The short term dynamics of behavioral thermoregulation of nymphs of the migratory grasshopper, Melanoplus sanguinipes
by Marni Gay Rolston

Abstract:
Nymphs of the migratory grasshopper Melanoplus sanguinipes (Fabricius) are active within a broad range of diurnal environmental temperatures on Montana rangeland. In this study, I determined the behavioral thermoregulatory strategies of nymphs of M sanguinipes, and assessed whether they are able to achieve and maintain body temperatures within a preferred temperature range. Preferred temperature ranges, or set-point ranges, were determined for each instar by estimating the interquartile range of body temperatures experienced by the nymphs on a thermal gradient. Dried grasshopper models were used to estimate body temperatures of the live nymphs. Behavioral observations in the field were compared with 1) set-point ranges established on the laboratory gradient, and 2) models that were positioned in a variety of microhabitat/posture combinations in the field. The set-point ranges and the model temperatures provided estimates of the body temperatures that the nymphs could achieve in particular microhabitats, and the soil temperatures at which they could maintain optimal body temperatures.

Estimates of body temperatures attained in the field often fell within the range of preferred body temperatures determined for grasshoppers on a thermal gradient in the laboratory. Perch height, insolation, and orientation of the body axes at the final chosen microhabitat were all related to surface temperature. Although there were some behavioral differences among the instars, they tended to. exploit similar thermal environments. They tended to move into microhabitats and assume body positions whereby they could increase their body temperatures as rapidly as possible in cool environments, and they tended to maintain body temperatures as close as possible to their estimated set-point ranges in hotter environments. Also, despite the structural differences between the grazed and ungrazed treatments, the nymphs' behavior was similar, suggesting that they are capable of finding suitable thermal microhabitats in a variety of habitats. This study illustrates that the behaviors of third, fourth, and fifth instar nymphs of M sanguinipes allow them to achieve and maintain body temperatures close to or within their set-point ranges, relative to nonthermoregulating nymphs.

# THE SHORT TERM DYNAMICS OF BEHAVIORAL THERMOREGULATION OF NYMPHS OF THE MIGRATORY GRASSHOPPER, *MELANOPLUS SANGUINIPES*

by

Marni Gay Rolston

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# ABSTRACT

Nymphs of the migratory grasshopper *Melanoplus sanguinipes* (Fabricius) are active within a broad range of diurnal environmental temperatures on Montana rangeland. In this study, I determined the behavioral thermoregulatory strategies of nymphs of *M. sanguinipes*, and assessed whether they are able to achieve and maintain body temperatures within a preferred temperature range. Preferred temperature ranges, or set-point ranges, were determined for each instar by estimating the interquartile range of body temperatures experienced by the nymphs on a thermal gradient. Dried grasshopper models were used to estimate body temperatures of the live nymphs. Behavioral observations in the field were compared with 1) set-point ranges established on the laboratory gradient, and 2) models that were positioned in a variety of microhabitat/posture combinations in the field. The set-point ranges and the model temperatures provided estimates of the body temperatures that the nymphs could achieve in particular microhabitats, and the soil temperatures at which they could maintain optimal body temperatures.

Estimates of body temperatures attained in the field often fell within the range of preferred body temperatures determined for grasshoppers on a thermal gradient in the laboratory. Perch height, insolation, and orientation of the body axes at the final chosen microhabitat were all related to surface temperature. Although there were some behavioral differences among the instars, they tended to exploit similar thermal environments. They tended to move into microhabitats and assume body positions whereby they could increase their body temperatures as rapidly as possible in cool environments, and they tended to maintain body temperatures as close as possible to their estimated set-point ranges in hotter environments. Also, despite the structural differences between the grazed and ungrazed treatments, the nymphs' behavior was similar, suggesting that they are capable of finding suitable thermal microhabitats in a variety of habitats. This study illustrates that the behaviors of third, fourth, and fifth instar nymphs of *M. sanguinipes* allow them to achieve and maintain body temperatures close to or within their set-point ranges, relative to nonthermoregulating nymphs.

# INTRODUCTION

The biology, life history, and behaviors of grasshoppers have been studied extensively because of their excessive destruction of valuable rangeland forage (Chapell and Whitman 1990). Many of these studies have examined adult thermoregulatory behavior within particular microhabitats (Pepper and Hastings 1952, Anderson et al. 1979, Joern 1982, Parker 1982, Kemp 1986, Gillis and Smeigh 1987, Whitman 1987, Whitman, 1988). However, only a few studies have investigated the effects of the thermal environment on grasshopper nymphs (Chapman 1965, Parker 1982, Whitman, 1986, With 1994). Because of the large size difference between adults and the smaller instars, and because optimal temperatures may change with age, preferred temperature ranges and thermoregulatory strategies may differ between adults and nymphs.

## Objectives

The purpose of this study was four-fold. The first objective was to determine the temperature ranges preferred by different instars of the migratory grasshopper, *Melanoplus sanguinipes* (Fabricus), by observing their locations on a thermal gradient in the laboratory. This information would provide estimates of each instar's set-point range, or preferred body temperatures. The second objective was to determine the various behavioral thermoregulatory strategies of third, fourth, and fifth instar *M. sanguinipes* in the field. The third objective was to estimate the approximate body temperatures attained by these nymphs by determining the body temperatures of grasshopper models placed in locations which live nymphs would use within the microhabitat. Finally, the fourth objective was to determine the effect of grazing on the thermoregulatory strategies employed by the three different developmental stages.

## Morphological and Physiological Means of Thermoregulation

All animals have specific temperature ranges within which they can function, but preferred body temperatures are not always easy or possible to achieve. This is especially true of insects and other ectotherms which require external sources of heat. Therefore, the thermal microclimate of an environment inhabited by an ectotherm is a major factor influencing its survival, growth, and reproduction.

The maintenance of a body temperature independent from that of the ambient temperature is referred to as thermoregulation. When environmental conditions allow, an active thermoregulator should exhibit body temperatures different from what would be expected of a passive, nonthermoregulating animal. Energy is transferred between the environment and an insect via three major pathways: 1) the absorption of radiant energy from the sun 2) loss or gain of heat by convective exchange with the surrounding air, and, 3) loss or gain of heat via conductive exchange with the substrate (Digby 1955, Chappell 1983). Various morphological, physiological, and behavioral attributes available to an ectotherm are used to regulate body temperature

Morphological characteristics that affect the thermoregulatory abilities of most insects include size, coloration, and texture of the animal's body. Because of their large surface area to volume ratio, smaller insects are more susceptible to convective exchange than larger insects, and are potentially subject to high rates of evaporative water loss (Anderson et al. 1979) that can drastically lower body temperatures in cool environments (May 1976), or have a desiccating effect in extremely hot, arid environments. This latter effect is due to the fact that the bodies of small insects heat up so rapidly that the maximum time of exposure to high temperatures is much less than that of insects with greater mass. Likewise, larger insects can maintain higher body temperatures in cool environments, and sublethal body temperatures in hot environments due to evaporative cooling (Prange and Pinshow 1994, Roxburgh et al. 1996).

Therefore, body temperatures of small ectotherms are not as stable as those of their larger counterparts (Whitman 1987). The high thermal inertia of large insects such as adult grasshoppers makes them relatively insensitive to temporary changes in the environment (Whitman 1987), and allows them to thermoregulate effectively (Gillis and Possai 1983, Chappell and Whitman 1990), resulting in stable body temperatures even under conditions of fluctuating ambient temperatures.

The exterior coloration of an ectotherm has an important effect on the absorption of solar radiation (Dearn 1990). Some studies indicate that insects with darker integument are able to heat at faster rates in higher, cooler habitats than their lighter counterparts because they absorb a greater proportion of incident radiation (Whitman 1988, Goulson

1993). Joern (1982) found that the body temperatures of dead grasshoppers painted black were an average 9°C higher than those painted white. Likewise, reflective, light-colored individuals tend to gain heat less rapidly (Willmer and Unwin 1981).

Some large insects with insolative hairs rely on metabolic production to warm up, using external heat sources secondarily (Heinrich 1975). Such endothermic insects often undertake preflight warm-up, in which they contract two sets of thoracic muscles against each other to temporarily generate enough heat for flight. Once airborne, the metabolic energy created by the continuous action of the thoracic muscles is sufficient to keep the insect warm.

Evaporative cooling is a physiological mechanism of heat loss for insects which is influenced by the temperature and humidity of the surrounding air. This strategy allows insects to keep their body temperatures below lethal levels by drawing heat out of the body through the process of evaporation. Grasshoppers usually use evaporative cooling only in lethally hot environments, where behavioral mechanisms are insufficient in maintaining body temperatures below critical thermal maxima (Prange 1996). However, this mechanism is limited to larger insects; small insects may be less able to regulate body temperatures by evaporative heat loss, due to smaller water reserves and faster heating rates (Prange and Pinshow 1994, Roxburgh et al. 1996).

## Behavioral Thermoregulation

Behavioral thermoregulation is considered the most important means by which ectotherms acquire or lose body heat. It has been documented in a large number of insects of different sizes, morphologies, and habitats (Watt 1968, May 1976, Anderson et al. 1979, Parker 1982, Chappell 1983, Gillis and Smeigh 1987, Whitman 1987, O'Neill and Kemp 1992, Rutowski et al. 1994). Because grasshoppers are ectothermic animals, they must live within the thermal constraints of their environment and have evolved behavioral methods of regulating their body temperatures so that they do not fall below the critical minimum or rise above the critical maximum temperatures (Chapman 1965). Within the range of these critical temperatures, there is also an optimal temperature range within which grasshoppers are efficient at walking, feeding, jumping, or digesting (Parker 1930, Whitman 1988). In cool environments, behavioral thermoregulation is an essential part of daily activities, because movement is limited without adequate accumulated body heat (Pepper and Hastings 1952, Anderson et al. 1979). Therefore, the maintenance of adequate body temperatures pre-empt critical activities such as foraging, predator avoidance, and mate-searching. In extremely hot environments, different behavioral thermoregulatory strategies are required for grasshoppers to maintain body temperatures which ensure survival.

When direct solar radiation is available, grasshoppers can dramatically influence their rates of heat gain by simply modifying the orientation of their body relative to the sun in order to control amounts of radiant energy absorbed. In cool environments, grasshoppers may orient the long axis and the dorsoventral axis of their body perpendicular to incoming solar rays, so that the maximum amount of direct radiation is intercepted. Solar exposure may be accentuated in this basking posture by lowering the hind leg that is toward the sun to reduce shade on the body. They may also raise the opposite hind leg out of the body's shadow, and adult grasshoppers may lower the abdomen below the shade of the wings. On cool mornings and afternoons, large numbers of grasshoppers commonly bask in this "flanking" position in vegetation or on the ground as they exploit the sun's low-intensity radiation (Anderson et al. 1979, Parker 1982, Whitman 1987).

In addition to orientating relative to the position of the sun, grasshoppers may increase their heat gain by adjusting their posture relative to the ground. Crouching against a solid substrate such as the soil surface or a rock allows a large proportion of the ventral thorax and abdomen to have direct contact with a warm conductive surface. This common behavior is usually initiated in cool environments when the substrate is warmer than the ambient temperature, and is used as a mechanism for heat gain (Anderson et al. 1979, Chappell 1983, Gillis and Smeigh 1987, Whitman 1987). Crouching also helps to reduce convective exchange by lowering the grasshopper's body into the nonturbulent boundary layer of air close to the soil surface (Whitman 1987).

In hot environments, grasshoppers have developed a wide repertoire of strategies which may prevent them from overheating. Those that are exposed to sunlight often orient the long axis of the body parallel to the sun's incoming rays, thereby reducing the total amount of radiation absorbed. Additionally, stilting postures, in which grasshoppers extend their legs to elevate their body off the warm surface of the soil, are utilized to avoid hot microhabitats close to the ground if shade is not available (Anderson et al. 1979, Chappell 1983, Gillis and Smeigh 1987, Whitman 1987, O'Neill et al. 1994). By stilting, a grasshopper also experiences an increased rate of convective exchange above the warm, static boundary layer (Whitman 1987).

Shade-seeking is an important strategy utilized by grasshoppers to avoid extreme temperatures during the hottest times of the day by diminishing exposure to solar radiation (Anderson et al. 1979, Parker 1982, Chappell 1983, Whitman 1987). This entails moving to shaded areas below leaves or within the shaded basal regions of the grass clumps (Parker 1982), climbing into the vegetation and clinging to the shady sides of stems (Whitman 1987), or retreating to cavities within dried livestock dung (O'Neill 1994). In addition, by moving above the ground, convective cooling is facilitated because of higher wind speeds (Anderson et al. 1979, Whitman 1987).

On clear days, the thermoregulatory behavior of many grasshoppers often follows a cyclical daily pattern. After spending the night in the upper regions of the vegetation, they often assume the basking position in the early morning light. Basking continues until they are warm enough to descend to the ground, where temperatures are beginning to exceed air temperatures, and where they crouch and bask on the soil surface. Later, if the ground temperatures approach lethal limits, grasshoppers may stilt, cling to vegetation, or seek shade. As the day progresses, temperature and solar radiation levels decrease, driving the grasshoppers back to the warm substrate or into the sun-lit vegetation until dusk.

## Thermoregulation by Nymphs

The repertoire of thermoregulatory behaviors of grasshopper adults has been well documented, but much less is known about how nymphs respond to their thermal environment. Because they are considerably smaller than adults, nymphs may use different forms of physiological and behavioral thermoregulation.

Grasshopper nymphs must rely almost exclusively on external mechanisms of heat gain and loss. Their small body mass and high surface area-to-volume ratios facilitate heat exchange and rapid achievement of equilibrium with the environment (Whitman 1987), enabling them to manipulate body temperatures quickly by simply shifting between sunny and shaded areas. Willmer and Unwin (1981) speculated that small insects may be more capable of enduring the often deadly hot midday temperatures because of their ability to rapidly cool down after they move to a shaded area. This may entail relocating to shaded areas on the ground, or climbing into the shade of protective vegetation.

Because small insects lose water more rapidly through evaporation, and since convective processes can significantly increase rates of water loss for nymphs, they may move into environments with lower wind speeds. In addition, the boundary layers that cover the surfaces of the ground and vegetation (Gieger 1965) may encompass a large proportion of a small insect such as an early instar grasshopper, and may therefore function in a protective manner by reducing their exposure to convection and, ultimately, desiccation. Moving into a boundary layer is probably not a viable strategy for older instars, since a large proportion of their bodies may extend outside of it. However, it is not as essential for older nymphs to avoid high wind speeds, since their larger bodies make them less vulnerable to temperature fluctuations (May 1976, Whitman 1987). Unlike smaller instars, whose legs may not be long enough to elevate them above the warm boundary layer, fourth and fifth instars may be able to stilt in order to avoid it (Whitman 1987).

## Effects of Grazing

The complex thermoregulatory behaviors of grasshopper adults and nymphs illustrate how they may be influenced by habitat structure. Grazing cattle often create a variety of microhabitats for grasshoppers, depending on grazing, and the variability of grazing within a site. In season-long grazing, selective feeding on highly palatable forage with higher protein content creates a pattern of short, heavily grazed areas versus tall, lightly grazed areas (Bakker et al. 1983, Willms et al. 1988). This mosaic of habitats results in increased structural diversity compared with ungrazed or uniformly grazed rangeland, and may enhance the survival of grasshoppers because of increased variability in the microclimate. A diverse habitat may simultaneously provide warm, open areas for ovipositing, a supply of food (Uvarov 1977), and an environment suitable for a wide range of thermoregulatory strategies.

Grazing has a number of quantifiable effects on the microclimate of rangeland. By opening up the vegetative canopy, grazing increases the amount of direct solar radiation, which increases the solar insolation of the soil surface, and the average wind speed

(Geiger 1965). Higher wind speeds near the ground may decrease the relative humidity of a habitat. Therefore, depending on a grasshopper's response to grazing (East and Pottinger 1983), decreasing the structural diversity of a habitat may increase or decrease the habitat's suitability for feeding, oviposition, or thermoregulation.

Nymphs may be more sensitive than adults to the change in habitat structure and microclimate caused by grazing. However, depending on the grazing intensity, this change may be advantageous in some respects and detrimental in others. Even though lightly grazed areas have lower forage quality (Bakker et al. 1983), they accumulate greater amounts of litter than heavily grazed areas. By creating humid conditions next to the ground (Waterhouse 1955), litter could provide important habitats for nymphs. Anderson et al. (1979) found that nymphs of *Eritettix simplex* (Scudder) are more abundant than adults in moist habitats, where they are less vulnerable to desiccation. However, nymphs may be more susceptible than adults to fungal and protozoan pathogens (Henry 1971, Bomar et al. 1993), which require high levels of humidity to germinate. In addition, native plants that are intensively grazed in early spring and then allowed to recover, sustain minimal effects on subsequent production (Blaisdell and Pechanec 1949). This represents a significant reduction in the amount of forage available as a source of shade or food at a time when most spring-hatching nymphs are emerging. Therefore, the timing of grazing, in addition to a grasshopper's life stage, may combine to create a low quality habitat for a nymph.

A long-term effect of behavioral thermoregulation for most ectotherms is an increased rate of development. Although specific ranges of body temperatures are required for daily movement and activities, the primary advantage of thermoregulatory basking is to increase rates of growth, development, and ultimately, reproduction. This is especially important in habitats that do not provide sufficient heat within a season for maturation and reproduction (Whitman 1988, Carruthers et al. 1992.) Basking and other behaviors that increase heat gain, allow grasshoppers to regulate their body temperatures within a preferred, or set-point range (Hertz et al. 1993), which is optimal for development. Therefore, according to Hertz et al., habitats which permit grasshoppers to easily attain body temperatures within their set-point range have a high "thermal quality", and habitats which require extensive thermoregulatory behaviors represent areas of low thermal quality.

## The Life History of *Melanoplus sanguinipes*

The grasshopper *M. sanguinipes* is a member of the subfamily Melanoplinae. It is commonly more abundant in areas that have been lightly grazed or ungrazed, with little or no bare ground (Jepson-Innes and Bock 1989, Quinn and Walgenbach 1990) and with a high percentage of total biomass (Capinera and Sechrist 1982). It is a mixed forb- and grass-feeding species that is a widespread pest throughout North America. The egg pods of this species are oviposited within clumps of vegetation an average of 1.2 cm below the soil surface at a vertical orientation (Kemp and Sanchez 1987). Springtime soil temperatures and moisture content are significant factors determining when the nymphs will emerge, and variation in oviposition locations may extend the hatching period.

The habitat preferences of *M. sanguinipes* on rangeland are governed by a wide range of environmental factors. Selections for particular habitat types may be influenced by available food (Campbell et al. 1974, Quinn and Walgenbach 1990), optimal thermoregulatory sites (Anderson et al. 1979, Chappell 1983, Whitman 1987), refuges from predators (Dempster 1963), and oviposition sites (Isely 1938, Fisher 1993), so there may be various reasons why a particular grasshopper chooses a specific habitat. However, the vegetative structure and microclimate of rangeland habitats are greatly modified when they are grazed heavily, and this has an important effect on the resident grasshoppers. This effect may be more pronounced for younger, smaller instars who are not as mobile as their older, larger counterparts.

Habitat selection may be correlated with a grasshopper's developmental stage. Gillespie and Kemp (1994) found significant age class differences within three *Melanoplus* species between a crop of winter wheat (*Triticum aestivium* L.) and rangeland from a sample taken early in the season. Early instar *Melanoplus* were more abundant on rangeland than on winter wheat, despite the higher forage quality of the wheat. However, numbers increased in the crop during the middle sample periods. They hypothesized that adults and older instar nymphs with greater dispersal capabilities than younger, less mobile nymphs moved into the less densely populated cropland with more abundant or higher quality forage than the rangeland (Gillespie and Kemp 1994). This study illustrated that small nymphs may encounter more problems when their habitat quality is reduced.

# MATERIALS AND METHODS

## The Thermal Gradients

I used thermal gradients to determine the preferred body temperatures (= set point temperatures of Hertz et al. 1993) of the different instars. The gradients created a stable environment in which most confounding variables could be controlled. They consisted of four parallel aluminum bars, each 106 cm long x 15 cm wide, and spaced 5 cm apart. At each end, the bars rested upon 2 hollow aluminum rods. A pump continuously passed water from a hot bath through the rod at one end, while another pump passed water from a cold bath through the other rod. As a result, a steep temperature gradient was maintained along the length of each bar. The gradient could be adjusted by changing the temperature of the water in each bath.

Surface temperatures on the bars ranged from approximately 16 to 63° C, with only slight deviations of 1 or 2 degrees over the course of a week. Gradients 1, 3, and 4 maintained similar temperature ranges, but because the range of gradient 2 was not consistent with the other three, it was not used in the analysis. Since bars 1 and 3 consistently exhibited similar temperatures, the regression equations generated from bar 1 were also used to estimate body temperatures for the grasshoppers on bar 3. I measured the surface temperatures of the gradient bars with 0.25 mm diameter copper/constantan thermocouples taped to each bar. They were taped every 10 cm on gradient 1, and every 20 cm on gradients 3 and 4. Therefore, I was able to determine the relationship between surface temperature and the distance along the bar.

Each of the four bars was enclosed with a clear plexiglass cover that allowed the grasshoppers to move along the surface of the gradient, yet prevented them from moving more than 3 cm above the surface. The covers also served to stabilize the temperatures along the length of each gradient. The entire apparatus was enclosed by an opaque outer cover, 130 cm x 95 cm x 80 cm. I observed the locations of the grasshoppers through two viewing windows, each 15 cm x 40 cm, on opposite sides of the box . When measurements were taken, the windows were opened, and a red light attached 55 cm above the center of the gradients was turned on so that the grasshoppers could be observed. At all other times, the grasshoppers were in complete darkness while they were on the gradient. The darkness ensured that they would not orient to any external light source, and that disturbance would be minimized.

On the day before the gradient observations were performed, I selected 100 *M. sanguinipes* of one developmental stage from the Bozeman, MT USDA lab, placed them in holding tubes in an incubator at 25° C, and provided them with ample amounts of fresh lettuce and wheat bran overnight. The following day, the grasshoppers were transferred from the holding tubes to the center of each gradient, and the plexiglass covers and outer cover were put in place. The first behavioral observations began approximately 20 minutes after I placed the grasshoppers on the gradients, so that the gradients could equilibrate, and the grasshoppers could habituate to their new environment.

I observed the nymphs of each of the six developmental stages on separate days, with between 5 and 44 individuals on each gradient bar. The number of grasshoppers observed per gradient varied due to factors such as grasshopper size, movements off the surface of the gradient, and mortality. They were observed each hour, for four hours, following the initial 20 minute equilibrium period for second, third, and fourth instars and adults. After the first observation, I observed the first and fifth instars at hours 2, 4, and 8. Each observation lasted approximately five minutes, during which time the windows to the outer box were opened, the red light was turned on, and the location of each grasshopper on each gradient was recorded. Individuals that climbed up the sides or to the top of the plexiglass were recorded, but were not included in the final analysis, because the temperatures at these locations were different than those found on the surface of the gradient By recording the position of each grasshopper along the gradient, I was able to estimate the preferred temperature range for each stage, and the differences between stages.

Operative body temperatures ($T_E$) are the predicted equilibrium temperatures of nonthermoregulating ectotherms (Hertz et al. 1993). In order to obtain $T_E$, I measured the body temperatures of dead grasshoppers of each stage that were placed on the gradients by inserting thermocouples into the membranous area directly posterior to the pronotum on the dorsal thorax. I used 0.08 mm diameter wire for the first, second, and third instars, and 0.25 mm wire for fourth and fifth instars. The thermocouples were glued in place, and the models were allowed to dry for approximately 24 hours before being placed on the gradient.

I used gradients 1 and 4 to measure the operative body temperatures, because they were the most accessible. All five stages were placed on each of the two gradients simultaneously, with each grasshopper model placed directly over a different thermocouple taped to the gradient. The plastic covers were put in place, and after a 20 minute equilibrium period, I measured $T_E$ for each model, and the surface temperature ($T_S$) at that location on the gradient. I then removed the plexiglass covers, and each model was moved to the next thermocouple location on the gradient. This was repeated every 20 minutes until each of the six models had been at every thermocouple location on its respective gradient. Two different trials were performed on two different days.

Estimates of the actual body temperatures of the live grasshoppers on the gradients were interpolated from the operative body temperatures, using two simple linear regression equations. The first equation expressed the linear relationship between specific locations on the gradient, and the respective surface temperatures. The second equation expressed the linear relationship between surface temperature and the operative body temperatures obtained from the grasshopper models on the gradient. This allowed me to determine the operative body temperatures of the models at specific locations on the gradient. The data could be further used to interpolate what the operative temperatures would be at locations on the gradient where $T_S$ was not known. Since grasshoppers are ectotherms, it can be further assumed that the operative temperatures obtained from the models may effectively approximate the actual body temperatures of living grasshoppers. Therefore, I was able to estimate the average body temperature for each hourly observation of every instar. A preferred body temperature range ($T_{SET}$) was also established for each grasshopper stage by determining the $T_B$'s of the interquartile range of the live grasshoppers on the gradient during the behavioral observations (Hertz et al. 1993).

## Study Sites and Species

I observed *M. sanguinipes* nymphs nine miles south of Three Forks, Montana, U.S.A. (latitude 45° 45' N, longitude 111°35' W), in a crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) pasture characterized by patches of bare soil. All field observations were recorded within two 35 m x 35 m plots. Since 1993, one plot (uniform) has been intensively grazed, so that, following grazing each year, the vegetation is at a low, uniform height. The ground between plants in this plot was covered with little or no plant litter, and was more exposed to direct solar radiation. Although this area had been heavily grazed by cattle for three consecutive years, before this study began, it was not grazed before these data were collected. The control plot has been ungrazed since 1992, so that the standing vegetation was dense, and composed of new, green stems and leaves, as well as old, dried stems from previous years. The bare spots between the grass clumps in the control treatment have accumulated a dense matt of plant litter. I observed third, fourth, and fifth instars of *M. sanquinipes* within both grazing treatments between 10 June - 24 July 1996. Data were collected only on sunny days with low wind speeds, so that environmental variation within and between days could be minimized.

## Field Behavioral Observations

I observed nymph behavior by slowly walking through one of the treatments until a nymph was flushed. After it landed, I determined whether it was a third, fourth, or fifth instar by noting its body size, and the presence/size of wing buds. The behavior of the nymph was observed and recorded for two minutes. Observations were discontinued and discarded if nymphs 1) moved to a new location during the last 30 seconds of the two minute observation period, 2) were disturbed by the observer or another insect, or 3) fed. This helped to ensure that the final location was one that was preferred by the nymph, and not a transitional area, or one chosen for reasons other than thermoregulation.

I recorded soil surface temperature ($T_S$) immediately after the two minute observation period ended, by inserting the tip of a 0.25 mm diameter copper/constantan thermocouple just under the surface of an area of sun-exposed soil, shading the tip from solar radiation, and recording the temperature after it had equilibrated. The ambient air temperature at the thoracic height of the nymphs ($T_{TH}$) was also measured at the beginning and end of the two minute observation period, and the two temperatures were then compared to determine whether nymphs moved into warmer or cooler microhabitats as a function of surface temperature. For each instar, a linear segmented model was used to estimate the threshold temperature where the nymphs changed thermoregulatory strategies and began to move into cooler microhabitats rather than warmer ones.

I also recorded perch height, and an index of the intensity of solar radiation (insolation) experienced by the grasshopper at the start and finish of the two minute period. All height data were subjected to a log transformation, because of the nonnormality of the data sets due to a prevalence of zeros. For the latter, focal observations were made to determine whether the nymph was in full sun, partial sun, or full shade. I also recorded the distance the nymph traveled, the total number of complete stops made, and whether the final body posture was crouched, normal, or stilted. In a crouched posture, the nymph pressed the venter of the thorax and abdomen against a warm substrate, such as the sun-heated soil surface. Stilting nymphs extended their legs, elevating the thorax and abdomen several millimeters above the hot substrate.

Finally, I observed the final orientation of the nymph's body relative to direct solar radiation. The orientation of the body in two different directions was recorded; the orientation of the longitudinal axis in the horizontal plane (LAH), and the orientation of the longitudinal axis in the vertical plane (LAV). To determine LAH, I recorded eight different horizontal orientations relative to the point on the horizon above which the sun was located, and estimated each angle to the nearest 45 degree; 0°, 45°, 90°, 135°, 180°, 235°, 270°, and 315°. If a grasshopper was perched at 0°, its head was facing the position on the horizon directly below the location of the sun. Similarly, if it was perched at 90° or 270°, its longitudinal axis would be perpendicular to the sun's radiation, thus maximizing the total body surface area exposed to solar incidence. The eight orientations were grouped into three categories (0°, 45°, and 90°) for ease in analysis, larger sample sizes, and consistency in the percentage of body surface area exposed to the sun. To determine LAV, I recorded three different vertical orientations of the grasshoppers' long axis relative to the soil surface: 1) parallel to the ground, 2) at a 45° angle to the ground, and 3) perpendicular (~90°) to the ground.

I used a nominal logistic regression for the analysis of the horizontal orientation data, so that I could model the probabilities that a randomly chosen grasshopper would be in a particular orientation of 0°, 45°, or 90° in relation to the sun as a function of soil surface temperature.

To assess the fit of the nominal logistic model, binary (individual) logistic regressions were also used to test for goodness-of-fit, and diagnostic procedures such as Delta Chi-Square and Delta Deviance discerned which particular observations were unusual. These results were then integrated in a descriptive way to determine the adequacy of the fit of the multivariate nominal model.

## Operative Body Temperatures in the Field

I measured the operative body temperatures ($T_E$) of *M. sanguinipes* models in the field so they could be compared with the behavioral observations of live *M. sanguinipes* in the field, and with the preferred body temperatures within the set point range ($T_{SET}$) estimated from the gradient observations. Using dried grasshoppers, I determined $T_E$ by implanting 0.25 mm diameter thermocouples into the membranous area directly posterior to the pronotum. Operative body temperatures were taken using third, fourth, and fifth instars. Approximately 40 operative body temperatures were randomly measured within both treatments. As with the field behavioral observations, measurements were only taken on cloudless days with limited wind.

I chose six different positions and orientations relative to the sun that are commonly observed for *M. sanguinipes* in the field. After arbitrarily tossing a 1 $m^2$ metal ring into a treatment area, I held a grasshopper model in each of the six positions within the ring, and recorded the $T_E$ of each position after the thermocouple had equilibrated. I alternated measurements between the grazed area (uniform) and the ungrazed area (control), using the same six positions for each instar. In the first position, I placed the model on unshaded bare soil, with its longitudinal axis oriented perpendicular to incoming solar radiation. In the second position, the model was placed on an area of bare soil or dried matted grass that had fallen to the ground from previous years, and it was oriented so that the body was aligned with the shadow of a standing stem. In this position, the model was either in partial sun or full shade. In the third position, I placed the model in full sun 5 cm above the surface of the ground on a horizontal stem or on elevated, matted grass, with the longitudinal axis of the body oriented perpendicular to solar radiation. In the fourth position, I held the model against the shady side of a vertical stem of varying thickness, approximately 5 cm above the ground. The fifth and sixth positions were similar to the fourth, except that the model was held 10 cm and 20 cm above the soil surface.

The amount of time required for equilibration varied with the size (instar) of the grasshopper model and the characteristics of the environment. Younger instars reached an equilibrium temperature more quickly than older ones, because of their smaller size. In addition, models that were placed on the hot mid-day surface of the soil took longer to reach a final temperature, due to the extreme temperatures, than did models that were placed higher up on the stems of grasses, where they were exposed to more moderate temperatures.

Surface temperature ($T_S$) was recorded prior to measuring the $T_E$ of each set of six model measurements. It was recorded by inserting the tip of a 0.25 mm diameter copper/constantan thermocouple just under the surface of an area of sun-exposed soil, shading the tip from solar radiation, and recording the temperature after it had equilibrated.

## Statistical Analysis

I used chi-square contingency tables to determine if the frequency distributions of nymphs adopting different behaviors varied with soil temperature. Data analyzed in this manner included the level of insolation that the nymphs moved into, and their horizontal and vertical orientations. I used a nominal logistic regression (Minitab) to find the expected probability that the horizontal orientation of a nymph was a function of soil temperature. I also used one-way ANOVA's (SAS Institute 1997) to determine whether there were significant differences, as a function of instar or grazing treatment, between 1) the mean final heights achieved by the nymphs, 2) their changes in height during the two-minute observation period, 3) the difference in mean final ambient temperature within the two minute period, and 4) the difference in total and linear distances that the nymphs traveled. I used a linear segmented regression model with two linear portions (SPlus). The point at which the two lines joined was used as an estimate of the ambient temperatures where the nymphs moved into cooler ambient temperatures rather than warmer ones. I also used simple linear regressions (Minitab) to determine the relationship between surface temperature and 1) the linear and total distances that were traveled, and 2) the total number of stops made by the nymphs within the two minute observation period. Finally, simple linear regressions were used to determine the relaionship between the operative body temperatures of the grasshopper models and soil temperature.

# RESULTS

## Set-Point Ranges on the Gradient

I found that there was a strong correlation between location on the gradient, and $T_S$, spanning a temperature range between 15-60°C (Table 1). There was also a strong, linear correlation between $T_S$ and the $T_E$ of models that were placed on the gradient (Table 1). These regressions provided a basis for estimating the $T_B$ experienced by live nymphs that were released onto the gradient. The nymphs tended to distribute themselves nonrandomly with respect to $T_S$ (Figures 1-3), indicating preferred set-point temperature ranges.

The set-point ranges that were generated after the second-hour observations were used to estimate the nymphs' preferred temperature ranges. Following suggestions of Hertz et al. (1993), the set-point ranges for each instar were determined by calculating the interquartile range of all estimated body temperatures on the gradient. The temperature ranges that were established indicated that the set-points did not vary as a function of instar, although there was a slight tendency for the set-point widths to decrease with larger instars (Table 2).

Table 1. Regression equations used to estimate the body temperatures of five instars of *M. sanguinipes* at four different hours on gradients 1, 3, and 4.

| Instar | Hour | Gradient | Regression of cm vs $T_S$ | | Regression of $T_S$ vs $T_E$ | |
|---|---|---|---|---|---|---|
| | | | Equation | $r^2$ | Equation | $r^2$ |
| 1 | 1 | 1 | y=54.12-0.40x | 1.00 | y=2.82+0.89x | 1.00 |
| | | 3 | - | - | y=2.82+0.89x | 1.00 |
| | | 4 | - | - | y=2.48+0.90x | 1.00 |
| | 2 | 1 | y=55.15-0.40x | 1.00 | y=2.82+0.89x | 1.00 |
| | | 3 | - | - | y=2.82+0.89x | 1.00 |
| | | 4 | - | - | y=2.48+0.90x | 1.00 |
| | 3 | 1 | y=55.68-0.41x | 1.00 | y=2.82+0.89x | 1.00 |
| | | 3 | - | - | y=2.82+0.89x | 1.00 |
| | | 4 | - | - | y=2.48+0.90x | 1.00 |
| | 4 | 1 | y=54.80-0.40x | 1.00 | y=2.82+0.89x | 1.00 |
| | | 3 | - | - | y=2.82+0.89x | 1.00 |
| | | 4 | - | - | y=2.48+0.90x | 1.00 |
| 2 | 1 | 1 | y=53.58-0.39x | 1.00 | y=3.70+0.85x | 1.00 |
| | | 3 | y=54.10-0.39x | 0.99 | y=3.70+0.85x | 1.00 |
| | | 4 | y=52.19-0.37x | 1.00 | y=3.07+0.88x | 1.00 |
| | 2 | 1 | y=54.08-0.39x | 1.00 | y=3.70+0.85x | 1.00 |
| | | 3 | y=56.42-0.40x | 1.00 | y=3.70+0.85x | 1.00 |
| | | 4 | y=55.62-0.38x | 1.00 | y=3.07+0.88x | 1.00 |
| | 3 | 1 | y=55.69-0.39x | 1.00 | y=3.70+0.85x | 1.00 |
| | | 3 | y=56.41-0.39x | 1.00 | y=3.70+0.85x | 1.00 |
| | | 4 | y=55.62-0.38x | 1.00 | y=3.07+0.88x | 1.00 |
| | 4 | 1 | y=54.50-0.39x | 1.00 | y=3.70+0.85x | 1.00 |

Table 1, continued.

| Instar | Hour | Gradient | Regression of cm vs $T_S$ | | Regression of $T_S$ vs $T_E$ | |
|---|---|---|---|---|---|---|
| | | | Equation | $r^2$ | Equation | $r^2$ |
| | | 3 | y=55.01-0.39x | 1.00 | y=3.70+0.85x | 1.00 |
| | | 4 | y=55.17-0.40x | 1.00 | y=3.07+0.88x | 1.00 |
| 3 | 1 | 1 | y=58.10-0.44x | 1.00 | y=2.74+0.89x | 1.00 |
| | | 3 | y=59.34-0.46x | 0.99 | y=2.74+0.89x | 1.00 |
| | | 4 | y=59.27-0.45x | 0.99 | y=3.14+0.87x | 1.00 |
| | 2 | 1 | y=58.29-0.43x | 1.00 | y=2.74+0.89x | 1.00 |
| | | 3 | y=59.64-0.46x | 0.99 | y=2.74+0.89x | 1.00 |
| | | 4 | y=59.53-0.44x | 0.99 | y=3.14+0.87x | 1.00 |
| | 3 | 1 | y=58.85-0.44x | 1.00 | y=2.74+0.89x | 1.00 |
| | | 3 | y=59.70-0.46x | 0.99 | y=2.74+0.89x | 1.00 |
| | | 4 | y=59.60-0.45x | 1.00 | y=3.14+0.87x | 1.00 |
| | 4 | 1 | y=58.59-0.44x | 1.00 | y=2.74+0.89x | 1.00 |
| | | 3 | y=59.60-0.46x | 0.99 | y=2.74+0.89x | 1.00 |
| | | 4 | y=58.59-0.44x | 1.00 | y=3.14+0.87x | 1.00 |
| 4 | 1 | 1 | y=54.30-0.40x | 1.00 | y=6.48+0.76x | 0.99 |
| | | 3 | y=54.48-0.39x | 0.99 | y=6.48+0.76x | 0.99 |
| | | 4 | y=54.91-0.40x | 0.99 | y=4.49+0.84x | 0.98 |
| | 2 | 1 | y=54.86-0.40x | 1.00 | y=6.48+0.76x | 0.99 |
| | | 3 | y=55.02-0.39x | 1.00 | y=6.48+0.76x | 0.99 |
| | | 4 | y=55.30-0.40x | 1.00 | y=4.49+0.84x | 0.98 |
| | 3 | 1 | y=54.89-0.40x | 1.00 | y=6.48+0.76x | 0.99 |
| | | 3 | y=55.30-0.39x | 1.00 | y=6.48+0.76x | 0.99 |
| | | 4 | y=55.54-0.40x | 1.00 | y=4.49+0.84x | 1.00 |

Table 1, continued.

| Instar | Hour | Gradient | Regression of cm vs $T_S$ | | Regression of $T_S$ vs $T_E$ | |
|---|---|---|---|---|---|---|
| | | | Equation | $r^2$ | Equation | $r^2$ |
| | 4 | 1 | y=54.66-0.39x | 1.00 | y=6.48+0.76x | 0.99 |
| | | 3 | y=55.08-0.39x | 1.00 | y=6.48+0.76x | 0.99 |
| | | 4 | y=55.41-0.40x | 1.00 | y=4.49+0.84x | 1.00 |
| 5 | 1 | 1 | y=54.81-0.40x | 1.00 | y=5.26+0.80x | 1.00 |
| | | 3 | - | - | y=5.26+0.80x | 1.00 |
| | | 4 | - | - | y=3.93+0.84x | 0.99 |
| | 2 | 1 | y=54.11-0.39x | 1.00 | y=5.26+0.80x | 1.00 |
| | | 3 | - | - | y=5.26+0.80x | 1.00 |
| | | 4 | - | - | y=3.93+0.84x | 0.99 |
| | 3 | 1 | y=54.86-0.40x | 0.99 | y=5.26+0.80x | 1.00 |
| | | 3 | - | - | y=5.26+0.80x | 1.00 |
| | | 4 | - | - | y=3.93+0.84x | 0.99 |
| | 4 | 1 | y=54.41-0.39x | 1.00 | y=5.26+0.80x | 1.00 |
| | | 3 | - | - | y=5.26+0.80x | 1.00 |
| | | 4 | - | - | y=3.93+0.84x | 0.99 |

Cells with no values indicate instars where regressions could not be conducted because the experiments were performed before thermocouples were placed on gradients 3 and 4.

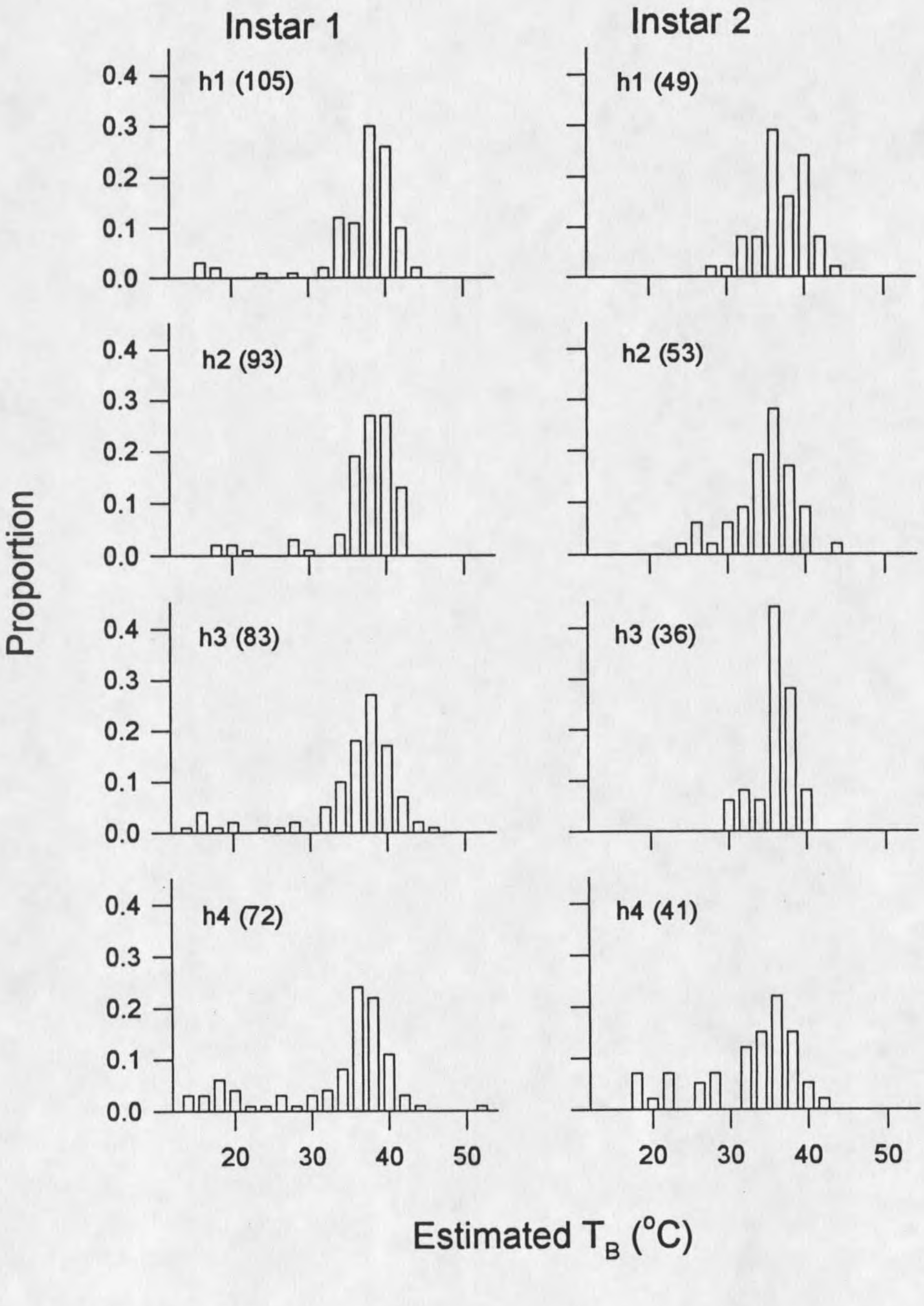

Figure 1. Proportion of first and second instars of *M. sanguinipes* on the gradient with estimated body temperatures at each of the four observational hours.

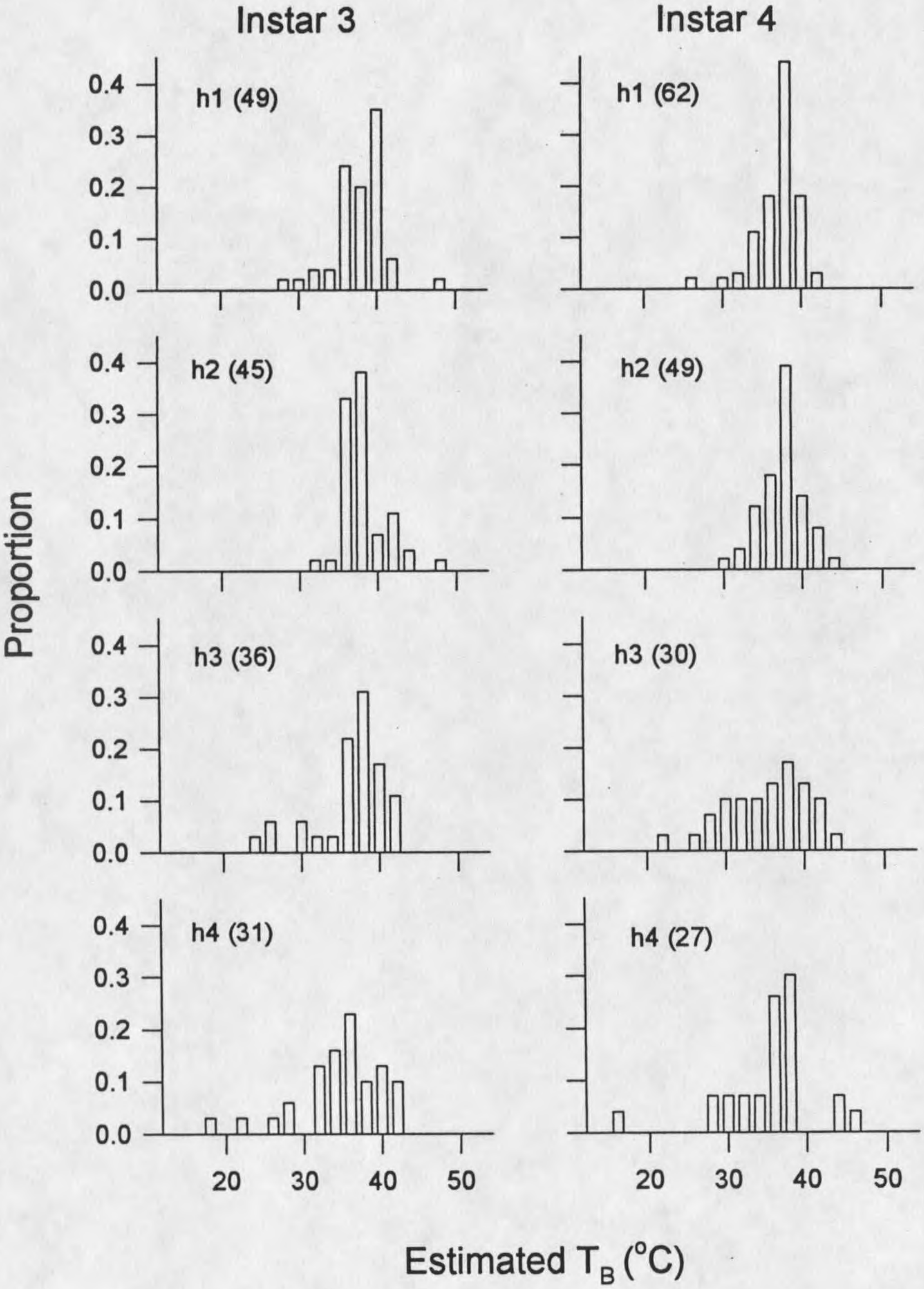

Figure 2. Proportion of third and fourth instars of *M. sanguinipes* on the gradient with estimated body temperatures at each of the four observational hours.

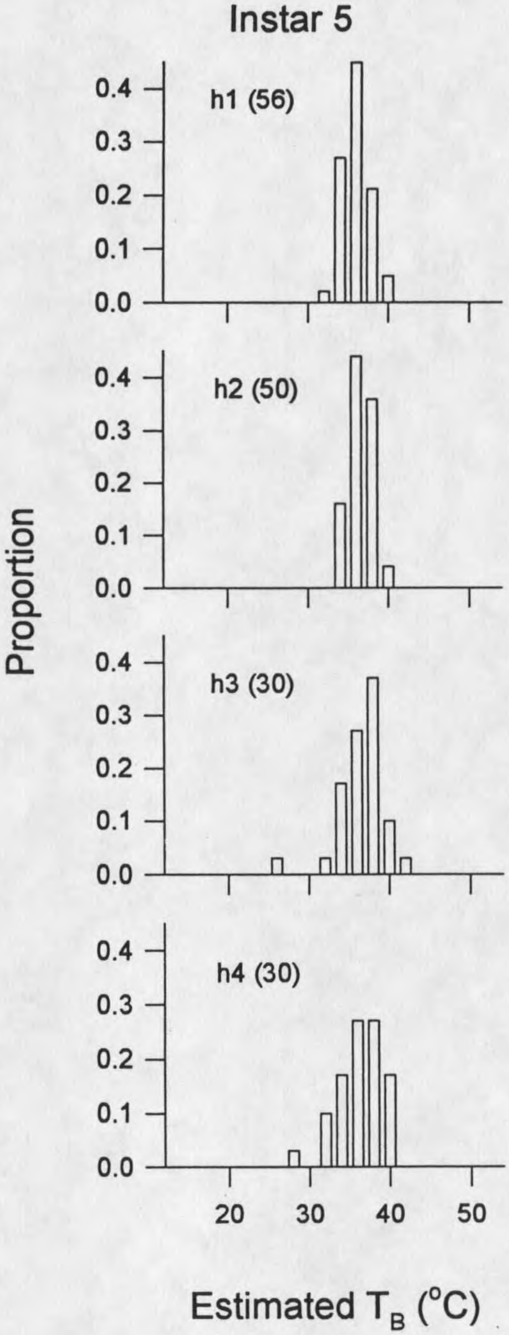

Figure 3. Proportion of fifth instar *M. sanguinipes* on the gradient with estimated body temperatures at each of the four observational hours.

Table 2. The estimated set-points and their respective widths after the second hour for all five instars of *M. sanguinipes* on a thermal gradient.

| Instar | N | Set-point Range (°C) | Setpoint Width (°C) |
|---|---|---|---|
| 1 | 93 | 36.77 - 40.52 | 3.75 |
| 2 | 53 | 34.28 - 37.77 | 3.49 |
| 3 | 45 | 37.04 - 39.34 | 2.30 |
| 4 | 49 | 36.51 - 39.43 | 2.92 |
| 5 | 49 | 35.99 - 37.83 | 1.84 |

The range of temperatures used by the nymphs tended to widen as the time spent on the gradient increased. Accordingly, the range of estimated body temperatures also widened with time, and was generally more narrow after the first and second hours than after the third and fourth hours (Table 3, Figures 1-3). Furthermore, the distribution was unimodal with a slight skew toward the cooler temperatures for the smaller instars.

Table 3. Width of the estimated body temperature ranges (°C) of five instars of *M. sanguinipes* as a function of time spent on the gradient.

| | Estimated Range of Body Temperatures | | | |
|---|---|---|---|---|
| Instar | Hour 1 | Hour 2 | Hour 3 | Hour 4 |
| 1 | 28.3 | 25.5 | 31.8 | 36.5 |
| 2 | 15.5 | 19.0 | 11.5 | 23.5 |
| 3 | 19.1 | 15.0 | 17.7 | 24.3 |
| 4 | 14.4 | 12.3 | 21.0 | 30.0 |
| 5 | 8.0 | 5.5 | 14.7 | 12.3 |

## Field Behavioral Observations

Because of heavy grazing in the uniform treatment from previous years, there were major microhabitat differences between the two treatments. These included the amount of standing and fallen vegetation present from past years, the proportion of the environment that was shaded, and wind speeds. Despite the differences in microhabitat structure and solar radiation reaching the soil surface, the range of surface temperatures that I recorded throughout the day for control and uniform treatments were similar. Temperatures from control were slightly warmer and slightly cooler (control range 13.5 - 61.6°C; uniform range 14.8 - 60.3°C).

### Insolation

The amount of direct solar radiation that reached a nymph influenced its behavior. Those that were flushed when surface temperatures were low tended to move into microhabitats with greater insolation during the following two minute observation period (Figures 4, 5, and 6). In cool mornings, nymphs that were flushed into shady areas usually walked or climbed immediately into insolated microhabitats, either on the soil surface, or up in the vegetation. In addition, as soil surface temperatures increased to 50°C and beyond, those that were flushed onto fully insolated surfaces near or on the ground tended to jump or walk quickly into cooler microhabitats. Therefore, as $T_S$ increased, a greater proportion of nymphs moved into microhabitats with less solar insolation. These trends were consistent among the three instars, as well as in the control and uniform treatments

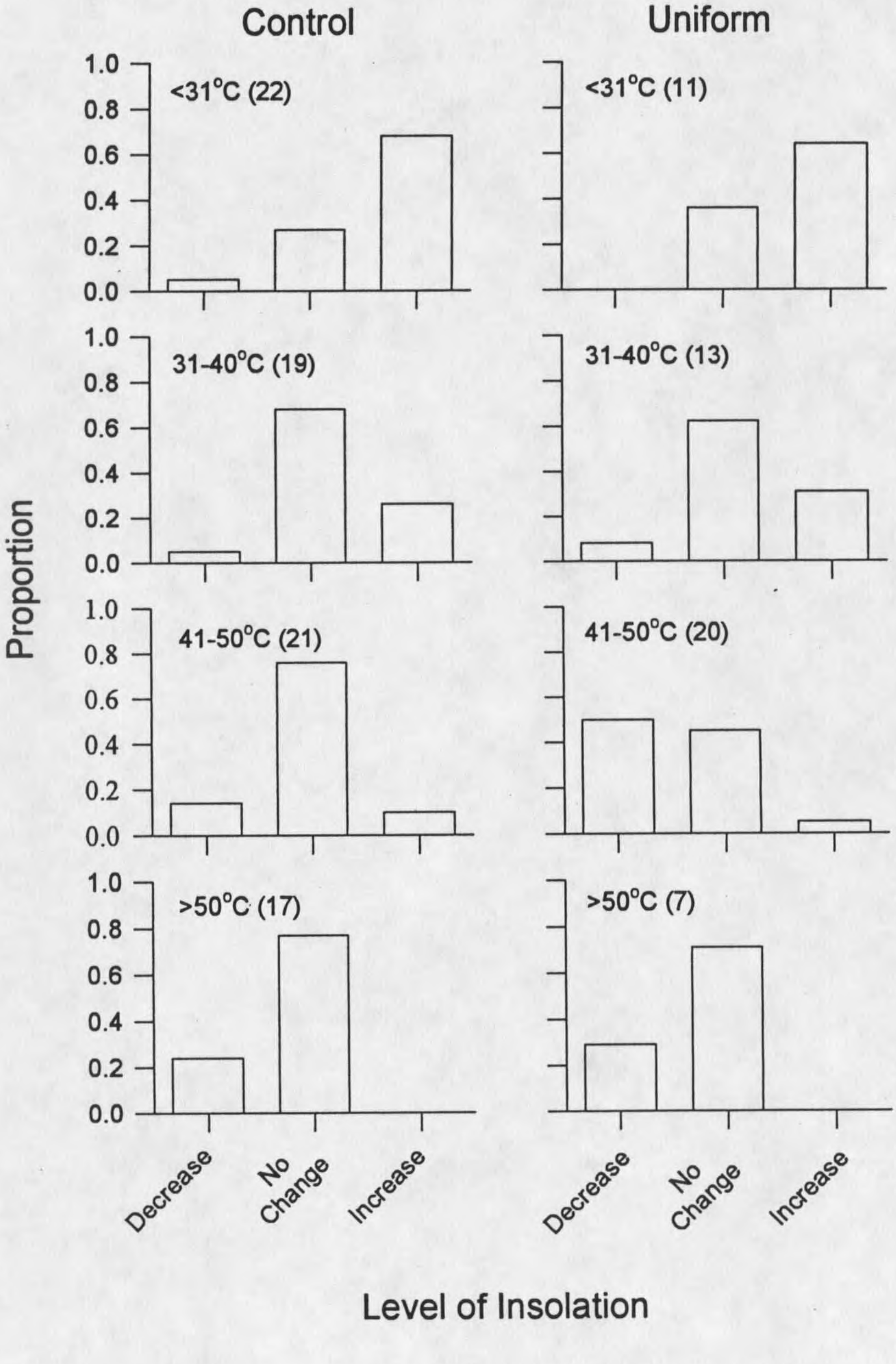

Figure 4. Magnitude of change in insolation at locations occupied by third instar *M. sanguinipes*.

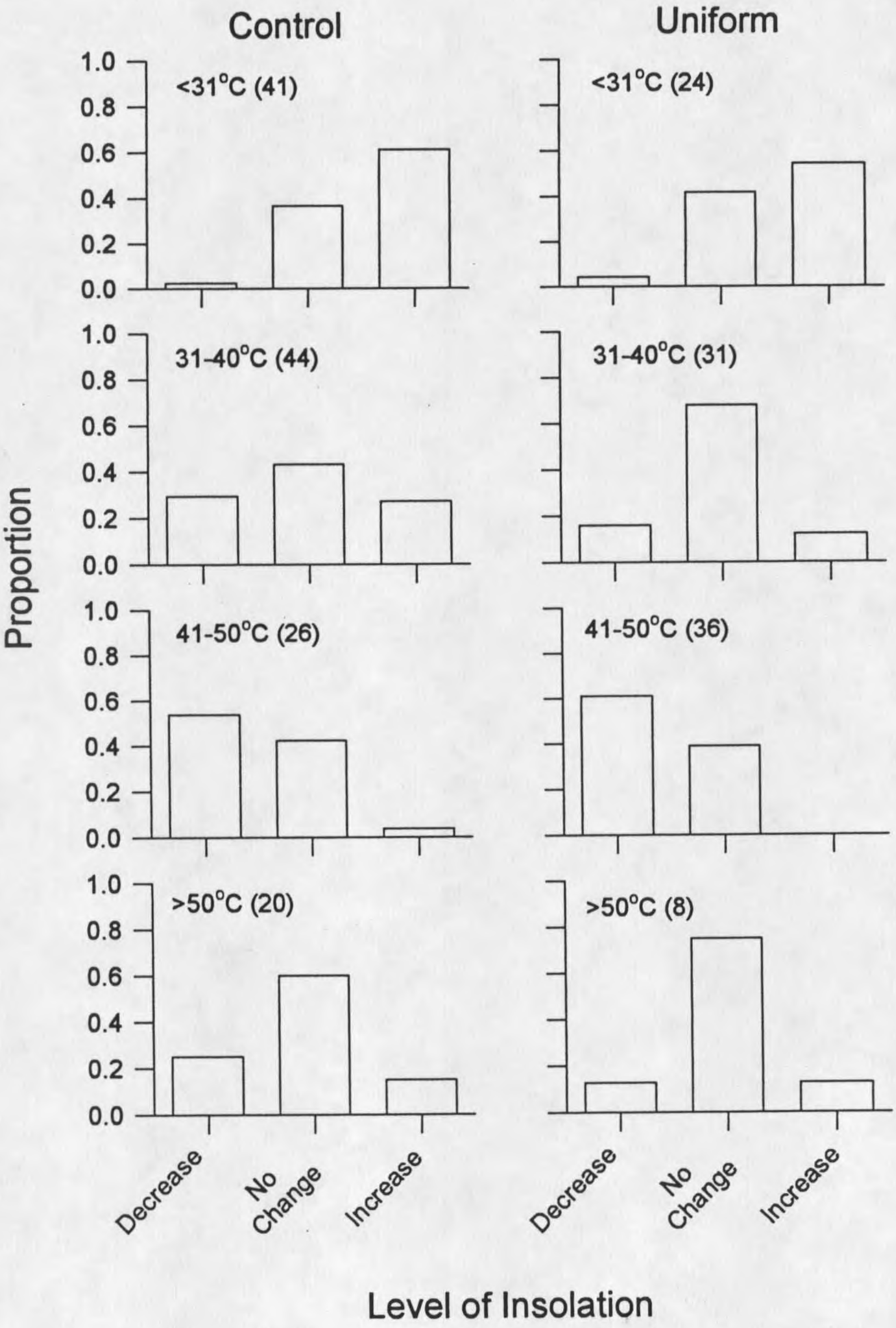

Figure 5. Magnitude of change in insolation at locations occupied by fourth instar *M. sanguinipes*.

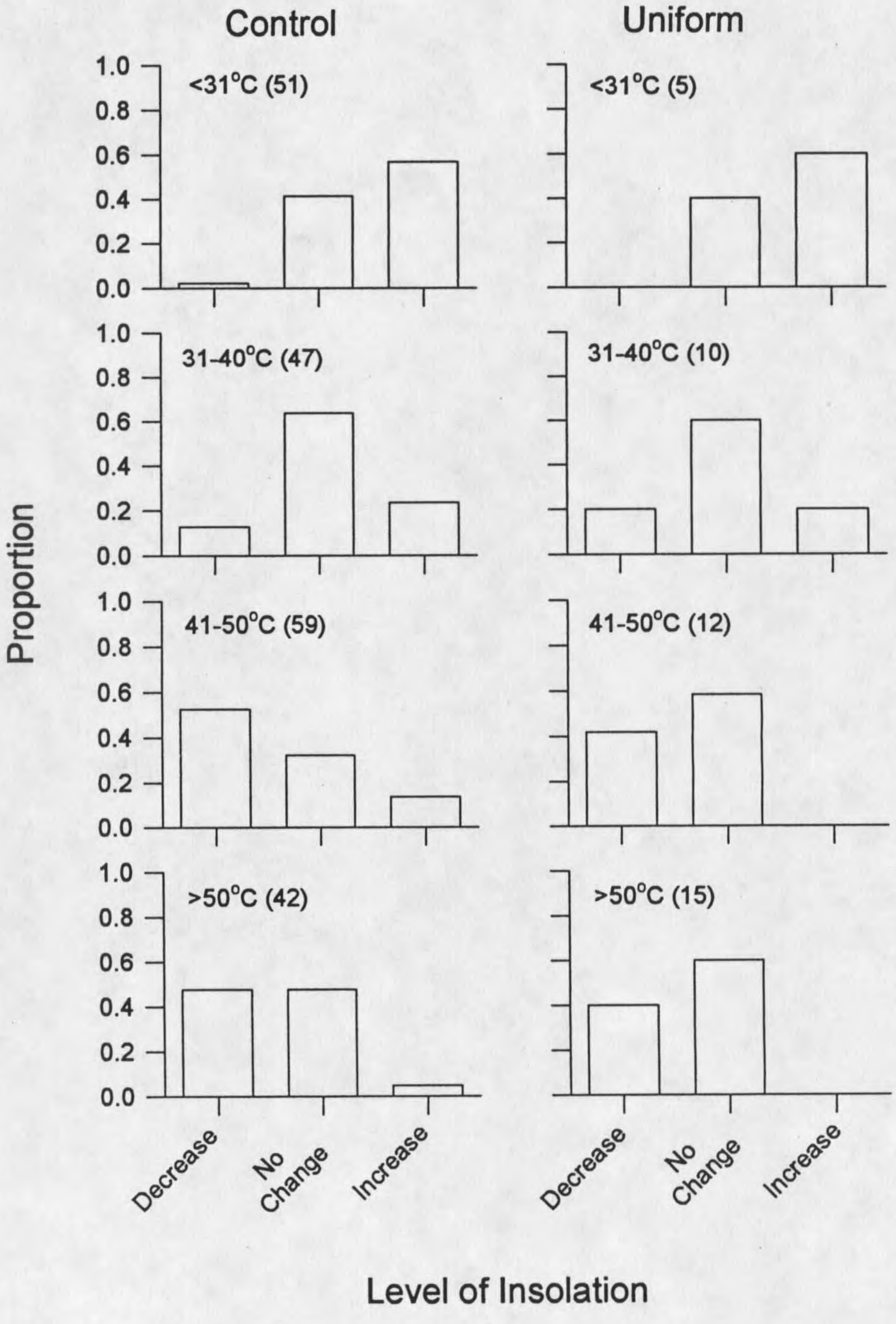

Figure 6. Magnitude of change in insolation at locations occupied by fifth instar *M. sanguinipes*.

(Table 4).

Table 4. Chi-square contingency table analysis of the change in insolation associated with four temperature categories for instars three, four, and five.

| Treatment | Instar | N | $\chi^2$ (6 df) | P-value |
|---|---|---|---|---|
| Control | 3 | 79 | 29.8 | 0.000 |
| | 4 | 131 | 38.7 | 0.000 |
| | 5 | 199 | 68.4 | 0.000 |
| Uniform | 3 | 51 | 23.0 | 0.001 |
| | 4 | 99 | 46.8 | 0.000 |
| | 5 | 42 | - | - |

Missing chi-square value represents an invalid test due to a large number of cells with 0.

<u>Orientation</u>

By orienting the body's longitudinal axis in a horizontal or vertical plane relative to the sun's position, a nymph can control the amount of solar radiation it intercepts. The temperature of the soil had a large impact on the speed at which they adjusted their orientations, and the angles that they preferred, with more conspicuous adjustments made in more extreme thermal environments.

The orientation of the nymphs in a horizontal plane differed significantly with surface temperature, except for fifth instars in the uniform treatment, in which case insignificant results may have been due to a small sample size (Table 5). At surface temperatures lower than 30°C, a large proportion oriented the long axis of the body approximately perpendicular (90°) to the direction of the sun (Figures 7, 8, and 9).

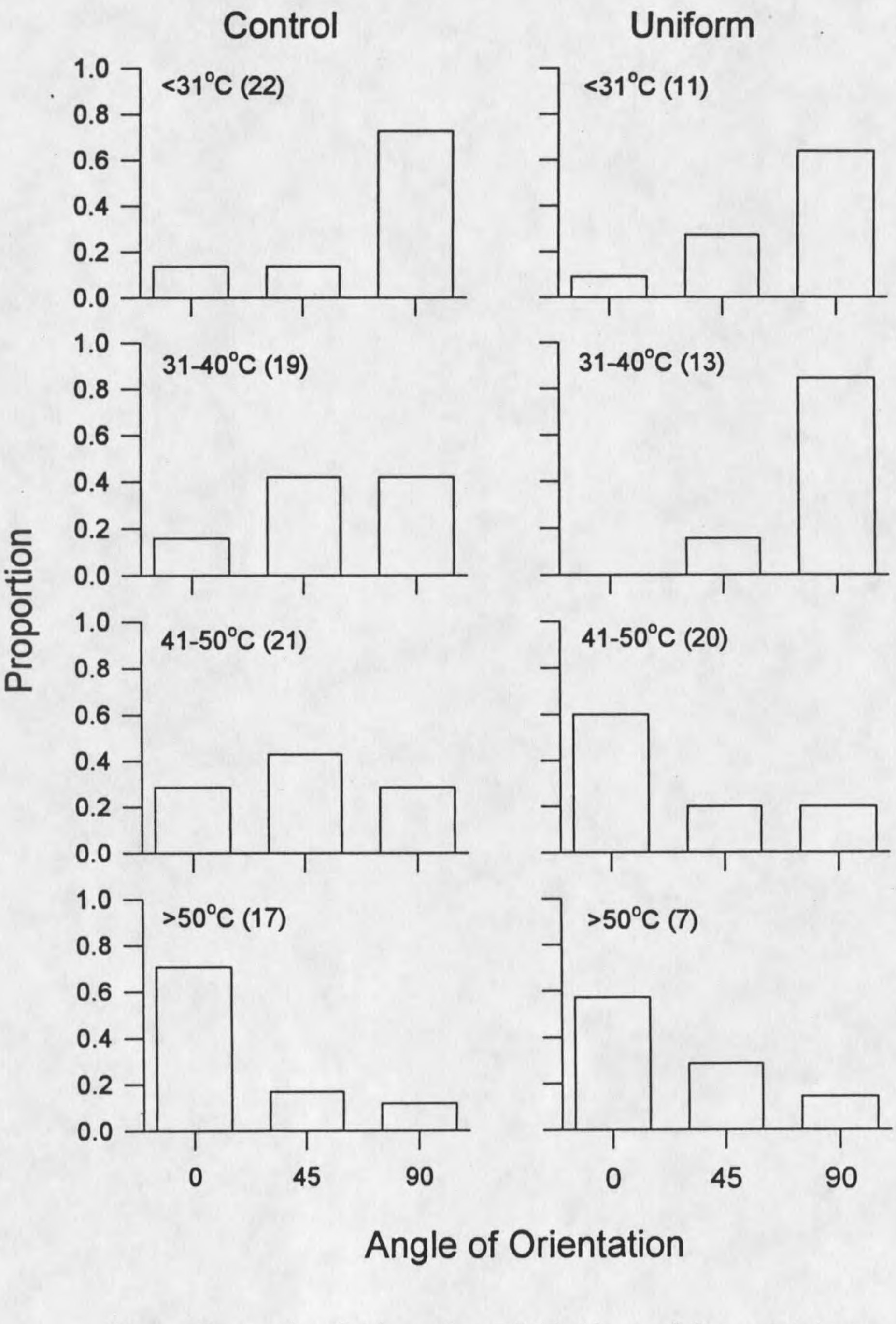

Figure 7. Angle of orientation of third instar *M. sanguinipes* relative to the position of the sun when the longitudinal axis is in a horizontal plane.

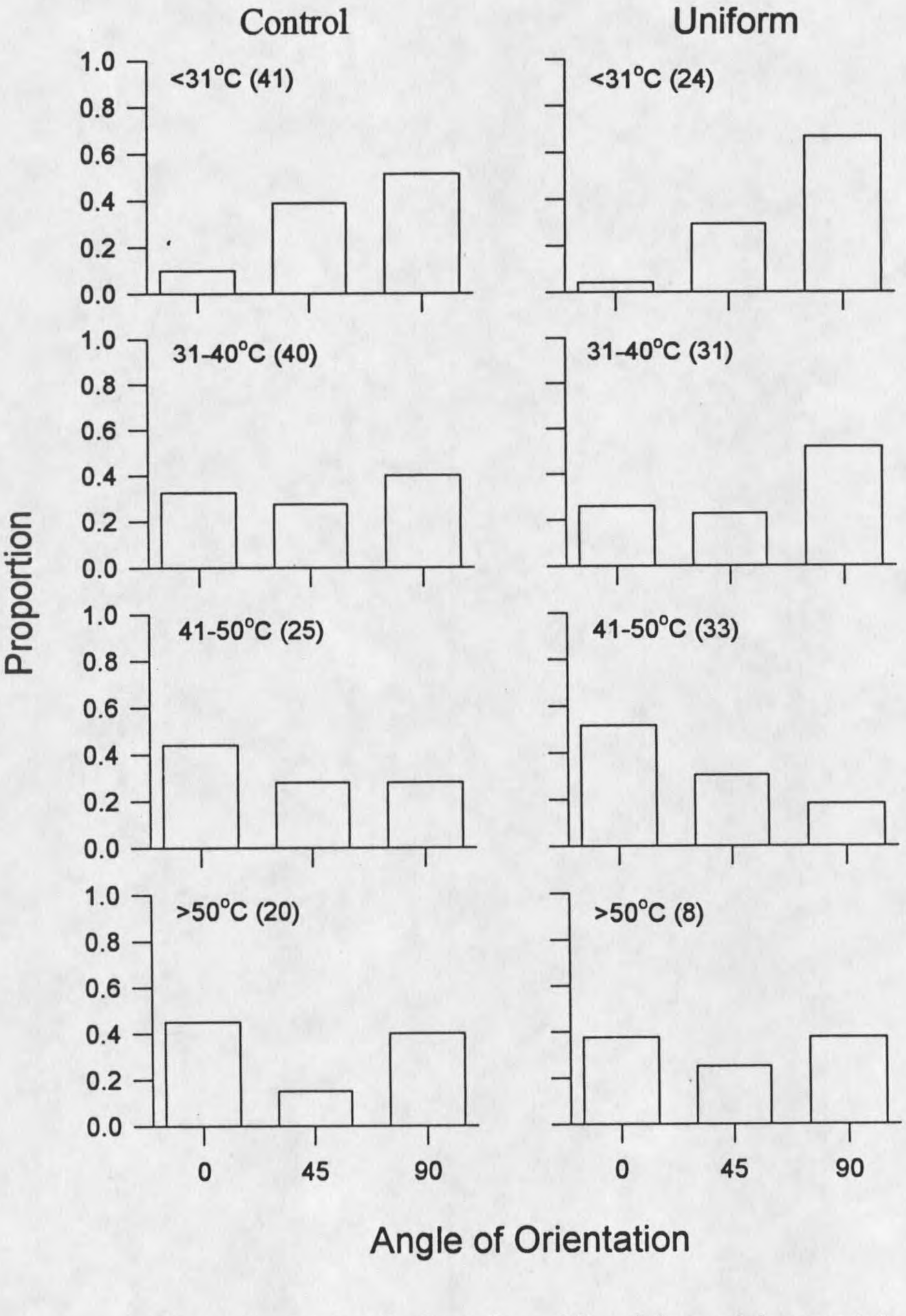

Figure 8. Angle of orientation of fourth instar *M. sanguinipes* relative to the position of the sun when the longitudinal axis is in a horizontal plane.

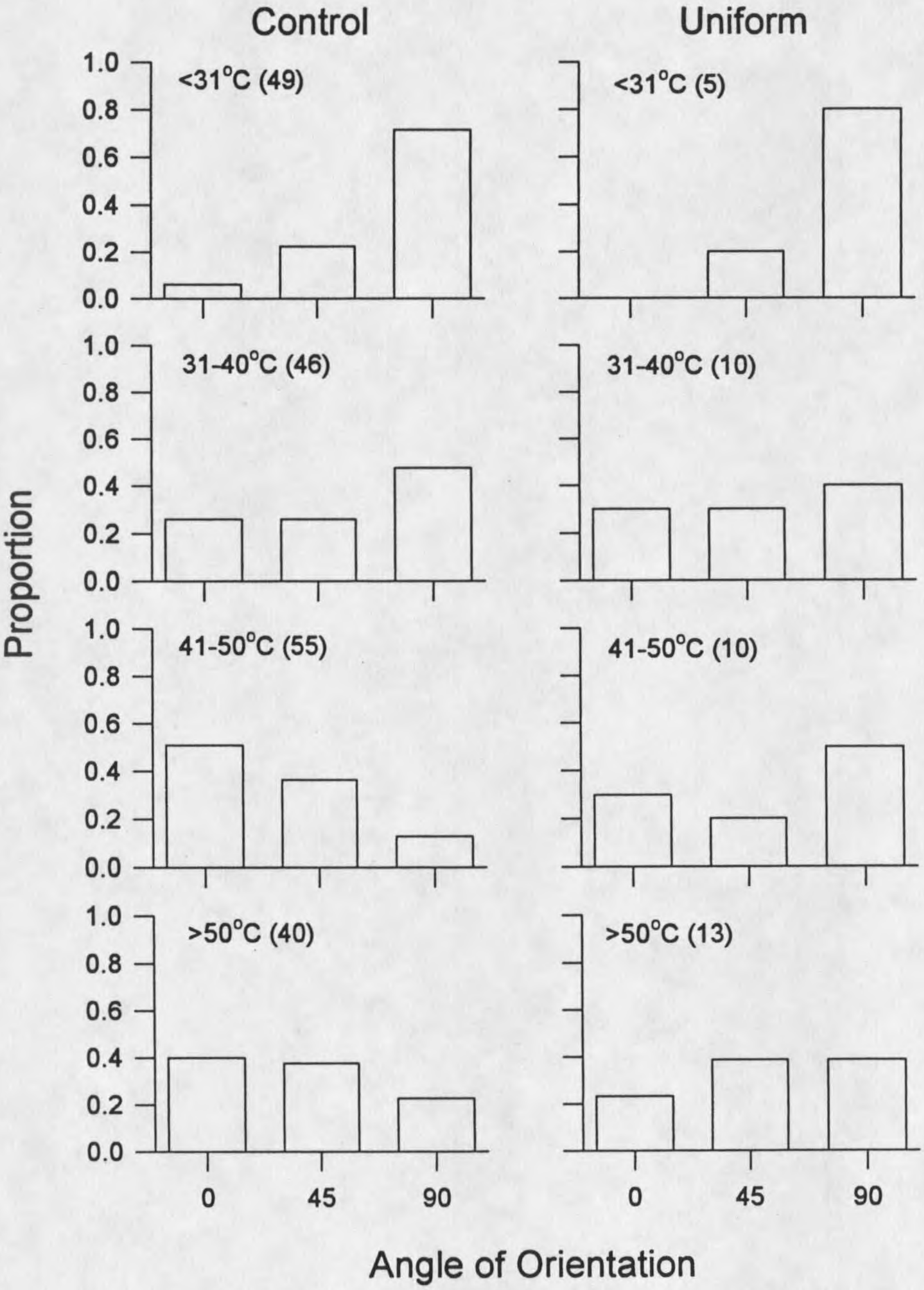

Figure 9. Angle of orientation of fifth instar *M. sanguinipes* relative to the position of the sun when the longitudinal axis is in a horizontal plane.

Conversely, at surface temperatures higher than 40°C, a relatively large proportion oriented parallel (0°) to the sun's incoming rays. However, the relationship between orientation and surface temperature was not as strong for high temperatures as it was for low temperatures. At temperatures between 30 and 40°C, the nymphs did not prefer any particular angle of orientation (Figures 7, 8, and 9).

Table 5. Chi-square contingency table analysis of the horizontal orientation of instars three, four, and five associated with four different temperature categories.

| Treatment | Instar | N | $\chi^2$ (6 df) | P-value |
|---|---|---|---|---|
| Control | 3 | 79 | 25.3 | 0.000 |
| | 4 | 126 | 13.8 | 0.031 |
| | 5 | 190 | 47.8 | 0.000 |
| Uniform | 3 | 51 | 21.2 | 0.002 |
| | 4 | 96 | 19.6 | 0.003 |
| | 5 | 38 | 3.8 | 0.701 |

The results of the logistic analysis support the hypothesis that the probability that a randomly chosen nymph will be found in a particular orientation of 0°, 45°, or 90° in relation to the position of the sun is a function of surface temperature (Table 6; Figure 10). The odds ratio value for third instars in the control treatment indicates that for every 0.1 degree rise in surface temperature, the nymphs are an estimated 1.06 times as likely to be oriented 45° compared with 90°, and an estimated 1.11 times as likely to be oriented 0° compared with 90°. The odds ratio values were consistently higher for the orientations of 0 versus 90°, as compared with 45 versus 90°, suggesting that as soil temperatures

Table 6. Results of a nominal logistic regression analysis in which the probability that a grasshopper is horizontally oriented at an angle of 0°, 45°, or 90° in relation to the position of the sun is correlated with the predictor, $T_S$.

| Treatment | Instar | N | Angle of Orientation | Slope Coefficient | SE | Odds Ratio | G Statistic (2 df) | P-Value |
|---|---|---|---|---|---|---|---|---|
| Control | 3rd | 56 | 0° vs 90° | 0.1020 | 0.004 | 1.11 | 16.305 | 0.000 |
| | | 55 | 45° vs 90° | 0.0536 | 0.003 | 1.06 | 16.305 | 0.000 |
| | 4th | 89 | 0° vs 90° | 0.0682 | 0.002 | 1.07 | 14.052 | 0.001 |
| | | 89 | 45° vs 90° | -0.0040 | 0.002 | 1.00 | 14.052 | 0.001 |
| | 5th | 132 | 0° vs 90° | 0.0201 | 0.002 | 1.12 | 45.035 | 0.000 |
| | | 131 | 45° vs 90° | 0.0185 | 0.002 | 1.08 | 45.035 | 0.000 |
| Uniform | 3rd | 40 | 0° vs 90° | 0.2173 | 0.010 | 1.24 | 23.260 | 0.000 |
| | | 34 | 45° vs 90° | 0.0563 | 0.007 | 1.06 | 23.260 | 0.000 |
| | 4th | 70 | 0° vs 90° | 0.1384 | 0.004 | 1.15 | 21.233 | 0.000 |
| | | 67 | 45° vs 90° | 0.0586 | 0.004 | 1.06 | 21.233 | 0.000 |
| | 5th | 27 | 0° vs 90° | 0.0501 | 0.008 | 1.05 | 1.766 | 0.414 |
| | | 29 | 45° vs 90° | 0.0386 | 0.007 | 1.04 | 1.766 | 0.414 |

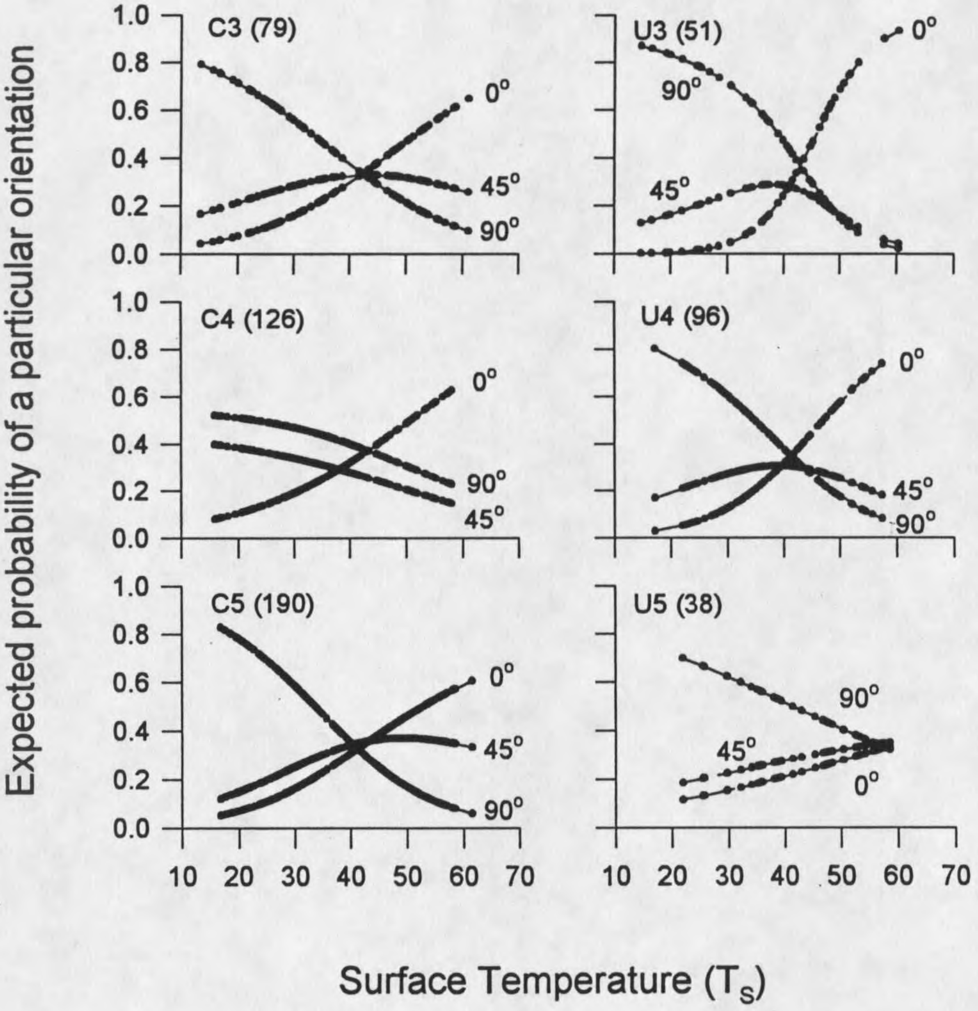

Figure 10. The expected probability that a nymph will orient in any of three horizontal orientations (0°, 45°, and 90°) relative to the direction of the sun as a function of soil surface temperature.

increase there is a stronger tendency for the nymphs to orient parallel (0°) to the sun's rays as opposed to orienting at a 45° angle. The G-value is a likelihood ratio test for the overall significance of the slope coefficients for the independent variable in the model (Hosmer and Lemeshow 1989). A large G-test statistic with a small P-value indicates that the model has a good fit. All of the tests were significant with a P-value of at least 0.001, except for instar five in the uniform treatment. Insignificance in this group is probably due to a small sample size.

Although the diagnostic procedures indicated that 21 of the 580 observations recorded for third, fourth, and fifth instars were unusual (Appendix), I kept them in the model because I had no reason to believe that the observations represented aberrant behaviors on the part of the nymphs due to observer presence. Most of the 21 outliers were created by grasshoppers oriented at a 0° angle relative to the position of the sun at low surface temperatures (48%), or oriented at a 90° angle at high surface temperatures (52%). However, such "outliers" are to be expected, because the behaviors of nymphs are influenced by factors other than thermoregulation, such as feeding and avoiding predators.

As with the horizontal orientations, I found that the vertical orientation of the nymphs's body also depended upon surface temperature. When $T_S$ was below 40°C, more nymphs tended to orient their body parallel to the soil surface, whereas when $T_S$ was above 40°C, the majority oriented perpendicular to the soil surface (Figures 11, 12, and 13; Table 7). The vertical orientation data are to a large extent a look at the substrate types utilized by the nymphs at different soil temperatures, rather than their responses to solar radiation on a vertical plane. In cool temperatures, the soil surface is the warmest

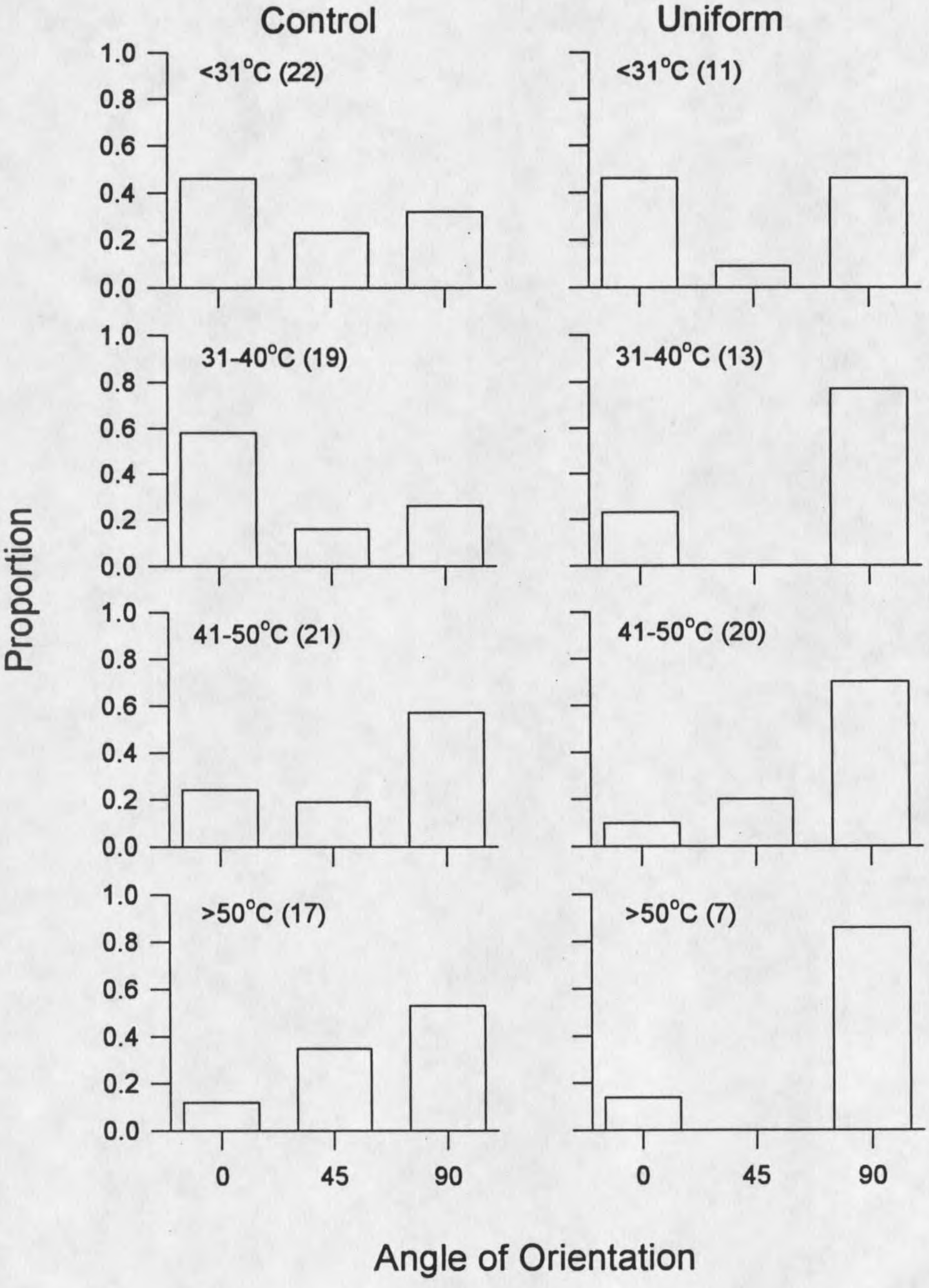

Figure 11. Angle of orientation of third instar *M. sanguinipes* relative to the soil surface when the longitudinal axis is in a vertical plane.

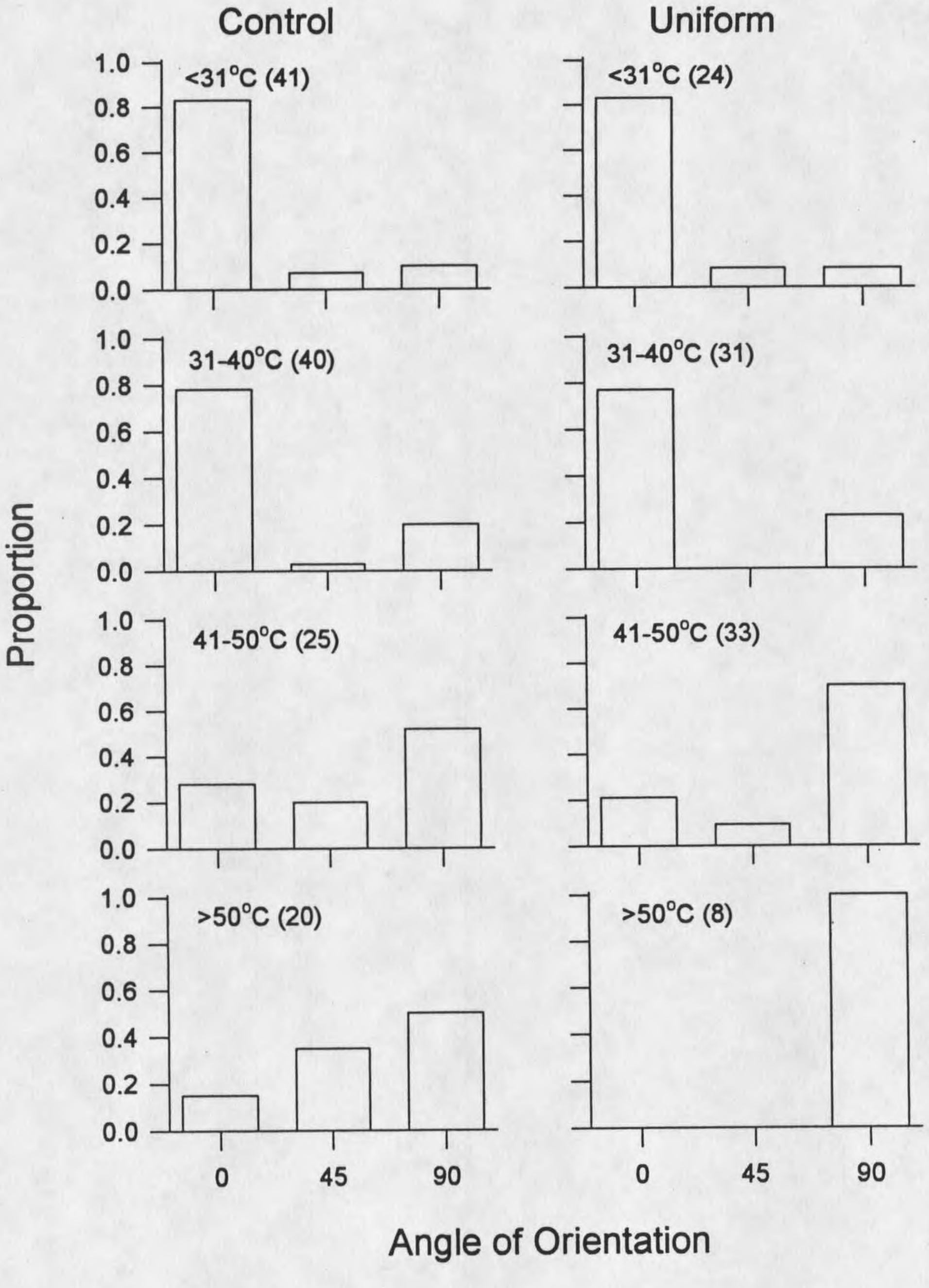

Figure 12. Angle of orientation of fourth instar *M. sanguinipes* relative to the soil surface when the longitudinal axis is in a vertical plane.

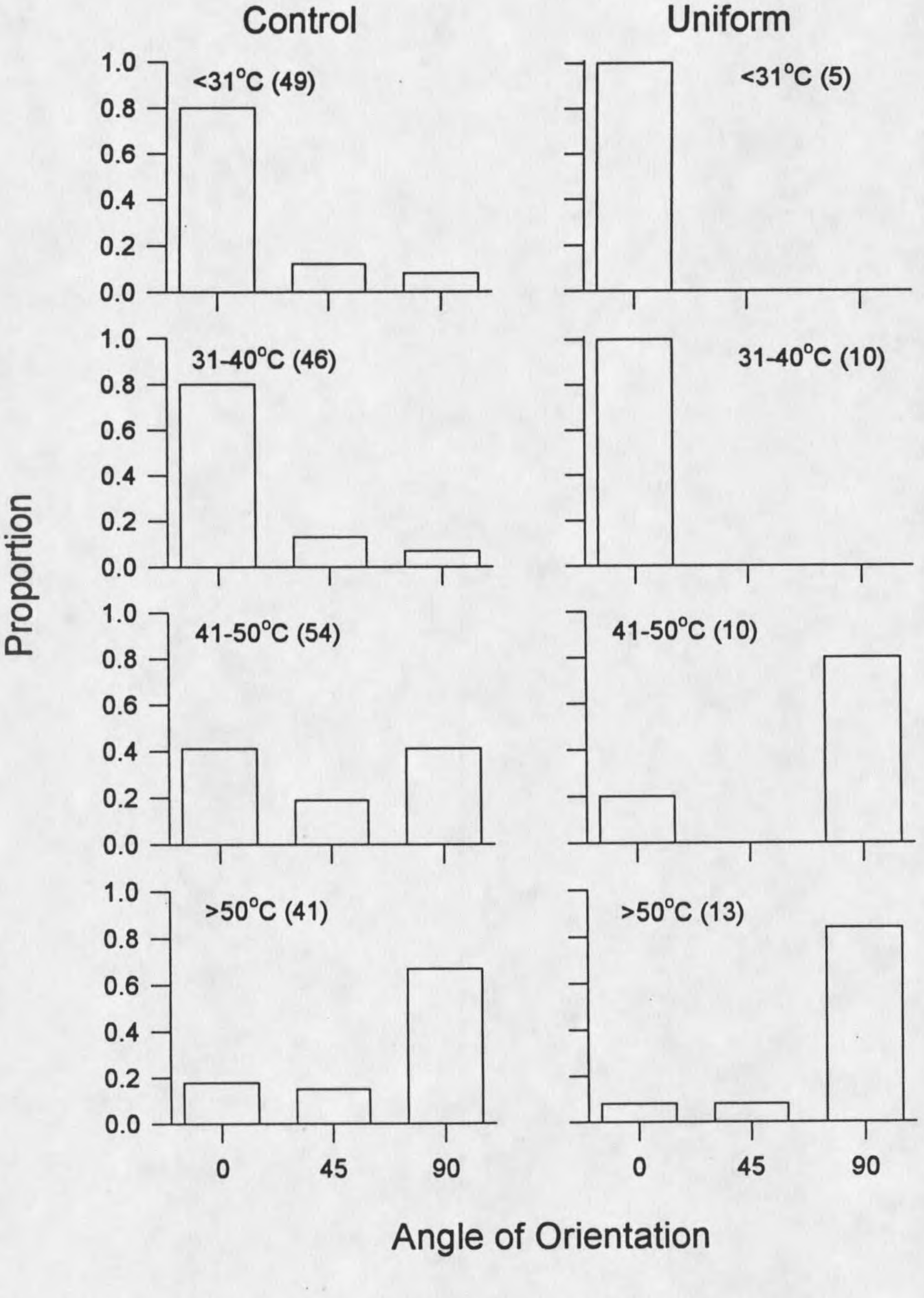

Figure 13. Angle of orientation of fifth instar *M. sanguinipes* relative to the soil surface when the longitudinal axis is in a vertical plane.

part of the environment, and so nymphs tend to remain on the ground. As temperatures increase, they move up into the stems and leaves of the vegetation, thus vertically orienting the longitudinal axis of their bodies perpendicular to the ground.

Table 7. Chi-square contingency table analysis of the vertical orientation of instars three, four, and five associated with four different temperature categories

| Treatment | Instar | N | $\chi^2$ (6 df) | P-value |
|---|---|---|---|---|
| Control | 3 | 79 | 11.824 | 0.066 |
| | 4 | 126 | 44.374 | 0.000 |
| | 5 | 190 | 61.830 | 0.000 |
| Uniform | 3 | 51 | - | - |
| | 4 | 96 | 49.860 | 0.000 |
| | 5 | 38 | - | - |

Missing chi-square values represent an invalid test due to a large number of cells with 0.

## Height

Nymphs were able to influence the air temperatures and wind speeds that they experienced by moving vertically within the vegetation in their microhabitat. Most changed height by either climbing onto the stems and leaves, or by jumping, which was more common during the warmer parts of the day. Because ambient temperatures were warmest near the soil surface, and became progressively cooler with increasing height and greater wind speeds, a range of environmental temperatures could be used. Therefore, nymphs tended to move up into the vegetation at higher temperatures, and there was a positive relationship between soil temperature and the final height attained by nymphs of each instar (Figure 14; Table 8). This relationship varied among instars. It was stronger in the fourth and fifth instars than in the third, with no significant relationship for third instars in uniform.. This discrepancy may reflect that when third instar nymphs were flushed, they tended to jump higher up into the vegetation, regardless of the soil temperature, whereas the older, larger instars tended to flush to the ground, and then walked up onto the vegetation with increasing soil temperatures. Consequently, the mean final heights differed among instars (ANOVA, $F=16.50_{(2.598)}$, $P<0.0001$); heights of third instars were significantly higher than fourth and fifth instars (Tukeys, $P<0.05$). However, there were only marginally significant differences in mean final height between control and uniform treatments (ANOVA, $F=3.63_{(1,599)}$, $P=0.06$); heights in uniform were slightly higher.

Table 8. Coefficients of determination for the relationship between soil temperature and the final heights attained by third, fourth, and fifth instars of *M. sanguinipes* in both treatments.

| Treatment | Instar | N | $r^2$ | P-value | Mean Final Ht (±SE) |
|---|---|---|---|---|---|
| Control | 3 | 79 | 0.08 | 0.010 | 8.05 (0.71) |
| | 4 | 131 | 0.15 | 0.000 | 4.52 (0.55) |
| | 5 | 199 | 0.23 | 0.000 | 4.27 (0.37) |
| Uniform | 3 | 51 | 0.01 | 0.417 | 9.00 (1.30) |
| | 4 | 99 | 0.19 | 0.000 | 5.17 (0.84) |
| | 5 | 42 | 0.18 | 0.005 | 7.27 (1.88) |

I expected a significant difference in height among the instars and between the

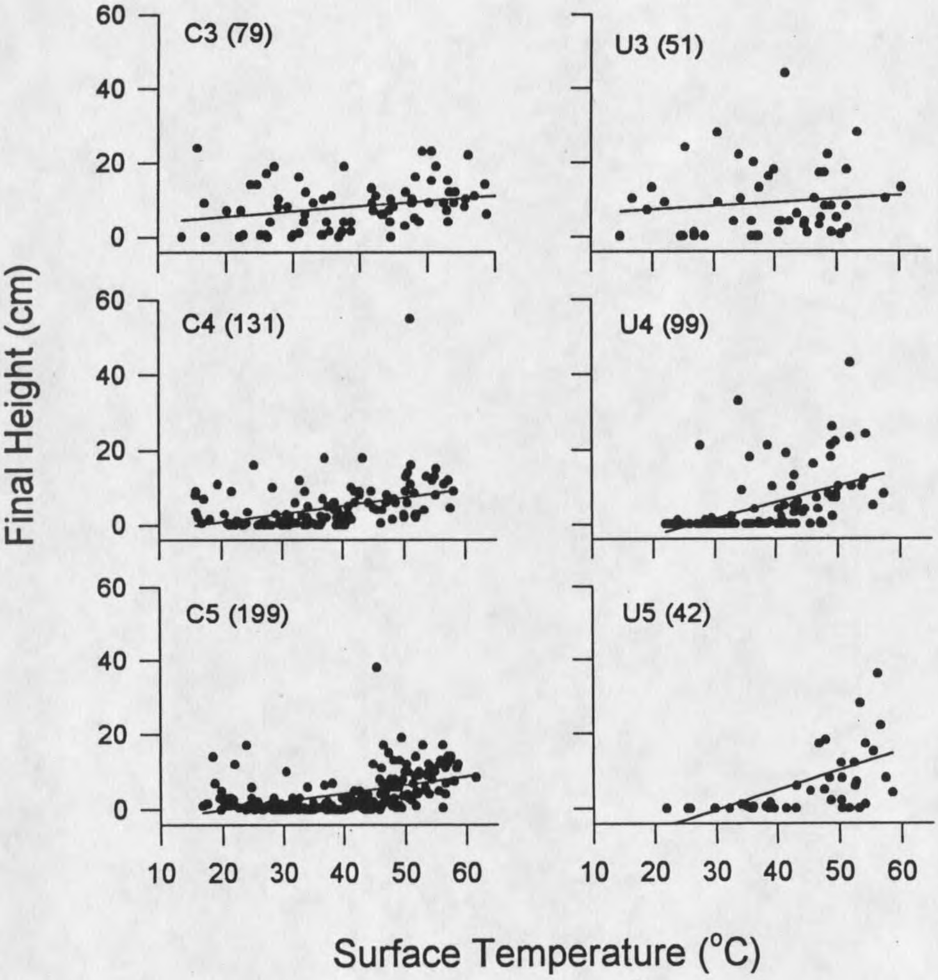

Figure 14. Final height as a function of $T_S$ for three instars of *M. sanguinipes*. C = control treatment and U = uniform treatment.

treatments during the two-minute observation period, because each instar prefered different landing sites after they were flushed, and because of the differences in habitat structure between control and uniform. However, the difference in height at the beginning and end of the two minute observation period varied only slightly as a function of soil temperature (Figure 15). Fourth and fifth instars tended to move higher with increasing temperatures, perhaps because of their tendency to initially flush to the ground before climbing up the vegetation. Likewise, third instars tended to move lower if they changed height at all. This may reflect their habit of flushing onto areas of the vegetation that were above the ground. As soil temperatures increased, third instars were already occupying microhabitats that were either suitable, or too cool, in which case they moved to warmer areas closer to the ground. There was a significant difference in the change in height from the beginning to end of the two minute observation period (ANOVA, $F=4.23_{(2, 598)}$, $P=0.015$) between the third and fifth instars (Tukeys, $P<0.05$), but not among the other instars. There was also a significant difference in height change between the control and uniform treatments (ANOVA, $F=18.18_{(1, 599)}$, $P<0.0001$).

## Ambient Temperature at Final Thoracic Height

Because the air temperature experienced by a nymph affects rates and direction of convective heat exchange, it influences body temperatures. Measurements of air temperatures at the nymphs' thoracic heights at their initial and final locations produced estimates of the ambient temperatures they exploited at particular soil temperatures. This allowed me to determine whether they moved into warmer or cooler microhabitats. The

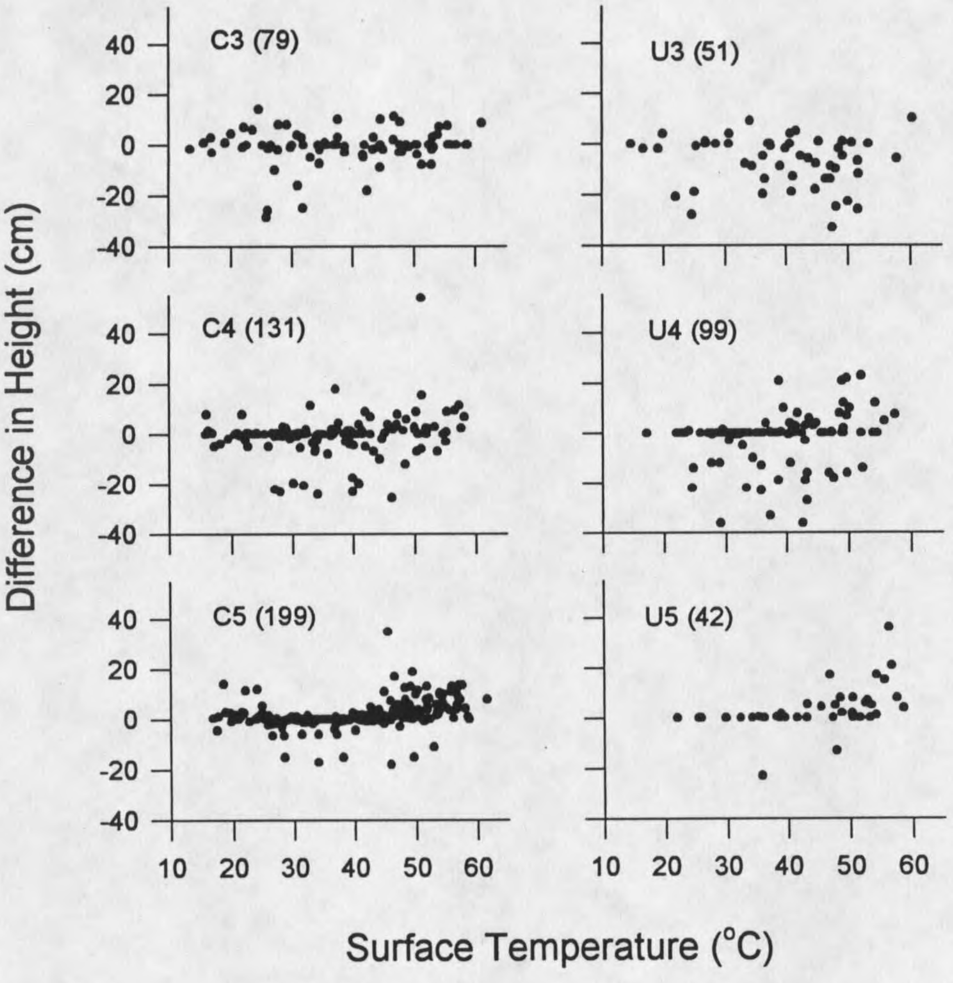

Figure 15. Difference in height as a function of $T_S$ for three instars of *M. sanguinipes.* C = control treatment and U = uniform treatment.

threshold temperatures (Table 9) estimated by the linear segmented models could be considered the body temperatures at which grasshoppers changed thermoregulatory strategies. Nymphs that were flushed into areas where the initial $T_{TH}$ was lower than the threshold temperature tended to move into areas where $T_{TH}$ was slightly higher. Nymphs that were flushed into microhabitats at or above the threshold attempted to stay within optimal ambient temperatures by avoiding $T_{TH}$'s that were too cool or too hot (Figure 16). These observations suggest that when initial $T_{TH}$ was below the threshold temperature, third, fourth, and fifth instars of *M. sanguinipes* remained in microhabitats with the same ambient temperatures. However, in areas where initial $T_{TH}$ was warmer than the threshold temperature, they actively moved into microhabitats of higher habitat quality (Hertz et al. 1993), where air temperatures were closer to their estimated set-point ranges.

Table 9. Initial $T_{TH}$ threshold temperatures and Final $T_{TH}$ mean temperatures of third, fourth, and fifth instars of *M. sanguinipes* in control and uniform treatment areas.

| Treatment | Instar | N | Initial $T_{TH}$ Threshold Temperature (±SE) | N | Mean Final $T_{TH}$ Temperature (±SE) |
|---|---|---|---|---|---|
| Control | 3 | 79 | 33.01 (0.84) | 15 | 33.20 (0.57) |
| | 4 | 131 | 30.90 (0.58) | 54 | 33.54 (0.28) |
| | 5 | 199 | 32.44 (0.53) | 88 | 32.82 (0.27) |
| Uniform | 3 | 51 | 30.83 (1.10) | 10 | 32.76 (0.49) |
| | 4 | 99 | 32.93 (0.85) | 38 | 34.49 (0.34) |
| | 5 | 42 | 34.74 (2.95) | 21 | 33.07 (0.60) |

The data also indicates that all three instars exploit similar ambient temperatures.

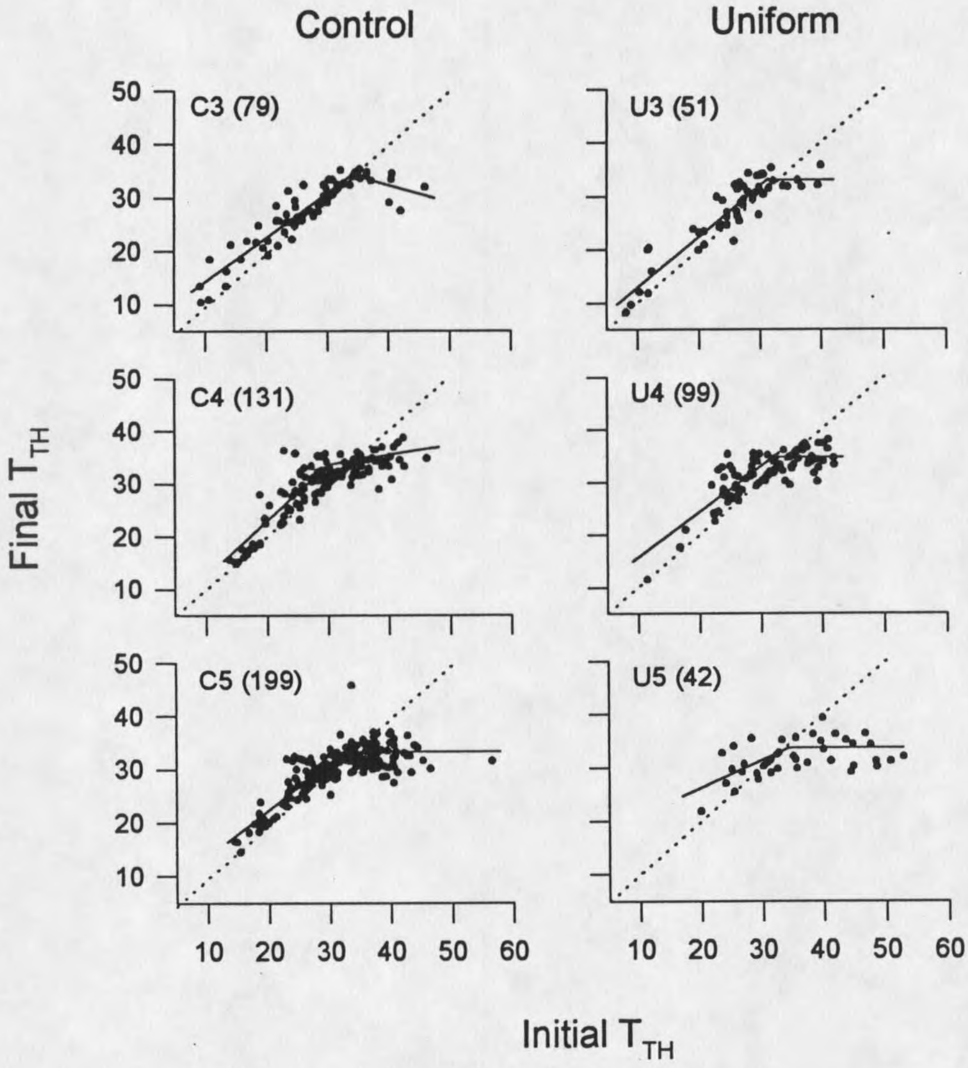

Figure 16. Final ambient temperature at thoracic height as a function of initial ambient temperature at thoracic height. C = control treatment and U = uniform treatment.

There was no large difference in the initial $T_{TH}$ threshold temperatures between any of the instars, nor between the control versus the uniform treatment (Table 9), implying that the nymphs responded similarly to specific ambient temperatures between approximately 30 to 35°C by changing thermoregulatory strategies, regardless of the instar or grazing regime. There was a significant difference in the mean final $T_{TH}$ as a function of instar (ANOVA, $F=5.59_{(2, 223)}$, $P<0.0043$) only between fourth and fifth instars (Tukeys, $P<0.05$), and there was also a significant difference in mean final $T_{TH}$ between the control versus the uniform treatment areas (ANOVA, $F=4.40_{(1, 224)}$, $P<0.037$). Therefore, after the initial threshold temperature was reached, nymphs experienced slightly higher mean final $T_{TH}$ in the control rather than in the uniform treatment.

## Distance Traveled

Most of the instars I observed did not travel in a straight line during the two-minute observation period after they were flushed, but rather followed sinuous paths while traveling various distances. They walked around or through clumps of vegetation, climbed or jumped onto the vegetation, or walked onto dead matted grass or bare soil. I found a significant difference in the linear distance traveled as a function of instar (ANOVA, $F=11.72_{(2, 598)}$, $P<0.0001$), with fifth instars moving the farthest, and third instars moving the least. The same trend was also apparent in the total distance traveled as a function of instar (ANOVA, $F=14.04_{(2, 598)}$, $P<0.0001$) between all three instars (Tukeys, $P<0.05$). However, there were no significant differences as a function of treatment in the total distances traveled (ANOVA, $F=0.73_{(1, 599)}$, $P=0.40$), or in the linear distances traveled

(ANOVA, $F=2.51_{(1, 599)}$, P=0.11). There may be questions concerning the validity of this analysis, because there was a seasonal increase in microhabitat height between the third and fifth instars, which may in turn increase the range of distances traveled by nymphs.

Soil temperature was not related to the distances traveled by each of the instars. There was no relationship between surface temperature and 1) the linear distance traveled by grasshoppers within the two-minute observation period (Figure 17, Table 10), 2) the total distance traveled (Figure 18, Table 11), or 3) the total number of stops made within the observation period (Figure 19; Table 12). As with the distance traveled, the number of stops made per grasshopper per observation was random with respect to soil temperature, and may perhaps be more closely associated with the availability of preferred microhabitats rather than soil temperature.

Table 10. Coefficient of determination for the relationship between soil temperature and the linear distance traveled.

| Treatment | Instar | N | $r^2$ | P-value |
|---|---|---|---|---|
| Control | 3 | 79 | 0.03 | 0.111 |
| | 4 | 131 | 0.01 | 0.190 |
| | 5 | 199 | 0.05 | 0.002 |
| Uniform | 3 | 51 | 0.01 | 0.633 |
| | 4 | 99 | 0.02 | 0.190 |
| | 5 | 42 | 0.04 | 0.218 |

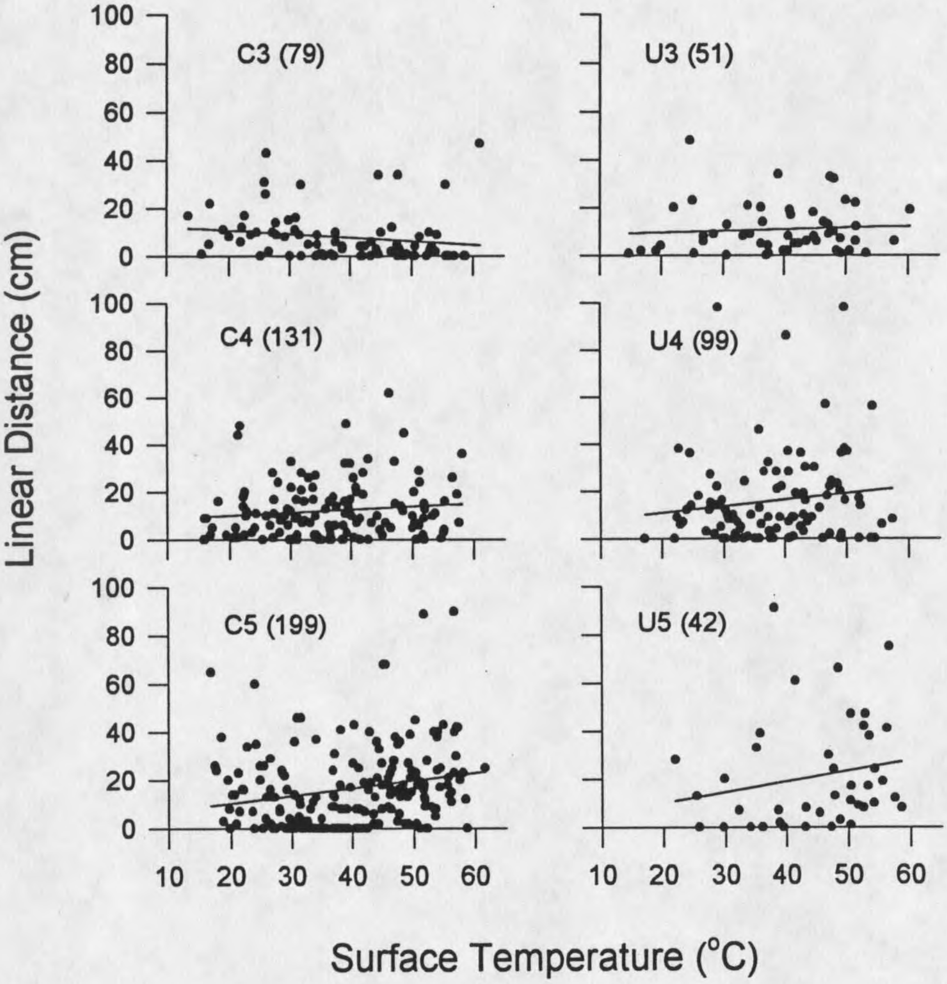

Figure 17. Linear distance traveled by third, fourth, and fifth instars of *M. sanguinipes* as a function of $T_S$ within the two minute observation period. C = control treatment and U = uniform treatment.

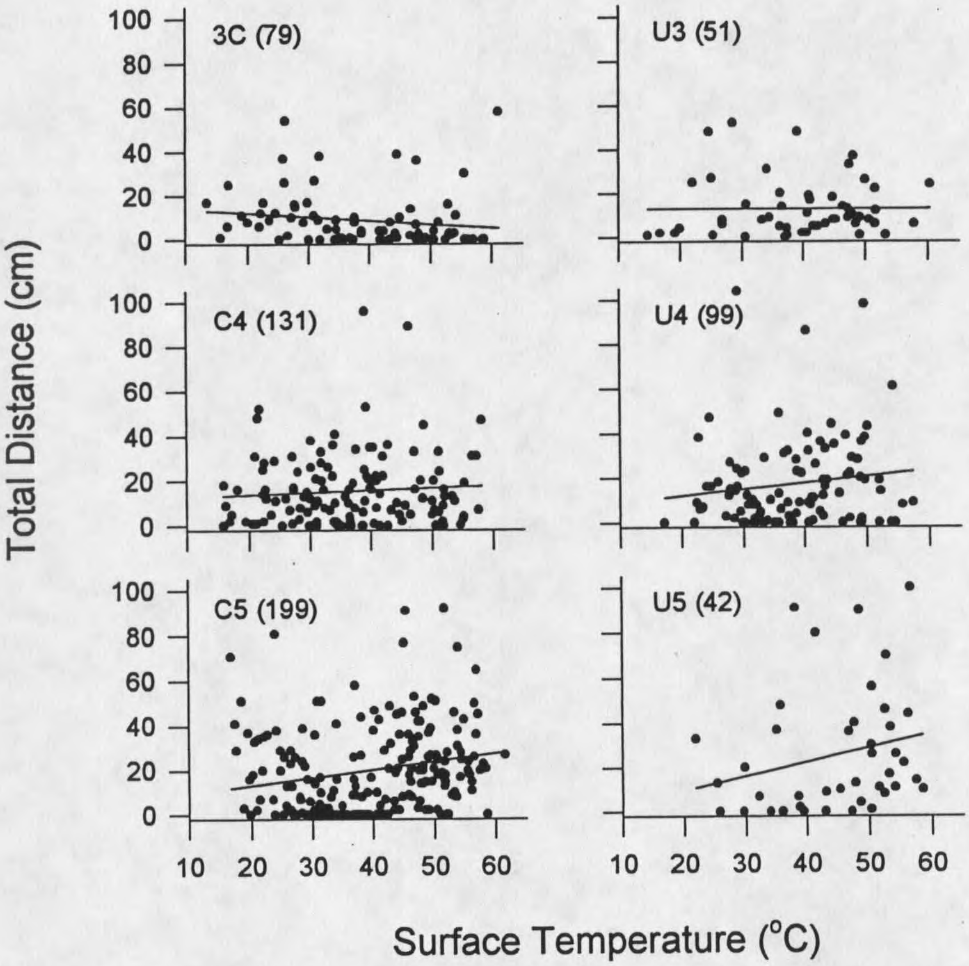

Figure 18. Total distance traveled by third, fourth, and fifth instars of *M. sanguinipes* as a function of $T_S$ within the two minute observation period. C = control treatment and U = uniform treatment.

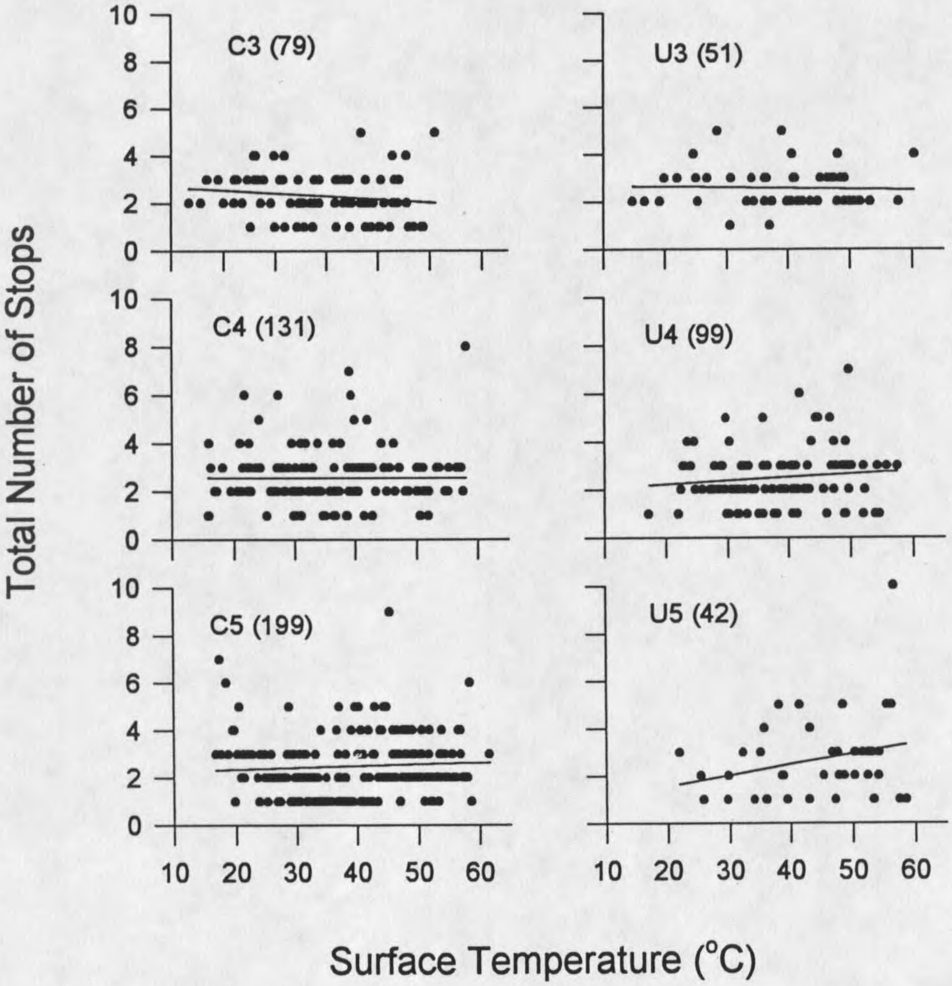

Figure 19. The total number of stops made by third, fourth, and fifth instars of *M. sanguinipes* as a function of $T_s$ within the two minute observation period. C = control treatment and U = uniform treatment.

Table 11. Coefficient of determination for the relationship between soil temperature and the total distance traveled.

| Treatment | Instar | N | $r^2$ | P-value |
|---|---|---|---|---|
| Control | 3 | 79 | 0.03 | 0.146 |
| | 4 | 131 | 0.01 | 0.387 |
| | 5 | 199 | 0.04 | 0.005 |
| Uniform | 3 | 51 | 0.00 | 0.965 |
| | 4 | 99 | 0.02 | 0.183 |
| | 5 | 42 | 0.05 | 0.142 |

Table 12. Coefficient of determination for the relationship between soil temperature and the total number of stops.

| Treatment | Instar | N | $r^2$ | P-value |
|---|---|---|---|---|
| Control | 3 | 79 | 0.02 | 0.176 |
| | 4 | 131 | 0.00 | 0.966 |
| | 5 | 199 | 0.00 | 0.390 |
| Uniform | 3 | 51 | 0.00 | 0.758 |
| | 4 | 99 | 0.01 | 0.243 |
| | 5 | 42 | 0.07 | 0.096 |

## Posture

The postures adopted by nymphs may play an important role in the amount of direct solar radiation that they intercept, and may be a significant thermoregulation strategy. However, there was little variation in the body postures adopted by third, fourth, and fifth instars, regardless of the type of substrate they were on, or the temperature of the soil. Despite evidence that adult *M. sanguinipes* commonly assume normal, stilted, or crouched postures relative to the soil temperature (O'Neill and Rolston, unpublished data), most nymphs were positioned mainly in the normal posture. However, the smaller size of the nymphs may make their postures more difficult to see from a distance.

## Operative Body Temperatures in the Field

Data on the field behavior of the nymphs, and on the corresponding range of $T_{TH}$ that they experienced, were used to design a set of experiments in which the potential range of body temperatures were estimated in the field. Models were placed in 6 different microhabitat/posture combinations in the control treatment, and 4 or 5 different combinations in the uniform treatment. There were fewer combinations in uniform due to a lack of appropriate microhabitats for the models to be placed in; the vegetation tended to be shorter than 20 cm, and it was rarely situated on a horizontal plane 5 cm above the ground (Table 13). For all microhabitat/posture combinations, $T_E$ tended to increase linearly with $T_S$ from early morning to mid-afternoon (Table 13, Figures 20-25).

However, the temperatures of the models varied relative to the particular microhabitat/posture combination they were placed in. For all instars, the models with the highest operative temperatures were positioned on bare soil, in full sun exposure, with the longitudinal axis of the body perpendicular to incident solar radiation (Figures 20-25). Models that were placed on the soil surface and aligned with the shade of a stem had operative temperatures that were slightly lower, and models placed 5 cm high in full sun

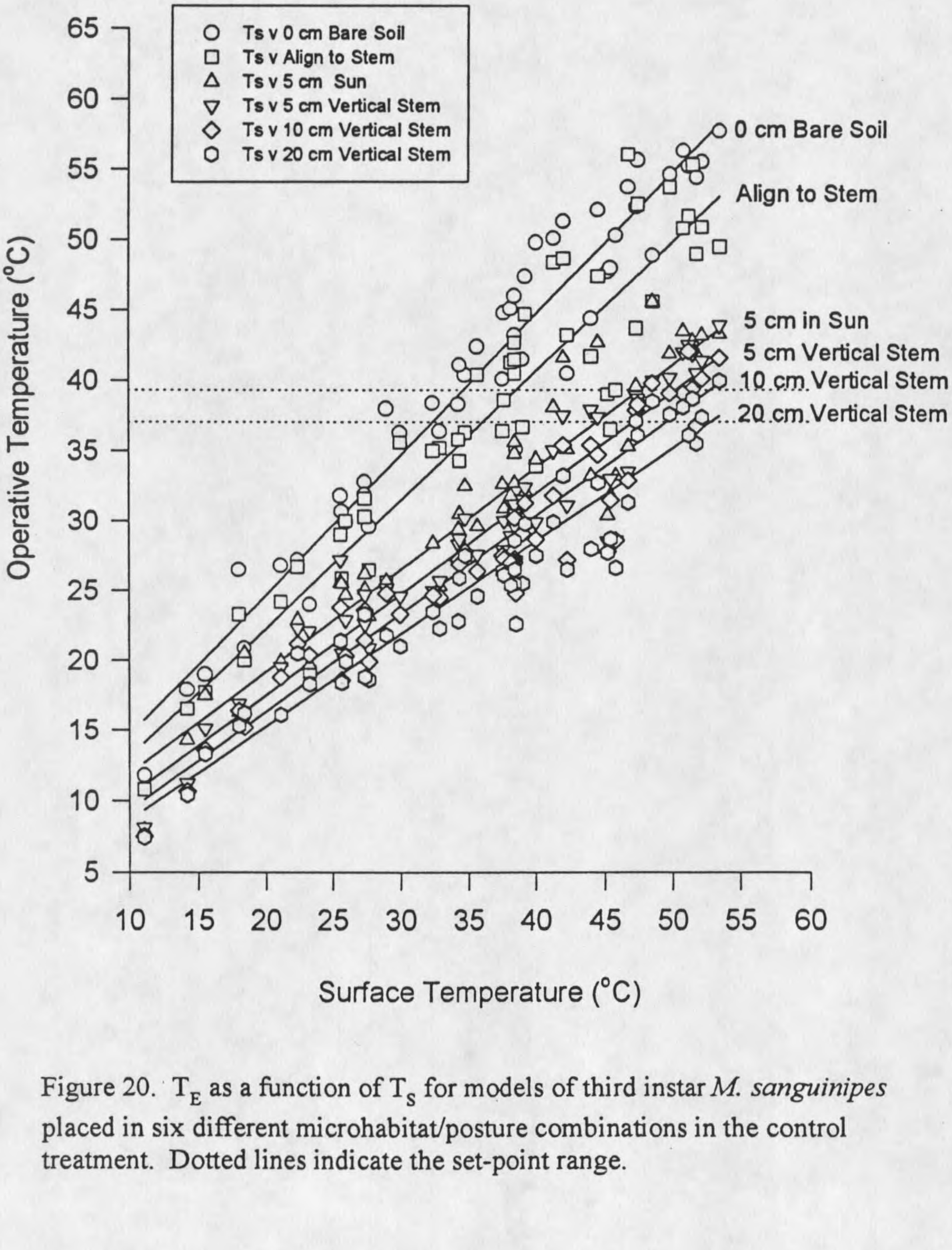

Figure 20. $T_E$ as a function of $T_S$ for models of third instar *M. sanguinipes* placed in six different microhabitat/posture combinations in the control treatment. Dotted lines indicate the set-point range.

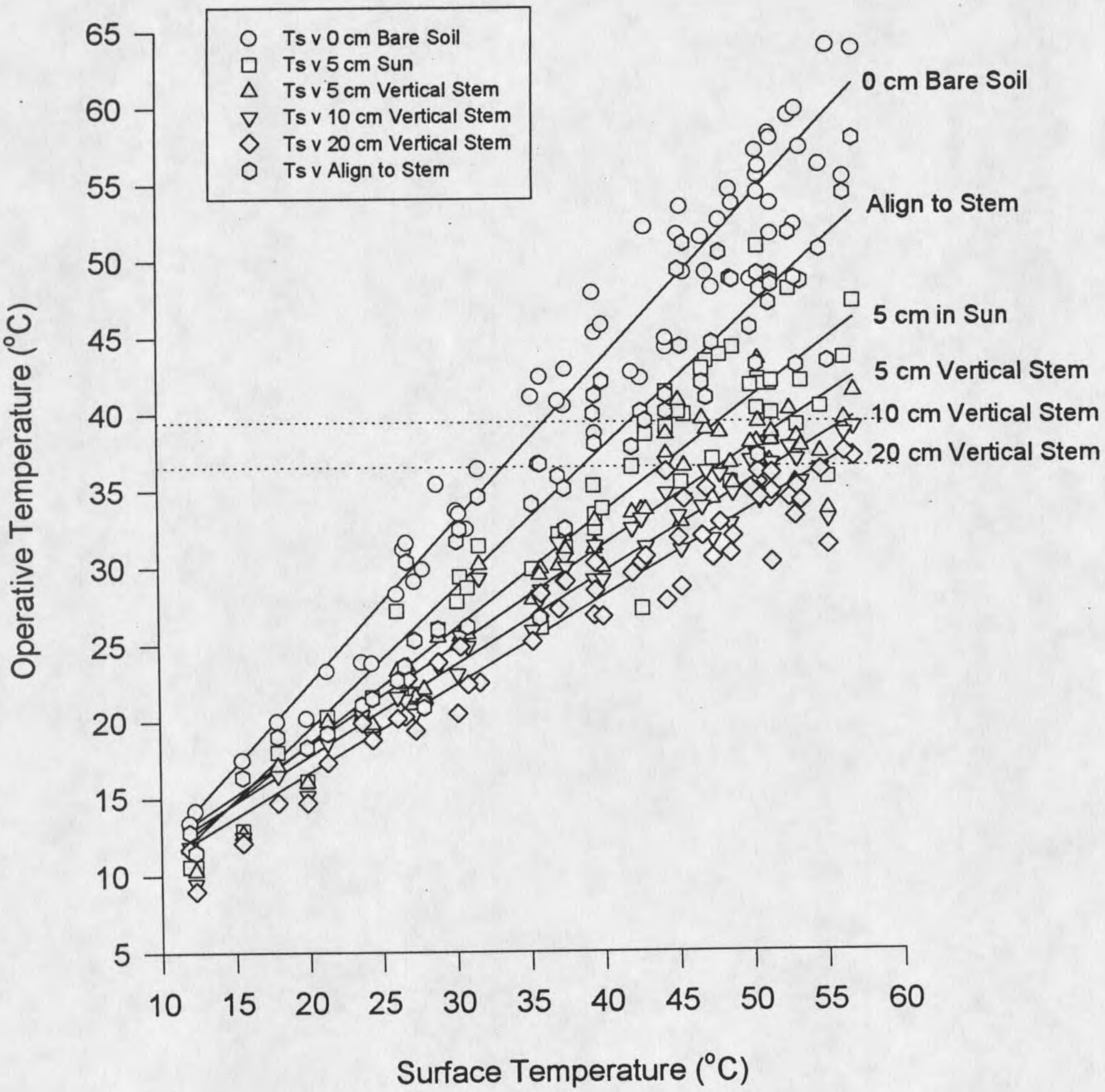

Figure 21. $T_E$ as a function of $T_S$ for models of fourth instar *M. sanguinipes* placed in six different microhabitat/posture combinations in the control treatment. Dotted lines indicate the set-point range.

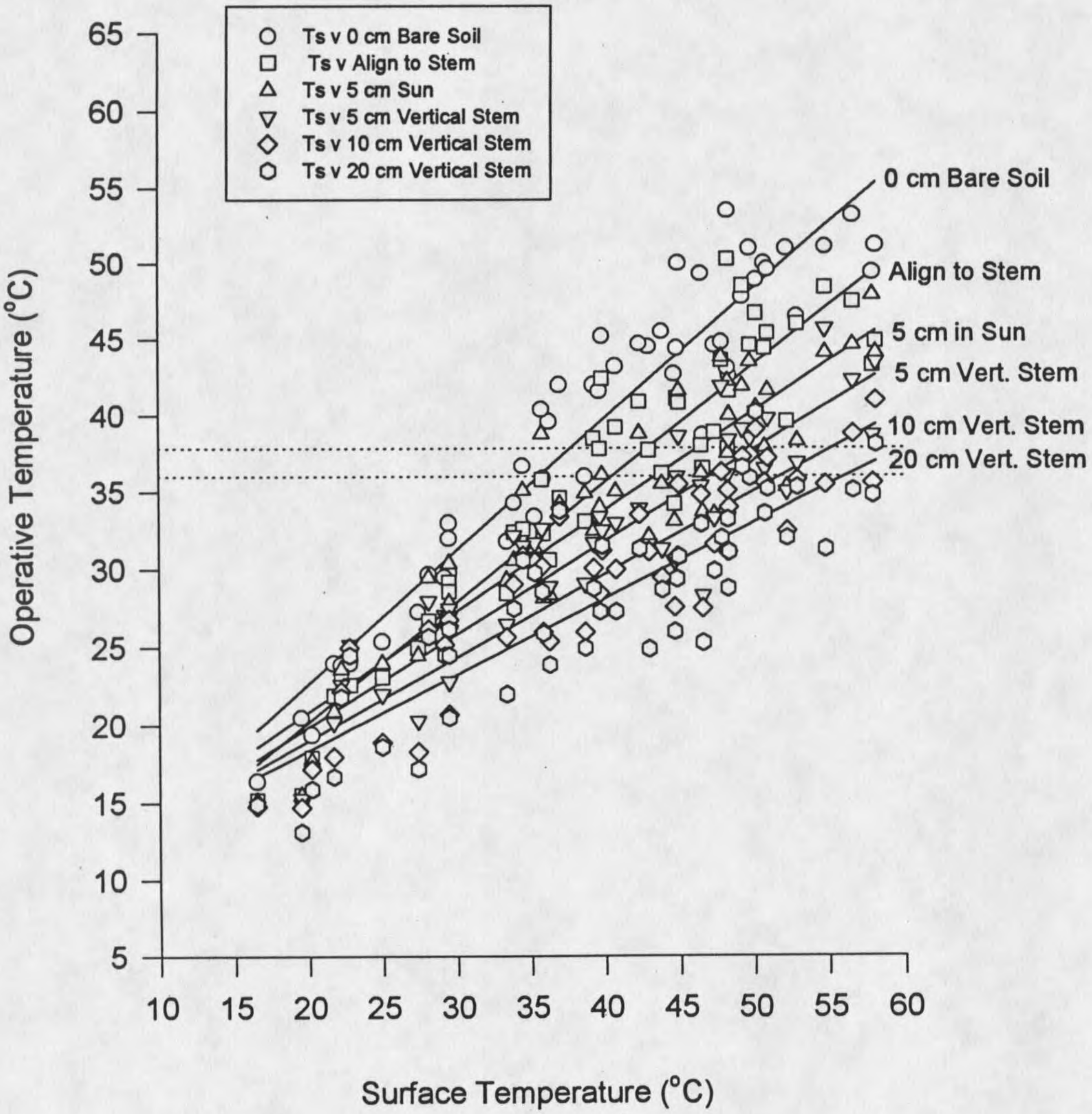

Figure 22. $T_E$ as a function of $T_S$ for models of fifth instar *M. sanguinipes* placed in six different microhabitat/posture combinations in the control treatment. Dotted lines indicate the set-point range.

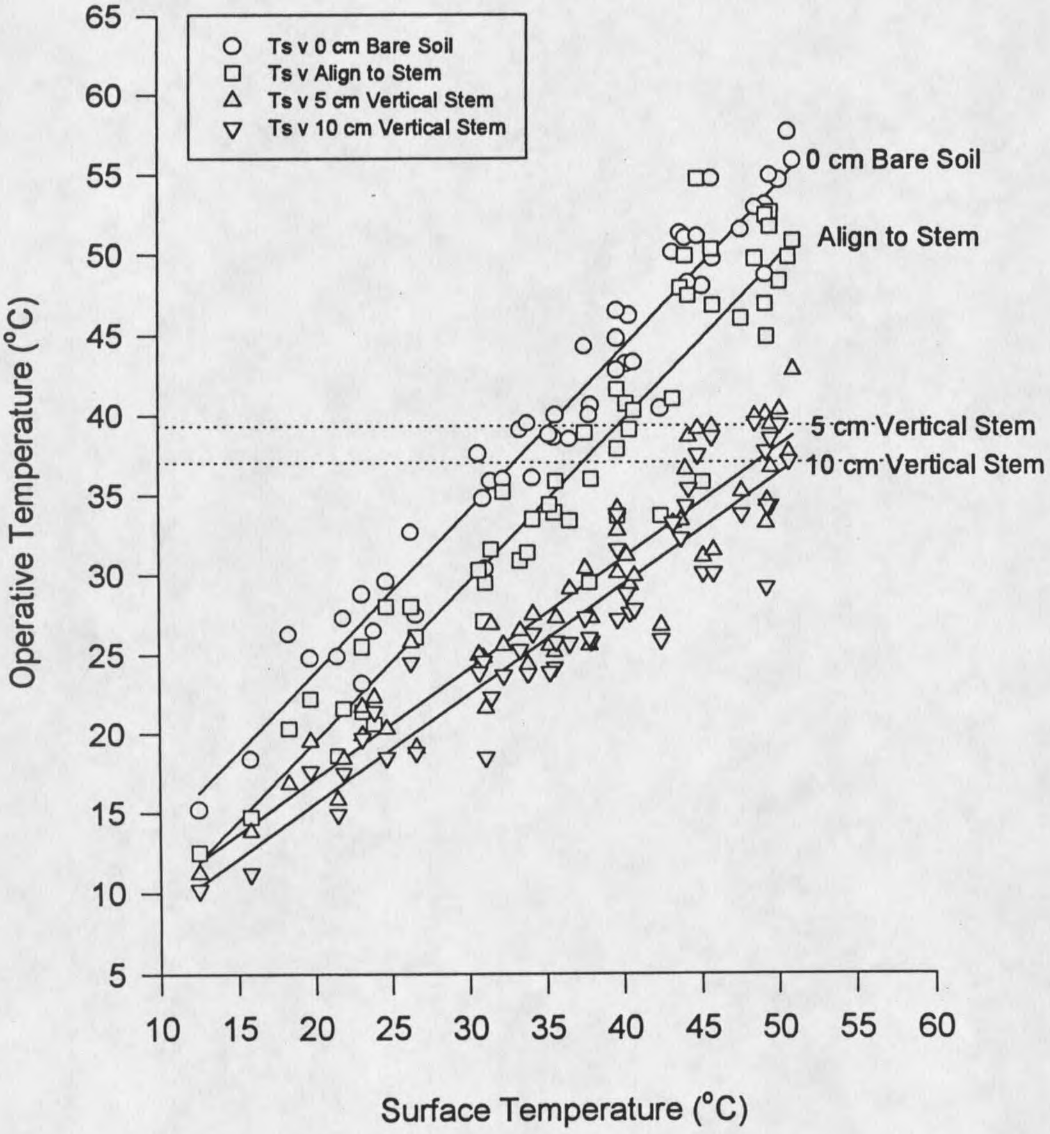

Figure 23. $T_E$ as a function of $T_S$ for models of third instar *M. sanguinipes* placed in six different microhabitat/posture combinations in the uniform treatment. Dotted lines indicate the set-point range.

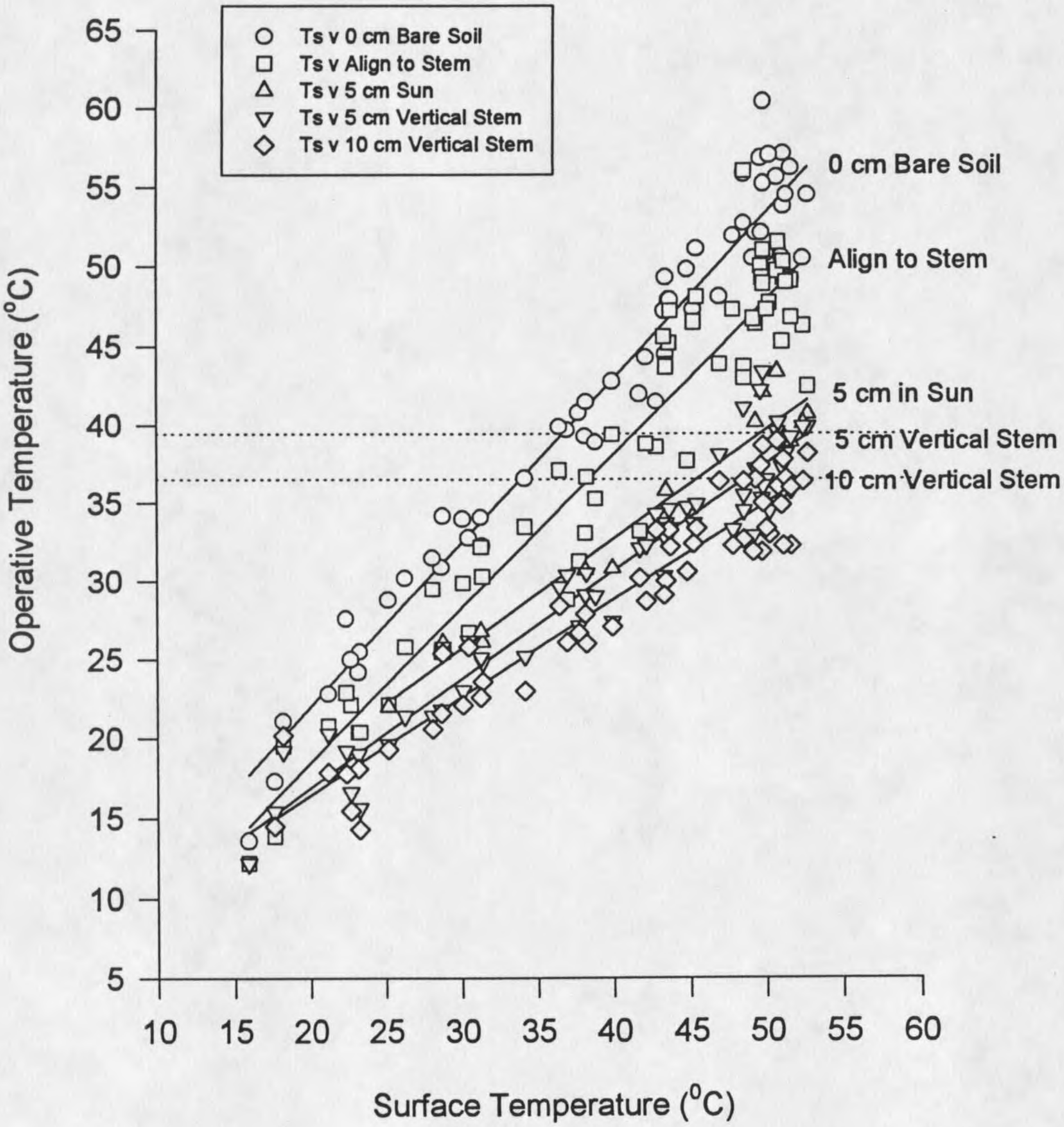

Figure 24. $T_E$ as a function of $T_S$ for models of fourth instar *M. sanguinipes* placed in six different microhabitat/posture combinations in the uniform treatment. Dotted lines indicate the set-point range.

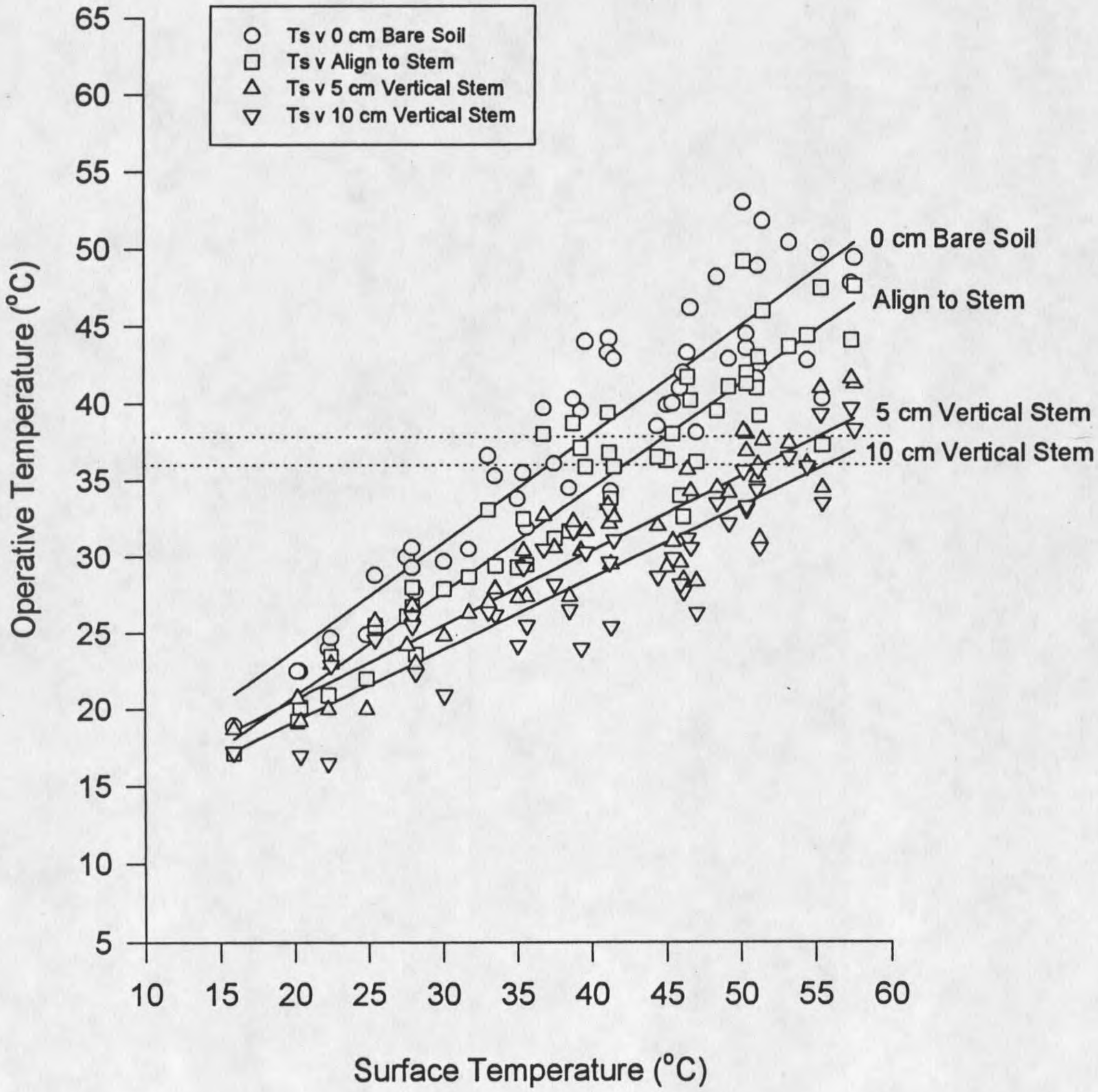

Figure 25. $T_E$ as a function of $T_S$ for models of fifth instar *M. sanguinipes* placed in six different microhabitat/posture combinations in the uniform treatment. Dotted lines indicate the set-point range.

Table 13. Coefficient of determination for the regression of operative temperature as a function of soil temperature for models of third, fourth, and fifth instars of *M. sanguinipes* placed in six different microhabitat/posture combinations within each treatment.

| Instar | Position | Control | | | Uniform | | |
|---|---|---|---|---|---|---|---|
| | | N | $r^2$ | P-value | N | $r^2$ | P-value |
| 3 | 0 cm Bare Soil | 52 | 0.94 | P<0.000 | 52 | 0.96 | P<0.000 |
| | Align to Stem | 52 | 0.87 | P<0.000 | 52 | 0.91 | P<0.000 |
| | 5 cm Horizontal | 52 | 0.90 | P<0.000 | 3 | - | P<0.000 |
| | 5 cm Vertical | 51 | 0.91 | P<0.000 | 52 | 0.89 | P<0.000 |
| | 10 cm Vertical | 52 | 0.91 | P<0.000 | 49 | 0.87 | P<0.000 |
| | 20 cm Vertical | 52 | 0.91 | P<0.000 | 3 | - | P<0.000 |
| 4 | 0 cm Bare Soil | 59 | 0.96 | P<0.000 | 59 | 0.96 | P<0.000 |
| | Align to Stem | 59 | 0.91 | P<0.000 | 59 | 0.92 | P<0.000 |
| | 5 cm Horizontal | 58 | 0.88 | P<0.000 | 14 | 0.95 | P<0.000 |
| | 5 cm Vertical | 59 | 0.92 | P<0.000 | 59 | 0.93 | P<0.000 |
| | 10 cm Vertical | 59 | 0.94 | P<0.000 | 55 | 0.91 | P<0.000 |
| | 20 cm Vertical | 59 | 0.93 | P<0.000 | 4 | - | P<0.000 |
| 5 | 0 cm Bare Soil | 50 | 0.91 | P<0.000 | 51 | 0.85 | P<0.000 |
| | Align to Stem | 50 | 0.89 | P<0.000 | 51 | 0.88 | P<0.000 |
| | 5 cm Horizontal | 50 | 0.85 | P<0.000 | 4 | - | P<0.000 |
| | 5 cm Vertical | 50 | 0.84 | P<0.000 | 51 | 0.85 | P<0.000 |
| | 10 cm Vertical | 50 | 0.82 | P<0.000 | 45 | 0.8 | P<0.000 |
| | 20 cm Vertical | 50 | 0.75 | P<0.000 | 1 | - | P<0.000 |

Missing $r^2$ value indicates a microhabitat that was not available for a model to be positioned on.

on horizontal vegetation were cooler still. Finally, models placed on vertical stems 5, 10, and 20 cm off the soil surface exhibited a trend of consecutively cooler operative temperatures (Figures 20-25). In addition, the operative temperatures of each of the combinations remained consistent among instars and between treatments.

By comparing the operative temperatures of the models in the field with the set-point ranges that were established on the gradient, I was able to estimate the range of surface temperatures within which the nymphs can maintain body temperatures. Generally, when $T_S \leq \sim 35$°C, third, fourth, and fifth instars in the field cannot achieve $T_B$s within their respective set-point ranges, and must instead attempt to minimize the difference between the two. However, at all $T_S > \sim 35$°C, body temperatures within the set-point range were available if the nymphs chose the correct microhabitat.

I predicted that a fairly large percentage of the operative temperatures that were determined in various microhabitats within the field would, at some point in the day, fall within the set point ranges that had been established on the gradient. However, only a small proportion of the operative temperatures were within the estimated set-point ranges (6%-11%), which implies that there may only be a limited number of opportunities or microhabitats available where optimal body temperatures can be attained (Table 14). This contrasts with Figures 20-25, which indicate that, when soil temperatures are between approximately 32 and 55°C, there are a large number of microhabitats available where nymphs of all three instars can maintain body temperatures within their respective set-point ranges. Additionally, in both treatments, third instars had the highest $D_E$'s (an index of thermal quality, calculated by finding the deviations between $T_E$ and the set-point range), followed by fourth instars, with fifth instars exhibiting the lowest (Table 14). Habitats with high $D_E$ are of lower thermal quality (Hertz et al 1993). This indicates that

Table 14. The percentage of operative temperatures that were below, within, or above each of the instars' set-point ranges in the six microhabitats chosen in the field, and the respective estimate of habitat quality, $D_E$.

| Treatment | Instar | N | % Below | % Within | % Above | $D_E \pm$ SE |
|---|---|---|---|---|---|---|
| Control | 3 | 311 | 0.68 | 0.07 | 0.25 | 9.27 (0.40) |
| | 4 | 353 | 0.61 | 0.11 | 0.27 | 8.00 (0.38) |
| | 5 | 300 | 0.65 | 0.06 | 0.29 | 6.93 (0.32) |
| Uniform | 3 | 211 | 0.65 | 0.08 | 0.27 | 8.85 (0.44) |
| | 4 | 250 | 0.57 | 0.10 | 0.33 | 7.74 (0.40) |
| | 5 | 203 | 0.64 | 0.06 | 0.30 | 6.25 (0.34) |

the habitat has a lower thermal quality for the third instars compared with the fourth and fifth instars, and suggests that smaller nymphs may have to spend more time or energy thermoregulating to achieve body temperatures close to or within their estimated set-point range.

# DISCUSSION

Body temperatures can be highly variable in ectotherms, and may fluctuate into dangerously high or low extremes. At the very least, non-optimal body temperatures compromise physiological processes. To counteract extreme temperatures in their environment, grasshoppers have evolved specific characteristics that help them to maintain body temperatures within preferred thermal ranges. Grasshoppers also achieve these temperatures by adjusting the location and position of their bodies within a microhabitat. Behavioral thermoregulation is a mechanism by which grasshoppers can modify their body temperatures to avoid death, digest food more efficiently (Harrison and Fewell 1995, Lactin and Johnson 1995), and, ultimately, to increase fecundity and the rate of development (Kemp 1986, Whitman 1988, Coxwell and Bock 1995). Nymphs are considerably more vulnerable to temperature extremes because of their high body surface area to volume ratio. Even though they experience smaller temperature excesses than adults overall, their body temperatures tend to increase or decrease more rapidly (Heinrich 1977, Willmer and Unwin 1981, Whitman 1987, Lactin and Johnson 1996b).

The results of this study indicate that behavioral adjustments enable small ectotherms such as third, fourth, and fifth instars of *M. sanguinipes* to maintain relatively consistent body temperatures throughout a variety of surface temperatures by shifting among microhabitats and by adopting various postures which maximize or minimize heat gain. When the set-point ranges from the gradient are superimposed onto the operative temperatures that were determined in the field, all three instars were capable of finding microhabitats within an optimal temperature range between surface temperatures 32-55°C. Furthermore, behavioral observations in the field indicate that these microhabitats and postures are commonly used by each of the instars.

## Gradient Data

The distribution of estimated body temperatures on the gradient was unimodal and nonrandom throughout the observation periods for all five instars. The estimated mean $T_B$ was consistently between 34-40°C during the second observational hour, indicating the nymphs prefer a particular range of temperatures. Similar results have been recorded from earlier studies. Using a thermal gradient, Chapman (1965) recorded the positions of second instar nymphs of *Schistocerca gregaria* (Scudder) on 30 minute intervals for five hours, and determined that the nymphs preferred temperatures between 34-45°C. In addition, when $T_B$ was estimated by observing the distance and orientation of *M. sanguinipes* nymphs relative to temperature gradients established from incandescent bulbs, Lactin and Johnson (1996a) found that nymphs tended to prefer body temperatures between 35-43 °C. They also determined that $T_B$ was not influenced by size, although they did not specify the instar of the nymphs they worked with.

In the present study, $T_B$ distributions for the smaller instars were slightly skewed to the cooler temperatures, whereas the larger nymphs tended to exhibit less variation and greater conformity in $T_B$. Perhaps this difference occurred because the bodies of younger, smaller instars were closer to the gradient, making them more prone to desiccation, and causing them to move to locations within and slightly below their set-point ranges.

As the time spent on the gradient increased, the nymphs became more mobile and exhibited a greater tendency toward randomness. There are several possible reasons for this. First, hunger may have played a role in the increased activity (Chapman 1965). Because the nymphs were not fed while they were on the gradient, the need to feed may have become more of a priority than thermoregulating precisely, so they may have moved about in search of food. Second, if food is not available, some nymphs may move to the cooler end of the gradient in an effort to conserve energy and slow down metabolic processes. Lastly, those instars that inadvertently moved to the cool end of the gradient may have lowered their body temperatures to such an extreme that they were unable to move onto warmer surface temperatures. This explanation is probably more relevant for the smaller instars, since they tend to gain and lose heat more rapidly.

I assumed that the set-point ranges generated for each instar on the gradient represented the target body temperatures that the nymphs were attempting to achieve in the field. A laboratory setting was used to collect this information because field data would be confounded with variables that would be difficult or impossible to measure, resulting in set-point ranges that do not accurately portray temperature-dependent preferences of the nymphs. For example, nymphs in the field are required not only to thermoregulate, but also to find food and avoid predators. The mean estimated body temperatures were only significantly different between instar two and instars one, three, and four. However, because the differences appeared random, and were not size-related, these results may not have any significant relevance. If this can be assumed, it would indicate that despite the considerable size differences between the nymphs, they all prefer similar temperatures on the gradient, and that differences in behavior among instars observed in the field represent distinct thermoregulatory strategies required by each size in an attempt to attain the same optimal temperatures.

## Field Behavioral Observations

### Insolation

Within the rangeland microhabitat of this study, the intensity of solar radiation varied extensively. A large amount of variation was associated with time of day, as the intensity of incident solar radiation peaked near midday. In addition, vegetation created a variety of shaded and unshaded microhabitats within the vegetation and on the soil surface (Waterhouse 1955). The ground was exposed to relatively little direct sun in the early morning and evening hours due to long shadows that were cast by the vegetation. Therefore, during these hours, more solar radiation could be intercepted higher up in the vegetation. However, as the angle of the sun's radiation became perpendicular to the ground, the shadows diminished, increasing the proportion of the microhabitat exposed to direct solar radiation.

Grazing had a tremendous impact on the intensity of solar radiation intercepted by the soil surface. The vegetation in the control area was much more dense than that in the uniform area, resulting in a greater proportion of shaded and partially shaded areas. Furthermore, since most of the ground in the control area was covered by a thick layer of matted grass deposited from previous years and shielded it from direct solar radiation, the soil was probably cooler. Likewise, a large proportion of the soil surface in the grazed treatment was completely bare and absorbed a greater amount of solar radiation.

Because sunny and shaded microhabitats were often available within the two treatment sites, nymphs could move into areas of greater or lesser solar radiation. During cool mornings, when surface temperatures were low, a large proportion of nymphs that were flushed into shaded or partly shaded regions immediately moved into microhabitats with higher levels of insolation. This behavior ensured that the amount of solar radiation absorbed could be maximized under the prevailing conditions.

Eventually, as surface temperatures increased, the nymphs tended to move into microhabitats with insolation levels lower than or equilavent to their initial level of insolation. Despite the increase in $T_S$, a large proportion of nymphs did not move in order to change the amount of solar radiation they intercepted. Shade-seeking is an important strategy used by other grasshoppers to avoid overheating (Whitman 1987), and so it seems unusual that it would not be used more frequently by this species. There are two possible explanations for this behavior. First, because the nymphs tended to move up into the vegetation, the increased wind speeds may have been sufficient in reducing $T_B$, and therefore shade was not needed. Furthermore, in the middle of the day there were relatively few microhabitats that exhibited low levels of insolation, and so the nymphs may have been unable to find more suitable areas.

Because of the coarse manner in which insolation was recorded, it was difficult to determine whether there was a difference in the amount of solar radiation exploited by the three nymphs as a function of instar or treatment. Lactin and Johnson (1996b) found that when *Melanoplus packardii* (Scudder) nymphs were illuminated in the laboratory, $T_B$ increased consistently with size, because the amount of energy intercepted increased with size. However, the rate of $T_B$ change varied with size, such that $T_B$ increased at a greater rate for smaller nymphs than for larger ones. This study suggests that larger instars will have higher body temperatures, and that smaller instars may be more susceptible to temperature extremes because they heat up much faster. Presumably, third instar nymphs in this study were more susceptible to high environmental temperatures and high levels of insolation than their older counterparts; this is implied later in the analysis on the orientation and height data.

### Orientation

Grasshopper nymphs were able to significantly increase the total amount of intercepted solar radiation by modifying the orientation of their bodies relative to the direction of the sun. Whitman (1987) determined that for the grasshopper *Taeniopoda eques* (Burmeister), orienting the long axis of the body (flanking) is the most important posture influencing heat gain. This is also true for nymphs of *M. sanguinipes* (Lactin and Johnson 1996b), for which the significance of body orientations relative to the sun was apparent in the speed at which the orientations were adjusted. In the field, the nymphs tended to adjust their body orientation rapidly in response to extreme hot or cold surface temperatures such as 10 or 50°C, whereas more moderate surface temperatures created weaker responses.. This conspicuous behavior illustrates the importance of maintaining appropriate body temperatures, and it emphasizes the significant role of body orientation for nymphs.

As the intensity of solar radiation increased throughout the day, $T_S$ also increased, resulting in significant changes in the horizontal and vertical body orientations exhibited by the nymphs. On a horizontal plane, body orientations perpendicular to the sun allowed the nymphs to absorb the greatest amount of solar radiation, thereby increasing the rate of heat gain. This behavior was usually observed in the cool mornings, and became less frequent as soil and body temperatures increased.

Although body orientations that reduced the total amount of insolation (45° and 0° relative to the sun) became more prevalent with increasing soil temperatures, they were rarely as common in warm temperatures as orientations of 90° were in cooler temperatures. This may have occurred because as $T_S$ increased, nymphs tended to move off the soil surface and up into the vegetation, where higher wind velocities may have contributed significantly to the reduction of their body temperatures, and orientations that minimized solar interception were not required. They could also move into the shade of a stem; however, only orientations in sun and partial sun were analyzed.

The correlation between $T_S$ and the horizontal angle of orientation adopted by the instars tended to be highly significant. The odds ratios from the logistic model were consistently larger for orientations of 0° versus 90°, compared with 45° versus 90°, indicating that as soil temperatures increased, there was a greater tendency for nymphs to orient parallel (0°) to solar radiation as opposed to orienting at a 45° angle. In addition, data on the frequencies of different orientations at $T_S$>50°C shows that there was a stronger tendency for third instars than for fourth and fifth instars to orient at 0°. In contrast, the logistic regression analysis of the horizontal orientations graphically predicted that nymphs of all three instars would equally orient at 0° in hot temperatures, implying that fourth and fifth instars behave similarly to third instars, and have a similar tendency to orient parallel to the sun when temperatures are high. This contradiction is probably due to the predictive rather than empirical nature of the logistic model.

Overall, horizontal orientations that increase the exposure of the body surface area to direct solar radiation may be equally important for all three instars when $T_S$ is low. However, horizontal orientations that decrease the amount of surface area exposed to solar radiation may be slightly more important for third instars than for fourth and fifth instars when $T_S$ is high. Because the orientation of the longitudinal axis of the body toward a heat source directly affects $T_B$, (Whitman 1987, Lactin and Johnson 1996a), and because smaller nymphs heat up more rapidly (Lactin and Johnson 1996b), it may be more important for third instars to orient away from solar radiation than for the larger instars.

Vertical orientations relative to the ground did not appear to be nearly as important as horizontal orientations were, possibly because on a vertical plane, there is a smaller discrepancy between the total amount of solar insolation that can be intercepted by the nymphs. Although there was a tendency for the nymphs to vertically orient parallel to the soil surface in cool temperatures, and to vertically orient perpendicular to the soil surface as $T_S$ increased, this behavior can be attributed to the type of substrate that they perched on at different soil temperatures, rather than to their responses to solar radiation on a vertical plane. Because the soil surface is the warmest part of the environment, the nymphs tended to remain on the ground when temperatures were cool, and then moved into the vegetation with higher temperatures. The small proportion of 45° vertical orientations are a testament to the smaller amount of vegetation found at this angle, especially in the uniform treatment, where there was very little matted grass.

There was a slight difference in the vertical orientations exhibited by third instar nymphs compared to fourth and fifth instars. Third instars tended to orient perpendicular to the soil surface when $T_S$ was low as well as when it was high. This can be explained by their preference for vegetation rather than the ground, regardless of $T_S$. In addition, there were noticeably more nymphs oriented vertically at high temperatures in uniform than in control. Due to a lack of shade or matted grass in this treatment, in high temperatures nymphs may have had no recourse other than to move onto the vegetation.

The effects of orientation are probably related to the size of the nymph. Even though the data in this study only suggests a difference in the horizontal orientations between third instars and their larger counterparts, there may be more discrete distinctions. On a laboratory gradient, Lactin and Johnson (1996a) found that when energy is expressed as the amount intercepted, small instars attained greater $T_B$ elevations than larger ones. This is largely because a flanking third instar exposes a greater surface area to radiation relative to the volume of the body that needs to be warmed. Therefore, small nymphs can not intercept as much energy overall, but their $T_B$ elevations increase at a faster rate.

## Height

The daily patterns of grasshopper movement within vegetation has been a well-documented behavior (Whitman 1987, Parker 1982). Typically, they begin the day perched in the tops of vegetation where early morning sun can be exploited, then move downward to the solar-heated ground as it is warmed by the sun. They then move back up the vegetation to avoid high temperature extremes in the middle of the day. However, in this study, the nymphs were flushed prior to being observed, so that any changes in microhabitat preference between the initial location and the final location could be recorded. Therefore, the nymphs were less likely to be recorded in vegetation in the early mornings when $T_S$ was low.

Nymphs of all three instars moved to similar heights throughout the day. They tended to exhibit directional movements up into the vegetation as $T_S$ increased, which was significant for most of the instars except third instars. They demonstrated an insignificant relationship between $T_S$ and final height in the uniform treatment, and the weakest relationship relative to the other instars in the control treatment. This difference probably reflects that when third instars were flushed, they tended to jump high up in the vegetation, regardless of $T_S$, whereas the older instars flushed to the ground and then walked up onto the vegetation as $T_S$ increased. With (1994) also observed this behavior in nymphs of *Opeia obscura* (Thomas). He found that nymphs leap between vegetative structures such as grass blades, whereas adults tend to walk on the ground.

Even though third instars had a significantly higher mean final height than fourth and fifth instars, all of the instars tended to orient perpendicular to the sun when $T_S$ was low. This implies that smaller nymphs do not need to utilize the conductive properties of the soil surface to gain heat, and can rely solely on radiative heat to maintain body temperatures within or near an optimal temperature range without being greatly influenced by higher wind speeds. However, convection increases proportionally with vegetation height, and the body temperatures of small insects fluctuate more with greater wind velocity (Whitman 1987). This behavior may be explained by the boundary layer. Perch surface temperatures above the ground are influenced by a thin layer of air that encompasses the substrate (Bakken 1989). Because of their small size, a large proportion of the bodies of third instars may be surrounded by this thin boundary layer, which covers the surface of vegetation and may help to protect the nymphs from the detrimental effects of high wind velocities. Likewise, the larger bodies of fourth and fifth instars probably extend outside of this protective layer, forcing them to retreat to the ground where the boundary layer is thicker and wind speeds are lower. Therefore, the boundary layer that covers the surface of vegetation may allow the small nymphs to remain on stems and grass blades without experiencing the cooling and desiccating effects of the wind.

Grazing had a peculiar effect on the heights attained by the nymphs. Mean final heights were marginally higher in the uniform treatment than in the control. Although this seems unusual, the field observations were recorded before grazing occurred in the uniform, and therefore the year's vegetation was high in both treatments. In addition, because the uniform area had no litter on the ground and fewer shaded regions, moving up into the vegetation may have been the only refuge from high soil temperatures available to nymphs in this treatment.

The change in height between the two-minute observation period was only significantly different between third and fifth instars. This difference may have occurred because third instars tended to move down from their initial perch in the vegetation into warmer temperatures, whereas fifth instars, which tended to flush to low heights, either remained at the same height, or moved up as $T_S$ increased.

## Ambient Temperature at Final Thoracic Height

The nymphs in this rangeland ecosystem were capable of exploiting a wide range of ambient temperatures. Unshaded ground absorbed a large amount of solar radiation, and because of its conductive properties, it rapidly heated up the surrounding air. Therefore, $T_A$ was warmest near the soil surface, and became progressively cooler higher up in the vegetation, due to increased wind speeds. Shaded regions of the microhabitat also had an influence on the surrounding air temperatures, although these effects were more pronounced in the morning when there were more shadows.

Ambient temperature has a direct influence on the body temperatures of small ectotherms such as grasshopper nymphs, because it affects the rate and direction of convective heat exchange between the animal's body and its environment. Kemp (1986) found that air temperature was the best predictor in determining the body temperatures of three different grasshoppers on rangeland. However, more recent sudies have questioned the appropriateness of regressing body temperature as a function of air temperature, because an insect's body temperature represents a variety of environmental and orientational factors (Lactin and Johnson 1996a) In the present study, air temperature was not recorded at a specific height above the substrate in conjunction with $T_B$, as is commonly done (Kemp 1986, Whitman 1987), but rather it was measured at places in the habitat where the nymph was located at the beginning and end of a two-minute observation period. The threshold temperature represents the initial ambient temperature at which the nymphs' final ambient temperature leveled off and remained within a relatively constant range.

There was a nonlinear relationship between air temperature at the initial thoracic height (initial $T_{TH}$), and air temperature at the final thoracic height (final $T_{TH}$) for all three nymphs. This relationship was only slightly greater than a 1:1 ratio until the initial $T_{TH}$ increased to temperatures that were closer to or above the threshold. This implies that ambient temperatures of final microhabitats were only slightly higher than those of the initial microhabitats until initial $T_{TH}$ was approximately 32°C. However, when nymphs were flushed into microhabitats where initial $T_{TH}$ was above the threshold, they actively moved into areas where the ambient temperatures were cooler, creating a line with a slope near zero.

These data indicate that when initial $T_{TH}$ was below the threshold, the nymphs were not thermoregulating as effectively as when initial $T_{TH}$ was above the threshold. This may occur because the range of ambient temperatures that the nymphs could exploit in the morning was narrow compared with the range in the afternoon. Early in the day, the soil surface was predominately shaded, making it cool due to limited sun exposure. Therefore, when $T_S$ was low, the nymphs could not find microhabitats with final ambient temperatures that were significantly warmer than the initial ambient temperatures. Although it appears that the nymphs were not thermoregulating, they actually were, because they moved to microhabitats where they could exploit the warmest ambient temperatures possible in their limited thermal environment. Even though the nymphs were not able to find suitable ambient temperatures when $T_S$ was low, they could still increase $T_B$ by moving into microhabitats with low levels of convection, and high levels of insolation where they could orient their bodies perpendicular to solar radiation.

After initial $T_{TH}$ became warmer than the threshold temperatures, the nymphs exhibited thermoregulatory behaviors by moving into microhabitats with cooler ambient temperatures. There was also more of a spread in the range of ambient temperatures that the nymphs could use as the day progressed and surface temperatures warmed up. Most of the nymphs tended to move from microhabitats with air temperatures between 33-58°C into microhabitats where the air temperatures were consistently close to 32-34°C, showing that the nymphs preferred a fairly narrow range of ambient temperatures, and tended to exploit this range once they were capable of reaching it. Therefore, the nymphs were capable of finding ambient temperatures which allowed them to maintain body temperatures close to or within their set-point ranges.

There was no clear reason why the mean final $T_{TH}$ between fourth and fifth instars was significantly different. This difference is not attributable to body size, because there was no significant difference between third and fifth instars. Likewise, it seems counterintuitive that the nymphs experienced significantly higher mean final $T_{TH}$'s in the control treatment compared with the uniform treatment, because the soil in uniform had greater exposure to solar radiation, and should have been warmer. However, Waterhouse (1955) found that in habitats where the vegetation growth is not very dense, the warm boundary layer of air is nearer to the ground due to higher wind speeds. This may explain the cooler temperatures in uniform. In addition, this difference may also be caused by the presence of an outlier in fifth instars in control.

## Distance Traveled

If third instar nymphs were required to travel the same distance as fifth instar nymphs to reach a suitable microhabitat, it can be suggested that, relative to body size, the smaller instars were moving farther. Therefore, small instars should travel the shortest total distance. Accordingly, Smith and Grodowitch (1987) and With (1994) found that adult grasshoppers move farther than nymphs.

Distance traveled provided an estimate of how much effort the nymphs expended before they found a suitable location. In some cases, the nymphs traveled far from their initial location on long, winding paths only to end up in an area near where they started. The total distance and the linear distance traveled increased with the age of the instar, implying that size has an influential role. However, this interpretation is confounded because different instars were observed at different times in the season. The height of the vegetation, and therefore the distances that were traveled, increased throughout the summer with older instars.

## Operative Body Temperatures in the Field

For small ectotherms such as grasshopper nymphs, operative temperatures can be considered valid estimations of body temperatures. The heat production of small nonflying animals such as nymphs is negligible (Bakken 1992), and can therefore be approximated by models in the field (Lactin and Johnson 1996b). Furthermore, because they have the same morphological characteristics as live nymphs, the $T_E$ of these models are influenced by, and respond similarly to, the same environmental variables that live nymphs experience. In this study, the various microhabitat/posture combinations of the models were chosen to represent the full range of common microhabitats and postures assumed by the nymphs of *M. sanguinipes*. Therefore, the models provided valid estimations of the potential range of body temperatures that could be experienced within the study site by nymphs (Hertz et al. 1993).

A comparison between field operative temperatures and the set-point ranges established on the gradient shows that nymphs of all three instars, and within both treatments, were capable of achieving body temperatures within their respective set-point ranges after $T_S$ reached approximately 30-35°C if they moved into the appropriate habitat. Figures 20-25 depict the body temperatures that nonthermoregulating nymphs would experience in each of the six microhabitat/posture combinations throughout a range of surface temperatures between 10-60°C. These figures also provide legitimate estimates of the particular combinations that would allow a nymph to remain within its set-point range at specific soil temperatures. The results predict that a perfectly thermoregulating nymph would remain on bare, sun-exposed soil with its body oriented perpendicular to solar radiation until $T_S$ was approximately 32°C, after which it would change strategies and either align to the shade of a stem or move onto a horizontally positioned stem positioned ~5 cm off the ground in full sun. After $T_S$ approached 40°C, the nymph would then move vertically up the vegetation, with the height of the nymph proportional to the increase in $T_S$.

Because there are probably a number of microhabitats on rangeland with thermal regimes similar to the six combinations that the $T_E$ models were placed in, the nymphs could have chosen a wide range of microhabitats, and a variety of body positions to maintain body temperatures close to or within their set-point range. However, the behavioral observations in this study describe strategies similar to the perfectly thermoregulating nymph, with slight variations according to instar. This indicates that the nymphs were thermoregulating, because they responded to temperature extremes in their environment by moving into more suitable thermal microhabitats, and by modifying their body positions in ways which allowed them to achieve more optimal body temperatures.

Despite differences in behavior among instars observed in the field, the models all exhibited similar operative temperatures relative to each of the microhabitat/posture combinations. Models placed on bare, sun-exposed soil consistently had the highest operative temperatures, whereas models placed 20 cm up on a vertical stem consistently had the lowest, and the remaining combinations were always in the same temperature categories. Overall, these results agree with the behavior of fourth and fifth instars, but they do not agree with observations of third instars that jump into the vegetation at low soil temperatures. Instead, the $T_E$ models show that third instars would be able to achieve higher body temperatures on the soil surface, rather than up in the vegetation. This implies that the smaller nymphs may be responding to environmental factors other than the thermal quality of their habitat.

Even though one or two of the microhabitat/posture combinations could not be used in uniform (i.e. when the models were 5 cm off the ground on horizontal vegetation, or 20 off the ground on vertical vegetation) there appeared to be no large differences in the operative temperatures between each treatment. Although it is highly probable that the nymphs will find microhabitat/posture combinations other than the six that were used in this study to achieve preferred body temperatures, this reduction in available optimal habitats demonstrates the detrimental effect that grazing may have on grasshoppers (Quinn and Walgenbach 1990, van Wingerden et al. 1991, Welch et al. 1991).

The estimates of habitat quality ($D_E$) determined that only 6-11% of the model operative temperatures were within the set-point ranges, implying that the six different microhabitat/posture combinations would provide only limited opportunities for the nymphs to achieve optimal body temperatures. However, this contrasts strongly with data which indicates that optimal body temperatures can be attained between a wide range of soil temperatures. There is also no apparent difference in the estimated habitat quality between grazing treatments. There was a tendency for habitat quality to decrease with smaller instars, implying that third instars were required to spend more time and energy thermoregulating to achieve optimal body temperatures than their larger counterparts. Because insects with higher surface area to volume ratios are more susceptible to temperature changes in their environment (Heinrich 1977, Willmer and Unwin 1981, Whitman 1987), smaller nymphs may have to modify their behavior more to maintain body temperatures within their estimated set-point range. However, the behavioral observations in the field provided no evidence in support of this.

## Summary

This study illustrates that the behaviors of nymphs of *M. sanguinipes* help them achieve and maintain body temperatures close to or within their set-point range, relative to nonthermoregulating nymphs. The operative temperatures and estimated set-point ranges provided an estimate of the body temperatures that the nymphs could achieve in particular microhabitats, and the surface temperatures at which they could maintain preferred body temperatures. Although there were some behavioral differences among the instars, they tended to exploit similar thermal environments. They tended to move into microhabitats and assume body positions whereby they could increase $T_B$ as rapidly as possible in cool environments, and to maintain $T_B$ close to their estimated set-point ranges in hotter environments. Also, despite the structural differences between the two treatments, the nymphs' behavior was similar in both uniform and control, suggesting that they are capable of finding suitable thermal habitats in a variety of habitats.

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# APPENDIX

Table 15. Diagnostic $\Delta\chi^2$ and $\Delta$Deviance for outliars of each of the three instars, and within both treatments. m is the total number of grasshoppers oriented at the specific temperature.

| Treatment | Instar | Angle of Orientation | N | Temperature (°C) | m | Estimated Probability | $\Delta\chi^2$ | $\Delta$Deviance |
|---|---|---|---|---|---|---|---|---|
| Control | III | 0° vs 90° | 56 | 15.7 | 1 | 0.0860 | 11.0981 | 5.3759 |
| | | | | 20.1 | 1 | 0.1213 | 7.5605 | 4.5367 |
| | | | | 22.0 | 1 | 0.1401 | 6.4000 | 4.1938 |
| | | | | 58.5 | 1 | 0.7971 | 4.1460 | 3.4075 |
| | | 45° vs 90° | 55 | - | - | - | - | - |
| | IV | 0° vs 90° | 89 | 18.2 | 1 | 0.1504 | 5.8428 | 3.9835 |
| | | 45° vs 90° | 89 | - | - | - | - | - |
| | V | 0° vs 90° | 132 | 19.9 | 1 | 0.0731 | 12.8950 | 5.4420 |
| | | | | 24.8 | 1 | 0.1239 | 7.1952 | 4.2986 |
| | | | | 53.3 | 1 | 0.8088 | 4.3139 | 3.3929 |
| | | | | 53.9 | 1 | 0.1896 | 4.6352 | 3.5171 |
| | | | | 54.2 | 1 | 0.8248 | 4.8047 | 3.5798 |
| | | | | 57.2 | 1 | 0.8707 | 6.8755 | 4.2328 |
| | | | | 58.0 | 1 | 0.8811 | 7.5635 | 4.4139 |
| | | 45° vs 90° | 131 | 19.6 | 1 | 0.1777 | 4.7204 | 3.5493 |

Table 15, continued.

| Treatment | Instar | Angle of Orientation | N | Temperature (°C) | m | Estimated Probability | $\Delta\chi^2$ | ΔDeviance |
|---|---|---|---|---|---|---|---|---|
| Uniform | III | 0° vs 90° | 40 | 25.3 | 1 | 0.0103 | 98.5438 | 11.5283 |
| | | | | 50.7 | 1 | 0.1504 | 5.8428 | 3.5636 |
| | | 45° vs 90° | 34 | 19.9 | 1 | 0.1557 | 5.9228 | 4.2204 |
| | | | | 25.0 | 1 | 0.2001 | 4.2628 | 3.4826 |
| | IV | 0° vs 90° | 70 | 29.5 | 1 | 0.1569 | 5.5262 | 3.8537 |
| | | | | 52.1 | 2 | 0.8269 | 10.4533 | 7.9128 |
| | | | | 52.3 | 1 | 0.8310 | 5.1388 | 3.7770 |
| | | 45° vs 90° | 67 | 17.2 | 1 | 0.1915 | 4.4805 | 3.5636 |
| | V | 0° vs 90° | 27 | - | - | - | - | - |
| | | 45° vs 90° | 29 | - | - | - | - | - |

Rows with no values indicate that there were no outliars for the orientation for that particular instar.