Sleep Site Selection in Three Species of *Norops* Lizards (Squamata: Polychrotidae) in the San Luis Valley, Puntarenas, Costa Rica

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ABSTRACT

The sleeping patterns of animals are important factors in fully understanding their behavior. I studied the sleeping site selection of three species of *Norops* lizards. I caught the lizards during the day, applied fluorescent powder, and tracked them to their eventual sleeping location at night using UV light. I measured multiple variables, including weather conditions, height, and level of protection provided by the site. Mean night heights of males and females, as well as among species, proved significantly different, thus maintaining daytime stratification. However, males and females were distributed opposite of that seen during the day, with females sleeping higher than males. Additionally, there were significant differences in protection preference across sex and species divisions, as only males and one species showed no preference for protected sites. Weather effects, such as precipitation and temperature, failed to show significance. Therefore, it appears that sex and species differences are the strongest factors driving sleeping site selection. Males seem driven more by sleeping site height, while females demonstrated greater desire for protection. Overall, the lizards were found to have a preference for protected sites.

RESUMEN

Para comprender todo sobre los acciones de un animal, es importante saber como duerme el animal. Yo estudié la selección de perchas para dormir en tres especies de *Norops*. Los atrapaba durante el día, les ponía polvo florescente, y los seguía por la noche con un foco de luz ultravioleta. Estudié algunos factores, incluyendo el tiempo, la altitud de percha, y el nivel de protección dado por la percha. Comparaciones de los promedios de los altitudes de los machos y las hembras y también entre las tres especies mostraron diferencias. Sin embargo, machos y hembras se distribuyeron diferente de lo que se ve durante el día. Las hembras duermen más arriba que los machos. También, habían diferencias en las preferencias por perchas con más proteccion entre los sexos y las especies; solo los machos y una especie no preferían perchas protegidas. Los efectos del tiempo no eran significantes. Por eso, parece que diferencias entre los sexos y especies son los factores más importantes para escoger perchas nocturnas. A los machos, les importa más la altitud de la percha, pero las hembras necesitan perchas protegidas. En general todos prefieren perchas con más protección.

INTRODUCTION

The behavior of sleep is a very important aspect of the natural history of an animal both because it takes up a significant amount of their time and because they are left extremely vulnerable as a result (Singhal *et al.* 2007). In order to fully understand the behavior and natural history of an organism, their sleeping patterns must be taken into account. The sleeping behavior of lizards, along with that of many other taxa, has been poorly documented. Though the concept of sleeping site selection by lizards has not been studied in great detail, there are a few theories concerning the motivations behind their selection patterns.

Some believe that predator avoidance is a determining factor in sleeping site selection, while others report that many factors such as abundance of certain plants, thermoregulation, or species differences are more important (Christian *et al.* 1984; Clark & Gillingham 1990; J. A. Pounds, pers. comm.). Christian *et al.* (1984) found that ambient temperature had a significant effect on the sleeping site selection of adult Galapagos land iguana (*Conolophus pallidus*), while juveniles selected sites based on greater protection. Clark & Gillingham (1990) noted that *Anolis* lizards were most often found sleeping on the most common understory plant in their study site, suggesting selection simply based on vegetation abundance. Finally, in an unpublished study of *Norops* sleeping site preference, Alan Pounds (pers. comm.) noticed strong trends corresponding to species differences.

Lizards from the genus *Norops* are small to medium size, primarily arboreal lizards, and are widespread throughout the Neotropics (Savage 2002). Past studies have demonstrated daytime perch height stratification across both sex and species differences in *Norops* lizards (Andrews 1983; Rummel & Roughgarden 1985). This stratification has been interpreted as resulting from inter-species competition for resources as well as from intra-species dominance hierarchies, with dominant males perching higher than other individuals. Females are typically found on lower perches. It is also known that male *Norops* go to lower perch heights to hunt and forage during the day in order to avoid competitive encounters with other males (Andrews 1983).

At night, they are often observed sleeping on leaf surfaces of understory forest plants, presumably in hopes that they would be awakened by the approach of a predator climbing up from below. Though this strategy appears common, few studies have investigated if significant numbers of *Norops* also sleep in other locations, or what factors are driving their sleeping site choices (Singhal *et al.* 2007, Pérez 2008). This study aims to determine the particular factors leading to sleeping site selection in three species of *Norops* from the San Luis Valley in Puntarenas, Costa Rica. The goal was to determine the strength of each variable in relation to its influence on sleeping site selection, in order to discover which factors drive the sleeping site selection of *Norops* lizards.

MATERIALS AND METHODS

Study Site

My study site consisted of a large area of dairy farm pasture and adjacent roadside habitat in the San Luis Valley, Puntarenas, Costa Rica, within the Premontane Wet Forest Holdridge Life Zone. The habitats consisted of pasture lands, with fence posts, small trees, and long grass, as well as roadside plant communities, which were mainly composed of rocks, small shrubs, and trees. The different sites provided a diverse array of vegetation, creating many possible sleeping sites.

Study Organisms

Norops biporcatus, *N. cupreus*, and *N. intermedius* are the three most abundant species in the study area, and were the object of this study. *N. biporcatus* is a large anole species (Mean SVL = 105.25 ± 5.25 mm) found primarily in and around trees. *N. intermedius* is a small to medium size species (Mean SVL = 43.38 ± 1.06 mm) commonly found in pasture habitat both on tree trunks and posts. *N. cupreus*, the most prevalent species in my study area ($N_{cupreus}$ = 43, N_{total} = 56; 77%), is also a small to medium size anole (Mean SVL = 40 ± 2.82 mm) that is common both on posts in pasture habitat and on rocks and sticks along the roadside.

This study was completed by observing these lizards in their natural habitat and measuring a wide range of variables. These variables were the following: type of sleeping site (e.g. on top of a leaf, under wood), height of perch, weather conditions (e.g. temperature, precipitation), level of protection provided by the sleeping site, and the size, sex, and species of each individual.

Daytime

Every day I did visual encounter surveys around pasture areas and along the road. For each *Norops* I caught, I recorded its snout-vent length (SVL), determined its sex and species, and described its perch site (e.g. tree trunk). I then drew two colored dots with Sharpie® paint pens on the dorsal surface, just in front of the hind leg joint. I drew one dot on each side of the spine, with each color corresponding to a specific number (Johnson 2005; M. A. Johnson, pers. comm.). This was done in order to identify individuals when found at night and to avoid recaptures. I placed the individual into a plastic bag, to allow time for the paint marks to dry and to minimize handling time. Next, I measured the height to the ground directly below the perch. I also marked the capture location with flagging tape, labeled with the individual's ID number, in order to find the location when returning at night. I then removed the individual from the bag, marked it with florescent tracking powder (procedure described below), and released it at the point of capture.

Nighttime

I returned at night to the capture sites in order to track each lizard and find its sleeping site. Using an ultraviolet flashlight, I followed the fluorescent powder trail of the lizard to its eventual sleeping site. Upon locating the individual, I described the type of site (e.g. tree branch) and measured the height as during the day. I also measured the ambient temperature, and described the amount of precipitation as either rain, mist, or none. Finally, I ranked the sleeping site as either Protected or Unprotected in relation to how easily a predator would be able to detect and access the individual. These lizards have a multitude of potential predators capable of attacking from above or below, such as snakes and birds. Therefore, it was necessary to take into account various avenues of attack and detection. Sleeping on a leaf within the canopy of a tree would therefore be considered protected due to the surrounding leaves, while sleeping on a leaf on a shrub would not. Though each might notify the lizard of a predator's approach from below, exposure to predation from above is greater for the lizard on the shrub.

Florescent Powder Application

Fluorescent powder pigments have increased in popularity in recent years and have been used for tracking the movements of many small mammals, amphibians, and reptiles (Mullican 1988; Fellers & Drost 1989; Kearney 2002; Birchfield & Deters 2005). It has been shown by numerous studies that the powder has no negative effects on the study individuals, even amphibians, which have very delicate skin that is important for respiration (Rittenhouse *et al.* 2006; Orlofske *et al.* 2009). The powder used in this study was purchased from the Radiant Color Company (Richmond, CA), from the RADGLO® JST Series; the two colors used were (JST-10 and JST-12).

After frustrated attempts to implement the methodologies described in numerous publications (Fellers & Drost 1991; Lindquist *et al.* 2007; Roe & Grayson 2008), I mixed alcohol-based hand sanitizer gel with the powder to create a paste. I applied the mixture with a small paintbrush over all ventral surfaces, including hands, feet, and arms. The gel evaporates quickly, leaving behind just the powder, and making application faster, easier, and more effective at covering all the ventral surfaces of the individual. This new method led to much greater tracking success due to the more consistent, longer-lasting trails left by the study individuals. It also provides benefits for the study animals; it ensures that no powder is blown into the eyes, nose, or mouth, and that none is placed on the dorsal surface, thereby not increasing their visibility to predators. Though there is a question of whether the hand sanitizer might harm the animals, each individual found at night exhibited no negative effects and appeared in prime condition. Due to their protective scales and the quick evaporation of the alcohol, it is probable that there would be no effect. However, this method would likely be hazardous to animals such as amphibians, and is not recommended.

Statistics
In order to determine if individuals slept above or below their daytime perch height, I subtracted the day perch height from the night perch height of a given individual (H_N-H_D). Any vales greater than one signified sleeping above (H_N-$H_D > 0$), and any values less than one meant the individual slept below (H_N-$H_D < 0$). I then analyzed the percentages of each sex and species sleeping above or below their day height and compared each to a random distribution using Chi-Square analysis. Here, the term random distribution refers to a 50/50 split that would be expected if the individuals had no preference in relation to the variable being analyzed.

RESULTS

Sleeping site selection seemed to be related mainly to two major factors: height of perch and level of protection provided by the site. For this reason, I will explain how variables of size, sex, and species relate to these factors.

Sleeping Site Height
Individual SVL was significantly related to night height, with a moderate positive correlation (Fig. 1). Larger lizards prefer to sleep higher up than their smaller counterparts. Between the sexes, males and females displayed significantly different mean night height values (97.44 ± 38.73 cm and 244.24 ± 280.33 cm, respectively) with females tending to sleep higher than males (Fig. 2). Among the three species, *N. cupreus* was found to sleep significantly lower (Mean = 44.61 ± 38.68 cm) than the other two species (Fig. 3; Tukey-Kramer, F = 23.0, p < 0.0001, DF = 2,43).

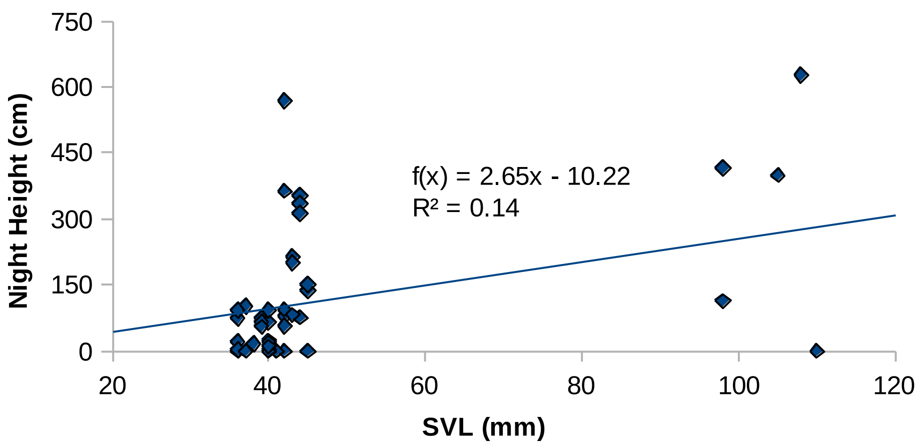

FIGURE 1. Snout-vent length of *Norops spp.* compared to sleeping site heights in the San Luis Valley, Puntarenas, Costa Rica. The regression is positively significant (F = 6.84, p = 0.012, N = 44).

In terms of sex, males and females displayed no difference in choosing to sleep above or below their day height (Fig. 4). However, when males were compared to a random distribution (i.e. 50% above, 50% below) they showed a significant difference, implying that they prefer to sleep below their day perch height ($\chi^2 = 7.84$, p = 0.0051, DF = 1). *N. cupreus* showed a significant difference from the other two species in sleeping below its day height (Fig. 5). Additionally, all three species individually displayed differences from a random distribution, suggesting that they each have a preference for either sleeping above or below their day height (*N. biporcatus*: $\chi^2 = 11.6$, p = 0.0007, DF = 1; *N. cupreus*: χ^2 =

31.4, $p < 0.0001$, DF = 1; *N. intermedius*: $\chi^2 = 57.8$, $p < 0.0001$, DF = 1).

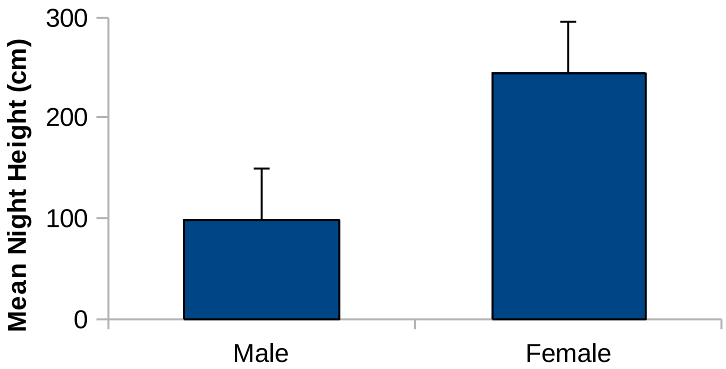

FIGURE 2. Mean night height comparison between male and female *Norops spp.* from the San Luis Valley, Puntarenas, Costa Rica. Male and female means were determined to be significantly different ($t = 2.46$, $p = 0.018$, DF = 44). Male N = 39, female N = 7.

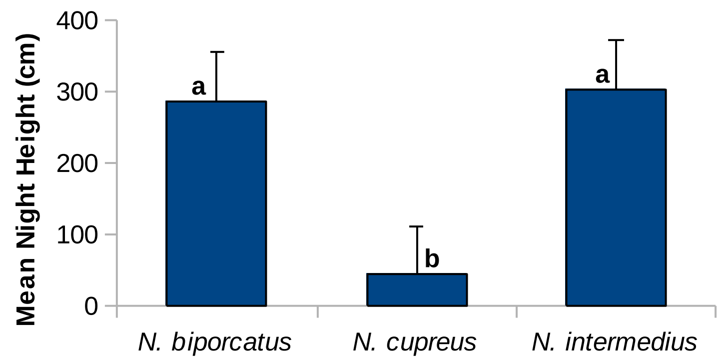

FIGURE 3. Mean night height comparison between *Norops* species from the San Luis Valley, Puntarenas, Costa Rica. Bars with different letters determined to be significantly different ($p < 0.05$). $N_{bip} = 6$, $N_{cup} = 32$, $N_{int} = 8$.

Protection Preference

Males differed from females in protection preference (Fig. 6). Females were never found sleeping in unprotected sites, yet males did not show a preference for protected sleeping sites ($\chi^2 = 1.0$, $p = 0.32$, DF = 1). The three species also showed differences in their preference for protected sites, and *N. biporcatus* was never found in an unprotected site (Fig. 7). *N. cupreus* was the only species that did not demonstrate a preference for protected sleeping sites ($\chi^2 = 0.040$, $p = 0.84$, DF = 1); *N. intermedius* strongly preferred protected sites ($\chi^2 = 57.8$, $p < 0.0001$, DF = 1). Finally I compared the total percentage of protected sites to a random distribution. The observed results were significantly different than what would be expected at random, which suggests that *Norops* prefer protected sleeping sites ($\chi^2 = 3.92$, $p = 0.048$, DF = 1).

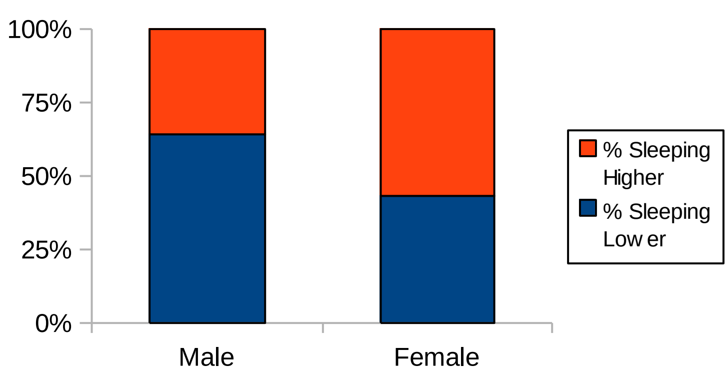

FIGURE 4. Percentage bars comparing the percent of individuals sleeping above or below their daytime perch height between male and female *Norops spp.* from the San Luis Valley, Puntarenas, Costa Rica. No differences were shown between males and females ($\chi^2 = 2.26$, $p = 0.13$, DF = 1). However, males were shown to significantly differ

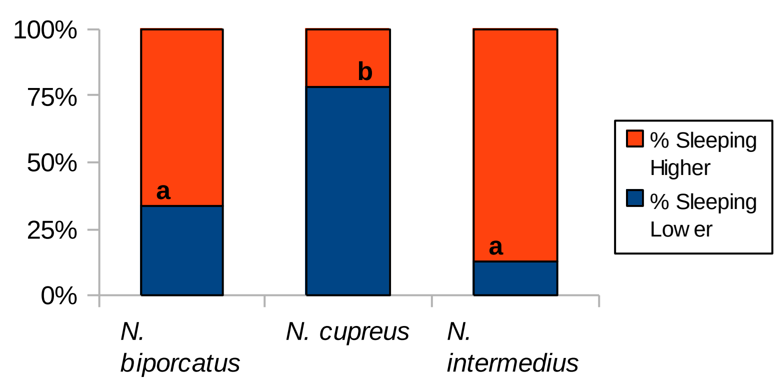

FIGURE 5. Percentage bars comparing the percent of individuals sleeping above or below their daytime perch height among *Norops* species from the San Luis Valley, Puntarenas, Costa Rica. Differences were shown between bars with different letters ($\chi^2 = 21.5$, $p < 0.0001$, DF = 2). All species were also shown to significantly differ from a

(p < 0.05) from a random distribution. Male N = 39, female N = 7.

random distribution ($p < 0.05$). $N_{bip} = 6$, $N_{cup} = 32$, $N_{int} = 8$.

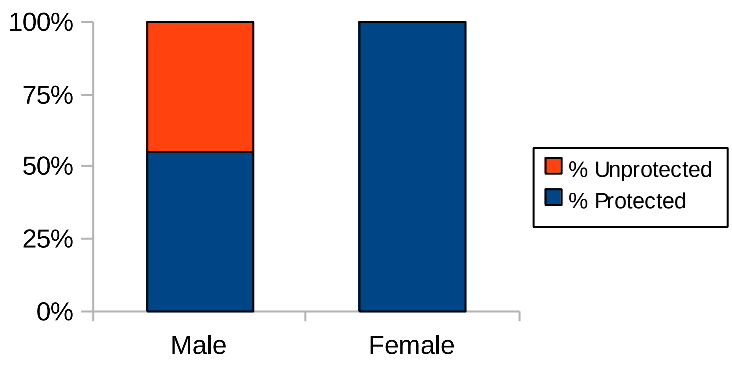

FIGURE 6. Percentage bars comparing the percent of individuals sleeping in protected or unprotected sites between male and female *Norops spp.* from the San Luis Valley, Puntarenas, Costa Rica. Males and females were shown to have different preferences for protected sites ($\chi^2 = 6.17$, $p = 0.013$, DF = 1). Females displayed non-random preference for protected sites ($p < 0.05$). Male N = 40, female N = 10.

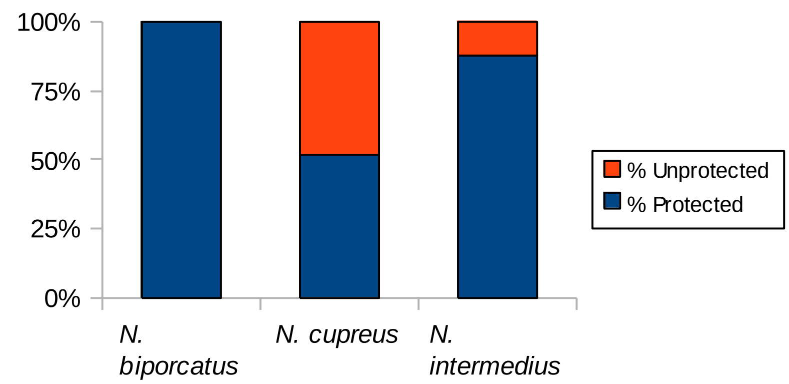

FIGURE 7. Percentage bars comparing the percent of individuals sleeping in protected or unprotected sites among *Norops* species from the San Luis Valley, Puntarenas, Costa Rica. Significant differences were shown between all three species ($\chi^2 = 8.26$, $p = 0.016$, DF = 1). Only *N. cupreus* failed to demonstrate a significant preference for protection against a random distribution ($p > 0.05$). $N_{bip} = 7$, $N_{cup} = 35$, $N_{int} = 8$.

DISCUSSION

Sleeping Site Height

My data suggest the same trend for sleeping site selection as is seen in daytime stratification, since larger individuals are found in higher sleeping sites. This trend could be due to larger individuals maintaining their dominant position during the night, and thus, sleeping higher than smaller individuals.

The results from sex comparisons suggest that the stratification seen during the daytime switches at night. Females were found in higher sites than males, which contradicts day perch trends. Males even chose to sleep below their daytime perches. In terms of female trends, it appears that they go higher in order to find more protected sites, as all females were found in protected sites. However, the reason for males to seek out lower sleeping sites is difficult to explain. This trend among males of sleeping low may be related to their daytime competition avoidance behavior while foraging, allowing them a period of time without the stress of competition while searching for an ideal sleeping site.

Size seems to be an important factor leading to nighttime stratification since the two similarly species, *N. cupreus* and *N. intermedius*, demonstrated significantly different nocturnal heights. *N. biporcatus* and *N. intermedius* showed no difference in mean night height, but because of their difference in size, they would be less likely to be competing for the same resources and would not require height stratification. This sleeping site height stratification among sympatric anole species has been noted before (Goto & Osborne 1989; Molina-Zuluaga & Gutierrez-Cardenas 2007), but the fact that each species displayed non-random selection of sites either above or below their day heights is further evidence. It also suggests that nocturnal site selection is more than just a consequence of their daytime perch height; that they choose to further stratify at night.

Protection Preference

Norops lizards are often encountered sleeping on leaves of understory plants, and it has been demonstrated that they prefer these relatively exposed sleeping sites in forest habitat (Singhal *et al.* 2007; Pérez 2008). However, the trends displayed here seem to show that there are many variables that work in concert to affect protection preference.

Both sex and species divisions displayed significant differences in protection preference, suggesting that level of protection provided by a sleeping site is an important factor between males and females, and among species. Females and members of *N. biporcatus* were always found in protected sites and obviously have a strong preference for being protected. Two groups, males and *N. cupreus*, did not show preference for protection; it is possible that selection within these groups is driven by other factors, such as height of sleeping site.

Ultimately, male sleeping site selection appears to be more driven by height, while females seem to choose based on protection provided by the site. Species differences also exert a significant effect on these preferences. As a whole, individuals displayed a non-random preference for protection, which contradicts commonly observed trends. At least in this disturbed habitat, protection is an important driving factor in sleeping site selection.

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