PLASTICITY OF THE RED HOURGLASS IN FEMALE WESTERN BLACK WIDOW SPIDERS (*LATRODECTUS HESPERUS*): URBAN ECOLOGICAL VARIATION, CONDITION-DEPENDENCE, AND ADAPTIVE FUNCTION

by

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved April 2014 by the
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ARIZONA STATE UNIVERSITY

May 2014

ABSTRACT

Urbanization provides an excellent opportunity to examine the effects of human-induced rapid environmental change (HIREC) on natural ecosystems. Certain species can dominate in urban habitats at the expense of biodiversity. Phenotypic plasticity may be the mechanism by which these 'urban exploiters' flourish in urban areas. Color displays and condition-dependent phenotypes are known to be highly plastic. However, conspicuous color displays are perplexing in that they can be costly to produce and may increase detection by enemies. The Western black widow spider (*Latrodectus hesperus*) is a superabundant pest species that forms dense aggregations throughout metropolitan Phoenix, Arizona, USA. Adult female *L. hesperus* display a red hourglass on their abdomen, which is speculated to function as a conspicuous warning signal to enemies. Here, I performed field studies to identify how widow morphology and hourglass color differ between urban and desert subpopulations. I also conducted laboratory experiments to examine the dietary sensitivity of hourglass coloration and to identify its functional role in the contexts of agonism, mating, and predator defense. My field data reveal significant spatial variation across urban and desert subpopulations in ecology and color. Furthermore, hourglass coloration was significantly influenced by environmental factors unique to urban habitats. Desert spiders were found to be smaller and less colorful than urban spiders. Throughout, I observed a positive correlation between body condition and hourglass size. Laboratory diet manipulations empirically confirm the condition-dependence of hourglass size. Additionally, widows with extreme body conditions exhibited condition-dependent coloration. However, hourglass obstruction and enlargement did not produce any effects on the outcome of agonistic encounters, male courtship, or predator deterrence. This work offers important insights into the effects of urbanization on the ecology and coloration of a superabundant pest species. While the function of the hourglass remains undetermined, my findings characterize the black widow's hourglass display as extremely plastic. Plastic responses to novel environmental conditions can modify the targets of natural selection and subsequently influence evolutionary outcomes. Therefore, assuming a heritable component to this plasticity, the response of hourglass plasticity to the abrupt environmental changes in urban habitats may result in the rapid evolution of this phenotype.

DEDICATION

To my partner Brian Amato: for his love, support, and patience throughout the completion of this work. You’re my best friend. Thank you.

To my parents Jim Gburek and Diane Bennett: for encouraging me to roam in the woods, catch bugs, and further my education. Thank you.

ACKNOWLEDGMENTS

I would like to thank my inspiring advisor, Dr. James Chadwick Johnson for constantly challenging me intellectually and encouraging me to go above and beyond in my research and writing efforts. I am deeply grateful for all his time, feedback, and encouragement throughout the completion of this work. I would also like to thank my co-chair, Dr. Kevin McGraw and committee member, Dr. Ronald Rutowski for offering their expertise in the field of animal coloration and the time they spent to help improve the quality of my research. I am overwhelmingly appreciative for my committee's continued enthusiasm and interest. Their guidance and participation in my work helped me to develop as a researcher and writer.

I would like to thank the following undergraduate students and friends whose assistance was pivotal in completing field and laboratory data collection: Kristina Aiello, Brian Amato, Katie Bratsch, Laura Dennis, Megan Grier, Rebecca Halpin, Joanna Jewel, Dale Stevens, Patricia Trubl, Lindsay Miles, Jesse Lam, Jennifer Larson, and Annika Vannan. Additionally, I would like to thank all the past and present members of the Johnson Laboratory for making my graduate experience so enjoyable with intellectual conversation, collaborative research, experimental trouble-shooting, and general spider shenanigans.

I would like to thank Lisa Taylor, Russell Ligon, and Melinda Weaver from the McGraw Laboratory and Kimberly Pegram and Brett Seymoure from the Rutowski Laboratory for their assistance with spectrophotometry techniques and in developing a protocol for acquiring spectral data from digital images. I would also like to thank the

School of Life Sciences staff, Yvonne Delgado, Kimberly Fuqua, and Wendi Simonsion for their assistance in traversing administrative and academic policies.

I would like to thank Dr. May Boggess and Maria Schaijik from the Arizona State University Statistical Consulting Department for their assistance in data analyses for Chapter 1 of this work. I would also like to thank Eric Moody, Dr. Stephen Pratt, and Dr. John Sabo for their assistance and patience in statistical analyses and developing my programming abilities.

This research was funded by several sources. Travel grants from the School of Life Sciences and the Animal Behavior Society enabled me to attend conferences to share my research and network with fellow biologists from across the globe. A research grant from the Animal Behavior Society was essential in funding my travel during field work and the purchase of equipment required for obtaining spectral data and housing experimental spiders. This material is based upon work supported by the National Science Foundation under grant nos. BCS-1026865, Central Arizona-Phoenix Long-Term Ecological Research (CAP LTER).

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CHAPTER 1

SPATIAL VARIATION IN THE SUBPOPULATION ECOLOGY AND COLOR OF BLACK WIDOW SPIDERS: INSIGHTS INTO THE EFFECTS OF URBANIZATION ON A SUPERABUNDANT PEST SPECIES

Abstract

Urbanization is an excellent example of human-induced rapid environmental change (HIREC). Urban habitats are characterized by habitat loss, invasion by exotic species, pollution, and climate change. Certain taxa termed ‘urban exploiters’ thrive in the wake of human disturbance and can out-compete other species, resulting in decreased biodiversity. Phenotypic plasticity may be the mechanism by which urban exploiters are able to dominate in urban habitats. For example, color displays can be highly plastic and fluctuate with foraging success and environmental variation. The Western black widow spider (*Latrodectus hesperus*) is a superabundant urban pest species. Urban widow subpopulations can be up to thirty times denser than subpopulations in the surrounding Sonoran desert. Adult female *L. hesperus* possess a brightly colored red hourglass on their abdomen, which is speculated to function as a conspicuous warning signal to enemies. To identify the effects of urbanization on black widow subpopulation ecology, body condition, and hourglass coloration, I conducted a field study where we monitored these variables in urban black widow subpopulations over the course of a breeding season. I found significant spatial variation across eight urban subpopulations in population ecology (i.e. population density, nearest neighbor distance, and web substrate), body condition, and hourglass coloration. Additionally, I found that hourglass saturation and brightness declined in individuals as the breeding season progressed. Body condition was a reliable predictor of hourglass size, but there were no distinct correlations between body condition and hourglass coloration among aggregations. Rather, the spectral qualities of the hourglass were correlated with the amount of impervious ground cover, prey abundance, and web substrate. Thus, my findings offer support for the contention that urbanization creates spatial heterogeneity and characterize the hourglass as a plastic color trait, capable of fluctuating with foraging success and variation in environmental factors unique to urban habitats.

Introduction

Urbanization is an excellent example of human-induced rapid environmental change (HIREC). Urban habitats are characterized by habitat loss, invasion by exotic species, pollution, and climate change (reviewed in Sih et al., 2010). Considering that over the next 40 years there is a projected 19% increase in human population density within urban centers, it is becoming increasingly important to understand the impacts of urbanization on natural ecosystems (United Nations Population Division, 2010). Urbanization is often thought of as producing biotic homogeneity (Blair, 1996; McKinney, 2006). Bird communities are well documented as decreasing in species diversity in urban habitats (Chace & Walsh, 2006; Marzulff, 2001). Indeed, certain species (termed ‘urban exploiters’) flourish in urban centers and out-compete other local species, resulting in decreased biodiversity (Blair, 1996). The mechanism by which urban exploiters are able to thrive in urban landscapes is not yet well understood.

However, recent findings suggest that certain groups of organisms actually exhibit increased diversity in areas with moderate levels of urbanization, due to the spatial heterogeneity of suburban landscapes (McKinney, 2008). Additionally, HIREC will likely have variable effects on different species (Schweiger et al., 2010). For example, urbanization has been shown to affect taxa differently based on their dispersal ability. Mobile species, such as birds, appear to be more sensitive to variation in vegetation structure (i.e., percent herbaceous cover, shrubbery cover, and tree cover) while less mobile species, such as beetles, seem to be more sensitive to increasing habitat fragmentation (i.e., reduced connectivity between habitat patches) (Croci et al., 2008). Thus, urbanization can create a variety of sub-habitats that vary with respect to species composition (McKinney, 2008; Van Keer et al., 2010).

The spatial heterogeneity of resource abundance and landscape structure in urban habitats can promote ecological variation among subpopulations of the same species. Many urban habitats are highly productive due to water supplementation and human-subsidized resource abundance (reviewed in McKinney, 2002). Urban management strategies often make resources that are typically spatially and temporally patchy more continuously available (Shochat et al., 2005). However, the relative abundance of resources available can vary within different types of urban landscapes, leading to spatial variation in intraspecific subpopulation densities and genetic composition (reviewed in Opdam & Wascher, 2004). Thus, the patchiness of urban landscapes may promote variation in the abundance, ecology, and success of organisms among subpopulations of the same taxa.

Much of our knowledge about the response of organisms to urbanization is limited to studies focusing on birds (Marzluff & Ewing, 2001). We know much less about the effects of anthropogenic disturbances on arthropod communities (McIntyre, 2000; Shochat et al., 2004). In 2000, McIntyre published a ‘call to action’ for ecologists to investigate the effects of urbanization on arthropod communities. In response to this there has been a growing body of research on arthropod populations in relation to urbanization. For example, Alaruikka et al. (2002) found carabid beetles to be most abundant in suburban and rural landscapes compared to strictly urban habitat, but no differences in the abundance or species richness of ground dwelling spiders across an urban-rural gradient. Christie et al. (2010) documented a strong compositional response of arboreal arthropods to urban fragmentation, in that communities were more diverse and densely populated in large patches of continuous vegetation compared to smaller patches with less vegetation.

Studies on key predatory arthropods such as spiders are particularly important as they may reflect changes in trophic structure among urban ecosystems (Shochat et al., 2004). Thus, spiders can serve as important ecological indicators of arthropod population dynamics in urban habitats. Additionally, many species of spiders are agriculturally important as they do not damage plants and control the exponential growth of herbivorous prey (Rajeswaron et al., 2005). Most spiders are generalist predators and thus are capable of positively responding to the superabundance of arthropod prey in urban areas (Pyle et al., 1981; McIntyre et al., 2001; Cook & Faeth, 2006). For example, Shochat at al. (2004) discovered that more productive urban habitats, such as agricultural fields and mesic yards, were characterized by large spider abundances and dominance by wolf spiders (*Lycosidae*) and sheet-web weaver spiders (*Linyphiidae*).

While these studies document changes in spider composition, diversity, and abundance, there is much less known about the important ways in which urban disturbances relate to spider phenotypes. This is surprising given that phenotypic plasticity (i.e., variation in the physical expression of a genotype due to environmental variation), may explain species responses to urbanization (Hendry et al., 2008; Whitman & Agrawal, 2009). Indeed, there are greater rates of phenotypic change in anthropogenically-altered habitats compared to natural habitats (Hendry et al., 2008), and the success of organisms in novel environments is often associated with phenotypic plasticity (Ehrlich, 1989; Holway & Suarez, 1999; Yeh & Price, 2004). Thus, plastic phenotypic responses to urbanization may be essential to the persistence and proliferation of certain spider taxa in urban environments.

Spider coloration can be highly plastic. Species from the families Theridiiae, Tetragnathidae, Linyphiidae, and Philodromidae can alter their color almost immediately when disturbed (reviewed in Oxford & Gillespie, 1998). Additionally, variation in diet, body condition, and environment are capable of inducing color changes in spiders. For example, varied prey type results dramatic changes in the base coloration of Hawaiian happy-face spiders (*Theridion grallator*) (Gillespie, 1989). Taylor et al. (2011) showed that male jumping spiders (*Habronattus pyrrithrix*) fed high-quality diets had enhanced body conditions as well as larger and redder facial ornamentation. The spider *Thomisus labefactus* (Thomisidae) can alter its UV reflectance to match its background in order to be less conspicuous to potential prey (Sato, 1987). Despite the growing body of work on spider coloration, relatively little research has been done addressing the relationship between urbanization and spider coloration.

The metropolitan region of Phoenix, Arizona, USA is an excellent area to investigate variation and plasticity in spider coloration and ecology in relation to urbanization. Phoenix is the fastest growing and sixth largest city in the United States, with exponential increases in urbanized area and human population (Jenerette & Wu, 2001; Luck & Wu, 2002). Following the completion of the Roosevelt Dam in 1911, Phoenix experienced dramatic land transformation from an agricultural area to an urban center (Knowles-Yánez et al., 1999; Luck & Wu, 2002). A recent gradient analysis of Phoenix landscape patterns showed high degrees of fragmentation and spatial complexity (Luck & Wu, 2002), leading to variation in arthropod abundance, community structure, and trophic dynamics among different habitats and land uses (McIntyre et al., 2011).

Phoenix is also home to dense aggregations of the Western black widow spider (*Latrodectus hesperus*), which exhibit significant spatial variation in prey abundance, female mass, and population density (Trubl et al., 2011). Black widow spiders are native to Western North America (Garb et al., 2004) and are considered a synanthropic species (i.e., associated with human habitats). Widow spiders also possess a potentially lethal neurotoxin, making them a medically-important species (Gonzales, 2001). Adult females possess a brightly colored red hourglass on their abdomen, which is in striking contrast to their dark brown or black abdomen, making the trait highly conspicuous (Figure 1.1). The hourglass is most apparent when spiders are foraging upside down in their webs at night. While the hourglass is thought to function as a warning signal to predators (Oxford & Gillespie, 1998), there is no evidence in the literature to support this claim.

I conducted a field study during the adult breeding season where I monitored the ecology of eight urban black widow subpopulations throughout metropolitan Phoenix.

Additionally, I recorded replicate measures of the body condition and hourglass color of individual spiders. Here, I predict that *L. hesperus* population ecology, body condition, hourglass size, and color exhibit high degrees of spatial variation. Specifically, I suggest that population density, distance between neighboring adult females, the presence of prey and/or males, web substrate, body condition, and hourglass coloration will vary significantly more between subpopulations than within subpopulations. Additionally, I predict that repeated measures of body condition, hourglass size, and color will decrease significantly during the course of the breeding season due to reduced resource abundance and predation pressure. I further hypothesize that body condition and the size and spectral qualities of the hourglass vary plastically as a function of habitat structure. Specifically, I expect that the presence of prey will positively correlate with enhanced body condition, hourglass size, and color. Lastly, I predict that widows with superior body conditions will produce larger and more colorful hourglass displays.

Materials and Methods

Site Selection and Description. I monitored eight *L. hesperus* subpopulations across metropolitan Phoenix, Arizona (Figure 1.2) for ten weeks during the course of the adult breeding season from May to October in 2012. I began monitoring sites during the months of May, June, and July as I located aggregations that met the following criteria: 1) sites had to be a minimum of 8km apart, and 2) sites had to contain a minimum of ten adult females (within 5,000 m^2). Sites were located in either commercial or residential habitats with xeric landscaping. During the initial census I determined the percent of impervious ground cover at each site by measuring the total area within sites (m^2)

occupied by concrete and/or urban infrastructure. Population density was determined weekly by counting the number of adult females present within each subpopulation (per m^2).

Focal Females. At each site I randomly selected ten adult female widows to monitor weekly (n=84). I uniquely marked focal females on the dorsum using Testor's ® non-toxic enamel paints to confirm identities during the ten week monitoring period. Each week I recorded the presence or absence of prey and/or males observed in each focal female's web and identification of web substrate. Web substrate was classified as belonging to one of three categories: 1) vegetation (i.e., web located on plant life), 2) urban infrastructure (i.e., web located on anthropogenically produced substrates such as cinderblock fences, drain holes, or light posts), or 3) a combination of vegetation and urban infrastructure (i.e., web located on both plant life and urban substrate). I also measured the distance of focal females to the nearest neighboring adult female (cm). Females were then lured from their webs using tethered live prey and captured to measure body condition (see below for calculations). Additionally, I recorded the following color measurements from the upper and lower half of the hourglass: area (mm^2), hue (°), saturation (%), and brightness (%) as well as abdomen brightness (%) (see below for color scoring protocol). In the event that a focal female went missing, she was replaced with another randomly selected local female. In statistical analyses I included data only from females present during the study for a minimum of three weeks.

Scoring Color and Body Condition. I acquired color data from digital images taken in the field. Prior to imaging the spiders were temporarily anesthetized with CO_2 gas and placed in a mesh restraint device. Each spider was photographed in raw NEF format, using a Nikon D50 equipped with a Micro NIKKOR 40mm lens. A Promaster RL60 LED macro ring light was used to standardize illumination. For each imaging session the camera and images were calibrated using an X-rite Colorchecker Passport with a white balance target and 24-patch classic color reference target (X-rite, Grand Rapids, MI, USA). Once spiders were recovered (i.e., fully mobile), they were released back into their respective webs. Images were later linearized and equalized using Adobe Photoshop CS5.1 in conjunction with PictoColor inCamera ICC profile software (Pike, 2011; Stevens et al., 2006). Hourglass coloration was scored along three conventional axes of color (hue, saturation, and brightness; Hill & McGraw, 2006). Due to the specular nature of the hourglass and abdomen, average color was calculated from three point samples taken from areas with no observable illumination reflectance from both the top and lower half of the hourglass at a tolerance level setting of 40 in Adobe Photoshop. Pilot spectrophotometry data indicated that the curve for mean abdomen reflectance does not display any dramatic spectral peaks (n=30) (Fig 1.3). Therefore, I only present data for average measures of abdomen brightness calculated from three point samples taken from either side of the hourglass at a tolerance level setting of 40 in Adobe Photoshop.

Hourglass area was obtained from digital images using public domain Image J software for Windows®. I spatially calibrated the software to recognize the pixel value of a known distance within an image as millimeters. I then outlined the hourglass using a tracing tool to obtain the pixel value of hourglass area in mm^2.

I calculated body condition using the residual index method as average body mass (mg) corrected for body size using residuals for the cube root of mass regressed on cephalothorax width. Using public domain Image J software for Windows®, I obtained measures of cephalothorax width from digital images. Each image included a reference scale to allow me to convert pixel values into millimeters. Residual index body conditions are recommended for detecting differences between groups drawn from the same population (Jakob et al., 1996; Moya-Laraño et al., 2008). I consider the eight subpopulations as belonging to one urban population.

Statistical Analysis. All statistical tests were performed in Stata (Ver. 13.0 for Windows® StataCorpLP, College Station, Texas, USA) and SPSS (Ver. 17.0 for Windows® SPSS, Chicago, IL, USA). Univariate ANOVAs were used to test for spatial variation in population density and nearest neighbor distance as well as spatial variation in body condition, hourglass size, and display color (site included as a random factor). I used a Fisher's exact test to determine spatial variation in prey and male abundance, and a Pearson Chi-square test to determine spatial variation in the proportions of the type of web substrate used at each subpopulation.

I performed a Spearman's rank order correlation test to identify associations between percent impervious ground cover, population density, nearest neighbor distance, the presence of prey, the presence of males, and web-building substrate, using site averages to account for spatial variation. To account for multiple tests I employed a Bonferroni correction (α=0.05/8, α=0.002).

To assess how ecological variables correlate with body condition and coloration, I performed a linear regression for each morphological variable (i.e., body condition, hourglass area, hue, saturation, brightness, and abdomen brightness) against all of the ecological variables (i.e., impervious ground cover, nearest neighbor distance, presence of prey and males, and web-building substrate) using backwards stepwise methods to arrive at a parsimonious model. I used clustered standard errors to account for probable correlations between observations on the same spider (Williams, 2000).

To determine if individual's body condition and hourglass size and color varied over multiple measures, I identified the largest number of individuals with the same amount of repeated measures (n=31). I then ran a repeated-measures ANOVA to evaluate if individual's body condition and hourglass size and color varied over the course of three measures.

To examine temporal effects on body condition and hourglass color I ran separate regressions using collection date as the predictive variable and nearest neighbor distance, population density, body condition, hourglass size, hue, saturation, brightness, and abdomen brightness as dependent variables. To account for multiple tests I employed a Bonferroni correction (α=0.05/6, α=0.008).

To account for variation among sites, I examined how body condition affects each of the hourglass color variables with linear regressions by site. I used a Wald test to determine the significance of each relationship. The Wald test uses a combination of variables (i.e., body condition and site) as predictors of dependent variables (i.e., hourglass area, hue, saturation, brightness, and abdomen brightness) in multiple regressions (Zar, 2010). I also ran regressions of body condition and hourglass area, hue, saturation, brightness, and abdomen brightness using site averages. To account for multiple tests I employed a Bonferroni correction (α=0.05/5, α=0.01).

Results

Field site characteristics (i.e., size and percent impervious ground cover), widow subpopulation ecology, and proportion of web substrate type varied significantly among subpopulations (Table 1.1). Specifically, I found significant spatial variation in population density, nearest neighbor distance, and web substrate (Figure 1.4a-c). There was no spatial variation in the presence of prey or males in focal females webs (Table 1.1). Additionally, I detected significant spatial variation in body condition, hourglass area, hourglass saturation, hourglass brightness, and abdomen brightness. Hourglass hue did not exhibit spatial variation (Figure 1.5a-f).

The presence of male(s) in a focal female's web was positively correlated with the presence of prey in a focal female's web (Figure 1.6). All other possible correlations between percent impervious surface, population density, nearest neighbor distance, the presence of prey, the presence of males, and web substrate failed to meet my conservative Bonferroni criteria (all $P > 0.002$).

I found that ecological factors influenced body condition, hourglass size, and coloration (Table 1.2). Specifically, spiders exhibited better body conditions when I observed prey in their webs and when they built their webs on a combination of vegetation and urban infrastructure. Hourglass area increased with impervious ground cover, but decreased when spiders built webs on exclusively urban infrastructure. Hourglasses were more orange (i.e., higher hue values) when prey was observed in their webs, and hourglass and abdomen brightness increased with impervious groundcover. Hourglass saturation was not influenced by any ecological factors. Additionally, population density, nearest neighbor distance, and the presence of males did not significantly influence body condition, hourglass size, or display coloration.

Hourglass saturation differed significantly among measurement time-points (Figure 1.7a). Bonferroni post hoc comparisons indicated that measure three was significantly lower than measure one (Figure 1.7a). Hourglass brightness also varied significantly among repeated measures (Figure 1.7b). Specifically, measure three was significantly lower than measure one and measure two (Figure 1.7b). I did not detect a repeated measures effect on body condition ($F_{2,29}$=1.403, P=0.262), hourglass area ($F_{2,29}$=2.451, P=0.104), hourglass hue ($F_{2,29}$=3.042, P=0.063), or abdomen brightness ($F_{2,29}$=0.279, P=0.759).

There was no temporal effect on nearest neighbor distance (R^2=0.002, $F_{1,375}$=0.830, P=0.363) or population density (R^2=0.028, $F_{1,72}$=2.082, P=0.153). Additionally, there was no temporal effect on body condition (R^2=0.008, $F_{1,375}$=3.180, P=0.075), hourglass hue (R^2=0.003, $F_{1,375}$=1.223, P=0.270), or abdomen brightness (R^2=0.001, $F_{1,375}$=0.209, P=0.648). I detected marginally non-significant trends (α=0.008) for a decrease in hourglass size (R^2=0.010, $F_{1,375}$=3.943, P=0.048), hourglass saturation (Figure 1.8a), and hourglass brightness (Figure 1.8b) over the course of the breeding season.

The relationship between body condition and the size and spectral qualities of the hourglass display varied among subpopulations. Body condition was positively correlated with hourglass size in subpopulations from Chandler ($F_{1,83}$=5.18, P=0.03), East Mesa ($F_{1,83}$=9.75, P<0.001), Glendale ($F_{1,83}$=8.36, P<0.001), South Phoenix ($F_{1,83}$=36.65, P<0.001), Tempe ($F_{1,83}$=21.61, P<0.001), and West Phoenix ($F_{1,83}$=9.89, P<0.001), but not for the Central Mesa ($F_{1,83}$=3.52, P=0.06) or Scottsdale subpopulations ($F_{1,83}$=0.36, P=0.55) (Figure 1.9).

With respect to coloration, I observed a significant positive correlation between body condition and hourglass hue in the Central Mesa subpopulation ($F_{1,83}$=6.15, P=0.02) and a negative relationship between body condition and hourglass hue in the Scottsdale subpopulation ($F_{1,83}$=31.17, P<0.001) (Figure 1.10a) There was a significant positive correlation between hourglass saturation and body condition in widows from the South Phoenix subpopulation ($F_{1,83}$=14.41, P<0.001) (Figure 1.10b). Hourglass brightness was negatively correlated with body condition in the East Mesa subpopulation ($F_{1,83}$=7.53, P=0.01), and positively correlated with body condition in the South Phoenix ($F_{1,83}$=86.55, P<0.001), and Scottsdale subpopulations ($F_{1,83}$=15.41, P<0.001) (Figure 1.10c). Abdomen brightness was positively correlated with body condition in the Scottsdale subpopulation ($F_{1,83}$=5.1, P=0.03) (Figure 1.10d). Slopes for regressions of body condition and color metrics indicated conflicting relationships these variables among sites (Figure 1.10a-d). There was no relationship between body condition and any color measures in subpopulations from Chandler, Glendale, Tempe, or West Phoenix (all P>0.05).

When using site averages I did not observe any correlations between body condition and hourglass area (R^2=0.166, $F_{1,7}$=1.196, P=0.316), hue (R^2=0.139, $F_{1,7}$=0.968, P=0.363), saturation (R^2=0.011, $F_{1,7}$=0.069, P=0.802), brightness (R^2=0.1001, $F_{1,7}$=0.003, P=0.958), or abdomen brightness (R^2=0.001, $F_{1,7}$=0.005, P=0.947).

Discussion

Spatial Variation in Black Widow Subpopulation Ecology, Body Condition and Color. My documentation of spatial variation in population ecology, body condition, and hourglass coloration are consistent with similar findings by Trubl et al. (2011) whose research indicated urban widow subpopulations are spatially distinct in terms of prey abundance, female mass, and population density. Resource availability can vary within different types of urban landscapes, leading to spatial variation in intraspecific subpopulation densities (reviewed in Opdam & Wascher, 2004). My data indicate urban subpopulations of black widow spiders exemplify this trend and offer support for the generalization that urbanization heightens spatial variation (Croci et al., 2008; Luck & Wu, 2002; Shochat et al., 2004).

My data also document significant spatial variation in body condition and the spectral qualities of the hourglass. Color displays can be especially sensitive to environmental factors such as temperature, diet, ambient light, background color, predator abundance, competition, and stress (Bradbury & Vehrencamp, 2011). Many of these environmental factors are highly variable in urban habitats, such as the relative abundance of human-subsidized resources and differences in landscape structure (Opdam & Wascher, 2004). Thus, the patchiness of urban environments can promote spatial variation in body condition and hourglass coloration.

Relationships Between Environmental Factors and Black Widow Body Condition and Color. I observed heightened body conditions when prey was observed in focal female's webs. Additionally, females were in superior condition when they built their webs on a combination of vegetation and urban infrastructure. However, I did not observe a relationship between the presence of prey and web substrate. I speculate that my measure of prey abundance may not accurately reflect the foraging success of focal females, as it was limited to weekly observations. Perhaps webs built on a combination of vegetation and urban substrate offer more opportunities for prey capture and subsequently result in improved body conditions.

Intriguingly, hourglasses were larger with increased impervious ground cover. The Chandler and East Mesa field sites had the largest amounts of impervious surface. Anecdotally, these sites were also frequently disturbed by human traffic and landscaping (Gburek, personal observation). Spiders at the Chandler location build webs along a stucco wall that ran parallel to a walkway frequently used for recreation by local residents. East Mesa spiders built webs within a regularly landscaped plot bordering a residential neighborhood. A great deal of research has been done suggesting that nonlethal disturbance stimuli caused by humans are analogous to perceived predation risks (reviewed in Frid & Dill, 2002). Moreover, the protective value of conspicuous warning coloration can be enhanced with color patch size (Forsman & Merilaita, 1999, Gamberale & Tullberg, 1996). Black widows at these subpopulations may have responded to human disturbance as an increased predation risk and subsequently produce larger hourglasses for improved protection from predation.

I also observed a trend for widows to produce smaller hourglasses when they built webs on exclusively urban infrastructure. This likely reflects the available amount of web substrate within sites. For example, widows producing the smallest hourglasses were from the Central Mesa and Scottsdale subpopulations where vegetation was scarce and females almost exclusively built their webs with the use of drain holes as a refuge (Gburek, personal observation). Perhaps drain holes offer superior protection from human disturbance (i.e., landscaping), potential enemies and/or provide more opportunities for foraging success, resulting in relaxed selection pressures on this phenotype.

Additionally, hourglass hue (i.e., spectral location on the color wheel) was enhanced with the presence of prey (i.e., hourglasses were more orange and less red or yellow). Changes in color resulting from variation in prey type are recorded in the Hawaiian happy face spider (*Theridion grallator*) (Gillespie, 1989). Moreover, the closely related Southern black widow spider (*Latrodectus mactans*), is capable of subtle changes in abdomen coloration in response to the ingestion of food coloring, indicating that diet can have discrete effects on the coloration of *Latrodectus* species (Gillespie, 1989). Thus, ingestion of particular prey types could potentially result in hourglasses which are more orange. The natural diet of *L. hesperus* consists of a variety of arthropod species from the orders of Coleoptera, Hymenoptera, Isopoda, Araneae, Dermaptera, Orthoptera, Lepidoptera, and Diptera (Salomon, 2011). This poses a particular challenge for the identification of specific prey items capable of influencing color. Perhaps a simpler approach would be to alter the color of one particular prey item though diet-manipulations, and determine if ingestion of colored prey items results in variation in hourglass color.

Furthermore, my results suggest that overall brightness was enhanced with percent impervious groundcover. Impervious ground cover from paving materials and light pollution are characteristics that are unique to urban habitats (Pickett et al., 2011; Verheijen, 1985). Certain types of concrete are substantially more reflective than vegetative ground cover (Taha, 1997), and thus capable of producing enhanced illumination at night in areas that are artificially lit. In a recent review Longcore and Rich (2011) distinguished astronomical light pollution (i.e., obstruction of viewing the night sky) from ecological light pollution (i.e., alteration of natural light regimes in terrestrial and aquatic ecosystems), which is capable of affecting the population ecology of organisms. Remarkably, many spiders have mechanisms for reversibly changing their body coloration in response to local lighting conditions and background coloration for the purposes of enhanced crypsis (Nelson & Jackson, 2011; Oxford and Gillespie, 1998; Théry & Casas, 2009). Thus, widows may produce brighter hourglasses to appear more cryptic to prey and potential enemies in areas with enhanced illumination resulting from the reflectance of artificial lighting off of reflective impervious groundcover.

Temporal Effects on Black Widow Ecology and Color. Population density and nearest neighbor distance did not display any variation with seasonality. This is consistent with similar findings by Trubl et al., (2011) that document a lack of temporal effects on widow prey abundance, female mass, or population density. Many studies suggest that urban habitats exhibit diminished seasonal variation in comparison to habitats undisturbed by human activity (reviewed in Shochat, 2005). This is often attributed to the dampening of seasonal variation in temperature (i.e., the urban heat island effect) (Hinkel et al., 2003) and year-round water supplementation (Shochat et al., 2004).

Conversely, the brightness and saturation of individual spider's hourglasses decreased across replicate measures. This suggests that hourglass coloration was not only variable among subpopulations, but also at the level of individual spiders. Thus, variation among the microhabitats and foraging success of individual's within subpopulations may be capable of influencing hourglass coloration. Alternatively, we uncovered a trend for all spiders to exhibit decreased hourglass saturation and brightness over the course of the breeding season. Therefore, seasonality may better explain the decrease across measures in individual spider coloration. For instance, the abundance of widow enemies may decline towards the end of the breeding season. Should the hourglass function as a conspicuous warning to predators (Oxford and Gillespie, 1998), the selective advantage of producing brighter and more colorful hourglasses should decrease along with declines in predator superfluity.

Relationships Between Body Condition and Hourglass Size and Color Among Subpopulations. My data show body condition to be a reliable indicator of hourglass size at most subpopulations with the exception of Central Mesa and Scottsdale. Body condition measures offer a snapshot of an individual’s physiological state and are typically calculated as a ratio or index controlling for fixed body size when comparing body mass across individuals (Jakob et al., 1996). Therefore, condition-dependent phenotypes can be incredibly plastic as their expression fluctuates with foraging success. The observed increase in hourglass size in response to heightened body condition was likely due to the stretching of the abdomen (Moya-Laraño et al., 2002).

My data document fewer correlations between body condition and the spectral qualities of the hourglass across sites. Surprisingly, the direction of this relationship was inconsistent among subpopulations that exhibited condition-dependence of coloration. Therefore, while body condition may be a reliable predictor for hourglass size, habitat structure and environmental variation within sites may be more effective at influencing hourglass coloration. As noted above hourglass size and brightness were significantly improved by the amount of impervious ground cover and the presence of prey.

Concluding Remarks and Future Directions. Urban Phoenix black widow subpopulations are spatially distinct in terms of their population ecology, body condition, and hourglass display coloration. Conversely, these variables exhibit minimal temporal variation across the breeding season. Thus, my findings offer additional support for the contention that urban habitats are spatial heterogeneous (reviewed in McKinney, 2008) and demonstrate reduced seasonality (reviewed in Shochat, 2005). Moreover, my data characterize the black widow's hourglass as a plastic color display, capable of fluctuating with foraging success and strongly influenced by environmental variables which are unique to urban disturbances. Future efforts will be aimed at identifying the mechanism by which black widows are able to proliferate in urban habitats, and addressing the condition-dependence and function of the red hourglass display. These studies will offer important insights into the mechanisms by which some species are able to thrive in urban areas at the expense of biodiversity, as well as add to the growing body of work on the ecology of urban pest species and spider coloration.

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Table 1.1

*Spatial variation in urban subpopulation ecology. Ecological variation by site. *true value **mean±SE. See Figure 1.2 for site locations.*

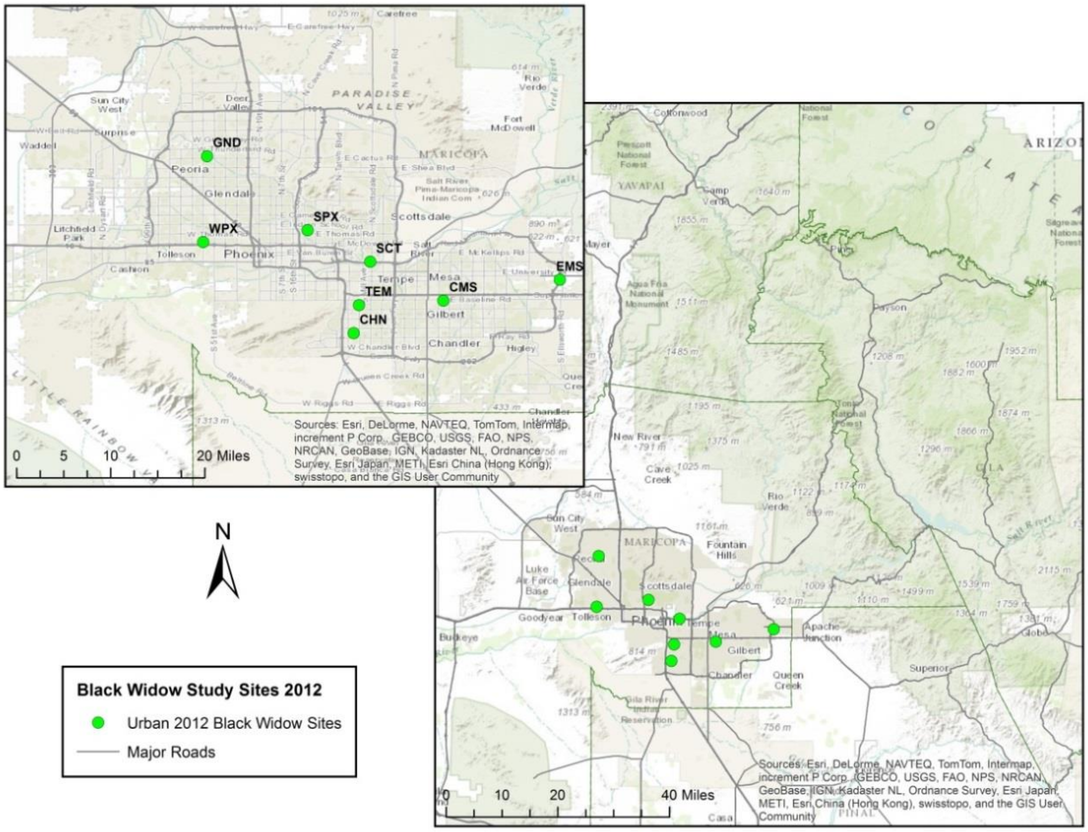
Site-Specific Ecology	Central Mesa (CMS)	Chandler (CHN)	East Mesa (EMS)	Glendale (GND)	South Phoenix (SPX)	Scottsdale (SCT)	Tempe (TEM)	West Phoenix (WPX)	Test Statistic	*P* -value
n	15	17	10	8	15	6	6	7	-	-
*Area (m^2)	233.17	829.95	1161.29	762	815.97	1463.93	1415.8	145.41	-	-
*% Impervious surface	0.8	65.34	53.74	26.65	0.85	20.92	13.97	1.26	-	-
Subpopulation Ecology										
**Population density (per m^2)	0.054 ± 0.004	0.014 ± 0.001	0.008 ± 0.001	0.009 ± 0.001	0.018 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.041 ± 0.003	**$F_{7,83}=228.12$**	**<0.001**
**Nearest neighbor distance (cm)	513.97 ± 227.22	568.42 ± 180.5	1440.42 ± 430.5	883.45 ± 409.57	1040.27 ± 604.28	2030 ± 1506	1946.52 ± 653.86	154.09 ± 38.21	**$F_{7,83}=9.43$**	**<0.001**
% Observed Prey in Webs	8	10	9	0	13	24	11	3	-	0.094
% Observed male(s) in webs	17	12	9	0	13	24	11	6	-	0.083
Web Substrate										
% Vegetation	18	0	47	38	29	0	44	45	**$\chi^2_{10}=190.71$**	**<0.001**
% Urban infrastructure	82	83	19	0	19	81	25	13		
% Vegetation & urban infrastructure	0	17	35	62	52	19	31	42		

Table 1.2

Regression model with urban ecological predictors of condition and color. Each row represents an individual model. Values represent model coefficients (i.e., slopes) for significant ecological predictors (t-value, P-value).

		Predictive Variables					Columns represent differences from using only vegetation as web-building substrate	
Response variables	df	% Impervious surface	Population density (per m^2)	Nearest neighbor distance (cm)	Presence of Prey	Presence of males	Urban infrastructure as web-building substrate	Combination of vegetation & urban infrastructure web-building substrate
Body condition (mg)	83	(0.7, 0.49)	(0.69, 0.49)	(0.81, 0.42)	**0.23 (2.13, 0.04)**	(0.01, 0.99)	(-1.08, 0.28)	**0.31 (2.43, 0.02)**
Hourglass area (mm^2)	83	**0.01 (2.04, 0.04)**	(-0.63, 0.53)	(-1.4, 0.16)	(-0.57, 0.57)	(-1.38, 0.17)	**-0.42 (-2.23, 0.03)**	(1.03, 0.31)
Hourglass hue (°)	83	(0.43, 0.67)	(0.05, 0.96)	(-0.66, 0.51)	**2.91 (2.79, 0.01)**	(0.88, 0.38)	(0.42, 0.67)	(0.61, 0.54)
Hourglass saturation (%)	83	(1.21, 0.23)	(-0.04, 0.96)	(-1.06, 0.29)	(1.62, 0.11)	(0.78, 0.44)	(-0.03, 0.98)	(0.47, 0.64)
Hourglass brightness (%)	83	**0.18 (4.41, <0.001)**	(1.94, 0.06)	(-0.67, 0.5)	(1.94, 0.06)	(0.99, 0.33)	(-0.18, 0.85)	(-0.08, 0.94)
Abdomen brightness (%)	83	**0.08 (4.15, <0.001)**	(-0.18, 0.86)	(-0.7, 0.48)	(1.53, 0.13)	(-0.98, 0.33)	(-0.59, 0.55)	(-0.09, 0.93)

Figure 1.1 Red hourglass of adult female black widows.



Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community

Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community

Figure 1.2 Location of urban subpopulations. CMS – Central Mesa, CHN – Chandler, EMS – East Mesa, GND – Glendale, SPX – South Phoenix, SCT – Scottsdale, TEM – Tempe, and WPX – West Phoenix.

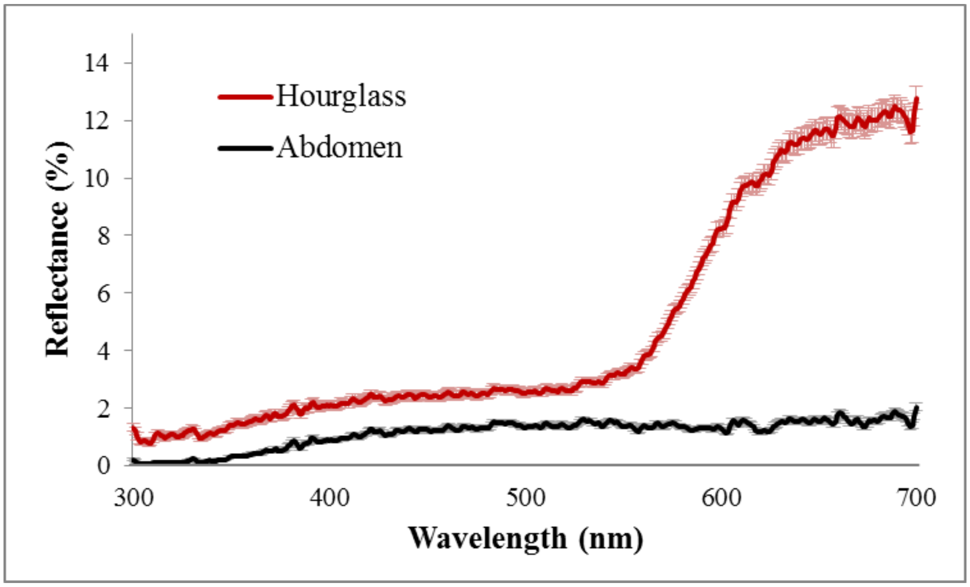


Figure 1.3 Average reflectance of the hourglass and abdomen. Variation from the mean represents standard error. Data was acquired from field caught urban spiders (n=30) using a standard UV-vis spectrophotometer (USB2000 with PX-2 pulsed xenon light source, Ocean Optics, Dunedin, FL, USA).

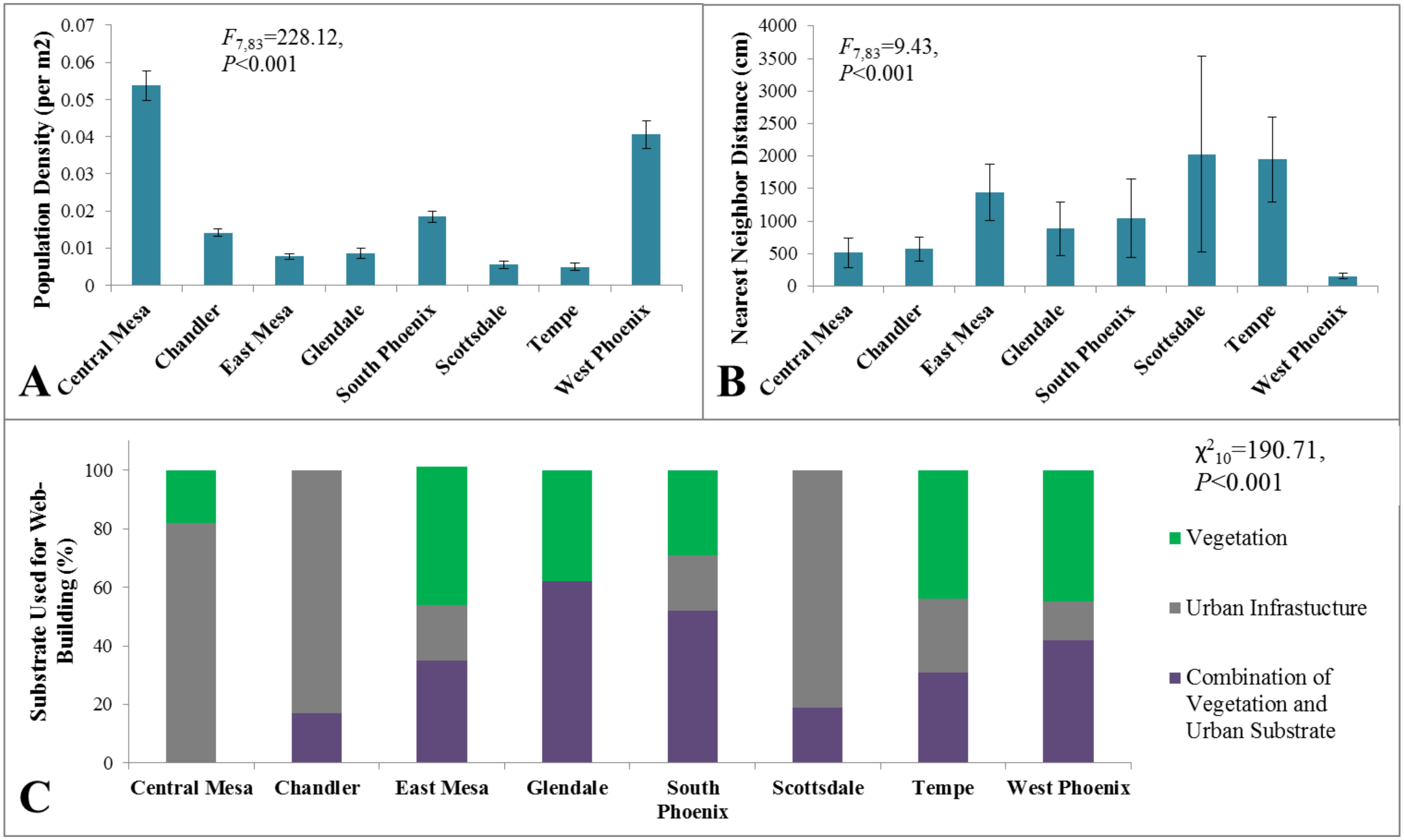


Figure 1.4 Spatial variation in urban subpopulation ecology. Specifically, spatial variation in A. nearest neighbor distance, B. population density, and C. web-building substrate. Values represent mean ± SE (Fig 1.4a, b). See Figure 1.2 for site locations.

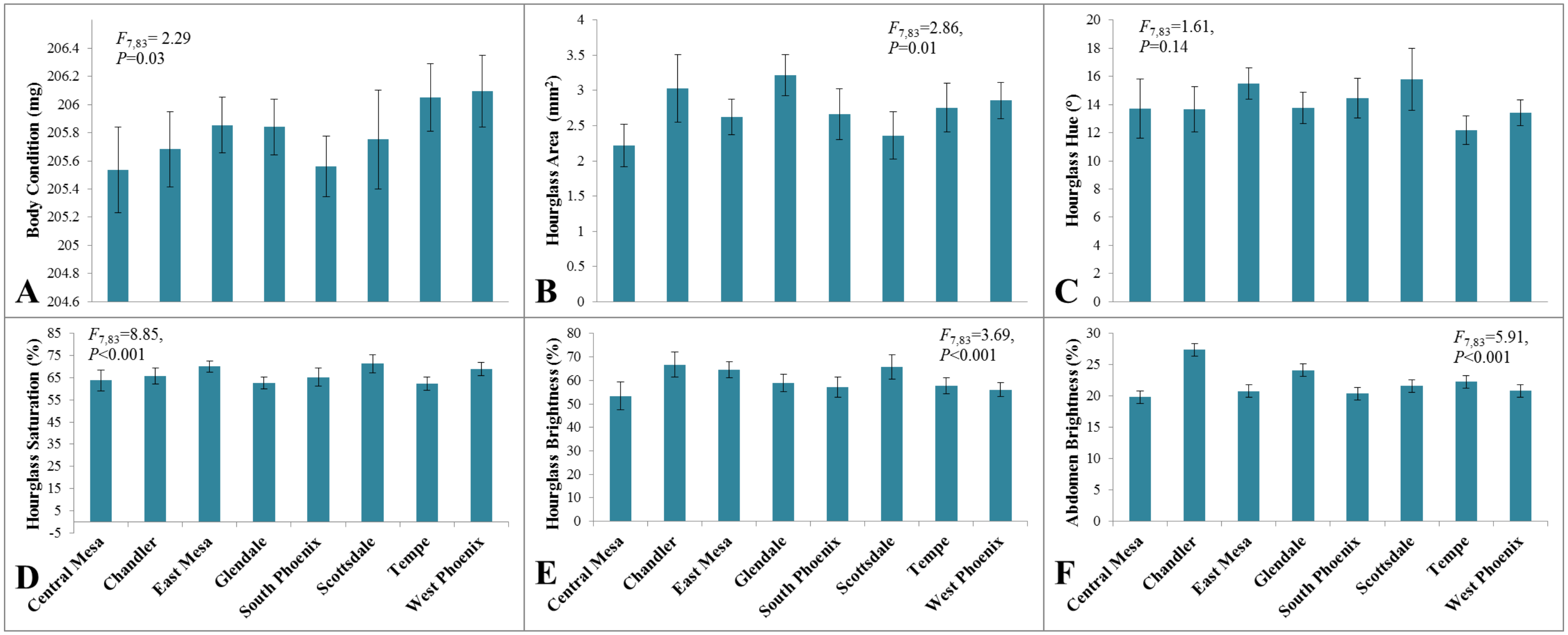


Figure 1.5 Spatial variation in urban subpopulation condition and color. Specifically, spatial variation in A. body condition, B. hourglass area, C. hourglass hue, D. hourglass saturation, E. hourglass brightness, and F. abdomen brightness. Values represent mean ± SE. See Figure 1.2 for site locations.

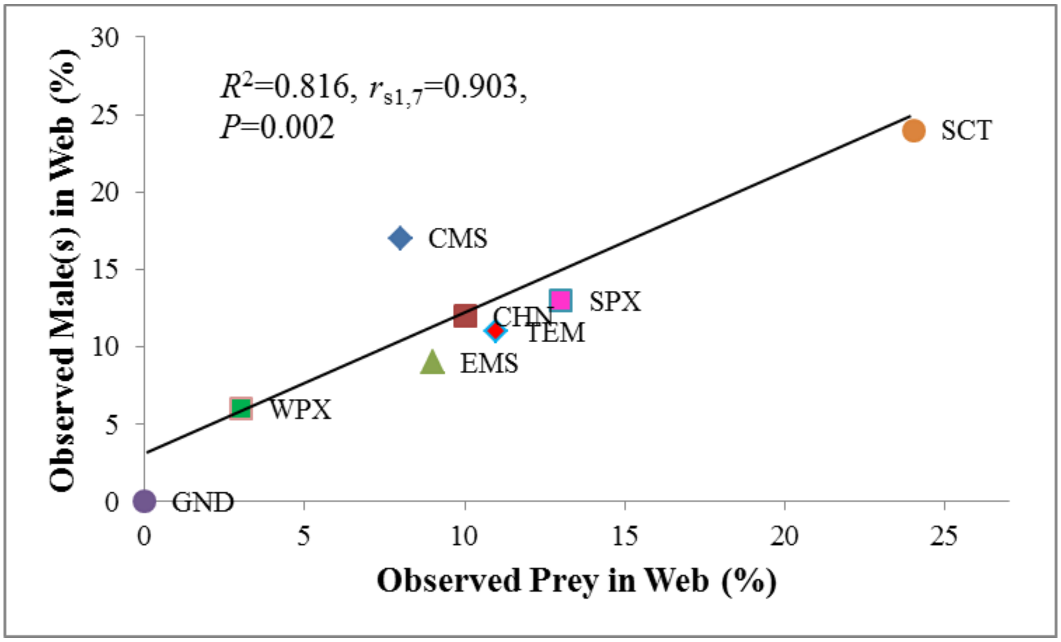


Figure 1.6 Correlation between the presence of males and prey. See Figure 1.2 for site locations.

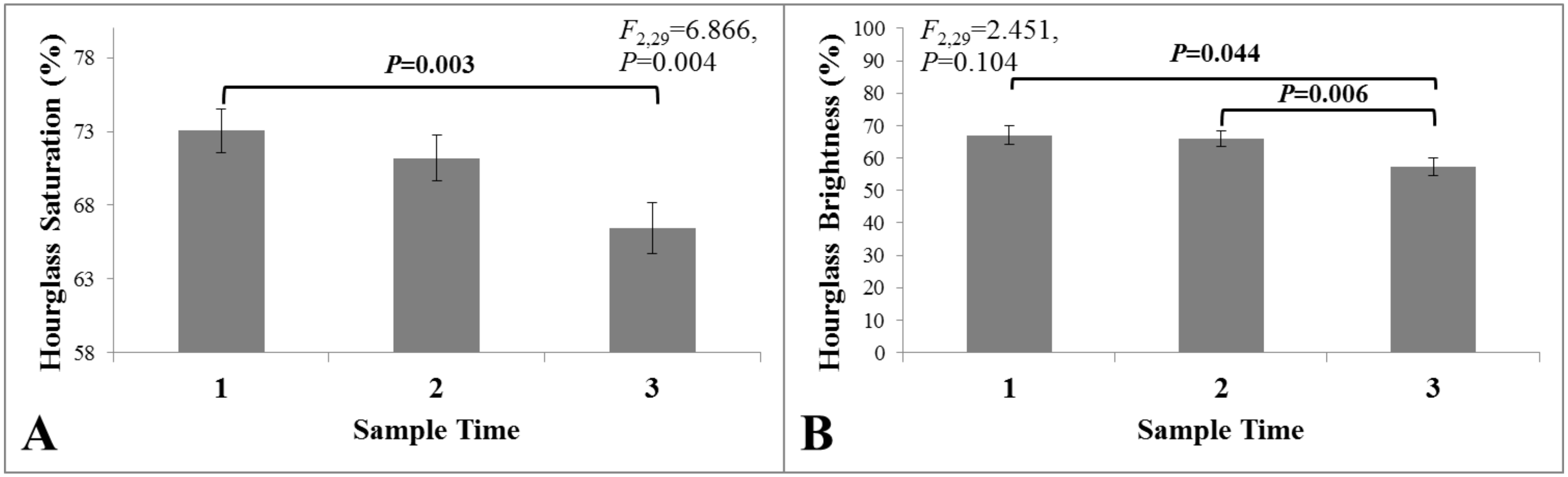


Figure 1.7 Repeated measures effect on hourglass color. Specifically, A. hourglass saturation and B. hourglass brightness.

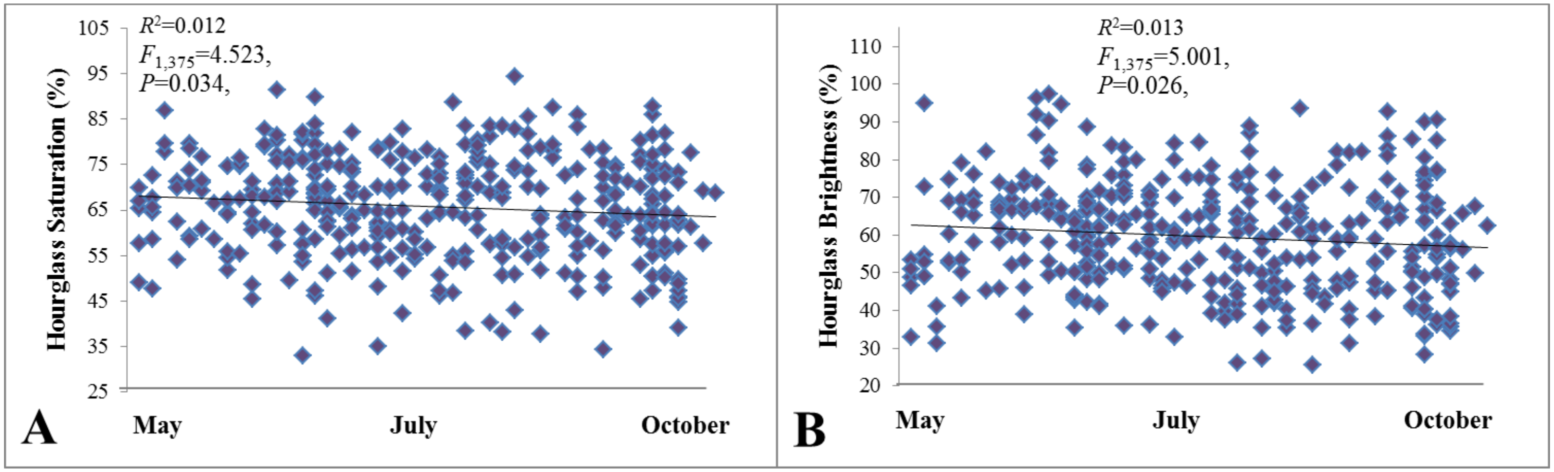


Figure 1.8 Temporal effects on hourglass color. There were marginally non-significant decreases over the breeding season in A. hourglass saturation and B. hourglass brightness.

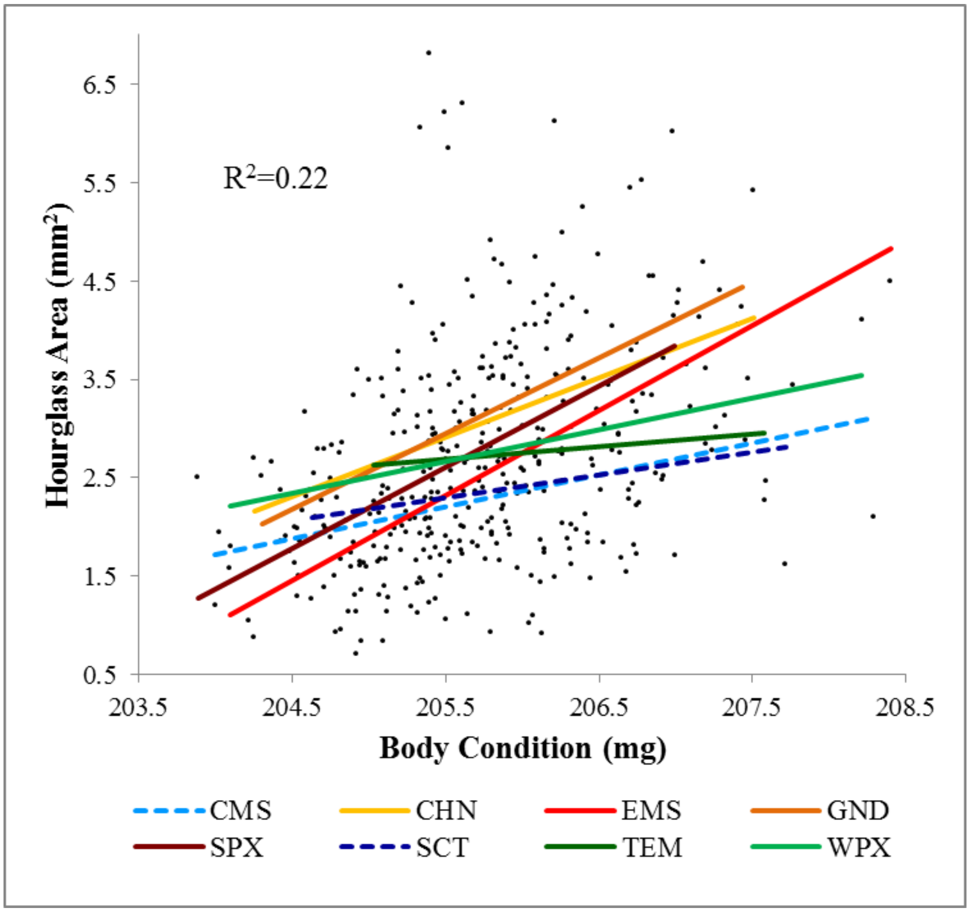


Figure 1.9 Correlation between body condition and hourglass size. Dashed lines represent non-significant correlations. See Figure 1.2 for site locations.

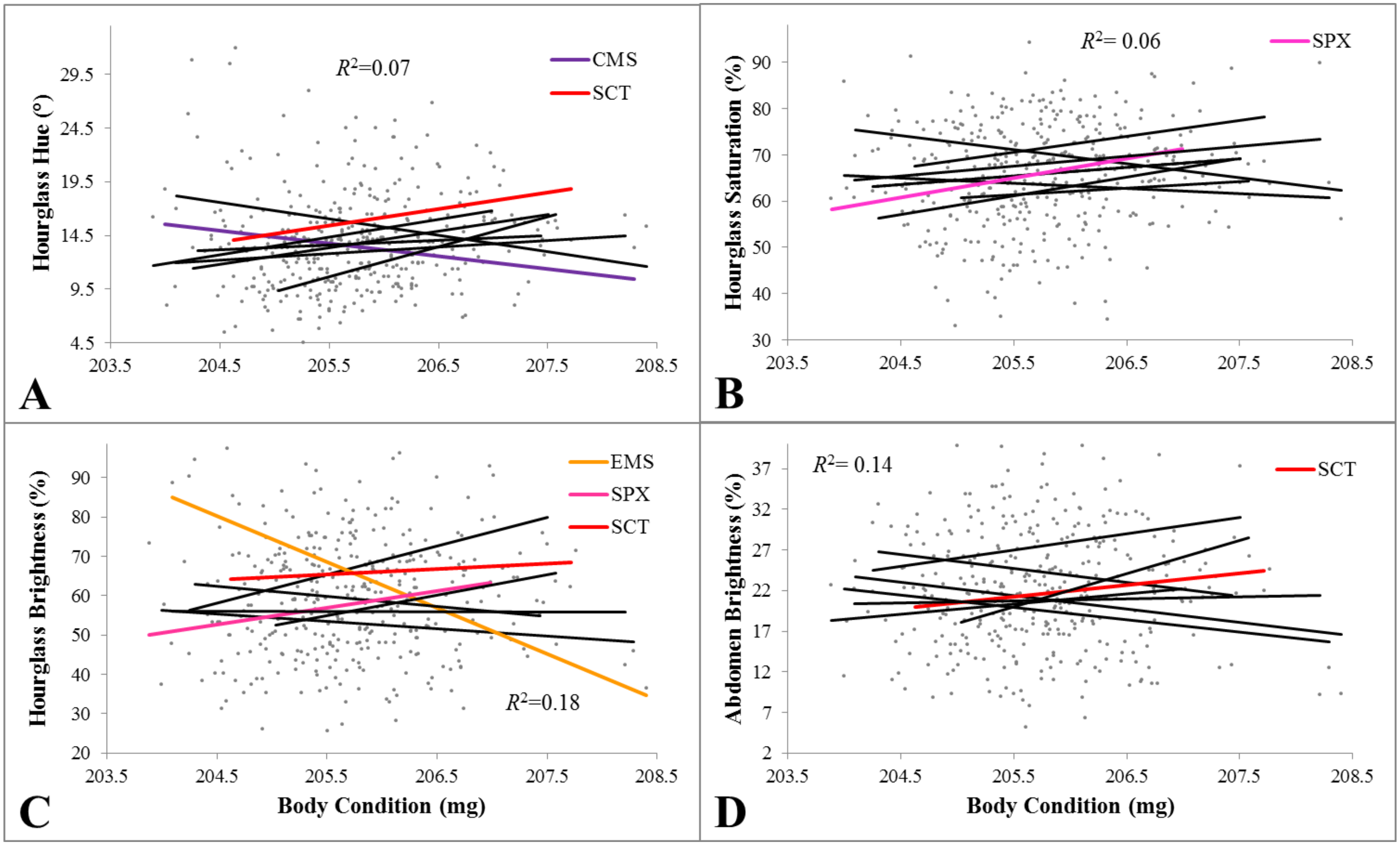


Figure 1.10 Correlations between body condition and hourglass color among sites. Specifically, between body condition and A. hourglass hue, B. hourglass saturation, C. hourglass brightness, and D. abdomen brightness. Colored lines represent significant correlations. See Figure 1.2 for site locations.

CHAPTER 2

VARIATION IN THE BLACK WIDOW'S HOURGLASS ACROSS AN URBAN DESERT AND DIET-INDUCED CONDITION-DEPENDENCE OF HOURGLASS SIZE AND COLOR

Abstract

Organisms vary in their capacity to cope with the effects of 'human-induced-rapid-environmental-change' (HIREC). Urbanization provides an excellent opportunity to examine the impact of HIREC on natural ecosystems. Certain species can dominate in urban habitats, while other species are less able to tolerate such drastic disturbances. This competitive asymmetry can result in the biotic homogenization of urban ecosystems. Phenotypic plasticity is thought to be a mechanism which could allow certain species to flourish in urban environments at the expense of biodiversity. Condition-dependent phenotypes can be highly plastic as their expression fluctuates with an organism's body condition. The Western black widow spider (*Latrodectus hesperus*) forms dense aggregations in urban habitats. In addition, black widows are perhaps best known for the bright red hourglass present on the abdomen. Here, I present field data documenting differences in fixed body size and hourglass coloration between widows residing in urban and desert habitats. Additionally, I identified spatial variation among urban and desert subpopulations in body condition and hourglass color. In general, my field data suggest a strong positive correlation between body condition and hourglass size. I followed this field study with a laboratory diet manipulation to examine the effects of foraging success on hourglass size and color. These empirical data confirm that the black widow's

hourglass is a highly plastic, condition-dependent trait. While the presumed enemy-deterrence function of the hourglass remains to be documented, I speculate that hourglass plasticity and condition-dependence could facilitate a rapid evolutionary response to the increase in arthropod abundance associated with urbanization.

Introduction

Understanding how organisms vary in their ability to cope with the effects of 'human-induced-rapid-environmental-change' (HIREC) was recently highlighted as a 'grand challenge' for organismal biologists (Sih et al., 2010). Urban areas are the most rapidly growing type of environment (Collins et al., 2000), leading to the loss and fragmentation of habitat (Buyantuyeu & Wu, 2009; Pyle et al., 1981). Despite such drastic habitat changes, certain species flourish in urban centers and have been termed 'urban exploiters' (Blair, 1996). These urban exploiters can out-compete other species (e.g. birds: Blair, 1996; spiders: Shochat et al., 2004; Trubl et al., 2011; and butterflies: Blair, 1999). Thus, urbanization and the superabundance of urban exploiters can result in the biotic homogenization of urban environments (Blair, 1996; McKinney, 2006).

The mechanism by which urban exploiters are able to thrive in urban ecosystems is not yet well understood (Shochat et al., 2010). While urban ecologists have developed a strong predictive theory at the level of systems ecology and community ecology (reviewed in Shochat et al., 2006), only rarely do organismal ecologists working at the level of the behavior and morphology of individual organisms address the important ways in which urban disturbance influences phenotypes. This is surprising, given that phenotypic changes in response to HIREC may be the result of plasticity (Hendry et al., 2008).

Phenotypic plasticity is variation in the expression of a genotype due to variation in environmental conditions (Whitman & Agrawal, 2009). Plastic phenotypes may be able to respond more quickly to the rapid and dramatic changes to the environment associated with urbanization (Hendry et al., 2008). In a meta-analysis of more than 3000 rates of phenotypic change in 68 systems, Hendry et al. (2008) documented greater rates of phenotypic change in anthropogenically altered habitats compared to natural habitats.

Human disturbance to natural environments can have not only profound ecological effects, but can also produce accelerated evolutionary responses (Palumbi, 2001). Intriguingly, if urbanization spurs phenotypic plasticity in some organisms, and this plasticity is to some extent grounded by heritable variation (Scheiner & Lyman, 1989), then the plastic traits of urban exploiters may be subjected to strong directional selection and can be expected to evolve rapidly. For example, Partecke & Gwinner (2007) present evidence that the tendency for urban birds to be more sedentary is, at least in part, a heritable trait. As such, the role of phenotypic plasticity in facilitating rapid evolutionary responses to urbanization needs to be better understood.

Behavioral phenotypes are well represented in the literature as being relatively plastic (reviewed in Sih et al., 2010). Behavioral adjustments in urban habitats may be important for expediting resource use, enhancing communication, and disturbance avoidance (reviewed in Sol et al., 2013). Some general behavioral responses to urbanization include switching the time of activity to crepuscular hours, decreased dispersal distances of juveniles, reduction in foraging efficacy, shifts in breeding times, and altered vocalizations, such as bird song (Ditchkoff et al., 2006).

Morphology is often considered to be less plastic than behavior. However, within the last decade there has been a growing body of work on how the plasticity of morphological phenotypes may be influenced by anthropogenically-altered environments. Color displays can be especially sensitive to environmental factors such as temperature, diet, ambient light, background color, predator abundance, competition, and stress (Bradbury & Vehrencamp, 2011). Urbanization can have dramatic impacts on coloration and this is well documented in avian species. For example, Northern Cardinal plumage decreases in brightness with urbanization (Jones et al., 2010).

Much less is known about how urbanization influences arthropod color displays. This is surprising given that a recent review by Umbers et al. (2014) reports up to 121 species of arthropods that exhibit reversible color changes in response to environmental variation. Thus, arthropod coloration can be surprisingly plastic and may be especially sensitive to the rapidly changing environment in urban areas. Urban arthropod communities are well suited for studying the effects of HIREC and phenotypic plasticity as they typically have short generation times and embody a range of trophic levels (McIntyre, 2000; Pyle et al., 1981). Studies focusing on the trophic effects of urbanization indicate that this type of disturbance results in a decrease in arthropod diversity and an increase in arthropod abundance (Pyle et al., 1981; McIntyre et al., 2001; Cook & Faeth, 2006). Therefore, understanding how arthropod coloration responds to urban disturbances could offer insight into how certain arthropod species are able to dominate in urban habitats at the expense of arthropod diversity.

Condition-dependent phenotypes can be highly plastic as their expression fluctuates with foraging success. Body condition measures offer a snapshot of an individual’s physiological state and are often calculated as some relationship between the body mass and body size of an organism (Jakob et al., 1996). Additionally, body condition is often used as an estimate of fitness (Moya-Laraño et al., 2008) because it is closely linked to immunity (Møller & Saino, 1994), fecundity (Moya-Laraño, 2002), and mating success (Cotton et al., 2006). The body condition of generalist predatory arthropods, such as spiders, can be especially sensitive to variation in prey availability and habitat fragmentation. For example, the body condition of orb weaving spiders is known to be closely linked with habitat fragmentation, population density, and prey availability (Bucher & Entling, 2011). Specifically, the body condition of *Araniella opisthographa* positively correlates with prey abundance, but is lowered with increasing isolation from woody habitats. The body condition of *Nuctenea umbratica* negatively correlates with population density, possibly due to competition (Bucher & Entling, 2011).

Additionally, there is a growing body of research suggesting that body condition is also closely associated with coloration in a variety of arthropods (e.g. butterflies: Kemp & Rutowski, 2007; ambush bugs: Punzalan et al., 2008; damselflies: Contreras-Garduno et al., 2008; jumping spiders: Taylor et al., 2011). For example, Taylor et al. (2011) found that the body condition and color of the jumping spider (*Habronattus pyrrithrix*) was significantly enhanced by increasing diet quality. Thus, increased arthropod abundance and decreased arthropod diversity in urban habitats may in turn affect the body condition and subsequent color of animals that prey primarily on arthropod populations.

The Western black widow spider, *Latrodectus hesperus,* is native to Western North America (Garb et al., 2004) and can be found densely aggregated in urban areas (Trubl et al., 2011). *L. hesperus* possesses a potent neurotoxin that is potentially lethal to small children and the elderly, making the black widow a medically important species (Gonzales, 2001). Recently, Trubl et al. (2011) documented a positive relationship between prey availability and spider mass in urban *L. hesperus* subpopulations. Additional findings included a significant effect of spatial variation (i.e. habitat fragmentation) on prey abundance, black widow mass, and population density (Trubl et al., 2011). These findings are contrary to the generalization that urbanization promotes biotic homogenization (Blair, 1996; McKinney, 2006), and instead suggest that habitat fragmentation is capable of producing spatially distinct subpopulations of urban-dwelling black widows.

Adult female *L. hesperus* possess a brightly colored red hourglass on the ventral surface of their abdomen (Foelix, 1996) (Figure 1.1). The hourglass is in stark contrast to the dark brown or black abdomen coloration of female black widows, making the hourglass conspicuous to animals capable of perceiving red wavelengths of light. The hourglass is most readily observed at night when spiders are foraging upside-down in their webs. Although the hourglass is speculated to serve as a warning signal to predators (Oxford & Gillespie, 1998), I can find no empirical evidence to support this claim. My previous research demonstrates a positive correlation between body condition and hourglass size (refer to Chapter 1).

I conducted a field study during the *L. hesperus* breeding season to identify potential differences in morphology, body condition, and hourglass coloration within and among urban and desert black widow populations. Additionally, I tested whether natural variation in the body condition of field-caught widows correlates with the size and spectral qualities of the red hourglass display. I hypothesize that the disparities in prey availability, predation pressure, and habitat fragmentation between urban and desert environments influence *L. hesperus* body condition and subsequent hourglass coloration. I followed this field study with a laboratory diet experiment to empirically examine the condition-dependence of hourglass size and color. Throughout, I predict that the hourglass is a condition-dependent color trait capable of fluctuating with foraging success and spatial variation.

Materials and Methods

Urban and Desert Field Study. I collected between four and ten adult female black widow spiders (n=59) from each of nine urban subpopulations across metropolitan Phoenix (Figure 2.1). These urban aggregations were located in either commercial or residential areas with xeric landscaping. Additionally, I collected between two and seven adult female spiders (n=30) from each of seven subpopulations located in the surrounding relatively undisturbed Sonoran desert (Figure 2.1). All aggregations were located a minimum of 16km apart from one another. Collections were done during the adult breeding season between May and September of 2013. Spiders were retrieved from their webs at night when they are most active. Within 24 hours of collection I recorded each spider’s mass (mg), cephalothorax width (mm), and body condition (see below for calculations). Additionally, I recorded each spider’s hourglass area (mm^2), hue (°), saturation (%), and brightness (%) as well as abdomen brightness (%) (see below for cephalothorax size and hourglass color scoring protocol).

Laboratory Diet Experiment. I randomly selected three adult female spiders from twelve different F-1 generation laboratory-reared families originating from urban, field-caught lineages. Prior to data collection one spider died, resulting in a sample size of 35 spiders across twelve lineages. I followed these individuals for a total of thirteen weeks. During weeks one through six I imposed one of three diet treatments on a randomly selected sister from each family: 1) low-frequency feeding in which spiders were hand fed one house cricket (*Acheta domesticus*) every other week, 2) intermediate feeding in which spiders were hand fed one *A. domesticus* once a week, and 3) high-frequency feeding in which spiders were hand-fed two house crickets *A. domesticus* twice a week. Crickets weighed 50 to 70% of the spider's mass. During week seven, diets were standardized such that all spiders received intermediate feedings (i.e. one *A. domesticus* weekly). During weeks eight through thirteen I switched high and low-frequency feeding treatments so that spiders originally receiving high-frequency feedings received low-frequency feedings and spiders originally receiving low-frequency feedings received high-frequency feedings. Twice a week I recorded each spider's body condition (see below for calculations) as well as the following color measurements from both the upper and lower half of the hourglass: area (mm^2), hue (°), saturation (%), brightness (%), and abdomen brightness (see below for color scoring protocol). Spiders did not respond as predicted to diet treatments. Results of a repeated-measures ANOVA indicated that there was no significant difference between average spider body condition for individuals receiving diet-manipulations (i.e., low-frequency feeding and high-frequency feeding)

($F_{1,18}$=3.445, P=0.08). However, all spiders exhibited extreme variation in body condition during the course of the experiment. Therefore, I identified the corresponding hourglass size and color of each individual's lowest, median, and highest body conditions to use in subsequent repeated-measures analyses.

Scoring Hourglass Coloration. I acquired color data from digital images (Pike, 2011; Stevens et al., 2006). In order to image the spiders they were temporarily anesthetized with CO_2 gas and placed in a mesh restraint device. Each spider was photographed in a dimly lit room in raw NEF format, using a Nikon D50 equipped with a Micro NIKKOR 40mm lens. A Promaster RL60 LED macro ring light was used to standardize illumination. For each imaging session the camera and images were calibrated using an X-rite Colorchecker Passport with a white balance target and 24-patch classic color reference target (X-rite, Grand Rapids, MI, USA). Images were linearized and equalized using Adobe Photoshop CS5.1 in conjunction with PictoColor inCamera ICC profile software (Pike, 2011; Stevens et al., 2006). Hourglass coloration was scored along three conventional axes of color (hue, saturation, and brightness; Hill & McGraw, 2006). Due to the specular nature of the hourglass and abdomen, color was calculated from three point samples taken from areas with no observable illumination reflectance from both the top and lower half of the hourglass at a tolerance level of 40 in Adobe Photoshop.

Previously acquired spectrophotometry data indicates that the curve for mean abdomen reflectance does not display any dramatic spectral peaks (n=30) (Figure 1.3). Therefore, I do not analyze abdomen hue or saturation and only present data for abdomen brightness. From digital images, average measures of brightness for the abdomen were calculated from three point samples taken from either side of the hourglass at a tolerance level setting of 40 in Adobe Photoshop.

Hourglass area was obtained from digital images using public domain Image J software for Windows®. I spatially calibrated the software to recognize the pixel value of a known distance within the image as millimeters. I then outlined the hourglass using a tracing tool to obtain the pixel value of hourglass area in mm^2.

Scoring Fixed Body Size and Body Condition. Cephalothorax width was used as an indicator of fixed adult body size because the cephalothorax is heavily sclerotized and does not change in size after maturation or with adult feeding (Jakob et al., 1996; Moya-Laraño, 2008). Using public domain Image J software for Windows®, I obtained measures of cephalothorax width from digital images. Each image included a reference scale, allowing us to convert pixel values into millimeters.

For urban-desert field comparisons, I calculated body condition as the ratio of mass (mg) to cephalothorax width (mm). This body condition ratio index is the preferred measurement used for population comparisons since it does not require the assumption that different populations have the same average mass (Jakob et al., 1996). To examine differences within urban and desert populations I calculated body condition using the residual index method (average body mass corrected for body size using residuals for the cube root of mass regressed on cephalothorax width). Residual index body conditions are recommended for detecting differences between groups drawn from the same population. Additionally, they are less sensitive to variation in fixed body size due to the standardization of body condition values using a pooled average mass (Moya-Laraño et al., 2008).

All spiders used in the laboratory diet study were F-1 generation from only urban lineages. Therefore, I consider my laboratory spiders as a single population and calculated body condition using the residual index method (Moya-Laraño et al., 2008). To compare the body condition of field spiders with the body condition of laboratory spiders I used body condition ratio indices (mg/mm).

Statistical Analysis. All statistical tests were performed in SPSS (Ver. 17.0 for Windows® SPSS, Chicago, IL, USA). Shapiro-Wilk goodness-of-fit tests were used to ensure the data were normally distributed. To compare color parameters from the upper and lower half of the hourglass I performed a two-tailed student's t-test for paired samples. Finding no significant differences (see below), subsequent analyses were performed using averages for total hourglass size and color.

For the urban-desert field comparison study, I used a two-tailed unequal variance t-test for independent samples to examine potential differences in body mass, cephalothorax width, body condition, hourglass area, and color metrics between spiders from urban and desert habitats. To account for multiple tests I employed a Bonferroni correction ($\alpha=0.05/8$, $\alpha=0.006$). Finding significant differences between habitat types (see below), I chose to perform subsequent analyses separately for urban and desert populations. To assess if body condition, hourglass area, and/or color vary with site I performed univariate ANOVAs, including population site as a random factor. Findings indicated significant spatial variation (see below). Thus, I chose to remain conservative and used site averages in linear regression analyses to identify correlations between body condition and hourglass area and/or color.

For the laboratory diet study, I ran a repeated-measures ANOVA to evaluate if an individual's lowest, median, and highest body conditions were significantly different from one another and to determine if hourglass area and color varied with body condition. I included family as a between-subjects factor to address possible family effects. To account for multiple tests, I used Bonferroni post-hoc comparisons. To assess how the natural range of body condition compares to the body condition of spiders reared on laboratory diets I compared body condition ratio indices (Jakob et al., 1996) using a univariate ANOVA with state (low, median, high, and field body condition) as a fixed factor. I used Bonferroni post-hoc comparisons to identify differences between groups.

Results

Urban and Desert Field Study. As noted in the methods, the upper and lower half of the hourglass did not differ in size or color (Table 2.1). Therefore, all further analyses of the hourglass involve values averaged across measurements of the upper and lower halves of the hourglass. I discovered a marginally non-significant trend for female spiders to be heavier in urban habitats (Figure 2.2a). Additionally, urban spiders were significantly larger than desert spiders (Figure 2.2b). Body condition and hourglass size did not differ with habitat type (Figure 2.2d). Hourglass hue did not differ between urban and desert populations (Figure 2.3a). There was a marginally non-significant trend for desert spiders to produce hourglasses that were more saturated values (Figure 2.3b). Hourglass brightness did not vary between habitats (Figure 2.3c). However, there was a marginally non-significant trend (α=0.006) for desert spiders to produce more reflective abdomens (Figure 2.3d).

I found significant variation in body condition and coloration among aggregations for both urban and desert habitats (Table 2.2). Specifically, hourglass brightness varied among subpopulations in both urban and desert habitats (urban: $F_{8,50}$=3.528, P=0.003; desert: $F_{6,23}$=3.42, P=0.015), while hourglass hue varied among only urban subpopulations ($F_{8,50}$=3.76, P=0.002) and body condition varied among only desert subpopulations ($F_{6,23}$=3.128, P=0.022). Other measures did not exhibit significant spatial variation in either urban or desert habitats (all P>0.05).

I also found body condition to be a strong predictor of hourglass size in both urban (Figure 2.4a) and desert populations (Figure 2.4b). There were no significant correlations between body condition and hourglass color parameters in urban subpopulations (hue: R^2=0.084, $F_{1,8}$=0.049, P=0.83, saturation: R^2=0.146, $F_{1,8}$=1.193, P=0.311 , brightness: R^2=0.154, $F_{1,8}$=1.276, P=0.296, abdomen brightness: R^2=0.427, $F_{1,8}$=5.207, P=0.056), or desert subpopulations (hue: R^2=0.037, $F_{1,8}$=0.194, P=0.678, saturation: R^2=0.067, $F_{1,8}$=0.356, P=0.577 , brightness: R^2=0.208, $F_{1,8}$=1.316, P=0.303, abdomen brightness: R^2=0.001, $F_{1,8}$=0.004, P=0.953).

Laboratory Diet Experiment. There was no effect of family on body condition or hourglass display coloration (all P>0.05). I was able to identify three significantly different body conditions (i.e., low, median, and high) for each individual spider across the duration of the laboratory diet manipulation (Figure 2.5a). Notably, individuals in low, median, and high body conditions produced hourglasses that varied significantly in size (Figure 2.6a). Post-hoc comparisons indicated that hourglass size increased in spiders from low to median to high body condition (Figure 2.6a). Hourglass hue also varied significantly with body condition (Figure 2.6b). Specifically, high-condition spiders had hourglasses that were more orange (i.e., higher hue values) than low-condition spiders (Figure 2.6b), but not more orange than median-condition spiders (Figure 2.6b). Additionally, hourglass and abdomen brightness varied with body condition (Figure 2.6c, d). Individuals in high-body condition had brighter hourglasses and abdomens than individuals in either low or median-condition (Figure 2.6c, d). Low and median-condition spiders did not differ with respect to hourglass and abdomen brightness (Fig 2.6c, d). There was no repeated measures effect on hourglass saturation across body conditions ($F_{2,22}$=0.1, P=0.905).

Additionally, I detected differences in body condition between field-caught and laboratory spiders (Figure 2.5b). Post-hoc comparisons revealed that average field-caught spider body condition was significantly lower than high-condition lab spiders (Fig 2.5b). The body condition of field-caught spiders was also marginally lower than median-condition laboratory spiders (Fig 2.5b). There was no significant difference between field-caught and low-condition laboratory spiders (Fig 2.5b).

Discussion

General Summary of Findings. My field data indicate that urban spiders are significantly larger but not heavier than spiders from undisturbed desert habitats. However, I found no significant difference in body condition between urban and desert spiders. I also discovered a strong trend for desert-dwelling spiders to produce more saturated hourglasses and more reflective abdomens than urban-dwelling spiders. Additionally, I documented spatial variation in body condition and hourglass coloration within urban and desert habitats. Specifically, hourglass brightness varies among urban and desert subpopulations, while body condition varies only among desert subpopulations and hourglass hue varies only among urban subpopulations. I observed a positive correlation between body condition and hourglass size in spiders from both habitats. The results of my laboratory experiment offer empirical evidence for the condition-dependence of hourglass size. Moreover, my findings suggest that when fed in way that produces extremely high body condition, black widows generate larger, brighter, and more orange hourglasses as well as brighter abdomens.

Urban and Desert Field Study. I observed urban black widows to be marginally heavier and significantly larger than desert widows. This may be explained by the increase in the productivity of urban habitats due to water supplementation and resource abundance (reviewed in McKinney, 2002). Urban management strategies often make human-subsidized resources available for urban-dwelling organisms (Shochat et al., 2006), resulting in an increase in the abundance of potential arthropod prey items for widows. Indeed, many arthropod species are well documented to be superabundant in urban habitats (cockroaches: Bateson & Dripps, 1972; Theridiid spiders: Crawford, 1979; house crickets: Garber, 1987; beetles: Lazenby, 1988) and urban arthropod densities up to 3,854 individuals per square meter have been recorded (Majzlan & Holecova, 1993).

Increases in urban prey abundance (Shochat et al., 2006) and longer times of the year when prey are available (Wilby, 2006), may heavily influence the body size of juvenile widows residing in urban habitats. Cephalothorax width was used as an indicator of fixed adult body size because the cephalothorax is heavily sclerotized and does not change in size after maturation or with adult feeding (Jakob et al., 1996; Moya-Laraño, 2008). Many urban-dwelling organisms begin breeding seasons earlier, due to warmer temperatures in cities (Erz, 1966; Wilby, 2006). Anecdotally, I have observed widows emerging as early as February and persisting as late as October in urban habitats. Moreover, the prey abundance of urban black widow subpopulations is known to exhibit minimal temporal variation during the course of the breeding season (Trubl et al., 2011). A typical response of spiders to food limitation is maturation at a smaller size (Moya-Laraño et al., 2003; Uetz, 1992; Uetz et al., 1992). Urban spiders are not constrained by limitations in prey availability and thus they likely mature at larger sizes.

Despite differences in mass and fixed body size, urban and desert spiders did not differ in body condition. Intriguingly, this implies that although urban and desert juvenile spiders may differ with respect to prey availability early in the breeding season, these differences are weakened during the peak of the breeding season when prey is available in both of these habitat types. Some studies suggest that the fixed body size of spiders is a more reliable predictor of fecundity than body condition (Arnquist & Hendriksson, 1997; Beck & Connor, 1992; Moya-Laraño, 2003). Therefore, urban widows may not only be able to grow larger due to more opportunities for foraging success, but they may also be more reproductively successful than desert spiders as they can begin breeding earlier in the season. This suggests a possible mechanism by which black widows are able to densely populate urban habitats.

These findings are contrary to work by Johnson et al. (2012) that documented urban black widows as being in worse condition and less fecund than their desert counterparts. This discrepancy may be due to the fact that I sampled nine urban subpopulations and seven desert subpopulations, while Johnson et al. (2012) sampled only one urban and one desert subpopulation. Thus, my sample may be more representative of variation in body condition within urban and desert environments. Indeed, urban widow subpopulations are known to be spatially distinct (present study; Trubl et al., 2011), requiring that multiple urban subpopulations be sampled to identify trends in widow population ecology.

With respect to coloration, I found a strong trend for desert spiders to produce more saturated hourglasses and brighter abdomens. Relatively little work has been done investigating how urbanization impacts spider coloration. This is surprising given that spider species belonging to the Thomisidae, Araneidae, Oxyopidae, Heteropodidae, and Linyphiidae families are known to alter their color in response to changes in the environment (reviewed in Oxford & Gillespie, 1998). For example, the flower-living crab spider (*Misumena vatia*) alters the color of its opisthosoma between white and yellow in order to match the color of flower on which it is foraging to be less conspicuous to prey and predators (Packard, 1905). Additionally, many *Poecilotheria* species have brightly colored hairs on their fangs and legs which they display to potential enemies when threatened (Bennie et al., 2011; reviewed in Oxford & Gillespie, 1998).

I speculate that urban black widows may be less colorful than their desert counterparts due to the release of natural enemies caused by human activities (reviewed in McKinney, 2002). The egg sacs of *L. hesperus* are known to be attacked by egg predators such as the fly *Pseudogaurax signatus* and insect parasitoids such as the wasp *Baeus latrodecti* (Pierce, 1942; Vetter et al., 2012). To date I have only recorded instances of egg sac predation and parasitism in desert widows (Gburek & Johnson, unpublished data). While the hourglass is speculated to function as a warning signal to enemies (Oxford & Gillespie, 1998), there is no empirical evidence supporting this claim.

Desert spiders may experience more pressure from enemies and thus, produce more saturated hourglasses and brighter abdomens. This is consistent with findings suggesting that red coloration as well as increased luminance (i.e., brightness) can promote initial predator avoidance and enhance avoidance learning (Prudic et al., 2007; reviewed in Stevens & Ruxton, 2012).

My findings also document significant spatial variation in body condition and hourglass coloration within urban and desert habitats. Urbanization is commonly thought to result in environmental homogenization due to the uniform nature of cities across the globe (Blair, 1996; McKinney, 2006). My findings are contrary to this in that I found significant differences in widow morphology and coloration within urban habitat. Recently, Trubl et al. (2011) uncovered spatial variation in female black widow body mass, population density, and prey availability across urban aggregations. Schweiger et al. (2010) suggests that biotic interactions in concert with climate change, habitat loss, and biological invasions affect different species differently. Thus, while certain taxa may indeed experience urban homogeneity, anthropogenic disturbances can also create a variety of sub-habitats, which can promote spatial heterogeneity across the landscape (McKinney, 2008; Van Keer et al. 2010). My data suggest that there are factors within urban and desert habitats (e.g., prey abundance) that vary spatially and may influence the body condition and coloration of black widows.

I observed a significant positive relationship between body condition and hourglass size in both urban and desert habitats. This may be due to the stretching of the abdomen with increased foraging success (Moya-Laraño et al., 2002). Thus, hourglass size may serve as an honest indicator of female body condition and past foraging success.

Therefore, a potential function of the hourglass may be a role in male mate choice. Johnson et al. (2011) documented a courtship bias of males towards high-condition females which reduced the threat of sexual cannibalism. Perhaps hourglass size is a condition-indicating signal males exploit to avoid sexual cannibalism.

Laboratory diet experiment. My laboratory diet manipulations offer empirical evidence for the condition-dependence of hourglass size. My data indicate that widows in low, median, and high body conditions produced significantly different and increasingly sized hourglasses. Surprisingly, I observed that high condition lab spiders produced brighter and more orange (i.e., higher hue values) hourglasses and brighter abdomens than either median or low-condition lab spiders. It’s important to note that high-condition and median-condition lab spiders produced significantly better body conditions than field-caught spiders. This suggests that some spectral qualities of the hourglass can exhibit condition-dependence when body condition exceeds natural variation.

My finding that hourglass color only exhibited condition-dependence when spiders were forced to produce extreme body conditions has implications for the potential direction of the evolution of this phenotype. A common hypothesis in the literature explaining the evolution of conspicuous traits is that inherent biases of perceptual systems respond strongly to exaggerated stimuli (Darwin, 1871). Many studies have documented how novel or artificial traits could be favored by natural selection (Arak & Magnus, 1993; Burley & Symanski, 1998). While the intended receiver of this signal is presently unknown, the generalization for stronger perceptual responses to exaggerated stimuli supports the idea that selection would favor the production of enhanced hourglass size and color. Alternatively, because my field collections were limited to the Phoenix metropolitan area and the surrounding Sonoran desert, Phoenix widows may not be a representative enough sample to observe the condition-dependence of hourglass coloration I observed in high-condition lab spiders. I have previously observed widows with extreme body conditions similar to those of our high-condition lab spiders in Northern California and New Mexico (Johnson, unpublished data).

Future Directions and Concluding Remarks. Future research should aim to document how variation in the site-specific ecology of aggregations in urban and desert habitats correlates with black widow body condition and hourglass coloration. Such data could help identify the mechanism by which black widows are able to flourish in urban environments and uncover possible trajectories for the evolution of the red hourglass phenotype. Additionally, the functional significance of the red hourglass is yet to be identified. A likely role of this trait is predator deterrence (Oxford & Gillespie, 1998), given their venomous nature. Thus, we need empirical studies testing the effect of hourglass exaggeration and obstruction on predation.

Human disturbance to natural environments has profound ecological effects and can also produce accelerated evolutionary responses (Palumbi, 2001). Evolutionary biologists contend that phenotypic plasticity can be important in evolutionary processes with respect to how environmental variation influences phenotypic variation (reviewed in Wund, 2012). My data characterize the black widow’s hourglass as extremely plastic and capable of fluctuating with foraging success and environmental variation. If the plasticity of hourglass size and color is grounded by heritable variation (Scheiner & Lyman, 1989), than it may be subjected to strong directional selection. Therefore, the response of hourglass plasticity to the drastic and abrupt changes in urban habitats (Buyantuyeu & Wu, 2009; Pyle et al., 1981) may result in the rapid evolution of this phenotype.

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Table 2.1

Comparision between the upper and lower halves of the hourglass. No significant differences in size or color.

Measure	*t* -value	df	*P* -value
Area (mm^2)	-1.384	212	0.168
Hue (°)	0.918	212	0.36
Saturation (%)	-0.165	212	0.869
Brightness (%)	-0.066	212	0.947

Table 2.2

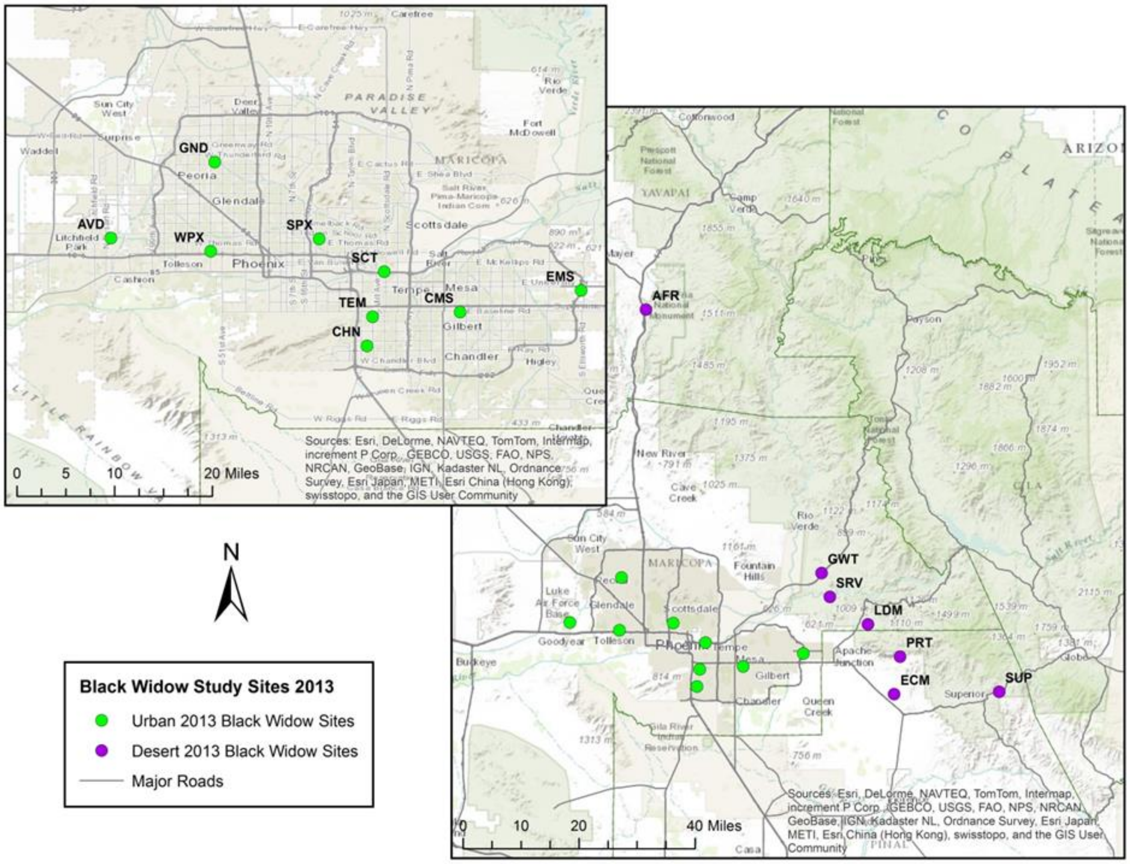
Spatial variation in urban and desert subpopulation condition and color. Univariate ANOVA testing for differences in body condition and coloration between sites. Values represent mean ± SE. See Figure 2.1 for site locations.

Urban populations

ANOVA, Site	n	Body condition (mg)	Hourglass area (mm^2)	Hourglass hue (°)	Hourglass saturation (%)	Hourglass brightness (%)	Abdomen brightness (%)
$F_{8,50}$	-	0.832	0.905	**3.76**	0.43	**3.528**	0.707
P -value	-	0.075	0.52	**0.002**	0.897	**0.003**	0.684
Chandler (CHN)	10	260.38 ± 0.48	3.71 ± 0.63	12.1 ± 5.35	66.58 ± 11.01	64.36 ± 7.13	22.26 ± 1.9
Scottsdale (SCT)	4	260.28 ± 0.22	3.48 ± 0.98	8.79 ± 2.77	56.16 ± 10.5	74.83 ± 9.14	22.37 ± 2.06
Tempe (TEM)	8	260.39 ± 0.4	3.26 ± 1.13	15.85 ± 5	64.85 ± 9.6	75.54 ± 13.9	21.1 ± 1.6
South Phoenix (SPX)	8	259.84 ± 0.18	2.96 ± 0.64	13.14 ± 3.46	62.29 ± 11.62	64.06 ± 11.03	21.66 ± 2.36
Glendale (GND)	7	260.3 ± 0.33	3.32 ± 0.97	10.52 ± 3.61	66.73 ± 10.72	83.66 ± 6.52	21.85 ± 1.99
West Phoenix (WPX)	6	260.36 ± 0.31	3.56 ± 1.27	12.33 ± 3.95	61.44 ± 20.16	70.5 ± 7.99	22.3 ± 2.5
Central Mesa (CMS)	7	260.2 ± 0.3	3.05 ± 0.76	12.14 ± 4.48	67.83 ± 20.59	69.47 ± 8.64	20.61 ± 1.97
East Mesa (EMS)	4	259.12 ± 0.49	2.45 ± 1.35	11.58 ± 6.5	69.62 ± 13.67	67.04 ± 3.19	20.25 ± 0.61
Avondale (AVD)	5	260.17 ± 0.21	3.43 ± 0.81	21.6 ± 1.26	66.6 ± 11.78	70.9 ± 3.19	21.53 ± 1.82

Desert populations

ANOVA, Site	n	Body condition (mg)	Hourglass area (mm^2)	Hourglass hue (°)	Hourglass saturation (%)	Hourglass brightness (%)	Abdomen brightness (%)
$F_{6,23}$	-	**3.128**	1.167	1.89	1.429	**3.42**	1.533
P -value	-	**0.022**	0.357	0.126	0.246	**0.015**	0.212
Agua Fria (AFR)	5	212.08 ± 0.28	3.8 ± 1.15	12.36 ± 4.73	71.5 ± 5.48	75.03 ± 2.14	21.73 ± 2.85
Peralta (PRT)	3	211.46 ± 0.68	3.74 ± 1.06	20.05 ± 5.49	62.94 ± 7.11	82.55 ± 13.27	21.61 ± 0.75
Great Western (GWT)	3	210.96 ± 0.80	3.21 ± 1.1	15.83 ± 4.8	76.16 ± 1.8	69.94 ± 8.49	24.66 ± 2.25
El Camino (ECM)	2	210.74 ± 0.16	2.27 ± 0.27	9.58 ± 1.29	60.08 ± 0.58	67.58 ± 1.53	24.66 ± 1.17
Superior (SUP)	7	210.88 ± 0.25	3.05 ± 1.07	15.71 ± 3.8	69.85 ± 9.4	65.57 ± 5.83	23.16 ± 6.13
Salt River (SRV)	7	210.38 ± 0.18	2.69 ± 0.94	12.14 ± 4.9	69.35 ± 11.48	69.21 ± 1.12	24.69 ± 1.95
Lost Dutchman (LDM)	3	211.62 ± 0.18	3.69 ± 0.73	12.94 ± 2.98	75.94 ± 1.71	66.33 ± 1.09	32.44 ± 13.54



Sources: Esri, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community

Figure 2.1 Location of urban and desert subpopulations. Urban sites: AVD – Avondale, CHN - Chandler, CMS – Central Mesa, EMS – East Mesa, GND – Glendale, SCT – Scottsdale, SPX – South Phoenix, TEM – Tempe, WPX – West Phoenix; and Desert sites: AFR – Agua Fria, ECM – El Camino, GWT – Great Western Trail, , LDM – Lost Dutchman Park, PRT – Peralta Trail, , SRV – Salt River Wash, SUP – Superior.

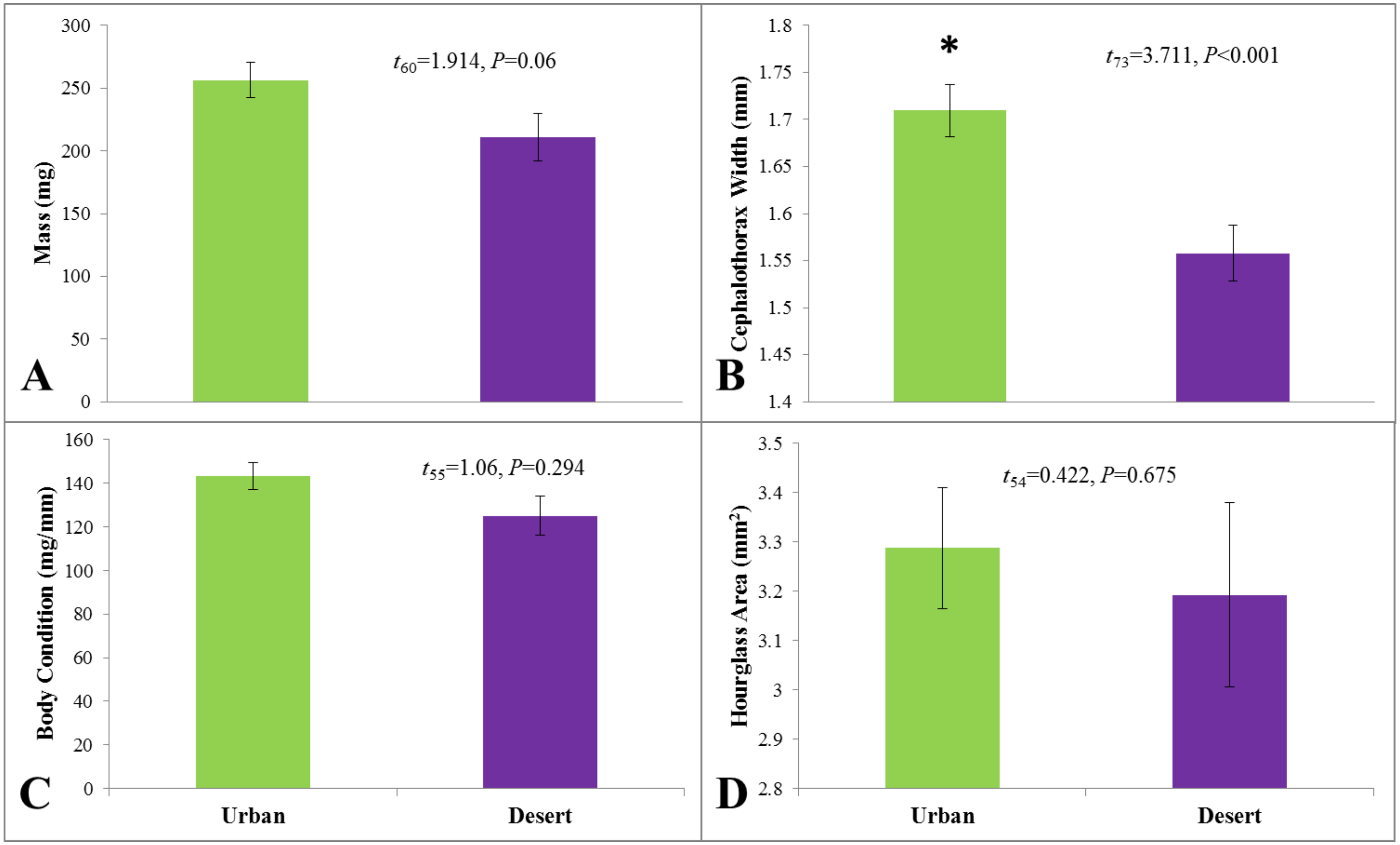


Figure 2.2 Differences between urban and desert spider's morphology. Specifically, differences in A. mass, B. cephalothorax width, C. body condition, and D. hourglass area. Values represent mean ± SE.

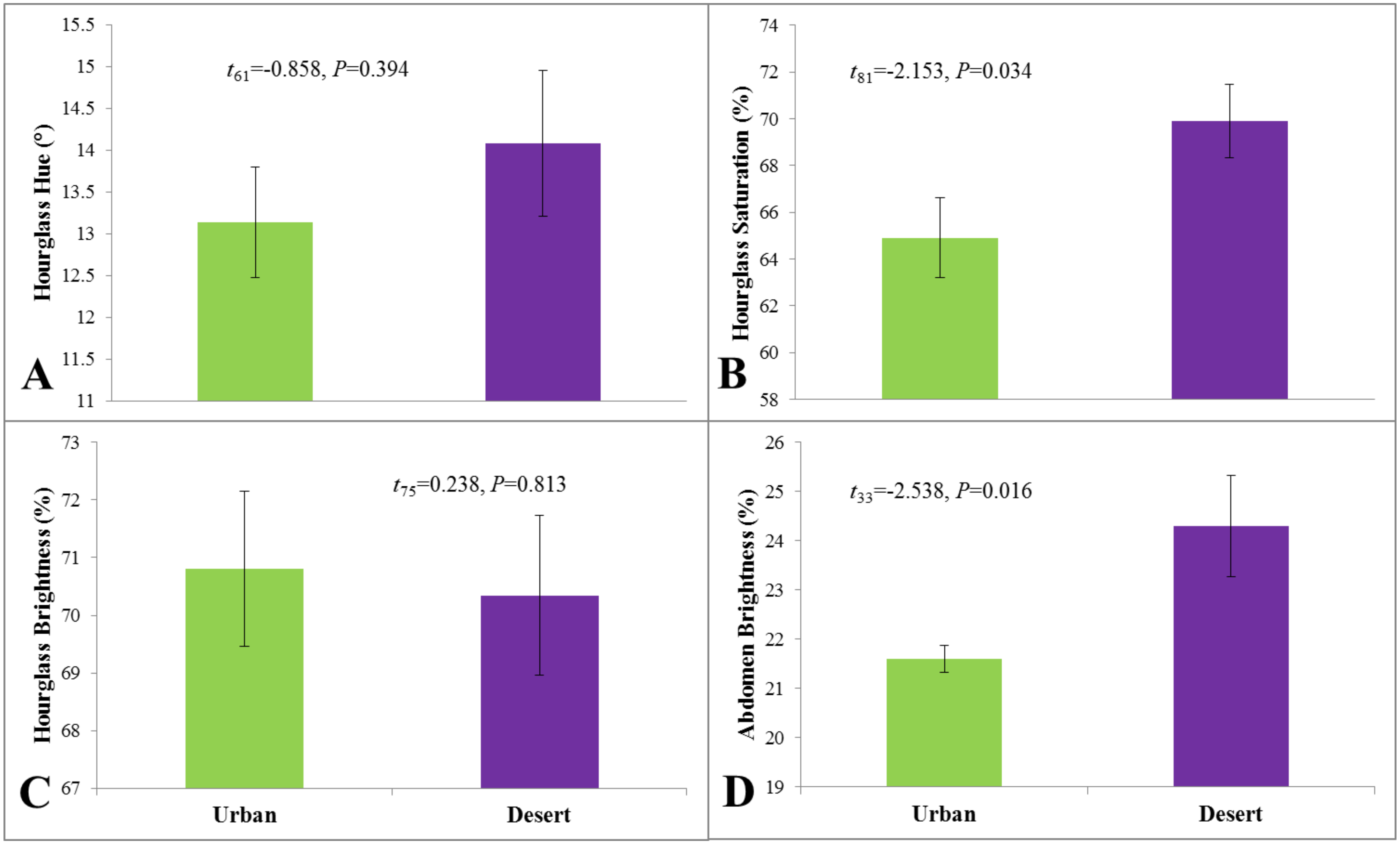


Figure 2.3 Differences between urban and desert spider's color in A. hourglass hue, B. hourglass saturation, C. hourglass brightness, and D. abdomen brightness. Values represent mean ± SE.

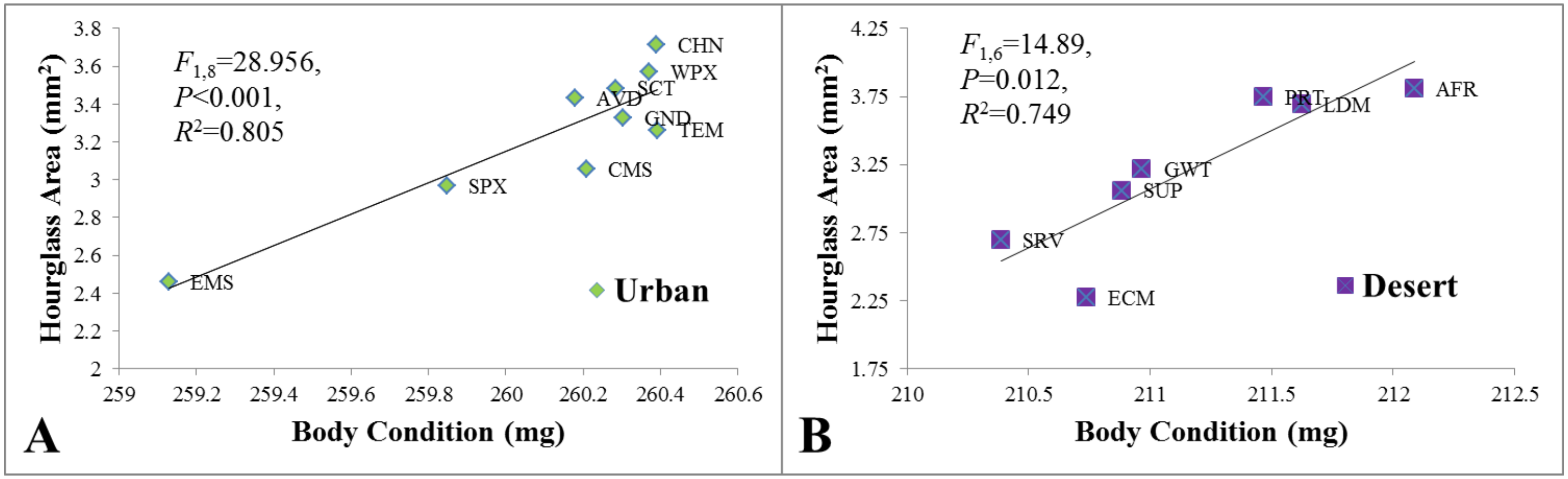


Figure 2.4 Condition-dependence of hourglass size in urban and desert habitats. Body condition was positively correlated with hourglass area in A. urban spiders and B. desert spiders. Values represent site averages. See Figure 2.1 for site locations.

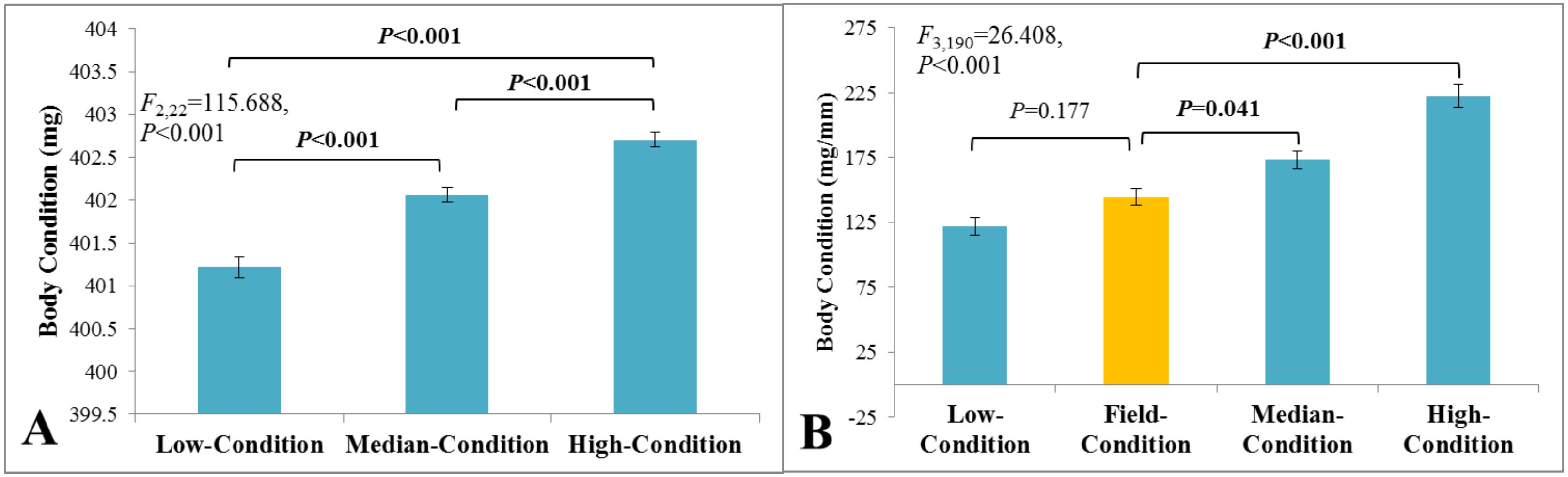


Figure 2.5 Diet-induced variation in body condition compared to field conditions. Individuals maintained on laboratory diets produced A. low, median and high-body conditions that differed significantly from one another. B. Field caught spiders average body condition is significantly different from laboratory median and high body conditions. Values represent mean ± SE.

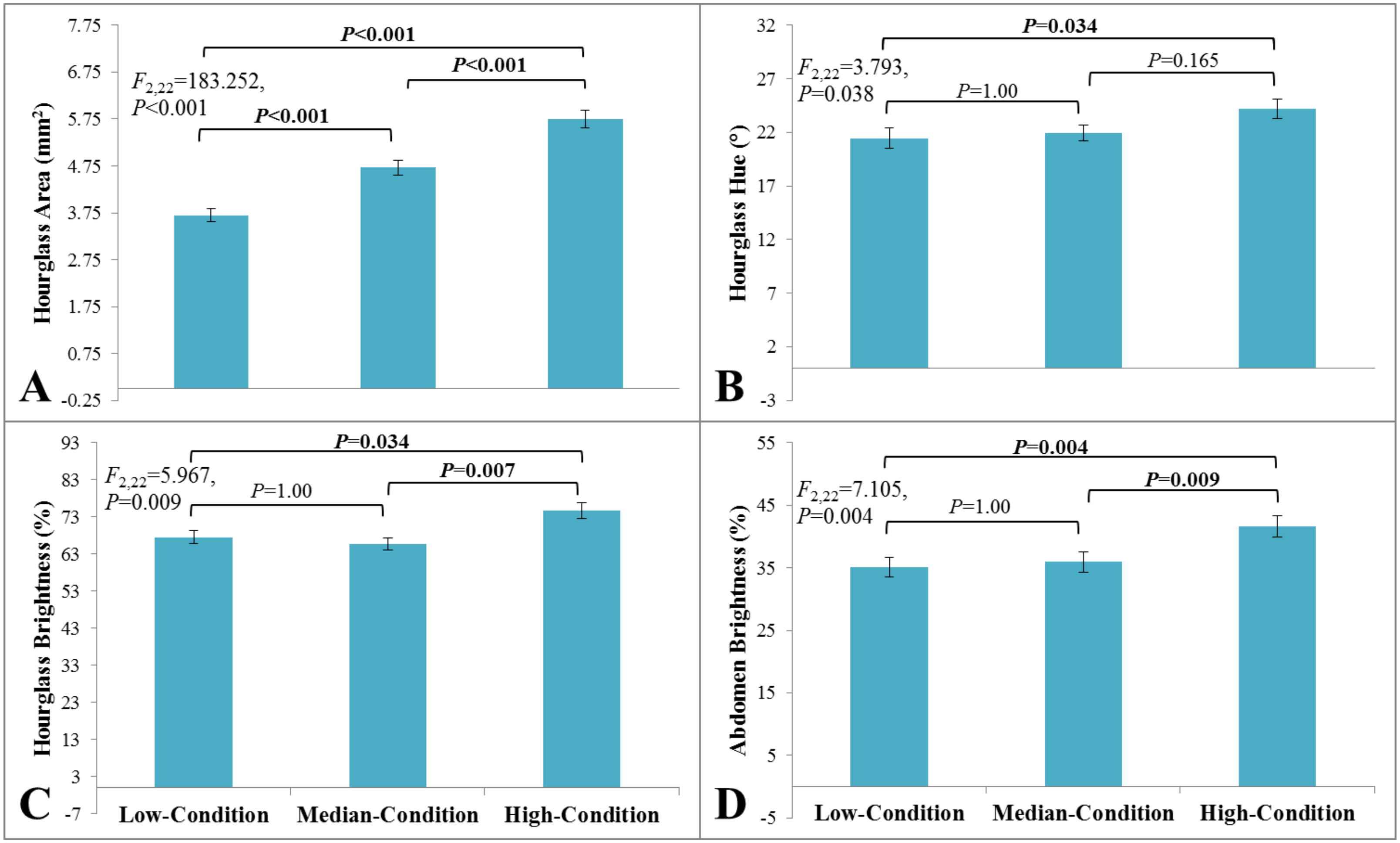


Figure 2.6 Color variation with variation in body condition. Variation with body condition in individual spider's A. hourglass area, B. hourglass hue, C. hourglass brightness, and D. abdomen brightness. Values represent mean ± SE.

CHAPTER 3

IN SEARCH OF A FUNCTIONAL ROLE FOR THE BLACK WIDOW'S RED HOURGLASS: NO EFFECT OF OBSCURING OR ENLARGING THE HOURGLASS IN AGONISM, MATING, OR ANTI-PREDATOR CONTEXTS

Abstract

The prevalence of conspicuous coloration is perplexing in that it presumably is costly to produce and may increase detection by enemies. Hypotheses explaining the function of bright coloration suggest that it signals some sort of information about an individual to the receiver and/or is selected as a mechanism to improve signal transmission. Many animals display conspicuous coloration during agonistic interactions, courtship, and predator deterrence. The Western black widow spider (*Latrodectus hesperus*) is an excellent system in which to examine the role of conspicuous coloration. Female widows are cannibalistic (i.e., agonism between females), sexually cannibalistic (i.e., agonism towards courting males), and bold towards enemies. In addition, adult female *L. hesperus* possess a brightly colored red hourglass on the ventral surface of the abdomen. While this trait is commonly speculated to function as a warning signal to predators, there are no published data to support this contention. I manipulated the hourglass by either obscuring it (using black paint) or enlarging it to twice the size of average hourglasses (using red paint). I then examined the role of hourglass presence and size in the context of agonism, mating, and predation (using the Mediterranean house gecko as a predator). I found that hourglass manipulation had no effect on the frequency or intensity of agonistic interactions, the proportion of male courtship, or predation by

geckos. Here, I discuss the implications of these findings and other hypotheses for the functional significance of this iconic color trait.

Introduction

Many animals are adorned with elaborate colors that they use in varying contexts (reviewed in Cott, 1940). Conspicuous coloration is perplexing, as it can often reduce survival due to the fact that it can increase both metabolic production costs and predation risk (reviewed in Andersson, 1994; Beddard, 1892; Hasson, 1994). A number of hypotheses have been proposed to explain the prevalence of conspicuous color traits, despite the potential costs. These hypotheses suggest that colors evolve as sexual or social signals (reviewed in Guilford & Dawkins, 1991; reviewed in Hebets and Papaj, 2005). For example, in the context of agonism, color displays can indicate fighting ability among individuals (Bond, 1989a; Bond, 1989b; Rohwer, 1982). The expected response of a rival to superior threatening color displays is reduced aggression or retreat to avoid the costs of conflict (Maynard & Parker, 1976; Maynard & Price, 1973).

Color plays important roles in aggressive interactions in a wide range of taxa including birds (Pryke et al., 2002), fish (Moretz, 2005), lions (West & Packer, 2002), and lizards (Baird et al., 1997). For example, the Midas cichlid (*Cichlasoma citrinellum*) exists in grey and gold color morphs, with the latter exhibiting heightened levels of aggression and dominance (Barlow, 1994). Additionally, the mane length and color of male African lions (*Panthera leo*) is used to assess fighting ability by rivaling males (West & Packer, 2002).

Color used during courtship can allow females to recognize males as the proper species (Estrada & Jiggens, 2008) and/or convey information regarding the quality of males as mates (reviewed in Andersson, 1994). However, quality-indicating hypotheses need not be limited to explaining the role of only male coloration in mating. For example, Weiss (2006) documented the stimulation of male courtship in striped plateau lizards (*Sceloporus virgatus*) by the orange throat patch produced by females during the reproductive season. Moreover, the spectral qualities of the orange throat patch were positively correlated with female body condition, mite load, and average egg mass (Weiss, 2006). These data characterized the female color display of *S. vigatus* as a quality-indicating signal influencing male mate choice. Indeed, recent work suggests that female color displays can function as quality-indicators even in the absence of sex role reversal (reviewed in Amundsen, 2000).

Color functioning as aposematic or warning signals to deter enemies can indicate the unpalatability of a prey item as well as exploit the visual biases of predators (reviewed in Stevens & Ruxton, 2012). For example, Bezzerides et al. (2007) documented the amount of red color on the elytra of Asian labybird beetles (*Harmonia axyridis*) as predicting the amount of alkaloids produced for chemical defense. Mallet & Jordon (1999) suggest that bright warning colors are favored by natural selection because they induce predator neophobia and are highly recognizable as well as enhance avoidance learning.

Adult female black widows (*Latrodectus hesperus*) possess a brightly colored red hourglass on the ventral surface of the abdomen (Foelix, 1996, Figure 1.1). The hourglass is oriented sky-wards when spiders are foraging upside down in their webs at night.

Presently, the function of the hourglass is unknown but is speculated to serve as a conspicuous warning signal to predators (Oxford & Gillespie, 1998). *L. hesperus* is native to Western North America (Garb et al., 2004) and can be found densely aggregated in urban areas (Trubl et al., 2011).

L. hesperus is an excellent system for testing hypotheses regarding the role of conspicuous coloration in the contexts of agonism, mating, and predator avoidance. Increased proximity between highly agonistic females in dense urban aggregations (Johnson et al., 2012) has led to frequent observations of intrasexual aggressive combat and cannibalism (Gburek, personal observation). Many *Latrodectus* species also participate in sexual cannibalism and readily consume males before, during, or after mating (Andrade, 1996; Forster, 1992; Johnson et al., 2011; Segoli et al., 2008). Recent work by Johnson et al. (2011) documented precopulatory attacks on males by poor-condition *L. hesperus* females as well as a male preference for courting high-condition females, which reduces the risk of sexual cannibalism. My previous work has identified hourglass size as tightly correlated with body condition, suggesting that hourglass size is an honest indicator of female condition (refer to Chapter 2).

In terms of enemies, urban *L. hesperus* subpopulations do not face the egg sac predators and parasitoids most commonly thought to limit widow population growth (Barnes et al., 1992; Pierce, 1942; Vetter et al., 2012). Instead, widows from urban Phoenix subpopulations often co-occur with the invasive Mediterranean house gecko (*Hemidactylus turcicus*) (Gburek, personal observation). Anecdotally, I have observed gecko predation on black widows in field and laboratory settings. Additionally, many gecko species are known to be capable of nocturnal color vision (Kelber & Roth, 2005;

Roth & Kelber, 2004; Yoshizawa, 1992). Thus, *H. turcicus* can prey on black widows and may be capable of perceiving the red hourglass.

Below I present a series of experiments in which I test the potential signaling role of the red hourglass in the context of female agonism, male courtship, and predation by geckos. Because hourglass size appears to be a plastic and condition-dependent aspect of the display (refer to Chapters 1 and 2), I chose to manipulate only the size and presence of the hourglass. I used paints matching the range of natural variation in hourglass and abdomen color to impose three treatments on focal females (see Figure 3.1a-c): 1) "hourglass obscured" in which I covered the hourglass with black paint matching average abdomen coloration, 2) "sham control" in which I painted over the naturally occurring size of the hourglass with paint matching average hourglass coloration, and 3) "hourglass enlargement" in which I painted an hourglass to be double the average hourglass size using paint matching average hourglass coloration. I predicted that, relative to hourglass enlargement, spiders with obscured hourglasses would lose agonistic encounters with other females, receive less courtship from males, and be preyed upon by geckos more often.

Materials and Methods

Focal Females. Ten focal female spiders were chosen at random from a total of thirty field-caught spiders to receive one of the aforementioned three treatments (Figure 3.1a-c). In the hourglass enlargement treatment, the size of average focal female's hourglass (3.182 ± 0.332 mm^2 (mean ± SE)) was doubled (6.5 mm^2) using a stencil and paint which matched average hourglass color (Figure 3.1a-c) (see below for paint matching and color scoring protocol). Spiders were temporarily aestheticized using CO_2 gas and placed in a mesh restraint device to apply paint treatments.

Focal female widows used in the experiment were caught from urban habitats throughout metropolitan Phoenix, Arizona during the summer of 2013 (n=30). Spiders were housed separately in transparent plastic containers (61 x 44 x 33 cm) outfitted with Styrofoam refuges (18 x 15 x 5 cm), two crossed 23 cm wooden dowels for web building substrate, and filled with sand to a height of 2 cm (Figure 3.2). Females were maintained on a diet of one house cricket (*Acheta domesticus*) weekly throughout the duration of the experiments. Crickets weighed 50 to 75% of the focal female's mass. No feedings were performed on the days trials were performed. Once released into their respective containers, I allowed females to build web for three weeks prior to beginning experiments.

Scoring Hourglass Color and Paint Matching Protocol. Using digital image analyses and spectrophotometry techniques, I acquired average hourglass color along three conventional axes (hue, saturation, and brightness; Hill & McGraw, 2006) and the average abdomen brightness of focal females prior to imposing treatments. Because spectrophotometry data from the abdomen did not indicate any dramatic peaks in abdomen reflectance (Figure 3.3), I do not report abdomen hue or saturation and only report abdomen brightness. Paints matching average hourglass and abdomen color were produced by blending various shades of Testor's® non-toxic enamel paint. I then painted six non-experimental F-1 generation laboratory-reared spiders (paint matching average hourglass color, n=3; paint matching average abdomen color, n=3). Digital images of painted non-experimental spiders were analyzed to confirm that my paint colors had spectral qualities falling within 95% confidence intervals of focal female's natural hourglass and abdomen coloration (Table 3.1). In order to immobilize spiders for imaging and spectrophotometry they were temporarily anesthetized with CO_2 gas and placed in a mesh restraint device.

To acquire spectral data from digital images spiders were photographed in raw NEF format, using a Nikon D50 equipped with a Micro NIKKOR 40mm lens. A Promaster RL60 LED macro ring light was used to standardize illumination. For each imaging session the camera and images were calibrated using an X-rite Colorchecker Passport with a white balance target and 24-patch classic color reference target (X-rite, Grand Rapids, MI, USA). Images were linearized and equalized using Adobe Photoshop CS5.1 in conjunction with PictoColor inCamera ICC profile software (Pike, 2011; Stevens et al., 2006). Due to the specular nature of my paint samples and the abdomens of un-painted spiders, average color was calculated from three point samples taken from areas with no observable illumination reflectance from both the upper and lower halves of the hourglass as well as either side of the hourglass at a tolerance level setting of 40 in Adobe Photoshop.

Spectrophotometry data was acquired using a standard UV-vis spectrophotometer (USB2000 with PX-2 pulsed xenon light source, Ocean Optics, Dunedin, FL, USA). I took a total of six reflectance measures of each painted and un-painted spider's hourglass and abdomen (three measures from the upper and lower half of the hourglass and three measures from either side of the hourglass) relative to a Spectralon diffuse reflectance white standard. These measurements were taken in a dark room with the probe positioned perpendicular to the area of interest. Distance between the spider's abdomen and the probe was standardized using a moveable stage mount.

Hourglass area was obtained from digital images using public domain Image J software for Windows®. I spatially calibrated the software to recognize the pixel value of one millimeter. I then outlined the hourglass using a tracing tool to obtain the pixel value of hourglass area in mm^2.

Scoring Body Condition. Female widow, male widow, and gecko body conditions were calculated using the residual index method as body mass (spider mass (mg); gecko mass (g)) corrected for fixed body size using residuals for transformed mass regressed on fixed body size. Residual body conditions indices are recommended for detecting differences between groups drawn from the same population since they are less sensitive to variation in fixed body size (Jakob et al., 1996; Moya-Laraño et al., 2008; Petren & Case, 1998). For measures of body size I used cephalothorax width for female widows (Moya-Laraño et al., 2008), leg tibia length for male widows (Jakob et al., 1996), and snout to vent length (SVL) for geckos (Green, 2001). I obtained measures of cephalothorax width and SVL from digital images. For regressions, spider mass (mg) was cube root transformed (Moya-Laraño et al., 2008) and gecko mass (g) was log transformed (Petren & Case, 1998). Body mass was obtained for spiders and geckos one hour prior to each experiment.

Experiment 1: Agonism. Rival females in this experiment were collected from urban habitats throughout metropolitan Phoenix, Arizona during summer 2013 (n=30). These females were housed individually in transparent plastic boxes (10 x 10x 12 cm) and maintained on the same diet regime as focal females (see above). To control for potential effects of body mass, I paired females that did not differ more than 50 mg in body mass. Prior to trials I marked rival spiders on the dorsum with yellow Testor's® non-toxic enamel paint in order to identify individuals during the experiment. To control for potential effects of painting, I also marked focal females with paint matching the average abdomen color of focal spiders.

Each pairing (i.e., focal and rival female) was observed for one hour. I recorded the proportion of rival spider's activity and aggressive behavior out of 31 observational scans. Activity was defined as any type of movement and aggressive behavior was defined as leg-waving, abdomen pounding against the web, or rapid expulsion of viscous silk in the direction of the other spider (Smith & Riechert, 1984). Additionally, I recorded the number and duration (secs) of aggressive interactions as well as the identity of the instigator and winner. I defined aggressive interactions as beginning when one spider charged the other and ending when one of the spiders retreated. In the event that neither spider retreated nor exhibited activity for four minutes after an aggressive interaction, I defined the outcome as a tie. Additionally, I recorded the proportion of scans that focal and rival females oriented their hourglasses towards one another.

Experiment 2: Mating. Male widows used in this experiment were F-1 laboratory-reared offspring of gravid females collected from urban habitats though out metropolitan Phoenix, Arizona during summer 2013 (n=27). Males were housed individually in transparent plastic boxes (3 x 3 x 6 cm) and maintained on a diet of three to five fruit flies (*Drosophila melanogaster*) weekly. Prior to mating trials one focal female from each of the treatment groups died (n=9 replicates per treatment). I introduced male spiders into a randomly selected focal female's container and made a total of 28 observational scans of each pairing over the course of three hours. I recorded the proportion of observational scans where males performed courtship behavior (i.e., web plucking and tapping (Forster, 1992)) and the proportion of scans where male courtship occurred on the female's abdomen. In order to prevent mating, males were removed in the event that courtship occurring on the abdomen was observed for twenty consecutive minutes, which is the typical duration of mounting required before males attempt to mate with a female (Johnson et al., 2011). Additionally, I recorded the proportion of scans that focal females oriented their hourglass towards males.

Experiment 3: Gecko Predation. The Mediterranean house geckos used in this experiment were collected from urban habitats throughout metropolitan Phoenix during the summer of 2013 (n=24). Geckos were housed individually in 10 gallon glass tanks (51 x 26 x 30 cm) in environmentally controlled walk-in chambers set to maintain their natural range of temperature (18-24°C) and humidity (20-35%). Each tank contained 2 cm of mixed sand and soil substrate and a brick refuge (13 x 13 x 4 cm). Geckos were offered one *A. domesticus* every other day and misted daily with distilled H_2O. The captive maintenance and experimental use of geckos was approved and monitored by Arizona State University Institutional Animal Care and Use Committee (IACUC) (protocol number: 12-1258R; see Appendix A).

Prior to predation trials three more focal females died of natural causes (hourglass obstructed: n=9; sham control: n=7; hourglass enlarged: n=8). Geckos were introduced into a randomly selected focal female's container and each pairing was observed for one hour. I recorded instances of gecko predation on widows and gecko activity directly after releasing the gecko and at two minute increments for a total of 31 observational scans.

Gecko activity was defined as any type of movement. Additionally, I recorded the proportion of scans during which focal females oriented their hourglass towards geckos. I allowed geckos and widows to co-habit for four weeks following the completion of the one-hour trial during which we checked each pairing daily for instances predation.

Statistical Analysis. All statistical tests were performed in SPSS (Ver. 17.0 for Windows® SPSS, Chicago, IL, USA). Shapiro-Wilk goodness-of-fit tests were used to assess the normality of data. All proportional data was arc sine square root transformed and the number of aggressive interactions during agonsim trials was plus one log transformed to improve normality.

I used mulitvariate ANOVAs (MANOVAs) to determine the effects of hourglass manipulation on agonism and mating data. I used a univariate ANOVA to determine the effect of hourglass manipulation on gecko activity. I used a Spearman's rank order correlation test to identify relationships between body conditions and agonistic interactions, male courtship behavior, and gecko activity. I employed a Bonferroni correction (agonism: $\alpha=0.05/16$, $\alpha=0.003$; mating: $\alpha=0.05/4$, $\alpha=0.0125$; gecko predation: $\alpha=0.05/2$, $\alpha=0.025$) to account for multiple tests. To verify that focal females and rival females did not differ in body condition I performed a two-tailed student's t-test for independent samples. To determine how web-residency influenced the outcome of agonistic interactions between females, I performed a two-tailed student's t-test for independent samples with residency as the grouping variable. Lastly, to identify differences in hourglass orientation towards predicted receivers among contexts (i.e., agonism, mating, and predation) I used a univariate ANOVA, including context as a fixed factor and Bonferroni post-hoc comparisons.

Results

Effects of Hourglass Manipulation Treatments on Agonsitic Encounters, Male Courtship, and Predation by Geckos.

There was no effect of hourglass manipulation treatment on rival females behavior, number or duration of aggressive interactions, or the outcome of interactions ($F_{16, 40}$=0.459, P=0.953, Figure 3.4a-d). Additionally, there was no effect of hourglass manipulation on total male courtship behavior, or the proportion of courtship behavior occurring on focal female's abdomens (Figure 3.5). I did not observe any instances of gecko predation on widows during initial trials. Furthermore, I did not identify an effect of hourglass manipulation on gecko activity (Figure 3.6). In the four weeks following the initial predation trials, I observed only two instance of gecko predation on females whose hourglass had been obscured.

Effects of Body Condition on Agonsitic Encounters, Male Courtship, and Predation by Geckos.

The body condition of focal females and rival females did not differ significantly (t_{58}=-0.834, P=0.410). Web-residency did not explain the outcome of aggressive interactions (t_{58}=0.209, P=0.835). There was a marginally non-significant trend (α=0.003) for intruders to win less often when paired with focal females exhibiting heightened body condition (R^2=0.243, $r_{s1,15}$=-0.493, P=0.006). However, there were no significant relationships between body condition and rival behavior, number or duration of aggressive interactions, or the outcome of aggressive interactions (all P>0.003). Additionally, there were no significant relationships between focal female and male body condition and male courtship behavior (all P>0.0125). Furthermore, there were no significant relationships between focal female and gecko body condition and gecko activity (all $P>0.025$).

Variation in Hourglass Orientation Among Agonism, Mating, and Anit-Predation Contexts. Lastly, I observed differences across contexts in hourglass orientation towards the predicted receiver (Figure 3.7). Specifically, focal females oriented their hourglass more towards courting males than rival females oriented their hourglass towards focal females ($P=0.004$). All other possible comparisons of positive hourglass orientation among contexts were non-significant (Figure 3.7).

Discussion

My data do not indicate any effect of hourglass obstruction or enlargement on agonistic encounters between females, the proportion of male courtship behavior, or gecko activity. However, I did observe two instances of gecko predation on females with obscured hourglasses during the four weeks following the initial predation trials. Body conditions were a poor indicator of rival spider behavior and the outcome of aggressive interactions. Moreover, web-residency did not predict the outcome of aggressive interactions. Similarly, there were no significant relationships between body conditions and male courtship behavior or gecko activity. I did observe focal females orienting their hourglass significantly more towards males than rival females oriented their hourglass towards focal females.

I speculate that the lack of response by rival females to obscured and enlarged hourglasses is likely due to the fact that historically in undisturbed environments, the opportunity for encounters between adult females has been limited. Indeed, Johnson et al.

(2012) documented distances between neighboring adult females in urban Phoenix to be as short as 1.9 m compared to 55.9 m in undisturbed, desert habitats. Following the completion of the Roosevelt Dam in 1911, Phoenix experienced dramatic land transformation from an agricultural area to an urban area (Knowles-Yánez et al., 1999; Luck & Wu, 2002). It is likely that widows began densely aggregating in the city during this period of rapid land transformation. Thus, it may be that urban-dwelling widows have not been faced with the prospect of agonistic encounters long enough to have evolved signals indicating aggression or fighting ability.

Additionally, my data suggest that the hourglass does not function as a sexual signal. I did not observe differences in the amount of courtship behavior performed by males paired with females with obscured hourglasses and males paired with females with enlarged hourglasses. Despite the fact that the hourglass size is a condition-dependent trait that fluctuates with foraging success (refer to Chapter 2) and males are known to preferentially court high-condition females (Johnson et al., 2011), males may not have the visual ability to discriminate among variation in hourglass size and color. This idea is substantiated by findings from Johnson et al. (2011) that indicate male widows can discriminate among variation in the recent foraging success of females through silken chemical cues, subsequently reducing the risk of sexual cannibalism that is only practiced by females in poor condition (Johnson et al., 2011). Indeed, many members of the Theridiidae family have poor vision, relying heavily on web-borne vibrations for information about their environment (Foelix, 1996).

I did uncover a trend for focal females to orient their hourglass towards courting males more often than rival females oriented their hourglass towards focal females. These data do not offer empirical evidence for the hourglass as a sexual signal. I suspect that this trend is largely due the location of the female's genital opening on the ventral surface of the abdomen directly above the hourglass. Males sometimes court on the abdomen of females for up to an hour before mating (Johnson et al., 2011). Thus, receptive females likely orient the ventral surface of their abdomens towards males during courtship.

Perhaps most perplexing is my finding that Mediterranean house geckos did not respond to obscured or enlarged hourglasses during initial predation trials. This is surprising, as the hourglass is often reported to be a warning signal to deter potential enemies (Oxford & Gilliespie, 1998). I do, however, note that the only gecko predation that occurred across this four-week experiment involved two females from the hourglass obstructed treatment. This suggests that if I had monitored pairings more long-term, I may have determined hourglass obstruction as having the predicted consequence of increased predation by geckos. Additionally, I did observe widows orienting hourglasses towards geckos (Figure 3.7) and I have made field observations of widows displaying their hourglass outwards from within their refuge (Gburek & Johnson, unpublished data). Future field and lab studied should focus on identifying the circumstances under which this behavior occurs.

Alternatively, *H. turcicus* may not be capable of discrimination between hourglass and abdomen coloration. The vision of Mediterranean house geckos is limited to discrimination of only UV, blue, and green light reflectance (300-550 nm) (Lowe, 1996). The reflectance spectrum of the hourglass and abdomen indicate only minimal differences between hourglass and abdomen reflectance at these wavelengths (Figure 3.3). Additionally, Mediterranean house geckos may not be the primary enemy of black widow spiders. *H. turcicus* is an invasive species, documented as arriving in the New World in 1910 (Rödder & Lötters, 2009). A more likely predator of black widows is the native Sonoran desert Western banded gecko (*Coleonyx variegatus*) (Parker, 1972).

Furthermore, the egg sacs of *L. hesperus* are known to be attacked by insect egg sac predators and parasitoids such as flies (e.g. *Pseudogaurax signatus*) and wasps (e.g., *Baeus latrodecti*) (Pierce, 1942; Vetter et al., 2012). Perhaps these insects pose a greater threat to black widows than geckos and thus are more likely to be the targeted receiver of the red hourglass signal. Future research should aim to identify the perceptual ability of these enemies.

Evolutionary biologists often attempt to explain the adaptive role of conspicuous coloration as functioning to improve an organism's success in agonistic encounters, courtship, predator avoidance, or foraging (reviewed in Guilford & Dawkins, 1991; reviewed in Hebets and Papaj, 2005). My study did not address the potential function of the hourglass display in terms of foraging success. Black widows are generalist arthropod predators (Salmon, 2011) and many insects are attracted to bright colors since their food sources are often colorful (e.g., flowers and fruit) (Pickering & Stock, 2003; Lebesa et al., 2011). A handful of studies have characterized the conspicuous coloration of orb-weaving spiders as a prey attractant (Craig & Ebert, 1994; Hauber, 2002; Hoese et al., 2006). For example, orb-weaving spiny spiders (*Gasteracantha fornicata*) exhibit contrasting white-yellow and dark dorsal stripes (Hauber, 2002; Mascord, 1970). Hauber (2002) observed lower rates of prey capture in spiny spiders whose white-yellow stripes were dyed black. Additionally, Craig & Ebert (1994) determined that *Trigona* stingless bees are attracted to the flower-like patterns of *Argiope argentata* spiders. However, black widows are typically observed foraging upside-down in their webs with the hourglass oriented sky-wards, and the majority of black widow prey items are ground-dwelling arthropods (Saloman, 2011). This offers little support for the idea that the hourglass serves to attract aerial insects which feed on pollen and nectar from colorful flowers.

Much of what we know about conspicuous color ornamentation comes from a few well-studied taxa such as birds, fish, and butterflies (reviewed in Andersson, 1994). Spiders differ in their biology from these groups in that females are often the larger sex and are typically highly aggressive and potentially cannibalistic (Herberstein, 2011). Black widow spiders are unique in that females are not only the larger gender, but also possess a colorful red hourglass (Foelix, 1996). Identification of the functional significance of the red hourglass display could offer novel insights for the field of animal coloration.

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Table 3.1

Paint matching average hourglass and abdomen color. Comparison between average field hourglass display color and matching paint color.

	Field hourglass display data (mean ± 95% confidence interval)	Matching paint data (mean ± SE)
Hourglass hue (°)	13.917 ± 1.927	12.333 ± 0.333
Hourglass saturation (%)	64.661 ± 3.408	64.333 ± 2.963
Hourglass brightness (%)	70.514 ± 2.719	73 ± 0.577
Abdomen brightness (%)	21.569 ± 0.544	21.667 ± 1.453

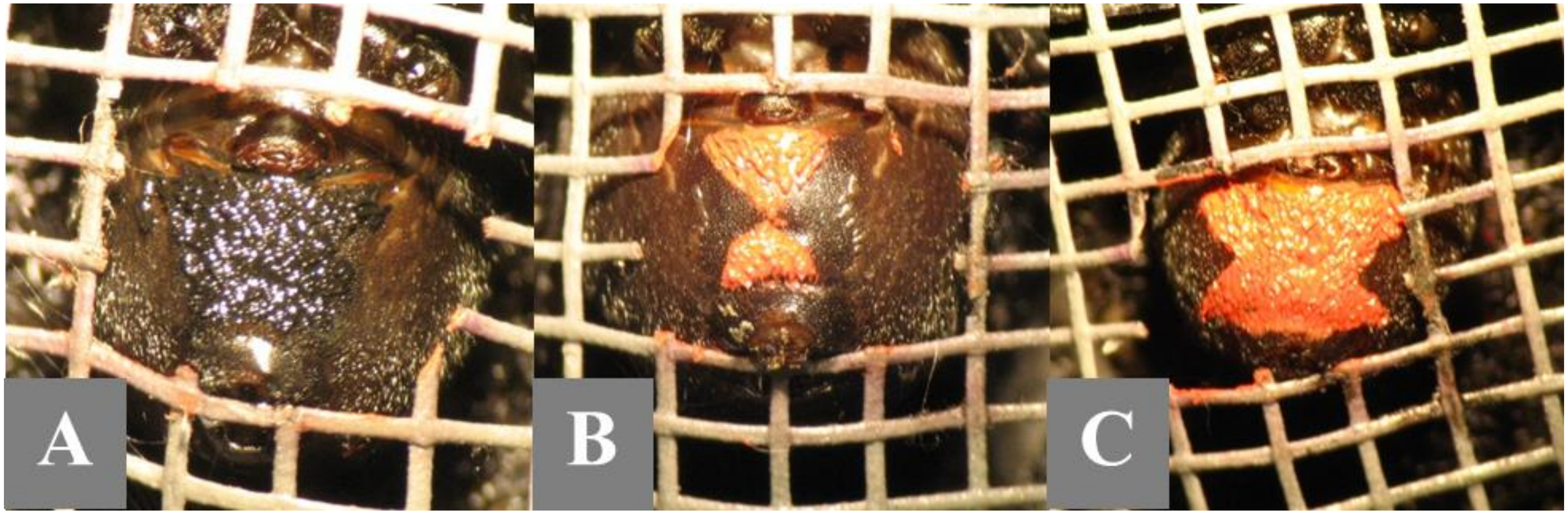


Figure 3.1 Hourglass manipulations treatments. Examples of: A. obscured hourglass, B. sham control, and C. enlarged hourglass.

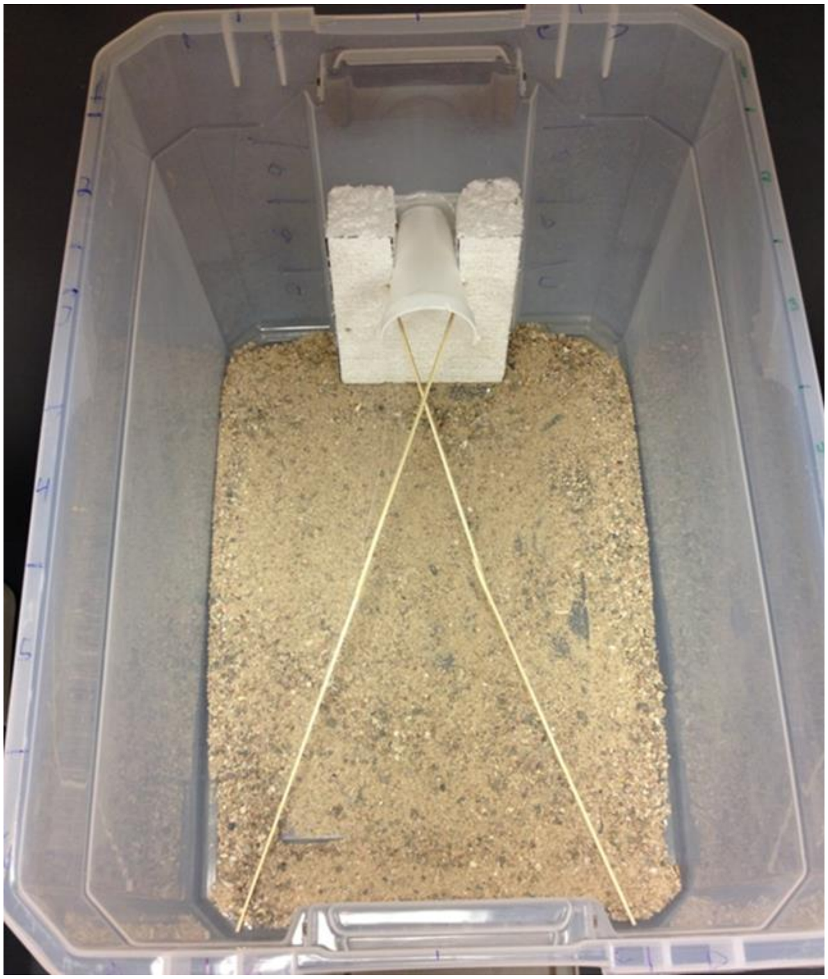


Figure 3.2 Housing for experimental spiders. Experimental spiders were housed in clear plastic containers outfitted with Styrofoam refuges and crossed wooden dowels for web building substrate.

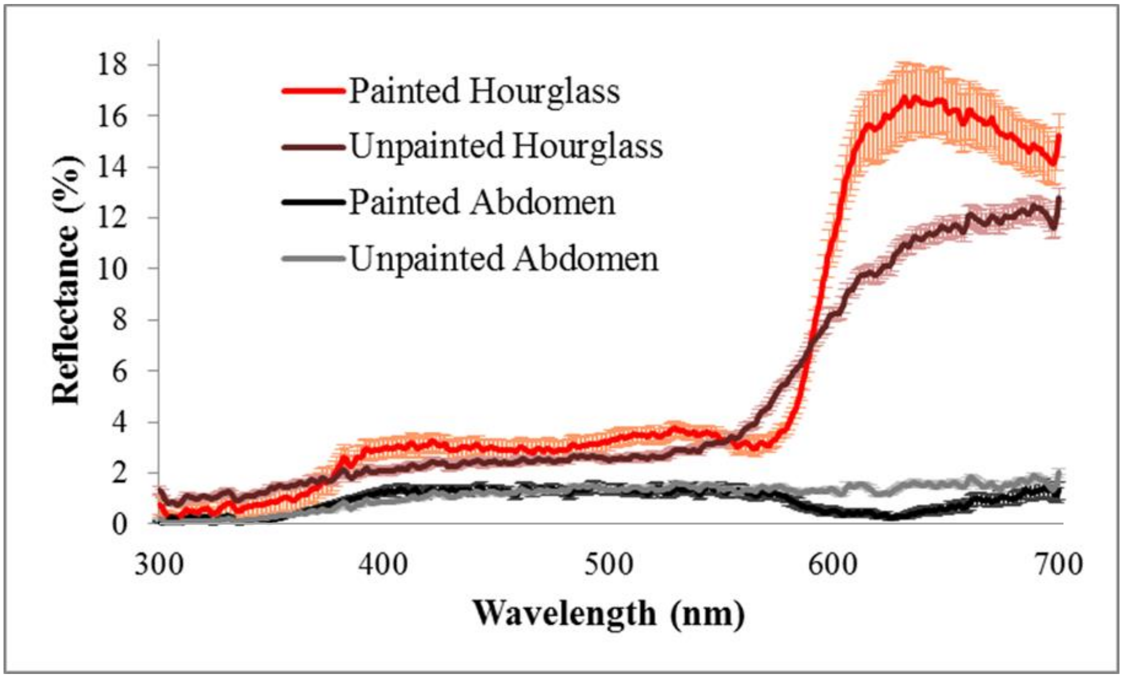


Figure 3.3 Average hourglass, abdomen, and paint reflectance. Variation from the mean represents standard error.

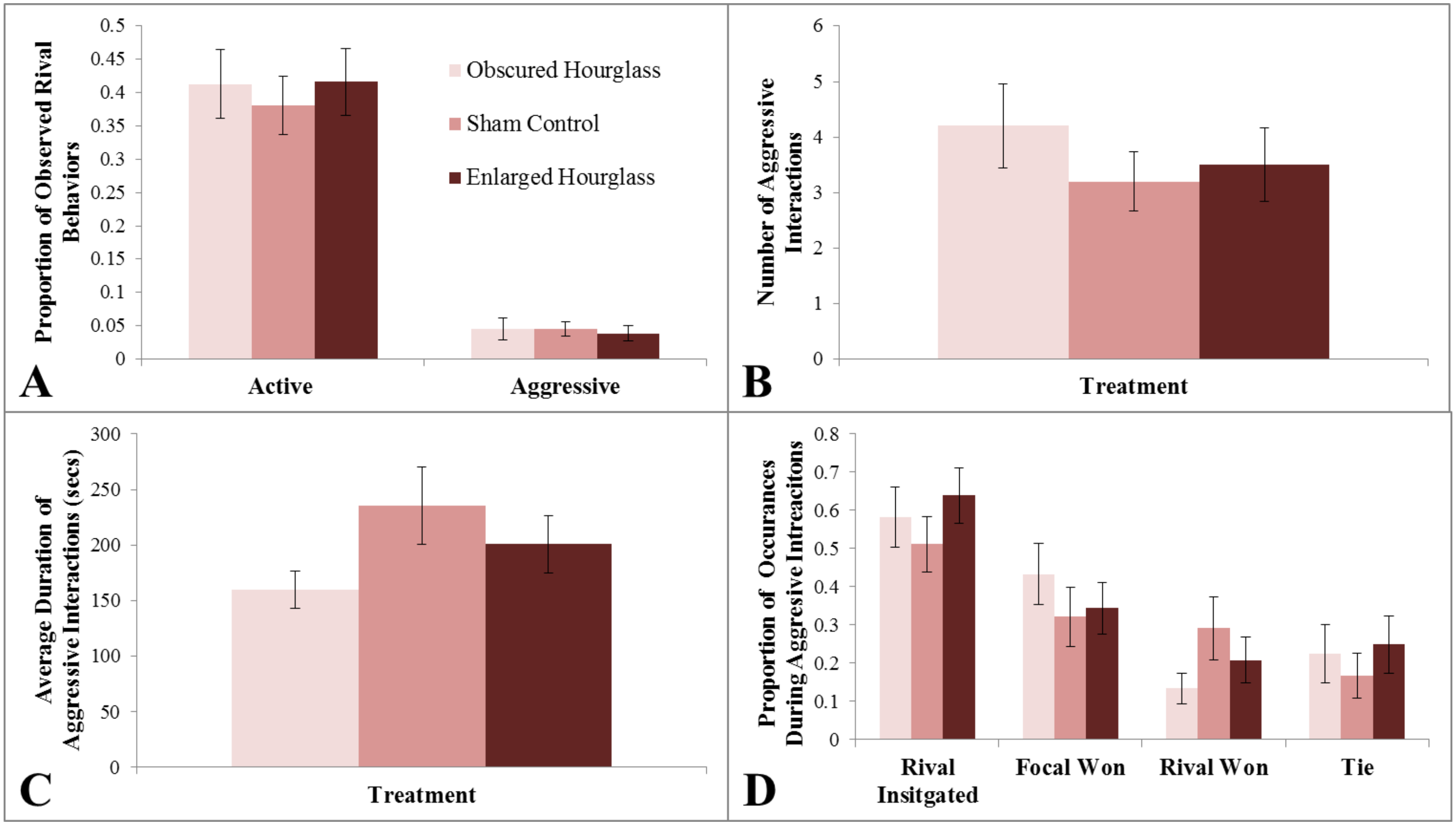


Figure 3.4 Hourglass manipulation effect on agonistic interactions. There was no effect of hourglass manipulation ($F_{16,40}$=0.459, P=0.953) on A. intruder activity and aggression, B. number of aggressive interactions, C. duration of aggressive interactions, and D. intruder instigation of interactions or outcome of interactions. Values represent mean ± SE.

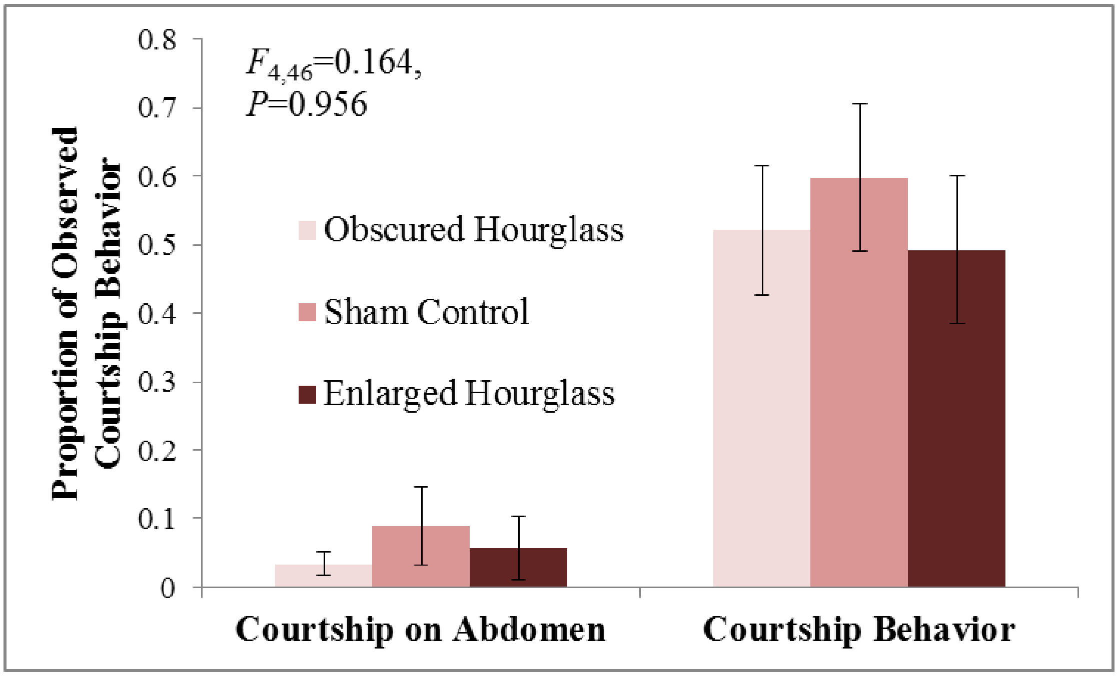


Figure 3.5 Hourglass manipulation effect on male courtship behavior. No significant effect of treatments on the proportion of courtship occurring on the abdomen or total courtship behavior. Values represent mean ± SE.

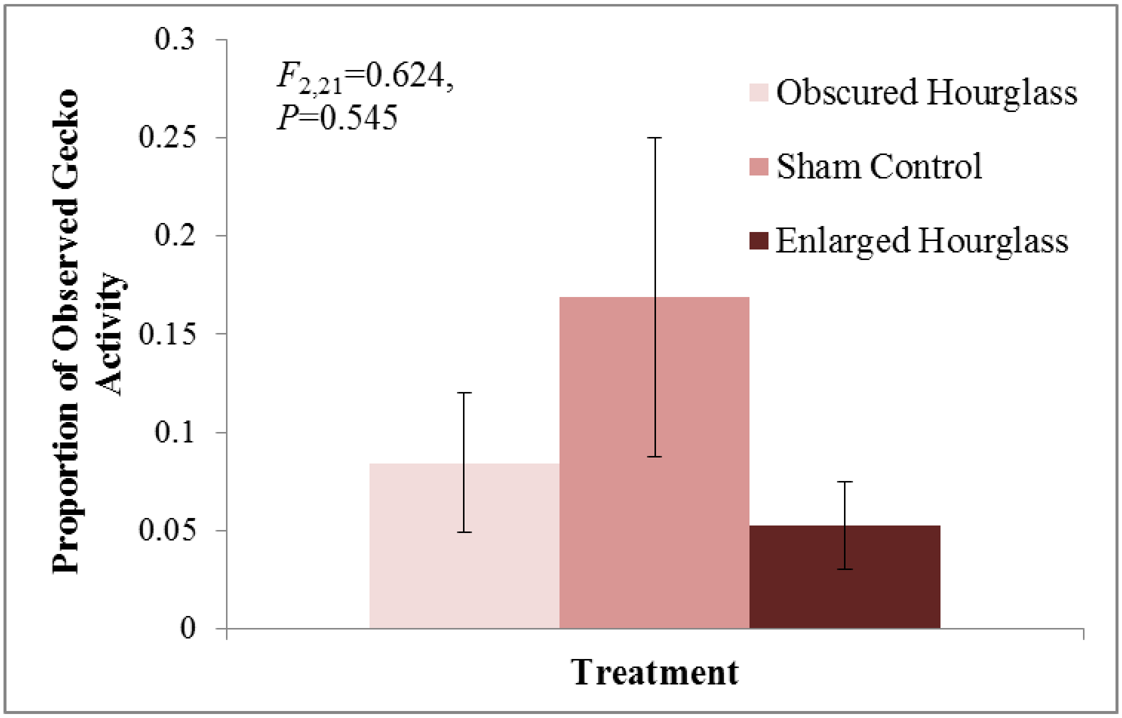


Figure 3.6 Hourglass manipulation effect on gecko activity. No significant effect of treatment on the proportion of gecko activity. Values represent mean ± SE.

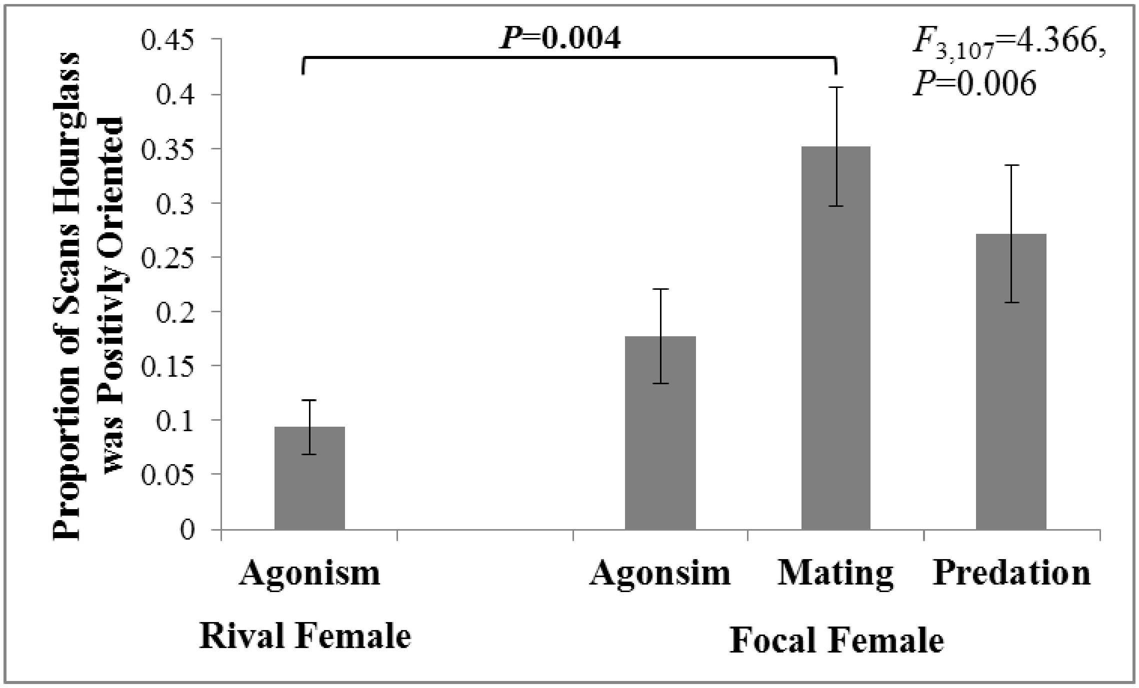
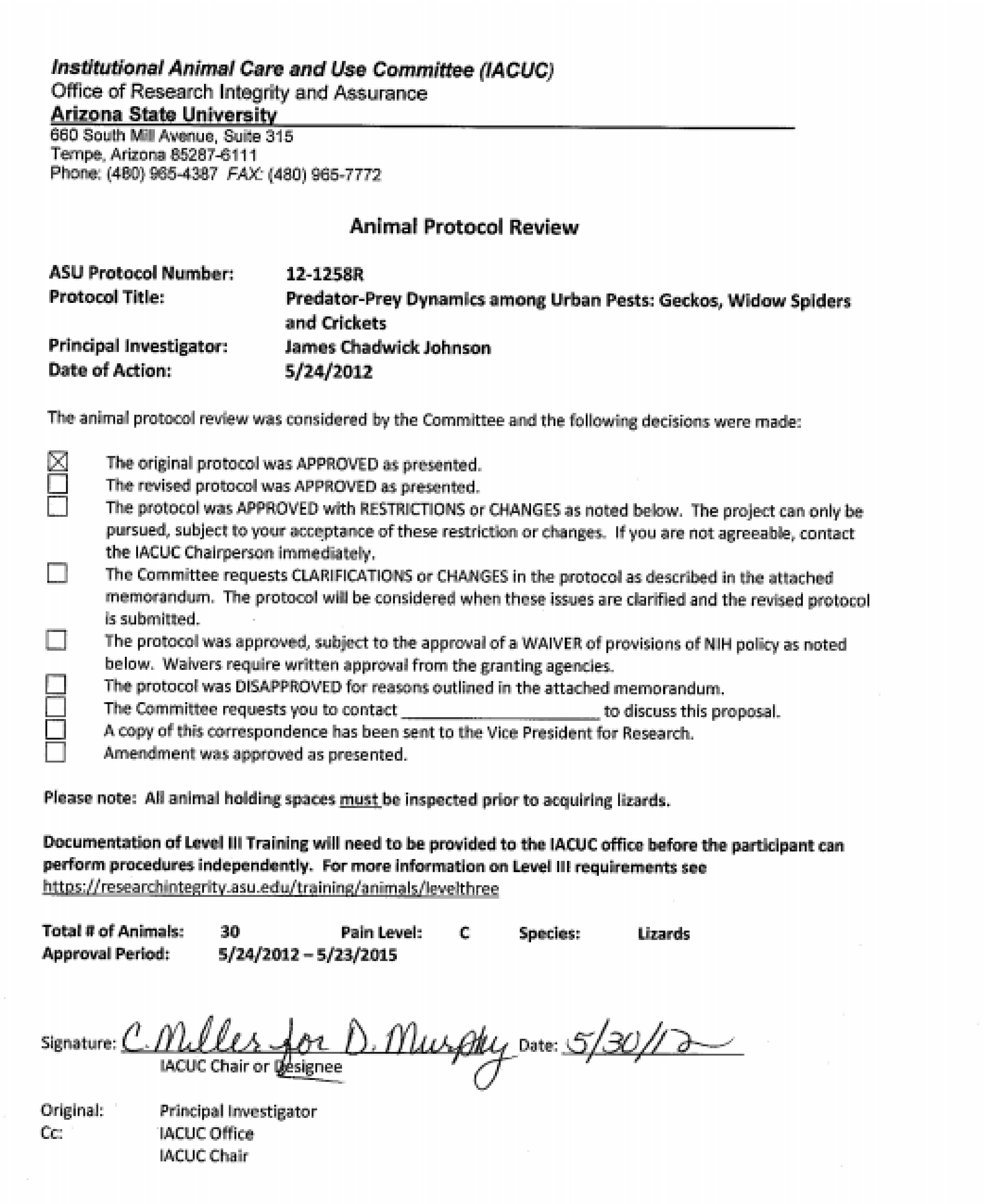


Figure 3.7 Variation in hourglass orientation across contexts. Values represent mean ± SE.

APPENDIX A

APPROVAL FOR CAPTIVE MAINTENANCE AND EXPERIMENTAL USE OF MEDITERRANEAN HOUSE GECKOS



Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 315
Tempe, Arizona 85287-6111
Phone: (480) 965-4387 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number:	**12-1258R**
Protocol Title:	**Predator-Prey Dynamics among Urban Pests: Geckos, Widow Spiders and Crickets**
Principal Investigator:	**James Chadwick Johnson**
Date of Action:	**5/24/2012**

The animal protocol review was considered by the Committee and the following decisions were made:

☒ The original protocol was APPROVED as presented.
☐ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be considered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☐ The Committee requests you to contact ______________________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☐ Amendment was approved as presented.

Please note: All animal holding spaces <u>must</u> be inspected prior to acquiring lizards.

Documentation of Level III Training will need to be provided to the IACUC office before the participant can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree

Total # of Animals: **30** **Pain Level:** **C** **Species:** **Lizards**
Approval Period: **5/24/2012 – 5/23/2015**

Signature: C. Miller for D. Murphy Date: 5/30/12
IACUC Chair or Designee

Original:	Principal Investigator
Cc:	IACUC Office IACUC Chair