FACTORS INFLUENCING ECTOPARASITISM ON WESTERN FENCE LIZARDS
(*SCELOPORUS OCCIDENTALIS*): HOST SEX, TESTOSTERONE, REPRODUCTIVE
CONDITION, AND BEHAVIOR

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Nicholas B. Pollock
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COMMITTEE MEMBERSHIP

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AUTHOR: Nicholas B. Pollock

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COMMITTEE CHAIR: Emily Taylor, PhD

COMMITTEE MEMBER: Larisa Vredevoe, PhD

COMMITTEE MEMBER: Candace Winstead, PhD

ABSTRACT

FACTORS INFLUENCING ECTOPARASITISM ON WESTERN FENCE LIZARDS (*SCeloporus OCCIDENTALIS*): HOST SEX, TESTOSTERONE, REPRODUCTIVE CONDITION, AND BEHAVIOR

Nicholas B. Pollock

Abstract

Host-parasite relationships are one of the most common symbiotic relationships present in a diverse array of ecosystems. There are numerous factors that impact the dynamics of these relationships. Major factors that can influence the degree of parasitism include host sex, hormonal state, reproductive condition, and behavior. It has been observed in several vertebrate taxa that males have higher ectoparasite intensities than females and males with increased testosterone have increased ectoparasite intensities. One potential reason for these observations is that testosterone concentrations are elevated in males, particularly during the breeding season, and when circulating concentrations increase males become more vulnerable to ectoparasitism. Here I first tested the hypothesis that higher circulating testosterone concentrations in male western fence lizards (*Sceloporus occidentalis*) induce higher tick intensities. To examine this hypothesis I implanted male lizards with either testosterone or blank implants in the field. The testosterone-implanted males had significantly higher tick intensities compared to the control males. However, in contrast, control males had significantly higher mite intensities compared to testosterone-implanted males. These results are consistent with other studies suggesting that testosterone impacts certain aspects of host-parasite relationships. However, the exact mechanism for how testosterone influences parasite intensities remains unclear.

There are two major current hypotheses for how testosterone influences ectoparasite intensities on males, the first involving immunosuppression and the second involving behavioral patterns and movement. However, another potential reason for why male lizards, particularly those with high circulating testosterone, have higher ectoparasite intensities than female and low testosterone male lizards is that the parasites preferentially choose their host. Furthermore, it has been demonstrated that vitellogenic female lizards have diminished immune function and this could potentially lead to increased ectoparasitism in much the same way that testosterone does in male lizards. Therefore, it is possible that a host preference is also present with vitellogenic versus non-vitellogenic female lizards. Although there have been a few interspecific studies done on this topic there have been no such studies on parasite host preference in reptiles to date. Here I tested three hypotheses: 1. Ticks prefer male lizards to female lizards. 2. Ticks prefer male lizards with high testosterone concentrations to male lizards with normal testosterone concentrations. 3. Ticks prefer vitellogenic female lizards to non-vitellogenic female lizards. All three experiments demonstrated no preference of host by ticks, which suggests they will attach to any suitable host they come across. However, during the male versus female host choice experiment ticks fed faster on vitellogenic

female lizards than male lizards and non-vitellogenic female lizards. These results, taken together with previous studies showing higher tick intensities on male lizards, lizards with experimentally elevated testosterone, and reproductive female lizards, provide evidence that ticks do not preferentially choose their host, but instead are found in higher numbers on certain hosts due to some other reason. Other potential explanations include differences in immune function, microhabitat use, and behavioral patterns.

One of the major hypotheses as to why male lizards, particularly those with high testosterone concentrations, have higher ectoparasite intensities than female lizards and male lizards with low testosterone concentrations is that these lizards perform more territorial behaviors, have increased movements, and larger home range sizes, thus exposing them to more parasites. Several studies have shown testosterone to increase the frequency of behaviors, movement, and home range size in lizards, but few, if any, have related it to ectoparasite intensities. Here I tested two hypotheses: 1. High testosterone male lizards have larger home ranges than male lizards with lower testosterone concentrations and female lizards. 2. High testosterone male lizards perform a higher frequency of territorial behaviors than male lizards with lower testosterone concentrations and female lizards. To test these hypotheses I implanted male lizards with either testosterone or blank-control implants, left female lizards unaltered, and performed behavioral observations in the field for 25 days. At the end of this time period, home range sizes were calculated as minimum convex polygons and ectoparasite intensities were quantified. Results of this study revealed no significant difference in ectoparasite intensities between high and low testosterone male lizards, but male lizards did have significantly higher ectoparasite intensities than female lizards. Furthermore, home range size and frequencies of territorial behaviors were not significantly different between high and low testosterone male lizards. However, male lizards did have larger home ranges and performed more territorial behaviors and movements than female lizards. These results suggest that home range, movement, and territorial behavior frequency contribute to higher ectoparasite intensities on male lizards, particularly those on males with high circulating testosterone. However, future studies need to address the behavioral and physiological mechanisms responsible for the observed effects of testosterone on parasitism, including parasite intensity, immunosuppression, and parasitic effects on host fitness.

Keywords: reptile, ticks, hormones, parasite, immune, reproduction, territorial

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CHAPTER I:

1. Introduction

One of the most interesting and well-studied biological relationships is that of a parasite and its host. Parasites can dramatically affect the fitness of the host by parasite-induced castration (Baudoin, 1975), decreased reproductive success (Hamilton and Zuk, 1982; Schall and Dearing, 1987; Møller, 1997; Lope et al., 1998; Møller, 1999), and transmission of diseases (Camin, 1948). Factors that influence parasite intensities include season and host age, genetic background, sex, and hormonal state. Seasonal shifts in parasite intensity have been observed across animal taxa, including fish (Mitchell, 1989), crickets (Zuk, 1987), sheep (Theodoropoulos et al., 1998), birds (Teel et al., 1998), and lizards (Schall et al., 2000; Eisen and Eisen, 1999; Eisen et al., 2001; Klukowski, 2004; Lumbad et al., in press). Furthermore, older and larger lizards have more parasites (Christian and Bedford, 1995; Schall and Marghoob, 1995; Sorci, 1996), specific genotypes of lizard species have increased resistance to parasites (Brown et al., 1995; Olsson et al., 2005), and parasites are more abundant in males of many animal species (Poulin, 1996; Zuk and McKean, 1996), including ball pythons (Aubret et al., 2005), lizards (Schall and Marghoob, 1995; Schall et al., 2000; Klukowski and Nelson, 2001; Salkeld and Schwarzkopf, 2005), salamanders (Anthony et al., 1994), reindeer (Folstad et al., 1989), and red jungle fowl (Zuk, 1990). The combination of seasonal, age, and sex effects on parasite intensities suggests a potentially important relationship between the endocrine system and parasitism.

Testosterone (T) is one of the most thoroughly studied hormones used to elucidate the relationship between the endocrine system and parasites. This androgenic hormone plays a role in numerous morphological, physiological, and behavioral processes. Testosterone, however, can be both beneficial and costly at the same time. It has been shown to increase reproductive success of male lizards by increasing territorial behaviors (Moore, 1986; Marler and Moore, 1988, 1989; Wingfield and Hahn, 1994; Klukowski and Nelson, 1998; Sinervo et al., 2000), home-range size (DeNardo and Sinervo, 1994; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009), endurance (Sinervo et al., 2000; John-Alder et al., 2009), movement (Olsson et al., 2000; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009), and secondary sex characteristics (Cooper et al., 1987; Zuk et al., 1995; Evans et al., 2000; Cox et al., 2005, 2005a).

In contrast, testosterone has also been shown to decrease immune function. For example, studies have indicated decreases in leukocyte counts (Zuk et al., 1995; Veiga et al., 1998; Uller and Olsson, 2003) and in the cell-mediated immune response (Duffy et al., 2000; Belliure et al., 2004; Oppliger et al., 2004) when testosterone is experimentally elevated. Furthermore, the humoral immune response has been shown to decrease when testosterone is elevated. In ocellated skinks (*Chalcides ocellatus*) testosterone treatment led to decreased IgG antibody titers against rat erythrocytes (Saad et al., 1990). In European starlings (*Sturnus vulgaris*) testosterone-implanted individuals had a significantly reduced IgG antibody response to keyhole limpet hemocyanin (KLH) (Duffy et al., 2000).

This reproductive-immune trade-off falls under the umbrella of the “immunocompetence handicap hypothesis” (Folstad and Karter, 1992). While higher

testosterone concentrations may make males more attractive to females due to stronger secondary sex characteristics, it may provide a handicap to those individuals because of immunosuppression. This compromising of immune function can lead to increased disease (Camin, 1948; Duckworth et al., 2001), parasitism (Saino et al., 1995; Salvador et al., 1996; Olsson et al., 2000; Hughes and Randolph, 2001; Klukowski and Nelson, 2001; Roberts et al., 2004; Cox and John-Alder, 2007), and mortality (Marler and Moore, 1988; Salvador et al., 1996; Sorci et al., 1996; Ros, 1999). Therefore, the secondary sex characteristics (i.e. territorial behaviors, color, etc.) serve as honest signals to females because only the most healthy and fit males with access to sufficient nutrients can afford to endure the costs of increased testosterone concentrations. Testosterone, required for the development of many secondary sex characteristics, tends to fall sharply during times of food shortage (Wilson et al., 1979; Pérez-Rodríguez et al., 2006; Ruiz et al., 2010). Therefore, nutrition may be considered a limiting factor for the expression of secondary sex characteristics (Lignon et al., 1990). Studies across multiple taxa have demonstrated that males with higher concentrations of circulating testosterone have higher parasite intensities (Saino et al., 1995; Salvador et al., 1996; Olsson et al., 2000; Hughes and Randolph, 2001; Klukowski and Nelson, 2001; Roberts et al., 2004; Cox and John-Alder, 2007). However, there have been studies that showed no significant relationship between circulating testosterone concentrations and parasite intensities in lizards (Lefcort and Blaustein, 1991; Salvador et al., 1997; Oppliger et al., 2004).

Western fence lizards (*Sceloporus occidentalis*) are major hosts for juvenile western black-legged ticks (*Ixodes pacificus*, Eisen and Eisen, 1999; Eisen et al., 2004; Slowik and Lane, 2004). The larvae and nymphs of this tick species commonly attach to

a small skin fold between the ear and shoulder of the lizard, termed the nuchal pocket (Arnold 1986). After feeding, the engorged (replete) larvae and nymphs drop off and molt into nymphs and adults, respectively. This species of tick is the vector of the Lyme disease spirochete, *Borrelia burgdorferi*, in the western United States. Insights into the ecology of this infectious disease can be gained by an understanding of relationships between host abundance and tick abundance (Swei et al., 2011) as well as host sex and physiological state and tick intensities.

The purpose of this study is to determine if testosterone influences tick intensities in free-ranging lizards. I hypothesize that testosterone causes male *S. occidentalis* to be more heavily parasitized by ticks. To test this hypothesis, I manipulated testosterone concentrations in free-ranging male *S. occidentalis* and measured their tick intensities. If the hypothesis is true, then I predict that testosterone-implanted male lizards will have heavier tick intensities than control lizards.

2. Methods

2.1 Experimental Design

We collected 54 male *S. occidentalis* on April 4-5, 2009 from Poly Canyon on the campus of the California Polytechnic State University, San Luis Obispo. This area is characterized by California oak woodland with occasional man-made structures. Upon capture by noose, snout-vent length (SVL, ± 0.5 cm) and body mass (± 0.5 g) were recorded and each lizard received a unique toe-clip for identification. Lizards were randomly placed into one of two treatment groups, testosterone-implanted ($n = 27$) or

blank-implanted ($n = 27$). Treatment groups did not significantly differ in SVL (two-tailed $T = 0.17$, $P = 0.864$) or body mass (two-tailed $T = 1.06$, $P = 0.299$). Implants were made from 5 mm pieces of silastic diffusion tubing (Dow Corning, Clarkesville, TN, U.S.A.: 1.47 mm inner diameter, 1.96 mm outer diameter), capped and sealed with silicon caulking, and filled with either 3 mm of crystalline testosterone propionate (Sigma-Aldrich, St. Louis, MO, U.S.A.) (testosterone-implanted lizards) or silicon caulking (blank-implanted). In the field, lizards were placed on ice to induce cold-induced surface anesthesia until they exhibited no foot-withdrawal reflex. Implants were placed into the coelomic cavity via a small ventrolateral incision that was then closed with absorbable suture. Lizards were then released back to their original sites of capture.

Four weeks later, we recaptured as many lizards as possible (14 testosterone-implanted and 12 blank-implanted lizards). Lizards were bled from the postorbital sinus with heparinized capillary tubes within 5 minutes after capture to determine post-treatment testosterone concentrations. Several hours later, blood samples were centrifuged for 5 minutes at 10,000 rpm, and plasma was extracted and frozen at -20°C . Plasma samples were shipped to Virginia State University, and circulating concentrations of testosterone were quantified by radioimmunoassay (RIA) according to the methods of Lind et al. (2010).

Lizards were then transported back to the laboratory at California Polytechnic State University in cloth bags. Immediately upon return the lizards were placed into individual 13 x 8 x 8 (cm) metal mesh cages elevated above tubs of water to collect all ectoparasites (mites and ticks) infesting lizards as they dropped from their hosts. Tubs were 30 x 16 x 8 (cm) and filled with 4 cm of water, such that any ticks and mites

dropping off the host lizards would fall into the tubs and float until they were retrieved daily by the investigators. The sides of the tubs were coated with Fluon (Bioquip, Rancho Dominguez, CA, U.S.A.) to prevent tick and mite escape. Tubs were placed in environmental chambers (27°C, 8:16 light:dark). Water was offered *ad libitum* and 2-3 crickets were offered per day. Numbers of replete tick larvae, replete tick nymphs, and total mites were quantified daily as they dropped off the lizard host and into the water. This procedure was repeated for each lizard every 24 hours until all ticks had dropped off. It was not possible to determine if all mites, especially larval mites, dropped off the lizards because some species, particularly trombiculids, may remain on the host for long periods of time (Klukowski, 2004). At the termination of the experiment the total number of replete tick larvae, replete tick nymphs, total replete ticks (larvae plus nymphs) and mites that had dropped off lizards was calculated for each lizard.

2.2 Data Analysis

Plasma testosterone concentrations of testosterone-implanted and blank-implanted lizards were compared using two-tailed t-tests. P-values were considered significant at the $\alpha = 0.05$ level. Parasite intensities (replete tick larvae, replete tick nymphs, total ticks, and mites) for the treatment groups were compared using two-sample Poisson rate tests. Statistical analyses were performed using Minitab Statistical Software version 10 (State College, PA, U.S.A.).

3. Results

3.1 Implants

Lizards with testosterone implants had significantly higher circulating concentrations of testosterone (means: testosterone-implanted males = 35.7 ± 2.0 , blank-implanted males = 21.1 ± 2.8 ng/ml; two-tailed $T = -4.25$, $P \leq 0.0001$). The experimentally elevated concentrations of testosterone were nonetheless within the physiological range of testosterone concentrations in spring (Taylor et al., unpublished) and were therefore not pharmacological doses.

3.2 Larval Tick Intensities

Testosterone-implanted males had significantly more replete tick larvae than control males (mean tick larvae: testosterone-implanted males = 17.1 ± 6.4 , blank-implanted males = 9.0 ± 3.0 ; $Z = 5.70$, $P \leq 0.0001$; Fig. 1).

3.3 Nymphal Tick Intensities

Testosterone-implanted males also had significantly more replete tick nymphs than control males (mean tick nymphs: testosterone-implanted males = 12.5 ± 2.6 , blank-implanted males = 6.1 ± 1.6 ; $Z = 5.33$, $P \leq 0.0001$; Fig. 1).

3.4 Total Tick Intensities

When overall total number of ticks (larvae and nymphs combined) recovered from lizards was calculated, testosterone-implanted males had significantly higher replete ticks than control males (mean total ticks: testosterone-implanted males = 29.6 ± 7.2 , blank-implanted males = 15.1 ± 3.9 ; $Z = 7.79$, $P \leq 0.0001$; Fig. 1).

3.5 Mite Intensities

Blank-implanted males had significantly higher mite intensities than testosterone-implanted males (mean mites: testosterone-implanted males = 14.8 ± 2.2 , blank-implanted males = 23.5 ± 9.4 ; $Z = -4.85$, $P \leq 0.0001$; Fig. 1).

4. Discussion

The results of this study suggest that testosterone influences ectoparasite intensities in *S. occidentalis*. Experimental elevation of testosterone concentrations led to increased intensities of both juvenile life stages of the tick *I. pacificus*, allowing me to support the hypothesis that testosterone causes this species of lizard to be more heavily parasitized by ticks. However, experimental elevation of testosterone had the opposite effect on mite intensities, with testosterone-implanted lizards having lower numbers of mites than blank-implanted lizards.

Positive relationships between testosterone and ectoparasite intensities have been demonstrated in several other species of lizards. Salvador et al. (1996) showed that by experimental elevation of testosterone in a free-living population of the large *Psammodromus* lizard (*Psammodromus algirus*) resulted in increased tick intensities. With respect to naturally occurring concentrations of testosterone, male Eastern fence lizards *Sceloporus undulatus* (Klukowski and Nelson, 2001) and striped plateau lizards *S. virgatus* (Cox and John-Alder, 2007) with higher circulating testosterone concentrations had higher mite intensities. Although numerous studies suggest a positive relationship between parasitism and testosterone concentrations, the precise mechanisms that drive the increased parasitism remain unclear.

One potential way that testosterone may influence parasitism is through a physiological trade-off. Testosterone is the major androgenic hormone responsible for increased frequency or intensity of sexual behaviors and secondary sexual characteristics during the breeding season, such as increased territorial behavior (Crews et al., 1978;

Sinervo et al., 2000) and increased breeding coloration (Cooper et al., 1987; Díaz et al., 1994; Salvador et al., 1996). Several studies that examined the relationship between testosterone, territorial behavior, and breeding coloration have investigated *Sceloporus* species. For example, studies on the mountain spiny lizard (*S. jarrovi*, Moore, 1986; Marler and Moore, 1989; Cox et al., 2008) and *S. undulatus* (Rand, 1992; Klukowski and Nelson, 1998; Cox et al., 2005, 2005a) have shown that increased concentrations of testosterone resulted in increased numbers or intensities of territorial displays, movements, and breeding coloration. While an increase in such characteristics may increase the reproductive success of the male (Ruby, 1978; DeNardo and Sinervo, 1994; Haenel et al., 2003, 2003a; John-Alder et al., 2009) they are energetically costly to maintain.

One cost of increased investment into reproduction is immunosuppression. Testosterone directly or indirectly suppresses various aspects of the immune system (Grossman, 1985). In phytohemagglutinin stimulation studies, where degree of wound swelling in response to injection with antigen is measured, more swelling indicates a more robust immune response. Testosterone-implanted males exhibited significantly less swelling compared to control males, in both lizards (Belliure et al., 2004; Oppliger et al., 2004) and birds (Duffy et al., 2000). Similar results were observed with respect to number of leukocytes, with testosterone-implanted males having significantly fewer leukocytes than control males, in lizards (Veiga et al., 1998; Uller and Olsson, 2003) and birds (Zuk et al., 1995). Furthermore, the humoral immune response has also been shown to decrease when testosterone is elevated. In ocellated skinks (*Chalcides ocellatus*), testosterone treatment led to decreased IgG antibody titers against rat erythrocytes (Saad

et al., 1990). In European starlings (*Sturnus vulgaris*), testosterone-implanted individuals had a significantly reduced IgG antibody response to keyhole limpet hemocyanin (KLH) (Duffy et al., 2000).

Testosterone-induced inhibition of immune function may affect the host's ability to respond to an ectoparasite like a tick. Upon attachment, ticks inject saliva into the feeding lesion of the host skin and initiate feeding. An effective host immune response can decrease the efficiency of this hematophagy by either preventing the tick from acquiring a complete blood meal or by killing the tick (Ribeiro, 1989; Wikel et al., 1994; Brossard and Wikel, 2004). The host response first involves blood coagulation. Damaged tissue cells release adenosine diphosphate, leading to the recruitment of platelets. A coagulation cascade is then initiated by numerous proteolytic enzymes and factors. Using surface adhesion proteins called integrins, the platelets begin to aggregate and adhere together, forming a plug (Champagne and Valenzuela, 1996). This plug is an attempt by the host's body to prevent blood from being lost. Additionally, serotonin and thromboxane A₂ are released to constrict the blood vessels, resulting in drop in blood flow to the area (Champagne and Valenzuela, 1996). Connected with host blood coagulation is a complex host immune response, involving antigen-presenting cells, cytokines, T lymphocytes, natural killer cells, complement, and antibodies (Wikel et al., 1996). These components can disrupt blood meal acquisition, impair physiological processes, and kill the tick (Wikel, 1996). Furthermore, in mammals it has been shown that after repeated exposure hosts develop increased resistance to ticks (Galbe and Oliver Jr., 1992). However, host immune reactivity to ticks is relatively poorly understood in reptiles. One study by Galbe and Oliver Jr. (1992) showed that after repeated infestations

of *I. scapularis* ticks, broad-headed skinks (*Plestiodon laticeps*) did not develop increased resistance to ticks. This lack of a development of resistance could be one reason why lizards can have high tick intensities even after multiple infestations. Further studies, however, are needed to examine the immunological relationship between reptile host-tick parasite relationships.

Tick saliva is composed of a complex array of compounds that function to increase blood flow to the feeding tick, decrease clotting, and combat the host immune response (Ribeiro, 1995; Schoeler and Wikel, 2001; Brossard and Wikel, 2004; Hovius et al., 2008). Counteracting the host blood coagulation response are tick apyrases, disintegrin-like peptides, and vasodilators (Champagne and Valenzuela, 1996). Tick apyrase hydrolyzes the ADP released from damaged host cells, which diminishes platelet recruitment. Disintegrin-like peptides function in preventing platelets from aggregating and forming plugs. Tick saliva also has vasodilators that increase the diameter of blood vessels, increase blood flow, and counteract the effects of host serotonin and thromboxane A₂. In addition to the salivary compounds that oppose the host blood coagulation response, there are also those compounds that oppose the host immune response. Tick modulation of the host immune response targets both “pre-programmed” and specific immune defenses of the host. This strategy allows the feeding tick to continue receiving a blood meal in the presence of both the rapidly activated “pre-programmed” immune response, such as complement and natural killer cells, and the slower activated specific immune response involving antibodies and cell-mediated defenses (Wikel et al., 1996). It has been found that tick saliva contains compounds that block the activation of the alternative complement pathway, inhibit the host antibody

response, and suppress cytokine production (Wikel et al., 1996). Inhibition of these host immune responses leads to a more favorable environment to feed in and limits the amount of damage done to the tick. Consequently, initiating a robust immune response against such ectoparasites by hosts would be expected to decrease ectoparasite intensity. However, if concentrations of testosterone are high and testosterone decreases immune function, higher feeding success rates and tick intensities would be expected.

Although testosterone has been implicated in an immune-reproductive trade-off in several species and has been associated with increased parasite intensities as well, some studies have found either no effect of testosterone on the immune system (Saino et al., 1995; Uller and Olsson, 2003) or no effect on parasite intensities (Salvador et al., 1997; Oppliger et al., 2004). Moreover, a study by Evans et al. (2000) found that testosterone actually increased antibody production in male house sparrows. Additionally, although the immune system is clearly important in defense against ectoparasites (Wikel, 1996; Wiel et al., 2006), there are few studies that connect immune response to ectoparasite intensities. Several studies in mammals have demonstrated a humoral immune response to tick salivary gland components (Wheeler et al., 1989; Canals et al., 1990; Galbe and Oliver, Jr., 1992). A link between testosterone, immune function, and ectoparasite intensity is therefore plausible, but more studies need to be done before definitive conclusions can be made.

Another potential way that testosterone may increase parasite levels on hosts is through altered behavioral patterns and daily activity periods, which could increase the host's probability of encountering parasites. Male lizards with higher testosterone concentrations have increased territorial behaviors, daily activity periods, and movement

patterns (Marler and Moore, 1988, 1989; DeNardo and Sinervo, 1994; Klukowski and Nelson, 1998; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009). However, there is little direct evidence that suggests these changes in behavioral patterns and daily activity actually increase the exposure to parasites. Olsson et al. (2000) demonstrated that male sand lizards (*Lacerta agilis*) had increased mobility when testosterone concentrations were increased, which led to an increase in tick intensities on those lizards. These diurnal lizards range throughout much of Europe, including southern England and southern Sweden, and inhabit heathland and open grasslands with interspersed shrubs (House and Spellerberg, 1983; Tjisse-Klasen et al., 2010), where they become infested with ticks (*I. ricinus*) while moving through vegetation. On the other hand, Lane et al. (1995) suggest that infestation of *S. occidentalis* by *I. pacificus* primarily occurs when lizards are nocturnally inactive beneath a light layer of soil or leaf litter, not when they are active during the day. One potential explanation for this observation is that larval and nymphal stages of this tick species do not ascend vegetation, but instead appear to quest beneath leaf litter and the topmost soil surface (Lane et al., 1995). The results of these studies are evidence that ectoparasite infestation of lizards can vary depending on the behavior of the host species and the location in which they encounter ectoparasites. Therefore, more studies need to be performed before a relationship between testosterone, behavior, movement, and parasite intensity can be concluded.

An interesting observation in this study is that blank-implanted male lizards had significantly higher mite intensities than testosterone-implanted male lizards. One potential explanation for this could be interspecific competition between the ticks and

mites, where ticks outcompete the mites for optimal sites of attachment on the host. As noted by Wharton and Fuller (1952), mites often have specific feeding sites they prefer, frequently attaching in large groups where skin layers of the host are thin. The major site of attachment for ticks and mites on *S. occidentalis* is the nuchal pocket, where parasites are unlikely to be brushed off as the host moves around (Arnold, 1986). It is possible that increased tick intensities in the nuchal pocket on testosterone-implanted lizards resulted in a crowding out of mites from this area, resulting in lower mite intensities than blank-implanted lizards. This reasoning is mostly speculative as there have been no studies to date that have investigated potential interspecific competition among ectoparasites for feeding locales. Factors influencing parasite community structure are relatively unexplored, in part because of the difficulty of manipulating and monitoring parasites in natural settings (Janovy, 2002).

Another potential explanation for why blank-implanted lizards had significantly higher mite intensities than testosterone-implanted lizards is that the effectiveness of the host immune response against mites versus ticks may be different. Parasites are known to compete interspecifically (Combes, 2001; Poulin, 2007); competitive interactions may be mediated by the host immune system. Mites tend to remain on reptilian hosts for longer periods of time than ticks (Arnold, 1986; Curtis and Baird, 2008); thus it would be beneficial for mites to invoke less of a host immune response. For example, species of trombiculid mites that normally infest mammals seem to cause only a slight immune response (Wrenn, 1996). Analogous comparisons can be made between long-term parasitic mites and lice that remain on the host for the duration of their lives, and must not elicit strong host immune responses that could compromise their ability to feed, and

short-term parasites such as mosquitoes (less than 10 minutes) (Ribeiro, 1987) and ticks (2-7 days for ticks, depending on life stage) (Sonenshine, 1991), which feed for a shorter time period and elicit a more intense host immune response. Furthermore, testosterone may have different effects on immune responses that are relevant to different parasites. Fuxjager et al. (2011) demonstrated that elevated testosterone increased trombiculid mite abundance, decreased the abundance of *Physaloptera retusa* gastrointestinal nematodes, and had no effect on *Spauligodon giganticus* gastrointestinal nematode abundance. It is possible that a similar relationship may be present with ticks and mites that feed *S. occidentalis*.

In summary, I have shown that free-ranging male lizards with elevated testosterone concentrations during the spring mating season harbor more ticks than their counterparts with lower testosterone concentrations. Additionally, I have demonstrated that free-ranging male lizards with lower circulating testosterone concentrations are infested more heavily with mites than male lizards with elevated circulating testosterone concentrations. The results of the present study are in agreement with numerous other studies on lizards, but the exact mechanism for how testosterone influences parasite intensities has yet to be determined. Future studies should focus on determining the behavioral and physiological mechanisms that give rise to the observed effects of testosterone on parasitism, including parasite intensity, immunosuppression, and parasitic effects on host fitness.

CHAPTER II:

1. Introduction

Host-parasite relationships are one of the most common symbiotic relationships in biology. Almost all organisms are vulnerable to some type of parasite and these parasites may exert numerous pressures on their hosts. Such pressures include altered behavioral patterns (Schall and Sarni, 1987; Dunlap and Schall, 1995; Bakker et al., 1997; Klein, 2003), decreased fitness (Baudoin, 1975; Hamilton and Zuk, 1982; Schall and Dearing, 1987; Lope et al., 1998; Møller, 1999), and decreased immune function and overall health (Rechav et al., 1980; Schall, 1990; Salvador et al., 1996). Additionally, there are numerous factors that influence the degree of parasitism. These factors include, but are not limited to, host sex, hormone concentrations, and reproductive effort.

The role of host sex in host-parasite relationships has been examined across multiple taxa. These studies have consistently shown males to have higher parasite intensities than females (Poulin, 1996; Zuk and McKean, 1996). This has been observed in several species, including ball pythons (Aubret et al., 2005), lizards (Schall and Marghoob, 1995; Schall et al., 2000; Klukowski and Nelson, 2001; Salkeld and Schwarzkopf, 2005), salamanders (Anthony et al., 1994), reindeer (Folstad et al., 1989), and red jungle fowl (Zuk, 1990). The explanation for this observation is not fully understood, but the androgenic hormone, testosterone (T), is believed to be a key factor. Testosterone concentrations are typically higher in males than females. Additionally, studies have demonstrated that males with higher concentrations of circulating testosterone have higher parasite intensities (Saino et al., 1995; Salvador et al., 1996;

Olsson et al., 2000; Hughes and Randolph, 2001; Klukowski and Nelson, 2001; Roberts et al., 2004; Cox and John-Alder, 2007). Testosterone concentrations are typically higher in males than females within a species and it has been shown to influence numerous aspects of an organism's behavior and physiology.

There are two major potential explanations for why male lizards have higher ectoparasite intensities compared to female lizards. Testosterone may stimulate increases in territorial behaviors and movement in males, which could expose them to more parasites. Studies on lizards have demonstrated that testosterone increases the frequency of territorial behaviors (Moore, 1986; Marler and Moore, 1988, 1989; Wingfield and Hahn, 1994; Klukowski and Nelson, 1998; Sinervo et al., 2000), home-range size (DeNardo and Sinervo, 1994; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009;), and movement (Olsson et al., 2000; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009). Additionally, testosterone may inhibit immune function, which, in turn, allows more parasites to infest, feed, and survive on males than on females. The inhibitory effect of testosterone on immune function has been observed in studies on lizards (Saad et al., 1990; Uller and Olsson, 2003; Oppliger et al., 2004; Belliure et al., 2004), birds (Zuk et al., 1995; Duffy et al., 2000; Casto et al., 2001; Grieves et al., 2006), and rodents (Benten et al., 1993; Hughes and Randolph, 2001; Stevenson and Riley, 2004). Furthermore, Veiga et al. (1998) showed that when testosterone concentrations are artificially increased, immune function decreases, and ectoparasite intensity increases.

Related to circulating testosterone concentrations, reproductive effort of the host may impact parasite intensities. Reproductive effort is defined as the proportion of resources invested in reproduction (Ricklefs, 1977; Vitt and Congdon, 1978; Tuomi et al.,

1983). In male lizards, peak reproductive effort occurs during the mating season when circulating testosterone concentrations are high (Goldberg, 1974; Tokarz et al., 1998; Brasfield et al., 2008; Taylor et al., unpublished). Testosterone has been shown to increase energy expenditure through longer daily activity periods and increases in frequency of territorial behavior. Furthermore, a study by Marler and Moore (1989) demonstrated that testosterone-implanted male lizards made fewer foraging attempts, caught fewer prey, had lower gut content mass, and had less stored energy in the form of lipids. This suggests that there is a time conflict between territorial behavior and foraging. These results have been corroborated by several other studies since (Marler and Moore, 1991; Klukowski et al., 1998; Cox et al., 2005) and it has also been shown that an increase in circulating testosterone leads to an increase in metabolic rate (Buchanan et al., 2001; Oppliger et al., 2004). The increased energy expenditure, as an indirect result of increased circulating testosterone, may lead to an insufficient amount of energy for immune function. This trade-off could lead to increased parasite intensities on males with high testosterone concentrations. Peak reproductive effort in female lizards occurs during vitellogenesis in oviparous species (Tinkle, 1969; Vitt et al., 1978; French et al., 2007). During vitellogenesis females produce the protein vitellogenin, which diverts energy to yolk production for follicles. This process is very energetically costly and can negatively impact immune function. When female birds have larger clutch sizes their immune function is significantly lowered (Norris et al., 1994; Nordling et al., 1998; Cichon et al., 2001). In female tree lizards (*Urosaurus ornatus*), immune activity is suppressed during vitellogenesis, but only under conditions when lizards are food-limited (French et al., 2007, 2007a, 2007b; French, 2008). Immunosuppression caused by increased

reproductive effort may result in increased parasite intensities, as observed in greater mouse-eared bats (*Myotis myotis*, Christe et al., 2000), collared fly catchers (*Ficedula albicollis*, Nordling et al., 1998), and common lizards (*Lacerta vivipara*, Sorci et al., 1996).

Although sex, hormone concentrations, and reproductive effort have been demonstrated to impact parasite load, very few studies have examined the role of host choice by parasites. The few studies that have been done on host choice have demonstrated that some species of ticks exhibit a preference for specific host species. For example, James and Oliver (1990) demonstrated that when juveniles of three species of Ixodid ticks (*I. dammini*, *I. scapularis*, and *I. pacificus*) were given a choice between a mouse, a lizard, and a chicken, the larvae of all three species preferred mice and the nymphs of all three species preferred mice and lizards. In another study (Slowik and Lane, 2009), larval and nymphal ticks of *Dermacentor occidentalis*, *I. pacificus*, and *I. jellisoni* were given a choice between a deer mouse (*Peromyscus maniculatus*), California kangaroo rat (*Dipodomys californicus*), western fence lizard (*Sceloporus occidentalis*), and California towhee (*Pipilo crissalis*). Both larval and nymphal *D. occidentalis* preferred rodents, *I. pacificus* preferred lizards, and *I. jellisoni* preferred kangaroo rats. We are only aware of one study that examined the preference of ticks for conspecific hosts with differing physiological states. In field and lab studies on two species of bats (*Myotis myotis* and *M. blythii*), parasitic mites had a clear preference for individuals that had unlimited access to food, thus having a higher nutritional status (Christe et al., 2003). These host choice studies suggest that there may be some cue emitted by hosts that

ectoparasites can detect in order to choose the most appropriate or beneficial host. The potential cue, however, is unknown.

The purpose of this study was to determine if larval western black-legged ticks (*Ixodes pacificus*) exhibit a preference for (*Sceloporus occidentalis*) in differing physiological conditions. This species of lizard is often parasitized by the larval and nymphal stages of western black-legged ticks (Eisen et al., 2004). The larvae and nymphs are commonly attached to a small skin fold between the ear and shoulder, termed the nuchal pocket (Arnold 1986). Ticks preferentially attach to regions of the lizard such as these that have fewer scales. Tick attachment occurs by insertion of the barbed hypostome into the skin, remaining attached for several days during feeding by secretion of a cement-like compound from the salivary glands to anchor them in place. After feeding, the engorged (replete) larvae and nymphs drop off and molt into nymphs and adults, respectively. Additionally, it has been observed that male lizards have higher tick intensities than female lizards of this species (Eisen and Eisen, 1999; Schall et al., 2000; Eisen et al., 2001; Lumbad et al., in press). The first experiments in the present study tested the hypothesis that ticks prefer male lizards over female lizards. If this is true, then a greater number of ticks will attach and successfully feed on male lizards than on female lizards exposed to larval ticks in the laboratory. The second experiment tested the hypothesis that ticks prefer male lizards with high testosterone concentrations (representative of the mating season) over male lizards with low testosterone concentrations. If this is true, then a greater number of ticks will attach and successfully feed on testosterone-implanted males than on control males. The final experiment tested the hypothesis that ticks prefer vitellogenic female lizards over non-vitellogenic female

lizards. If this is true, then a greater number of ticks will attach and successfully feed on vitellogenic female lizards than on female non-vitellogenic lizards.

2. Methods

2.1 Study Animals

Sceloporus occidentalis were collected by hand-held noose from the Chimineas Ranch unit of the Carrizo Plain Ecological Reserve in San Luis Obispo, California. This area is characterized as semi-arid grassland with areas of scattered oak trees and numerous rock outcrops. In contrast to nearby coastal regions (Lumbad et al. in press), *I. pacificus* has not been found to infest *S. occidentalis* at Chimineas, likely because this habitat would not be expected to provide adequate humidity for *I. pacificus* development, though it is possible that isolated microhabitats may be permissive for this tick species. Lizards were transported back to the California Polytechnic State University, San Luis Obispo in cloth bags, and snout-vent length (SVL, +/- 0.5 cm) and body mass (g, +/- 0.5 g) were measured.

Laboratory populations of larval *Ixodes pacificus* were generated for use in the study by feeding field-collected adults on bulls. Ticks were collected using the blanket dragging method (Falco and Fish, 1992) along a forest trail in Montaña de Oro State Park, California. Collected ticks were maintained in 20 ml plastic vials (Wheaton Science Products, Millville, NJ, U.S.A.) containing a mixture of plaster of Paris with activated charcoal to prevent desiccation and retard mold growth. Vials were suspended above distilled water (100% humidity) within glass desiccator jars at 23°C and maintained under

an 8:16 light:dark photoperiod until feeding. Pairs of male and female ticks (approximately 20 of each sex per animal) were fed to repletion on bulls (*Bos primigenius taurus*) at the California Polytechnic State University beef unit and fed until fully replete (engorged with blood). Fully engorged female ticks were housed individually in vials as above until oviposition and larval emergence at 6-8 weeks. Larvae were held in the laboratory for approximately 4 weeks post-emergence before placement on lizards to ensure readiness for host feeding. For some trials, larval age was up to three months post-emergence.

2.2 Experiment 1 Design: Male Versus Female Host Choice

Male-female host choice experiments were performed at three different time periods during 2009: spring (April 20 - May 9, $n = 13$), early summer (May 10 - June 4, $n = 12$), and mid-summer (June 21 - July 13, $n = 13$), chosen to examine lizards at times of varying reproductive effort. In this species, circulating testosterone concentrations are high in males in the spring and low in the summer (Taylor, unpublished). Female lizards were deemed vitellogenic if follicles were detected by palpation. All spring and early summer females were non-vitellogenic, and mid-summer females were vitellogenic.

For tick host preference trials, male and female lizards were paired by size and placed into 2,500 ml beakers, each containing a microcentrifuge tube of 100 tick larvae. To permit host basking, beakers were placed close to a 60W incandescent lamp for approximately 12 h. Fine mesh was secured around the top of the beaker with rubber bands to prevent tick escape from the beakers. Male and female lizard pairs were not significantly different in SVL (spring: two-tailed $T = 0.24$, $P = 0.814$, early summer: two-tailed $T = 0.31$, $P = 0.760$, mid-summer: two-tailed $T = 1.81$, $P = 0.089$) and body mass

(spring: two-tailed $T = -0.80$, $P = 0.429$, early summer: two-tailed $T = 0.20$, $P = 0.842$).

However, male and female lizards paired together in mid-summer were significantly different in body mass with females being larger (two-tailed $T = 3.52$, $P = 0.002$).

Infestation trials spanned 48 hours during which beakers were periodically misted with distilled water to prevent desiccation of the larvae. Lizards were then removed and placed into individual 13 x 8 x 8 (cm) metal mesh cages elevated above tubs of water to collect mites and ticks infesting lizards as they dropped off these hosts. Tubs were 30 x 16 x 8 (cm) and filled with 4 cm of water, such that any ticks dropping off the host lizards would fall into the tubs and float until they were retrieved daily by the investigators. The sides of the tubs were coated with Fluon (Bioquip, Rancho Dominguez, CA, U.S.A.) to prevent ticks escape. Tubs were placed in environmental chambers (27°C, 8:16 light:dark). Water was offered *ad libitum* and 2-3 crickets were offered per day. Replete and unfed tick numbers were quantified daily as ticks dropped off into the water. The number of residual ticks that did not attach was recorded for each lizard. The experiments continued for approximately three weeks, until all ticks were collected. The numbers of replete ticks and unfed ticks were quantified daily as the ticks dropped off of the lizard hosts and into the water tub. At the termination of the experiment the total number of replete ticks and repletion rate (the average time it took for ticks to become engorged with a blood meal and drop off) were calculated for each lizard. The overall total number of ticks (replete and unfed combined) on lizards was also calculated.

2.3 Experiment 2 Design: High Testosterone Versus Low Testosterone Male Host Choice

This study was performed during late summer, when male lizards have low circulating testosterone concentrations (Taylor, unpublished). On September 18, 2009,

twenty-eight male lizards were captured, brought to the laboratory, and measured as above. Lizards were randomly assigned to one of two treatment groups, testosterone-implanted ($n = 14$) or blank-implanted ($n = 14$). The treatment groups did not significantly differ in SVL (two-tailed $T = -0.23$, $P = 0.821$) or body mass (two-tailed $T = 0.60$, $P = 0.554$). Implants were made from 5 mm pieces of silastic diffusion tubing (Dow Corning, Clarkesville, TN, U.S.A.: 1.47 mm inner diameter, 1.96 mm outer diameter), capped and sealed with silicon caulking, and filled with either 3 mm of crystalline testosterone propionate (Sigma-Aldrich, St. Louis, MO, U.S.A.) (testosterone-implanted) or silicon caulking (blank-implanted). Prior to implantation, lizards were placed on ice to induce cold-induced surface anesthesia until they exhibited no foot-withdrawal reflex. Implants were placed into the coelomic cavity via a small ventrolateral incision that was then closed with absorbable suture. Lizards were housed outdoors for a 14-day period (to allow the implants to take effect) in 2.4 m diameter snapset kiddie pools (Intex Recreation Corp., Long Beach, CA, U.S.A.) containing sand substrate, plants for shade, and cinder blocks for basking.

At the end of the treatment period, male lizards were bled from the postorbital sinus with heparinized capillary tubes within 5 minutes after capture to determine post-treatment testosterone concentration. Several hours later, blood samples were centrifuged for 5 minutes at 10,000 rpm, and plasma was extracted and frozen at -20°C . Plasma samples were shipped to Virginia State University, and circulating concentrations of testosterone were quantified by radioimmunoassay (RIA) according to the methods of Lind et al. (2010). Testosterone-implanted and blank-implanted lizards were then paired

together by size and infested with tick larvae as in Experiment 1. Data collection proceeded as in Experiment 1.

2.4 Experiment 3 Design: Vitellogenic Versus Non-Vitellogenic Female Host Choice

This study was performed during late summer when female lizards were not vitellogenic, confirmed by palpation. On August 6, 2009, twenty-two female lizards were captured, brought to the laboratory, and randomly placed into one of two treatment groups, vitellogenic ($n = 12$) or non-vitellogenic ($n = 12$). The two groups were not significantly different in SVL (two-tailed $T = 0.21$, $P = 0.836$) or body mass (two-tailed $T = 0.88$, $P = 0.393$). Vitellogenic lizards were injected intracoelomically with 0.10 ml of follicle stimulating hormone (FSH, Sigma-Aldrich, St. Louis, MO, U.S.A.) dissolved in Reptile Ringers solution every other day for 24 days, until palpation indicated they were vitellogenic. Vitellogenesis was confirmed after the experiment by post-mortem dissection. Non-vitellogenic lizards were injected intracoelomically with an identical volume of Reptile Ringers every other day for 24 days. Lack of vitellogenesis was confirmed by post-mortem dissection. Throughout the treatment period the lizards were housed outdoors in kiddie pools as in Experiment 2.

At the end of the 24-day treatment period female lizards were paired together by size and infested with ticks as in Experiments 1 and 2.

2.5 Data Analysis

Plasma testosterone concentrations of testosterone-implanted and blank-implanted lizards were compared with two-sample t-tests. Numbers of replete ticks and repletion rates for treatment groups (male versus female, Testosterone-implanted lizards versus blank-implanted, and vitellogenic versus non-vitellogenic) were compared using paired t-

tests. For the male versus female host choice studies, a one-way ANOVA with Tukey pair-wise comparisons assuming equal variances was performed to compare repletion rates between the three different time periods (spring, early summer, and mid-summer) for male and female lizards. All t-tests were two-tailed and p-values were considered significant at the $\alpha = 0.05$ level. Statistical analyses were performed using Minitab Statistical Software version 10 (State College, PA, U.S.A.).

3. Results

3.1 Male Versus Female Host Choice

3.1.1 Tick Intensities

There was no significant difference in replete tick intensities on male versus female lizards in any of the three time periods: spring (mean replete ticks: males = 23.7 ± 4.5 , females = 17.9 ± 3.4 ; two-tailed $T = 0.95$, $P = 0.360$; Fig. 2a), early summer (mean replete ticks: males = 25.3 ± 2.9 , females = 19.9 ± 2.8 ; two-tailed $T = 1.09$, $P = 0.299$; Fig. 2b), and mid-summer (mean replete ticks: males = 27.8 ± 4.9 , females = 23.7 ± 4.6 ; two-tailed $T = 0.50$, $P = 0.629$; Fig. 2c).

3.1.2 Male Versus Female Repletion Rates

Repletion rates between male and female lizards did not significantly differ during spring (mean repletion rate: males = 12.8 ± 0.5 days, females = 13.0 ± 0.4 days; two-tailed $T = -0.54$, $P = 0.600$) and early summer (mean repletion rate: males = 12.9 ± 0.3 days, females = 12.7 ± 0.4 days; two-tailed $T = 0.34$, $P = 0.744$) time periods. However, ticks fed significantly faster on female lizards than on male lizards during the

mid-summer time period (mean repletion rate: males = 13.4 ± 0.3 days, females = 11.5 ± 0.2 days; two-tailed $T = 4.53$, $P = 0.001$).

3.1.3 Repletion Rates Across Time Periods

Repletion rates for male lizards across the three time periods did not significantly differ from each other ($F = 0.54$, $P = 0.589$; Fig. 3). Repletion rates for female lizards were significantly faster in mid-summer than in spring and early summer time periods ($F = 5.52$, $P = 0.008$; Fig. 4).

3.2 High Testosterone Versus Low Testosterone Male Host Choice

3.2.1 Implants

Testosterone implantation almost doubled circulating concentrations of testosterone (testosterone-implanted males = 35.7 ± 2.0 ng/ml, blank-males = 21.1 ± 2.8 ng/ml; two-tailed $T = -4.25$, $P \leq 0.0001$).

3.2.1 Tick Intensities

There was no significant difference in replete tick intensities (mean replete ticks: testosterone-implanted males = 29.7 ± 3.1 , blank-implanted males = 26.6 ± 4.2 ; two-tailed $T = -0.59$, $P = 0.566$; Fig. 5) on testosterone-implanted versus control males.

3.2.2 High Testosterone Versus Low Testosterone Male Repletion Rates

Ticks fed significantly faster on control males compared to testosterone-implanted males (mean repletion rate: testosterone-implanted males = 13.8 ± 0.3 days, blank-implanted males = 13.2 ± 0.3 days; two-tailed $T = -2.30$, $P = 0.038$).

3.3 Vitellogenic Versus Non-Vitellogenic Female Host Choice

3.3.1 Tick Intensities

There was no significant difference in replete tick intensities (mean replete ticks: vitellogenic = 32.8 ± 4.0 , non-vitellogenic = 26.6 ± 4.1 ; two-tailed $T = -0.83$, $P = 0.427$; Fig. 6) on vitellogenic versus non-vitellogenic females.

3.3.2 Vitellogenic Versus Non-Vitellogenic Female Repletion Rates

There was no significant difference in repletion rate between vitellogenic and non-vitellogenic lizards (mean repletion rate: vitellogenic = 14.8 ± 0.4 days, non-vitellogenic = 13.8 ± 0.3 days; two-tailed $T = -2.13$, $P = 0.059$).

4. Discussion

Several studies have demonstrated a host preference by ticks when given a choice between a mammal, lizard, and bird host (James and Oliver, 1990; Slowik and Lane, 2009). However, no studies to date have examined whether ticks exhibit preference for hosts of either sex, varying hormonal concentrations, or reproductive effort. The results from this study suggest that *I. pacificus* larvae do not exhibit a preference when given a choice among *S. occidentalis* individuals within the same species, regardless of sex, hormonal state, and reproductive condition. Therefore, it may be that larval ticks are unable to detect host physiological condition prior to attachment.

Our hypothesis that larval ticks prefer male lizards to female lizards was rejected. This was shown at three different times of the year, spring (males have high testosterone and females are not reproductive), early summer (males have low testosterone and females are not reproductive), and mid-summer (males have low testosterone and females are vitellogenic). These findings suggest that ticks are not able to detect some sort of cue,

such as chemicals in skin lipids, emitted by host lizards that would indicate its sex or reproductive condition. Free-ranging male lizards have higher tick loads than female lizards (Eisen and Eisen, 1999; Schall et al., 2000; Eisen et al., 2001; Lumbad et al., in press); this sex difference must be due a factor other than a preference for male host over females. Rather than preferentially choosing a particular host, it may be more beneficial for questing ticks to attach to any lizard that passes by because they may never encounter another host and, as a result, will starve to death. There are numerous factors that influence the rate at which ticks encounter hosts and influence survival and mortality rates. Such factors include temperature, day length, moisture, predation, and, in particular, the abundance of potential hosts (Randolph, 2004). These factors fluctuate often and, as a result, so do the host encounter, survival, and mortality rates of ticks. A study by Daniel et al. (1976) demonstrated that tick survival and daily mortality rates varied depending on the life stage, time of the year, and on the density of potential hosts available. Additionally, Randolph (1998) postulated that for a tick species with a mean fecundity of 2000 eggs, on average 5 %, 10%, and 20% of ticks will survive from eggs to larvae, larvae to nymphs, and nymphs to adults, respectively.

When tick repletion rates were compared between male and female lizards, the only significant difference was observed during the mid-summer time period when male lizards have low circulating testosterone and female lizards are vitellogenic. Ticks fed more quickly on females than males during this time period, and indeed ticks fed more quickly on female lizards in mid-summer than in the other time periods. One explanation for this difference is that vitellogenic female lizards may have decreased immune function, which permitted increased tick feeding rates. Upon attachment, ticks inject saliva into the feeding lesion of the host skin and initiate feeding. Tick saliva is composed

of a complex array of compounds that function to increase blood flow to the feeding tick, decrease clotting, and combat the host immune response (Ribeiro, 1995; Schoeler and Wikel, 2001; Brossard and Wikel, 2004; Hovius et al., 2008). Hosts respond by mounting a response against these compounds in the tick's saliva, thereby decreasing the efficiency of hematophagy by either prematurely shortening feeding duration, leading to early drop-off (partial repletion), or by killing the tick, often *in situ* (Ribeiro, 1989; Wikel et al., 1994; Brossard and Wikel, 2004).

The host response first involves blood coagulation. Damaged tissue cells release adenosine diphosphate, leading to the recruitment of platelets. A coagulation cascade is then initiated by numerous proteolytic enzymes and factors. Using surface adhesion proteins called integrins, the platelets begin to aggregate and adhere together, forming a plug (Champagne and Valenzuela, 1996). This plug is an attempt by the host's body to prevent blood from being lost. Additionally, serotonin and thromboxane A₂ are released to constrict the blood vessels, resulting in drop in blood flow to the area (Champagne and Valenzuela, 1996). Counteracting the host blood coagulation response are tick apyrases, disintegrin-like peptides, and vasodilators (Champagne and Valenzuela, 1996). Tick apyrase hydrolyzes the ADP released from damaged host cells, which diminishes platelet recruitment. Disintegrin-like peptides function in preventing platelets from aggregating and forming plugs. Tick saliva also has vasodilators that increase the diameter of blood vessels, increase blood flow, and counteract the effects of host serotonin and thromboxane A₂.

Connected with host blood coagulation is a complex host immune response, involving antigen-presenting cells, cytokines, T lymphocytes, natural killer cells,

complement, and antibodies (Wikel et al., 1996). These components can disrupt blood meal acquisition, impair physiological processes, and kill the tick (Wikel, 1996). Similar to the salivary compounds that oppose the host blood coagulation response, there are also compounds that oppose the host immune response. Tick modulation of the host immune response targets both “pre-programmed” and specific immune defenses of the host. This strategy allows the feeding tick to continue receiving a blood meal in the presence of both the rapidly activated “pre-programmed” immune response, such as complement and natural killer cells, and the slower activated specific immune response involving antibodies and cell-mediated defenses (Wikel et al., 1996). It has been found that tick saliva contains compounds that block the activation of the alternative complement pathway, inhibit the host antibody response, and suppress cytokine production (Wikel et al., 1996). Inhibition of these host immune responses leads to a more favorable environment to feed in and limits the amount of damage done to the tick. Consequently, initiating a robust immune response may prevent ticks from feeding to repletion or at least slow down the rate at which ticks feed. Vitellogenesis is an energetically expensive process (Braña et al., 1992; Bonnet et al., 1994) and if a female individual does not have appropriate resources to support both reproductive and immune systems during this time, then a trade-off may occur, leading to suppression of the immune response (French et al., 2007, 2007a, 2007b; French, 2008). This may enhance the ability and speed of attached ticks to feed to repletion.

There were no differences in the number of ticks feeding on males with experimentally elevated testosterone and control males, causing us to reject the hypothesis that ticks would exhibit a preference for male lizards with higher circulating

testosterone concentrations. Studies in free-ranging lizards in the field have documented increased ectoparasite intensities in males with experimentally elevated testosterone (Saino et al., 1995; Salvador et al., 1996; Olsson et al., 2000; Klukowski and Nelson, 2001; Cox and John-Alder, 2007), including *S. occidentalis* (Pollock et al., chapter 1 of this thesis). These results suggest an interesting role of testosterone in mediating ectoparasite loads. If ticks do not prefer males with high testosterone, then testosterone must influence tick loads in some other way. We suggest two hypotheses to explain this, the first involving the effect of testosterone on immune function (Saad et al., 1990; Veiga et al., 1998; Uller and Olsson, 2003; Oppliger et al., 2004; Belliure et al., 2004) and the second involving the effect of testosterone on territorial behaviors and movements (Moore, 1986; Marler and Moore, 1988, 1989; Klukowski and Nelson, 1998; Olsson et al., 2000; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009). Males generally have lower immune function compared to females, often due to inhibitory effects of testosterone (Folstad and Karter, 1992; Zuk and McKean, 1996; Mondal and Rai, 1999; Klein, 2000; Mondal and Rai, 2002; Tschirren et al., 2003). This could leave males more vulnerable to ectoparasitism by permitting more parasites to infest, feed, and survive. Additionally, testosterone has been shown to increase male territorial behaviors (Moore, 1986; Marler and Moore, 1989; Klukowski and Nelson, 1998; Sinervo et al., 2000) and movement (Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009), thus potentially exposing male lizards to more parasites. Therefore, testosterone, whether via inhibition of immune function or stimulation of behaviors that increase exposure to parasites, or a combination of the two, is an important factor that could mediate differences in parasite intensity between lizard sexes.

Ticks fed significantly faster on the blank-implanted lizards versus testosterone-implanted lizards. This is contrary to what was expected because if testosterone diminishes the immune response, this should permit ectoparasites to feed more rapidly on hosts (Ribeiro, 1989; Wikel et al., 1994; Brossard and Wikel, 2004). Although the majority of studies have shown an inhibitory effect of testosterone on the immune system, there have been a few studies showing the opposite effect (Tschirren et al., 2005). For example, Saino et al. (1995) demonstrated that while testosterone was positively related to ectoparasite load, antibody levels and number of leukocytes actually increased with experimentally elevated testosterone.

Similar to the results from the sex and testosterone host preference experiments, there was no evidence of a preference for host when reproductive effort in female lizards was manipulated, leading us to reject the hypothesis that ticks prefer vitellogenic lizards over non-vitellogenic lizards. This again suggests that questing ticks do not choose their host, but rather attach to any suitable host available.

Lastly, there was no significant difference in repletion rates between vitellogenic and non-vitellogenic female lizards. Under food-limited conditions the reproductive system draws energy from the immune system to support the costly process of vitellogenesis, which in turn may benefit ectoparasites on the host because of decreased host immune response. However, as shown by French et al. (2007, 2007a, 2007b) the immune response of vitellogenic female lizards is only impacted under conditions where there is not enough energy to support both immune and reproductive systems. The studies above found a decrease in immune function in vitellogenic lizards only when they were energy-limited; when food was available *ad libitum*, no difference were observed. All

female lizards in the present experiment were fed 2-3 crickets every day and were therefore not energy limited, which may explain why differences in tick repletion rates between vitellogenic and non-vitellogenic female lizards were not observed.

The experiments described here are the first to explore the potential interplay between tick host preference in response to sex, hormonal concentrations, and reproductive effort in reptiles. It has been widely shown that males have higher parasite intensities than females (Schall and Marghoob, 1995; Zuk and McKean, 1996; Schall et al., 2000; Aubret et al., 2005; Salkeld and Schwarzkopf, 2005), that experimental elevation of testosterone in males increases parasite intensities (Salvador et al., 1996; Olsson et al., 2000; Klukowski and Nelson, 2001; Cox and John-Alder 2007; Pollock et al., chapter 1 of this thesis), and a few studies have shown reproductive females to have higher parasite intensities than non-reproductive females (Sorci et al., 1996; Nordling et al., 1998; Christe et al., 2000). It is important to determine if ectoparasites can preferentially choose their hosts because it would be to the benefit of the parasite to attach and feed on the best host possible. Furthermore, evidence for a host preference would provide one explanation for the observed differences in parasite intensities among these groups. The fact that no preference for hosts was observed in any of the host choice experiments in this study suggests that ticks do not choose their host, but instead attach and feed on whatever host they come across. This allows us to rule out the possibility that ticks are attracted to a cue emitted by a potentially more beneficial host of a given species. Future studies should focus on determining the behavioral and physiological mechanisms that give rise to the observed effects of testosterone on parasitism in males.

Also, studies should be performed focusing on the role of female reproductive effort on parasite load, immunosuppression, and fitness.

CHAPTER III:

1. Introduction

Competition for mates and survivorship represent opposing selective forces that can optimize the level of territorial behavior since both are important for lifetime reproductive success (Thornhill and Alcock, 1983; Marler and Moore, 1989). The theory of sexual selection suggests one benefit of increased aggressive territorial behaviors is greater success in male-male competition for access to females. However, this reproductive benefit can be balanced out by costs of natural selection, such as increased risk of injury, increased exposure to predators, and increased energy use. While this theory of costs and benefits of male behavior can easily be rationalized, there have been relatively few studies to directly examine this type of relationship. By performing experiments where hormone concentrations are manipulated some costs of male territorial behavior may be revealed.

In many species, testosterone, the male steroid hormone, is the main mediator of territorial behavior. Increased testosterone concentration in males has been shown to increase territorial behavior in several species of lizards (Crews et al., 1978; Moore, 1986; Marler and Moore, 1988, 1989; Salvador et al., 1996; Klukowski and Nelson, 1998; Watt et al., 2003) and birds (Moore, 1984; Wingfield and Hahn, 1994; Hau et al., 2000; Mougeot et al., 2005). Furthermore, testosterone has been shown to influence movement and home range size in lizards. Lizards with higher circulating concentrations of the hormone have increased movement patterns (Olsson et al., 2000; Sinervo et al., 2000; Klukowski et al., 2004; Cox et al., 2005; John-Alder et al., 2009) and increased home range size (DeNardo and Sinervo, 1994; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009). It has been suggested that these testosterone-induced increases in territorial behavior and overall movement may improve a male's fitness by both

increasing access to females and increasing the ability to defend those females (Ruby, 1978; DeNardo and Sinervo, 1994; Haenel et al., 2003, 2003a; John-Alder et al., 2009). As a result, an increase in testosterone in male lizards during the breeding season may be reproductively beneficial.

Countering the reproductive benefits of increased testosterone are the costs on survivorship and health. Although male lizards with high testosterone have increased reproductive success, these males have diminished health because of increased parasitism and decreased body mass (through increased energy use). First, males with higher testosterone concentrations have been shown to have higher parasite intensities. This relationship has been shown in numerous studies on numerous species (Saino et al., 1995; Salvador et al., 1996; Olsson et al., 2000; Hughes and Randolph, 2001; Klukowski and Nelson, 2001; Roberts et al., 2004; Cox and John-Alder, 2007; John-Alder et al., 2009; Pollock et al., chapter 1 of this thesis). Increased parasitism can bring on costs to the health of the host in a wide variety of ways, such as anemia (Rechav et al., 1980; Schall 1982; Dunlap and Mathies, 1993; Pfäffle et al., 2009). This in turn can dramatically decrease survivorship of the infected host. Second, males with higher testosterone concentrations have been shown to have a significantly decreased body mass compared to males with lower testosterone concentrations (Marler and Moore, 1988, 1989, 1991; Ketterson et al., 1991; Olsson et al., 2000; Klukowski et al., 2004). Performing the territorial behaviors, having increased movement patterns, and increased home ranges is energetically expensive for individuals. If there is not enough energy coming in then health is impacted through a drastic decrease in body mass. This unhealthy decrease in body mass under high testosterone conditions, however, does not occur when resources

are plentiful (Marler and Moore, 1991). Due to these costs of testosterone on body mass and susceptibility to parasitism it can be assumed that there is also an impact on survivorship when testosterone is high. In fact, it has been demonstrated that male lizards with high concentrations of testosterone exhibit higher mortality than male lizards with lower concentrations of testosterone (Marler and Moore, 1988; Salvador et al., 1996; Sorci et al., 1996; John-Alder et al., 2009).

In a previous study it was shown that male *S. occidentalis* have more ticks than females (Lumbad et al., in press) and I proceeded to demonstrate a role of testosterone in tick parasitism in this species, where male lizards with experimentally elevated testosterone had higher tick intensities compared to control male lizards (Pollock et al., chapter 1 of this thesis). Although this testosterone-parasitism relationship has been demonstrated in several other studies the exact mechanism has yet to be determined. There have been numerous cited studies that suggest a role of testosterone-caused immunosuppression of the host, which leaves individuals more vulnerable to parasitism (Folstad and Karter, 1992; Veiga et al., 1998; Olsson et al., 2000; Perez-Orella and Schulte-Hostedde, 2005). It is also very plausible that increases in territorial behavior, movement, and home range size caused by testosterone expose individuals to more parasites.

Western fence lizards (*Sceloporus occidentalis*) are major hosts for juvenile western black-legged ticks (*Ixodes pacificus*, Eisen and Eisen, 1999; Eisen et al., 2004; Slowik and Lane, 2004). The larvae and nymphs of this tick species commonly attach to a small skin fold between the ear and shoulder of the lizard, termed the nuchal pocket (Arnold 1986). After feeding, the engorged (replete) larvae and nymphs drop off and

molt into nymphs and adults, respectively. This species of tick is the vector of the Lyme disease spirochete, *Borrelia burgdorferi*, in the western United States. Insights into the ecology of this infectious disease can be gained by an understanding of relationships between host abundance and tick abundance (Swei et al., 2011) as well as host sex and physiological state and tick intensities.

The aim of the present study is to determine if an increase in circulating testosterone drives male *S. occidentalis* to perform more territorial behaviors, have increased movements, and have increased home ranges, thus causing an increase in tick intensities. I hypothesize that testosterone increases ectoparasite intensities of male lizards by stimulating them to occupy larger home ranges and exhibiting more territorial behaviors. If this hypothesis is supported then it can provide one explanation for why male lizards have higher ectoparasite intensities than female lizards and why male lizards with higher circulating testosterone have higher ectoparasite intensities than male lizards with lower circulating testosterone. The occupation of larger home ranges may expose lizards to more ectoparasites and the exhibition of more territorial behaviors may place these lizards into a negative energy balance, thus decreasing immune function.

2. Methods

2.1 Testosterone Implants

Thirty-two male lizards and 13 female lizards were captured by noose in early April 2010 from Poly Canyon on the campus of California Polytechnic State University, San Luis Obispo. This area is characterized by rolling grass hillsides with scattered oak

trees, rock outcrops, and occasional man-made structures. Snout-vent length (SVL, $\pm$ 0.5 cm) and body mass ($\pm$ 0.5 g) were measured and each lizard received a unique toe-clip and whiteout-painted number on their dorsal surface for identification. Female lizards were then released at the site of capture and male lizards were randomly placed into one of two treatment groups, testosterone-implanted ($n = 16$) or blank-implanted ($n = 16$). The treatment groups did not significantly differ in SVL ($T = 1.22$, $P = 0.234$) and body mass ($T = 0.40$, $P = 0.692$). Implants were made from 5 mm pieces of silastic diffusion tubing (Dow Corning, Clarkesville, TN, U.S.A.: 1.47 mm inner diameter, 1.96 mm outer diameter), capped and sealed with silicon caulking, and filled with either 3 mm of crystalline testosterone propionate (Sigma-Aldrich, St. Louis, MO, U.S.A.) (testosterone-implanted) or silicon caulking (blank-implanted). In the field, lizards were placed on ice to induce cold-induced surface anesthesia until they exhibited no foot-withdrawal reflex. Implants were placed into the coelomic cavity via a small ventrolateral incision that was then closed with absorbable suture. Lizards were then released back at the site of capture.

2.2 Experimental Design: Effect of Testosterone on Home Range Size

For a period of 25 days lizards were observed for 15-minute time trials and had home range size recorded. Home ranges were marked by flagging the initial location of the marked lizard during each trial and subsequent locations were marked at the end of each trial for all locations the lizard moved to. Home range markers were made out of 50 cm pieces of wire (Home Depot, Atlanta, GA, U.S.A.) with white taped ends for easier visibility. These markers were kept out of the view of lizards so as not to disturb them. At the end of the study, after lizards were recaptured to quantify ectoparasites intensities, the

distance around the outermost markers was measured using a rolling tape measure. The area (m^2) was calculated by hand as minimum convex polygons. This technique was repeated for all observed marked male and female lizards.

2.3 Experimental Design: Effect of Testosterone on Territorial Behaviors

For a period of 25 days lizards were observed for 15-minute observation trials and had behaviors recorded. For each day there were two observation periods, AM (10-2 pm) and PM (2-5 pm). Behaviors were recorded using Olympus VN-7000 digital recorders (Olympus, Center Valley, PA, U.S.A.) from a distance of greater than 3 m. Behaviors recorded included push-ups, full shows, movements, prey consumption, shuddering, and chases. Push-ups were characterized as raising/lowering the head and trunk by leg extension/flexion. Full shows were characterized as the same motion as push-ups, but with back arched and body laterally compressed. Movements (non-specific) were characterized as any time an individual moved on its own and was not chased by another lizard. Prey consumption (eat) was any time an individual captured and ingested a prey item. Shuddering was characterized by holding the head low and moving it rapidly up and down. This behavior was most often in the presence of a female. Chases were when an individual chased another lizard, whether a female or male. The total number of each behavior was recorded for each observation trial for each lizard. At the end of the study the grand total of each behavior and behavioral rates (# behavior/minute) were calculated for each lizard for each time period. Total behavioral rates were also calculated by combining both AM and PM time periods. Behavioral rates were calculated by multiplying the number of observation trials by 15 minutes and then dividing the total

number of the particular behavior by the total amount of minutes that the lizard was observed for.

2.4 Experimental Design: Effect of Testosterone on Ectoparasite Intensity

Approximately 32 days later, 19 male individuals (8 testosterone-implanted and 11 blank-implanted) and 3 female individuals were recaptured. An additional 5 unmarked female lizards were also recaptured. Lizards were then transported back to the laboratory at California Polytechnic State University in cloth bags. Immediately upon return the lizards were placed into individual 13 x 8 x 8 (cm) metal mesh cages elevated above tubs of water to collect all ectoparasites (mites and ticks) infesting lizards as they dropped from their hosts. Tubs were 30 x 16 x 8 (cm) and filled with 4 cm of water, such that any ticks and mites dropping off the host lizards would fall into the tubs and float until they were retrieved daily by the investigators. The sides of the tubs were coated with Fluon (Bioquip, Rancho Dominguez, CA, U.S.A.) to prevent tick and mite escape. Tubs were placed in environmental chambers (27°C, 8:16 light:dark). Water was offered *ad libitum* and 2-3 crickets were offered per day. Numbers of replete tick larvae, replete tick nymphs, and total mites were quantified daily as they dropped off the lizard host and into the water. This procedure was repeated for each lizard every 24 hours until all ticks had dropped off. It was not possible to determine if all mites, especially larval mites, dropped off the lizards because some species, particularly trombiculids, may remain on the host for long periods of time (Klukowski, 2004). At the termination of the experiment the total number of replete tick larvae, replete tick nymphs, total replete ticks (larvae plus nymphs) and mites that had dropped off lizards was calculated for each lizard.

2.5 Data Analysis

Home range sizes were compared between treatment groups (testosterone-implanted, blank-implanted, and female) by using two-sample t-tests. T-tests were two-tailed and p-values were considered significant at the $\alpha = 0.05$ level. Behavioral frequencies among treatment groups were compared using one-way ANOVAs with Tukey pair-wise comparisons, assuming unequal variances. Parasite intensities (replete tick larvae, replete tick nymphs, total ticks, and mites) for the treatment groups were compared using two-sample Poisson rate tests. Statistics were performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, U.S.A.). All p-values were considered significant at the $\alpha = 0.05$ level.

3. Results

3.1 Effect of Testosterone on Home Range Size

3.1.1 Home Range Sizes of Testosterone-Implanted Versus Control Males

Variation in home range sizes were high for all treatment groups and ranged from 7.3 to 253.2 m² for control males, 0.6 to 862.8 m² for testosterone-implanted males, and 0.1 to 175.8 m² for females. Home range size was not significantly different between testosterone-implanted and control males (mean territory size: testosterone-implanted males = 138.4 ± 65.8 m², blank-implanted males = 119.6 ± 22.9 m²; $T = 0.26$, $P = 0.798$; Fig. 7a).

3.1.2 Home Range Sizes of Males Versus Females

Home range size of females was also found to be not significantly different from testosterone-implanted males (mean territory size: testosterone-implanted males = 138.4

$\pm 65.8 \text{ m}^2$, females = $36.7 \pm 20.2 \text{ m}^2$; $T = 1.43$, $P = 0.180$; Fig. 7a). Females did have a significantly smaller home range size compared to control males (blank-implanted males = $119.6 \pm 22.9 \text{ m}^2$, females = $36.7 \pm 20.2 \text{ m}^2$; $T = 2.72$, $P = 0.013$; Fig. 7a).

3.1.3 Home Range Sizes After Correcting for Extreme Outliers

Analyses were repeated again after removing an extreme outlier from the testosterone-implanted group lizard and two extreme outliers from the female group. The outlier lizard from the testosterone-implanted group had a home range size of 862.8 m^2 , more than 8x larger than the next largest territory size. The outlier lizards from the female group had home range sizes of 96.4 and 175.8 m^2 , more than 3x and 5x larger than the next largest home range size, respectively. After removal of the extreme outliers home range size was still not significantly different between testosterone-implanted and control males (mean territory size: testosterone-implanted males = $72.6 \pm 18.9 \text{ m}^2$, blank-implanted males = $119.6 \pm 22.9 \text{ m}^2$; $T = -1.53$, $P = 0.141$; Fig. 7b). However, home range size of females was significantly smaller than testosterone-implanted males (mean territory size: testosterone-implanted males = $72.6 \pm 18.9 \text{ m}^2$, females = $8.3 \pm 3.7 \text{ m}^2$; $T = 3.06$, $P = 0.012$; Fig. 7b). Females also had significantly smaller home range size compared to control males (blank-implanted males = $119.6 \pm 22.9 \text{ m}^2$, females = $8.3 \pm 3.7 \text{ m}^2$; $T = 4.78$, $P \leq 0.0001$; Fig. 7b).

3.2 Effect Of Testosterone On Territorial Behaviors

3.2.1 AM Territorial Behaviors of Testosterone- Implanted Versus Control Males

Rates for all behaviors during the AM time period were not significantly different between testosterone-implanted and control males (Fig. 8): push-ups (two-tailed $T = -0.36$, $P = 0.930$), full shows (two-tailed $T = -1.39$, $P = 0.369$), movements (two-tailed T

= 0.14, $P = 0.989$), prey consumption (two-tailed $T = 0.02$, $P = 1.000$), shuddering (two-tailed $T = -0.58$, $P = 0.833$), and chases (two-tailed $T = -0.53$, $P = 0.860$).

3.2.2 AM Territorial Behaviors of Testosterone-Implanted Males Versus Females

Behavioral rates between testosterone-implanted males and females (Fig. 8) were significantly different for push-ups (two-tailed $T = -3.50$, $P = 0.006$) and full shows (two-tailed $T = -2.91$, $P = 0.028$), with testosterone-implanted males performing more of these behaviors. All other behavioral rates for these two treatment groups were not significantly different: movements (two-tailed $T = -1.58$, $P = 0.276$), prey consumption (two-tailed $T = -1.08$, $P = 0.541$), shuddering (two-tailed $T = -2.06$, $P = 0.133$), and chases (two-tailed $T = -0.14$, $P = 0.990$).

3.2.3 AM Territorial Behaviors of Control Males Versus Females

Behavioral rates between control males and females (Fig. 8) were significantly different for push-ups (two-tailed $T = 3.03$, $P = 0.018$) and full shows (two-tailed $T = 2.64$, $P = 0.047$), with control males performing more of these behaviors. All other behavioral rates for these two treatment groups were not significantly different: prey consumption (two-tailed $T = 2.55$, $P = 0.053$), movements (two-tailed $T = 1.72$, $P = 0.222$), shuddering (two-tailed $T = 1.28$, $P = 0.429$), and chases (two-tailed $T = -0.25$, $P = 0.966$).

3.2.4 PM Territorial Behaviors of Testosterone- Implanted Versus Control Males

Rates for all behaviors during the PM time period were not significantly different between testosterone-implanted and control males (Fig. 9): push-ups (two-tailed $T = -0.68$, $P = 0.781$), full shows (two-tailed $T = -0.73$, $P = 0.751$), movements (two-tailed T

= 0.69, $P = 0.773$), prey consumption (two-tailed $T = -1.41$, $P = 0.364$), shuddering (two-tailed $T = 1.03$, $P = 0.570$), and chases (two-tailed $T = 0.94$, $P = 0.627$).

3.2.5 PM Territorial Behaviors of Testosterone-Implanted Males Versus Females

Behavioral rates between testosterone-implanted males and females (Fig. 9) were significantly different for push-ups (two-tailed $T = -2.95$, $P = 0.025$) only, with testosterone-implanted males performing more of these behaviors. All other behavioral rates for these two treatment groups were not significantly different: full shows (two-tailed $T = -2.27$, $P = 0.090$), movements (two-tailed $T = -1.78$, $P = 0.208$), prey consumption (two-tailed $T = -0.85$, $P = 0.677$), shuddering (two-tailed $T = 0.15$, $P = 0.988$), and chases (two-tailed $T = -1.88$, $P = 0.181$).

3.2.6 PM Territorial Behaviors of Control Males Versus Females

Behavioral rates between control males and females (Fig. 9) were significantly different for push-ups (two-tailed $T = 4.35$, $P = 0.002$) only, with control males performing more of these behaviors. All other behavioral rates for these two treatment groups were not significantly different: full shows (two-tailed $T = 1.81$, $P = 0.197$), movements (two-tailed $T = 1.89$, $P = 0.174$), prey consumption (two-tailed $T = -0.46$, $P = 0.891$), shuddering (two-tailed $T = 0.76$, $P = 0.733$), and chases (two-tailed $T = 1.02$, $P = 0.576$).

3.2.7 Total Territorial Behaviors of Testosterone- Implanted Versus Control Males

Total rates for all behaviors were not significantly different between testosterone-implanted and control males (Fig. 10): push-ups (two-tailed $T = -0.77$, $P = 0.723$), full shows (two-tailed $T = -1.46$, $P = 0.322$), movements (two-tailed $T = 0.57$, $P = 0.837$),

prey consumption (two-tailed $T = -0.39$, $P = 0.919$), shuddering (two-tailed $T = -0.60$, $P = 0.824$), and chases (two-tailed $T = 0.91$, $P = 0.640$).

3.2.8 Total Territorial Behaviors of Testosterone-Implanted Males Versus Females

Behavioral rates between testosterone-implanted males and females (Fig. 10) were significantly different for push-ups (two-tailed $T = -4.41$, $P = 0.0002$) and full shows (two-tailed $T = -3.71$, $P = 0.002$), with testosterone-implanted males performing more of these behaviors. All other behavioral rates for these two treatment groups were not significantly different: movements (two-tailed $T = -2.27$, $P = 0.072$), prey consumption (two-tailed $T = -1.38$, $P = 0.363$), shuddering (two-tailed $T = -1.48$, $P = 0.311$), and chases (two-tailed $T = -0.93$, $P = 0.625$).

3.2.9 Total Territorial Behaviors of Control Males Versus Females

Behavioral rates between control males and females (Fig. 10) were significantly different for push-ups (two-tailed $T = 5.01$, $P \leq 0.0001$), full shows (two-tailed $T = 2.94$, $P = 0.012$), and movements (two-tailed $T = 2.54$, $P = 0.039$), with control males performing more of these behaviors. All other behavioral rates for these two treatment groups were not significantly different: prey consumption (two-tailed $T = 1.82$, $P = 0.178$), shuddering (two-tailed $T = 1.13$, $P = 0.504$), and chases (two-tailed $T = 1.00$, $P = 0.584$).

3.3 Ectoparasite Intensities

3.3.1 Larval Tick Intensities

Replete tick larval intensities were not significantly different between testosterone-implanted and control males (mean tick larvae: testosterone-implanted males = 4.4 ± 1.5 , blank-implanted males = 3.0 ± 0.9 ; $Z = 1.52$, $P = 0.129$; Fig. 11). However,

testosterone-implanted males (mean tick larvae: testosterone-implanted males = 4.4 ± 1.5 , females = 0.6 ± 0.2 ; $Z = 4.74$, $P \leq 0.0001$; Fig. 11) and control males (mean tick larvae: blank-implanted males = 3.0 ± 0.9 , females = 0.6 ± 0.2 ; $Z = 4.01$, $P \leq 0.0001$; Fig. 11) had significantly more replete tick larvae than females.

3.3.2 Nymphal Tick Intensities

Replete tick nymphal intensities were not significantly different between testosterone-implanted and control males (mean tick nymphs: testosterone-implanted males = 3.3 ± 1.4 , blank-implanted males = 2.6 ± 0.7 ; $Z = 0.88$, $P = 0.378$; Fig. 11). However, testosterone-implanted males (mean tick nymphs: testosterone-implanted males = 3.3 ± 1.4 , females = 0.4 ± 0.3 ; $Z = 4.27$, $P \leq 0.0001$; Fig. 11) and control males (mean tick nymphs: blank-implanted males = 2.6 ± 0.7 , females = 0.4 ± 0.3 ; $Z = 4.11$, $P \leq 0.0001$; Fig. 11) had significantly more replete tick nymphs than females.

3.3.3 Total Tick Intensities

When taking replete tick larvae and nymphs into account, testosterone-implanted males were still not significantly different from control males (mean total ticks: testosterone-implanted males = 7.6 ± 2.1 , blank-implanted males = 5.6 ± 1.2 ; $Z = 1.72$, $P = 0.085$; Fig. 11). However, testosterone-implanted males (mean total ticks: testosterone-implanted males = 7.6 ± 2.1 , females = 1.0 ± 0.3 ; $Z = 6.38$, $P \leq 0.0001$; Fig. 11) and control males (mean total ticks: blank-implanted males = 5.6 ± 1.2 , females = 1.0 ± 0.3 ; $Z = 5.73$, $P \leq 0.0001$; Fig. 11) had significantly more total ticks than females.

3.3.4 Mite Intensities

Mite parasitism was significantly different between all treatment groups. Testosterone-implanted males had significantly more mites than control males (mean

mites: testosterone-implanted males = 25.8 ± 4.3 , blank-implanted males = 17.7 ± 3.8 ; $Z = 3.65$, $P \leq 0.0001$; Fig. 11) and significantly more mites than females (mean mites: testosterone-implanted males = 25.8 ± 4.3 , females = 9.6 ± 3.5 ; $Z = 7.67$, $P \leq 0.0001$; Fig. 11). Furthermore, control males had significantly more mites than females (mean mites: blank-implanted males = 17.7 ± 3.8 , females = 9.6 ± 3.5 ; $Z = 4.83$, $P \leq 0.0001$; Fig. 11).

4. Discussion

It has been demonstrated that male lizards have higher ectoparasite intensities than female lizards (Schall and Marghoob, 1995; Eisen and Eisen, 1999; Schall et al., 2000; Klukowski and Nelson, 2001; Salkeld and Schwarzkopf, 2005; Lumbad et al., in press). Furthermore, male lizards with high circulating concentrations of testosterone have increased ectoparasite intensities relative to low testosterone counterparts (Salvador et al., 1996; Olsson et al., 2000; Klukowski and Nelson, 2001; Cox and John-Alder, 2007; Pollock et al., chapter 1 of this thesis). It has been suggested that increased frequency of territory behavior, movement, and home range size, due to testosterone, is a potential explanation for higher observed ectoparasite intensities in male lizards with high circulating testosterone (Klukowski and Nelson, 2001; Cox and John-Alder, 2007). However, this relationship between behavior, movement, and ectoparasite intensity is often difficult to test. The study discussed here focused on the influence of testosterone on home range size, territorial behavior, and the role of these factors in ectoparasite intensities on male western fence lizards and female western fence lizards.

Ectoparasite intensities, both tick and mite, were significantly higher on male lizards than female lizards. These results agree with those of several other studies that show males having higher parasite infestations than females. The explanation for this observation is not fully understood as of yet, but the androgenic hormone, testosterone, is believed to be a key factor. Studies have demonstrated that males with higher concentrations of circulating testosterone have higher parasite intensities (Saino et al., 1995; Salvador et al., 1996; Olsson et al., 2000; Hughes and Randolph, 2001; Klukowski and Nelson, 2001; Roberts et al., 2004; Cox and John-Alder, 2007). Testosterone concentrations are typically higher in males than females within a species and it has been shown to influence numerous aspects of an organism's behavior and physiology.

A prominent hypothesis that could explain higher parasite intensities in males, especially those with high circulating testosterone concentrations, is that testosterone increases territorial behaviors and movement in males, which exposes them to more parasites. Several studies have demonstrated testosterone increasing territorial behaviors (Moore, 1986; Marler and Moore, 1988, 1989; Wingfield and Hahn, 1994; Klukowski and Nelson, 1998; Sinervo et al., 2000), home-range size (DeNardo and Sinervo, 1994; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009), and movement (Olsson et al., 2000; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009). These testosterone-induced behavioral changes may lead to high ectoparasite intensities by exposing male lizards to more parasites.

Contrary to an earlier field study performed in 2009, testosterone-implanted male lizards and control male lizards were not significantly different in tick intensities. Furthermore, in the 2009 study mites were significantly more abundant on the control

lizards, but the opposite was seen in the present study where mites were more abundant on testosterone-implanted male lizards. These differences observed between years may be explained by differences in study site habitat composition. The habitat composition between the two sites is different to where it is possible that tick populations are lower at the site of the present study. Western black-legged tick populations are positively correlated with relative humidity, negatively correlated with ambient temperature, and more abundant in woodland habitats than grassland habitats (Loye and Lane, 1988; Eisen and Eisen, 1999). A study by Eisen et al. (2001) showed that lizards inhabiting leaf litter areas harbored more larval ticks than those in areas lacking leaf litter and lizards captured on logs and rocks harbored more nymphal ticks than those captured on other substrata. The site from the 2009 study had higher moisture content, more leaf litter, more logs, more shaded rocks, and was significantly more wooded than the present site. As a result, the overall tick abundance may have been significantly lower during this study because of less suitable tick habitat, which prevented me from observing a difference in tick intensity between lizard treatment groups.

Results from the territory experiment suggest that male *S. occidentalis* have larger home range sizes than females, but males with higher testosterone concentrations do not have significantly different home range sizes from males with lower testosterone concentrations. Males of the genus *Sceloporus* have been shown to have larger home range sizes than females (Rose, 1982; Davis and Ford, 1983; Sheldahl and Martins, 2000; Haenel et al., 2003; John-Alder et al., 2009). This could be due to the fact that male individuals are the ones defending territories within these home range for access to resources and females. The larger the home range size, the more females the male will

have access to during the breeding season (Ruby, 1978; DeNardo and Sinervo, 1994; Haenel et al., 2003, 2003a; John-Alder et al., 2009). Although male and female differences in home range size were expected, it was not expected that home range sizes of high testosterone males and low testosterone males to not be different.

In the present study male lizards with high testosterone concentrations did not have significantly different home range sizes from males with low testosterone concentrations. This was an unexpected finding considering testosterone has been shown to increase movement and home range size in male lizards (DeNardo and Sinervo, 1994; Olsson et al., 2000; Sinervo et al., 2000; Klukowski et al., 2004; Cox et al., 2005; John-Alder et al., 2009) and birds (Wingfield, 1984; Chandler et al., 1994; Moss et al., 1994). There is a lack of studies, however, showing a different relationship, that is, testosterone having no impact on movement and home range size (Frazier et al., unpublished). A study on Greek tortoises (*Testudo graeca graeca*) by Sereau et al. (2010) resulted in similar conclusions to the present study on western fence lizards. The authors first found that peaks of testosterone corresponded to periods of inactivity and secondly, that although there were strong contrasts in testosterone concentration between treatment groups, there was no significant difference in activity (walking, foraging, digging, and sexual behaviors) and space use. These results coupled with those described here suggest that testosterone may not always be correlated with increased movement and territory size, but may be, instead, species-specific.

Analyses of territorial behaviors revealed male western fence lizards perform more push-ups and full shows and have increased movements than female lizards. However, other behaviors, such as chasing, prey consumption, and shuttering were not

significantly different among treatment groups. Push-ups are the most common territorial display of fence lizards followed by the more aggressive display of full shows. It is thought male *Sceloporus* perform these displays for territorial maintenance and defense. Additionally, male lizards moved around more frequently than females, which has been demonstrated in several other studies examining lizard behavior (DeNardo and Sinervo, 1994; Olsson et al., 2000; Wone and Beauchamp, 2003; John-Alder et al., 2009). The observation that there was no difference in frequency of shutter behavior between male and female lizards is another unexpected. Shuttering is believed to be a behavior performed by a male lizard in the presence of a female lizard in order to stimulate female reproductive behavior.

When territorial behaviors were compared between high testosterone and low testosterone male western fence lizards there were no significant differences found for any of the behaviors. Studies across birds and reptiles have demonstrated that territorial behaviors and aggression increase with an increase in testosterone (Wingfield, 1984; Marler and Moore, 1988, 1989; Moss et al., 1994; Wingfield and Hahn, 1994; DeNardo and Sinervo, 1994; Klukowski and Nelson, 1998; Smith and John-Alder, 1999; Watt et al., 2003). The best explanation for the lack of a difference observed for territorial behavior and home range size between testosterone-implanted and control lizards involves below optimal climatic conditions during the time of the study.

Optimal climatic conditions for *S. occidentalis*, like most basking reptiles, involve warm, sunny days with no precipitation and little wind. Under these conditions lizards are out basking, moving around, and performing everyday behaviors. However, during this study there were several days where conditions were well below these optimal conditions

and it was difficult to find any lizards out, male or female. During these days it was cool and fully overcast with high winds and precipitation. It is, therefore, possible that high testosterone males were prevented from performing their full degree of behaviors and from moving around as much as they would under better conditions. Similar observations were seen in tortoises (Lambert, 1981; Hailey et al., 1984; Sereau et al., 2010). Although it may be argued that because I failed to measure circulating testosterone concentrations in the lizards it is not possible to conclude that the testosterone-implanted male lizards actually had higher hormone concentrations than the control male lizards. However, the implants used in this study have been proven to work in two previously completed studies (Pollock et al., unpublished).

In summary, male western fence lizards had significantly larger home ranges, had more movements, and performed more territorial behaviors than female western fence lizards. Additionally, male lizards had higher tick intensities than female lizards. When comparing high testosterone to low testosterone male western fence lizards there were no significant differences in home range size, movement, and frequency of territorial behaviors. Corresponding to those results is the observation that there was no significant difference in tick intensities between high testosterone and low testosterone male lizards. These results suggest that home range, movement, and territorial behavior frequency contribute to higher ectoparasite intensities seen on male lizards, particularly those on males with high circulating testosterone. This could be through increased exposure to ectoparasites. However, a study by Lane et al. (1995) suggests infestation primarily occurs when lizards are inactive in their burrows, not when they are active during the day. More studies need to be performed in order to determine if lizards do in fact become

infested during periods of inactivity and also to further elucidate the role of home range size, movement, and territorial behavior on ectoparasite intensities.

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Fig. 1. Ectoparasite intensities on testosterone-implanted (gray) and blank-implanted (white) male lizards. Values are reported as means $\pm$ SEM. Parasite groupings with asterisks indicate a significant difference between treatment groups ($P \leq 0.0001$).

Fig. 2. Cumulative replete tick intensities on male (black $\blacklozenge$) and female (gray $\blacksquare$) lizards at three times of the year: spring (a), early summer (b), and mid-summer (c). Values are reported as means $\pm$ SEM. There was no significant difference in tick intensities between sexes at any time point [spring (a): $T = 0.95$, $P = 0.360$; early summer (b): $T = 1.09$, $P = 0.299$; mid-summer (c): $T = 0.50$, $P = 0.629$].

Fig. 3. Cumulative replete tick intensities on male lizards at three times of the year: spring (dark gray ♦), early summer (light gray ■), and mid-summer (black ▲). Values are reported as means $\pm$ SEM. There was no significant difference in repletion rate between time points ($F = 0.54$, $P = 0.589$).

Fig. 4. Cumulative replete tick intensities on female lizards at three times of the year: spring (dark gray ♦), early summer (light gray ■), and mid-summer (black ▲). Values are reported as means $\pm$ SEM. Repletion rate was significantly different between time points ($F = 5.52$, $P = 0.008$), with ticks feeding significantly faster on female lizards during mid-summer compared to spring and early summer.

Fig. 5. Cumulative replete tick intensities on testosterone-implanted (black ■) and blank-implanted (gray ◆) male lizards. Values are reported as means $\pm$ SEM. There was no significant difference in tick intensity ($T = -0.59$, $P = 0.566$) between treatment groups.

Fig. 6. Cumulative replete tick intensities on vitellogenic (black ■) and non-vitellogenic (gray ◆) female lizards. Values are reported as means $\pm$ SEM. There was no significant difference in tick intensity ($T = -0.83$, $P = 0.427$) between treatment groups.

Fig. 7. Home range sizes of testosterone-implanted male, control male, and female lizards with extreme outliers (see text for description) included (a) and without extreme outliers (b). Values are reported as means $\pm$ SEM. (a) Females had significantly smaller home ranges than control males ($T = 2.72$, $P = 0.013$), but were not significantly different from testosterone-implanted males ($T = 1.43$, $P = 0.180$). Home ranges of control and testosterone-implanted males were not significantly different ($T = 0.26$, $P = 0.798$). (b) Females had significantly smaller home ranges than control males ($T = 4.78$, $P \leq 0.0001$) and testosterone-implanted males ($T = 3.06$, $P = 0.012$). Home ranges of control and testosterone-implanted males were not significantly different ($T = -1.53$, $P = 0.141$).

Fig. 8. Behavioral rates of testosterone-implanted male (light gray), control male (white), and female (dark gray) lizards during the AM time period. Values are reported as means $\pm$ SEM. Treatment groups with different letters are significantly different from each other ($P < 0.05$).

Fig. 9. Behavioral rates of testosterone-implanted male (light gray), control male (white), and female (dark gray) lizards during the PM time period. Values are reported as means $\pm$ SEM. Treatment groups with different letters are significantly different from each other ($P < 0.05$).

Fig. 10. Total behavioral rates of testosterone-implanted male (light gray), control male (white), and female (dark gray) lizards. Values are reported as means $\pm$ SEM. Treatment groups with different letters are significantly different from each other ($P < 0.05$).

Fig. 11. Ectoparasite intensities on testosterone-implanted (light gray), control (white) male, and female (dark gray) female lizards. Values are reported as means $\pm$ SEM. Parasite groupings with different letters are significantly different from each other ($P \leq 0.0001$).