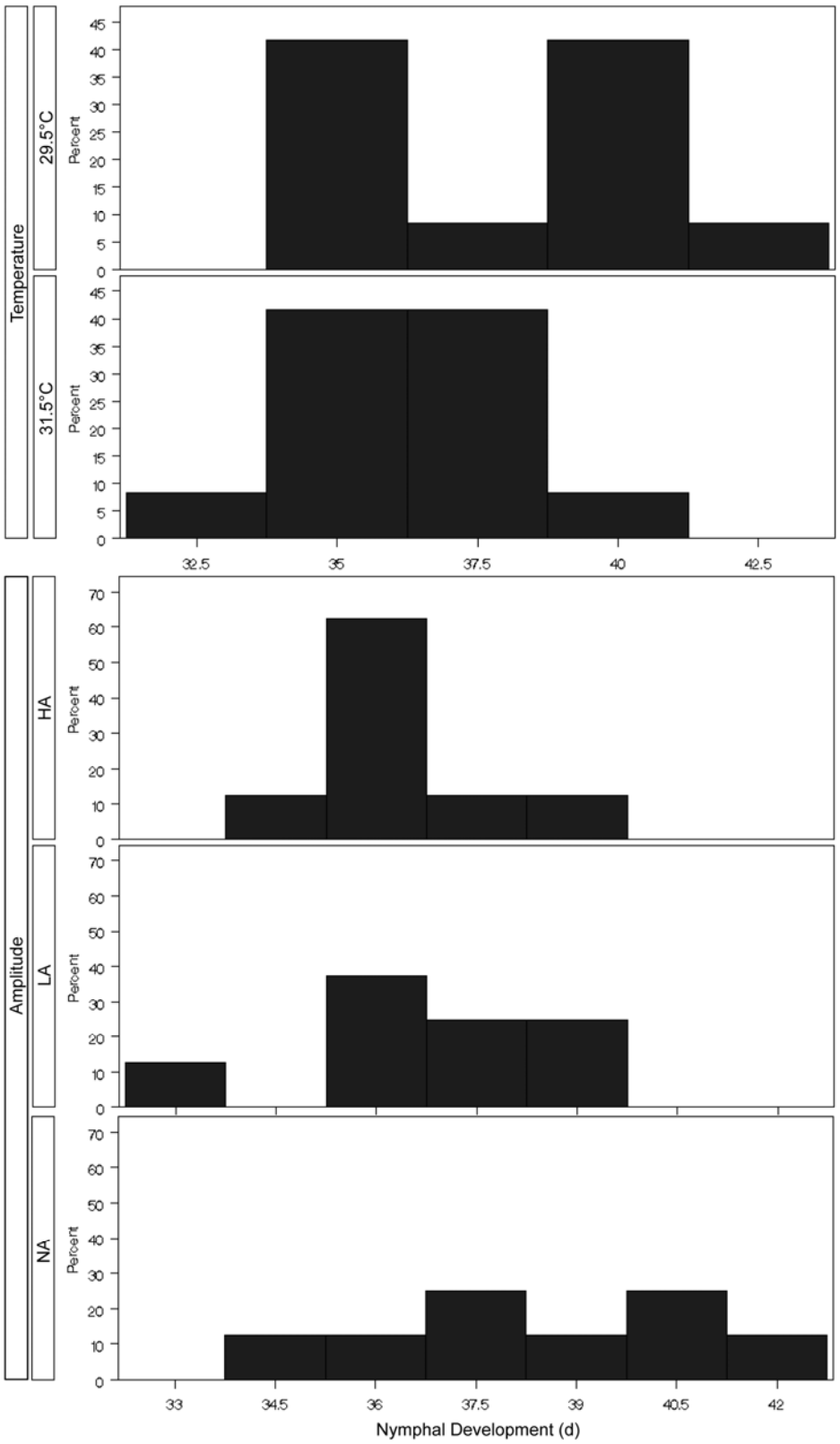

Figure 3-3. Nymphal development time (d) of low frequency treatments at fixed levels of temperature and amplitude.

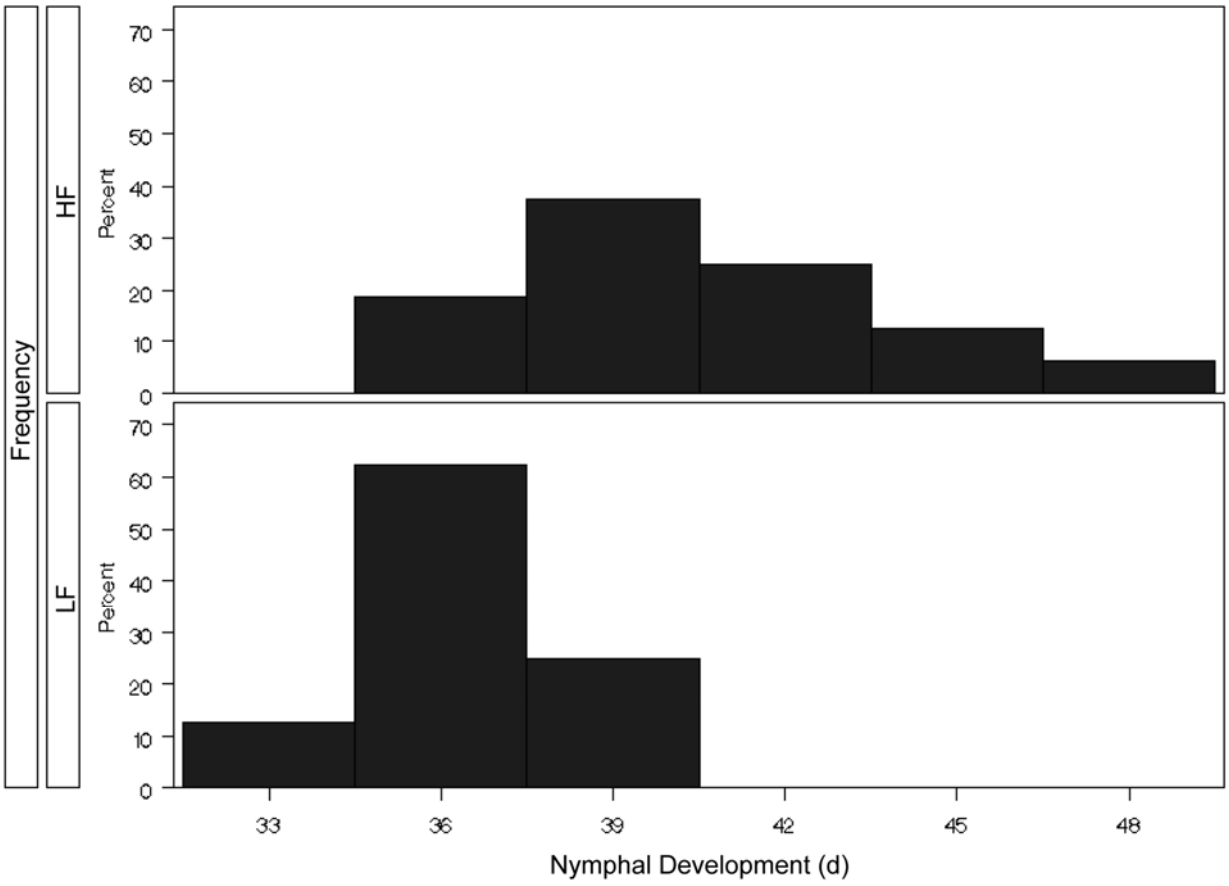

Figure 3-4. Nymphal development time (d) for high frequency and low frequency treatments.

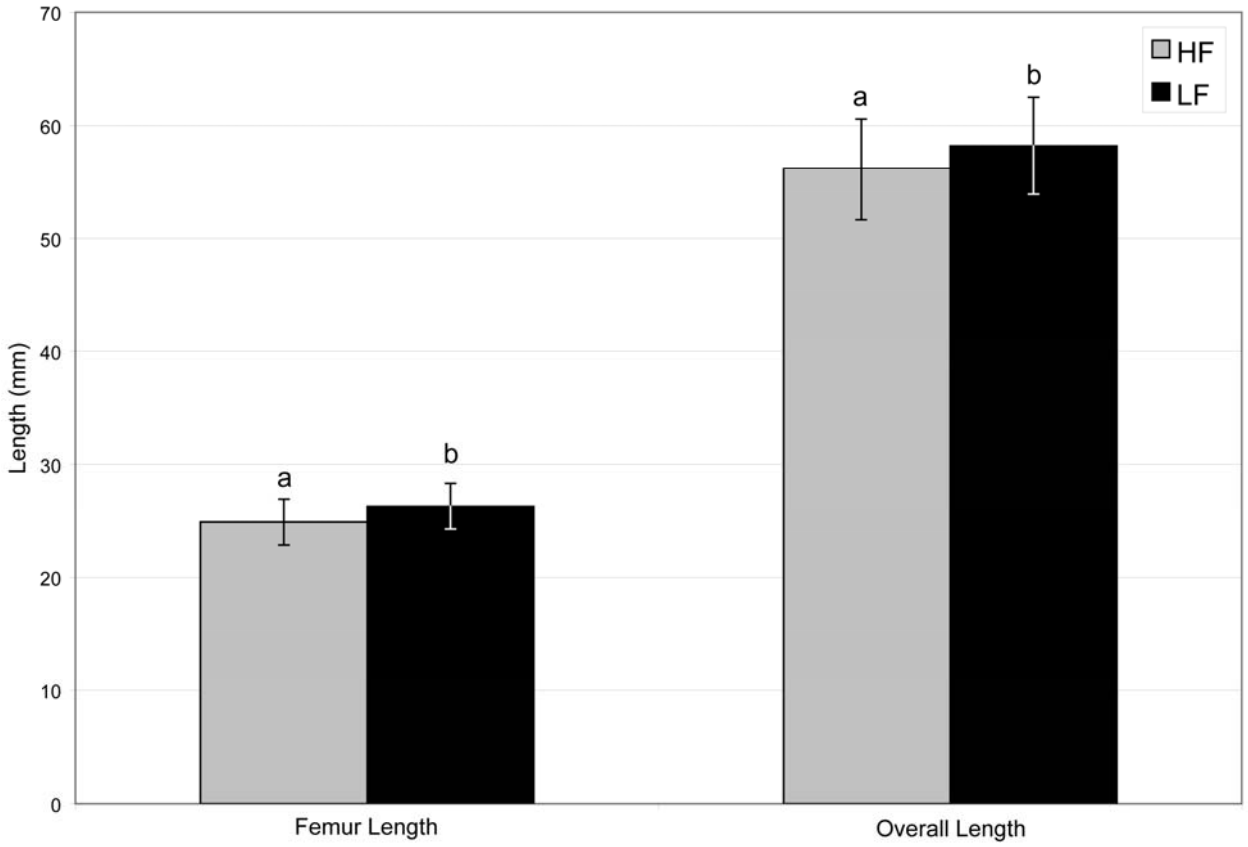

Figure 3-5. Mean (± SD) femur and overall length at high and low frequency treatments (HF = high frequency, LF = low frequency). Columns designated by a different letter under their respective category are considered different at the 0.05 level.

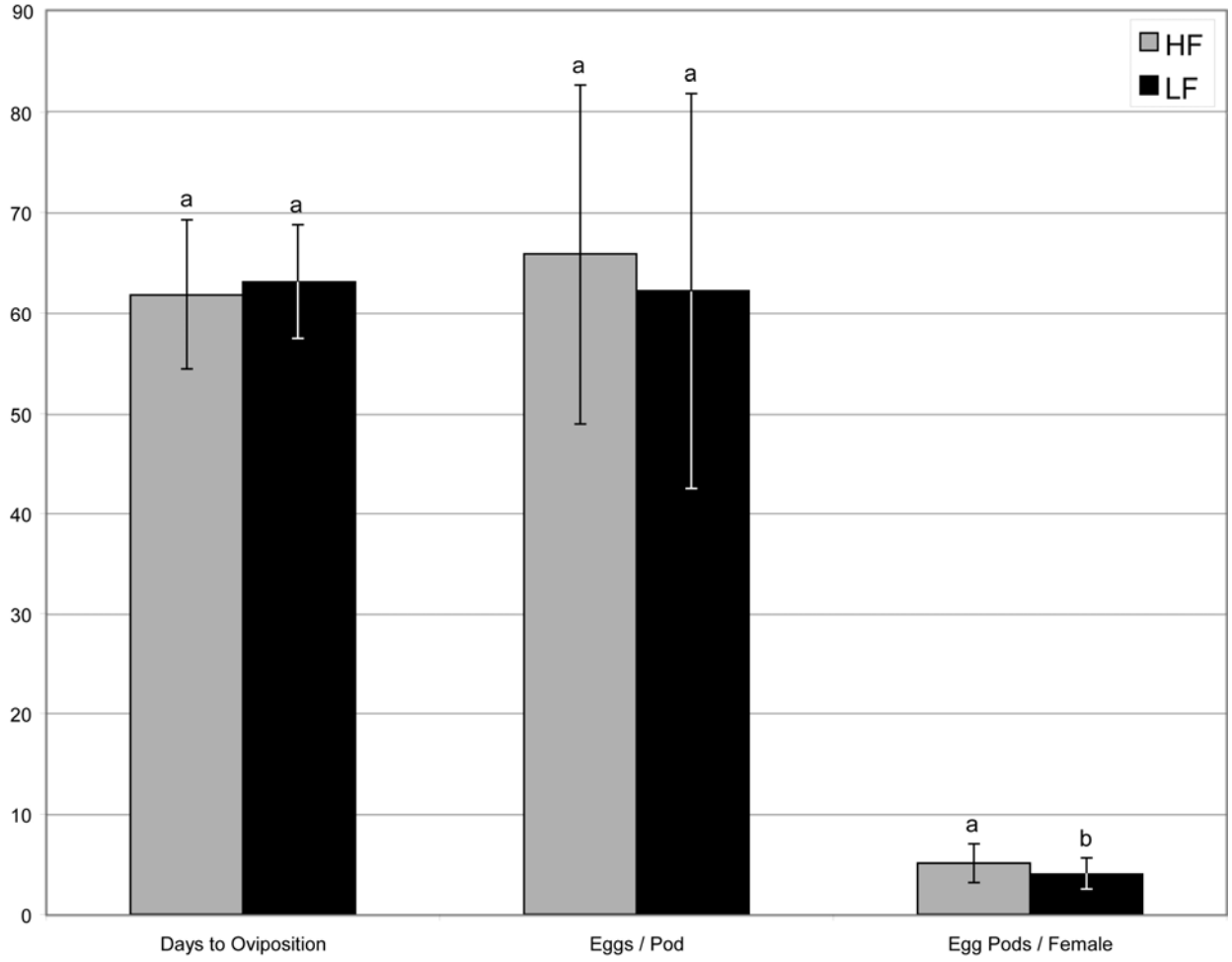

Figure 3-6. Mean (± SD) number of days to first oviposition, egg pods / female, and eggs / pod at high and low frequency treatments (HF = high frequency, LF = low frequency). Columns designated by a different letter under their respective category are considered different at the 0.05 level.

CHAPTER 4
CONCLUSIONS

Overall, the linear plots of body temperature (Figs. 2-2 – 2-9) show a general dependence of body temperature on sunlight intensity. This relationship can also be seen in the multiple linear regression analysis of grasshopper body temperatures . Data from this study have shown how important sunlight availability can be to *S. americana* for maintaining body temperatures above ambient and that there is a positive linear relationship between these environmental factors and body temperature. The rates of temperature increase measured in this study suggest that grasshoppers have the ability to raise their body temperature by 2.5°C per minute, if not faster, by basking in direct sunlight. At this rate, grasshoppers could more than double their body temperature in less than ten minutes and reach optimal temperature in even a shorter period of time. Furthermore, body temperatures of sun constrained grasshoppers were 12.4-19.6°C above ambient temperature, often around 75% above ambient temperature and even reaching 50°C**.** Thus, sunlight plays a vital role in the achievement and maintenance of optimal body temperatures above ambient temperature.

Shade constrained grasshoppers usually had body temperatures above ambient, up to 26.9% above and even reaching 41.3% above ambient. In addition, body temperatures of all grasshoppers were shown, at times, to be significantly higher than ambient temperature even in the absence of direct sunlight. If this is generally true, it would cause problems with phenology modeling and might explain why there often are discrepancies between predicted development rates or population numbers and actual ones. Current phenology models are often based on mean daily ambient temperature and developmental and reproductive data obtained at constant temperatures. In order to develop accurate phenology models, it may be necessary to obtain more

accurate body temperatures either by direct measurement or by estimation using measurements of sunlight intensity, temperature and possibly wind speed.

As expected, body temperatures of free roaming grasshoppers were often between those of shade and sun constrained grasshoppers. This study has shown that behavioral orientation toward direct solar radiation has a significant positive effect on body temperature and that there is a positive relations ship between basking behavior and body temperature and as basking increased body temperature increased. On the hottest days tested, when free roaming grasshoppers remained in the shade, their body temperatures were very similar to those of shade constrained grasshoppers. This further exemplifies the effects of behavior on body temperature. In addition, grasshopper behavior was found to be affected by ambient temperature and as ambient temperature increased basking behavior decreased. This helps prove that grasshoppers do not utilize all the heat available to them and that basking is only used to raise body temperature when ambient temperatures are not high enough to provide sufficient heat.

The differences between free roaming grasshoppers and constrained grasshoppers, along with the regression analysis conducted on behavioral observations, especially the effect of ambient temperature on mean behavioral rating (Fig. 2-14), provides evidence showing that grasshoppers are behaviorally thermoregulating. While the above discussion might suggest that it would benefit those modeling phenology to measure more than just ambient temperature, accurately measuring behavior in the field for the purpose of improving models will likely prove to be a daunting task. A simpler solution may be to monitor environmental conditions to ascertain whether or not optimal body temperature can be achieved by thermoregulating grasshoppers. It can be assumed that grasshoppers will attempt to maximize fitness whenever

possible, and knowing when they are able to do so and how development and reproduction are affected, could help to better predict development time and population numbers in the field.

Another important facet of this study, which is directly related to the above discussion, was to examine how adverse weather conditions could affect body temperature and to assess whether even summer conditions in Florida could be sub-optimal for the development and reproduction of *S. americana*. This study showed how frequently and quickly body temperature in grasshoppers can be affected by adverse weather conditions, and how mean temperatures of free roaming grasshoppers were significantly reduced on cloudy or rainy days, compared to those on predominately sunny days. Adverse weather conditions could cause causing up to 38% decreases in body temperature at a rate of up to 0.0269 °C/sec and reduce mean body temperatures by up to 8ºC a day. It is easy to imagine that any prolonged occurrence of these adverse weather conditions could have severe negative implications on development and reproduction in *S. americana,* and grasshoppers in general. In Florida, average ambient temperature is high during the months when grasshoppers are most active, and sunlight is not required to complete development. However, periods of prolonged cloud cover and rain occur during the summer months in Florida, which can prevent grasshoppers from reaching optimal temperatures, and therefore optimal fitness. Knowing how weather affects grasshopper body temperature is part of knowing when grasshoppers will be able to optimize body temperature. Including the effects of adverse weather conditions on a grasshopper's ability to optimize body temperature into phenology models would likely help to improve a model's predictive power.

At more optimal temperatures, nymphal development time seemed to be little affected by temperature changes in this study, and there was little evidence of a Kaufmann effect in *S. americana* at the 31.5°C temperatures tested. The few effects temperature fluctuations had on

nymphal development time occurred in the lower temperature treatments and at the low amplitude and constant regimes. It is likely that in 29.5°C treatments, temperature fluctuations provided enough of a rise in temperature to counteract some of the negative effects of rearing at suboptimal temperatures. The fact that the 29.5°C constant treatment showed a deceleration in developmental rate compared to other alternating 29.5°C treatments suggest a nonlinear relationship between body temperature and developmental rate, and subsequently a Kaufmann effect at suboptimal temperatures. More tests at suboptimal conditions would be necessary to confirm this. While the results do not provide a clear explanation, what is clear is that at least at lower temperatures, temperature fluctuations have some significant effect on development time in *S. americana*, even if it may be modest.

The mean temperatures of 31.5ºC and 29.5ºC chosen for this study were taken from the field portion of this study and represent 24hr mean body temperatures for mostly cloudy and mostly sunny days. The small differences in development time detected between the two temperatures, and those caused by temperature fluctuations, will likely have little effect on phenology modeling for pest management purposes, and for predicting development time in the field. Grasshoppers in the field will not all hatch at the same time, and therefore will not complete development at the same time, making differences in development time of 5 d practically irrelevant. However, it is still suggested that phenology modeling be based on body temperature rather than ambient temperature.

Data involving female fecundity were more heavily affected by temperature changes and differences in temperature than development time, and could be more sensitive to small changes in temperature. Together, the data for egg pods / female and eggs / pod give an accurate view of how fecundity might be affected by differences in temperature and temperature fluctuations.

Why reproduction was affected more during the months of February to June than October to February remains unclear. Differences in most of the other parameters showed that differences between frequencies were likely due to trial effects, and suggested that differences in reproduction would also be due to trial effects. One plausible explanation for the differences is that there was some experimental error involved, and there were differences in the rearing methods between the two studies, possibly food quality.

Overall, temperature was shown to affect nymphal development time, reproductive development time, and female fecundity. With few exceptions, higher temperature treatments increased developmental rate and female fecundity. Amplitude was also shown to affect nymphal development time, reproductive development time, and female fecundity; however, the effects are less clear. Amplitude caused few differences between 31.5ºC treatments, with the exception for the 31.5ºC constant treatment. The effects of amplitude seemed to be more pronounced at the sub-optimal 29.5ºC treatments. Still, there is no clear pattern. The low amplitude 29.5ºC treatment within the high frequency treatments was often the only treatment affected by amplitude, while in low frequency treatments the 29.5ºC low amplitude treatment was often not different from the 29.5ºC high amplitude treatment and it was only the 29.5ºC constant treatment that was different from the other 29.5ºC treatments. Frequency affected all parameters except the number of days to oviposition and eggs / pod, causing increased developmental rates and egg pod production. Again, it must be stressed that these effects cannot be separated from those that might be caused by differences between trials.

However, there is almost certainly some factor, likely season, which is interacting with the treatments and producing such puzzling results. High frequency treatments were conducted during the late fall and winter months, while low frequency and constant temperature treatments

were conducted during the spring and early summer months. If there was a seasonal effect, one might easily conclude that high frequency treatments would have laid fewer eggs. However, this is not the case and they seemed to have a higher fecundity than those females reared in low frequency treatments. The effect of mean temperature also seems to be more pronounced in low frequency treatments. If temperature affects reproduction equally, regardless of season, the results reported here make little sense. However, it might be possible that temperature does not affect fecundity equally and that optimal or critical temperatures for reproduction change with regard to season. While this has not been observed in grasshoppers it has been observed in termites (Hu and Appel 2004). If the optimal temperature for reproduction in *S. americana* did change with season, and during the winter months optimal temperatures were lower than those for summer months, it would easily explain why the number of egg pods / females is less affected by the 29.5°C temperatures in high frequency treatments. However, there is little evidence proving season caused differences between the trials and all that can be stated is that either frequency or an unknown trial effect is responsible.

Another possibility is that our lack of knowledge about the specifics of *S. americana* reproduction may be preventing the correct interpretation of the data. While it is know that there are two generations of *S. americana* a year in Florida and that the second generation overwinters as an adult, it is not known if there are any differences in development and fecundity between these two generations. Consider that females reared in high frequency treatments during the winter months could be the laboratory equivalent of the generation that overwinters as adults. It may be possible that females of *S. americana* surviving the winter lay more eggs than those females of the second generation as programmed response to reduced population size due to

lower survival rates of overwintering adults. A possible mechanism for such a response could be the reduction in optimal temperature as discussed above or some other unknown factor

The 1991 *S. americana* outbreak in Florida was attributed to a 5 yr drought (Capinera 1993a) which caused available sunshine to be very high and possibly increased grasshopper fitness. The data from this study provide some tangible evidence as to how weather conditions can affect grasshopper fitness and why a 5 yr drought might be beneficial for grasshopper populations in Florida. Drought conditions would be expected to be close to those represented by July 5 or July 8, when grasshopper body temperature averaged around 38ºC, while normal conditions might be represented by days like June 29, with mean body temperatures of free roaming grasshoppers around 30ºC. Obviously there will be advantages to extended periods with an 8ºC difference in daytime body temperatures. These extended periods of increased body temperature are an excellent example of how periods of drought might benefit grasshopper development and reproduction leading to outbreak populations.

If drought was thought to cause outbreaks of *S. americana* at the time of the 1991 outbreak, scientists might have tried to use population modeling to estimate the scale of the outbreak. Using a traditional modeling method, they would have likely used the daily mean for ambient temperatures for the period being monitored. For supposed drought conditions or in this case, July 5 or July 8, mean ambient temperature was around 33ºC for daytime temperatures, and after averaging in nighttime temperatures it would be near 29.5ºC. Development and reproductive estimates would likely be based on data from laboratory studies conducted at constant temperatures, essentially basing the model on something similar to the 29.5ºC constant treatment from this study. However, grasshoppers on these days would experience body temperatures which would not be best represented by the 29.5ºC constant treatment.

Grasshoppers on these days experienced body temperatures much higher than ambient and with few fluctuations, and instead would be best represented by the 31.5ºC low frequency low amplitude treatment.

If the phenology model was based on mean ambient temperature and used the data obtained from the 29.5ºC constant treatment in this study, the population would take 93.5 days to reach reproduction and females would produce 1.3 egg pods per female and 40.6 eggs per pod. In contrast, if the model was based on more accurate body temperatures and used the data obtained from the 31.5ºC low frequency low amplitude treatment, the population would take 63.3 days to reach reproduction and females would produce 5.6 egg pods per female and 66.7 eggs per pod. *S. americana* has a spring or early summer generation followed by an autumn generation in Florida. In order for the population based on ambient temperature and the 29.5ºC constant treatment to remain stable we must assume that 2% of all eggs laid during the early summer generation survive to be reproductive females. For purposes of this discussion winter generations will be disregarded and assumed to maintain a stable population. The phenology model based on mean ambient temperatures and data from this study would predict that after 5 yr of drought each female from the original population before the drought began and her successive offspring would have multiplied into 1.16 females over the 5 yr period, essentially a static population. If a phenology model was based on more accurate body temperatures and the data from the 31.5ºC low frequency low amplitude treatment and the same assumptions were applied, each female and her successive offspring would multiply into 22,800 females over the 5 year period of drought. Such an extreme scenario is very unlikely, because initial survival rates are likely to be much lower and would continue to decrease because of increasing population size

and competition. However, it is easy to see how phenology models based on mean daily temperatures and laboratory data collected at constant temperatures could under predict a population outbreak.

There are several issues that caused problems with this study. First, the study might have tried to examine too many parameters and sacrificed a more in depth study of each individual parameter under more treatments and different temperatures. Another problem with the study is that collection of reproduction data was discontinued because of pesticide contamination of grasshopper food and lifetime fecundity could not be assessed. Lifetime fecundity data may have been able to better show the effects of temperature and temperature fluctuations. However, the biggest problem with this study is the separation of high and low frequency treatments that caused severe problems when analyzing data and negated the study's ability to analyze any effects of frequency on development and reproduction. This study has raised more questions that it has answered. Why does amplitude have a greater effect on development and reproduction at lower temperatures? An even more puzzling question is, why did grasshoppers lay more eggs per pod at lower temperatures in high frequency treatments but fewer in low frequency treatments? A more in depth focused study of the affects of temperature fluctuation on development and reproduction in *S. americana* would answer many of these questions.

In conclusion, this study has provided novel data from the field relating to grasshopper body temperature, and has shown that temperature fluctuations (other than scotophase fluctuations) can have an effect on development (at least at sub-optimal temperature at or below 29.5ºC) and reproduction in *S. americana* and most likely other grasshoppers. Most importantly, this study has shown that, under most conditions, grasshopper body temperatures are very different from ambient temperature. While the study provides no exact method for improving

phenology modeling, it does suggest that the most common method currently used can be inaccurate, and that use of more relevant mean body temperatures or data from temperature treatments similar to those temperatures experienced in the field would likely improve our ability to predict population sizes of not only grasshoppers, but other insects as well. While using sunlight intensity to estimate body temperatures for modeling would be appropriate for basking insects, such as grasshoppers, it is not appropriate for those insects not utilizing sunlight as their primary heat source. Each insect's biology should be considered on an individual basis and an appropriate method to best estimate actual body temperature should be used.

LIST OF REFERENCES

Ashamo, M.O., and O.O. Odeyemi. 2000. Effect of rearing temperature on the fecundity and development of *Euzopherodes vapidella* Mann (Lepidoptera: Pyralidae), a pest of stored yam. J. Stored Prod. Res.. 37:253-261.

Beck, S.D. 1983. Insect thermoperiodism. Annu. Rev. Entomol. 28:91-108.

Begon, M. 1983. Grasshopper populations and weather: the effects of insolation on *Chorthippus brunneus*. Ecol. Entomol. 8:361-370.

Berner, D., C. Körner, and W.U. Blanckenhorn. 2004. Grasshopper populations across 2000 m of altitude: is there life history adaptation? Ecography 27: 733-740.

Blanford, S., and M.B. Thomas. 2000. Thermal behavior of two acridid species: effects of habitat and season on body temperature and potential impact on biological control agents. Environ. Entomol. 29:1060-1069.

Brillon, S., Y. Lambert, and J. Dodson. 2005. Egg survival, embryonic development, and larval characteristics of northern shrimp (*Pandalus borealis*) females subject to different temperature and feeding conditions. Marine Biol. 147:895-911.

Capinera, J.L. 1993a. Host-plant selection by *Schistocerca americana* (Orthoptera: Acrididae). Environ. Entomol. 22:127-133.

Capinera, J.L. 1993b. Differentiation of nymphal instars in *Schistocerca americana* (Orthoptera: Acrididae). Florida Entomol. 76:175-179.

Capinera, J.L., L.F. Wiener, and P.R. Anamosa. 1980. Behavioral thermoregulation by late-instar range caterpillar larvae *Hemileuca oliviae* Cockerell (Lepidoptera: Saturniidae). J. Kansas Entomol. Soc.. 53:631-638.

Carruthers, R. I., T. S. Larkin, H. Firstencel, and Z. Feng. 1992. Influence of thermal ecology on the mycosis of a rangeland grasshopper. Ecology 73:190-204.

Casey, T. M. 1981. Behavioral mechanisms of thermoregulation, pp. 79-114. In B. Heinrich, Insect Thermoregulation. John Wiley & Sons, New York, New York.

Castillo, J., J.A. Jacas, J.E. Peña, B.J. Ulmer, and D.G. Hall. 2006. Effect of temperature on life history of *Quadrastichus haitiensis* (Hymenoptera: Eulophidae), an endoparasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Biol. Contr. 36:189-196.

Chappell, M. A., and D. W. Whitman. 1990. Grasshopper thermoregulation, pp. 143-172. In R.F. Chapman and A. Joern (eds.), Biology of Grasshoppers. John Wiley & Sons, New York, New York.

Fielding, D.J. 2004. Developmental time of *Melanoplus sanguinipes* (Orthoptera: Acrididae) at high latitudes. Environ. Entomol. 33:1513-1522.

Forsman, A. 1997. Thermal capacity of different colour morphs in the pygmy grasshopper *Tetrix subulata*. Ann. Zool. Fennici 34: 145-149.

Forsman, A. 2000. Some like it hot: intra-population variation in behaivoral thermoregulation in color-polymorphic pygmy grasshoppers. Evol. Ecol. 14:25-38.

Gardner, K.T., and D.C. Thompson. 2001. Development and phenology of the beneficial grasshopper *Hesperotettix viridis*. Southwest. Entomol. 26:305-313.

Gündüz, N.E., and A. Gülel. 2002. Effect of temperature on development, sexual maturation time, food consumption and body weight of *Schistocerca gregaria* Forsk (Orthoptera: Acrididae). Turkish J. Zool. 26:223-227.

Harrison, J. F., and J. H. Fewell. 1995. Thermal effects on feeding behavior and net energy intake in a grasshopper experiencing large diurnal fluctuations in body temperature. Physiol. Zool. 68:453-473.

Heinrich, B. 1993. Grasshoppers and other Orthoptera, pp. 143-190. In B. Heinrich, Hot- Blooded Insects. Harvard University Press, Cambridge, Massachusetts.

Heinrich, B. 1996. The Thermal Warriors. Harvard University Press, Cambridge, Massachusetts.

Hu, X.P. and A.G. Appel. 2004. Seasonal variation of critical thermal limits and temperature tolerance in Formosan and Eastern subterranean termites (Isoptera: Rhinotermitidae). Physiol. Ecol. 33:197-205.

Huey, R.B. and R.D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. Amer. Zool. 19:357-366.

Inglis, G. D., D. L. Johnson, and M. S. Goettel. 1996. Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. Biol. Contr. 7:131-139.

Jordaan, A., S.E. Hayhurst, and L.J. Kling. 2006. The influence of temperature on the stage at hatch of laboratory reared *Gadus morhuaI* and implications for comparisons of length and morphology. J. Fish Biol. 86:7-24.

Kemp, W. P. 1986. Thermoregulation in three rangeland grasshopper species. Can. Entomol. 18:335-343.

Kemp, W. P., and B. Dennis. 1989. Development of two rangeland grasshoppers at constant temperatures: development thresholds revisited. Can. Entomol. 121:363- 371.

Kührt, U., J. Samietz, and S. Dorn. 2005. Thermoregulation behavior in codling moth larvae. Physiol. Entomol. 30:54-61.

Kuitert, L.C., and R.V. Connin. 1952. Biology of the American grasshopper in the southeastern United Sates. Florida Entomol. 35:22-33.

Lactin, D. J., and D. L. Johnson. 1996. Behavioral optimization of body temperature by nymphal grasshoppers (*Melanoplus sanguinipes,* Orthoptera: Acrididae) in temperature gradients established using incandescent bulbs. J. Therm. Biol. 21:231-238.

Lactin, D. J., and D. L. Johnson. 1997. Response of body temperature to solar radiation in restrained nymphal migratory grasshoppers (Orthoptera: Acrididae): influences of orientation and body size. Physiol. Entomol. 22: 131-139.

Lactin, D. J., and D. L. Johnson. 1998a. Convective heat loss and change in body temperature of grasshopper and locust nymphs: relative importance of wind speed, insect size, and insect orientation. J. Therm. Biol. 23:5-13.

Lactin, D. J., and D. L. Johnson. 1998b. Environmental, physical, and behavioral determinants of body temperature in grasshopper nymphs (Orthoptera: Acrididae). Can. Entomol. 130:551-557.

Miles, C.I. 1985. The effects of behaviorally relevant temperatures on mechanosensory neurons of the grasshopper, *Schistocerca americana.* J. Exp. Biol. 116:121-139.

Nakahira, N., R. Nakahara, and R. Arakawa. 2005. Effect of temperature on development, survival, and adult body size of two green lacewings, *Mallada desjardinsi* and *Chrysoperla nipponensis* (Neuroptera: Chrysopidae). Appl. Entomol. Zool. 40:615-620.

Parker, J.R. 1930. Some effects of temperature and moisture upon *Melanoplus mexicanus mexicanus* Saussure and *Cammula pellucida* Scudder (Orthoptera). Mont. Agric. Exp. Sta. Bul. 223:1-132.

Petavy, G., J.R. David, P. Gibert, and B. Moreteau. 2001. Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. J. Therm. Biol. 26:29-39.

Pitt, W. C. 1999. Effects of multiple vertebrate predators on grasshopper habitat selection: trade-offs due to predation risk, foraging, and thermoregulation. Evol. Ecol. 13:499-515.

Prange, H.D. 1990. Temperature regulation by respiratory evaporation in grasshoppers. J. Exp. Biol. 154:463-474.

Putnam, L. G. 1963. The progress of nymphal development in pest grasshoppers (Acrididae) of western Canada. Can. Entomol. 95:1210-1216.

Satar, S., U. Kersting, and M.R. Ulusoy. 2005. Temperature dependent life history traits of *Brevicoryne brassicae* (L.) (Hom., Aphididae) on white cabbage. Turk. J. Agric. For. 29:341-346.

Squitier, J.M., and J.L. Capinera. 2002a. Observations on the phenology of common Florida grasshoppers (Orthoptera: Acrididae). Florida Entomol. 85:227-234.

Squitier, J.M., and J.L. Capinera. 2002b. Habitat associations of Florida grasshoppers (Orthoptera: Acrididae). Florida Entomol. 85:235-244.

Uvarov, Sir B. 1966a. Grasshoppers and Locusts: A Handbook of General Acridology, Vol. 1. Cambridge University Press, London, England.

Uvarov, Sir B. 1966b. Grasshoppers and Locusts: A Handbook of General Acridology, Vol. 2. Cambridge University Press, London, England.

Whitman, D.W. 1986. Developmental thermal requirements for the grasshopper *Taeniopoda eques* (Orthoptera: Acrididae). Ann. Entomol. Soc. Am. 79:711-714.

Whitman, D. W. 1988. Function and evolution of thermoregulation in the desert grasshopper *Taeniopoda eques*. J. Anim. Ecol. 57:369-383.

Willott, S. J. 1997. Thermoregulation in four species of British grasshoppers (Orthoptera: Acrididae). Funct. Ecol. 11:705-713.

Woodson, W.D., and J.V. Edelson. 1988. Developmental rate as a function of temperature in a carrot weevil, *Listronotus texanus* (Coleoptera: Curculionidae). Ann. Entomol. Soc. Am. 81:252-254.

BIOGRAPHICAL SKETCH

Jason G. Froeba was born on August 20, 1982 in Metairie, Louisiana, where he was also raised with his younger brother. He graduated from Archbishop Rummel High School in 1999. After high school, he attended the University of New Orleans and graduated with a B.S. in biological sciences in 2003. His undergraduate coursework took him to Costa Rica for 5 weeks of Spanish and Environmental study and leadership. This is where his love for insects first took root. After college he spent a year working for Dial One Franklynn Pest control in Metairie, Louisiana.

Jason moved to Gainesville, Florida in 2004 to pursue his M.S. in entomology. Jason is currently living in New Roads, Louisiana (near Baton Rouge). He currently works for Louisiana's Department of Wildlife and Fisheries, performing data analysis for the Marine Fisheries division. Upon completion of his M.S. degree, Jason will continue to work for Louisiana's Department of Wildlife and Fisheries. Jason has been married to Emily Froeba for 3 years. They have a daughter Annemarie, age 1, and are expecting a second daughter this summer.