The Effects of UV Reflectance on Mate Searching in *Enallagma carunculatum*

Stephanie Hatchew, GaHee Kim, Vanessa Sheu

University of Michigan Biological Station

August 16, 2007

Abstract

Several studies on damselflies have examined potential causes for female-limited polymorphisms and how males distinguish between the morphs. Recent spectral analyses have shown that in some species, such as *Enallagma carunculatum*, the blue morph reflects ultraviolet wavelengths, like males, whereas the green morph does not. Therefore, UV reflectance may be a visual cue used by mate-searching males to detect females. Our study gauged male reaction toward blue females modified with sunscreen to decrease UV reflectance, blue female controls, green female controls, male controls, and UV-modified males. Blue female controls had many more sexual reactions than any other control treatment. UV females and male controls experienced some reactions. From this we speculated that males use multiple visual cues, including coloration pattern and hue as well as UV reflectance. Female variation within these traits suggests that female morphs have evolved in response to other female morphs and not as male mimics.

Introduction

As a mechanism for evolutionary change, sexual conflict can result in sex-specific adaptations that are continuously being "upgraded" in response to a change by the other sex. In several large odonate families, female polymorphisms are quite common and may be one such adaptation against sexual harassment by males (Fincke 2004, Fincke et. al 2005). The male mimicry hypothesis suggests that blue females avoid sexual interactions by looking like males, which makes them less detectable by males. An alternative suggests that as visual signaling is particularly noisy compared to other sensory cues (Greenfield 2002), the presence of two morphs may prevent males from forming a stable search image (Fincke et. al 2007). Faced with two morphs, a male cueing to the majority morph may increase his searching efficiency (Miller & Fincke, 1999, Van Gossum et. al 2001). Thus, one expected response to female polymorphism is that males develop a search image for the majority female morph (Fincke et. al 2007).

Enallagma carunculatum has two female morphs. The blue female morph and the male share similar light reflectance curves, and both curves reflect ultraviolet light. However, the green female morph does not (T. Shultz & O.M. Fincke, unpublished data, Fig. 1). These differences in UV may be used by males as visual signals while searching for mates; our study is novel in that UV has never been examined as a visual cue. *E. carunculatum* generally mate from the late morning to early evening. Sunlight composition varies in the amount of UV over the day, with more UV from 10:00-15:00 and less UV in the late afternoon (T. Shultz, unpublished data). If UV is a visual reproductive signal, then the changing amount of available UV should change a blue female's appearance throughout the day. Additionally, removing UV reflectance by covering a female's thorax and abdomen with sunscreen may also make her less conspicuous to a mate-searching male. Our hypothesis is that UV light reflected by blue females is a visual signal that helps males in mate searching. We predict that more male sexual reactions towards blue females without sunscreen than towards females with sunscreen, and male sexual reactions will be observed towards blue females earlier in the day than later in the day. Alternatively, the presence of UV may increase a female's similarity to a male, so its removal may make her more detectable. Such differences in detectability by males would be reflected by a difference in male sexual behavior toward late-appearing and UV-modified blue females.

Materials and Methods

Male density tests were run from 9:00-17:00 on two days to determine a baseline for local male activity. At each hour, we recorded the total number of males captured in two minutes.

This study occurred on five days between 28 July and 12 August at the University of Michigan Biological Station (UMBS) Douglas Lake, Michigan in Cheboygan County (T37N, R3W, S33/34). Here, *E. carunculatum* is reproductively active from mid-July to early September (O.M. Fincke, unpublished data). In female and male controls, the natural range of UV reflectance had a lower bound of approximately 380 nanometers^{-1} (T. Shultz & O.M. Fincke, unpublished data, Fig. 2 and Fig. 3 respectively). UV-modified females and males possessed a thin coat of Coppertone oil and fragrance-free sunscreen (SPF 45) spread on their thoraxes and abdomens, which modified the lower bound of UV reflectance to approximately 310 nanometers^{-1} (T. Schultz& O.M. Fincke, unpublished). Spectral analyses were performed using an Ocean Optics spectroradiometer.

Testing was run throughout the timeframe of 11:00-17:00, because the amount of UV composition in sunlight is greater between 11:00-15:00 than 15:00-17:00 (T. Shultz & O.M. Fincke). Trials were run using the "damselfly on a stick" method in which a live damselfly's legs were glued onto the edge of a dowel using Duco cement (Fincke et. al 2007). The individual was placed at the testing site, and male reactions to the individual were observed for ten minutes. Sexual reactions were compared to the total number of flybys, when a male was within 5 cm of the damselfly, and hovering, when a male was immediately over the damselfly for a longer time. Sexual reactions were defined as grabbing, when the male attached to the damselfly in a vertical position, and tandem, when the male attempted to maneuver the damselfly into a horizontal formation. If males attempted any type of reaction, they were collected to prevent inflated numbers and were released prior to the second trial. Nineteen UV-modified *E. carunculatum* experimental females were used. Thirteen blue *E. carunculatum* females, two green *E. hageni females*, twenty-one *E. carunculatum* males, and eleven UV-modified *E. carunculatum* males were also used as controls.

Statistical Analyses

We used a 2-tailed independent sample t-test in SPSS to analyze the rates of male reactions to blue female controls in comparison to blue UV-modified females. We also used this test to compare the male reactions to blue female controls and male controls. Finally, we contrasted male reactions to UV-modified females and male controls. We also used 3 t-tests to compare the means of these groups two at a time.

Results

Male Density

Peak male was between 12:00 and 15:00 (Fig. 4). In the area in full sun with dense vegetation, male density was lowest at 9:00 with 1 male/minute, increasing to 8 males/minute at 13:00, then decreasing to 5 males/minute at 17:00. In the partially shaded area with sparse vegetation, male density generally increased from 1.5 males/minute to 5 males/minute at 14:00, then decreased to original levels at 16:00. The combined transect of the two areas remained low between 1 and 2 males/minute, with the exception of a sudden increase to 3.5 males/minute at 11:00.

Observations

We observed three copulating pairs and found that following copulation, the pair rested on the beach for about 6 minutes (4 minutes, 6 minutes, 8 minutes). Al pairs flew in tandem across the lake. Only blue females were found in this later part of the season. The thoracic and abdominal pattern on females is different from that of males: males have bright blue stripes and a bright tip of the male, females have darker backs with no tip.

We found four solo females in an area of tall, dense vegetation (mostly milkweed) two meters from shore. The females perched at the border of the vegetation and the beach. Males were observed perching or actively searching along the beach. Male behavior showed signs scramble competition: several males were often seen harassing a tandem pair.

Male Reaction Trials

UV-reflecting female controls elicited the most sexual interactions from males (Fig. 1), five times as many sexual interactions as UV-modified females (n=13 and n=19 respectively, x=1.08 and x=0.21 respectively, F=14.243, p<0.001). Both female morphs had more interactions than male controls (n=21, female controls: F=29.69, p<0.001; UV-modified females: F=12.05, p<0.001). No males reacted to UV-modified male controls or green *E. hageni* females (n=11 and n=2 respectively).

Fourteen male reactions toward control females occurred in the afternoon between 12:55-15:16. Two male reactions toward UV-modified females occurred in the noon and early afternoon between 11:27-14:21. A single male reaction toward the control male occurred in the evening at 17:21.

Discussion

As in other damselfly species, *Enallagma carunculatum* males were observed to search in sunny, grassy areas for mates. UV reflectance was a visual cue used by mate-searching males. UV-reflecting female controls had the most male reactions. Also, all male interactions to blue female controls occurred mid-afternoon, when the amount of available UV was relatively high, and the reflected UV signal was relatively strong. The only search "error" that occurred was a single sexual reaction toward a male control in the evening. The male may have reflected less UV, making his signal similar in appearance to a blue female's signal. Furthermore, no males reacted to male controls without UV reflectance.

UV reflectance may be used in conjunction with other visual factors. One factor is coloration pattern, used by searching males as a visual cue (Miller and Fincke 1999). Our treatment groups represented different combinations of two visual cues: UV reflectance and coloration pattern. The relative number of sexual reactions elicited by each group may indicate the importance of each visual component. The most optimal combination for male mate recognition was the blue female pattern with UV reflectance, present in female controls. The second most optimal combination occurred in UV-modified females, which had the female pattern but no UV reflectance. Only one reaction occurred toward male controls, which had UV reflectance but no female pattern. No males reacted toward UV-modified male controls, which had neither UV reflectance nor the female pattern. Similarly, no males reacted toward the green *E. hageni* female, which lacked UV reflectance and the blue background with the female pattern. The differences in male reactions suggest an order in visual cues in the male search image: coloration pattern is most important, followed by the presence of UV reflectance. During our study, blue females were the majority morph, so males may be most responsive to blue female visual cues.

The difference in these two visual cues highlights variation in female polymorphism: coloration patterns have diverged to having blue and green backgrounds, and UV reflectance has also diverged into either present or absent. Females seeking to avoid harassment may derive highly different appearances consisting of multiple opposing factors, making it more difficult for males to form a stable search image. For example, if UV reflectance was absent in both morphs, males may link the shared lack of UV reflectance to female appearance, and both morphs would be identified as female by that trait. However, if both morphs possess opposing traits, then no single trait could be generalized to all females, and males may subsequently experience difficulty in forming an ideal search-image.

Our study is more consistent with the hypothesis that female-limited polymorphisms evolved in response to male harassment and is maintained by negative frequency-dependent selection (Fincke et al. 2005), than with the male mimicry hypothesis (Johnson 1975, Sherratt 2001). The presence of UV increased visual detectability instead of functioning as camouflage in blue females. The difference in male reactions to individuals with variations of multiple traits suggests that the male search image may not be a single general snapshot but a jigsaw puzzle consisting of multiple elements. While blue females may share some of these elements with males in UV reflectance and color, only one male was grabbed, indicating that males have little confusion distinguishing between females and males. In another damselfly species, *E. civile*, males likewise rarely interacted sexually with other males (Fincke et. al 2007). On the other hand, males had difficulty in recognizing UV-modified females, indicating that differences between females is a larger source of confusion. Similarly, the presence of interspecific females confused *E. civile* males (Fincke et. al 2007). Female variance with other females seems to be the cause of male confusion, as opposed to female similarity to males. In other words, it does no good for a rabbit to look like the hunter, only to look unlike a rabbit.

Males, however, may have a chance to increase their success by capitalizing on environmental conditions that enhance the strength of a particular visual cue, e.g. the time of day and UV reflectance. In our study, noon to mid-afternoon was the time window in which the greatest number of male reactions occurred, and when the greatest density occurred. Since males preferentially grabbed UV control females at these times, males may search for mates at the time of day when the UV signal was the strongest.

This study was limited in that it occurred at the end of *E. carunculatum*'s flight season, when male density was lower and only the blue female morph was present. It is thought that green morphs may emerge earlier in the season, to be replaced by blue morphs later on (Fincke, personal comment). Future studies could examine the expected increase and decrease in the significance of particular visual cues in male mate recognition as the morph with that trait increased and decreased in frequency. Other studies could test individuals with different coloration patterns, hue, and UV reflectance to determine the most optimal combination of visual cues. UV could also be added to the green morph by means of fly floatant.

Female choice and male-male competition has been though to drive dimorphism between the sexes, but recently it has been suggested that sexual conflict is another impetus (Fincke et. al 2005). In *E. carunculatum* and other polymorphic damselfly species, sexual conflict could also create intrasexual dimorphism, in which females exhibit variation in several traits. Thus, sexual conflict may function similarly to a disruptive selection. The divergence may not only be in physical but behavioral traits; blue females are thought to exhibit more aggressive behavior (Cordero and Andrés 2001). Another trait may be secondary genitalia, suggesting speciation as an extreme version of intrasexual dimorphism (Fincke et. al 2007). For all its costs to both sexes, sexual conflict may be a source of intraspecific diversity, even diversity at the extreme of speciation.

Acknowledgements

We thank Dr. Ola Fincke for borrowing her experimental method as well as her scientific zeal. We also thank Tom Schultz and his spectral analysis work for the extensive knowledge that produced a small light-bulb moment, which started the study. Many thanks go to the Biology 381 Natural History and Evolution class for field work assistance and for its patience.

Literature Cited

Fincke, O.M. 2004. Polymorphic signals of harassed female odonates and the males that learn them support a novel frequency-dependent model. Animal Behavior 67: 833-845.

Fincke, O.M., R. Jödicke, D. R. Paulson, & T. D. Schultz. 2005. The evolution and frequency of female color morphs in Holarctic Odonata: why are male-like females typically the minority? International Journal of Odonatology 8 (2) 2005: 183-212

Fincke, O.M., A. Fargevieille, & T.D. Schultz. 2007. Lack of innate preference for morph and species identity in mate-searching *Enallagma* damselflies. Behav Ecol Sociobiol.

Greenfield, M. D. 2002. Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication. Oxford: Oxford University Press.

Johnson, C., 1975. Polymorphism and natural selection in ischnuran damselflies. Evolutionary Theory 1: 81-90.

Miller, M.N. & O. M. Fincke. Cues for mate recognition and the effect of prior experience on mate recognition in *Enallagma* damselflies. Journal of Insect Behavior (12) 6 1999.

Sherratt, T.N., 2001. The evolution of female-limited polymorphisms in damselflies: a signal detection model. Ecology Letters 4: 22-29.

Van Gossum, H., R. Stoks & L. De Bruyn, 2001b. Reversible frequency-dependent switches in male mate choice. Proceedings of the Royal Society of London (B) 268: 83-85.

Appendix

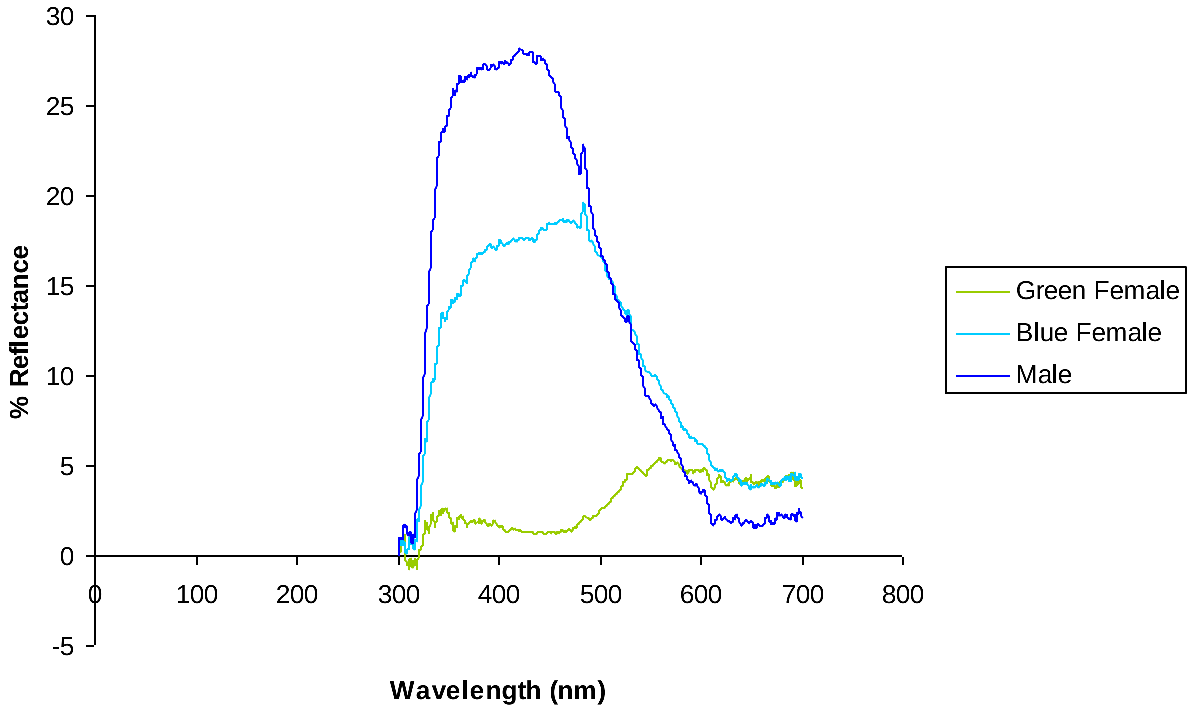

Figure 1. Mean reflectance spectra of *Enallagma* damselflies, showing the color (wavelength) and the intensity (% reflectance) of the male (top curve), the blue female (middle curve), and the green female (lowest curve). Wavelengths of UV light are approximately between 300-400 nm.

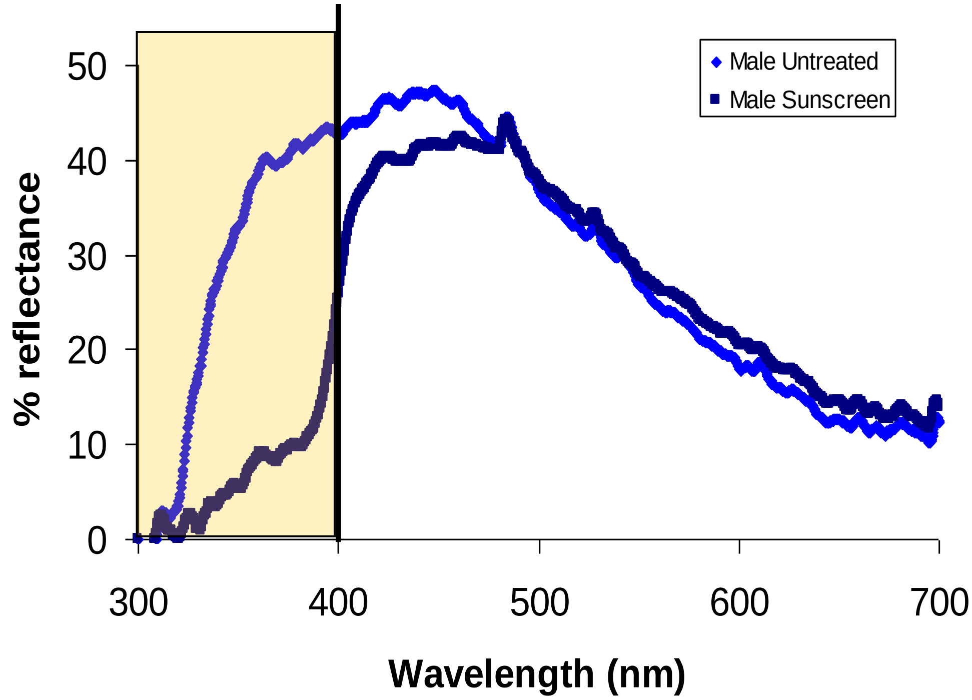

Figure 2. Reflectance spectra of *E. carunculatum* male, showing the color (wavelength) and the intensity (% reflectance) of the male (top curve), the blue female (middle curve), and the green female (lowest curve). Wavelengths of UV light are approximately between 300-400 nm.

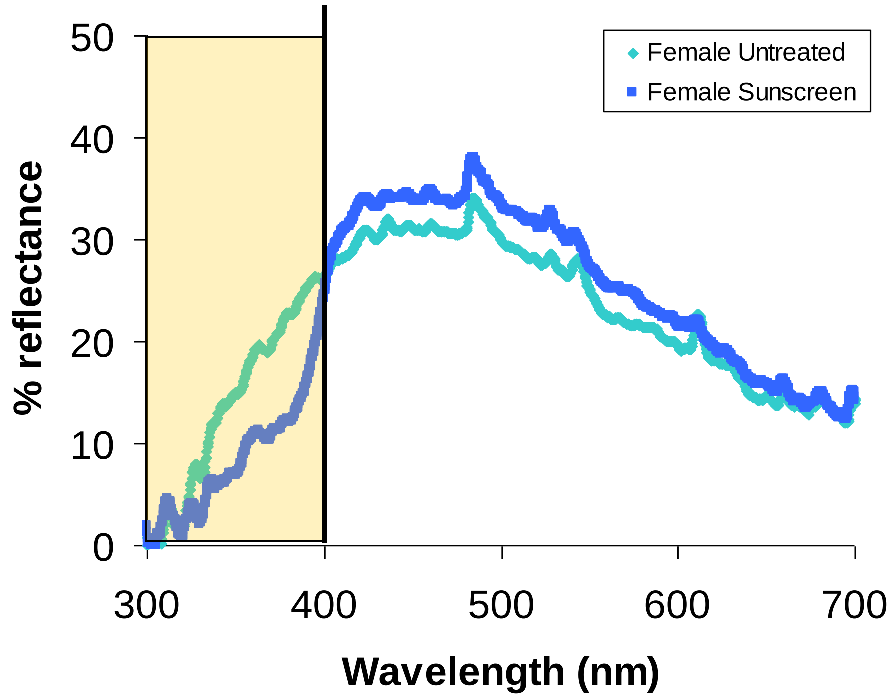

Figure 3. Figure 2. Reflectance spectra of *E. carunculatum* female, showing the color (wavelength) and the intensity (% reflectance) of the male (top curve), the blue female (middle curve), and the green female (lowest curve). Wavelengths of UV light are approximately between 300-400 nm.

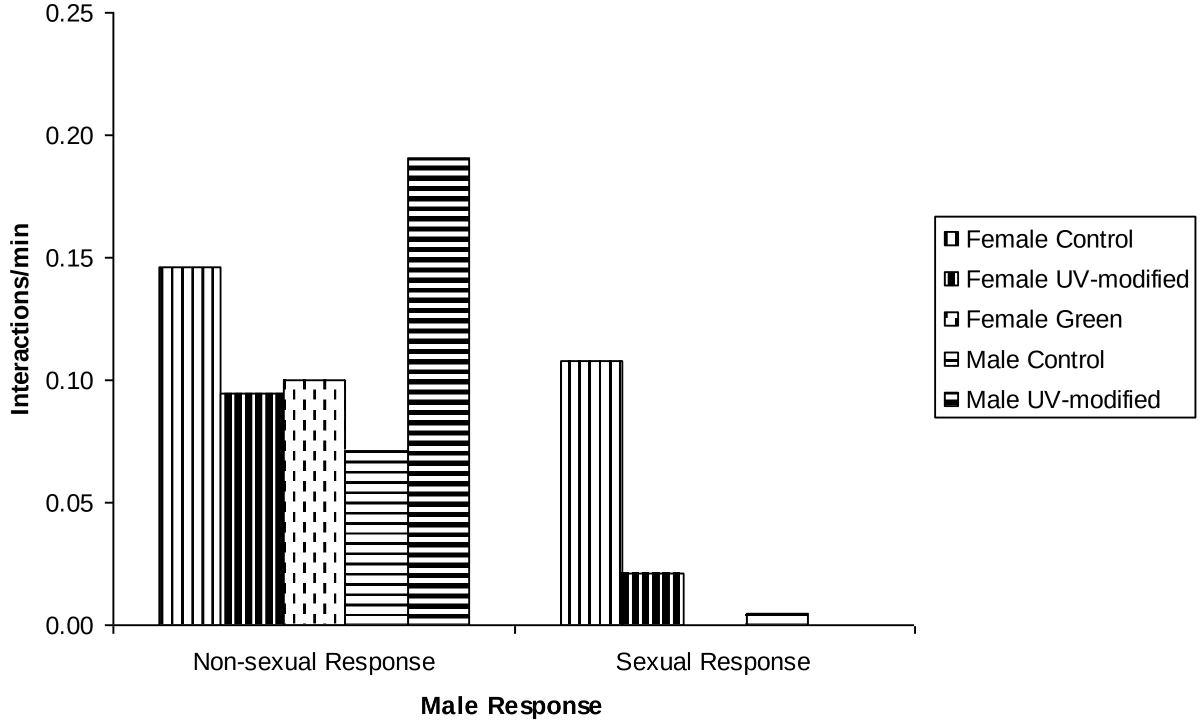

Figure 4. Average rates of male sexual reactions (grabs and tandems) and non-sexual reactions (flybys and hovers) towards UV-modified females, blue female controls, green female controls, male controls, and UV-modified males.

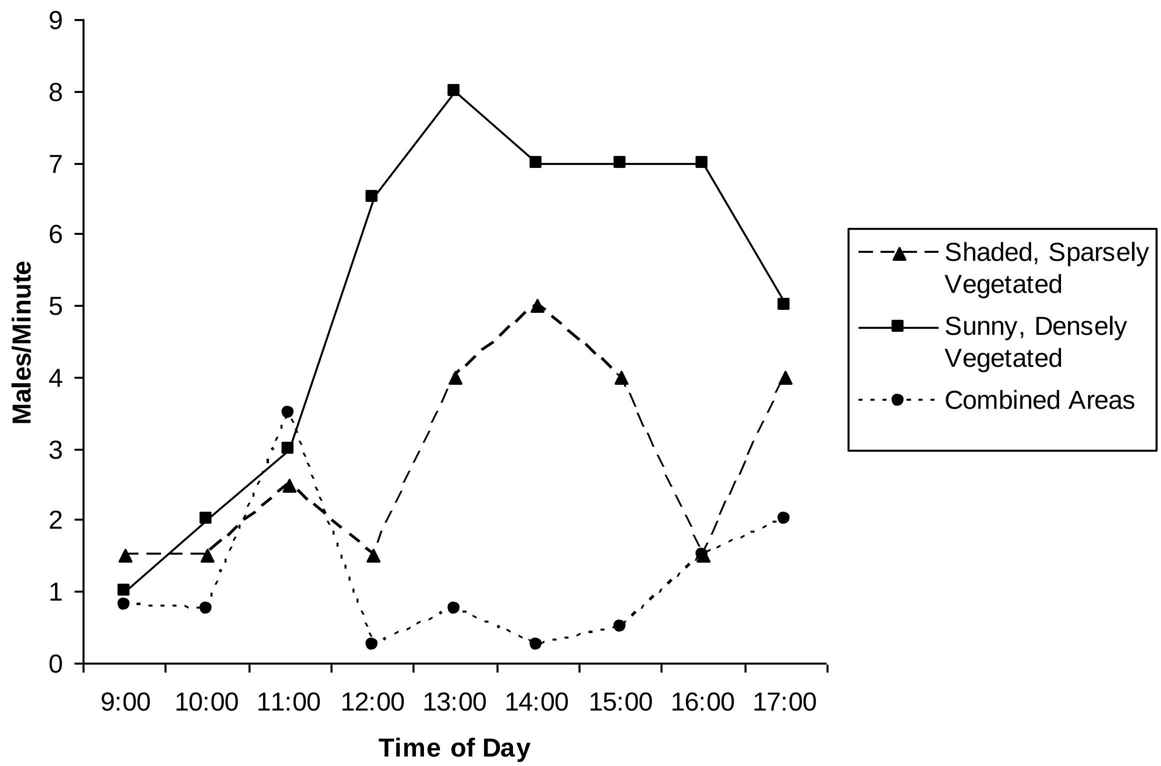

Figure 5. Male density trends in trial site throughout the day. Shown is the range of male densities in varying light and vegetation conditions. Multiple combinations of these conditions were present within our trial site.