INFESTATION INTENSITIES, ATTACHMENT PATTERNS, AND THE EFFECT ON HOST CONTEST BEHAVIOR OF THE TICK *IXODES PACIFICUS* ON THE LIZARD *SCELOPORUS OCCIDENTALIS*

A Thesis

presented to

the Faculty of California Polytechnic State University

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biological Sciences

by

Dylan Michael Lanser

August 2019

COMMITTEE MEMBERSHIP

TITLE:	Infestation Intensities, Attachment Patterns, and the Effect on Host Contest Behavior of the Tick Ixodes pacificus on the Lizard Sceloporus occidentalis
AUTHOR:	Dylan Michael Lanser
DATE SUBMITTED:	August 2019
COMMITTEE CHAIR:	Gita Kolluru, Ph.D. Professor of Biological Sciences
COMMITTEE MEMBER:	Larisa Vredevoe, Ph.D. Professor of Biological Sciences
COMMITTEE MEMBER:	Emily Taylor, Ph.D. Professor of Biological Sciences

ABSTRACT

Infestation Intensities, Attachment Patterns, and the Effect on Host Contest Behavior of the Tick *Ixodes pacificus* on the Lizard *Sceloporus occidentalis*

Dylan Michael Lanser

Parasites often have profound effects on the survival and evolution of their hosts, and hence on the structure and health of entire ecosystems. Yet basic questions, such as the degree of virulence of a given parasite on its host, and factors influencing which hosts in a population are at the greatest risk of infection, are vexingly difficult to resolve. The western blacklegged tick-western fence lizard (*Ixodes pacificus*-*Sceloporus occidentalis*) system is important, primarily because *I. pacificus*, a vector of the Lyme disease spirochete *Borrelia burgdorferi*, is dependent on *S. occidentalis* for blood meals in its subadult stages, and this lizard possesses an innate immune response that removes the Lyme disease pathogen from attached ticks. My study focused on two aspects of the *I. pacificus*-*S. occidentalis* interaction.

In Chapter 1, I investigated factors correlating with the intensity of *I. pacificus* infestations on *S. occidentalis*. Infection intensity (parasites per host) is often highly variable within a host population, though certain individuals, such as males, tend to be more heavily infected. Previous work in the *I. pacificus*-*S. occidentalis* system suggests that differences in behavior, such as the frequency of territorial patrols, may contribute to variation in tick intensity among lizards. I therefore hypothesized that lizard traits that correlate with dominance would also correlate with infestation intensity. Specifically, I predicted that larger and more colorful males would have higher infestation intensities than less impressive animals. In this chapter, I also focused on site selection by ticks infesting *S. occidentalis*. Skin folds on the necks of these lizards (nuchal pockets) may function to divert ectoparasites away from eyes, ears, and other potentially vulnerable structures. I therefore also looked for factors correlating with tick attachment in these pockets. I sampled ticks on adult male *S. occidentalis* in the spring and summer, which is the seasonal peak for both *S. occidentalis* territorial behavior and subadult *I. pacificus* abundance. After determining the site of infestation and intensity of ticks on these lizards, I re-infested lizards with laboratory-reared *I. pacificus* larvae, and again quantified tick intensity and attachment location. Contrary to expectation, no host traits correlated with tick intensity among ticks naturally infesting lizards, and lab-reared larval intensity was negatively correlated with lizard body size. As expected, ticks acquired by lizards naturally concentrated inside nuchal pockets, and I also observed this site preference among ticks in lab-based experimental infestations. Although the general pattern, lab-reared ticks were more varied in the sites on which they fed. There was a negative correlation between infestation intensity and the proportion of ticks attached in nuchal pockets. Unsurprisingly, the most reliable predictor of tick intensity and site selection was the season.

In Chapter 2, I explored how tick attachment affects male *S. occidentalis* contest behavior. *I. pacificus* infestation has been shown to have negative physiological impacts on *S. occidentalis*, but mechanisms linking physiological changes to ultimate fitness consequences have been largely underexplored. I hypothesized that tick infestation

reduces male *S. occidentalis* fighting ability by reducing O_2 carrying capacity, or by obstructing or damaging vulnerable structures on their hosts. I held fifty half-hour trials between pairs of size- and ventral badge-matched male *S. occidentalis*, with one male in each pair infested with lab-reared *I. pacificus* larvae. I found that tick infestation negatively correlated with aggressive behavior in these staged contests. In support of reduced O_2 capacity as the mechanism of reduced aggression, my ecologically relevant infestation intensities seemed to cause significant declines in hematocrit among experimentally infested lizards relative to controls. However, the site at which ticks attached did not significantly correlate with the aggressiveness of their lizard hosts.

This is one of only a handful of studies to address the direct effect of *I. pacificus* on *S. occidentalis.* My study demonstrates that tick infestation can be detrimental to the fitness of their lizard hosts even without the transmission of pathogens. This insight may prove informative in future work on the ecology of *I. pacificus*-borne diseases in the western United States. This study is also one of only a few to use parasite infection to induce an asymmetry in fighting ability in intrasexual contests.

ACKNOWLEDGMENTS

I am extremely grateful to my committee cochairs, Drs. Gita Kolluru and Larisa Vredevoe, for their extensive advice and support over the past few years. Many thanks to Dr. Emily Taylor for serving on my committee, and also for the insight on matters herpetological shared by her and her graduate students in the Physiological Ecology of Reptiles Laboratory, especially Heather Neldner for teaching me how to noose lizards. Thanks to the many undergraduates who worked on this project, including Rebeca Almeida, Eric Gonzales, Josh Hallmark, Teo Humkey, Kei Iemori, Fiona Kelly, Julius Larion, Connor Maldonado, Jamie Marcos, Laura Murray, Lucy Ramirez, Rachel Rivera, Jenna Van Mouwerik, and especially to Andrew Morris, for assistance during many critical phases of this project. Dr. Christy Strand loaned the security cameras used to record fights between lizards. Thanks to Dave Clendenen for lending equipment and assistance in numerous photography sessions. Amber Nichols facilitated the use of the roof of Science North for more than a year for this project. Thanks to Doug Brewster, for lending his expertise and assistance in the fabrication of arenas, and to Rob Brewster, for 3D printed mounts for surveillance cameras, as well as for his advice on animal enclosures. I am very grateful for the support provided by the rest of the faculty, staff, and graduate students of the Cal Poly Biological Sciences Department. Allan Hancock College and the NSF Bridges to the Baccalaureate program funded Esmeralda Sanchez to help with this project during summer 2016. Aaron Lazanoff and the students at the Cal Poly Beef Unit provided cattle for rearing ticks. Thanks to Sarah Bailey for providing samples of *Plasmodium*-infected reptile blood. Dr. John Walker provided input on statistical methods The Baker/Koob Endowment and Cal Poly Cal Poly College Based Fees provided funds. This research was conducted with Cal Poly IACUC and CDFW (SC-12412) approval.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

1. Infestation Intensities and Attachment Patterns of *Ixodes pacificus* on *Sceloporus occidentalis* under Field and Laboratory Conditions

1.1 Introduction

Parasites reduce the ability of their host to perform fitness-enhancing functions, such as attracting a mate (Hamilton and Zuk 1982; McLennan and Brooks 1991; Møller et al. 1999), delivering oxygen to muscle tissue (Dunlap and Mathies 1993), sprinting (Garrido and Pérez-Mellado 2014), gaining mass (Klukowski and Nelson 2001), and defending a territory (Rau 1984; Schall and Dearing 1987; Maksimowich and Mathis 2000). But what determines which individuals in a host population acquire parasites, the intensity of those parasites on their host, and where on their hosts the parasites settle? Might there be patterns across the tremendous diversity of host-parasite interactions? Many parasites, including ticks, hookworms, and flukes, have patchy spatial distributions, adding a considerable random element to infections (Sousa and Grosholz 1991; Schall et al. 2000; Utzinger et al. 2003; Poulin 2007). Despite this element of chance, a few correlations between host characteristics and infection intensity have been repeatedly documented. In some systems there is a positive correlation between the intensity of parasites infecting a host in one season and the same season the following year (Bull and Burzacott 1993; Brunner and Ostfeld 2008). Larger animals in a host population tend to be more heavily infected than smaller individuals (Dunlap and Mathies 1993; Korner and Schmid-Hempel 2005; Halliday et al. 2014; Knapp et al. 2018). The parasite intensity experienced by a host individual is related to the diversity of parasites infecting it (Schall et al. 1982; Dunlap and Mathies 1993; Eisen et al. 2001; Lello et al. 2004). Territory size also positively correlates with parasite intensity, possibly as a result

of animals with larger territories engaging in behaviors (such as patrolling those territories) that expose them to infection (Olsson et al. 2000; Klukowski and Nelson 2001; Salvador et al. 1996; Pollock et al. 2012b). Animals that live in homogenous environments may also harbor more parasites than hosts with highly diverse home ranges (Thamm and Wells 2009). It may therefore be possible to identify those hosts in a population that are likely to have relatively high parasite intensities.

Some of the above-mentioned host traits associated with higher parasite intensities are also traits that tend to be concentrated in males, and the males of many species are more heavily infected than females (Tälleklint-Eisen and Eisen 1999; Schall et al. 2000; Klukowski and Nelson 2001; Brunner and Ostfeld 2008; reviewed in Zuk and McKean 1996, but see Roberts et al. 2004). Given the nearly ubiquitous presence of parasites in the environment and their aggregation on males, Hamilton-Zuk (1982) hypothesized a deep evolutionary connection between parasite infection and male sexual displays: costly (Zahavi 1975) male traits have evolved as the product of female mate choice and the cyclical evolutionary arms races between parasites and hosts, with females using male-associated traits as honest indicators of the bearer's resistance to common, virulent parasites in an environment (Hamilton and Zuk 1982). Hamilton and Zuk (1982) observed that (1) elaborate male-typical ornamentation in birds is positively correlated with high diversity of parasites in interspecific comparison, and that (2) within a host population, males with brilliant coloration tend to host fewer parasites than duller males. Color ornaments commonly displayed by males can be expensive to produce and maintain, whether they are achieved with pigments (e.g., carotenoids; Baeta et al. 2008) or structurally (e.g., ordered guanine-crystal-containing vesicles in iridophores; Morrison

and Frost-Mason 1991; de Lanuza et al. 2014; Megía-Palma et al. 2018). Brilliant male color displays produced in an environment brimming with fitness-siphoning parasites therefore advertise the bearer's possession of hearty alleles to discriminating females (Hamilton and Zuk 1982; review in Møller et al. 1999). The Hamilton-Zuk hypothesis has inspired a large body of work spanning many animal taxa (reviews in in McLennan and Brooks 1991, Zuk and McKean 1996). Immunosuppressive properties of testosterone have been proposed as a mechanism linking male-typical traits (even beyond coloration, e.g., size and territorial aggression, Olsson et al. 2000; Klukowski and Nelson 2001) and parasite burden (Folstad and Karter 1992; but see Boonekamp et al. 2008). A causal link in which testosterone directly suppresses the immune system has been demonstrated in some taxa (e.g., in chickens, Verhulst et al. 1999), but the precise mechanism linking testosterone and immune function (and therefore parasite intensity) is not settled (Boonekamp et al. 2008). In addition to testosterone-mediated coloration, darker coloration also results from the activity of the POMC gene, the pleiotropic effects of which include suppression of immune responses (Ducrest et al. 2008).

In the process of infecting a new host, motile parasite life stages must navigate the exterior surface, tissues, or body cavities of their hosts to inhabit locations that will allow them to maximize their fitness. For instance, in order to facilitate their transmission to new hosts, microfilaria increase their presence in human capillary beds during hours when mosquitoes feed (Dreyer et al. 1996), adult male ticks loiter at sites on lizards that allow the male ticks to respond quickly to the presence of a female tick (Chilton et al. 1992), and competition between trematodes over individual snail intermediate hosts can be intense (Sousa 1992), leading some trematode species to evolve a "soldier" caste that

localizes to sites in the snail where competitors are likely to enter (Hechinger et al. 2010). A wide range of parasites manipulate host behavior to enhance their odds of transmission; some migrate into the host's CNS and exert control by damaging neural tissue, causing inflammation, interfering with the neural proteome, or directly modulating neurotransmitter activity (reviews in Klein 2003, Thomas et al. 2005, Lafferty and Shaw 2013, Herbison 2017). Host exteriors present ectoparasites with diverse landscapes abounding with opportunities and hazards, upon which they must find attachment locations that allow them to balance their needs to extract resources from their host, shelter from adverse environmental conditions, find mates, compete with other parasites, and survive grooming/predation attempts (Pilosof et al. 2012). Selection pressure imposed by these factors can lead to a high degree of site-specificity. For instance, hundreds of thousands of northern fowl mites (*Ornithonyssus sylviarum*) may infest an individual chicken (*Gallus gallus domesticus*) while staying within a small region around the host's vent, behavior that most likely results from the ectothermic mite's narrow range of temperature tolerance (Devaney and Augustine 1988; De La Riva et al. 2015). Ectoparasites also tend to localize at sites that are difficult for the host to groom, and for many ectoparasites shelter from dislodgment may be a more important factor contributing to site selection than interspecific competition or other pressures (Reiczigel and Rózsa 1998; Pilosof et al. 2012). Pilosof et al. (2012) found that a taxonomically diverse group of arthropod ectoparasites consistently select sites on mammal hosts that are difficult for the host to groom. As another example, ticks attached on *Lacerta agilis* and *L. vivipara* are extremely likely to be found on the forelimbs, presumably because this is a secure location with soft scales that shelter ticks from dislodgment (Bauwens et al. 1983).

Attachment site selection by parasites can have consequences for the host, and some host species have apparently evolved defenses against parasites attaching at sites at which they might be especially detrimental to host fitness. Lesions caused by ticks on lizards can cause considerable inflammation and can be very persistent (Goldberg and Bursey 1991; Goldberg and Holshuh 1993). Diversion of ticks away from sense organs and other vulnerable sites may therefore benefit lizards (Arnold 1986; Salvador et al. 1999, but see Bauer et al. 1990). Lizards in many families have special "pockets" (sometimes termed "acarodomata") that are the target of attachment for ticks and mites (Arnold 1986). These structures are characterized by a fold of skin over a region of soft scales covering a section of the dermis containing a high concentration of lymphocytes and dense vascularization (Arnold 1986). In those species that possess them, pockets are present in hatchlings that have never been exposed to ectoparasites, are absent in many species that are not heavily infested with ectoparasites, and have arisen multiple times independently, suggesting they serve a role in coping with the deleterious effects of ectoparasite infestation (Arnold 1986; Salvador et al. 1999). These pockets can be located on the posterior of hindlimbs, in axillary areas, or on either side of the neck between the jaw and forelimb; pockets in the last of these locations are commonly known as "nuchal pockets" (Arnold 1986). If ticks are prevented from attaching within nuchal pockets on a lizard, they will attach at other sheltered locations, such as the external auditory meatuses and in the limb axillae (Salvador et al. 1999). Ticks that attach in these pockets are likely sheltered from abiotic conditions, while the host lizards may benefit from diversion of ticks from vulnerable structures (Arnold 1986). At least in the case of parasites that do

not spend their entire life on the host (such as ticks), structures such as nuchal pockets may therefore benefit both species in the host-parasite relationship.

Lizard-tick interactions are extremely common models of parasite-host interactions, with examples abounding on every continent where both occur (e.g. Europe: Olsson et al. 2000; Salvador et al. 1999; Dudek et al. 2016; Megía-Palma et al. 2016b, Australia: Bull and Burzacott 1993, North America: Tälleklint-Eisen and Eisen 1999; Eisen et al. 2004; Pollock et al. 2012a,b, Lane et al. 2013, South America: Labruna et al. 2002; Viana et al. 2012, Africa: Nowak et al. 2010; Mihalca 2012, Asia: Mihalca 2012; Keskin et al. 2013). In the present study, I focus on the Western blacklegged tick-western fence lizard system (*Ixodes pacificus-Sceloporus occidentalis*). *I. pacificus* is a three-host hard (Ixodid) tick found throughout the western United States (Furman and Loomis 1984). *I. pacificus* is capable of completing its life cycle in one year under laboratory conditions, but most often takes three years in field conditions (Peavey and Lane 1996; Padgett and Lane 2001; Fig. 1). Each life stage feeds once, and must do so within one year of reaching that life stage (Padgett and Lane 2001). Adult female *I. pacificus* produce approximately 1,400 viable larvae under laboratory conditions (Kurlovs et al. 2014). In California, *I. pacificus* is the most commonly reported tick found on humans (Furman and Loomis 1984), as well as on many reptile and bird species (Eisen et al. 2004) (Fig. 2). Lizards, predominantly *S. occidentalis*, but also *Elgaria multicarinata, E. coerulea*, and *Uta stansburiana*, among others, account for > 80% of subadult (larval and nymphal) tick blood meals in California (Goldberg and Bursey 1991; Eisen et al. 2004). Subadult *I. pacificus* abundance on *S. occidentalis* is highest in early spring (Lumbad et al. 2011), coinciding with peak questing behavior among subadult ticks (Clover and Lane

1995; MacDonald and Briggs 2016). Peak larval abundance occurs in March through early April, either slightly preceding (Lane and Loye 1989; Clover and Lane 1995) or directly overlapping with peak nymphal activity (MacDonald and Briggs 2016). In California, micro- and macroclimatic conditions are highly variable, often leading to narrower windows of subadult tick activity in the drier southern half of the state relative to the wetter, cooler northern half (which happens to be the location of most studies on the interaction between *I. pacificus* and *S. occidentalis*) (MacDonald and Briggs 2016). The high annual variability of climatic factors means that the precise timing of peak larval activity at a single site may change year to year.

Sceloporus occidentalis (Phrynosomatidae) is a medium-sized [adults 60-80 mm snout-vent length (SVL)] lizard common throughout California and other states in the American far west. These lizards have sexually dimorphic coloration, most notably in the black and deep blue patches on the ventral side of male throats and abdomens (when present, female ventral badges are less saturated and smaller than male badges, Engbretson and Livezey 1972; Ressel and Schall 1989). The pattern of ventral blue and black scales seen in *S. occidentalis* is typical of its genus (Ossip-Drahos et al. 2016). This coloration is the product of thin-layer interference, in which uniformly spaced guanine platelets within a top layer of iridophores selectively reflect blue wavelengths (Morrison and Frost-Mason 1991). Wavelengths transmitted through the layer of iridophores are absorbed by an underlying layer of melanophores, which are also responsible for the black portions of the badge (Quinn and Hews 2003). Maintenance of structural color badges may be energetically costly for lizards (de Lanuza et al. 2014). Coloration in lizards is associated with the rate of wound healing (Seddon and Hews 2016b), and may

convey information about parasite infection to conspecifics (Ressel and Schall 1989; Megía-Palma et al. 2018). Male lizards display their badges towards conspecifics during courtship and agonistic encounters, which frequently occur during the mating season (Sheldahl and Martins 2000). The onset of the mating season of *S. occidentalis* coincides with the mid-spring peak of subadult *I. pacificus* activity. It therefore seems plausible (after Hamilton and Zuk 1982) that brilliant blue ventral coloration of male *S. occidentalis* serves to convey information about the bearer's parasite infection status.

I. pacificus is of medical and economic interest because it is a vector of *Anaplasma phagocytophilum* (which causes human granulocytic anaplasmosis), *Babesia odocoilei* (cervid babesiosis), *Borrelia miyamotoi* (tick-borne relapsing fever), and, most importantly from a human medical perspective, *Borrelia burgdorferi*, a spirochete responsible for Lyme disease (Burgdorfer et al. 1982; Brown and Lane 1992; Eshoo et al. 2015). Transovarial (vertical) passage of *B. burgdorferi* has very rarely been reported in *Ixodes pacificus* (Lane and Burgdorfer 1987), and ticks primarily acquire *B. burgdorferi* by feeding on infected hosts (Lane and Burgdorfer 1987; Lane and Brown 1991; Salkeld and Lane 2010). *S. occidentalis* blood contains anti-*B. burgdorferi* complement proteins (Kuo et al. 2000), and *B. burgdorferi* infection is therefore exceedingly rare in ticks found on lizards (including *S. occidentalis*) in California (Lane et al. 2013). Along with abiotic factors (MacDonald and Briggs 2016), the fact that a majority of blood meals taken by subadult *I. pacificus* come from Lyme-refractory *S. occidentalis* could contribute to the low prevalence of *B. burgdorferi* in *I. pacificus* in California, which may contribute to the low incidence of Lyme disease in the human population of the western

United States compared to the eastern United States (Lane and Loye 1989; Eisen et al. 2004, but see Swei et al. 2011).

S. occidentalis also hosts a variety of parasites in addition to *I. pacificus*, raising the interesting potential for interactions between parasites in hosts that are multiply infected. These other parasites include trombiculid mites (Bonorris and Ball 1955; Schall et al. 2000; Lumbad et al. 2011; Pollock et al. 2012b; Seddon and Hews 2016a), the apicomplexan hemoparasites *Plasmodium mexicanum* (Schall 1982), *Schellackia (Lankesterella) occidentalis* (Schall 1982; Megía-Palma et al. 2014, 2017b), and *Acroeimeria spp.* intestinal apicomplexans (Megía-Palma et al. 2018). The prevalence of tick infestation is lower among *S. occidentalis* that are infected with *P. mexicanum* than would be expected at random (Schall 1982; Dunlap and Mathies 1993; Eisen et al. 2001 [intensity of nymphs only]). *P. mexicanum*, the most thoroughly studied parasite of *S. occidentalis*, imposes fitness costs on infected animals (Schall et al. 1982; Schall and Dearing 1987; Dunlap and Mathies 1993). The exact way in which hemo- and ectoparasites of this lizard interact has not, to the best of my knowledge, been examined in much depth, though increased mortality among coinfected animals may be responsible (Dunlap and Mathies 1993; but see Spence et al. 2017). Lizards infected with *P. mexicanum* might be less active than noninfected animals, decreasing their risk of acquiring ticks (Eisen et al. 2001). Additionally, tick infestation affects the immune profile of iguanid lizards (Knapp et al. 2018). Knapp et al. (2018) found that rock iguanas (*Cyclura cychlura*) mount an innate immune response against ticks, though they found no correlation between tick intensity and hemogregarine parasitemia. *I. pacificus* infestation may therefore be tied to hemoparasite infection through alterations to the host immune

system, competition over the shared resource of host erythrocytes, or increased morbidity and mortality in coinfected hosts.

In the present study, I identified patterns of infestation intensity and site of attachment of subadult *I. pacificus* on *S. occidentalis* in a coastal region of central California, and I attempted to identify factors contributing to those patterns. I studied these patterns in both naturally occurring tick infestations of adult male lizards, as well as experimental infestations of these lizards conducted with purpose-reared larval ticks (ticks originating from these two sources will be referred to as "wild-acquired" and "lab-reared", respectively). For determinants of the intensity of wild-acquired ticks on lizards, I hypothesized that 1a) season plays a predominant role in infestation intensities, possibly as a result of seasonal patterns in humidity and temperature (MacDonald and Briggs 2016) and subadult ticks exhausting their energy reserves prior to feeding, 1b) male-associated traits in lizards (such as large body size and brilliant blue ventral coloration), are related to parasite infection, either through physiological (e.g., individuals with brilliant badges are also immunocompromised) or through their behavioral correlates (e.g., larger animals possessing larger territories containing more parasites), and 1c) lizards infected with hemoparasites have lower tick intensity because of the combined effects of lethargy decreasing tick encounter rate, decreased hematocrit in coinfected animals, and increased immune response as a result of coinfection (Spence et al. 2017). I therefore predicted that 1a) the intensity of wild-acquired ticks infesting *S. occidentalis* would decrease following a mid-spring peak, 1b) tick intensities would positively correlate with lizard size (length [SVL], mass, and jaw width) and blue badge coloration (blueness [hue], saturated, and blue area), and that 1c) animals infected with either of two

apicomplexan hemoparasites (*Plasmodium mexicanum* and/or *Schellackia (Lankesterella) occidentalis*) would be infested with fewer ticks than noninfected animals.

For determinants of the intensity of lab-reared ticks on lizards, I had the same hypothesis and prediction for the relationship of tick intensity and season as the one proposed above for wild-acquired ticks (2a). I further hypothesized that 2b) past parasite intensity positively correlates with future tick intensity 2c) larger animals provide better habitats for ectoparasites through their larger surface area and ability to provide shelter, 2d) the immune systems of animals with high-quality color badges are compromised, increasing the susceptibility of these individuals to parasites 2e) hematophagous parasites (such as ticks) are more successful in attaching to hosts with a high concentration of erythrocytes because they are able to extract resources from host blood with greater efficiency. For lab-reared tick intensity, I therefore predicted that 2b) there would be a positive correlation between the intensity of wild-acquired and lab-reared ticks on an individual, 2c) there would be a positive correlation between lizard size (length [SVL], mass, and jaw width) and lab-reared tick intensity, 2d) there would be a positive correlation between the color of a lizard's ventral surface (blueness [hue], saturation, and blue area) and lab-reared tick intensity, and 2e) there would be a positive correlation between host hematocrit at the time of capture and the intensity of lab-reared larvae infesting that lizard.

I also generated hypotheses for attachment site selection by ticks on lizards, and these were nearly identical with respect to wild-acquired and lab-reared ticks. I hypothesized that 3a) ticks compete over the limited resource of attachment space in the nuchal pocket, 3b) as the area of the nuchal pocket increases, so too does its capacity for

ticks, and 3c) lizards coinfected with hemoparasites may be less able (either through lower activity levels reducing tick dislodgment or altered immune response) to keep tick infestation confined to the nuchal pocket. In addition to these hypotheses, for wild-acquired ticks I hypothesized that 3d) ticks become increasingly reliant on the nuchal pocket as ambient conditions (temperature, humidity) depart from the optimum for subadult ticks. I also hypothesized that 3e) tick attachment in structures such as the nuchal pocket is the product of ticks responding to factors such as host grooming behavior, abrasion against objects in the environment, and a need to shelter from direct sunlight (i.e., factors present in a natural environment but mostly absent in a controlled setting). For attachment site, I therefore predicted that 3a) there would be a negative correlation between the odds of finding ticks attached in the nuchal pocket and the overall infestation intensity, 3b) there would be positive correlation between the odds of finding ticks attached in the nuchal pocket and the size (length [SVL], mass, and jaw width) of the host, and 3c) hemoparasite infection would reduce the odds of ticks being attached in the nuchal pocket. For wild-acquired ticks, which were exposed to natural environmental stresses while questing for hosts, I predicted that 3d) there would be a positive correlation between the day of collection and the odds of ticks attaching in the nuchal pocket. I predicted that 3e) lab-reared ticks would attach at more varied sites on lizard hosts than those observed among wild-acquired ticks.

1.2 Methods

Adult Ixodes pacificus *Collection and Rearing*

In this study, I investigated attachment site selection and infestation intensity of *I. pacificus* ticks on *S. occidentalis* lizards using both wild-acquired and lab-reared *I. pacificus*. In order to generate subadult ticks for laboratory infestations, I collected adult *I. pacificus* between December 2016 and January 2017 by flagging in livestock pastures, Eucalyptus groves, oak woodlands, chaparral, and riparian areas adjacent to the California Polytechnic State University core campus (San Luis Obispo County, CA). Flags were microfleece or flannel sheets measuring approximately 1 m^2. Once collected, ticks (23 males, 21 females) were confined beneath a modified bucket hat (DALIX Bucket Hat, Rancho Cucamonga, CA, with Velcro opening) to the shaved barrel of a cow at the Cal Poly Beef Unit on January 28th, 2017 (Fig. 3). Replete female ticks that detached were trapped in the bottom of this hat. I removed replete female ticks daily until none were left, yielding sixteen gravid female ticks (five died prior to repletion, possibly as a result of entrapment in the tag cement used to affix the hat). All larvae used in this study were generated in this single feeding, which concluded in early February 2017. Following the protocol described in Pollock et al. (2012a), both before and after feeding I kept ticks in mesh-covered plastic scintillation vials, partially filled with a mixture of plaster of Paris and activated charcoal (to prevent desiccation). Vials were placed in desiccators above sterile water (to maintain ~100% humidity) inside of incubators at 23°C. Adult ticks spent up to two months in incubators prior to feeding, and continued in these conditions after feeding and through oviposition and eclosion. Thirteen of the sixteen recovered adult females produced viable larvae. Although I did not attempt a

direct count of larvae, a conservative estimate based on Kurlovs et al. (2014) suggests I generated at least 12,000 through this method. Larvae were maintained in the vials in which they hatched until required for experimental infestations of male *S. occidentalis*, which took place between 38 and 119 days post-eclosion.

Adult Male Sceloporus occidentalis *Collection and Housing*

I collected adult male *S. occidentalis* ('lizards' hereafter, unless noted otherwise) between April 23rd and July 9th, 2017, from a population in San Luis Obispo County, CA. Lizards in this population are frequently infested by *I. pacificus* (Lumbad et al. 2011). Lizards were captured from five locations in the general vicinity of Cal Poly: 1) riparian areas along Stenner Creek, 2) Brizzolara Creek north of the Cal Poly core campus (which was also the primary site of adult *I. pacificus* collection), 3) the Cal Poly Arboretum, 4) among boulders, isolated oak trees, and man-made structures in Poly Canyon, and 5) among pathways and ornamental plantings in the core Cal Poly campus. The furthest distance between capture locations was approximately 5 km. Because of the need to collect lizards within timeframes spanning only a few days for a related set of experiments (see Chapter 2), I made no attempt to capture equal numbers of lizards at each location. I noose-captured lizards with heavy-duty line attached to fishing rods (B'n'M Pole Company, West Point, MS, USA). Noose-capturing is an extremely common method of collecting lizards, and one that has been used in previous investigations of this system (e.g., Eisen et al. 2001). Males lizards were identified by their enlarged post-anal scales. All retained males had snout-vent lengths (SVL) $\geq$ 60 mm, which is a commonly used threshold for sexual maturity in this species (e.g., Schall and Sarni 1987; Lane and

Loye 1989; Eisen et al. 2001). Lizards in this population have been the subject of numerous studies, and I therefore screened captured males in the field for signs of previous handling, immediately releasing individuals bearing clipped digits or other artificial markings. I transported lizards in cloth bags to the Cal Poly Medical Entomology Lab within a few hours of capture.

Within a few hours of collection, I verified SVL measurements taken in the field, and recorded mass, hematocrit (% packed cell volume in blood), wild-acquired tick intensity, and number of ticks at various attachment sites on each lizard. In combination with variables extracted from photographs of lizards (described below), these served as the variables used in models of tick intensity and site selection. Mass was measured to the nearest 0.5 g with a spring scale (Pesola AG, Schindellegi, Switzerland). I drew between 60 and 90 μl of blood from lizard right post-orbital sinuses with a heparinized microhematocrit tube, which I then centrifuged at 10,000 rpm for 5 minutes to determine hematocrit. A small volume this blood sample was diverted for hemoparasite screening (described below). Approximately half (49/100) of the lizards were infested with subadult *I. pacificus* at the time of capture, and I recorded the number of ticks attached at various sites on these lizards in the process of removing the ticks with jeweler's forceps and an Olympus SZ60 stereoscope (63X maximum magnification). In my classification of tick attachment location, I categorized ticks as being attached to either the eyelids, external auditory meatuses, nuchal pockets (i.e., laterally on the neck), on limbs, or a catch-all "other" location on their hosts. I did not differentiate between larvae and nymphs while determining the intensity at each attachment site. I also clipped distal phalanges of each lizard to permanently and uniquely identify individuals on the day they were captured.

Sceloporines and other lizard taxa are not negatively affected by toe clipping, and toe clipping is therefore common in herpetological work (e.g., Schall et al. 1982; Schall 1983; Schall and Dearing 1987; Olsson 1994; Salvador et al. 1996; Haenel et al. 2003; Zamora-Camacho et al. 2015).

Throughout the majority of their time in captivity, lizards were housed outdoors in open-top enclosures constructed from large plastic storage bins (202.8 L, height 49.5 cm, width 54.25 cm, and length 116.5 cm at the rim; Fig. 4). For purposes related to a concurrent study of the behavior of these lizards (see Chapter 2), lizards were isolated in territories measuring 447 cm^2 in the approximately one-week period prior to infestation, and in 1343 cm^2 territories in the week following infestation. The steepness of the enclosure walls prevented lizards from escaping. All surfaces of the enclosure were painted white to reflect sunlight and thereby reduce the temperature inside enclosures. White aquarium gravel substrate (~1 cm depth) and custom-made white-clay hides were employed for the same reason. Shade-cloth draped lengthwise over the rim of these enclosures cast regions of partial shade on the substrate, permitting lizards to thermoregulate. Lizards were offered three crickets (*Acheta domesticus*) daily, along with drinking water. Lizards were assigned enclosures at random. On days with either rain or temperatures in excess of 35°C, I sheltered enclosures beneath tents made of silvered tarps. Enclosures were elevated on cinderblocks to further reduce temperatures inside of bins, as well as to mitigate the risk of flooding.

Measurement of Lizard Blue Abdominal Badges and Jaw Width from Photographs

In order to investigate a potential relationship between ventral blue badge characteristics and tick intensity/attachment location, I extracted badge characteristics (size, hue, saturation, and brightness) from photographs of each lizard's ventral surface (Fig. 5). I photographed each animal within its first week of captivity (i.e., after wild ticks had been removed, but before experimental infestation). Because structural coloration in these lizards is temperature-dependent (Morrison et al. 1996; Langkilde and Burns 2012), I conducted all photography in a room maintained at 25°C (+/- 1°C). I gave animals several minutes to thermally equilibrate to these surroundings prior to each photography session. All photographs were taken with a Nikon D800 digital camera (Nikon Corporation, Tokyo, Japan) shooting in RAW (NEF) format. In order to minimize lighting and temperature variation between photography sessions, I used a custom photography stage consisting of a flat-white painted wooden panel with four full-spectrum (5000 K) LEDs lamps facing inwards from the corners of the panel (Fig. 6). This stage remained in the same location within the temperature-controlled, fluorescently lit lab throughout the study. The digital camera was mounted on a tripod pointing down towards the center of the stage. I held lizards by their snout and tail base directly beneath the camera, photographing their dorsum (for jaw width measurements) and venter (for blue ventral badge measurements) in rapid succession. Coloration in sceloporine lizards seems to be independent of stress responses (MacLeod et al. 2019), so handling time likely did not affect photographic measurements. To ensure consistency across shoots, every photograph included a ruler and image calibration target with color standard and grayscale (Past Horizon Tools, East Lothian, UK). The assistance of an experienced

photographer (Dave Clendenen, Cal Poly Biological Sciences Department) was crucial for this portion of the study.

I extracted blue badge size, hue, saturation, and brightness in Fiji (an extension of ImageJ, Schindelin et al. 2012; Schneider et al. 2012), in which I also measured jaw width from photographs of each animal's dorsum. For ease of use in Fiji, NEF files were converted to TIF format (without compression) using ViewNXi software (Nikon Corporation, Tokyo, Japan). Pixel scale was set using the Fiji calibration tool with the ruler included in each photograph. Jaw width of each lizard was measured in the dorsal-surface photograph by dragging the straight tool across the widest section at the base of the head. The area of the blue ventral badge was measured in the ventral surface photographs using the color threshold, polygon, and threshold tools. Color threshold was used to identify all pixels in the image with hue values from 106^{o} to 289^{o} (green to blue) on a hue wheel, and saturations >20%. This effectively selected blue and green pixels in the ventral badges, while excluding low-saturation blue pixels in the flat white background and black scales bordering the blue badge. These pixels were highlighted in black, and the color TIF was converted to a BW 8-bit image. The polygon tool was then used to select the region of the photo containing each lizard's abdomen, using the armpits and femoral pores to define the cranial and caudal extent of the abdominal region, respectively. I then used the threshold tool to calculate the badge area (cm^2) as the sum of pixels passing this threshold in the size-calibrated image.

Hue, saturation, and brightness values of the blue component of the ventral badge were measured by converting RGB TIF images to HSB stacks in Fiji. I used the oval tool to select seven scales at the same position in the ventral blue badge in each photograph

(the anterior most and posterior most scales, the medial and lateral scales midway along the badge, the scale at the center of the badge, and the two scales midway between the central scale and the anterior-most and posterior-most scales). I then applied a macro function to cycle among the hue, saturation, and brightness layers in the stack, while recording mean values within the selected scales. Hue was measured on a 0˚-360˚ (red-violet) scale, while saturation and brightness were both represented as a percentage (0 – 100%), with 100% representing a white/fully saturated pixel for the saturation and brightness channels, respectively.

Infestation of Lizards with Lab-reared Ixodes pacificus *larvae*

Within each group of lizards collected within a few days of each other time, I selected half of the lizards at random (fifty overall) for infestation with larval ticks (the other half were used as controls in another experiment, see Chapter 2). Two-sided permutation tests (R package: 'coin'; function: 'independence_test'; Hothorn et al. 2006) found no significant differences ($\alpha = 0.05$, no corrections applied) in SVL ($Z = 0.13$, $p = 0.89$), mass ($Z = 0.089$, $p = 0.93$), jaw width ($Z = 0.81$, $p = 0.42$), blue ventral badge area ($Z = 1.03$, $p = 0.30$), hue ($Z = 0.73$, $p = 0.47$), saturation ($Z = 0.78$, $p = 0.44$), brightness ($Z = 1.07$, $p = 0.28$), and the intensity of naturally acquired ticks ($Z = 0.73$, $p = 0.47$) between these randomly selected groups.

Following a protocol similar to the one described in Pollock et al. (2012a), between May 4 and July 24, 2017, I infested these fifty adult male *S. occidentalis* with lab-reared larvae. All the larvae I used in this study came from the single event described above, so within each infestation event larvae were the same age. Larvae were between

38 and 119 days old at the time of experimental infestation. Prior to placement on lizards, I chilled larvae on ice to facilitate handling and counting. I inspected individual larvae under a stereoscope (Olympus SZ60) for motility, transferring motile larvae with a fine paintbrush from their vials to 1.5 ml microcentrifuge tubes (fifty/tube). Lizards occupied 2.8 L glass beakers lined on the exterior with paper, ensuring animals were minimally disturbed by their surroundings throughout infestation (Fig. 7). I dispensed 100 larval ticks (in two microcentrifuge tubes) into each lizard-containing beaker by first gently shaking tubes over lizards, then taping them to the interior of the beakers to permit the remainder of the larvae to crawl out. I covered each beaker opening with fine mesh to prevent ticks from escaping. I positioned a 50 W heat lamp approximately 0.5 m above beakers. Ticks were allowed 48 hours to attach and initiate feeding in this setting. After 48 hours, I transferred lizards to the previously described outdoor enclosures, where they remained for a median of seven days before I removed all the larvae that had attached.

Quantification of Lab-reared Tick Intensity and Attachment Site

I removed lab-reared ticks feeding on lizards with the aid of a stereoscope (Olympus SZ60, 60X magnification) after a week in outdoor enclosures (immediately following the conclusion of the experiment described in Chapter 2) (Fig. 8), preserving them in 70% ethanol. During tick removal, I recorded the location of each attached tick, using the same categories used previously for wild-acquired ticks (i.e., eyelids, external auditory meatuses, nuchal pockets, limbs, or “other”). Following visual inspection, I placed lizards in wire cages over enamel pans partially filled with water. Pan edges were coated with Fluon (polytetrafluorethylene) to prevent replete larvae afloat in the water

from escaping. I kept these cages in an incubator set to 23°C for one week, after which I counted any previously missed larvae that dropped from lizards. The few ticks collected from the pans were assumed to have attached at a site other than the eyelids, external auditory meatuses, nuchal pockets, or limbs, as I had extensively examined these attachment locations. After briefly inspecting lizards for any remaining ticks, I euthanized all lizards. Animals were handled in accordance with protocols approved by the California Polytechnic State University Institutional Animal Care and Use Committee.

Hemoparasite Screening

I screened all lizards for the apicomplexan hemoparasites *P. mexicanum* and *S. (L.) occidentalis* using blood drawn from lizard post-orbital sinuses on the day of collection. *P. mexicanum* was screened for with Giemsa-stained blood-smears and nested PCR. Following protocols modified from Schall (1983) and Eisen et al. (2001), I fixed a thin film of blood on a microscope slide in 100% methanol for one minute. I then stained slides in 10% Giemsa (J.T. Baker Brand, Avantor Performance Materials, Center Valley, Pennsylvania, USA) in a pH 7.0-7.2 phosphate-buffered solution (Table 1). Two assistants scanned each slide at 1000X magnification for hemoparasites for at least 3 minutes, recording presence or absence of parasite intracellular life stages. I used PCR to verify infections detected in blood smears, generally following the methods described in Perkins and Schall (2002). I deposited a few drops of blood from every lizard onto Whatman filter paper. Once dry, DNA in blood-saturated squares of filter paper (3-4 mm^2) was extracted using DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany).

DNA extracted from blood samples known to contain *P. mexicanum* was used as a positive control. I used Illustra™ PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare, Chicago, Illinois) to amplify a 673 bp region of *Plasmodium* cytochrome *b* in two nested PCR reactions, using the primers and general procedure described in Vardo et al. (2005). The first (outer) reaction (running conditions: 94℃ 4 min, [94℃ 20 s, 60℃ 20 s, 72℃ 90 s] x 35, 72℃ 7 min) min used the primers DW4 (5'-TGT TTG CTT GGG AGC TTG TAA TCA TAA TGTG-3') and DW2 (5'-TAA TGC CTA GAC GTA TTC CTG ATT ATC CAG-3') with DNA extracted from lizard blood samples (in 1 µl of Qiagen Elution Buffer) for a total reaction volume of 25 µl. The second (inner) reaction (running conditions: 94℃ 1 min, [94℃ 20 s, 50℃ 20 s, 72℃ 90 s]x40, 72℃ 7 min) used the primers DW1 (5'-TCA ACA ATG ACT TTA TTT GG-3') and DW3 (5'-TGC TGT ATC ATA CCC TAA AG-3') plus 1 µl of template from the first round. DNA extractions from known *Plasmodium*-infected and non-infected eastern fence lizards (*Sceloporus undulatus*) served to verify the specificity of my nested PCR. Negative controls using PCR water in lieu of template DNA were run alongside all reactions. I ran products from the second PCR reaction in 1.5% agarose gels to identify samples containing the 673 bp target amplicon. DNA was visualized in gels with ethidium bromide under UV light.

I also screened Giemsa-stained blood smears for the presence of *Schellackia (Lankesterella) occidentalis* hemoparasites, another apicomplexan hemoparasite of *S. occidentalis* (Bonorris and Ball 1955, Megía-Palma et al. 2014). A recent molecular phylogenetic analysis groups many *Schellackia* species infecting New World lizards within *Lankesterella,* including the species formerly known as *Schellackia occidentalis* (Megía-Palma et al. 2017b). This recent reclassification is reflected in the name used for

this parasite within this text. Sporozoites of this species are characterized by their negligible displacement of host erythrocyte nuclei, and by their band-shaped nucleus midway along the length of the sporozoite (Bonorris and Ball 1955). *Schellackia (Lankesterella) occidentalis* was the only parasite fitting the general description of *Schellackia (Lankesterella)* spp. parasites reported by Megía-Palma et al. (2017b) infecting a *Sceloporus occidentalis* population geographically close and climatically similar to my study site (Santa Cruz and San Luis Obispo Counties, CA, respectively). I therefore assumed that all intraerythrocytic parasites with banded nuclei that did not significantly displace host nuclei were *S. (L.) occidentalis*. Unlike *P. mexicanum* infections, I did not confirm the identity of *S. (L.) occidentalis* with PCR.

Statistical Analysis

I used exploratory factor analysis (R package: psych; functions: fa, fa.parallel; Revelle 2018) on lizard SVL, mass, jaw width, blue abdominal badge size, hue saturation, and brightness to create composite variables describing lizard morphological traits. I used an orthogonal (varimax) rotation and minimum residual factoring method. I interpreted factors with eigenvalues > 1, using variables with |loadings| > 0.4 to describe the factors (Table 2). The two retained factors retained under these criteria were interpreted as '(factor 1) body size' and '(factor 2) badge color'. Broadly, individual scores on the first factor positively correlated with size in terms of SVL, mass, jaw width, and the area of the ventral badge size, while individuals scores on the second factor positively correlated with blue ventral badge saturation, hue, and area (corresponding

with individuals with higher scores on this factor possessing comparatively impressive badges). I used these factors as independent variables in the models described below.

I created a pair of linear models for the intensity of subadult tick infestation. The first of these was a linear mixed-effects model (R package: lme4; function: lmer; Bates et al. 2015) with wild-acquired tick intensity as the response variable, and the day of collection (2017-04-23 = 0), each lizard's score on factor 1 body size, factor 2 badge color, and each lizard's infection with *S. (L.) occidentalis* (presence/absence of sporozoites in blood films) entered as explanatory variables. Collection date was only included as a first-order term, as lizard collection began in the latter half of the subadult *I. pacificus* season at this location (Lumbad et al. 2011; MacDonald and Briggs 2016), and the observed trend in wild-acquired tick intensity on lizards was monotonically decreasing over the study period (see Results). The response variable was natural log (+1) transformed to ensure normality of residuals in the final model. I included the collection site of each lizard as a random effect. The prevalence of *P. mexicanum* was too low among this group of lizards to be used in this or any other model. I did not use hematocrit measurements as an explanatory variable in this model because lizard hematocrit might be a product of, and hence collinear with, infestation intensity (Dunlap and Mathies 1993, Chapter 2 of this thesis). I selected this model over a zero-inflated negative binomial mixture model fit with the same terms (with collection site as the source of zero-inflation) based on a comparison of AIC between the two models.

The model I fit for lab-reared tick intensity was a linear model (R package: stats; function: lm; R Core Team 2018) with the number of lab-reared ticks that had not attached to their lizard hosts (i.e., 100 – lab-tick-intensity) as the response variable. Wild-

acquired tick intensity, *S. (L.) occidentalis* infection, the date of the experimental infestation (2017-05-04 = 0), hematocrit at collection, factor 1 body size, and factor 2 badge color were included as predictor variables. No random effects were used in this model. The response variable was square-root transformed to normalize model residuals.

I created a second pair of models for attachment site selection by wild-acquired and lab-reared ticks. The primary purpose of these models was to test the hypothesis that intraspecific competition exists among ticks for preferred attachment sites, and to determine if other host characteristics, as well as the season affect, tick attachment site. Both of these models were logistic regressions performed in R 3.5.1. The model for wild-acquired ticks (package: stats; function: glm; R Core Team 2018) had the counts of wild-acquired ticks found in the nuchal pocket versus outside lizard's nuchal pocket at the time of collection as the response variable. Fixed effects in this model included each of the 49 naturally infested lizard's overall wild-acquired tick intensity, scores on factor 1 body size, scores on factor 2 badge color, collection day, and *S. (L.) occidentalis* infection status. The related model for lab-reared tick attachment site (package: lme4; function: glmer; Bates et al. 2015) used the number of lab-reared ticks attached inside of the nuchal pocket versus all other attachment locations on the fifty larvae-treated lizards as the response variable. Predictor variables in this model were identical to those in the model for wild-acquired tick attachment site, with the exception of collection day, which was omitted from the second model. I included infestation group in this model as random effect.

1.3 Results

Summary

In terms of the predictions outlined above, I found support for 1a, 2a, and 3a, 3e, and marginal support for 3c (in lab-reared tick infestations only). I found no support for 1b, 1c, 2b, 2d, 2e, 3b (in wild-acquired infestations only), and 3c (also in wild-acquired infestations only). For 2c, 3a (in lab-reared tick infestations only), 3b (also in lab-reared tick infestations only) and 3d (in both wild-acquired and lab-reared tick infestations) I found significant correlations suggesting relationships opposite my predictions.

Wild Subadult Tick Site Selection on Lizard Hosts

As expected, the most frequent attachment location for both wild-acquired and lab-reared ticks was inside the nuchal pocket (Fig. 9). The odds of a wild-acquired tick attaching in the nuchal pocket were negatively correlated with the overall infestation intensity of that animal (Table 3; Figs. 10-14). Unexpectedly, the odds of wild-acquired ticks attaching in the nuchal pocket were negatively correlated with collection date (i.e., wild-acquired ticks removed from lizards towards the end of the study tended to be more dispersed on their hosts). Other predictor variables in this model (factor 1 body size, factor 2 badge color, and *S. (L.) occidentalis* infection) did not correlate with the odds of wild-acquired ticks being attached in the nuchal pocket. I also observed mites (probably *Geckobiella* sp.: Bonorris and Ball 1955; Schall et al. 2000; Seddon and Hews 2016a) on

lizards, though these seemed to concentrate on the posterior of hindlimbs rather than inside nuchal pockets.

Lab-reared Larval Tick Site Selection on Lizard Hosts

Lab-reared larvae were more varied than wild-acquired ticks in their attachment location, with many more lab-reared ticks attaching around the eyelid. In parallel with the model for wild-acquired tick attachment site, in the logistic regression model I created for lab-reared larval attachment in the host nuchal pocket, the odds of larvae attaching at this site negatively correlated with the overall infestation intensity and factor 1 body size (Table 4; Figs. 15-18). Additionally, factor 2 badge color negatively correlated with the odds of larval attachment in the nuchal pocket. Infection with *S. (L.) occidentalis* correlated (marginally) non-significantly with the response variable in this model.

Patterns in Wild Subadult Tick Infestation Intensity

Contrary to expectation, there were no significant relationships between lizard factor 1 body size, factor 2 badge color, hemoparasite infection, or hematocrit and wild-acquired tick intensity (Tables 5 and 6, Figs. 19-22). As expected for this system, wild-acquired tick intensities peaked early in the collection season (i.e., late April 2017), and decreased over the summer (Fig. 23). In my model for wild-acquired tick infestation intensity, collection date was the only significant predictor of wild-acquired tick intensity.

Patterns in Lab-reared Larval Tick Infestation Intensity

I recovered 3442 of the 5000 lab-reared larvae I placed on lizards during infestation (Table 7). As was the case in the model for wild-acquired tick intensity, the intensity of lab-reared larvae correlated significantly with the date of infestation, with infestation intensity decreasing as larvae aged (Table 8; Figs. 24-29). Unexpectedly, infestation intensity was also negatively correlated with the size of the host (factor 1 body size). Wild-acquired tick intensity, factor 2 badge color, *S. (L.) occidentalis* coinfection, and host hematocrit did not significantly correlate with tick intensity in this model.

Infection with Non-tick Parasites

Two lizards (2%) were infected with *P. mexicanum* (Fig. 30). Both were collected in early May. Because of the small number of infected individuals, analysis of the interaction between *P. mexicanum* infection and tick infestation was not conducted. Twelve lizards (12%) collected between late-April and late-June (covering most of the collection period) were infected with *S. (L.) occidentalis*. Of these twelve infected lizards, seven were selected at random for experimental tick infestation. Hemoparasite infection did not significantly correlate with any of the response variables in these models, though this may have been a product of the low prevalence of this parasite among lizards in this study.

1.4 Discussion

My observations conform with the expectation that *I. pacificus* have a high degree of site-specificity on their *S. occidentalis* hosts. The nuchal pocket was heavily favored by both lab-reared and wild-acquired ticks, an observation consistent with previous observations of the site selection by this tick on lizard hosts (e.g., *S. occidentalis*: Lane and Loye 1989; Schall et al. 2000; Pollock et al. 2012b, *Uta stansburiana*: Goldberg and Bursey 1991). Shelter from dislodgment is probably a major contributor to ectoparasite site-selection (Reiczigel and Rózsa 1998; Pilosof et al. 2012), and ticks may find shelter from dislodgment by attaching in the nuchal pocket, and may also find shelter from abiotic conditions there (Arnold 1986, but see Salvador et al. 1999, in which lizards with blocked nuchal pockets did not host fewer ticks than control animals). Lizard hosts may also benefit from ticks congregating at this site through the diversion of ticks away from sense organs (Arnold 1986). In this study, ticks placed on lizards in a laboratory environment, which happened to be free from the wide variety of abrasive surfaces abundant in a natural setting, frequently attached around the eyes, an attachment location never used by wild-acquired ticks on these lizards. This observation is consistent with Andrews et al. (1982), in which attachment sites among experimentally placed ticks on lizards subtly differed from sites selected by ticks in naturally occurring infestations. Engbretson and Livezey (1972) observed potential grooming behavior directed against ectoparasites by *S. occidentalis*, and the difference in observed site selection by wild-acquired and lab-reared ticks in my study may therefore result from reduced grooming effectiveness in the lab as compared to the field.: possibly the smoothness and small size of the glass beakers used in infestations provided less

opportunities to dislodge ectoparasites as compared with the dense vegetation present in the field (Andrews and Petney 1981). Because lizards were placed in enclosures containing abrasive surfaces after the initial 48-hour infestation period, my recovery of lab-reared ticks from eyelids approximately a week after tick placement suggests that any pressure towards attachment in the nuchal pocket due to abrasion may only be present for a short period after ticks find a host. Ixodid ticks secrete fast-setting salivary cement within minutes of penetrating a host's integument at a location acceptable to the tick (Kemp et al. 1982). The risk of dislodgment therefore declines after about 24 hours, when the salivary cement has fully hardened. Field studies also suggest that ticks may avoid sites outside of the nuchal pocket because of abrasion; Lane and Loye (1989) found that ticks infesting lizards in dense chaparral were lower intensity and highly concentrated in the nuchal pocket. It could be that these ticks were exclusively found in the nuchal pocket because of the higher risk of dislodgment in this habitat type, or because of the increased risk of desiccation due to the lack of ground cover and corresponding reduced humidity in the soil microclimate.

The observed negative correlation between the odds of a tick (lab-reared or wild-acquired) being attached in the nuchal pocket and tick intensity suggests intraspecific competition for attachment in the nuchal pocket. Karbowiak et al. (2019) observed a similar trend in mammals infested with two species of ticks; when both tick species were present on a host at high intensities, ticks attached at increasingly diverse attachment locations, suggesting competition between ectoparasites. Such a relationship is also consistent Lane and Loye's (1989) results mentioned above. Another possible explanation for this correlation could be that lizards with high infestation intensities have

poor immune responses to ticks, a soft scale phenotype, wide gaps between scales (Dudek et al. 2016), or some other trait I did not measure that permits both high tick intensities and lessens the need for ticks to attach inside the nuchal pocket.

Although *I. pacificus* is the only species of tick commonly found on *S. occidentalis* in California (Eisen et al. 2004), mites (*Geckobiella* sp.) are frequently found on these lizards (Bonorris and Ball 1955; Schall et al. 2000; Seddon and Hews 2016a), including among the lizards collected for my study. I did not focus on mite ectoparasites in this study, partially because others have also observed that these mites select slightly different locations on lizards as compared with ticks in that they do not aggregate in nuchal pockets (Schall et al. 2000; Pollock et al. 2012b). Additionally, tick and mite intensities are uncorrelated, at least on lizards in the population sampled for this study (Lumbad et al. 2011). In contrast to the evidence I found for intraspecific competition between ticks, subadult *I. pacificus* attachment site selection is therefore not likely to be influenced by the presence of mites. It may be that mites are inferior competitors for attachment sites, and there is some anecdotal evidence that ticks displace mites from *S. occidentalis* nuchal pockets (Pollock et al. 2012b).

Ticks sample host fluids with receptors located in their chelicerae in order to determine the quality of a potential feeding site before allowing salivary cement to fully harden (Waladde and Rice 1982). Surprisingly, wild-acquired tick attachment in the nuchal pocket negatively correlated with the date of collection. This was unexpected because one hypothesized role of the nuchal pocket is to lure ectoparasites away from vulnerable sensory structures by providing more shelter from abiotic conditions than other attachment locations. Increasingly hostile environmental conditions (decreasing

humidity in summer months; MacDonald and Briggs 2016) should therefore drive ticks towards shelter in the nuchal pocket. One possible explanation for the observed trend *away* from the nuchal pocket may be increasing keratosis at this site from infestations earlier in the season (Goldberg and Bursey 1991; Goldberg and Holshuh 1992; Goldberg and Holshuh 1993). Although I believe this plausibly explains my observation, I did not examine the histology of lizard nuchal pockets in this study, and more work is therefore necessary to confirm that the host integument in the nuchal pocket changes over summer in response to repeated tick feedings. As the proportion of mixture of subadult life stages changes over the summer months (Eisen et al. 2001), another possible explanation is that the trend away from the nuchal pocket reflects different attachment site preferences by the two subadult *I. pacificus* life stages that feed on lizards, possibly because fewer nymphs can simultaneously fit in nuchal pockets compared to larvae..

Puzzlingly, the negative correlation I observed between host body size and lab-reared tick attachment in the nuchal pocket contradicts the expectation that competition between ticks would be less intense when space in preferred attachment locations is abundant. Although my measurement of lizard body size did not directly incorporate the area of the nuchal pocket, the incorporation of jaw width in my measure of body size almost certainly resulted in a close, positive correlation between body size and nuchal pocket area , as the jaw forms the anterior of the nuchal pocket. I therefore expected apparent competition between ticks (reflected in high proportions of ticks attached outside the nuchal pocket) to decrease with host size. It could be that other potential tick attachment locations also increase in area, or begin to provide adequate shelter, as lizard size increases, reducing any pressures driving ticks toward their host's nuchal pocket.

Behavior resulting in tick dislodgment (such as grooming behavior) may also vary with host size. The tissue beneath nuchal pockets contains highly concentration of lymphoid cells (Arnold 1986), so it is possible that variation in the immune systems of hosts influence the suitability of attachment in this pocket. As discussed previously, immune cell infiltration and dermal thickness beneath the nuchal pocket increase as a result of repeated ectoparasite infestation in these lizards (Goldberg and Bursey 1991; Goldberg and Holshuh 1992), and nuchal pockets of larger (older) lizards may be less suitable to ticks because of this.

The honesty of the information conveyed in animal displays is thought to be maintained by the cost paid by the bearer (Zahavi 1975). If, for example, brilliant coloration is correlated with high levels of testosterone or the proopiomelanocortin gene (POMC) expression, part of the cost associated with the coloration may be a suppressed immune system (Folstad and Karter 1992; Ducrest et al. 2008). If the brilliant blue coloration of male *S. occidentalis* ventral badges is kept honest through immunosuppression, one would expect a negative correlation between the quality of the badge and the immune reaction (e.g., polymorphonuclear leukocyte infiltration; Ducrest et al. 2008) at the most heavily ectoparasite-infested sites. increased aggregation of parasites at attractive attachment locations on high badge-quality animals. I observed the opposite trend, with ticks attaching at sites other than the nuchal pocket more frequently as apparent badge quality increased. One possible explanation for this trend may be that badge quality in this species is not linked to testosterone; in a comparison of two *S. occidentalis* populations, Seddon and Hews (2016a) found that the populations with higher average melanization (i.e., those with more impressive coloration) actually had a

lower average plasma testosterone concentration. Even if testosterone is linked to badge coloration in this lizard, studies have not found a link between *S. occidentalis* testosterone and ectoparasitism (Pollock 2012b; Seddon and Hews 2016a). As an alternative to testosterone, male-typical coloration in this species may depend on expression of the *POMC* gene. To the best of my knowledge, *POMC* has not been investigated in sceloporine lizards, though it is highly conserved in vertebrates (Schiöth et al. 2005; Ducrest et al. 2008), and influences coloration in other lizard species (Baeckens and Van Damme 2018), so it can reasonably be expected to play a role in *S. occidentalis* coloration. If the *POMC* gene influences both coloration and immune function in *S. occidentalis*, the negative correlation between tick aggregation in the nuchal pocket and ventral badge coloration remains puzzling. However, if the nuchal pocket has evolved to be an attractive attachment site for ectoparasites in terms of the immune response in addition to the shelter from dislodgment, immunosuppression could lower its relative attractiveness, which would result in the negative correlation between tick aggregation and ventral badge coloration I observed. Unfortunately, speculation in this direction rests on a shaky foundation given the uncertainty in the function of the nuchal pocket, and more work is needed to establish causal links between coloration, immune response, and the role of the nuchal pocket with respect to ectoparasites.

It is extremely common for all types of parasites to be highly aggregated on hosts (Poulin 2007), and, in agreement with previous work (e.g., Eisen et al. 2001; Brunner and Ostfeld 2008; Lumbad et al. 2011), my study demonstrates the *I. pacificus*-*S. occidentalis* system is like other systems in terms of a small number of hosts feeding a disproportionate number of parasites. Among the lizards I sampled, this pattern was

observed even at the peak of tick prevalence, the maximum intensity of wild-acquired ticks I observed was less than a third of that recorded by Lumbad et al. (2011) in the same population nine years previously. There are several possible explanations for the difference in maximum intensity between these studies. I sampled lizards later (by two weeks) and less intensively in April than Lumbad et al. (2011). Extrapolating the negative correlation between collection date and tick intensity backward, the lizards in my population may have had higher intensities earlier in the season (although extrapolating too far backwards is obviously not justified, as the peak of subadult tick activity is brief [Eisen et al. 2001; MacDonald and Briggs 2016]). Other studies have reported wild-acquired subadult tick intensities on western fence lizards as high as 80 (Schall et al. 2000, larvae and nymphs on adults), 130 (Lumbad et al. 2011, larvae and nymphs on adults), and 114 larvae / 42 nymphs (Eisen et al. 2001, on adults and juveniles). Ixodid ticks are extremely susceptible to desiccation (Padgett and Lane 2001), and the lower maximum tick intensity in 2017 may reflect a decrease in the tick population resulting from a multi-year drought in the years preceding my study. Although the average tick intensity on my lizards following experimental infestations was greater than the average intensity when I collected the lizards, my maximum lab-reared tick intensity fell well within the range observed in field surveys conducted by others during peak tick intensity, including the survey conducted by Lumbad et al. (2011) at the same location. My lab-reared tick intensities were therefore at ecologically relevant levels for male lizards from this population.

The correlation between collection date and the intensity and prevalence of wild-acquired ticks on lizards strongly reflects the seasonal nature of this parasite. Wild-

acquired tick intensities declined dramatically between my initial collection dates in late April and those in mid-July. This aligns with the known phenology of this tick in California (Lane and Loye 1989; Tälleklint-Eisen and Eisen 1999; Eisen et al. 2001; Lumbad et al. 2011; MacDonald and Briggs 2016). There is a sharp decline in subadult tick activity in late spring, and in flagging surveys of subadult *I. pacificus* at study sites approximately 100 km SE of mine (Sedgwick and Paradise Reserves in Santa Barbara County, CA), MacDonald and Briggs (2016) found no nymphs or larvae after mid-May. At the University of California Hopland Field Station (Mendocino Co., CA), 460 km NNW of my site, the number of lizards infested with subadult *I. pacificus* rises rapidly in April (Tälleklint-Eisen and Eisen 1999), with prevalence verging on 100% during the peak season (late-April for nymphs, early-May for larvae) before dropping precipitously over summer (Lane and Loye 1989; Eisen et al. 2001). Nymphs can be found questing in Northern California as late as September and October (Clover and Lane 1995; Rejmanek et al. 2011). In the San Francisco Bay area, which is closer climatically and geographically to my study site than Mendocino Co., the peak season for nymphal ticks begins as early as March and ends in June or July, with nymphs still present at low levels towards the end of the year (Salkeld et al. 2014, references therein). I found subadult ticks on lizards at low intensities through mid-July, and Lumbad et al. (2011) found ticks on lizards as late as September in San Luis Obispo Co., though in both of our studies the majority of lizards collected after April were not infested. The length of the season for subadult *I. pacificus* at my study site may be intermediate between the longer season of peak activity at Hopland and in the San Francisco Bay area, and Santa Barbara Co., CA. However, changes in factors such as rainfall also complicate comparisons between years

and study sites. For instance, the absence of tick activity after May observed by MacDonald and Briggs (2016) in locations south of San Luis Obispo Co. might reflect low rainfall in 2014 and 2015 rather than differences due to latitude. Finally, it is possible that estimates for the timing of peak tick activity depend on sampling method, with some studies drawing inferences from ticks recovered from hosts (e.g., this study) and others basing estimates on ticks collected through flagging (e.g., MacDonald and Briggs 2016).

I did not find a correlation between wild-acquired tick intensity and lizard body size. Host size positively correlates with intensity in other lizard-parasite systems (e.g., Bauwens et al. 1983; Bull and Burzacott 1993; Molnár et al. 2013; Dudek et al. 2016), but, based on this study and Pollock et al. (2012a), this correlation is not present in *I. pacificus*-*S. occidentalis*. In other lizard-parasite systems, possible factors contributing to larger individuals having higher parasite intensities include larger individuals possessing more surface area for attachment (though space does not seem to be a limiting factor for some ectoparasites on lizards, see Chilton et al. 1992), larger individuals accumulating parasites with age, and larger individuals maintaining larger territories (Haenel et al. 2003) in which they may encounter parasites (Bauwens et al. 1983; Klukowski and Nelson 2001, but see Halliday et al. 2014). At present I have no explanation for why the *I. pacificus*-*S. occidentalis* system should be an exception to the correlation reported in other systems, and perhaps a study with more intensive sampling during peak subadult tick activity would uncover a significant correlation between lizard size and tick intensity.

Badge color did not significantly with tick intensities. Colorful displays likely play a role in sexual selection in sceloporine lizards (Cooper and Burns 1987; Pruett et al.

2016; but see Swierk +et al. 2012), and my findings therefore do not support the intraspecific prediction of the Hamilton-Zuk (1982) hypothesis. At least some components of the ventral coloration in sceloporine lizards are dependent on testosterone production (Cox et al. 2005). Because testosterone is thought to suppress immune investment (the immunocompetence handicap hypothesis [ICHH; Folstad and Karter 1992]), multiple studies have investigated potential correlations between testosterone and parasite intensity and prevalence in lizards (Salvador et al. 1996; Veiga et al. 1998; Salvador et al. 1999; Olsson et al. 2000; Klukowski and Nelson 2001; Cox and John-Alder 2007; Pollock et al. 2012b; Halliday et al. 2014; Seddon and Hews 2016a). Others have demonstrated that testosterone positively correlates with wild-acquired tick intensity in male *S. occidentalis* (Pollock et al. 2012b). However, the mechanism linking tick intensity and male *S. occidentalis* testosterone may involve factors only present the wild, as larval *I. pacificus* show no preference for, or increased feeding success on, lizards with experimentally elevated testosterone levels compared to control animals when infestation are carried out in a laboratory environment (Pollock et al. 2012a). To the extent that the 'factor 2 badge color' variable in my study correlated with male lizard testosterone, my findings do not support a relationship between testosterone, badge color, and ectoparasite intensity in this system.

Some caution is required in a discussion of the links between immune competence and male traits that correlate with testosterone. When studies testing the ICHH have found a correlation between increased testosterone and parasite prevalence or intensity, a causal relationship in which testosterone directly suppresses the immune system is often not sufficiently established (Roberts et al. 2004). Additionally, the

applicability of my findings to the ICHH are limited because the coloration of ventral surfaces in *Sceloporus* is likely influenced by many factors in addition to testosterone. Seddon and Hews (2016a) found that individuals in the more melanistic of two *S. occidentalis* populations had lower average testosterone. Blue coloration (the component of the ventral badge I measured) is the product of a layer of iridophores selectively reflecting blue wavelengths, while underlying melanophores absorb other wavelengths (Morrison and Frost-Mason 1991; Quinn and Hews 2003; Megía-Palma et al. 2016b). Melanocortins (α-, β-, and γ-melanocyte stimulating hormones and adrenocorticotropin) influence not only the production of eumelanin, but also immune function, behavior, and many other traits through melanocortin receptors (MCRs) (reviewed in Ducrest et al. 2008). Grooming activity is increased by melanocortin 4 receptor activation in neural tissue (reviewed in Ducrest et al. 2008), and increased melanocortin production could therefore lower ectoparasite intensities. A melanistic island population of the lizard *Podarcis siculus* hosted fewer ectoparasites (ticks and mites) than non-melanistic conspecifics on a nearby mainland, suggesting that melanistic lizards may actually be less susceptible to infestation (Baeckens and Van Damme 2018). At the same time, melanocortins may dampen immune responses (Ducrest et al. 2008), which could result in increased parasite intensities. Seddon and Hews (2016a) found higher mite intensities among the more melanized of two *S. occidentalis* populations, and no intrapopulation relationship between ventral badge darkness and ectoparasite (mite) intensity. The lack of intrapopulation correlations between melanism and ectoparasite infestation reported by Seddon and Hews (2016a) is consistent with the absence of a relationship between badge color and tick intensity among male lizards in the population I studied. More work is

needed to determine if there is a relationship between ectoparasites of *S. occidentalis* and male coloration, especially in light of the reported effects of endoparasites on coloration in *S. occidentalis* (Ressel and Schall 1989; Megía-Palma et al. 2018).

Parasite intensity is also determined by the biology of the parasite. This might explain the decline in both wild-acquired and lab-reared tick intensity over the approximately three months this study lasted. Ixodid tick life stages feed once, with each life stage surviving for up to a year unfed, though the ability to successfully feed on a host decreases the longer a tick has gone since its last molt (Padgett and Lane 2001). In my study lab-reared ticks were sheltered from environmental conditions during the initial 48-hour infestation period, but they were less sheltered from the elements over the following week. Increasing daytime heat and decreasing humidity over the course of this study may have contributed to attrition among larval ticks on lizards, in addition to larvae exhausting their energy reserves. It is also possible that some ticks successfully fed and dropped off of lizards in warmer weather before they could be counted, though this would require larvae to feed at about twice the rate previously reported for ticks on these lizards in controlled laboratory conditions (Pittman et al. 2013).

The negative correlation I found between lizard body size and lab-reared tick intensity is curious because of the absence of precisely this relationship in a previous study of this host-parasite system (Pollock et al. 2012a), as well as field studies of other tick-lizard systems in which tick intensity is positively correlated with host size (Dudek et al. 2016). Some reptiles mount adaptive immune responses to tick infestation (e.g., Fielden et al. 1992, Rulison et al. 2014a; Mugabo et al. 2015, but see Galbe and Oliver 1992), so the lower infestation intensity among larger (older) lizards in my study could

result from an acquired immune response. , Ectoparasite feeding produces granulomas and hyperkeratosis in the nuchal pockets of sceloporine lizards (Goldberg and Holshuh 1992), so a related explanation for the trend I observed may be that the integument at preferred ectoparasite attachment locations on older animals (i.e., individuals that have hosted many seasons of ectoparasites) becomes thick and unsuitable for larvae.

Although *S. occidentalis* males host higher tick intensities than females (Tälleklint-Eisen and Eisen 1999; Eisen et al. 2001), a difference potentially attributable to sex differences in territorial behavior (Pollock et al. 2012b), it is interesting that I did not observe a correlation between parasite intensity and the coloration of blue ventral badges used by male lizards in territorial displays. This finding aligns with Seddon and Hews (2016a), who found no correlations between male aggressive behavior, coloration, and (wild-acquired) ectoparasitic mite infestation within *S. occidentalis* populations (their study did not report tick infestations). At least for mites, this seemingly indicates that behavior has little effect on ectoparasite intensity on *S. occidentalis*. However, mite and tick intensities are not correlated on *S. occidentalis* (Lumbad et al 2011; Pollock et al. 2012b), suggesting that different factors may drive their intensities on hosts. In a related study, I found no correlations between lizard aggressiveness, body size or badge coloration (chapter 2). It is therefore possible that territorial behavior does expose lizards to a higher risk of ectoparasite infestation, but that I did not detect a relationship between traits used in territorial encounters and wild-acquired tick intensity because coloration is not closely linked with the behavior they putatively support (Wiens 2000).

Larvae used in experimental infestations were remarkably successful in attaching to lizards. The rate for lab-reared ticks in my study is consistent with the one reported by

Pollock et al. (2012a), with an average larval tick intensity of circa sixty-nine ticks (of one-hundred) in my study versus 60% reported in theirs (though the methods in their study differed slightly in that 100 ticks were permitted to choose between male lizards). The attachment rate among ticks in my study is also similar to the one observed by Padgett and Lane (2001) for periodically disturbed lab-reared *I. pacificus* larvae on mice.

I expected lizard morphological variables (body size and badge color, namely) to correlate with lab-reared and wild-acquired tick intensity. Although such a correlation was found between body size and lab-reared tick intensity, correlations between lab-reared tick intensity and badge color, and wild-acquired tick intensity and body size and badge color were nonsignificant. Although I predicted that there would be correlations between these factors, the absence of correlations may not be surprising, as illustrated by the contrast in tick feeding success between *S. occidentalis* and *S. undulatus. S. undulatus* and *I. scapularis* are closely related and resemble *S. occidentalis* (Martins 1993; Ossip-Drahos et al. 2016) and *I. pacificus,* respectively, yet less than 10% of wild *S. undulatus* harbor ticks (*Ixodes scapularis*) during the peak of subadult *I. scapularis* activity (at least in the northern portion of the range of this lizard in New Jersey, USA) (Rulison et al. 2014a). Additionally, lab infestations of *S. undulatus* with *I. scapularis* are difficult and result in low rates of feeding (Rulison et al. 2014a), at least relative to the feeding rates I observed in my system. Future studies, perhaps involving experimental infestations of *S. occidentalis* with *I. scapularis* subadults, and *S. undulatus* infestations with *I. pacificus* subadults, may be useful in identifying non-morphological factors correlating with tick intensity.

In contrast to previous studies demonstrating a significant correlation between *P. mexicanum* infection and tick intensity (Dunlap and Mathies 1993, but see Eisen et al. 2001 in which *P. mexicanum* infection had a significant correlation with nymphal, but not larval, prevalence on lizards), hemoparasite infection did not significantly correlate with any response variables in any of my models. There was a marginally nonsignificant correlation between infection with the hemoparasite *S. (L.) occidentalis* and lab-reared larvae attaching in the nuchal pocket (namely, there was some indication that ticks were more highly aggregated in the nuchal pocket on *S. (L.) occidentalis* infected animals). Given that others have identified interactions between ecto- and hemoparasites of this lizard, the lack of significant correlations in my study may be due to the small sample size of hemoparasite-infected lizards. The 12% prevalence of *S. (L.) occidentalis* infection among my lizards was much lower than the 42.2% prevalence reported in a nearby *S. occidentalis* population (Santa Cruz, CA; Megía-Palma et al. 2018). *P. mexicanum* prevalence was also much lower at my site (2% prevalence) than among lizards at Hopland, CA (Schall et al. 1982 [25% prevalence]; Ressel and Schall 1989 [14.4% prevalence]). However, the scarcity of plasmodium infected lizards at my site has precedent; no *P. mexicanum* infection was detected in a recent survey of a *S. occidentalis* population in Santa Cruz, CA (Megía-Palma et al. 2018), underscoring the patchy distribution of this parasite. If there is an interaction between hemo- and ectoparasites of this lizard, whether it results from competition between parasites or from increased mortality in animals harboring both ecto- and hemoparasites has not been methodically addressed, as far as I am aware. It is highly likely that the differences in the vectors of these parasites contributes to their differing prevalence. Mites (most likely *Geckobiella*

sp.), the putative vector of *S. (L.) occidentalis* (Bonorris and Ball 1955), infests lizards at my study site year-round (Lumbad et al. 2011), whereas female *Lutzomyia vexator,* the sandfly vector of *P. mexicanum,* only feed in a narrow temperature range, and only on lizards in or near squirrel burrows (Fialho and Schall 1995).. I screened all lizards for *P. mexicanum* using blood films and PCR (Vardo et al. 2005; Fig. 31), so the relative prevalences of these two parasites is unlikely to be the result of false negatives from potentially lower parasitemia in *P. mexicanum* infections compared to *S. (L.) occidentalis* infections.

My study represents one of the first attempts to compare attachment intensity and site selection of wild-acquired and lab-reared subadult ticks on lizards. Both lab-reared and wild subadult *I. pacificus* gravitated towards, and possibly competed over, attachment space in the nuchal pocket, suggesting ticks benefit from attaching at this location. The benefit derived by ticks could be directly addressed with a manipulative experiment with treatments excluding ticks from nuchal pockets, perhaps along the lines of the methods used by Salvador et al (1999). It may also be informative to undertake histological observations of the nuchal pocket (and other common tick attachment sites) to determine if hyperkeratosis rapidly develops in these locations with repeated tick feedings. Such an effort would address the mechanism behind the surprising negative correlation I observed between host body size and intensity, or whether a different mechanism (e.g., acquired immunity, increased grooming, selection favoring animals with better innate immunity to a common parasite) is responsible could be the subject of future studies. Future work involving naturally acquired tick infestations may benefit from sampling lizards earlier in the season, when subadult tick intensities are greater, and

thus more directly comparable to laboratory intensities. Additionally, this study was limited in scope to include only male lizards, and studies of site selection and tick intensity should be extended to include female lizards. As female ventral blue badges are much less saturated and usually lack the mid-ventral black component, it would be especially interesting to investigate whether the trends I observed with respect to badge color and tick site-selection apply to female lizards as well. Finally, future experimental infestations of *S. occidentalis* should investigate genetic variation in innate immunity to tick infestation, as such an investigation could help determine if there is an evolutionary arms race between ticks and lizards, and whether genes affecting immunity against ectoparasites have pleiotropic effects on coloration. As lizards are largely responsible for maintaining high population densities of *I. pacificus* in California (Swei et al. 2011) while also clearing attached ticks of *Borrelia* spirochetes (Lane and Loye 1989; Lane and Quistad 1998), it is my hope that the investigation presented here will contribute to future work on this important host-parasite system.

2. The Effects of Tick Parasitism on Contest Behavior in the Lizard *Sceloporus occidentalis*

2.1 Introduction

In the complex relationship between host and parasite, one of the most basic questions can be surprisingly difficult to answer: how harmful is infection to the host (Lehman 1993)? The difficulty in determining the cost of a given parasite is clear from the many parasites that have no discernable impact on their hosts (Clayton 1991; McLennan and Brooks 1991; Bull and Burzacott 1993; Raveh et al. 2011). Because most parasites affect host survival and reproduction indirectly via the proximate damage caused to host tissues, changes to host physiological capacities, and altered host behavior (McElroy and de Buron 2014), even selecting a yardstick with which to measure harm can present a challenge. Because of the wide variety of ways in which parasite infection can alter host behavior (reviews in Hart 1988; Dantzer 2008; McElroy and de Buron 2014) and because of the importance of behavior for host fitness, investigating such changes provides an especially useful and ecologically relevant window into the cost of infection.

Behavioral shifts following parasite infection may be mediated by either the host or the parasite, and in many systems it is unclear which species (if either) benefits from the change (Hart 1988; Poulin and Maure 2015; Herbison et al. 2018).Examples of parasite-manipulators include species that alter host anti-predator behavior to facilitate trophic transmission (e.g., Lafferty and Morris 1996; Gatkowska et al. 2012; Wesołowska and Wesołowski 2013), parasites that subvert host parental behavior (Innocenti et al.

1998), and parasites that alter host habitat preference (e.g., Thomas et al. 2002; Finnerty et al. 2018; Morris et al. 2018), among many others (Klein 2003; Herbison 2017). The mechanisms used by parasites to manipulate host behavior can be highly sophisticated and specific to a given host (Klein 2003; Lafferty and Shaw 2013), but parasites may also benefit from altered host behavior when the mechanism of 'manipulation' is as crude as the damage caused in the course of infection (Lafferty and Shaw 2013). For instance, anemia and fever in parasite-infected hosts can reduce grooming behavior and render hosts susceptible to new infections (Day and Edman 1983). The numerous examples of parasitic manipulation of host behavior (Herbison 2017) underscores just how common behavioral changes are in host-parasite interactions, and demonstrates how parasites may evolve to cause their hosts more harm than the amount strictly necessary for their growth.

Shifts in host behavior may also be initiated by the host as part of an adaptive response to parasite infection (Hart 1988). Common responses to infection among vertebrates include elevated body temperature, iron sequestration, entering a state of low energy expenditure, and decreased response to hunger and thirst, presumably in order to devote resources to fighting infections and thereby increasing long-term fitness (Hart 1988; Dantzer 2004). These host-mediated shifts are also often accompanied by reduced effort in reproduction (Forbes 1993), among other shifts in behavior.

Some host behaviors may be more reflective of the costs of parasite infection than others. Behaviors associated with attracting a mate or vying for social dominance often bring animals to the outer envelope of their physiological capacities (Marler et al. 1995; Robson and Miles 2000; Husak and Fox 2006), making such behaviors promising candidates for measuring the impact of parasite infection. Infected animals that are not

visibly sick under normal circumstances may betray signs of infection during agonistic interactions against a healthy rival (Schall and Dearing 1987). Parasite infection is known to influence the outcome of intrasexual contests, through which it can upend social hierarchies and disrupt access to mates (Freeland 1981; Rau 1984; Howard and Minchella 1990; Maksimowich and Mathis 2000). Competitive social behaviors often involve the display of sexual characters that are informative to conspecifics precisely because they are costly to produce and maintain (Zahavi 1975). Because parasites are almost ubiquitous in nature, a primary function of sexual displays may be to demonstrate the signaler's ability to efficiently handle common infections (Hamilton and Zuk 1982; reviews in Clayton 1991; Møller et al. 1999). Over evolutionary time, sexual selection translates the lesions and anemia produced by parasites into hosts bearing colorful badges and other expensive advertisements of genetic quality (Clayton 1991), and the presence of an elaborate display may therefore indicate that a species is under selection pressure from parasites (Hamilton and Zuk 1982). Although numerous studies have found that the quality of the badge displayed to conspecifics reflects the parasite infection status of the bearer (e.g., Ressel and Schall 1989; Torio 1992; Megía-Palma et al. 2016a), behavior may be an even more sensitive indicator of the magnitude of the fitness costs incurred by infection (Folstad and Karter 1992, but see Møller et al. 1999).

Perhaps because parasites inside a host (endoparasites) can directly access resources throughout the volume of the host, whereas those outside (ectoparasites) are limited to the resources accessible in their lower-dimension habitat, conventional wisdom suggests that endoparasites, having already breached the host's first defense, are more virulent than ectoparasites confined to the exterior (Bauwens et al. 1983; Lehman 1993).

But such an assumption would be misguided, as ectoparasites can be quite damaging to their hosts. Even in primates, lesions and inflammation associated with ectoparasite attachment can cause significant mortality (Brain and Bohrmann 1993). Factors that may lead to even higher virulence of ectoparasites compared to endoparasites include the fact that ectoparasites are much more likely to survive the death of a host, and ectoparasites are much less reliant on living hosts for dispersal (Ewald 1983). Additionally, because the vast majority of ectoparasites reproduce sexually, they tend to have a much lower coefficient of relatedness to conspecifics infesting a host compared to asexually reproducing endoparasites, and ectoparasites are therefore expected to compete with each other more intensively, potentially at the host's expense (Ewald 1983). Ectoparasites are therefore expected to be under less selective pressure to evolve towards commensalism with their hosts, compared to endoparasites (Ewald 1983; Lehman 1993).

Perhaps the most well-known way in which ectoparasites affect hosts is through vectoring highly virulent pathogens (*Plasmodium* spp., trypanosomes, *Yersinia pestis*, etc.), but the mechanisms by which they can reduce host fitness are actually quite diverse (Lehman 1993). Some of the harm wreaked by ectoparasites arises directly from attachment at particular locations on the host exterior. Depending on the location, ectoparasites can interfere with olfaction (Maksimowich and Mathis 2000), vision (Borucinska et al. 1998), feeding (Brain and Bohrmann 1993). Inflammation beneath attachment sites may also decrease the performance of underlying muscle (Goldberg and Bursey 1991; Maksimowich and Mathis 2000). In addition to local harm at the site of attachment, hematophagous ectoparasites can extract a sufficient quantity of blood to induce anemia (Dunlap and Mathies 1993; Salvador et al. 1996; Musante et al. 2007).

Ectoparasites frequently suppress the host immune response to facilitate their own feeding, producing a vulnerability that can be exploited by opportunistic pathogens (Yang and Cox-Foster 2005; reviews in Schoeler and Wikel 2001, Brossard and Wikel 2004). Ectoparasite infestation may also be visible to conspecifics, who may avoid interacting with infested individuals in order to reduce their risk of becoming infested, further reducing the fitness of the parasite-afflicted host through social exclusion (Freeland 1976; reviewed in Clayton 1991).

In this study, I investigated the *Sceloporus occidentalis* (western fence lizard) - *Ixodes pacificus* (western blacklegged tick) host-parasite system. *S. occidentalis* and *I. pacificus* are sympatric throughout large portions of California and other regions of the far-western United States (Furman and Loomis 1984; Clover and Lane 1995; Stebbins 2003, although there are populations of lizards that are rarely, if ever, tick-infested [E. Taylor, personal comm.]). The *S. occidentalis-I. pacificus* system is well studied because of its key potential role in the ecology of Lyme disease in the western United States (e.g., Lane and Loye 1989; Salkeld and Lane 2010; Swei et al. 2011). *S. occidentalis* possesses an innate defense against *Borrelia burgdorferi* (the agent of Lyme disease), eradicating the spirochete in both the lizard's tissues and in the feeding tick (Lane and Quistad 1998; Kuo et al. 2000). There has been considerable interest in the importance of *S. occidentalis* for maintaining and distributing juvenile life-stages of *I. pacificus* (e.g., Eisen et al. 2004; Swei et al. 2011), but few studies to date have investigated the effect *I. pacificus* on *S. occidentalis*.

S. occidentalis (Phrynosomatidae) is an insectivorous (Sabo and Power 2002) lizard with a maximum snout-vent length (SVL) of approximately 90 mm (Stebbins

2003). *S. occidentalis* exhibit sexually dimorphic coloration, with males bearing patches of vibrantly blue scales on their throats and abdomens (Stebbins 2003). Color badges play a role in sex recognition in sceloporine lizards (Smith and John-Alder 1999), and probably sexual selection as well (Cooper and Burns 1987; Swierk et al. 2012). Intrasexual contests between male sceloporine lizards has been well studied (e.g., Carpenter 1978; Rothblum and Jenssen 1978; Cooper and Burns 1987; Martins 1993; Marler et al. 1995; Quinn and Hews 2000; Haenel et al. 2003; Robbins et al. 2010), including in *S. occidentalis* (e.g., Garland et al. 1990; Schall and Houle 1992; Sheldahl and Martins 2000),, making the behavior of this lizard especially tractable for manipulative studies. Agonistic behaviors typical of *S. occidentalis* include pushup displays and lateral flattening, both of which appear to present the displayer's normally hidden blue ventral patches to conspecifics (Ressel and Schall 1989). Male *S. occidentalis* are most active during the peak of the April-May breeding season (Tsuji 1988). Both sexes maintain semi-exclusive territories within larger home-ranges (Sheldahl and Martins 2000). The primary resource acquired by male sceloporine lizards by maintaining territories seems to be access to females (Haenel et al. 2003).

The structurally-produced blue coloration of *S. occidentalis*¸ which is typical of most sceloporine lizards (Quinn and Hews 2000; Pruett et al. 2016), is the product of the reflection of a narrow range of wavelengths of light by a layer of guanine-crystal containing vesicles within iridophores, the size and spacing of which determine the hue of the badge (Morrison and Frost-Mason 1991; Langkilde and Boronow 2012). A layer of black melanophores beneath the iridophores absorbs any photons transmitted through the iridophores (Quinn and Hews 2003). The net effect is a badge consisting of brilliant blue

scales fringed in black (Fig. 5). Although sparsely studied, the energetic costs to male lizards associated with maintaining structurally based colorations may be non-trivial (Doucet and Meadows 2009; de Lanuza et al. 2014). Many male *S. occidentalis* also bear smaller pterine-based yellow patches on their limbs (Megía-Palma et al. 2018). Ventral badge characteristics correlate with *S. occidentalis* morphology such that larger males bear darker, more vibrantly blue badges compared to smaller males (Ressel and Schall 1989). Because of potential expenses associated with producing, blue and black badges, and because of the known role of these badges in sex recognition by conspecifics, many have reasonably supposed that coloration conveys information on fighting ability (Langkilde and Boronow 2010, 2012). However, some studies have cast doubt on the assumption that male coloration is informative in intrasexual contests in *S. undulatus* (Langkilde and Boronow 2010; Swierk and Langkilde 2013), a species morphologically, behaviorally, and phylogenetically close to *S. occidentalis* (Martins 1993, 1994; Conant and Collins 1998; Wiens 2000; Stebbins 2003). To the best of my knowledge, the correlation between badge traits and performance in agonistic interactions remains somewhat uncertain in *S. occidentalis*.

I. pacificus is a three-host Ixodid (hard-bodied) tick with larval, nymphal, and adult stages. Its range extents throughout the far-western United States, northern Baja California, and southern British Columbia (Furman and Loomis 1984; Brown and Lane 1992; Scott et al. 2016). Its life cycle takes three years in a natural setting, though it can be completed in less time if nymphs feed immediately after molting in late summer, or if the process occurs in controlled laboratory conditions (Peavey and Lane 1996; Padgett and Lane 2001) (Fig. 1). Larvae and nymphs (collectively ‘subadults’) typically take

blood meals on small reptiles, birds, and mammals, whereas adults feed and mate on large mammals (Furman and Loomis 1984; Lane and Brown 1991; Eisen et al. 2004). The majority of blood meals taken by subadult ticks come from lizards, and these overwhelmingly from *S. occidentalis* (Eisen et al. 2004). Each life stage finds and attaches to a host, feeds, detaches from the host, and either molts to the next stage or deposits eggs, resulting in approximately 1400 eggs per adult female (Padgett and Lane 2001; Kurlovs et al. 2014). Larvae require approximately fourteen days to feed on lizard hosts under constant laboratory conditions (Pollock et al. 2012a). In addition to vectoring *B. burgdorferi*, *I. pacificus* is medically and economically important because it vectors *Anaplasma phagocytophilum* (which causes human granulocytic anaplasmosis), *Babesia odocoilei* (cervid babesiosis), and *Borrelia miyamotoi* (tick-borne relapsing fever) (Burgdorfer et al. 1982; Brown and Lane 1992; Eshoo et al. 2015). Transovarial transmission of spirochetes is extremely rare, and almost all ticks therefore acquire the pathogen through feeding on infected hosts (Schoeler and Lane 1993). Relationships between *I. pacificus* and its hosts are therefore important for human and animal health.

There are several reasons to believe *I. pacificus* infestation affects *S. occidentalis* fitness. Tick intensity (number of ticks on a host) negatively correlates with hematocrit (percent cell volume in blood) on these lizards (Dunlap and Mathies 1993). Tick infestation may also exacerbate the detrimental impacts of *Plasmodium mexicanum* (lizard malaria parasite) infection, as coinfected individuals have poorer body condition than lizards infected with only one of these parasites (Dunlap and Mathies 1993). *P. mexicanum* infection prevalence exceeds 25% in some lizard populations (Ayala 1970; Schall et al. 1982), so interactions between ticks and hemoparasites may have

considerable impact on *S. occidentalis*, especially during the seasonal peak of tick infestation. Tick attachment causes scaring and hyperkeratosis on lizard hosts (Goldberg and Bursey 1991), which may be detrimental to lizards, especially if it occurs on vital sensory structures, and several anatomical features of *S. occidentalis* may reflect coevolution with virulent ectoparasites. Hamilton and Zuk's (1982) influential hypothesis concerning the correlation between male sexual displays in a given species and the diversity of parasites infecting that species predicted that species with colorful displays will have a higher diversity of costly parasites than those with dull displays (but see Lefcort and Blaustein 1991). As coevolution with harmful parasites is a possible evolutionary mechanism leading to the production of brilliant displays, coloration in *S. occdientalis* may serve as an indication that this species is burdened by its parasites (Ressel and Schall 1989). Additionally, *I. pacificus* tend to attach inside folds in the dermis on the lateral part of *S. occidentalis* necks ("nuchal pockets", Lane and Loye 1989; Chapter 1). These structures may be attractive to ticks because they provide ectoparasites with shelter from harsh abiotic features of the environment, and also contain soft scales (Arnold 1986). Lizards may produce these pockets because they divert ectoparasites away from sense organs (Arnold 1986). If these structures do serve the diversionary role proposed by Arnold (1986), the presence of nuchal pockets on *S. occidentalis* may be a clue to the potential harm inflicted by these parasites on their lizard hosts.

If *I. pacificus* imposes fitness costs on *S. occidentalis*, such costs may be evident in the behavior of infested animals. Infection with a lizard-malaria hemoparasite (*Plasmodium mexicanum*) negatively correlates with dominance in males of this species

(Schall and Dearing 1987). Ticks and *P. mexicanum* share a potential mechanism of behavioral alteration by reducing the hematocrit of infected animals (Schall et al. 1982; Dunlap and Mathies 1993), so it is reasonable to suppose that, if infection with *P. mexicanum* is sufficient to reduce dominance in male *S. occidentalis*, ticks may similarly affect host behavior. Displays towards rivals are aerobically demanding (Brandt 2003), so any reduction in O_2 carrying capacity due to parasites consuming erythrocytes will likely manifest in altered territorial behavior.

In this study, I looked for changes in male *S. occidentalis* contest behavior following experimental infestations of lizards with lab-reared larval *I. pacificus*. With respect to lizard contest behavior and tick infestation, I hypothesized that 1) tick infestation is detrimental to the fighting ability of male lizards, either because it reduces host physiological capacities or obstructs host sense organs, as outlined above. I therefore predicted that 1a) male *S. occidentalis* experimentally infested with ticks would be less aggressive and 1b) infested animals would perform more submissive behaviors relative to controls in staged contests between the two. In support of reduced aerobic capacity as the mechanism of reduced fighting ability, I predicted that experimentally infested animals would 1c) have reduced hematocrit compared to control animals.

I hypothesized 2) that tick attachment around sense organs affects *S. occidentalis* fighting ability by reducing the host's ability to react to rival aggression. Among experimentally infested animals, I therefore predicted 2a) that there would be a negative correlation between aggressive behaviors and the numbers of ticks attached outside of the nuchal pocket (i.e., around sensory structures such as the eyelids and external auditory

meatuses), and 2b) a positive correlation between evasive behaviors and tick attachment outside the nuchal pocket.

I further hypothesized that 3) the magnitude of the effect of tick parasitism would negatively correlate with host condition (in terms of body size and/or badge characteristics) because animals in good relative body condition also have ample resources to compensate for the detrimental impacts of infestation. I therefore predicted that contests between infested and non-infested animals would be closer to parity when the animals involved were in good condition, and therefore that there would be a positive correlation between aggression and 3a) lizard body size and 3b) ventral badge coloration.

Many of the male lizards used in this study had been infected by parasites in the wild, including wild *I. pacificus* and apicomplexan hemoparasites (namely *P. mexicanum* and *Schellackia (Lankesterella) occidentalis*). Dominance in male *S. occidentalis* is known to negatively correlate with *P. mexicanum* infection (Schall and Dearing 1987), and I therefore predicted 4a) a negative correlation between *S. (L.) occidentalis* infection and aggressiveness among male *S. occidentalis*.

2.2 Methods

Generation of Ixodes pacificus *Larvae*

Larvae reared to infest male *S. occidentalis* were the offspring of adult ticks I collected by flagging in livestock pastures, oak woodlands, and riparian areas in San Luis Obispo County, CA, in winter 2016-2017. I placed adult ticks (23 males, 21 females) beneath a confinement device (a 100% cotton bucket hat modified with a resealable opening, affixed with tag cement) on the shaved barrel of a cow (female *Bos taurus*,

provided courtesy of the Cal Poly Beef Unit). I removed any females that had engorged, detached, and fallen to the bottom of the confinement device during daily checks of the cow. Ticks were maintained before and after feeding in mesh-covered scintillation vials partially filled with a mixture of activated charcoal and plaster of Paris. These I stored in desiccators filled with sterile water, all inside incubators set to 23°C. These conditions prevent desiccation and encourage rapid egg development (Peavey and Lane 1996; Padgett and Lane 2001; Ogden et al. 2004, generally following the protocol described in Pollock et al. 2012a). Thirteen of the sixteen female ticks recovered from the cow generated viable larvae. All of these females oviposited around two weeks after being removed from the cow. Eggs hatched around forty days after oviposition. Larvae remained in vials until they were used for experimental infestation. I made no attempt to count the larvae available for infestation, but based on estimates made by Kurlovs et al. (2014), a minimum of 10,000 viable larvae were produced, or far more than required for this study.

Capture and Initial Data Collected on Male S. occidentalis

Between April 23rd and July 9th, 2017, I collected 100 adult male *S. occidentalis* by noose at California Polytechnic State University in San Luis Obispo County, California. Collection locations spanned approximately 5 km around the campus, including ornamental plantings in the core campus, green spaces among student housing, oak woodlands, and riparian areas. Lizards missing digits or bearing any other sign of previous handling were released immediately. Male lizards were distinguished from female lizards through inspection of post-anal scales. Because my hypotheses concerned

the behavior of adult male lizards only, I immediately released females and lizards with snout-vent lengths (SVL) ≤ 60 mm, which is a widely used threshold for sexual maturity in this species (e.g., Schall and Sarni 1987; Lane and Loye 1989; Eisen et al. 2001). I transported lizards meeting these criteria to the Cal Poly Medical Entomology Lab in cloth bags within a few hours of capture. I clipped the digits of each lizard in a unique pattern, allowing for identification of individuals throughout this study. I weighed each lizard, obtained blood samples, and removed and counted wild-acquired ticks on the day of capture. Mass was measured to the nearest 0.5 g with a spring scale (Pesola AG, Schindellegi, Switzerland). Blood (60-90 µl per animal) was obtained by puncturing each lizard's right infraorbital sinus with a heparinized microhematocrit tube (Fisher Scientific, Chino, CA, USA). In order to identify lizards infected with *Plasmodium mexicanum* via PCR (see below), a small volume of blood from each animal was blotted onto filter paper and stored at -20°C. A small volume of blood from each animal was also used to prepare slides to screen for hemoparasites (*P. mexicanum* and *Schellackia (Lankesterella) occidentalis*). The remainder of the blood from each lizard was used to determine hematocrit (% cellular volume) by centrifugation for 5 min at 10,000 rpm in a microhematocrit centrifuge. In order to quantify wild tick (larvae and nymph) intensity, I thoroughly inspected lizards under a dissecting microscope (Olympus SZ60) at 60X magnification, removing all ticks with forceps and preserving them in 70% ethanol. I recorded the attachment location (eyelid, external auditory meatus, nuchal pocket, limb, or 'other') for each tick.

Photographic measurements of lizard badges

In order to quantify the area, hue, saturation, and brightness of each lizard's blue abdominal badge, and also to measure jaw width, I photographed lizards within a few days of capture. Equipment and assistance were kindly donated by an experienced photographer (Dave Clendenen, Cal Poly Biological Sciences Department). In order to reduce variation in the lighting environment, and because the hue of the structurally produced blue coloration in sceloporine lizards varies with temperature (Langkilde and Boronow 2012), all photography sessions were conducted at a single station within a temperature-controlled (25 +/-1°C) room. A Nikon D800 camera (Nikon, Japan) shooting in RAW (NEF) format was mounted on a tripod in the center of four full-spectrum (5000K) LED lamps, which were bolted to the corners of a 72 X 58.4 cm flat-white painted plywood panel (Fig. 6). These lamps provided omnidirectional lighting. I held lizards by their snout and tail base, photographing their venter (for badge measurements) and dorsum (for jaw width) in rapid succession (Fig. 5). Handling time per lizard was less than a minute, though coloration in sceloporine lizards seems to be independent of stress responses (MacLeod et al. 2019), so handling time likely did not affect photographic measurements. To ensure consistency across photography sessions, each photograph included a ruler and photography standard with color targets and grayscale (Past Horizon Tools).

I analyzed photos in Fiji (an extension of ImageJ) (Schindelin et al. 2012; Schneider et al. 2012). NEF files were first converted to TIF format (without compression) using ViewNXi software (Nikon), then imported into Fiji. RGB TIFs were converted to HSB stacks in Fiji, from which I extracted hue, saturation, and brightness

values by averaging seven scales in the blue region of each lizard's ventral badge (Fig. 5b). These scales were in the same relative position on each lizard: the anterior most and posterior most fully blue scale, three scales across the middle of the badge (i.e., the halfway point between the anterior and posterior scales), and one scale each at the halfway points between the middle three scales and the anterior and posterior ends. Hue was measured on a 0˚-360˚ (red-violet) scale, and saturation and brightness were represented as a percentage (0 – 100%), with 100% representing a white/fully saturated pixel for the saturation and brightness channels, respectively.

Testing Arenas and Animal Care

Lizards were housed throughout this study in outdoor semi-enclosures, excepting the period immediately after capture, during the 48-hour infestation period (see below), and for one week following trials (the last of these only if the animal was treated with ticks). Enclosures also served as the arenas in which I staged contests. I constructed enclosures from large plastic storage bins (202.8 l, height 49.5 cm, width 54.25 cm, and length 116.5 cm at the rim, Fig. 4). Enclosures were constructed in two basic types, which differed only in the area occupied by each lizard. Each lizard had approximately 447 cm^2 in the pre-infestation design, and a 1343 cm^2 territory in post-infestation/trial arenas, with transverse corrugated plastic partitions dividing the enclosures and isolating animals. I filled bins with approximately 1 cm of white aquarium gravel substrate to permit burrowing. I draped shade-cloth lengthwise over half of each enclosures, which cast a patch of partial shade on the substrate and allowed lizards to thermoregulate. Each sub-compartment also contained a custom-made clay hide. In order to control the

temperature inside these enclosures, the gravel, partitions, hides, and arenas themselves were white. This also improved contrast in video recordings (see also Sheldahl and Martins 2000). I oriented bins such that each side received approximately equal sunlight in the afternoon when trials were held (see below). Lizards were offered 2-3 crickets (*Acheta domesticus*) daily. The sides of the bins were heavily misted with water during feedings, as lizards occasionally drank droplets off the arena walls. I erected silvered tarps over enclosures on days when temperatures exceeded 35°C, or when rain was forecasted. Enclosures were elevated approximately 20 cm above the ground, which served to further reduce internal temperatures and decrease the risk of flooding.

Pairing and Infestations

In order to maximize parity between male lizards in all aspects of fighting ability except for tick infestation, I paired lizards on the basis of tail autotomy status (i.e., lizards with broken tails were only paired with each other), SVL (mean difference = 0.1 mm, median = 0, max = 2 mm), mass (0.04 g, 0.25 g, 5 g), and the proportion of the ventral surface occupied with blue scales (1.6%, 2.1%, 19.2%), with priority given to pairing criterion in that order. After forming pairs, I randomly assigned one lizard within each pair to the experimental treatment and one lizard to the control group. Two-sided permutation tests (R package: 'coin'; function: 'independence_test'; Hothorn et al. 2006; with tests stratified by lizard pair) confirmed that infested and non-infested lizards did not differ significantly in SVL ($Z = 0.73$, $p = 0.47$), mass ($Z = -0.2$, $p = 0.84$), jaw width ($Z = -0.99$, $p = 0.32$), ventral blue badge area ($Z = -1.56$, $p = 0.12$), number of wild ticks ($Z = -1.24$, $p = 0.21$), ventral badge hue ($Z = 0.69$, $p = 0.49$), saturation ($Z = -1.04$, $p = 0.30$),

and brightness ($Z = 1.19$, $p = 0.23$). Although hemoparasite infection status was unknown for each lizard until after the conclusion of behavioral trials, paired permutation after trials revealed no significant difference in *S. (L.) occidentalis* infection rates between treatment groups ($Z = -0.63$, $p = 0.53$, seven and five infected animals assigned at random to infested and control treatments, respectively).

I infested lizards between May 4 and July 24, 2017, when larval ticks were between thirty-eight and 119 days post-eclosion. Within twenty-four hours of a planned infestation, motile larvae were chilled and individually counted into centrifuge tubes using a stereoscope and paintbrush, with each tube containing exactly fifty larvae. I placed lizards in 2.8 L beakers, above which I positioned 50 W reptile basking lights (Zoo Med Laboratories Inc., San Luis Obispo, CA), and around which I wrapped paper towels to maintain visual isolation between lizards. This ensured that males were strangers during trials, thereby minimizing variability in aggression due to 'dear enemy' effects during trials (Smith and John-Alder 1999; Husak and Fox 2003) (Fig. 7). I dispensed 100 larvae (i.e., two microcentrifuge tubes) onto the randomly selected lizard, first by gently shaking and tapping tubes above the animal, then by taping tubes to the beaker walls to permit any remaining larvae to leave the tube. Lizards and tick larvae shared beakers for forty-eight hours. I placed mesh above beakers to prevent ticks that did not immediately attach to lizards from escaping. Non-infested control animals were exposed to identical conditions, though without larval ticks. After forty-hours hours I removed lizards and placed them in the arena-type (1343 cm^2 territory) enclosures. The arena occupied by the pair and the side given to each member of the pair was also assigned at random.

Staged Contests

Between May 13th and August 4th, 2017, I staged fifty interactions between infested and non-infested lizards in outdoor enclosures. In each trial, the lizards in each pair had been in the arenas a mean of seven days (SD = 0.81, min = 6, max = 9). This acclimation period is roughly equivalent to the one used in Robbins et al. (2010) for two species of sceloporine lizards, and in Martin et al. (2016) for *Zootica tropica*, and considerably longer than the acclimation period used in other studies of *Sceloporus occidentalis* aggressive behavior (e.g., Garland et al. 1990; Sheldahl and Martins 2000). Although I hoped that lizards would completely acclimate to their surroundings in this time, the primary purpose of this period was to allow larvae to begin feeding on their hosts (though not so long as to allow ticks engorge and detach). Trials lasted 30 minutes and were held in the afternoon between 13:50 and 17:00, with most starting between 14:30 and 16:00. Surveillance cameras (Lorex, Markham, ON, Canada) mounted on tripods were positioned at either end of the arena prior to testing. The entire arena was visible in the combined view of both cameras. As lizards seemed disturbed by my presence when I positioned cameras before each trial, I observed a ten-minute waiting period between camera placement and partition removal. After this period, I used a long wooden pole to remove partitions while staying out of the view of lizards. With the partition removed, a slit cut in the shade-cloth that had accommodated the partition cast a sunfleck in the middle of the arena. I hoped that the presence of this sunfleck at the new border of the lizard "territories" would motivate contests between lizards (others have used a single, centrally located basking light to provide a resource for males to lizards to contest [Garland et al. 1990]). I was able to monitor trials as they occurred, though

behaviors were scored from recordings. At the end of each trial I took the temperature in arenas by burying a thermometer in the partially shaded region in the middle of the arena. Each lizard was used once. I minimized any odor cues left by previous occupants by washing arena interiors with soap and water between trials (Brandt 2003; Thompson et al. 2008; Swierk and Langkilde 2013), and also by pooling and mixing substrate from all arenas during cleaning events after each group of trials.

After trials, I removed lizards from arenas and quantified tick intensity and attachment location. I also drew a second blood sample for hematocrit determination, following the same procedure described above. In order to verify that non-attached ticks had not transferred from the designated infested lizard to the non-infested lizard during the acclimation period or trial, I inspected both infested and non-infested lizards. To ensure a complete tick count, I placed lizards in wire cages above Fluon (polytetrafluorethylene)-coated, water-filled trays within a 23°C incubator. Lizards were held in this setup for one week after trials, in which time any larvae that I had missed during my initial inspection detached and became trapped in the tray beneath each lizard. I categorized the few ticks recovered from trays as having attached in the 'other' location category, as I had thoroughly inspected the eyelids, external auditory meatuses, nuchal pockets, and limbs on each lizard. I then euthanized lizards by first inducing deep anesthesia with isoflurane, followed by decapitation.

Trial Review

After the conclusion of all trials, footage exported from the LOREX surveillance cameras was converted to MP4 720p format with WinFF file conversion software

(https://www.biggmatt.com/p/winff.html). These files were then edited with Avidemux video editing software (http://avidemux.sourceforge.net/) to exactly 30 minutes from the raising of partitions. These files were then imported into BORIS (Friard and Gamba 2016). I created an ethogram consisting of twenty-five behaviors using the tool available in BORIS (Table 9). I randomized the order in which trials and individual lizards within pairs were reviewed by permuting a list of the trial files in R. Most trial playbacks were reviewed by at least two people. Reviewers were not aware of which lizard in the pair had been infested, as the list containing information on which lizard in each pair was infested was not consulted during the scoring process, and the resolution of the trial playbacks was too low for the reviewers to distinguish ticks directly. As I did not mark lizards in a way that would make them individually identifiable in playbacks, reviewers tracked lizards from the side of the arena the lizard started in, working in slow-motion whenever lizards in playbacks were in close proximity.

Hemoparasite Screening

Because *S. occidentalis* morphology, physiology, and behavior is affected by hemoparasite infections (Schall and Dearing 1987; Ressel and Schall 1989; Megía-Palma et al. 2018) I screened lizards for *Plasmodium mexicanum* and *Schellackia (Lankesterella) occidentalis* (recent phylogenetic analysis placed *S. (L.) occidentalis* within *Lankesterella* rather than *Schellackia* [Megía-Palma et al. 2017b], and the name used here for this parasite reflects this reclassification). Blood used for hemoparasite screening derived from the samples drawn for hematocrit determination on the day of collection. Tests for *P. mexicanum* included both scans of blood films and nested PCR,

whereas only blood films were used to screen for *S. (L.) occidentalis*. Blood films were prepared and stained in a protocol similar to procedures described in Schall (1983) and Eisen et al. (2001) (Table 1). In brief, I fixed blood films in 100% methanol for one minute, then stained the films in 10% Giemsa (J.T. Baker Brand, Avantor Performance Materials, Center Valley, Pennsylvania, USA) in pH 7.0-7.2 phosphate-buffered solution for fifty minutes (Table 1). Slides were viewed for at least three minutes for both *S. (L.) occidentalis* and *P. mexicanum*. *P. mexicanum* gametocytes were distinguished from *S. (L.) occidentalis* sporozoites by the displacement of host erythrocyte nuclei, which can be seen in erythrocytes infected with *P. mexicanum* but not *S. (L.) occidentalis*, and by the equatorially banded nucleus in *S. (L.) occidentalis* sporozoites (Bonorris and Ball 1955; Ayala 1970; Megía-Palma et al. 2014) (Fig. 30). *P. mexicanum* trophozoites, schizonts, and merozoites, which also inhabit *S. occidentalis* peripheral blood, were also searched for in this screening (Ayala 1970; Schall 1982).

Nested PCR, in which the amplicon from a first PCR reaction is used as the template in a second reaction, was used as a second method to verify the presence or absence of *P. mexicanum.* Nested PCR effectively detects even very low parasitemia infections (Vardo et al. 2005). Briefly, DNA was extracted from dried blood dots on filter paper with a DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany), using the manufacturer-provided protocol for nucleated blood samples. I then amplified a 673 bp region of the *Plasmodium* cytochrome *b* gene with outer primers DW4 (5’-TGT TTG CTT GGG AGC TTG TAA TCA TAA TGTG-3’) and DW2 (5’-TAA TGC CTA GAC GTA TTC CTG ATT ATC CAG-3’), and inner primers DW1 (5’-TCA ACA ATG ACT TTA TTT GG-3’) and DW3 (5’-TGC TGT ATC ATA CCC TAA AG-3’) (Vardo et al.

2005). Running conditions were 94℃ 4 min, [94℃ 20 s, 60℃ 20 s, 72℃ 90 s]x35, 72℃ 7 min and 94℃ 1 min, [94℃ 20 s, 50℃ 20 s, 72℃ 90 s]x40, 72℃ 7 min, for outer and inner reactions, respectively. All reactions were run with Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Chicago, Illinois) and 22 µl of PCR-grade water. I used 1 µl of template in each round, with DNA extracted from lizard blood samples in AE buffer (Qiagen) as the template in the outer reaction, and the products of the outer reaction as the template in the inner reaction. I ran a reaction with additional PCR water in lieu of DNA template as a negative control in every batch of reactions. Positive controls were derived from DNA extractions from lizards that had been identified as infected in a previous study (Vredevoe et al., unpublished). I verified the specificity of the primers for *Plasmodium* by running blood samples from a pair of infected and non-infected *Sceloporus undulatus* through this protocol (Fig. 31). The presence of the 673 bp *Plasmodium cytochrome b* amplicon was determined in1.5% agarose gels. *Plasmodium* infection status was inferred from the presence or absence of an amplicon of this size.

Data Analysis

I created composite scores from lizard morphological variables using exploratory factor analysis (R package: 'psych'; functions: 'fa', 'fa.parallel'; Revelle 2018) for use as predictors in further analyses. I retained two varimax-rotated factors using standard factor-analytic criteria (Costello and Osborne 2005), and interpreted these as '(factor 1) body size' and '(factor 2) badge color' ('body size' and 'badge color' hereafter) from the variables with |loadings|>0.4 on each factor (Table 2). Scores on the first factor positively correlated with lizard size in terms of SVL, mass, jaw width, and blue ventral badge area.

Scores on the second factor positively correlated with more visually striking blue ventral badges in terms of area, hue (with higher hue scores corresponding to higher wavelength light), and saturation.

A second factor analysis was used to reduce the number of behavior variables and explore underlying patterns of behavior. Prior to running a factor analysis on behaviors, I used a Kaiser-Meyer-Olkin test (Kaiser & Rice 1974; R package: 'psych'; function: 'KMO'; Revelle 2018) to determine which behaviors had been sampled adequately. Of the 25 behaviors in my ethogram, eleven had individual measures of sampling adequacy indicating that they were suitable for factor analysis (MSAi $\geq$ 0.6). Parallel analysis and scree plots run on the correlation between these suitable behaviors suggested retaining two factors. Factors after these first two also had eigenvalues < 1, which is a commonly used threshold for factor retention (but see Costello and Osborne 2005). I therefore retained two factors and interpreted them as 'factor 1 aggressiveness' and 'factor 2 reposition-escape', using the same interpretation criteria applied in the factor analysis on morphology variables (Table 10). Scores on the first factor positively correlated with the frequency or duration of behaviors that are widely interpreted as being 'aggressive' (e.g., Garland et al. 1990; Sheldahl and Martins 2000; Seddon and Hews 2016a), such as pushups (two- and four-legged), shudders, approaches, nudges, and charges. Scores on the second factor positively correlated with the number of small movements, escape attempts, nudges, and approaches.

I used a stratified permutation test (R Package: 'coin'; function: 'independence_test'; Hothorn et al. 2006) to test the hypothesis that there would be a greater decrease in hematocrit among infested lizards compared to control animals. Pair

ID was entered as a block term. I used a permutation test rather than a *t*-test as changes in hematocrit were not normally distributed among control animals.

I created a pair of linear mixed models for the behavior of lizards in trials (R package: 'lme4'; function 'lmer'; Bates et al. 2015). Each models included scores on one of the behavior factors (i.e., aggressiveness or reposition-escape) as the response variable, with tick treatment, temperature inside the enclosure at the end of the trial, factor 1 body size, factor 2 badge color, the intensity of ticks on lizards at the time of collection, and *S. (L.) occidentalis* infection as first-order predictors. I included temperature in the arena in this model as body temperature is important for male *S. occidentalis* contest behavior (Engbretson and Livezey 1972). Before fitting the final mode, I fit models with all possible two-way interactions between predictors, and iteratively re-fit these models until only significant interactions remained. I used an α of 0.05 for determining which interactions to include. In both models, this process resulted in the retention of only first-order terms. Both models included pair ID as a random effect. Factor 1 aggressiveness was log(+1) transformed in an attempt to normalize residuals, though normality, as assessed with a Shapiro-Wilk test (R package: 'stats'; function: 'shapiro.test'; R Core Team), was ultimately unattainable. The response variable in the factor 2 reposition-escape model required no transformation.

In order to determine possible effects of tick attachment to sensitive structures outside of the nuchal pocket, I fit a second pair of linear models for the two behavior factors (aggressiveness and reposition-escape) exclusively among infested animals. These models included the proportion of ticks that attached in the nuchal pocket as a predictor. Other predictors were shared with the previously described models, including lizard body

size, badge color, and the temperature inside the arena on the day of the trial. Just as with the first pair of models, I determined which interactions to include by first fitting models with all two-way interactions, then performing a manual backward selection based on significance (with $\alpha = 0.05$). As correlations between the behavior factors and some of these predictors had already been tested in the first pair of linear mixed models, I interpreted significant correlations as those with $p < 0.0125$ in the four models described thus far.

In order to explore how infested and non-infested animals potentially differed in the way in which they engaged rivals, I created (first order) Markov chains from behavioral observations (Fig. 34). These depicted the probability of one behavior following another, and provided a general sense of the sequence of agonistic behaviors, and how specific patterns of behavior may have differed between infested and non-infested animals. I included two-leg pushups, slow approaches, four-leg pushups, charges, bites or nudges, full-shows, and retreats (Table 9) in this part of my analysis, as these behaviors have been classified as 'aggressive' or 'submissive' in previous studies of sceloporine lizards (e.g., Schall and Dearing 1987; Garland et al. 1990; Sheldahl and Martins 2000; Robbins et al. 2010; Swierk and Langkilde 2013). Due to inadequate sampling of some of the behaviors for factor analysis (see above), the behaviors used to construct the Markov chains were not necessarily the same behaviors used in the factor analysis on lizard behaviors. Information contained in Markov matrices allowed me to identify broad differences between infested and non-infested animals in terms of the progression of behaviors, but I did not subject specific differences between the transition frequencies for infested and non-infested animals to statistical analysis.

2.3 Results

Summary

I found that tick-infested lizards were significantly less aggressive than non-infested opponents (support for prediction 1a), and that a possible explanation for decreased aggressiveness among infested lizards was the larger average decline in hematocrit among these animals compared to non-infested controls (support for 1c). However, infestation was not correlated with submissive behaviors (escape-reposition) than non-infested controls (no support for 1b), nor did the proportion of ticks attached outside the nuchal pocket correlate with infested lizard behavior (no support for 2a or 2b). Lizard body size and badge color did not correlate with behavior either (no support for 3a or 3b). *Schellackia (Lankesterella) occidentalis* correlated with neither aggressiveness nor reposition-escape (no support for 4a). All possible interactions between the predictors described above were excluded from final models due to lack of significance.

Infestation Intensity and Attachment Location

The mean tick intensity on lizards was 68.84 (SD: 23.81; median: 76; maximum: 99; minimum: 13). Intensity varied temporally over the study (Table 7). Ticks attached in the nuchal pocket far more frequently than any other location (Fig. 9). Factors correlating with tick attachment site selection and infestation intensity are discussed in depth in Chapter 1.

Aggressiveness

As predicted, tick-infested male lizards had significantly lower scores on factor 1 aggressiveness than non-infested animals (Table 11, Fig. 32). Contrary to expectation, there were no significant interactions in this model, including those between tick infestation and *S. (L.) occidentalis* infection, body size, badge color, trial temperature, or the intensity of ticks at the time of capture. In addition to tick infestation, trial temperature was significantly correlated with aggressiveness scores (Fig 33); as expected, factor 1 aggressiveness increased with temperature. Infested animals had lower aggression scores than the non-infested animal in 30 of 50 trials (Fig. 37). No terms besides temperature and tick infestation significantly correlated with factor 1 aggressiveness.

Reposition-escape

Contrary to expectation, there were no significant correlations between factor 2 reposition-escape and lab-tick treatment, trial temperature, body size, badge color, *S. (L.) occidentalis* infection, or the number ticks infesting lizards at the time of collection, after correcting for multiple tests in the linear mixed model (Table 12). Before correction for multiple tests, badge color was significantly positively correlated with reposition-escape.

Attachment Site Effects

There were no significant correlations in either of the linear models concerning the proportion of ticks that attached inside the nuchal pockets and lizard behavior (Tables 13 and 14). This was contrary to the expectation that lizards with a high proportion of

ticks obstructing limbs or impairing sense organs would behave less aggressively and attempt disengage from contests. As in the model including all lizards, badge color correlated positively with reposition-escape before correcting for multiple tests.

Hematocrit

Hematocrit dropped significantly for both infested and non-infested animals between capture and behavior trials (paired tests: $t = 7.16$, df = 49, $p < 0.001$ and $t = 12.11$, df = 49, $p < 0.001$ for non-infested and infested animals, respectively) (Fig. 35). This reduction was significantly greater among infested than non-infested subjects (one-sided permutation test $Z = 3.44$, $p < 0.001$; R package: 'coin'; function: 'independence_test'; Hothorn et al. 2006).

General Characteristics of Agonistic Interactions

Both infested and non-infested animals engaged in agonistic behaviors (i.e., those behaviors that have historically been interpreted as aggressive or submissive, e.g., Carpenter 1978; Garland et al. 1990; Sheldahl and Martins 2000; Seddon and Hews 2016a, see Table 9) in forty-five out of fifty trials. In five of these trials one lizard in the pair retreated without reciprocating aggression, whereas in forty of fifty pairs both lizards engaged in putatively aggressive behaviors. The progression of these forty trials that involved some degree of reciprocal aggression were generally as follows: several minutes after I raised the partition, one lizard (the 'intruder') would approach the other (either slowly or rapidly). The most typical response from the other animal (the 'defender') was to enter a full-show posture, exposing its ventral badge to the intruder. The defender also

often performed several sets of pushups. Behavior diverged from this point. There were often repeated rounds of pushup displays interspersed with brief physical contact; in twenty-nine trials lizards nudged (and possibly nipped, though the camera resolution did not allow reviewers to distinguish between these actions) at, kicked, or wrestled (bit and held) each other. In one trial a subject bit the forelimb of its opponent and used its grasp to push the other around the arena, though such highly escalated contests were rare. Aggressive displays by both lizards in a pair rarely lasted more than a few minutes in the 30-minute trial, after which one or both animals would begin circling the arena and attempting to scale its walls, or burrow in the substrate. The most common opening aggressive behavior among non-infested animals was an approach (seen in nineteen of fifty trials, combining both slow approaches with charges), whereas the most common first aggressive behavior among infested animals was full-show (twenty of fifty trials).

I created Markov chains from selected agonistic behaviors to describe the difference between infested and non-infested animals (Fig. 34). The most notable difference between infested and non-infested lizards was in the behavior that following retreats: non-infested animals were twice as likely as infested animals to re-approach the other lizard after a retreat compared to infested lizards, and infested animals were more likely to follow retreats by entering a full-show posture. Non-infested animals were also more likely than infested animals to approach opponents following a series of pushups, and performed more approaches than infested animals. Infested animals were approximately twice as likely as non-infested animals to retreat following displays. Although these particular differences in behavior between infested and non-infested animals complement the general conclusion that infested animals behaved less

aggressively than their non-infested opponents, the overall sequence of behaviors was similar between infested and non-infested animals. Infested and non-infested animals also spent similar amounts of time motionless during trials (Fig. 36).

2.4 Discussion

The significant reduction in aggressive behavior I observed among tick-infested male *S. occidentalis* in contests against non-infested animals suggests that there is a substantial cost associated with *I. pacificus* infestation, which likely influences the ability of tick-infested individuals to acquire mates and defend territories in the wild. Infested animals were both significantly less aggressive than non-infested lizards on average, and were also the less aggressive animal when paired against non-infested individuals in a majority of trials. Aggressiveness is an important determinant of the ultimate victor in conflicts between sceloporine lizards, with one analysis ranking it second only to SVL in terms of predicting the winner of a contest between lizards (Robbins et al. 2010). Because the main benefit derived by male sceloporines from defending territories seems to be access to females (Haenel et al. 2003), it is likely that, under field conditions, tick-infestation decreases reproductive success for male lizards.

Laboratory studies are sensitive to resolving specific aspects of a host-parasite system, but may also miss a broader picture, such as the contribution of factors that could amplify or diminish the detrimental impact of a parasite on its host in the wild (Lehmann 1993). For instance, territory loss resulting from infestation could increase exposure to predators if losers are forced to venture into unfamiliar territory (Metzger 1967). Acknowledging the limitations of lab-based studies, there is ample reason to believe that

the results of my study are applicable to lizards in the wild. Male lizards in my study engaged in most of the agonistic behaviors recorded in field studies (e.g., Carpenter 1978; Sheldahl and Martins 2000; Seddon and Hews 2016a), including pushups, bites, shudders, and displays of the ventral color badges. Lizards actively displayed and engaged in physical combat for only a few minutes within the 30-minute trial period, a similar duration to contests between *S. occidentalis* and other sceloporines in more natural settings (Schall and Sarni 1987; Haenel et al. 2003). The factor analysis I performed on behaviors can also be compared to the principal component analysis (PCA) conducted by Seddon and Hews (2016a) on behaviors performed by *S. occidentalis* in staged territorial encounters in the wild. As in my analysis, Seddon and Hews (2016a) retained two components: the first composed primarily of aggressive behaviors and the other of chemosensory and Full-Show. As in my analysis, Head Bobs, Approaches ("move towards") and Bites ("physical contact") had higher magnitude loadings on their first component compared to their second component, whereas Chemosensory behaviors had a higher magnitude loading on their second orthogonal component. In contrast to my analysis, Seddon and Hews (2016a) reported a positive correlation between full-show and other putatively aggressive behaviors, which I did not find. My second factor also differs from the second component derived by Seddon and Hews (2016a) in that slow approaches, an aggressive behavior, positively correlated with chemosensory behaviors in my study, and negatively correlated in theirs. The difference in the relationship between specific agonistic behaviors in our studies may be the product of the difference in the experimental setting, and possibly because Seddon and Hews (2016a) terminated encounters at the onset of physical combat.

The intensity of ticks on infested *S. occidentalis* during trials were well within the range of tick intensities on wild *S. occidentalis*. Several studies have found peak *I. pacificus* intensities on *S. occidentalis* exceeding 100 ticks/lizard (Eisen et al. 2001; Lumbad et al. 2011), which was the maximum possible lab-reared larvae intensity in my study. In the field, *I. pacificus* nymphs may make up the majority of ticks infesting lizards outnumbering larvae 2:1 at certain times of the year (Eisen et al. 2001; Lumbad et al. 2011). Nymphs are far larger than larvae and ingest considerably more blood (Dunlap and Mathies 1993). The larger size of nymphs and larvae may also make nymphs more capable of obstructing sense organs. Because I this study was limited to larvae to simplify tick rearing and analysis, and because many reports of tick intensities on wild *S. occidentalis* report combined nymph and larvae intensities comparable to the larvae-only infestations in this study, the effect of tick infestation I report may underestimate the actual impact of *I. pacificus* on *S. occidentalis* in the wild.

Greater declines in hematocrit among tick-infested lizards may be sufficient to explain their reduced aggression relative to non-infested animals. A decline of 25% in hemoglobin corresponds to a 20% reduction in stamina (Schall and Sarni 1987). Infested and non-infested animals spent approximately equivalent lengths of time active within 30-minute contests, implying that it was the intensity of aggressive behaviors that was reduced among infested animals during the relatively brief periods of spent in vigorous activity. Garland et al. (1990) found that dominance is linked to locomotor capacity, but found no correlation between stamina and dominance in male *S. occidentalis*. If tick infestation does reduce aerobic scope in infested *S. occidentalis* via reductions in blood oxygen carrying capacity, it would not be the only example among the parasites of *S.*

occidentalis; infection with *P. mexicanum* correlates with reductions in hemoglobin and aerobic scope in *S. occidentalis* (Schall 1982; Schall et al. 1982).

Host behavioral responses to parasite infection that maximize lifetime reproductive success should be favored by evolution, even if such responses tradeoff short-term reproductive success (Hart 1988; Forbes 1993). *S. occidentalis* can live for several years, and every adult lizard is likely to be infested by *I. pacificus* at some point in their lives, as many *S. occidentalis* populations verge on 100% tick infestation prevalence in the spring (e.g., Eisen et al. 2001). Much of the decline in aggressiveness among tick-infested *S. occidentalis* may therefore be attributable to an adaptive behavioral response to infections that prioritizes long-term fitness, in addition to the direct negative consequences of the infestation.

Animals may become conditioned through past success to act aggressively in future encounters (Stuart-Fox and Johnston 2005; Fawcett and Johnstone 2010). The highest quality males in a population would therefore act aggressively because of a history of success. Even though lizards in my study had no social interaction for at least two weeks prior to trials, at least some lizard species seem to have the ability to remember the outcomes of contests that happened weeks before, meaning winner-loser effects could still have been present in trials between animals in my study (Stuart-Fox and Johnston 2005). Perhaps the most parsimonious explanation for the lack of correlation between size and aggression is that male *S. occidentalis* are capable of assessing their fighting ability relative to their opponent based on information they gather about opponent size and quality in the early stages of a contest, but there are other possible explanations. For instance, a winner effect might be counterbalanced if young

adult lizards benefit from increased aggression more than older adults, which is a strategy that is likely to be favored in some species (Fawcett and Johnstone 2010). Assuming *S. occidentalis* do have the capacity to assess the relative fighting ability of their rival, my practice of pairing lizards based on SVL and badge size would mask any effect of body size or badge color on aggressive behavior.

Displays involving colorful badges and armaments may be used by males to advertise fighting ability and genetic quality to rivals and potential mates, respectively (reviewed in Berglund et al. 1996). In species possessing both colorful displays and contest behavior, some have argued that color badges may have evolved first as an advertisement of fighting ability, and only later adopted an intersexual role (Howard and Minchella 1990; Berglund et al. 1996). In lizards, both body size and badge color may be used by conspecifics to gather information about the fighting ability of the bearer (Olsson 1994; Huyghe 2004; but see Stuart-Fox and Johnston 2005; Langkilde and Boronow 2010). Signals such as colorful badges may serve to settle costly conflicts by providing honest information about potential asymmetries before any blood is shed (Berglund et al. 1996). It is therefore curious that ventral badge color did not correlate with aggression in my study, and also that the full-shows, in which the blue ventral badges become visible to rivals, was not a significant component of factor 1 aggression. These findings are not unique; Seddon and Hews (2016a) found that ventral badge melanization did not correlate with intra-population variation in aggressive behavior in *S. occidentalis*. The role of badges in agonistic behavior in the closely related (and morphologically similar) *S. undulatus* is also questionable (Langkilde and Boronow 2010; Swierk and Langkilde 2013). This suggests that badge color may not be used in contests between male *S.*

occidentalis, whereas pushups and other locomotor behaviors, and possibly physical combat, seem to be crucial components of contests (Garland et al. 1990).

Agonistic encounters in which there is an asymmetry in fighting ability, such as the ones experimentally created in my study, are the norm in nature (Parker 1974a). Selection should favor the ability to assess the direction of any asymmetry and adjust the level of aggression accordingly, such that the assessor does not waste resources on a contest they are unlikely to win (Maynard Smith and Parker 1976; Arnott and Elwood 2009). As tick infestation seems to introduce such an asymmetry, infested *S. occidentalis* with such a capacity should temporarily reduce aggressive output against non-infested rivals. There is an extensive literature describing the ways in which animals gather (or fail to gather) information on relative fighting ability when deciding how much to invest in a particular contest (Arnott and Elwood 2009). Individuals in species lacking the ability to assess a rival's fighting ability may simply fight until an internal limit is reached (reviewed in Arnott and Elwood 2009). If the contests engaged in by such a species does not include costs that become more severe with the strength of the rival (i.e., if physical combat is uncommon), the length of the trial should be determined by the capabilities of the weaker contestant (Taylor and Elwood 2003; Arnott and Elwood 2009; Elwood and Arnott 2012). As the intensity of trials among my *S. occidentalis* did not correlate with either body size or badge color, it would seem that *S. occidentalis* have some rival-assessment capability, that rivals impose costs on each other during contests in proportion with the imposer's quality, or both. Many animals likely have the required cognitive ability to assess their own ability relative to that of an opponent (Fawcett and Mowles 2013), and lizards seem capable of performing such assessments (Martin et al.

2016). In a study in which the apparent level of symmetry of contests was experimentally manipulated through changes to UV-color badges, Martin et al. (2016) demonstrated that common lizards (*Zootoca vivipara*) are capable of assessing their fighting ability relative to that of the rival. Identifying the assessment strategy utilized by male *S. occidentalis* would require an experimental design tailored to answering that question, so full resolution of this question is beyond the scope of my study. Interactions of parasites and assessment of self vs rival fighting ability is an interesting area for future study, especially given the role parasites may play in the signals evolved for assessment of potential mates.

The significant positive correlation between temperature and aggressive behavior I observed conforms to the trend expected for ectotherms. I attempted to control temperatures in arenas such that they never much exceeded the preferred resting temperature of *S. occidentalis* (34°C, readings exceeded 35°C in only one trial), temperatures were lower than the peak of *S. occidentalis* performance curve during trials. The body temperature for male *S. occidentalis* engaging in agonistic encounters can reach as high as 40°C (Engbretson and Livezey 1972), considerably higher than their preferred resting temperature. Interestingly, this increase in body temperature may be accomplished by metabolic means at the onset of agonistic interactions (Engbretson and Livezey 1972). If the ability to metabolically elevate temperature during social encounters is critical to *S. occidentalis*, this suggests an additional mechanism by which parasites might affect these lizards. *S. occidentalis* infected with *P. mexicanum* spend less time visibly basking, though infected animals do not seem to differ in their preferred temperature or ability to thermoregulate (Schall and Sarni 1987). My method of measuring temperature during

trials only included a reading of the substrate temperature, as the inclusion of this variable was primarily intended to provide a control in my models of the effect of tick infestation. Future work might use more sensitive methods to investigate possible interactions between *S. occidentalis* body temperature, tick infestation, and agonistic behavior. Curiously, temperature did not correlate significantly with reposition-escape, even though this factor included behaviors that would seem to demand considerable energy expenditure, namely making many small movements and attempting to scale the walls of the arena.

I found no significant correlation between the proportion of ticks attached outside the nuchal pocket and aggressiveness or reposition-escape. Because most ticks that did not attach in the nuchal pocket attached to eyelids and external auditory meatuses (chapter 1), this implies that disruption of senses by ticks may not affect lizard contest behavior. My findings therefore do not support the hypothesis that nuchal pockets evolved to divert ectoparasites from sensitive structures (Arnold 1986). However, because tick attachment inside or outside the nuchal pocket correlates with host traits that influence fighting ability (chapter 1), future studies should take a manipulative approach to determining whether tick attachment to sense organs affects host fighting ability. Also, my study leaves open the possibility that ticks attaching to sense organs impose fitness costs on lizards by, for instance, disrupting the lizard's ability to detect predators or prey. Nymphs, which are far larger and were not used in this study due to the length of time needed to rear them relative to larvae, may still affect lizard behavior through obstruction of sense organs.

Parasites coinfecting a host can interact in complex ways, with implications for host fitness (Alizon et al. 2013, but see Knapp et al. 2018). The presence of such interactions may therefore complicate attempts to isolate the effect of a single parasite, and the possibility of such interactions arising from coinfection was a distinct possibility in my study. There is some evidence that *P. mexicanum* infected *S. occidentalis* also infested with ticks have lower aerobic capacity than lizards infected with only one of these parasites (Dunlap and Mathies 1993). Additionally, the behavior of *S. occidentalis* infected with *P. mexicanum* differs from that of non-infested animals (Schall and Dearing 1987; Schall and Houle 1992). In my study, the prevalence of both *S. (L.) occidentalis* and, especially, *P. mexicanum* within the *S. occidentalis* population I studied was low relative to other populations in California (Ayala 1970; Schall et al. 1982; Megía-Palma et al. 2018). Although the effects of coinfection with ticks and *Plasmodium* were not relevant for the lizards sampled in this study, due to the patchy spatial distribution of *P. mexicanum* infections (Schall 1982) it is possible that such effects may be relevant to nearby lizard populations. Very little is known about the effects of *S. (L.) occidentalis* compared to *P. mexicanum* on their lizard hosts. Like *P. mexicanum*, badge coloration of *S. occidentalis* infected with *S. (L.) occidentalis* hemoparasites subtly differs from non-infected animals (though in both cases, male lizards naturally infected with these parasites appear [to human observers, at least] to be higher quality than non-infected animals) (Ressel and Schall 1989; Megía-Palma et al. 2018). However, I found no correlations between *S. (L.) occidentalis* infection and behavior. If future studies confirm my finding, it may be fruitful to examine what differs between *P. mexicanum* and *S. (L).*

occidentalis infections (both apicomplexan hemoparasites), such that there are behavioral changes associated with infection with the former but not the latter.

Because most parasites, including *I. pacificus* (Lane et al. 2010), rely on their hosts for long-distance dispersal, the host's habitat preferences determine where parasites are found. Shifts in host habitat preference may therefore affect the distribution of parasites in the environment. Because of this, host habitat preference may become a target for manipulation by parasites (Finnerty et al. 2018). Shifts in microhabitat preference following parasite infection are known in *S. occidentalis*; lizards with malaria spend less time in prominent basking positions and more time in shaded areas than non-infected animals, possibly as a result of reduced aggression (Schall and Sarni 1987). If decreased aggression among tick-infested *S. occidentalis* results in similarly decreased access to basking sites as is observed among *P. mexicanum* infected lizards, ticks may benefit from reducing the aggression of their hosts, as sheltered habitats are favored by these desiccation-prone ticks (Lane et al. 1995; Peavey and Lane 1996; Padgett and Lane 2001). If reduced aggression in tick-infested *S. occidentalis* results in lower activity (perhaps in the form of fewer territorial patrols), ticks may also benefit from a reduced probability of being brushed off as infested lizards negotiate dense vegetation. *S. occidentalis* seems to attempt to groom itself of ectoparasites (Engbretson and Livezey 1972), and ticks infesting *S. occidentalis* may also benefit from reduced directed grooming behavior from lethargic hosts. If ticks benefit from behavioral shifts in their lizard hosts resulting from infestation, such effects may represent a barrier to the lizards and ticks coevolving towards commensalism.

The effects of host-parasite interactions often transcend the host and parasite populations to affect the surrounding ecosystem. Indeed, understanding the parasites found in a particular ecosystem is indispensable to understanding the ecosystem as a whole (Dunne et al. 2013). Ecosystem-level effects can take many forms, including parasite infection increasing the predation risk of a host and thereby affecting trophic interactions many links removed from the hosts and parasites themselves (Lafferty et al. 2008). Ecosystems are also affected when parasites exclude or limit certain hosts. For instance, African trypanosomiasis (sleeping sickness, nagana) has historically rendered parts of Africa unsuitable for humans and livestock (Steverding 2008; Brun et al. 2010). Although ticks are probably not in the same league in terms of virulence to *S. occidentalis* as more infamous parasites are to certain hosts, my finding that tick infestation is likely detrimental to lizards may still have implications for ecosystems in the western United States. The *Ixodes-Sceloporus-Borrelia* system has attracted interest because of possible implications for human public health (e.g., Lane and Loye 1989; Clover and Lane 1995; Lane and Quistad 1998; Kuo et al. 2000; Eisen et al. 2004; Salkeld and Lane 2010; Swei et al. 2011; Rulison et al. 2014). As *S. occidentalis* is a crucial host for subadult *I. pacificus* (Swei et al. 2011), future investigations of *I. pacificus*, their, hosts, and the many pathogens transmitted between them (Eshoo et al. 2015) should consider detrimental impact of *I. pacificus* on *S. occidentalis*.

3. Tables

Table 1: Giemsa Staining Materials.

Solution	Components	Final Volume	Notes
Acid Stock	9.07 g (0.66 mol) KH_2PO_4	1000 ml	
Alkaline Stock	9.5 g (0.66 mol) Na_2HPO_4	1000 ml	
Working Buffer	61 ml Alkaline Stock 39 ml Acid Stock	1000 ml	Adjusted to pH 7.0 – 7.2 through small additions of Acid/Alkaline Stock
Buffered Giemsa Solution	3 ml Giemsa Blood Staining Solution (J.T. Baker brand) 30 ml Working Buffer	33 ml	

Table 2: Results of a Factor Analysis on Lizard Morphological Variables. I used variables with |loadings| > 0.4 (in boldface) to name and interpret these factors. The eigenvalue of each factor, as well as the percent of the total variance it explained, are shown below.

Factors / Morphological Variables	Factor 1 (body size)	Factor 2 (badge color)
SVL	**0.8940**	-0.0469
Mass	**0.9505**	0.0020
Jaw Width	**0.7073**	-0.1572
Blue Abdominal Area	**0.5429**	**0.7029**
Blue Badge Hue	-0.2141	**0.5991**
Blue Badge Saturation	0.1073	**0.7797**
Blue Badge Brightness	-0.1380	0.3928
Eigenvalue	2.5926	1.6242
Percent Variance Explained	36.8	23.5
Cumulative Percent Variance Explained	36.8	60.2

Table 3: Results of the Logistic Regression Model of the Relationship between the Odds of Wild-acquired Ticks Attaching in the Nuchal Pocket (Versus at Other Locations on Lizard Hosts) and Predictor Variables, Including the Day on which Lizards Were Collected, Factor 1 Body Size, Factor 2 Badge Color, *S. (L.) occidentalis* Coinfection, and Wild-acquired Tick Intensity. This model was created with a logit link, and the estimates in this table remain in that form.

	Estimate	SE	Z	P > |z|
Day Collected	**-0.028**	**0.01**	**-2.746**	**0.006**
Factor 1 Body Size	-0.035	0.201	-0.176	0.86
Factor 2 Badge Color	-0.182	0.286	-0.639	0.523
S. (L.) occidentalis (Infected)	0.078	0.704	0.111	0.912
Wild-Acquired Tick Intensity	**-0.052**	**0.019**	**-2.761**	**0.006**

Table 4: Results of the Mixed Effects Logistic Regression Model of the Relationship between Lab-reared Ticks Attaching in the Nuchal Pocket (Versus at Other Locations on Lizard Hosts) and Predictor Variables, Including Factor 1 Body Size, Factor 2 Badge Color, *S. (L.) occidentalis* Coinfection, and Lab-reared Tick Intensity. Infestation group was added as a random effect. This model was created with a logit link, and the estimates in this table remain in that form.

	Estimate	SE	Z	P > |z|
Factor 1 Body Size	**-0.246**	**0.043**	**-5.739**	**< 0.001**
Factor 2 Badge Color	**-0.214**	**0.046**	**-4.66**	**< 0.001**
S. (L.) occidentalis (Infected)	0.205	0.112	1.83	0.067
Lab-reared Tick Intensity	**-0.009**	**0.002**	**-3.791**	**< 0.001**
Random Effect	Variance Explained			
Infestation Group	0.215			

Table 5: Summary Statistics of Wild-acquired Tick Intensity. Means (+/- SD) apply only to infested lizards.

	No. Lizards Sampled	Infestation Prevalence (%)	Mean Intensity (+/-SD)	Mean Nymphs	Mean Larvae	Max.
All	100	49	5.1 (7.6)	2.4 (3.9)	2.6 (4.3)	39
Collection Location						
Arboretum	14	14	1.5 (0.7)	0 (0)	1.5 (0.7)	2
Brizzolara Creek	22	91	8.9 (10.5)	4.2 (5.1)	4.8 (6.1)	39
Core Campus	25	32	2.1 (1.1)	0.6 (1.1)	1.5 (1.5)	4
Poly Canyon	11	64	4.4 (3.7)	3.1 (3.6)	1.3 (1.1)	11
Stenner Creek	28	43	1.7 (0.8)	0.8 (1)	0.9 (0.8)	3
Month (Days)						
April (23-25)	16	69	11.7 (13.3)	5.8 (6)	5.9 (7.9)	39
May (13, 23, 24, 26, 27)	32	53	3.1 (3.7)	1.7 (2.5)	1.4 (1.9)	14
June (6-9, 17-21, 25-29)	34	38	3.8 (2.7)	1.5 (2.6)	2.3 (1.7)	9
July (9-12)	18	44	2.1 (1.4)	0.8 (1.2)	1.4 (1.6)	4

Table 6: Output from the Linear Mixed Model of the Relationship between Wild acquired Tick Intensity and Predictors Variables, Including the Date on which the Lizard Was Collected, Factor 1 Body Size, Factor 2 Badge Color, and Coinfection with *S. (L.) occidentalis* Hemoparasites.

	Estimate	SE	Df residual	t value	P > |t|
Day Collected	**-0.009**	**0.003**	**94.778**	**-2.62**	**0.01**
Factor 1 Body Size	0.049	0.074	91.234	0.655	0.514
Factor 2 Badge Color	0.057	0.082	92.239	0.688	0.493
S. (L.) occidentalis (Infected)	-0.238	0.213	91.595	-1.115	0.268
Random Effect	Variance Explained				
Location Category	0.368				

Table 7: Summary Statistics of Lab-reared Tick Intensity.

Group	Dates Sampled	No. Lizards	Mean Intensity (+/-SD)	Max.
1	May 13, 16-19	8	76 (13.54)	92
2	May 30 - June 3	7	80.71 (17.33)	95
3	June 12 – 15	9	83.89 (11.87)	99
4	July 2 – 5	9	60 (28.94)	92
5	July 15 – 17	8	39.25 (21.14)	67
6	August 1 – 4	9	73.33 (17.33)	91
All	May 13 – August 4	50	68.84 (23.81)	99

Table 8: Output from the Linear Model of the Relationship between the Intensity of Lab-reared Ticks on Lizards and the Date (i.e., the Age of Larval Ticks), Factor 1 Body Size, Factor 2 Badge Color, Hematocrit, Coinfection with *S. (L.) occidentalis*, and Wild-acquired Tick Intensity. Because of the transformation applied to the dependent variable, positive coefficients correspond to a negative correlation between lab-reared tick intensity and that variable.

	Estimate	SE	t value	P >|t|
Day Infested	**0.033**	**0.013**	**2.641**	**0.011**
Factor 1 Body Size	**0.685**	**0.309**	**2.22**	**0.032**
Factor 2 Badge Color	-0.05	0.348	-0.144	0.886
Hematocrit	0.029	0.049	0.601	0.551
S. (L.) occidentalis (Infected)	0.124	0.857	0.145	0.885
Wild-Acquired Tick Intensity	0.006	0.089	0.07	0.944

Table 9: Ethogram Used to Review Trials. Behaviors with KMO MSAi > 0.6, which were used in factor analysis, are indicated in boldface. Behaviors that have been interpreted as aggressive or submissive behaviors in previous studies of *S. occidentalis* behavior are indicated with (+) or (-), respectively. The sum of the count or time (seconds) spent in each behavior across the study is indicated in the rightmost column.

Behavior	Description	Observations/Time
Scratch	Scratch/kick substrate. Attempt to burrow	331
Twitch (+)	Tail twitches	72
Enter Hide	Lizard disappears into hide	20
Contact (non-aggressive)	Focal lizard contacts other lizard without biting, nudging, wrestling, kicking, or any other apparent aggressive act	61
Kick (+)	Hits other lizard with hindlimbs, usually while rotating. Tail lash	42
Escape Attempt	Attempts to scale arena walls (no such attempts were successful)	922
Chemosensory	Licks or rubs nostrils on gravel substrate	453
Reposition	Rotates >90^{o} or moves less than one body length in any direction	1079
Startle	Jumps away from opponent from a flattened (non-vigilant) position	9
Vigilant (non-basking)	In shade with head up, not moving	97624 (sec)
Burrowed (-)	Covered at least to shoulders by substrate	14217 (sec)
Retreat (-)	Moves directly away from other lizard after being in close proximity	326
Wrestle	Bites and holds other lizard	109 (sec)
Perching	Atop hides	3117 (sec)
Vigilant (basking)	In sun with head up, not moving	13282 (sec)
Flattened (basking)	In sun with head down, not moving	2899 (sec)
Exploring	Moving around arena, but neither approaching nor moving away from other lizard. >5 seconds of inactivity ends this state	24877 (sec)
Flattened (non-basking) (-)	In shade with head down, not moving	7448 (sec)
Approach (slow) (+)	Moving deliberately towards other lizard, but not running	343
Charge (+)	Runs directly towards other lizard	69
Full-Show (+)	Posture with extended legs, arched back, and lateral compressions exposing blue ventral scales	12510 (sec)

Two-Leg Pushup (+)	Single pushup involving only the forelimbs	2570
Four-Leg Pushup (+)	Single pushup involving both fore- and hindlimbs	1022
Nips/Nudges	Focal lizard taps other lizard with head. Due to camera resolution brief bites could not be distinguished from head smacks without biting.	379
Shudders (+)	Rapid up and down movements involving only the head	138

Table 10: Results of a Factor Analysis on Lizard Contest Behaviors. I used variables with |loadings| > 0.4 (boldface) to name and interpret these factors. The eigenvalues of each factor, as well as the percent of the total variance explained by each factor, are shown at the bottom of the table.

Factors / Behaviors	Factor 1 (Aggressiveness)	Factor 2 (Reposition-escape)
Contact (non-aggressive)	0.03	0.23
Escape Attempt	-0.05	**0.53**
Chemosensory	0.12	**0.39**
Reposition	0.24	**0.76**
Full-Show	0.34	0.15
Two-Leg Pushup	**0.79**	-0.03
Four-Leg Pushup	**0.45**	0.15
Nips/Nudges	**0.42**	**0.44**
Charges	**0.53**	0.25
Approaches (Slow)	**0.78**	**0.40**
Shudders	**0.78**	-0.22
Eigenvalue	3.08	1.18
Percent Variance Explained	0.25	0.14
Cumulative Percent Variance Explained	0.25	0.39

Table 11: Results of a Linear Mixed Model of the Relationship between Factor 1 Aggressiveness and Various Predictor Variables, Including Tick Infestation (Binary), The Temperature at the End of Trials, Lizard Body Size, Badge Color, The Number of Ticks Infesting the Lizard in the Field, And *S. (L.) occidentalis* Infection (Binary). The response variable was log(+1) transformed. Pair ID was entered as a random effect. The p-value and F statistic are taken from Type III Wald F tests on the model output. Predictors that correlated significantly with the response are indicated in boldface ($\alpha = 0.0125$).

	Estimate	SE	F	Df residual	P
Tick Infestation (Non-Infested)	**0.37**	**0.14**	**7.31**	**47.97**	**0.009**
Trial Temperature	**0.05**	**0.02**	**10.23**	**46.41**	**0.002**
Factor 1 Body Size	-0.07	0.07	1.00	53.32	0.321
Factor 2 Badge Color	0.09	0.08	1.27	82.20	0.262
Ticks at Collection	0.00	0.01	0.49	92.96	0.485
S. (L.) occidentalis Infection (Infected)	0.19	0.22	0.78	92.76	0.379
Random Effect	Variance Explained				
Pair ID	0				

Table 12: Results of a Linear Mixed Model of the Relationship between Factor 2 Reposition-escape and Various Predictor Variables, Including Tick Infestation (Binary), The Temperature at End of Trials, Lizard Body Size, Lizard Badge Color, The Number of Ticks Infesting the Lizard in the Field, And *S. (L.) occidentalis* Infection (Binary). Pair ID was entered as a random effect. The p-value and F statistic are taken from Type III Wald F tests on the model output.

	Estimate	SE	F	Df residual	P
Tick Infestation (Non-Infested)	0.09	0.11	0.65	46.92	0.425
Trial Temperature	0.03	0.02	2.28	47.66	0.138
Factor 1 Body Size	-0.03	0.10	0.07	71.12	0.796
Factor 2 Badge Color	0.18	0.09	3.97	85.24	0.495
Ticks at Collection	-0.02	0.01	1.97	69.86	0.164
S. (L.) occidentalis Infection (Infected)	-0.03	0.22	0.03	71.49	0.867

Random Effect	Variance Explained
Pair ID	0.42

Table 13: Results of a Linear Mixed Model of the Relationship between Factor Aggressiveness and Various Predictor Variables, Including the Proportion of Ticks Attaching in the Nuchal Pocket, The Temperature at the End of Trials, And Lizard Body Size. The response variable was log(+1) transformed. The p-value and F statistic are taken from Type III Wald F tests on the model output.

	Estimate	SE	F	Df residual	P
Proportion of Ticks in NP	0.47	0.68	0.48	45	0.491
Trial Temperature	0.04	0.02	2.47	45	0.123
Factor 1 Body Size	-0.01	0.10	0.01	45	0.915
Factor 2 Badge Color	0.16	0.11	2.04	45	0.160

Table 14: Results of a Linear Mixed Model of the Relationship between Factor 2 Reposition-escape and Various Predictor Variables, Including the Proportion of Ticks Attaching in the Nuchal Pocket, The Temperature at the End of Trials, And Lizard Body Size. The p-value and F statistic are taken from Type III Wald F tests on the model output.

	Estimate	SE	F	Df residual	P
Proportion of Ticks in NP	0.31	0.35	0.78	45	0.382
Trial Temperature	0.02	0.01	3.26	45	0.078
Factor 1 Body Size	0.01	0.05	0.01	45	0.914
Factor 2 Badge Color	0.14	0.06	5.52	44	0.023

4. Figures

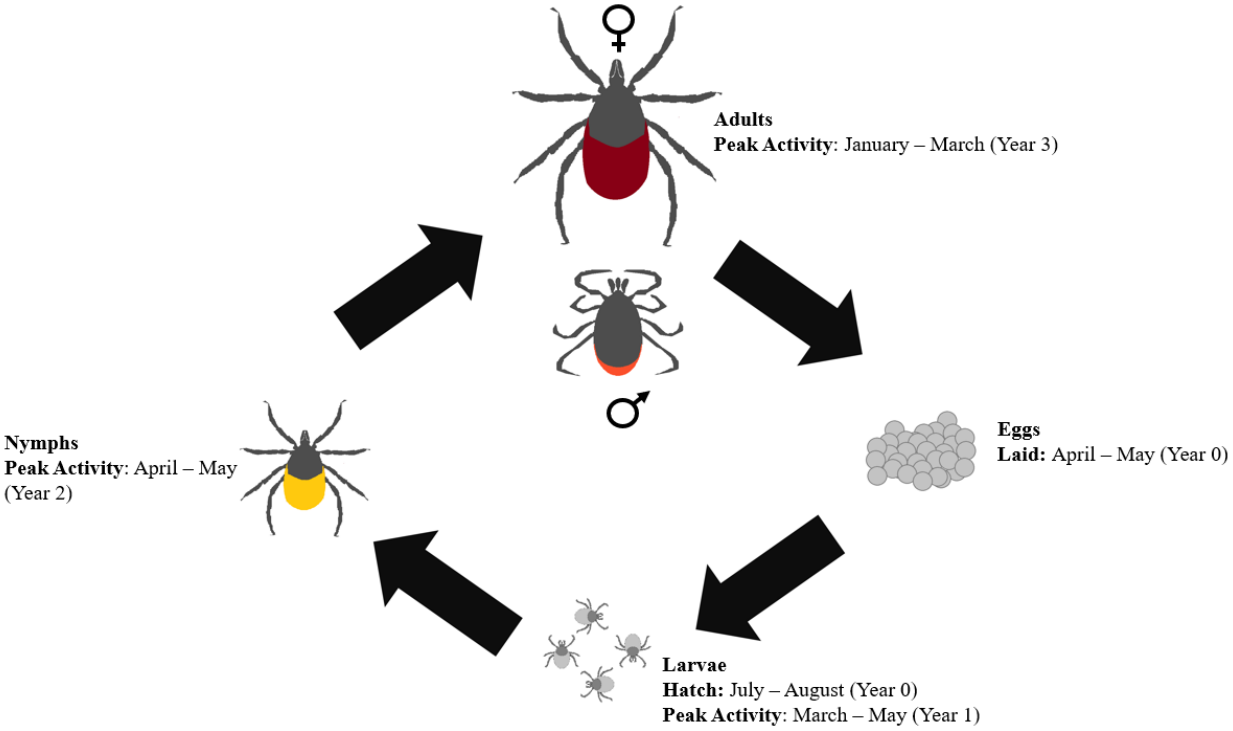

Figure 1: A Life Cycle Diagram of the Western Black-legged Tick (*Ixodes pacificus*) Showing the Timing of Peak Activity for Each Life Stage (Padgett and Lane 2001; MacDonald and Briggs 2016).

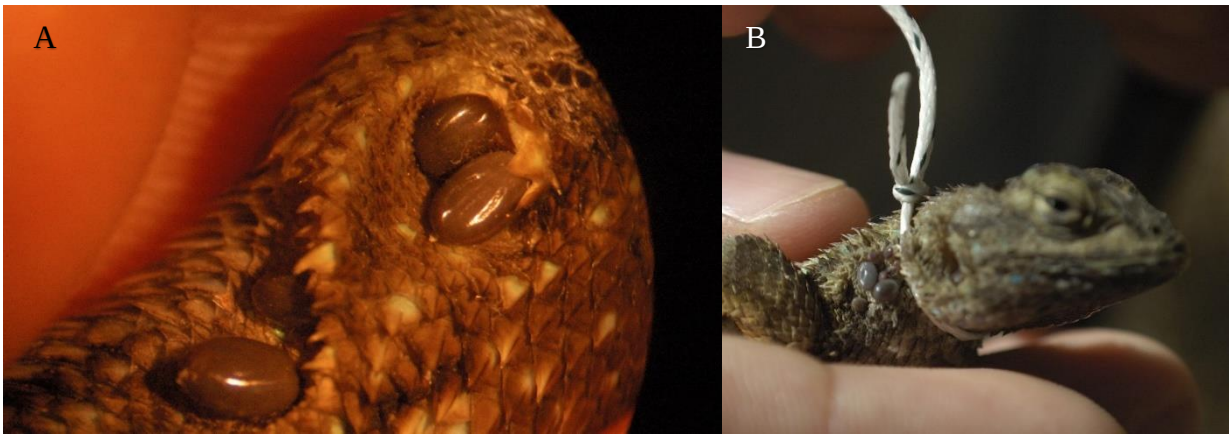

Figure 2: Wild *Ixodes pacificus* Infesting *Sceloporus occidentalis*. A) Two nymphs attached in the nuchal left pocket (lower left) and two nymphs completely obstructing the left tympanic membrane (upper right). B) Nymphs and larvae in the right nuhcal pocket of a lizard, pictured immediately after being captured by noose.

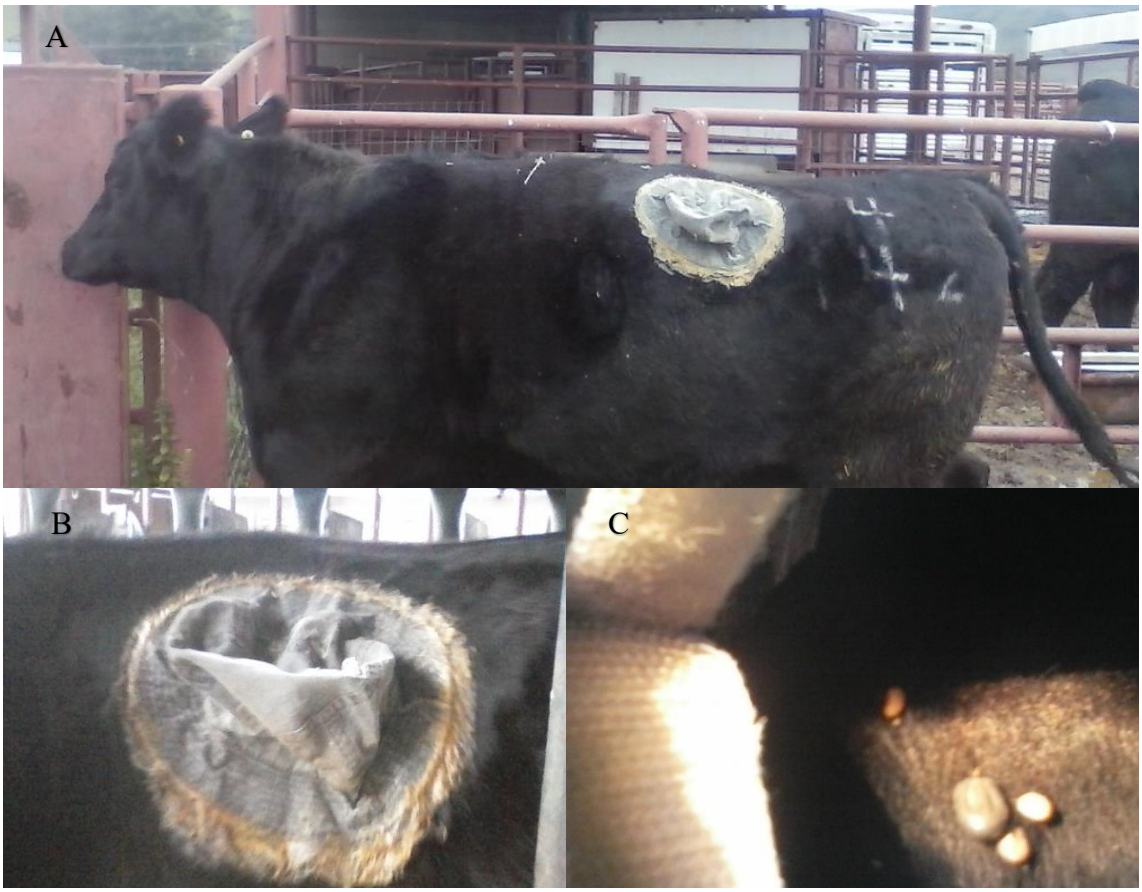

Figure 3: Three Images of the Process of Larval Tick Generation from Feeding Adult Ticks on a Female *Bos taurus*. A) I monitored the cow on which ticks fed at the Cal Poly Beef Unit daily to ensure the health of the host and to rapidly recover replete ticks. B) I glued a cotton bucket hat modified with a Velcro-sealed opening to the cow's flank using tag cement. This hat confined and protected the ticks I placed on the cow. C) An image of female ticks feeding beneath the hat.

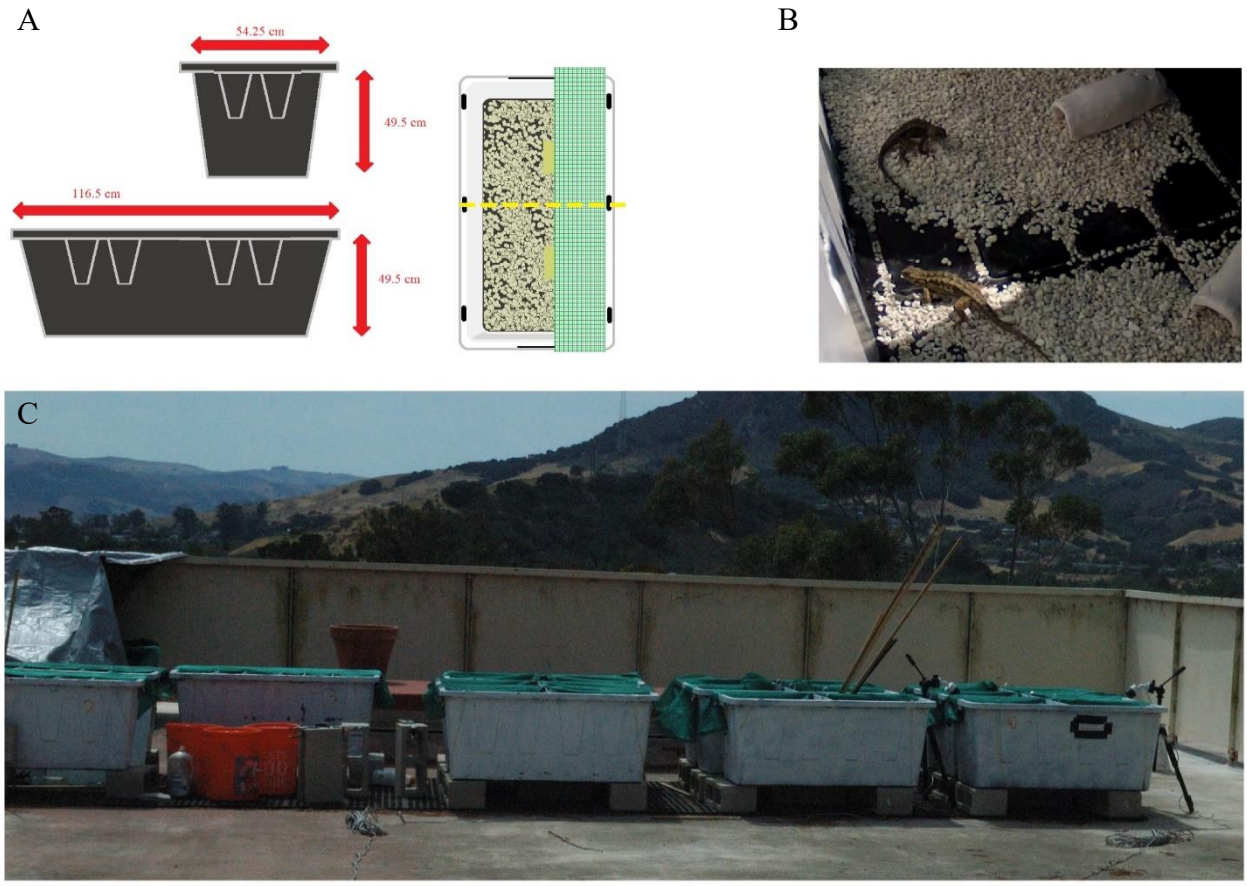

Figure 4: Outdoor Semi-enclosures A) A diagram showing the dimensions and positions of gravel substrate, hides, and shadecloth in bins. B) Two lizards display towards each other in a staged encounter inside of their semi-enclosures. C) Outdoor semi-enclosures for lizard housing in situ. The three leftmost enclosures were used for housing lizards prior to infestation, while the remaining nine housed lizards after experimental infestations.

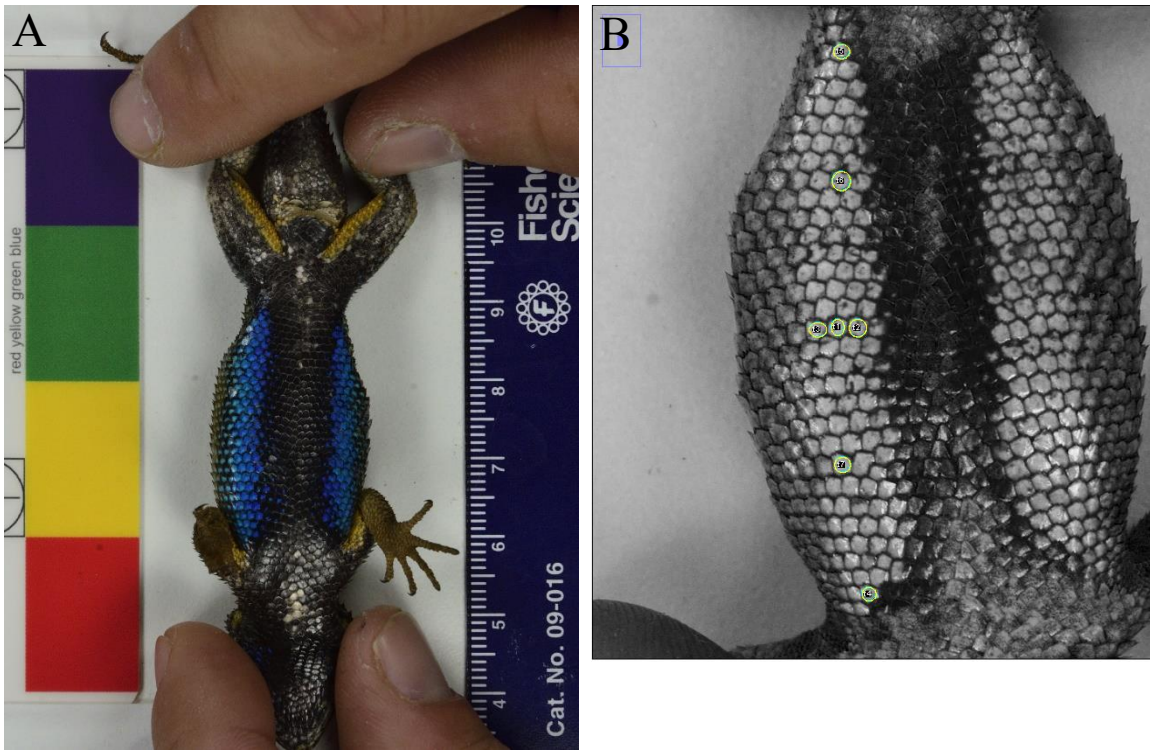

Figure 5: Lizard Photography A) A male lizard being held down in order to photographs its ventral blue badge. I measured badge area between the armpits (bottom of image)and femoral pores (top of image). The color standard, ruler, and flat white background of the photography stage used in every photography session are visible in this picture. B). The blue layer of an RGB stack in Fiji (ImageJ) of a male lizard (not the same individual pictured in A). I created a macro function to record average pixel values of selected regions in a stack. The selected regions (yellow circles) were seven scales at the same position within the blue component of the ventral badge of each lizard.

Figure 6: Photography Stage Used to Photograph Lizards *in situ.*

Figure 7: Image of the Beakers Used to Infest Male *S. occidentalis* with *I. pacificus* Larvae. Some of these beakers contained control (non-experimentally-infested) animals.

Figure 8: Western Black-legged Tick Larvae Attached to the Left Eyelid of a Western Fence Lizard Approximately 10 Days Post-infestation. The position of one tick is indicated with an arrow. There are at least eighteen larvae in this image.

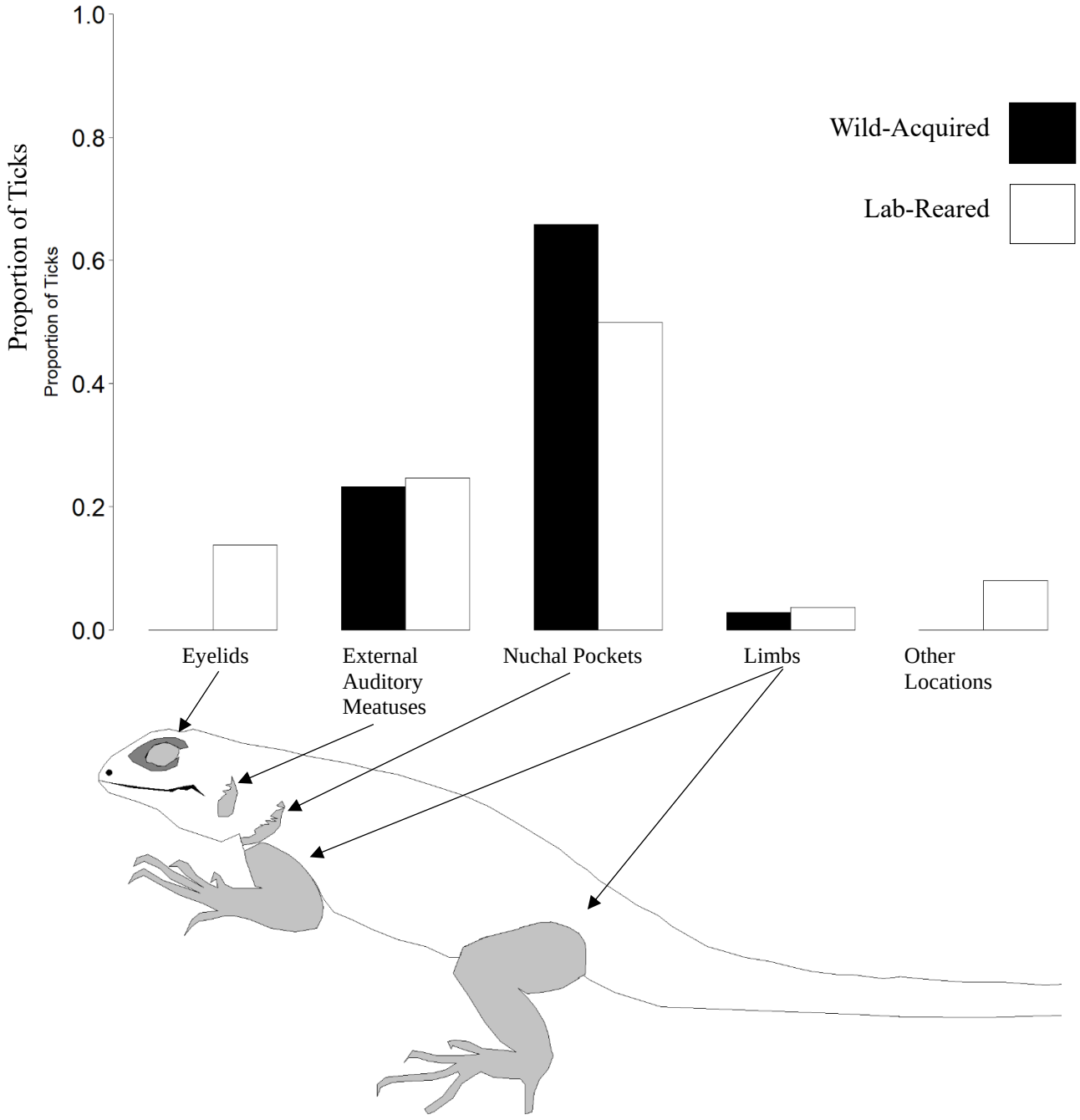

Figure 9: The Proportion of Wild-acquired (Black Bars) and Lab-Reared (Light Bars) Ticks that Attached at Each Location on Their Lizard Hosts. Shaded regions on the lizard represents the extent of each of the first four sites. Ticks found on unshaded regions were classified as "other".

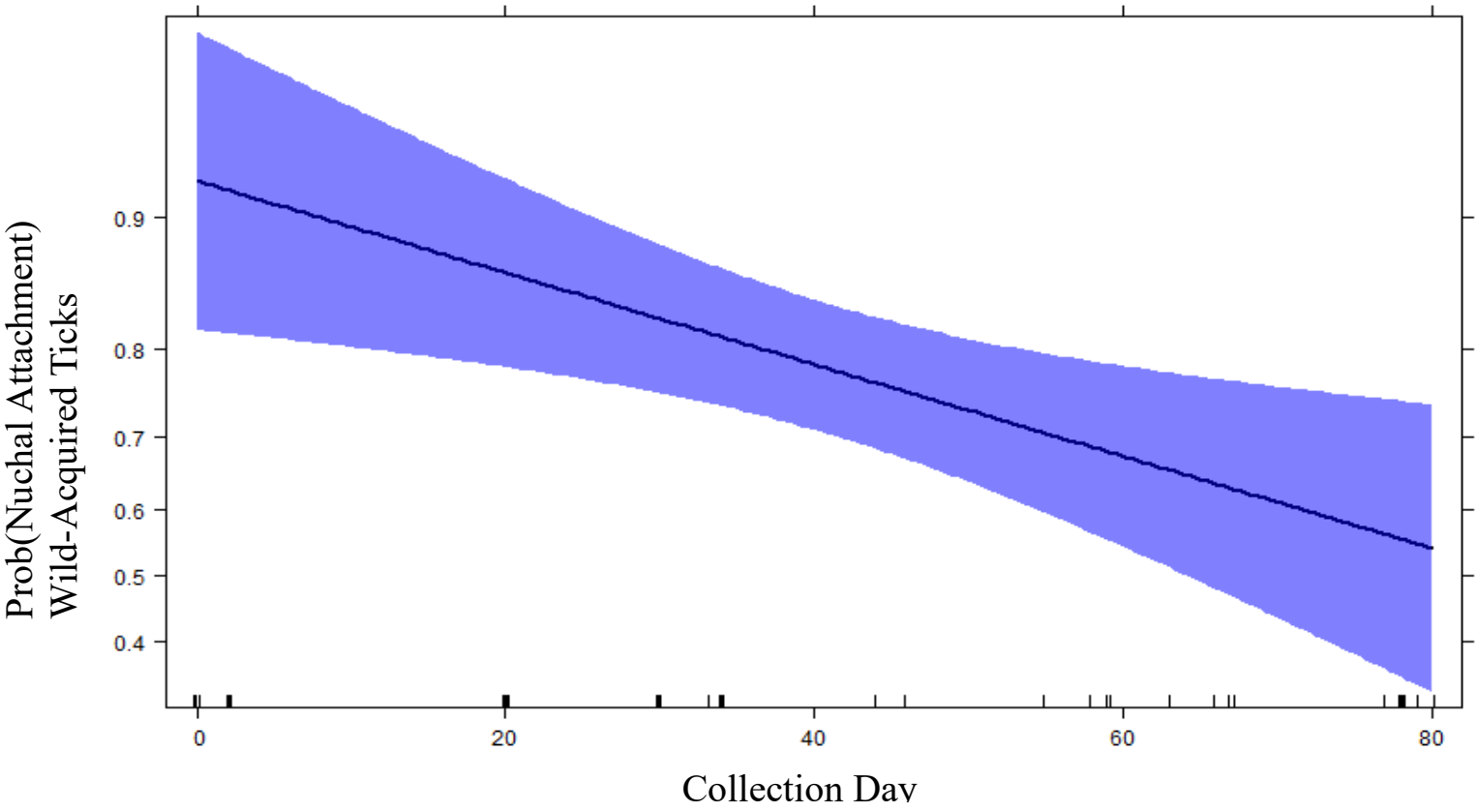

Figure 10: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Wild-acquired Ticks Attaching in the Nuchal Pocket and Collection Day. The shaded region represents the 95% CI. Predictions have been converted from log odds to probabilities for plotting purposes.

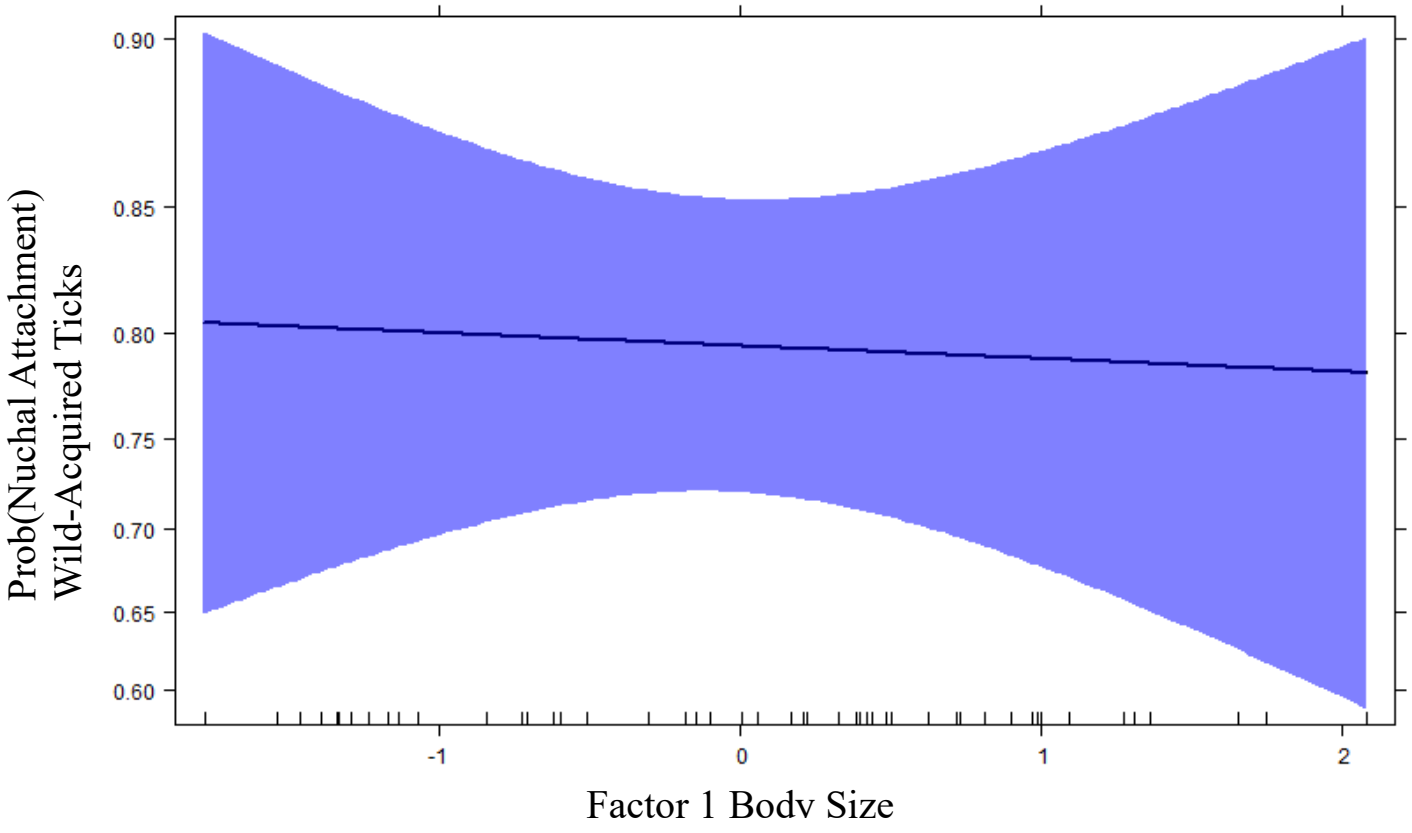

Figure 11: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Wild-acquired Ticks Attaching in the Nuchal Pocket and Factor 1 Body Size. The shaded region represents the 95% CI. Predictions have been converted from log odds to probabilities for plotting purposes.

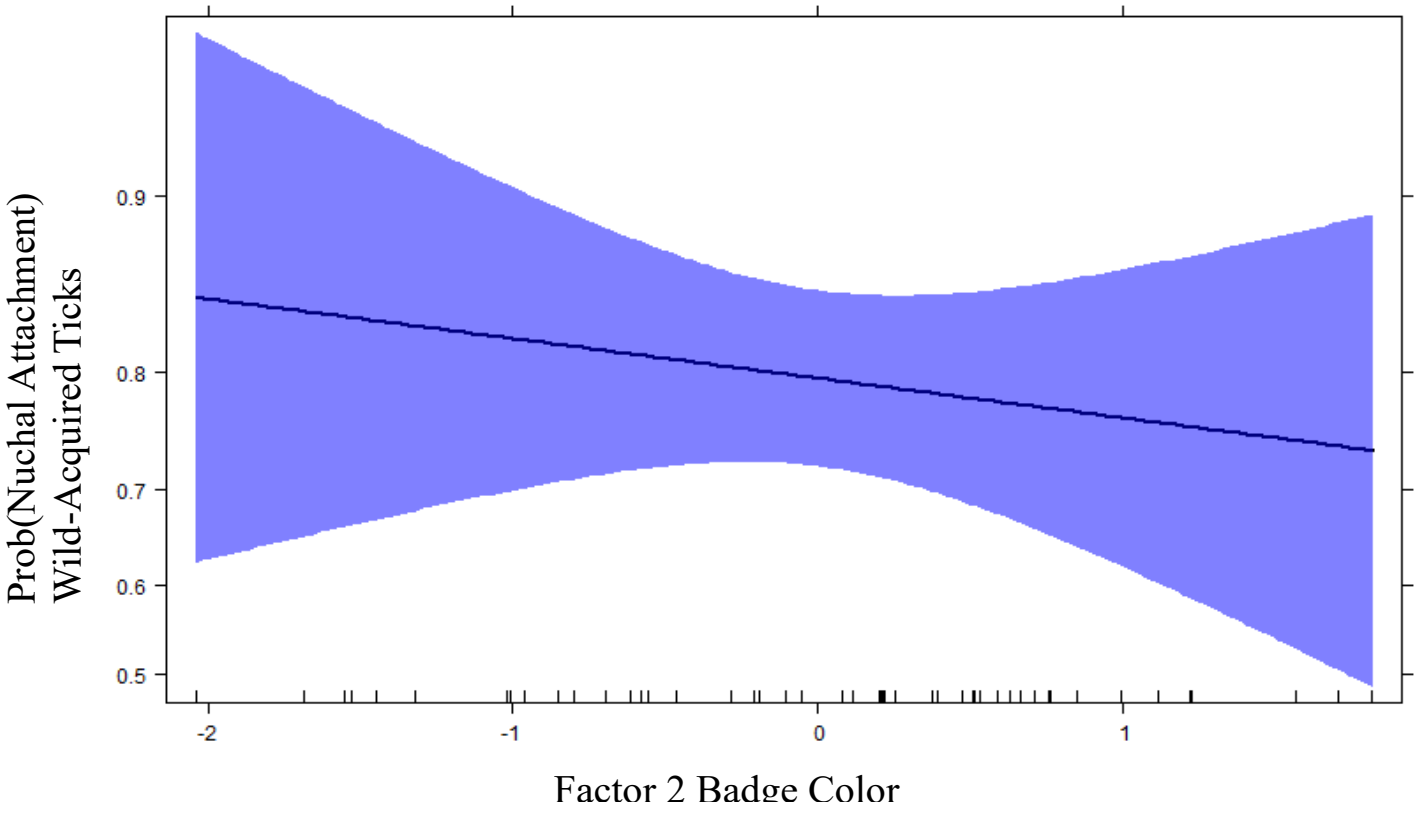

Figure 12: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Wild-acquired Ticks Attaching in the Nuchal Pocket and Factor 2 Badge Size. The shaded region represents the 95% CI. Predictions have been converted from log odds to probabilities for plotting purposes.

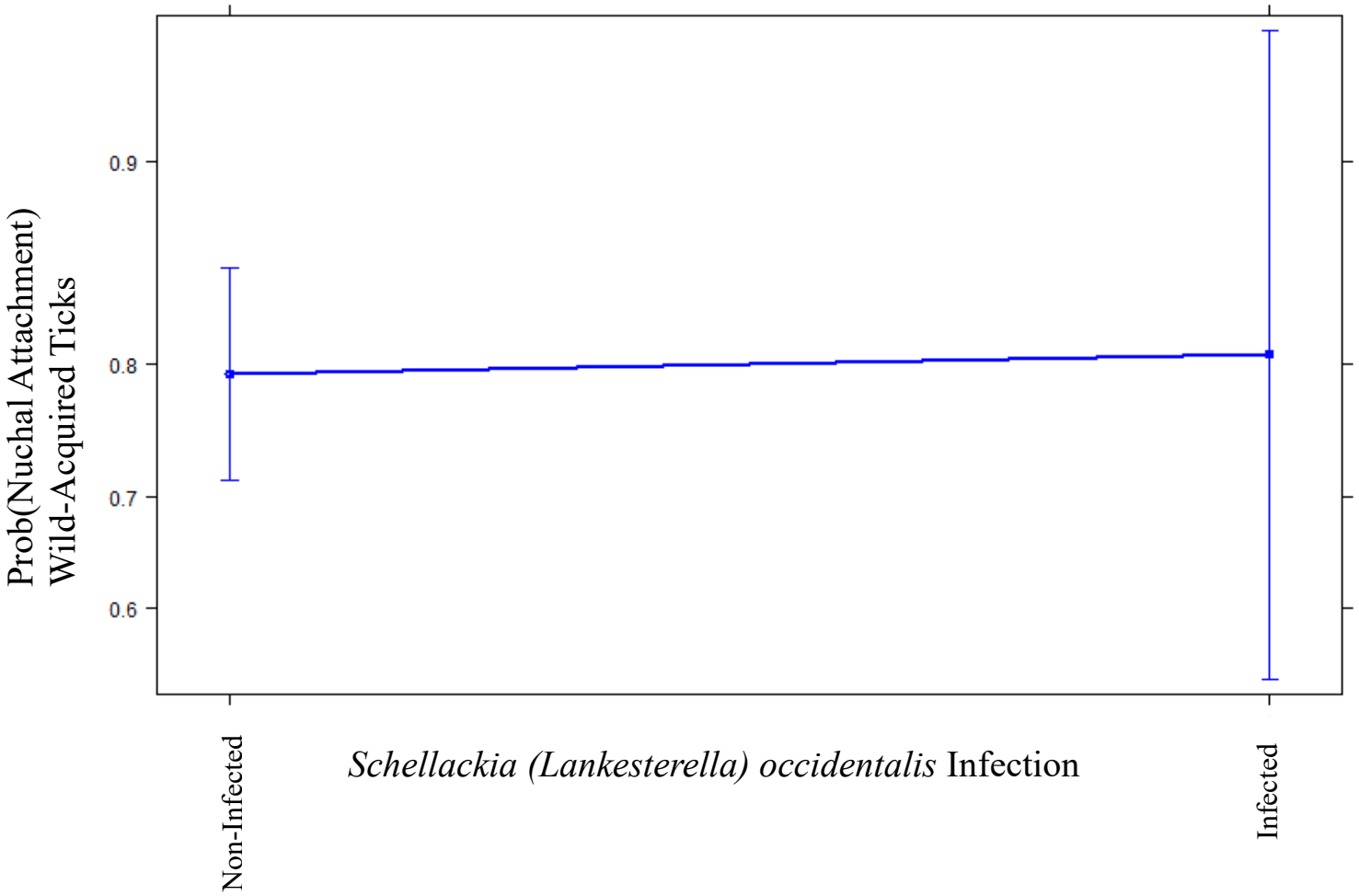

Figure 13: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Wild-acquired Ticks Attaching in the Nuchal Pocket and *Schellackia (Lankesterella) occidentalis* Infection. Predictions have been converted from log odds to probabilities for plotting purposes. Bars represent standard error.

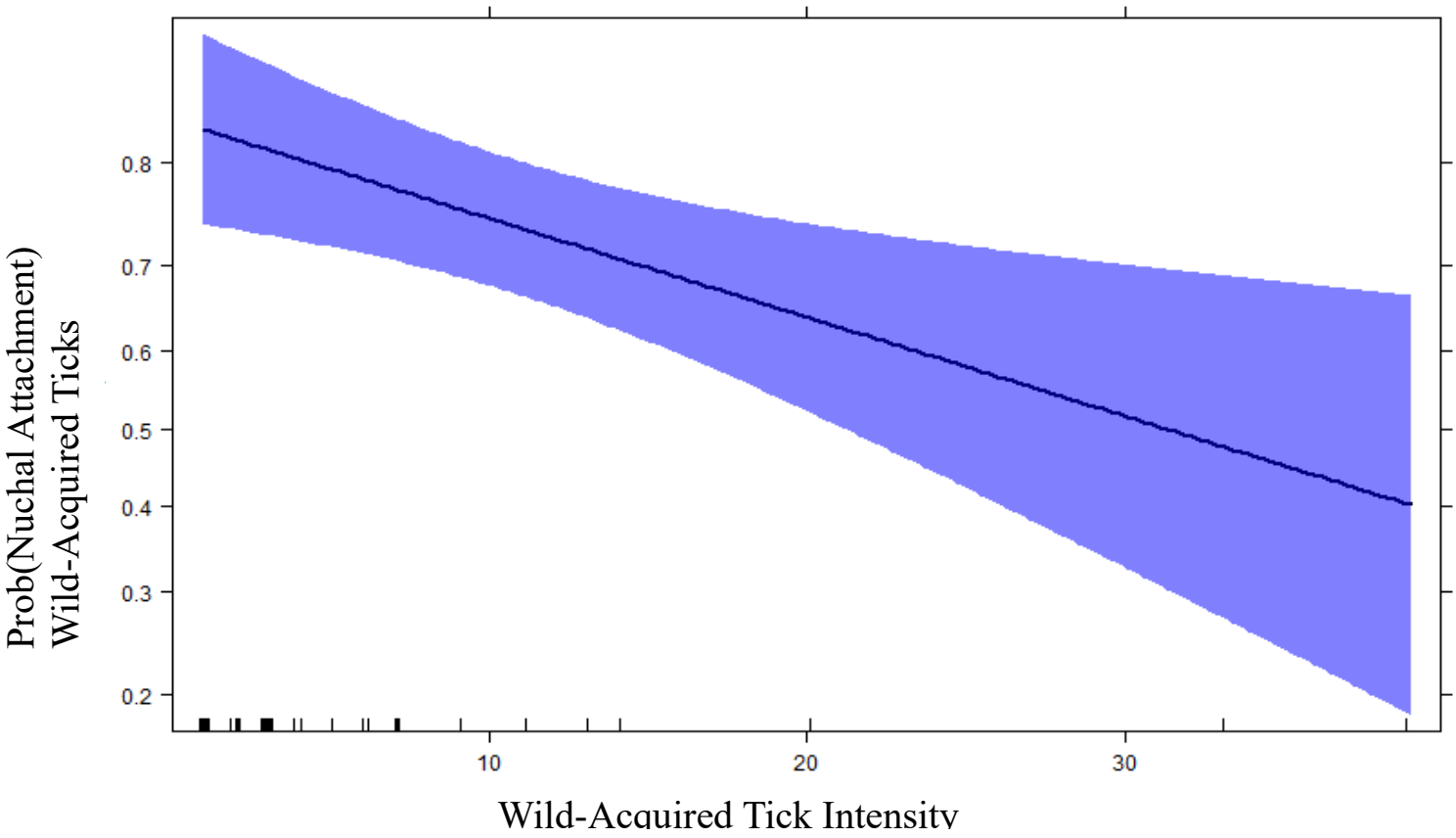

Figure 14: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Wild-acquired Ticks Attaching in the Nuchal Pocket and Wild-acquired Tick Intensity. The shaded region represents the 95% CI. Predictions have been converted from log odds to probabilities for plotting purposes.

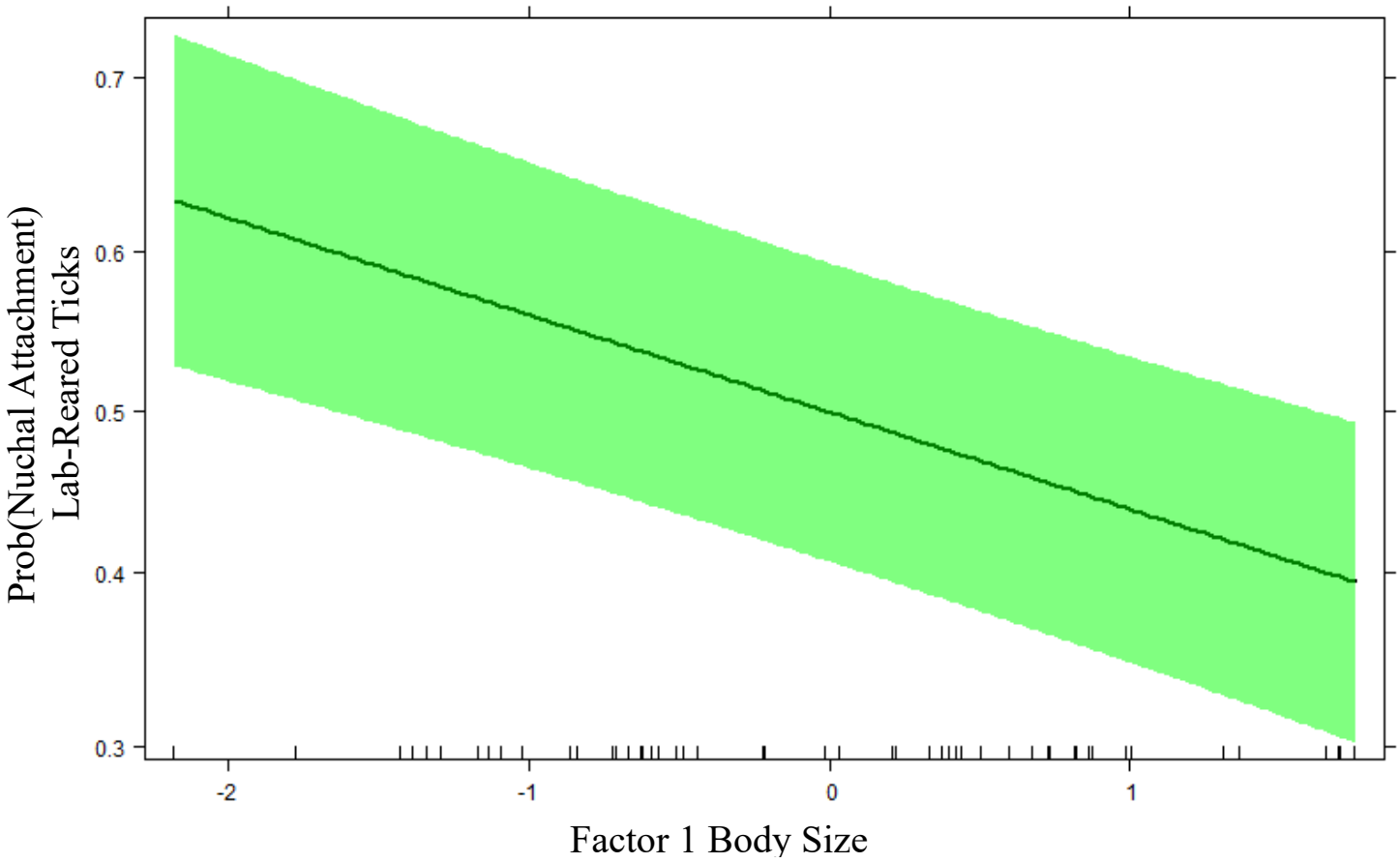

Figure 15: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Lab-reared Ticks Attaching in the Nuchal Pocket and Factor 1 Body Size. The shaded region represents the 95% CI. Predictions have been converted from log odds to probabilities for plotting purposes.

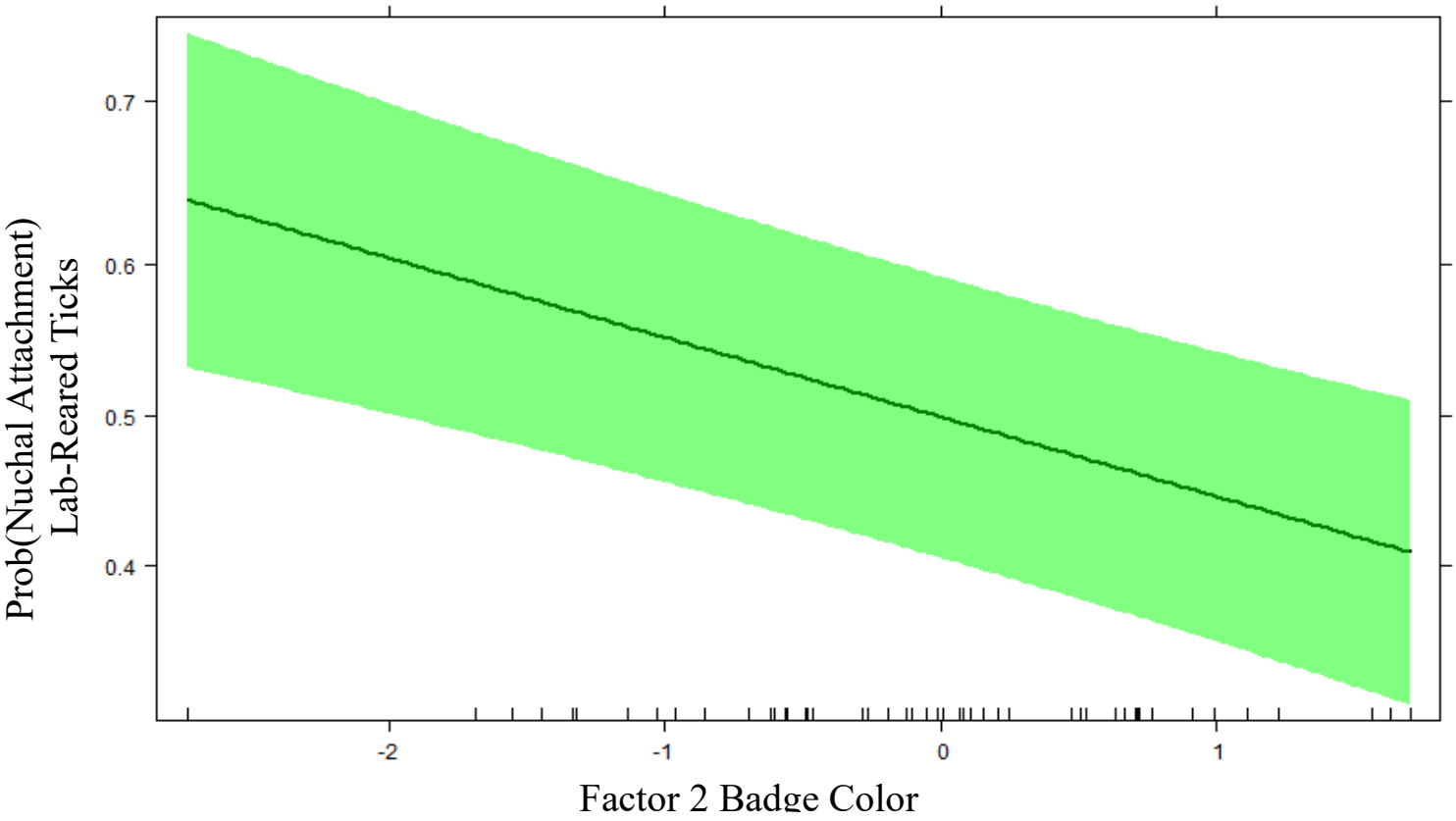

Figure 16: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Lab-reared Ticks Attaching in the Nuchal Pocket and Factor 2 Badge Color. The shaded region represents the 95% CI. Predictions have been converted from log odds to probabilities for plotting purposes.

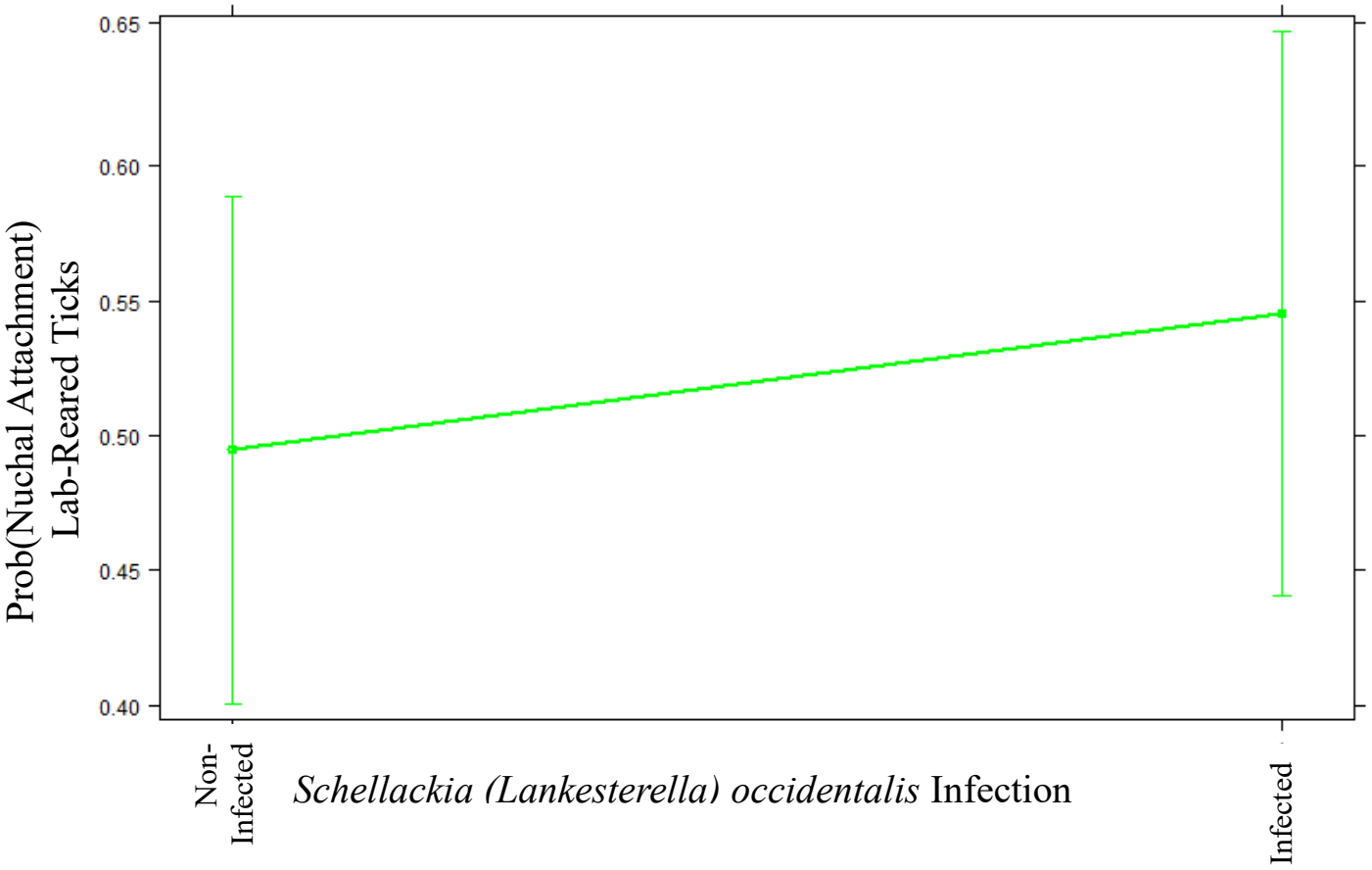

Figure 17: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Lab-reared Ticks Attaching in the Nuchal Pocket and *Schellackia (Lankesterella) occidentalis* Infection. Predictions have been converted from log odds to probabilities for plotting purposes. Bars represent standard error.

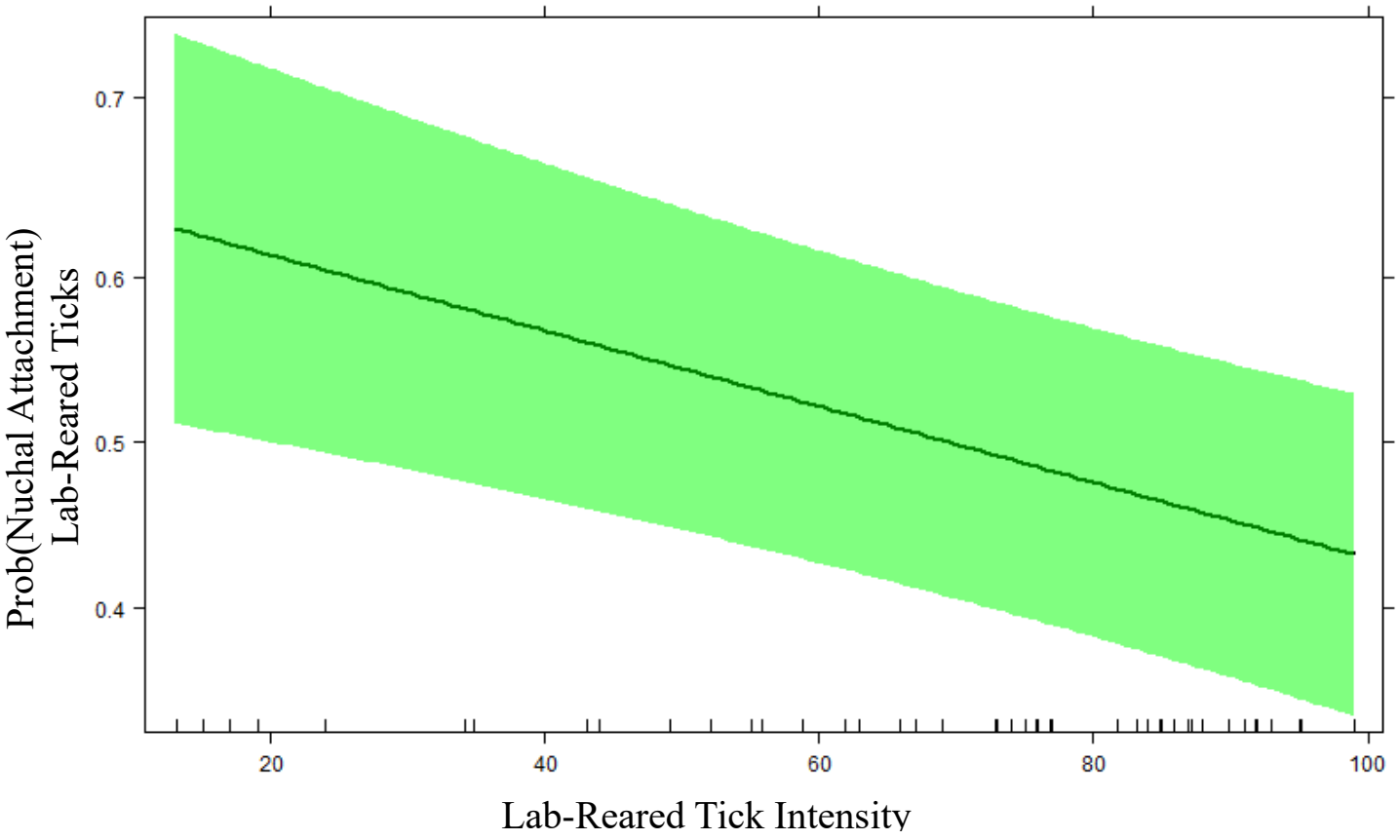

Figure 18: Effects Plot from the Logistic Regression Showing the Relationship between the Probability of Lab-reared Ticks Attaching in the Nuchal Pocket and Lab-reared Tick Intensity. The shaded region represents the 95% CI. Predictions have been converted from log odds to probabilities for plotting purposes.

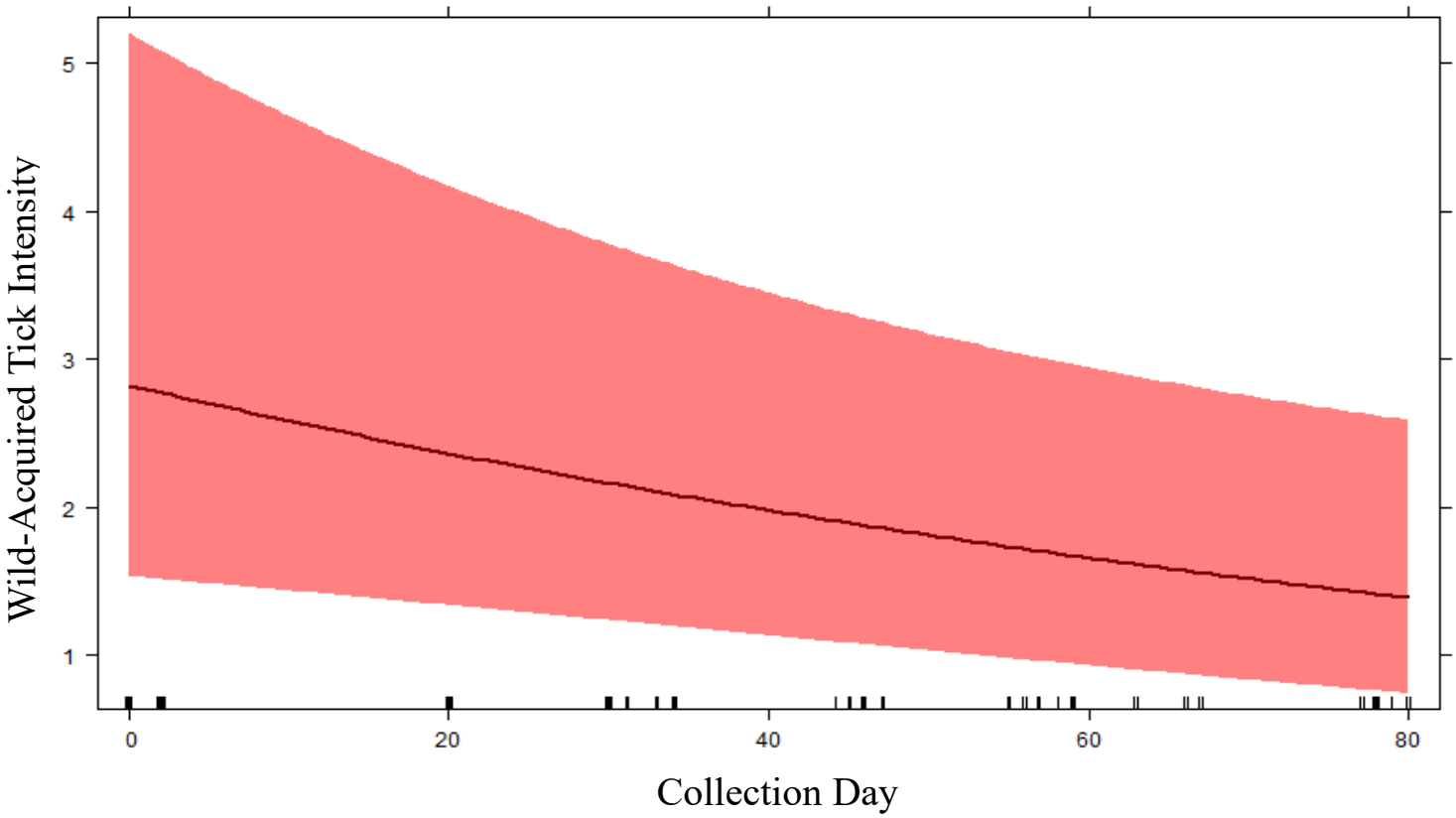

Figure 19: Effects Plot from the Linear Mixed-effects Model Showing the Relationship between the Wild-acquired Tick Intensity and Collection Day. The shaded region represents the 95% CI.

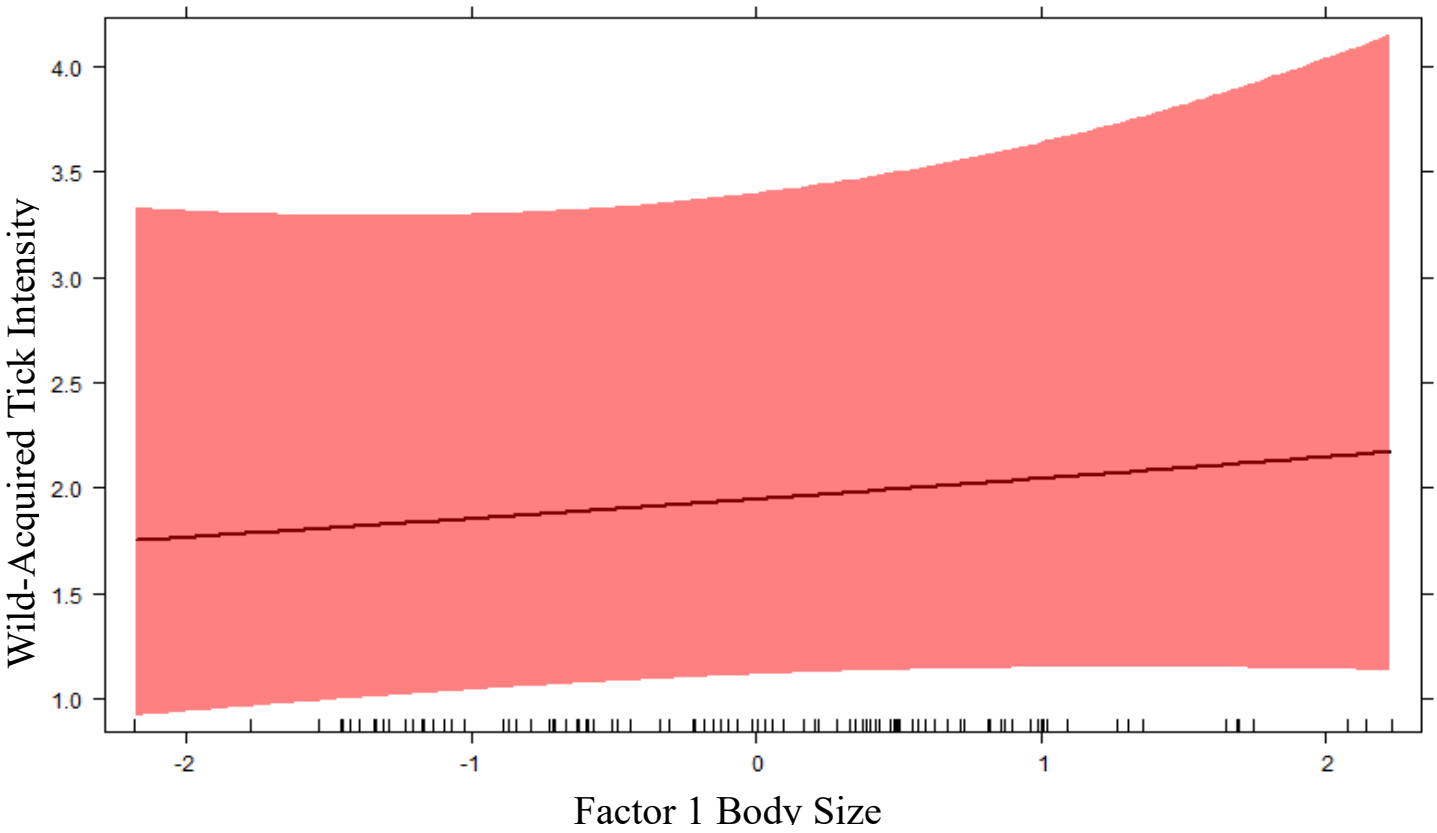

Figure 20: Effects Plot from the Linear Mixed-effects Model Showing the Relationship between the Wild-acquired Tick Intensity and Factor 1 Body Size. The shaded region represents the 95% CI

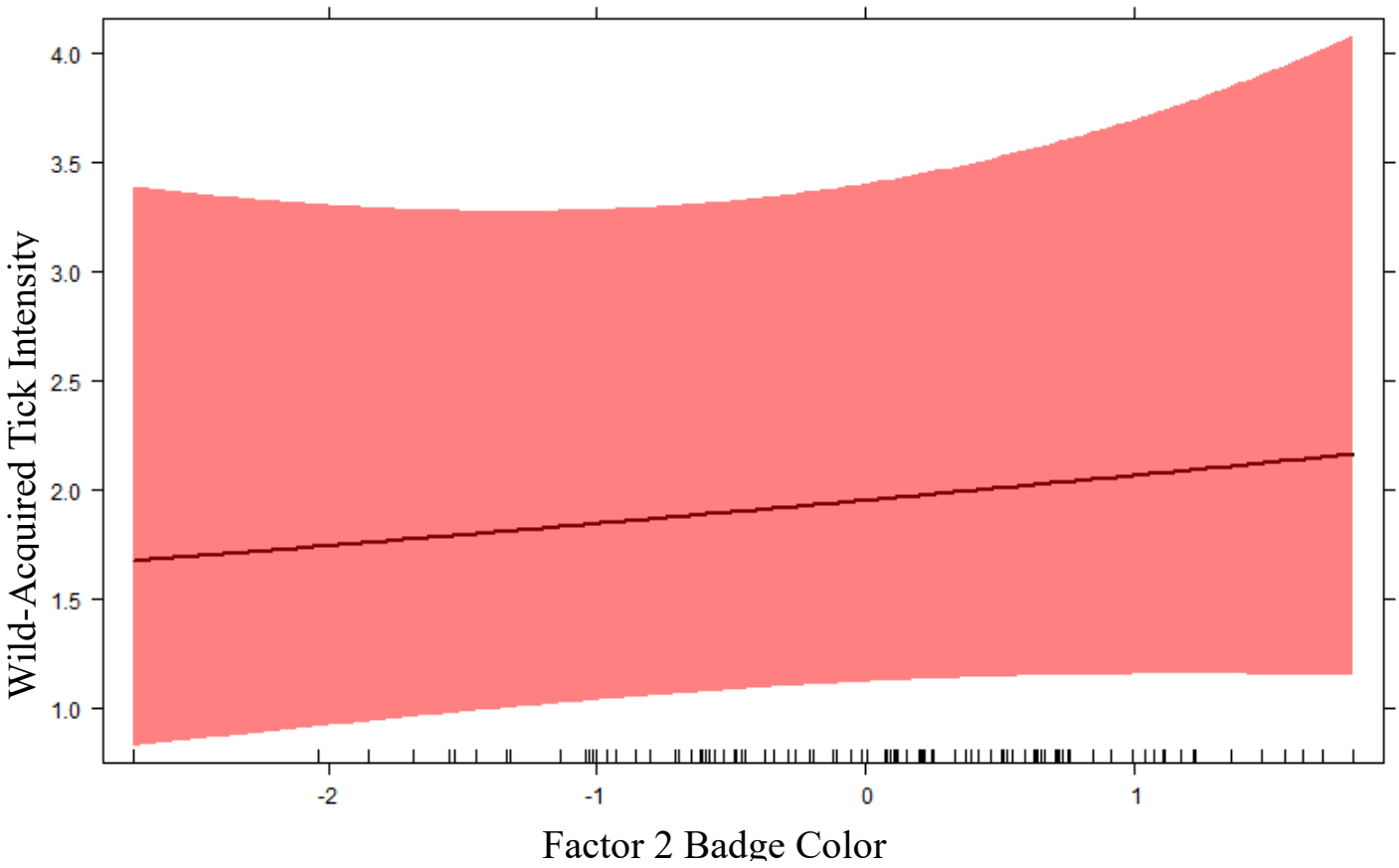

Figure 21: Effects Plot from the Linear Mixed-effects Model Showing the Relationship between the Wild-acquired Tick Intensity and Factor 2 Badge color. The shaded region represents the 95% CI.

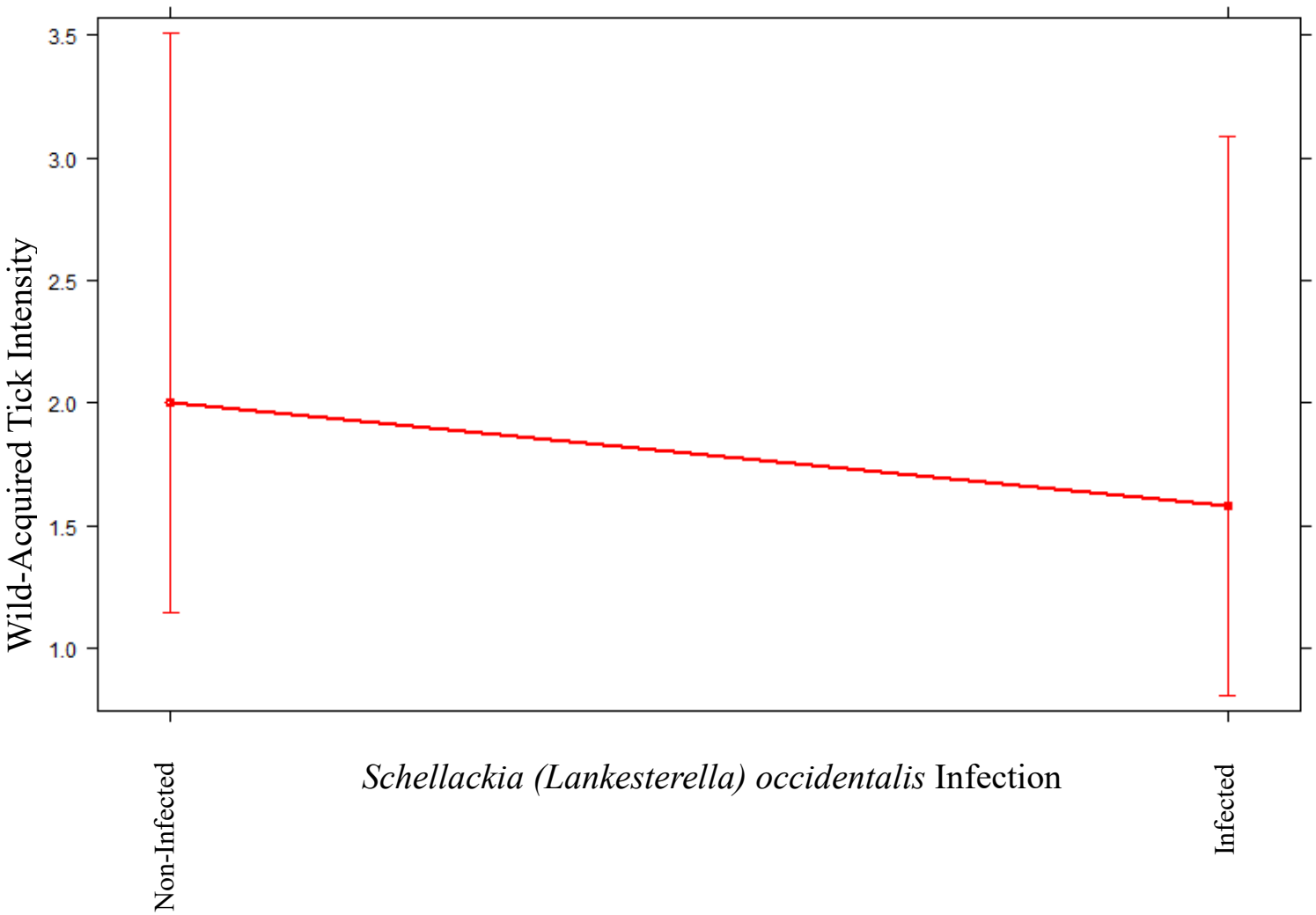

Figure 22: Effects Plot from the Linear Mixed-effects Model Showing the Relationship between the Wild-acquired Tick Intensity and *Schellackia (Lankesterella) occidentalis* Infection. Bars represent standard error.

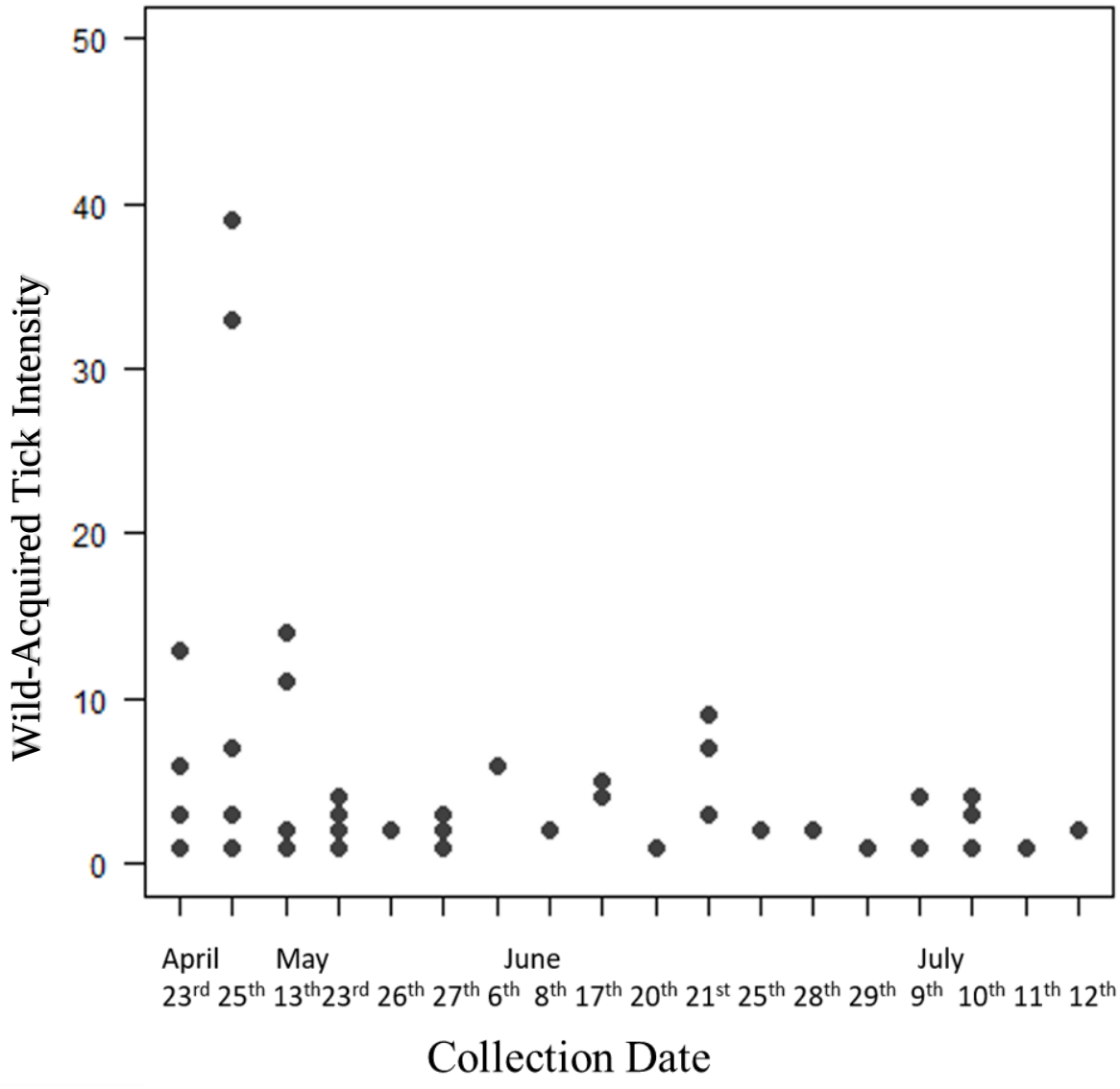

Figure 23: The Distribution of Wild Tick Intensities on Lizards I Collected over My Field Season (April 23 – July 12, 2017). The counts of Tick Intensity include both larvae and nymphs. Each point represents one of the 48 lizards I collected that harbored Wild Ticks (out of 100 lizards total).

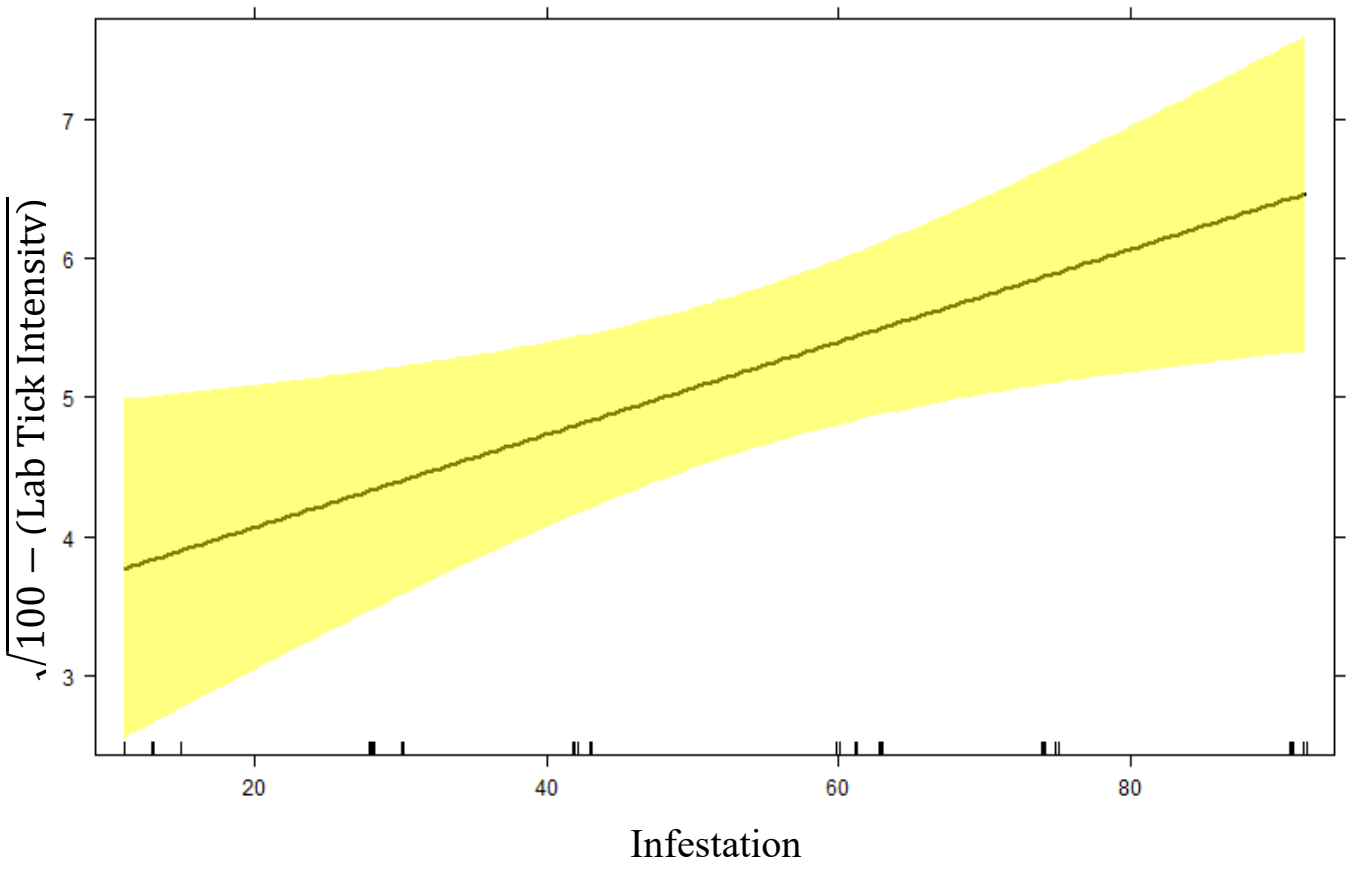

Figure 24: Effects Plot from the Linear Model Showing the Relationship between (Transformed) Lab-reared Tick Intensity and Infestation Day. The shaded region represents the 95% CI.

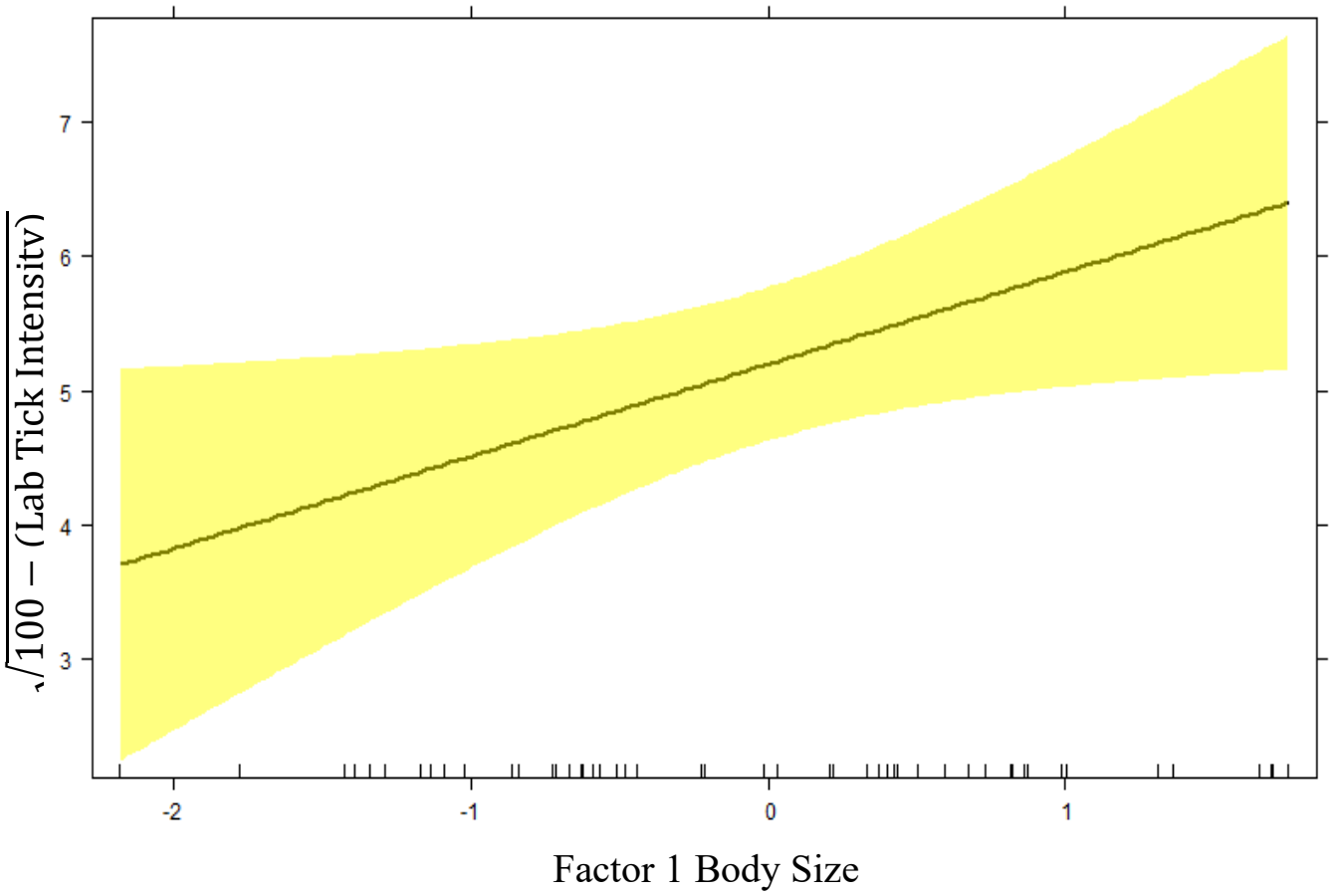

Figure 25: Effects Plot from the Linear Model Showing the Relationship between the Probability of (Transformed) Lab-reared Tick Intensity and Factor 1 Body Size. The shaded region represents the 95% CI.

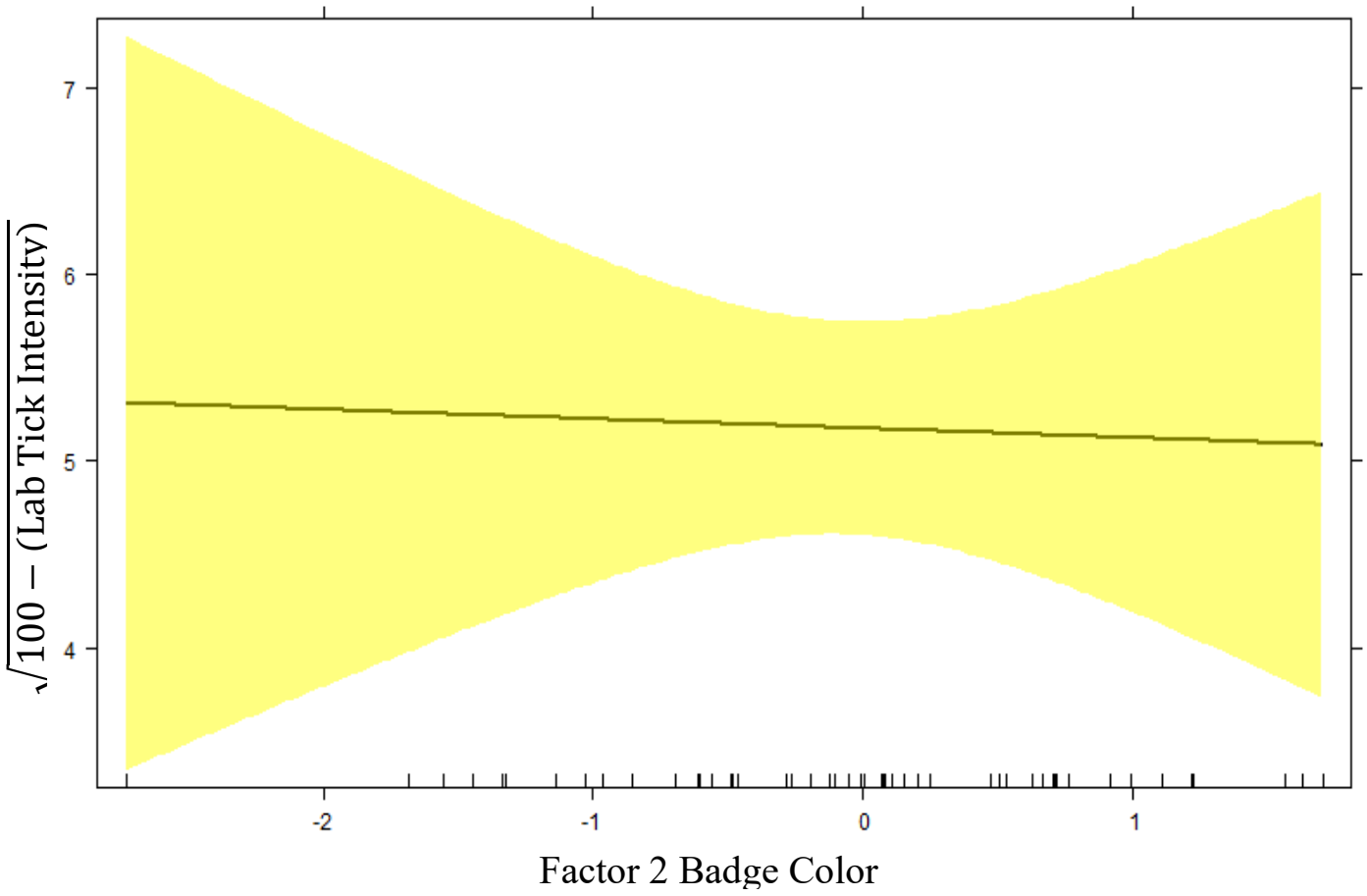

Figure 26: Effects Plot from the Linear Model Showing the Relationship between the Probability of (Transformed) Lab-reared Tick Intensity and Factor 2 badge Color. The shaded region represents the 95% CI.

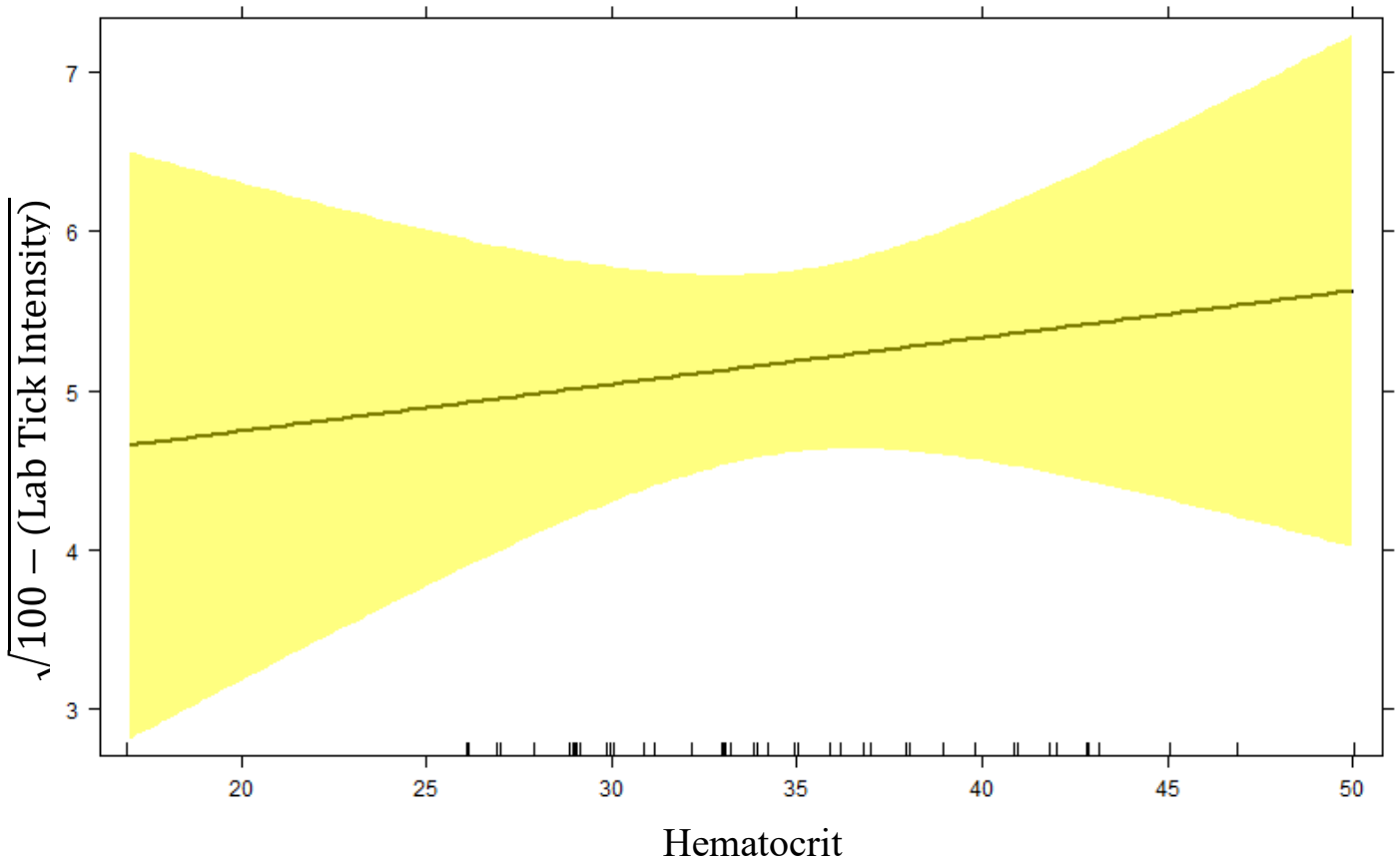

Figure 27: Effects Plot from the Linear Model Showing the Relationship between the Probability of (Transformed) Lab-reared Tick Intensity and Hematocrit. The shaded region represents the 95% CI.

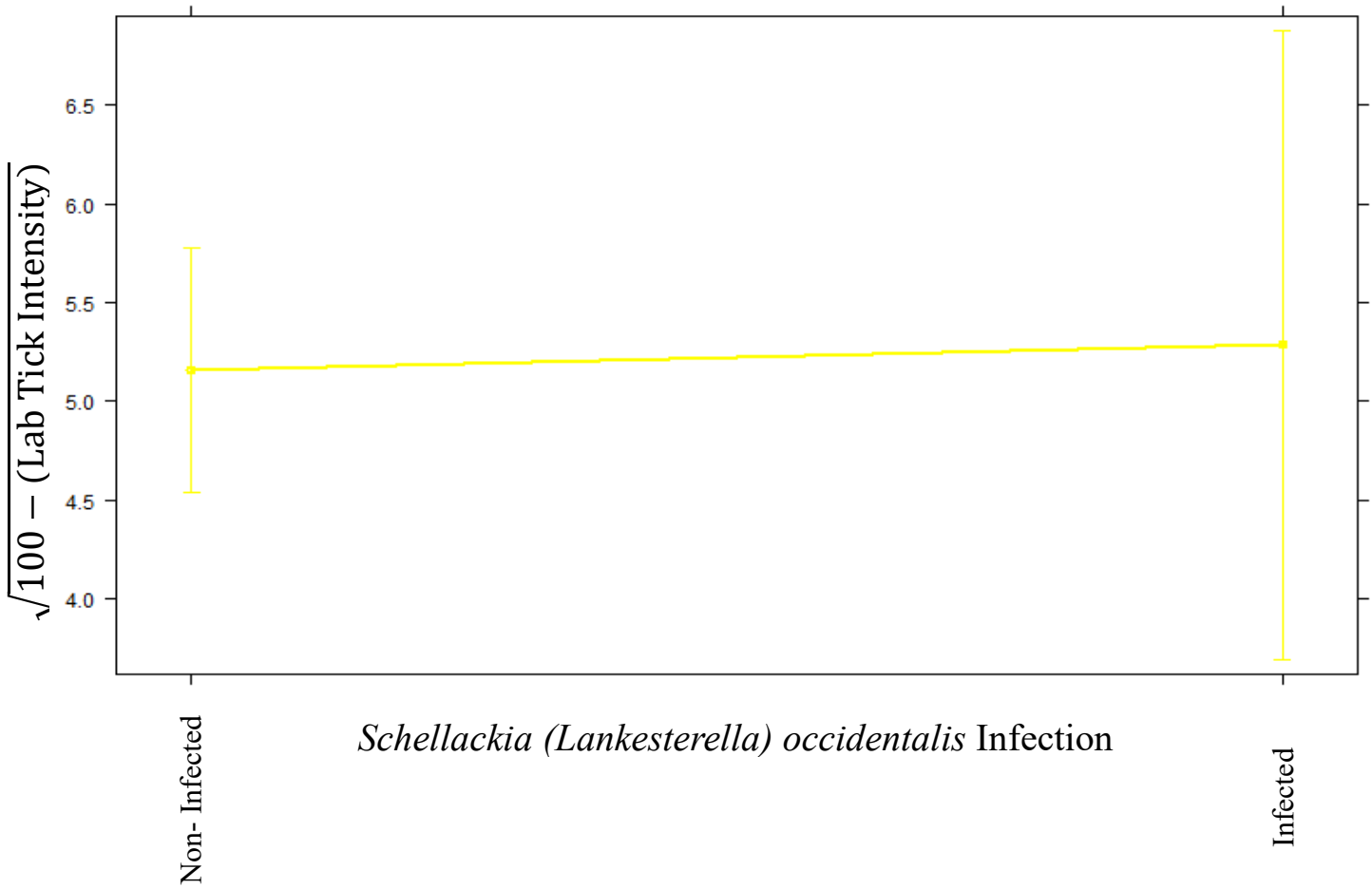

Figure 28: Effects Plot from the Linear Model Showing the Relationship between the Probability of (Transformed) Lab-reared Tick Intensity and *Schellackia (Lankesterella) occidentalis* Infection. Bars represent standard error.

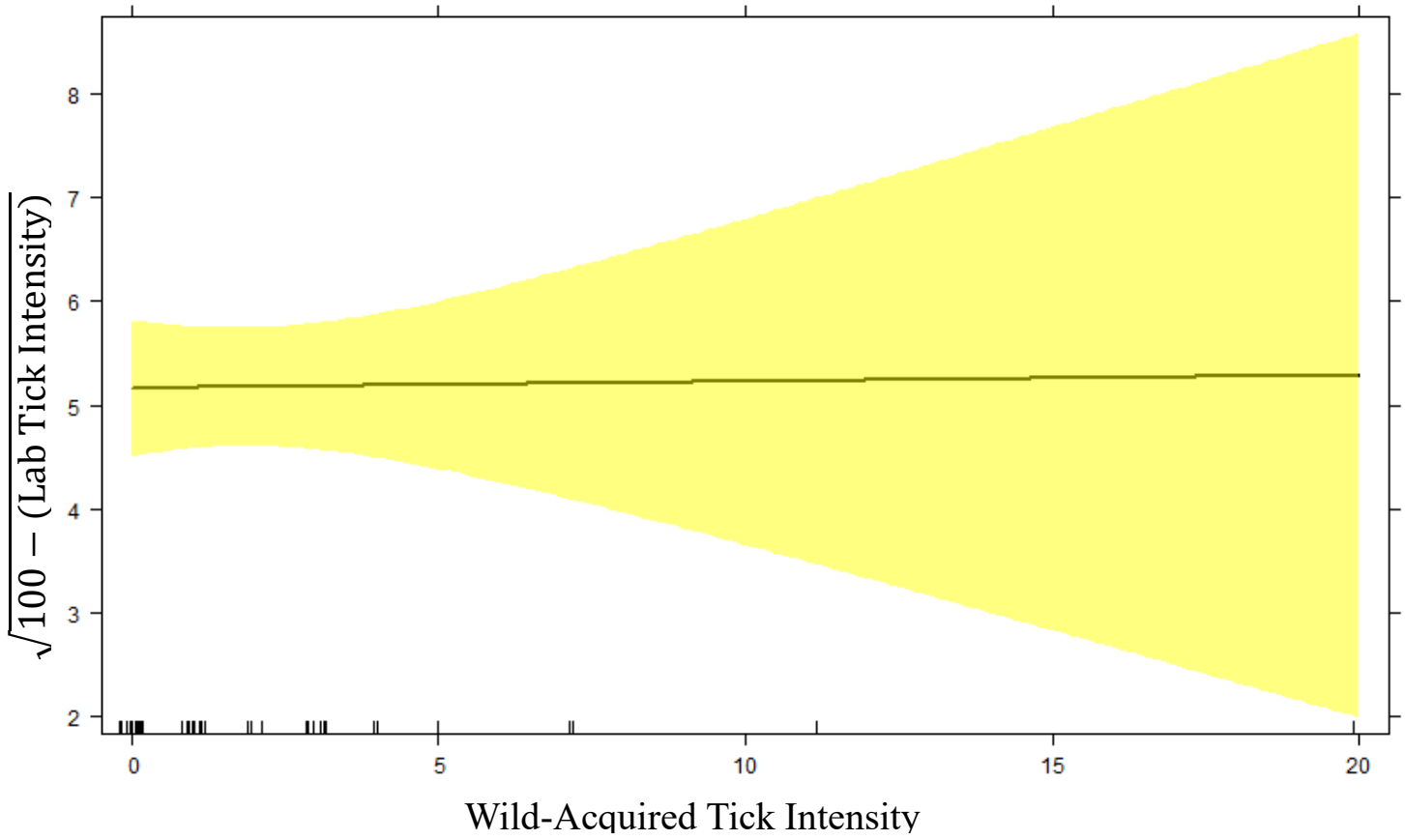

Figure 29: Effects Plot from the Linear Model Showing the Relationship between the Probability of (Transformed) Lab-reared Tick Intensity and Wild-acquired Tick Intensity. The shaded region represents the 95% CI.

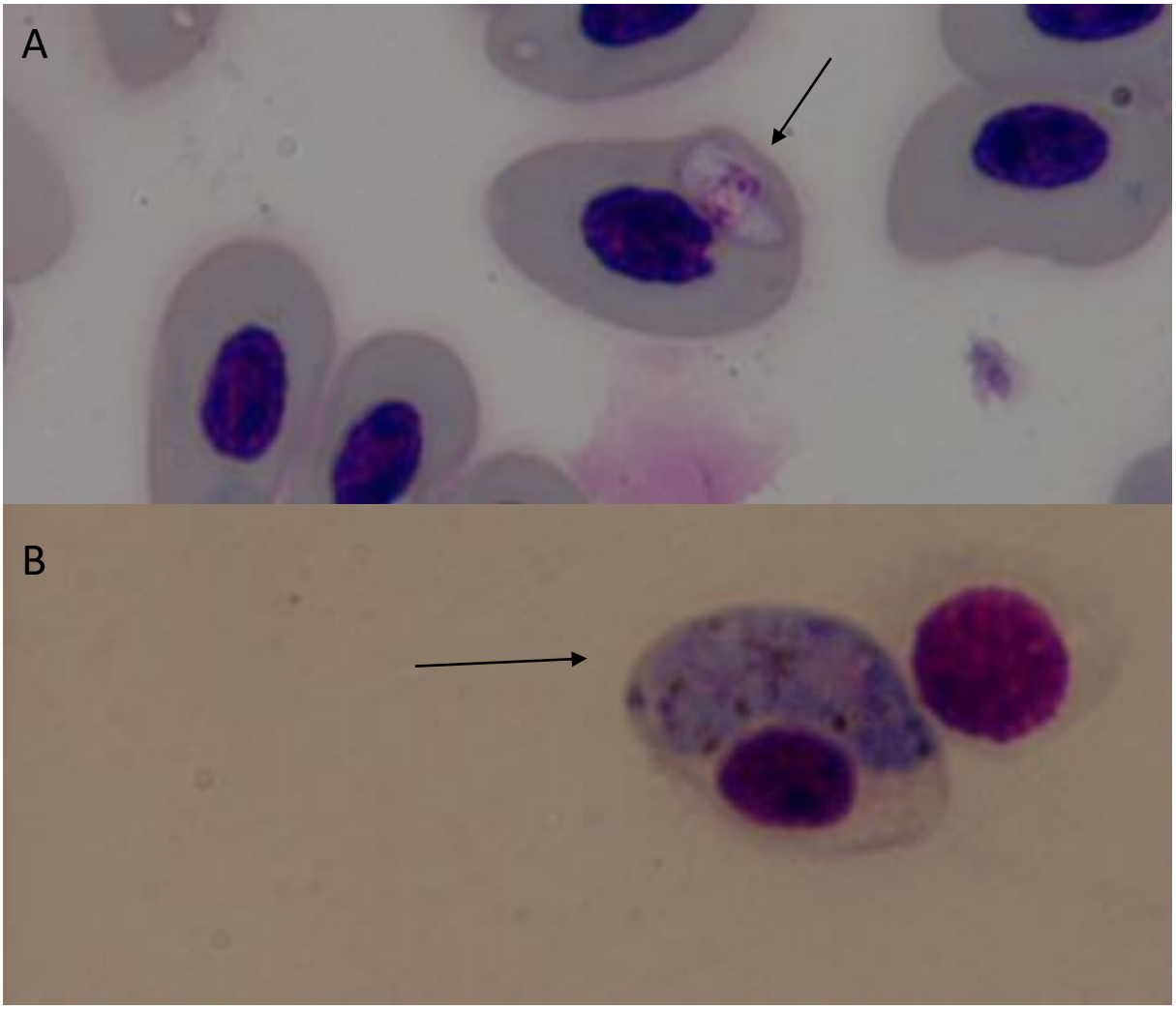

Figure 30: Western Fence Lizard Blood Smears with Giemsa Staining. A) A *Schellackia (Lankesterella) occidentalis* sporozoite (arrow) in the cytoplasm of a lizard erythrocyte. B) *Plasmodium mexicanum* (arrow, probably a female gametocyte) inside a lizard erythrocyte, displacing the erythrocyte nucleus.Images taken through an Olympus BX51 microscope at 400X magnification.

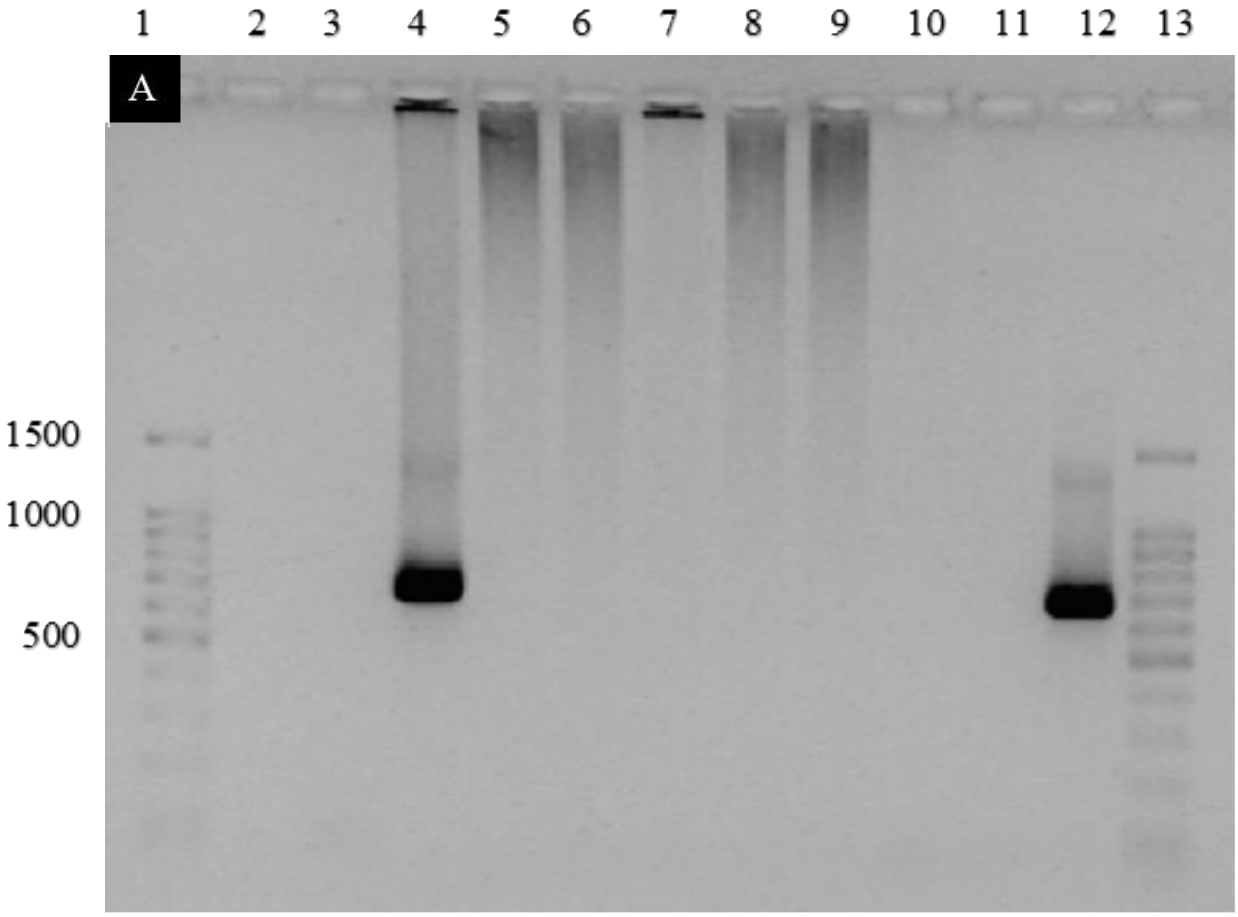

Figure 31: Agarose Gel Containing Products of a Nested PCR Targeting a Region of the *Plasmodium* Cytochrome b Gene. The second and third lanes contain negative controls from the first and second PCR reaction. The fourth lane contains the cytochrome b amplicon (673 bp) from a *P. mexicanum*-infected *S. occidentalis* found in a previous study in San Luis Obispo County, CA. Lane 12 contains the PCR products from a *Plasmodium*-infected *S. undulatus*. Lanes 1 and 13 (the left and rightmost lanes in this image) were loaded with 100 bp ladder (Promega, Madison, WI). The absence of a DNA band at 673 bp in lanes 5-11 indicates that these lizards were not *Plasmodium*-infected.

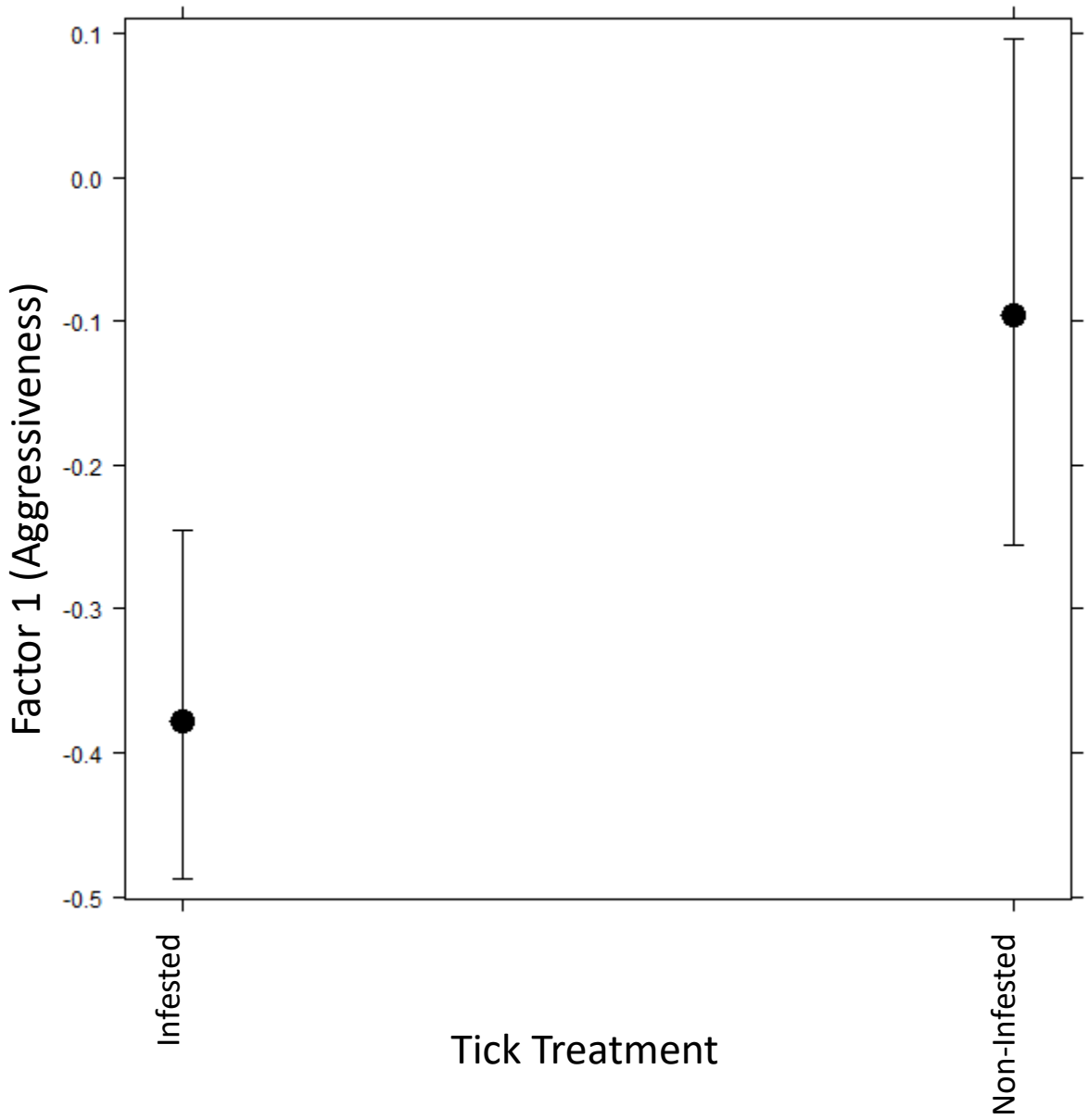

Figure 32: Effects Plot from the Linear Mixed-effects Model Showing the Relationship between Factor 1 Aggressiveness and Tick Infestation. The log(+1) transformation applied to the response variable (y-axis) has been undone for this plot. Bars represent standard error.

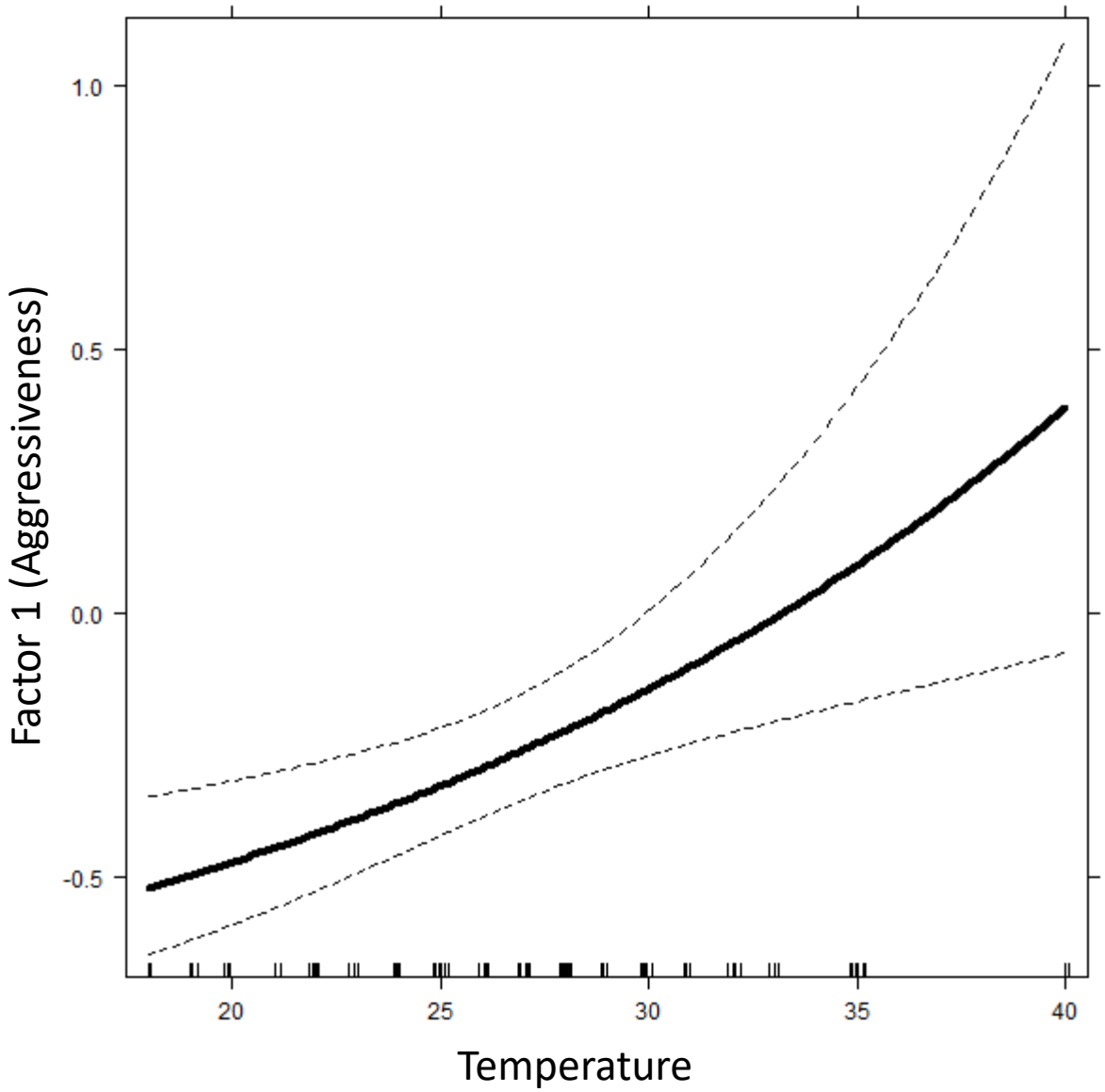

Figure 33: Effects Plot from the Linear Mixed-effects Model Showing the Relationship between Temperature in Arenas during Trials and Factor 1 Aggressiveness. The region within the dashed lines represents the 95% CI.

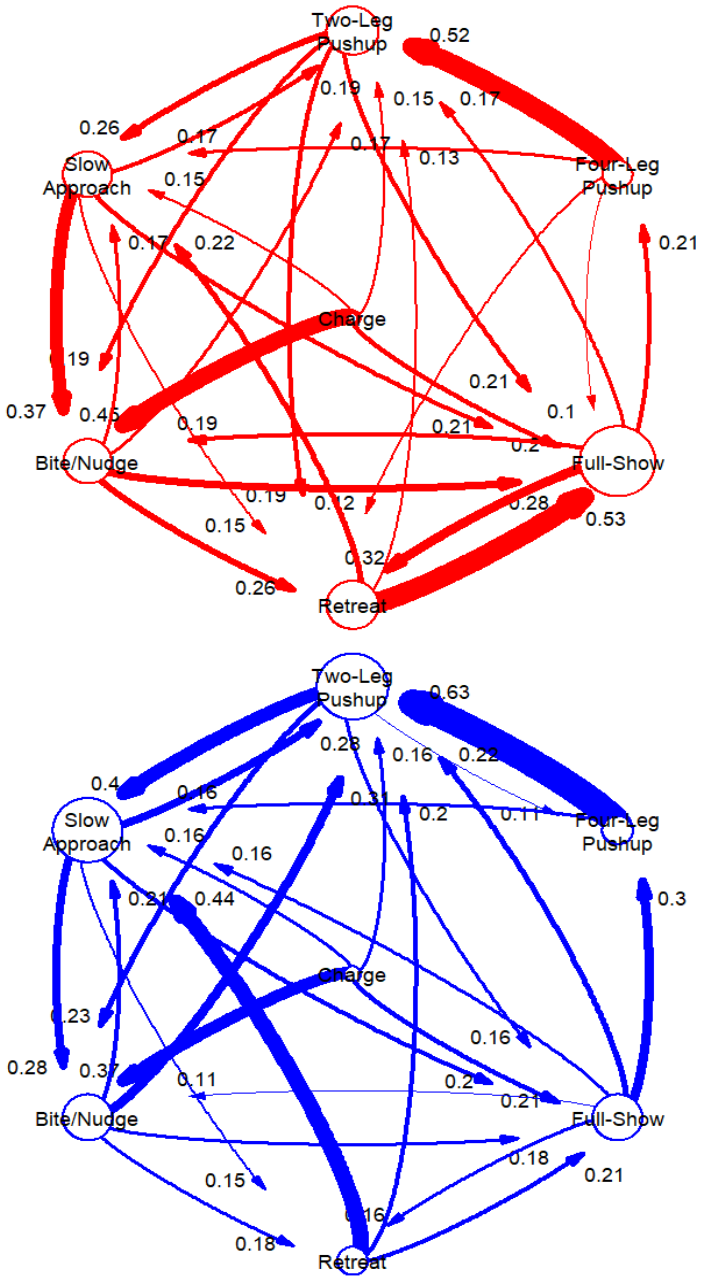

Figure 34: Diagram of a Markov Chain Created for Selected Agonistic Behaviors Exhibited by Infested Animals (Top) and Non-infested Animals (Bottom). Arrows depict the transition frequency between these behaviors, with arrow thickness proportional to this frequency. The diameter of each node is proportional to how many observations were made of that behavior. Transition frequencies below 0.1 are not depicted. The frequencies of repeated behaviors were excluded from analysis because pushups were highly repetitive.

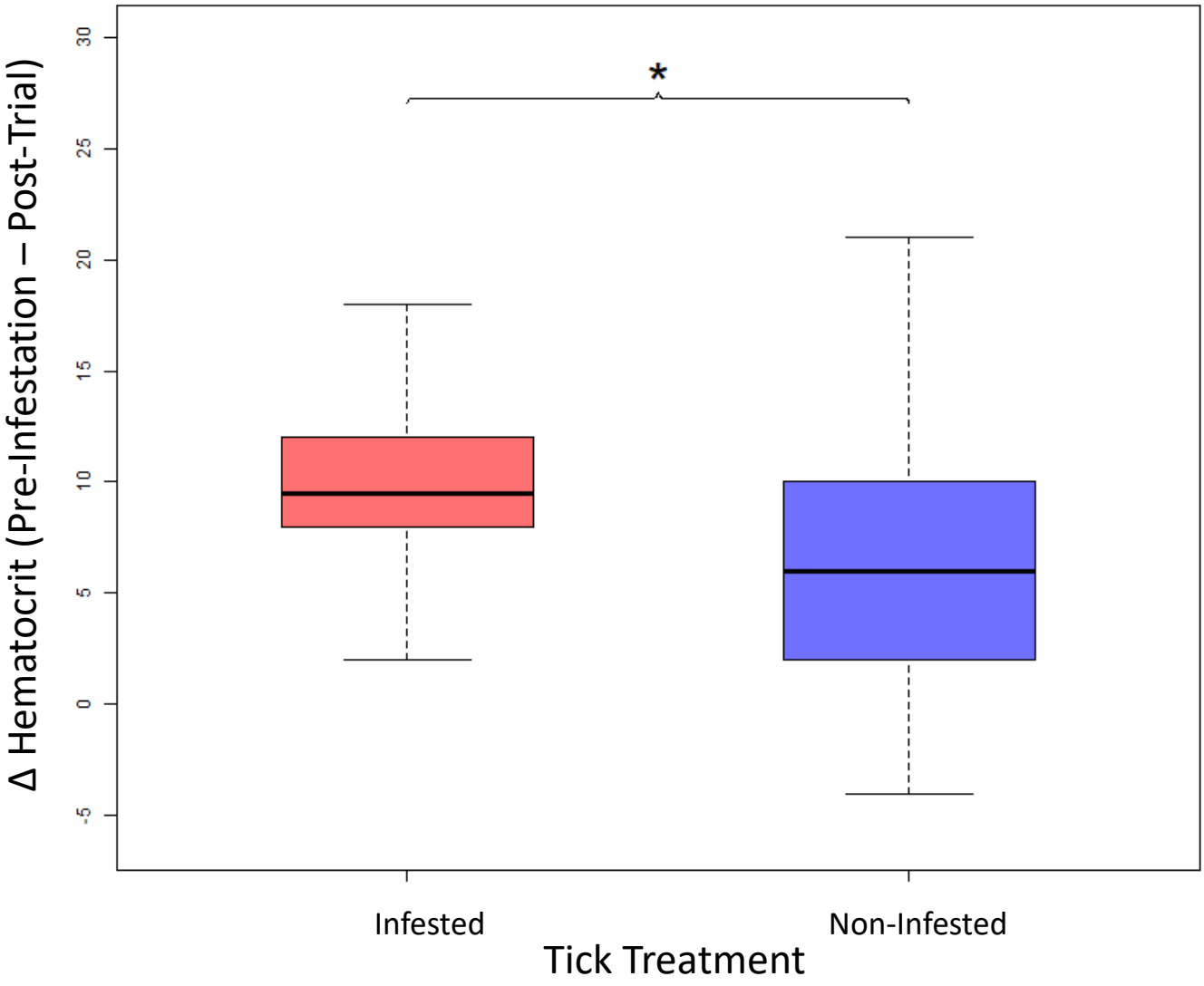

Figure 35: Change in Hematocrit between Capture and the Conclusion of Trials for Infested and Non-infested *S. occidentalis*. The asterisk denotes a significant difference. Whiskers represent quartiles

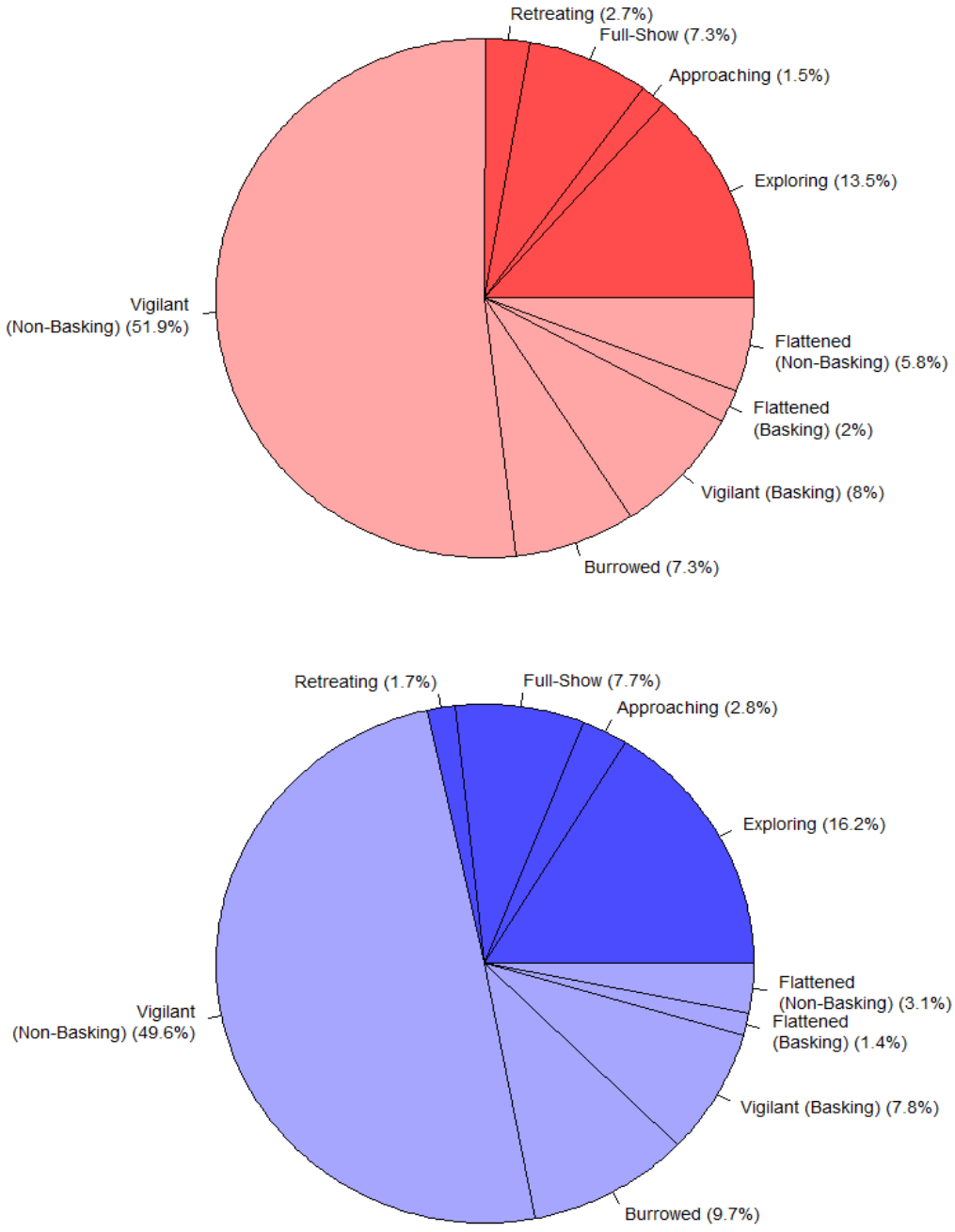

Figure 36: Division of Time Spent in Each State Behavior by Infested Animals (Top) and Non-infested Animals (Bottom). Behaviors are shaded by apparent intensity; lightly shaded behaviors represent different categories of motionlessness.

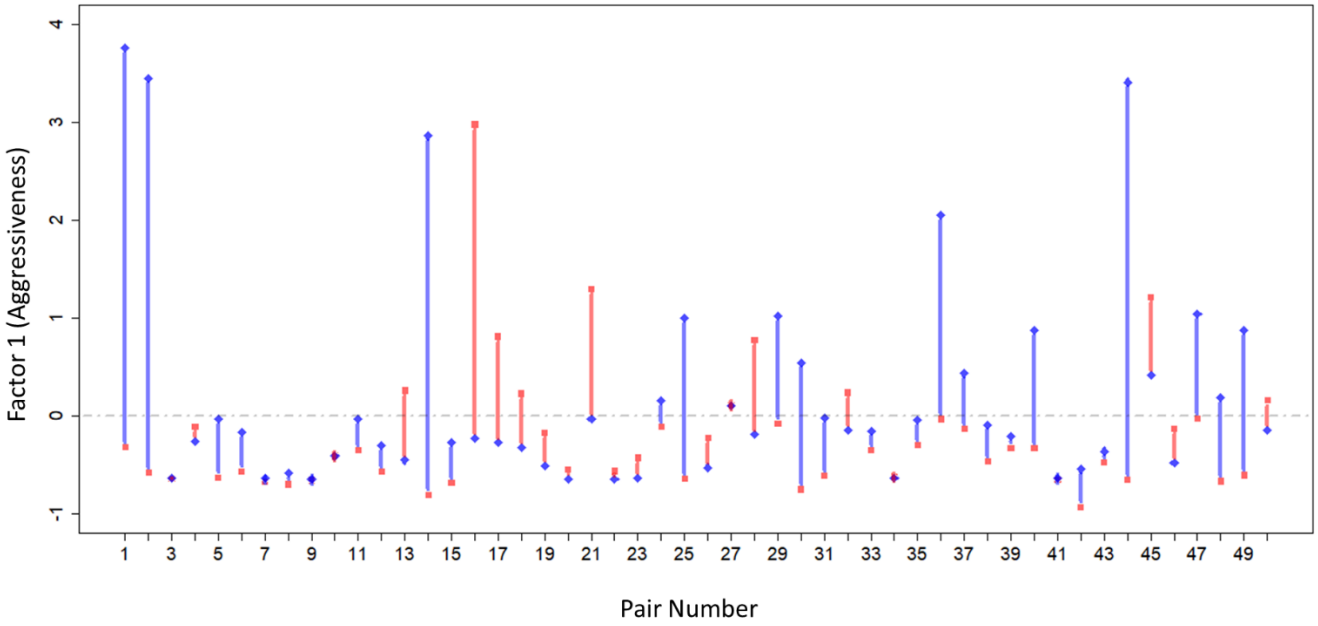

Figure 37: Pair Differences in Factor 1 Aggressiveness Scores. Each point represents the aggressiveness of an individual lizard within its pair, with infested lizards depicted in red and non-infested lizards in blue. Red and blue lines represent trials in which the infested or non-infested lizard was more aggressive, respectively.

References

Alizon, S., Roode, J. C., & Michalakis, Y. (2013). Multiple Infections and the Evolution of Virulence. *Ecology Letters, 16(4),* 556-567.

Andrews, R. H., & Petney, T. N. (1981). Competition for Sites of Attachment to Hosts in Three Parapatric Species of Reptile Tick. *Oecologia, 51(2),* 227-232.

Andrews, R. H., Petney, T. N., & Bull, C. M. (1982). Niche Changes between Parasite Populations: An Example from Ticks on Reptiles. *Oecologia, 55(1),* 77-80.

Arnold, E. N. (1986). Mite Pockets of Lizards, A Possible Means of Reducing Damage by Ectoparasites. *Biological Journal of the Linnaean Society, 29,* 1-21.

Arnott, G., & Elwood, R. W. (2009). Assessment of Fighting Ability in Animal Contests. *Animal Behaviour, 77,* 991-1004.

Ayala, S. C. (1970). Lizard Malaria in California; Description of a Strain of *Plasmodium mexicanum*, and Biogeography of Lizard Malaria in Western North America. *The Journal of Parasitology*, 56(3), 417-425

Baeckens, S., & Van Damme, R. (2018). Immunocompetence and Parasite Infestation in a Melanistic and Normally-coloured Population of the Lacertid Lizard, *Podarcis siculus*. *Amphibia-Reptilia,* DOI:10.1163/15685381-20181008.

Baeta, R., Faivre, B., Motreuil, S., Gaillard, M., & Moreau, J. (2008). Carotenoid Trade-off between Parasitic Resistance and Sexual Display: An Experimental Study in the Blackbird (*Turdus merula*). *Proceedings of the Royal Society B, 275,* 427-434.

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67(1),* 1-48.

Bauer, A. M., Russel, A. P., & Dollahon, N. R. (1990). Skin Folds in the Gekkonid Lizard Genus *Rhacodactylus:* A Natural Test of the Damage Limitation Hypothesis of Mite Pocket Function. *Canadian Journal of Zoology, 68(6),* 1196-1201.

Bauwens, D., Strijbosch, H, & Stumpel, A. H. P. (1983). The Lizard *Lacerta agilis* and *L. vivipara* as hosts to larvae and nymphs of the tick *Ixodes Ricinus. Holarctic Ecology, 6(1),* 32-40.

Berglund, A., Bisazza, A., & Pilastro, A. (1996). Armaments and Ornaments: An Evolutionary Explanation of Traits of Dual Utility. *Biological Journal of the Linnean Society, 58,* 385-399.

Bonorris, J. S., & Ball, G. H. (1955). *Schellackia occidentalis* n.sp., a Blood-inhabiting Coccidian Found in Lizards in Southern California. *Journal of Protozoology, 2,* 31-34.

14 Boonekamp et al. 2008

Boonekamp, J. J., Ros, A. H. F, and Verhulst, S. (2008). Immune Activation Suppresses Plasma Testosterone Level: A Meta-Analysis. *Biology Letters, 4(6),* 741-744.

Borucinska, J. D., Benz, G. W., & Whiteley, H. E. (1998) Ocular Lesions Associated with Attachment of the Parasitic Copepod *Ommatokoita elongate* (Grant) to Corneas of Greenland Sharks, *Somniosus microcephalus* (Block & Schneider). *Journal of Fish Diseases, 21(6),* 415-422.

Brain, C., and Bohrmann, R. (1992). Tick Infestation of Baboons (*Papio ursinus*) in the Namib Desert. *Journal of Wildlife Diseases, 28(2),* 188-191.

Brandt, Y. (2003). Lizard Threat Display Handicaps Endurance. *Proceedings: Biological Sciences, 270(1519),* 1061-1068.

Brossard, M., & Wikel, S. K. (2004). Tick Immunobiology. *Parasitology, 129,* S161-S176.

Brown, R. N., & Lane, R. S. (1992). Lyme Disease in California: A Novel Enzootic Transmission Cycle of *Borrelia burgdorferi*. *Science, 256(5062),* 1439-1442.

Brun, R., Blum, J., Chappuis, F., & Burri, C. Human African Trypanosomiasis. (2010). *Lancet, 375,* 149-159.

Brunner, J. L. & Ostfeld, R. S. (2008). Multiple Causes of Variable Tick Burdens on Small-Mammal Hosts. *Ecology, 89(8),* 2259-2272.

Bull, M., & Burzacott, D. (1993). The Impact of Tick Load on the Fitness of Their Lizard Hosts. *Oecologia, 96,* 415-419.

Burgdorfer, W., Barbour, A. G., Hayes, S. F., Benach, J. L., Grunwaldt, E., & Davis, J. P. (1982). Lyme Disease – A Tick-Borne Spirochetosis? *Science, 216(4552),* 1317-1319.

Carpenter, C. C. (1978). Comparative Display Behavior in the Genus *Sceloporus* (Iguanidae). *Contributions in Biology and Geology, 18,* 1-72.

Chilton, N. B., Bull, C. M., and Andrews, R. H. (1992). Niche Segregation in Reptile Ticks: Attachment Sites and Reproductive Success of Females. *Oecologia, 90(2),* 255-259.

Clayton, D. H. (1991). The Influence of Parasites on Host Sexual Selection. *Parasitology Today, 7(12),* 329-334.

Clover, J. R. & Lane, R. S. (1995). Evidence Implicating Nymphal *Ixodes pacificus* (Acari: Ixodidae) in the Epidemiology of Lyme Disease in California. *American Journal of Tropical Medicine and Hygiene, 53(3),* 237-240.

Conant, R., & Collins, J. T (1998). *A Field Guide to Reptiles and Amphibians of Eastern and Central North America,* 3rd. ed. pp. 232. New York: Houghton Mifflin.

Cooper, W. E., & Burns N. (1987). Social significance of ventrolateral coloration in the fence lizard, *Sceloporus undulatus*. *Animal Behaviour, 35,* 526-532.

Costello, A. B., & Osborne, J. W. (2005). Best Practices in Exploratory Factor Analysis: Four Recommendations for Getting the Most from Your Analysis. *Practical Assessment, Research & Evaluation, 10(7),* 1-9.

Cox, R. M., & John-Alder, H. B. (2007). Increased Mite Parasitism as a Cost of Testosterone in Male Striped Plateau Lizards *Sceloporus virgatu*s. *Functional Ecology, 21,* 327-334.

Cox, R. M., Skelly, S. L., Leo, A., & John-Alder, H. B. (2005). Testosterone Regulates Sexually Dimorphic Coloration in the Eastern Fence Lizard, *Sceloporus undulatus. Copeia, 2005(3),* 597-608.

Dantzer, R. (2004). Cytokine-Induced Sickness Behaviour: a Neuroimmune Response to Activation of Innate Immunity. *European Journal of Pharmacology, 500,* 399-411.

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From Inflammation to Sickness and Depression: When the Immune System Subjugates the Brain. *Nature Reviews Neuroscience, 9(1),* 46-57.

Day, J. F., Edman J. D. (1983). Malaria Renders Mice Susceptible to Mosquito Feeding When Gametocytes Are Most Infective. *Journal of Parasitology, 69(1),* 163-170.

De La Riva, D. G., Soto, D., & Mullens, B. A. (2015). Temperature Governs On-host Distribution of the Northern Fowl Mite, *Ornithonyssus sylviarum* (Acari: Macronyssidae). *Journal of Parasitology, 101(1),* 18-23.

de Lanuza, G., Carazo, P., & Font, E. (2014). Colours of Quality: Structural (But Not Pigment) Coloration Informs about Male Quality in a Polychromatic Lizard. *Animal Behaviour, 90,* 73-81.

Devaney, J. A., & Augustine, P. C. (1988). Correlation of Estimated and Actual Northern Fowl Mite Populations with the Evolution of Specific Antibody to a Low Molecular Weight Polypeptide in the Sera of Infested Hens. *Poultry Science, 67(4),* 549-555.

Doucet, S. M., & Meadows, M. G. (2009). Iridescence: A Functional Perspective. *Journal of the Royal Society Interface, 6,* S115-S132.

Dreyer, G., Pimentael, A., Medeiros, Z., Béliz, F., Moura, I., Coutinho, A., de Andrade, L. D., Rocha, A., da Silva, L. M., & Piessens, W. F. (1996). Studies on the Periodicity and Intravascular Distribution of *Wuchereria bancrofti* microfilariae in Paired Samples of Capillary and Venous Blood from Recife, Brazil. *Tropical Medicine and International Health, 1(2),* 264-272.

Ducrest, A., Keller, L., & Roulin, A. (2008). Pleiotropy in the Melanocortin System, Coloration and Behavioural Syndromes. *Trends in Ecology and Evolution, 23(9),* 502-510.

Dudek, K., Skórka, P., Sajkowska, Z. A., Ekner-Grzyb, A., Dudek, M., & Tryjanowski, P. (2016). Distribution Pattern and Number of Ticks on Lizards. *Ticks and Tick-borne Diseases, 7,* 172-179.

Dunlap, K. T., & Mathies, T. (1993). Effects of Nymphal Ticks and Their Interaction with Malaria on the Physiology of Male Fence Lizards. *Copeia, 1993(4),* 1045-1048.

Dunne, J. A., Lafferty, K. D., Dobson, A. P., Hechinger, R. F., Kuris, A. M., Martinez, N. D., McLaughlin, J. P., Mouritsen, K. N., Poulin, R., Reise, K., Stouffer, D., B., Thieltges, D. W., Williams, R. J., & Zander, C. D. Parasites Affect Food Web Structure Primarily through Increased Diversity and Complexity. *PLOS Biology, 11(6).*

Eisen, R. J., Eisen, E. L., & Lane, R. S. (2001). Prevalence and Abundance of *Ixodes pacificus* Immatures (Acari: Ixodidae) Infesting Western Fence Lizards (*Sceloporus occidentalis*)

in Northern California: Temporal Trends and Environmental Correlates. *The Journal of Parasitology, 87(6),* 1301-1307.

Eisen, L., Eisen, R. J., & Lane, R. S. (2004). The Roles of Birds, Lizards, and Rodents as Hosts for the Western Black-Legged Tick *Ixodes pacificus*. *Journal of Vector Ecology, 29(2),* 295-308.

Elwood, R., & Arnott, G. (2012). Understanding How Animals Fight with Lloyd Morgan's Canon. *Animal Behaviour, 84,* 1095-1102.

Engbretson, G. A., & Livezey, R. L. (1972). The Effects of Aggressive Display on Body Temperature in the Fence Lizard *Sceloporus occidentalis occidentalis* Baird and Girard. *Physiological Zoology, 45(3),* 247-254.

Eshoo, M. W., Carolan, H. E., Massire, C., Chou, D. M., Crowder, C. D., Rounds, M. A., Philipson, C. A., Schutzer, S. E., & Ecker, D. J. (2015). Survey of *Ixodes pacificus* Ticks in California Reveals Diversity of Microorganisms and a Novel Widespread *Anaplasmataceae* Species. *PLoS ONE, 10(9),* e0135828.

Ewald, P. W. (1983). Host-Parasite Relations, Vectors, and the Evolution of Disease Severity. *Annual Review of Ecology and Systematics*, *14,* 465-485.

Fawcett, T. W., & Johnstone, R. A. (2010). Learning Your Own Strength: Winner and Loser Effects Should Change with Age and Experience. *Proceedings of the Royal Society B, 277,* 14237-1434.

Fawcett, T. W. & Mowles, S. L. (2013). Assessment of Fighting Ability Need Not be Cognitively Complex. *Animal Behaviour, 86,* e1-e7.

Fialho, R. F., & Schall, J. J. (1995). Thermal Ecology of a Malarial Parasite and its Insect Vector: Consequences for the Parasite's Transmission Success. *Journal of Animal Ecology, 64,* 553-562.

Fielden, L. J., Rechav, Y., & Bryson, N. R. (1992). Acquired Immunity to Larvae of *Amblyomma marmoreum,* and *A. hebraeum* by Tortoises, Guinea-pigs and Guinea-fowl. *Medical and Veterinary Entomology, 6,* 251-254.

Finnerty, P. B., Shine, R., & Brown, G. P. (2018). Survival of the Feces: Does a Nematode Lungworm Adaptively Manipulate the Behavior of Its Cane Toad Host? *Ecology and Evolution, 8,* 4606-4618.

Folstad, I., & Karter, A. J. (1992). Parasites, Bright Males, and the Immunocompetence Handicap. *The American Naturalist, 136(3),* 603-622.

Forbes, M. R. L. (1993). Parasitism and Host Reproductive Effort. *Oikos, 67(3),* 444-450.

Freeland, W. J. (1976). Pathogens and the Evolution of Primate Sociality. *Biotropica, 8(1),* 12-24.

Freeland, W. J. (1981). Parasitism and Behavioral Dominance among Male Mice. *Science, 213(4506),* 461-462.

Friard, O., & Gamba, M. (2016). BORIS: A Free, Versatile Open-Source Event-Logging Software for Video/Audio Coding and Live Observations. *Methods in Ecology and Evolution, 7,* 1325-1330.

Furman, D. P., & Loomis, E. C. (1984). *The Ticks of California (Acari: Ixodida).* Berkeley: University of California Press.

Galbe, J., & Oliver, J. H. (1992). Immune Response of Lizards and Rodents to Larval *Ixodes scapularis* (Acari: Ixodidae). *Journal of Medical Entomology, 5(1),* 774-783.

Garland, T., Hankins, E., & Huey, R. B. (1990). Locomotor Capacity and Social Dominance in Male Lizards. *Functional Ecology, 4(2),* 243-250.

Garrido, M., & Pérez-Mellado, V. (2014). Sprint Speed is Related to Blood Parasites, But Not to Ectoparasites, in an Insular Population of Lacertid Lizards. *Canadian Journal of Zoology, 92,* 67-72.

Gatkowska, J., Wieczorek, M., Dziadek, B., Dzitko, K., & Dlugonska, H. (2012). Behavioral Changes in Mice Caused by *Toxoplasma gondii* Invasion of Brain. *Parasitology Research.*

Goldberg, S. R., & Bursey, C. R. (1991). Integumental Lesions Caused by Ectoparasites in a Wild Population of the Side-Blotched Lizard (*Uta stansburiana*). *Journal of Wildlife Diseases, 27(1),* 68-73.

Goldberg, S. R., & Holshuh, H. J. (1992). Ectoparasite-induced Lesions in Mite Pockets of the Yarrow's Spiny Lizard, *Sceloporus jarrovi,* (Phrynosomatidae). *Journal of Wildlife Diseases, 28(4),* 537-541.

Goldberg, S. R., & Holshuh, H. J. (1993). Histopathology in a Captive Yarrow's Spiny Lizard, *Sceloporus jarrovi* (Phrynosomatidae), Attributed to the Mite Hirstiella sp. (Pterygosomatidae). *Transactions of the American Microscopical Society, 112(3),* 234-237.

Haenel, G. J., Smith, L. C., & John-Alder, H. B. (2003). Home-Range Analysis in *Sceloporus undulatus* (Eastern Fence Lizard). I. Spacing Patterns and the Context of Territorial Behavior. *Copeia, 2003(1),* 99-112.

Halliday, W. D., Paterson, J. E., Patterson, L. D., Cooke, S. J., & Blouin-Demers, G. (2014). Testosterone, Body Size, and Sexual Signals Predict Parasite Load in Yarrow's Spiny Lizards (*Sceloporus jarrovi*). *Canadian Journal of Zoology, 92,* 1075-1082.

Hamilton, W. D., & Zuk, M. (1982). Heritable True Fitness and Bright Birds: A Role for Parasites? *Science, 218(4570),* 384-387.

Hart, B. J. (1988). Biological Basis of the Behavior of Sick Animals. *Neuroscience and Biobehavioral Reviews, 12(2),* 123-137.

Hechinger, R. F., Wood, A. C., & Kuris, A. M. (2010). Social Organization in a Flatworm: Trematode Parasites form Soldier and Reproductive Castes. *Proceedings of the Royal Society B, 278,* 656-665.

Herbison, R. E. H. (2017). Lessons in Mind Control: Trends in Research on the Molecular Mechanisms behind Parasite-Host Behavioral Manipulation. *Frontiers in Ecology and Evolution, 5,* 102.

Hothorn, T., Hornik, K., van de Wiel, M. A., & Zeileis, A. (2006) A Lego System for Conditional Inference. *The American Statistician, 60(3),* 257-263.

Howard, R. D., & Minchella, D. J. (1990). Parasitism and Mate Competition. *Oikos, 58(1),* 120-122.

Husak, J. F., & Fox, S. F. (2003). Adult Male Collared Lizards, *Crotaphytus collaris*, Increase Aggression Towards Displaced Neighbors. *Animal Behaviour, 65,* 391-396.

Husak J. F., & Fox, S. F. (2006). Field Use of Maximal Sprint Speed by Collared Lizards (*Crotaphytus collaris*): Compensation and Sexual Selection. *Evolution, 60(9),* 1888-1895.

Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M., & Van Damme, R. (2005). Morphology, Performance and Fighting Capacity in Male Lizards, *Gallotia galloti*. *Functional Ecology, 19,* 800-807.

Innocenti, G., Vannini, M., & Galil, B. S. (1998). Notes on the Behaviour of the Portunid Crab *Charybdis longicollis* Leene Parasitized by the Rhizocephalan *Heterosaccus dollfusi* Boschma. *Journal of Natural History, 32,* 1577-1585.

Kaiser, H. F., & Rice, J. 1974. Little Jiffy, Mark IV. *Educational and Psychological Measurement. 34,* 111-117.

Karbowiak, G., Miklisova, D., Stanko, M., Werszko, J., Hajdul-Marwicz, M., Szewczyk, T., & Rychlik, L. (2019). The Competition between Immatures of *Ixodes ricinus* and *Dermacentor reticulatus* (Ixodida: Ixodidae) Ticks for Rodent Hosts. *Journal of Medical Entomology, 56(2),* 448-452.

Kemp, D. H., Stone, B. F., & Binnington, K. C. (1982). Tick Attachment and Feeding: Role of the Mouthparts, Feeding Apparatus, Salivary Gland Secretions and the Host Response. In *Physiology of Ticks* (ed. Obenchain, F. D., & Galun, R.), pp. 119-168. Oxford: Pergamon Press.

Keskin, A. Bursali, A., Kumlutas, Y., Ilgaz, C., & Tekin, S. (2013). Parasitism of Immature Stages of *Haemaphysalis sulcate* (Acari: Ixodidae) on Some Reptiles in Turkey. *Journal of Parasitology, 99(5),* 752-755.

Klein, S. L. (2003). Parasite Manipulation of the Proximate Mechanisms that Mediate Social Behavior in Vertebrates. *Physiology & Behavior, 79,* 441-449.

Klukowski, M., & Nelson, C. E. (2001). Ectoparasite Loads in Free-Ranging Northern Fence Lizards, *Sceloporus undulatus hyacinthinus*: Effects of Testosterone and Sex. *Behavioral Ecology and Sociobiology, 49(4),* 289-295.

Knapp, C. R., Perez-Heydrich, C., Zachariah, T. T., Jollay, J., Schnelle, A. N., Buckner, S. D., Lattin, C. R., & Romero, L. M. (2018). Host Sex, Size, and Hemoparasite Infection Influence the Effects of Ectoparasitic Burdens on Free-ranging Iguanas. *Ecology and Evolution, 9,* 1946-1956.

Korner, P., & Schmid-Hempel, P. (2005). Correlates of Parasite Load in Bumblebees in an Alpine Habitat. *Entomological Science, 8,* 151-160.

Kuo, M. M., Lane, R. S., & Giclas, P. C. (2000). A Comparative Study of Mammalian and Reptilian Alternative Pathway of Complement-Mediated Killing of the Lyme Disease Spirochete (*Borrelia burgdorferi*). *The Journal of Parasitology, 86(6),* 1223-1228.

Kurlovs, A. H., Li, J., Cheng, D., & Zhong, J. (2014). *Ixodes pacificus* Ticks Maintain Embryogenesis and Egg Hatching after Antibiotic Treatment of *Rickettsia* Endosymbiont. *PLoS ONE, 9(8),* e104815.

Labruna, M. B., Camargo, L. M. A., Terrassini, F. A., Schumaker, T. T. S., & Camargo, E. P. (2002). Notes on Parasitism by *Amblyomma humerale* (Acari: Ixodidae) in the State of Rondônia, Western Amazon, Brazil. *Journal of Medical Entomology, 39(6),* 814-817.

Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., De Leo, G., Dobson, A. P., Dunne, J. A., Johnson, P. T. J., Kuris, A. M., Macrogliese, D. J., Martinez, N. D., Memmott, J., Marquet, P. A., McLaughlin, J. P., Mordecai, E. A., Pascual, M., Poulin, R., & Thieltges, D. W. (2008). Parasites in Food Webs: The Ultimate Missing Links. *Ecology Letters, 11,* 533-546.

Lafferty, K. D. & Shaw J. C. (2013). Comparing Mechanisms of Host Manipulation across Host and Parasite Taxa. *The Journal of Experimental Biology, 216,* 56-66.

Lane, R. S., & Brown, R. N. (1991). Wood Rats and Kangaroo Rats: Potential Reservoirs of the Lyme Disease Spirochete in California. *Journal of Medical Entomology, 28(3),* 299-302.

Lane, R. S., & Burgdorfer, W. (1987). Transovarial and Transstadial Passage of *Borrelia burgdorferi* in the Western Black-Legged Tick, *Ixodes pacificus* (Acari: Ixodidae). *American Journal of Tropical Medicine and Hygiene, 37(1),* 188-192.

Lane, R. S., Kleinjan, J. E., & Schoeler, G. B. (1995). Diel Activity of Nymphal *Dermacentor occidentalis* and *Ixodes pacificus* (Acari: Ixodidae) in Relation to Meteorological Factors and Host Activity Periods. *Journal of Medical Entomology, 32(3),* 290-299.

Lane, R. S., Fedorova, N., Kleinjan, J. E., & Maxwell, M. (2013). Eco-epidemiological Factors Contributing to the Low Risk of Human Exposure to Ixodid Tick-borne Borreliae in Southern California, USA. *Ticks and Tick-borne Diseases, 4,* 377-385.

Lane, R. S., & Loye, J. E. (1989). Lyme Disease in California: Interrelationship of *Ixodes pacificus* (Acari: Ixodidae), the Western Fence Lizard (*Sceloporus occidentalis*), and *Borrelia burgdorferi*. *Journal of Medical Entomology*, *26(4)*, 272-278.

Lane, R. S., & Quistad, G. B. (1998). Borreliacidal Factor in the Blood of the Western Fence Lizard (*Sceloporus occidentalis*). *The Journal of Parasitology, 84(1),* 29-34.

Lane, R. S., Mun, J., & Stubbs, H. A. (2010). Horizontal and Vertical Movements of Host-seeking *Ixodes pacificus* (Acari: Ixodidae) Nymphs in a Hardwood Forest. *Journal of Vector Ecology, 34(2),* 252-266.

Langkilde, T., & Boronow, K. E. (2010). Color as a Signal: The Relationship between Coloration and Morphology in Male Eastern Fence Lizards, *Sceloporus undulatus. Journal of Herpetology, 44(2),* 261-271.

Langkilde, T., & Boronow, K. E. (2012). Hot Boys Are Blue: Temperature Dependent Color Change in Male Eastern Fence Lizards. *Journal of Herpetology, 46,* 461-465.

Langkilde, T., & Burns, K. E. (2012). Hot Boys Are Blue: Temperature Dependent Color Change in Male Eastern Fence Lizards. *Journal of Herpetology, 46,* 461-465.

Lefcort, H., & Blaustein, A. R. (1991). Parasite Load and Brightness in Lizards: An interspecific Test of the Hamilton and Zuk Hypothesis. *Journal of Zoology, 224,* 491-499.

Lehmann, T. (1993). Ectoparasites: Direct Impact on Host Fitness. *Parasitology Today, 9(1),* 8-13.

Lello, J., Boag, B., Fenton, A., Stevenson, I. R., & Hudson, P. J. (2004). Competition and Mutualism among the Gut Helminths of a Mammalian Host. *Nature, 428(6985),* 840-844.

Lumbad, A. S., Vredevoe, L. K., & Taylor, E. N. (2011). Season and Sex of Host Affect Intensities of Ectoparasites in Western Fence Lizards (*Sceloporus occidentalis*) on the Central Coast of California. *The Southwestern Naturalist, 56(3),* 369-377.

MacDonald, A. J., & Briggs, C. J. (2016). Truncated Seasonal Activity Patterns of the Western Blacklegged Tick (*Ixodes pacificus*) in Central and Southern California. *Ticks and Tick-borne Diseases, 7,* 234-242.

MacLeod, K. J., McCormick, G. L., & Langkilde, T. (2019). Glucocorticoids Do Not Influence a Secondary Sexual Trait or Its Behavioral Expression in Eastern Fence Lizards. *Scientific Reports, 9,* 5229.

Maksimowich, D. S., & Mathis, A. (2000). Parasitized Salamanders are Inferior Competitors for Territories and Food Resources. *Ethology, 106,* 319-329.

Marler, C. A., Walsberg, G., White, M. L., & Moore, M. (1995). Increased Energy Expenditure Due to Increased Territorial Defense in Male Lizards after Phenotypic Manipulation. *Behavioral Ecology and Sociobiology, 37(4),* 225-231.

Martin, M., Meylan, S., Haussy, C., Decencière, B., Perret, S., & Le Galliard, J. (2016). UV Color Determines the Issue of Conflicts But Does Not Covary with Individual Quality in a Lizard. *Behavioral Ecology, 27(1),* 262-270.

Martins, E. P. (1993). A Comparative Study of the Evolution of *Sceloporus* Push-Up Displays. *The American Naturalist, 142(6),* 994-1018.

Martins, E. P. (1994). Phylogenetic Perspectives on the Evolution of Lizard Territoriality. In *Lizard Ecology: Historical and Experimental Perspectives* (ed. L. J. Vitt, E. R. Pianka), pp. 117-144. Princeton: Princeton University Press.

Maynard Smith, J., & Parker, G. A. (1976). The Logic of Asymmetric Contests. *Animal Behaviour, 24,* 159-175.

McElroy, E. J., & de Buron, I. (2014). Host Performance as a Target of Manipulation by Parasites: A Meta-Analysis. *The Journal of Parasitology, 100(4),* 399-410.

McLennan, D. A., & Brooks, D. R. (1991). Parasites and Sexual Selection: A Macroevolutionary Perspective. *The Quarterly Review of Biology, 66(3),* 255-286.

Megía-Palma, R., Martínez, J., & Merino, S. (2014). Molecular Characterization of Haemococcidia Genus *Schellackia* (Apicomplexa) Reveals the Polyphyletic Origin of the Family Lankesterellidae. *Zoologica Scripta, 43(3),* 304-312.

Megía-Palma, R., Martínez, J., & Merino, S. (2016a). A Structural Colour Ornament Correlates Positively with Parasite Load and Body Condition in an Insular Lizard Species. *Science of Nature, 103(7-8),* 52.

Megía-Palma, R., Martínez, J., & Merino, S. (2016b). Structural- and Carotenoid-Based Throat Colour Patches in Males of *Lacertan schreiberi* reflect different parasitic diseases. *Behavioral Ecology and Sociobiology, 70,* 2017-2025.

Megía-Palma, R., Martínez, J., & Merino, S. (2018a). Manipulation of Parasite Load Induces Significant Changes in the Structureal-Based Throat Color of Male Iberian Green Lizards. *Current Zoology, 64(3),* 293-302.

Megía-Palma, R., Martínez, J., Paranjpe, D., Damico, V., Rocío, A., Palacios, M. G., Cooper, R., Ferri-Yanez, F., Sinervo, B., & Merino, S. (2017b). Phylogenetic Analyses Reveal that *Schellackia* Parasites (Apicomplexa) Detected in American Lizards are Closely Related to the Genus *Lankesterella*: Is the Range of *Schellackia* Restricted to the Old World? *Parasites & Vectors, 10,* 470.

Megía-Palma, R., Paranjpe, D., Reguera, S., Martínez, J., Cooper, R. D., Blaimont, P., Merino, S., and Sinervo, B. (2018b). Multiple Color Patches and Parasites in *Sceloporus occidentalis*: differential relationships by sex and infection. *Current Zoology, 64(6),* 703-711.

Metzgar, L. H. (1967). An Experimental Comparison of Screech Owl Predation on Resident and Transient White-Footed Mice (*Peromyscus leucopus)*. *Journal of Mammology, 48(3),* 387-391.

Mihalca, A. D. (2015). Ticks Imported to Europe with Exotic Reptiles. *Veterinary Parasitology, 213,* 67-71.

Møller, A. P., Christe, P., & Lux, E. (1999). Parasitism, Host Immune Function, and Sexual Selection. *The Quarterly Review of Biology, 74(1),* 3-20.

Molnár, O., Bajer, K., Mészáros, B., Török, J., & Herczeg, G. (2013). Negative Correlation Between Nuptial Throat Colour and Blood Parasite Load in Male European Green Lizards Supports the Hamilton-Zuk Hypothesi. *Naturwissenschaften, 100*, 551-558.

Morris, A., Green, M., Martin, H., Crossland, K., Swaney, W. T., Williamson, S. M., Rae, R. (2018). A Nematode That Can Manipulate the Behaviour of Slugs. *Behavioural Processes, 151,* 73-80.

Morrison, R. L., & Frost-Mason, S. K. (1991). Ultrastructural Analysis of Iridophore Organellogenesis in a Lizard, *Sceloporus graciosus* (Reptilia: Phrynosomatidae). *Journal of Morphology, 209,* 229-239.

Morrison, R. L., Sherbrooke, W. C., & Frost-Mason, S. K. (1996). Temperature-Sensitive, Physiologically Active Iridophores in the Lizard *Urosaurus ornatus*: An Ultrastructural Analysis of Color Change. *Copeia, 1996(4),* 804-812.

Mugabo, M., Perret, S., Decencière, B., Meylan, S., and Le Galliard, J. (2015). Density-dependent Immunity and Parasitism Risk in Experimental Populations of Lizards Naturally Infested by Ixodid Ticks. *Ecology, 96(2),* 450-460.

Musante, A. R., Pekins, P. J., & Scarpitti, D. L. (2007). Metabolic Impacts of Winter Tick Infestations on Calf Moose. *Alces, 43,* 101-110.

Nowak, M., Cieniuch, S., & Stańczak, J. (2010). Detection of *Anaplasma phagocytophilum* in *Amblyomma flavomaculatum* ticks (Acari: Ixodidae) Collected from Lizard *Varanus exanthematicus* imported to Poland. *Experimental and Applied Acarology, 51,* 363-371.

Ogden, H. H., Lindsay, L. R., Beauchamp, G., Charron, D., Maarouf, A., O'Callaghan, C. J., Waltner-Toews, D., & Barker, I. K. (2004). Investigation of Relationships Between Temperature and Developmental Rates of *Ixodes scapularis* (Acari: Ixodidae) in the Laboratory and Field. *Journal of Medical Entomology, 41(4),* 622-633.

Olsson, M. (1994). Nuptial Coloration in the Sand Lizard, *Lacerta agilis*: An Intra-Sexually Selected Cue to Fighting Ability. *Animal Behaviour, 48,* 607-613.

Olsson, M., Wapstra, E., Madsen, T., & Silverin, B. (2000). Testosterone, Ticks and Travels: A Test of the Immunocompetence-Handicap Hypothesis in Free-Ranging Male Sand Lizards. *Proceedings: Biological Sciences, 267(1459),* 2339-2343.

Ossip-Drahos, A. G., Oyola Morales, J. R., Vital-García, C., Zúñiga-Vega, J. J., Hews, D. K., & Martins, E. P. (2016). Shaping Communicative Colour Signals Over Evolutionary Time. *Royal Society Open Science, 3,* 160728.

Padgett, K. A., & Lane, R. S. (2001). Life Cycle of *Ixodes pacificus* (Acari: Ixodidae): Timing of Developmental Processes Under Field and Laboratory Conditions. *Journal of Medical Entomology, 38(5),* 684-693.

Parker, G. A. (1974). Assessment Strategy and the Evolution of Fighting Behaviour. *Journal of Theoretical Biology, 37,* 223-243.

Peavey, C. A., & Lane, R. S. (1996). Field and Laboratory Studies on the Timing of Oviposition and Hatching of the Western Black-Legged Tick, *Ixodes pacificus,* (Acari: Ixodidae). *Experimental & Applied Acarology, 20,* 695-711.

Perkins, S. L., & Schall, J. J. (2002). A Molecular Phylogeny of Malarial Parasites Recovered from *Cytochrome b* Gene Sequences. *The Journal of Parasitology, 88(5),* 972-978.

Pilosof, S., Lareschi, M., and Krasnov, B. R. (2012). Host Body Microcosm and Ectoparasite Infracommunities: Arthropod Ectoparasites Are Not Spatially Segregated. *Parasitology, 139,* 1739-1748.

Pittman, W., Pollock, N. B., & Taylor, E. N. (2013). Effect of Host Lizard Anemia on Host Choice and Feeding Rate of Larval Western Black-Legged Ticks (*Ixodes pacificus*). *Experimental and Applied Acarology, 61(4),* 471-479.

Pollock, N. B., Vredevoe, L. K., & Taylor, E. N. (2012a). How Do Host Sex and Reproductive State Affect Host Preference and Feeding Duration of Ticks? *Parasitology Research, 111(2),* 897-907.

Pollock, N. B., Vredevoe, L. K., & Taylor, E. N. (2012b). The Effect of Exogenous Testosterone on Ectoparasite Loads in Free-Ranging Western Fence Lizards. *Journal of Experimental Zoology, 317A,* 447-454.

Poulin, R. (2007). Are there General Laws in Parasite Ecology? *Parasitology, 134,* 763-776.

Poulin, R., & Maure, F. (2015). Host Manipulation by Parasites: A Look Back before Moving Forward.

Pruett, J. A., Zúñiga-Vega, J. J., Campos, S. M., Soini, H. A., Novotny, M. V., Vital-García, C., Martins, E. P. & Hews, D. K. (2016). Evolutionary Interactions Between Visual and Chemical Signals: Chemosignals Compensate for the Loss of a Visual Signal in Male *Sceloporus* Lizards. *Journal of Chemical Ecology, 42(11),* 1164-1174.

Quinn, V. S., & Hews, D. K. (2000). Signals and Behavioural Responses Are Not Coupled in Males: Aggression Affect by Replacement of an Evolutionary Lost Colour Signal. *Proceedings of the Royal Society London B, 267,* 755-758.

Quinn, V. S., & Hews., D. K. (2003). Positive Relationship between Abdominal Coloration and Dermal Melanin Density in Phrynosomatid Lizards. *Copeia, 2003(4),* 858-864.

R Core Team (2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://R-Project .org/.

Rau, M. E. 1984. Loss of Behavioural Dominance in Male Mice Infected with *Trichinella spiralis. Parasitology, 88,* 371-373.

Raveh, S., Heg, D., Dobson, F. S., Coltman, D. W., Gorrell, J. C., Balmer, A., Röösli, S., & Neuhaus, P. (2011). No Experimental Effects of Parasite Load on Male Mating Behaviour and Reproductive Success. *Animal Behaviour, 82,* 673-682.

Reiczigel, J., & Rózsa, L. (1998). Host-Mediated Site Segregation of Ectoparasites: An Individual-Based Simulation Study. *The Journal of Parasitology, 84(3),* 491-498.

Rejmanek, D., Nieto, N. C., Barash, N., & Foley, J. E. (2011). Temporal Patterns of Tick-borne Granulocytic Anaplasmosis in California. *Ticks and Tick-borne Diseases, 2,* 81-87.

Ressel, S., & Schall, J. J. (1989). Parasites and Showy Males: Malarial Infection and Color Variation in Fence Lizards. *Oecologia, 78,* 158-164.

Revelle, W. (2018). Psych: Procedures for Personality and Psychological Research, Northwestern University, Evanston, Illinois, USA, https://CRAN.R-project.org/package=psych Version = 1.8.4.

Robbins, T. R., Pruitt, J. N., Straub, L. E., McCoy, E. D., & Mushinsky, H. R. (2010). Transgressive Agression in *Sceloporus* Hybrids Confers Fitness through Advantages in Male Agonistic Encounters. *Journal of Animal Ecology, 79,* 137-147.

Roberts, M. L., Buchanan, K. L., & Evans, M. R. (2004). Testing the Immunocompetence Handicap Hypothesis: A Review of the Evidence. *Animal Behaviour, 68,* 227-239.

Robson, M. A. & Miles, D. B. (2000). Locomotor Performance and Dominance in Male Tree Lizards, *Urosaurus ornatus. Functional Ecology, 14(3),* 338-344.

Rothblum, L., & Jenssen, T. A. (1978). Display Repertoire Analysis of *Sceloporus undulatus hyacinthinus* (Sauria: Iguanidae) from South-Western Virginia. *Animal Behaviour, 26,* 130-137.

Rulison, E. L, Kerr, K. T., Dyer, M. C., Hans, S., Burke, R. L., Tsao, J. I., & Ginsberg, H. (2014a). Minimal Role of Eastern Fence Lizards in *Borrelia burgdorferi* Transmission in Central New Jersey Oak/Pine Woodlands. *The Journal of Parasitology, 100(5),* 578-582.

Sabo, J. L., & Power, M. E. (2002). River-watershed Exchange: Effects of Riverine Subsidies on Riparian Lizards and Their Terrestrial Prey. *Ecology, 83(7),* 1860-1869.

Salkeld, D. J., Castro, M. B., Bonilla, D., Kjemtrup, A., Kramer, V. L., Lane, R. S., & Padgett, K. A. (2014). Seasonal Activity Patterns of the Western Black-Legged Tick, *Ixodes pacificus,* in Relation to Onset of Human Lyme Disease in Northwest California. *Ticks and Tick-borne Diseases, 5,* 790-796.

Salkeld, D. J., & Lane, R. S. (2010). Community Ecology and Disease Risk: Lizards, Squirrels, and the Lyme Disease Spirochete in California, USA. *Ecology, 91(1),* 293-298.

Salvador, A., Veiga, J. P., & Civantos, E. (1999). Do Skin Pockets of Lizards Reduce the Deleterious Effects of Ectoparasites? An Experimental Study with *Psammodromus algirus*. *Herpetologica, 55(1),* 1-7.

Salvador, A., Veiga, J. P., Martin, J., Lopez, P., Abelenda, M., and Puerta, M. (1996). The Cost of Producing a Sexual Signal: Testosterone Increases the Susceptibility of Male Lizards to Ectoparasite Infestation. *Behavioral Ecology, 7(2),* 145-150.

Schall, J. J. (1982). Lizard Malaria: Parasite-Host Ecology. In *Lizard Ecology: Studies on a Model Organism* (ed. R. B. Huey, E. R. Pianka and T. W. Schoener), pp. 84-100. Cambridge: Harvard University Press.

Schall, J. J. (1983). Lizard Malaria: Cost to Vertebrate Host's Reproductive Success. *Parasitology, 87,* 1-6.

Schall, J. J., Bennett, A. F., & Putnam, R. W. (1982). Lizards Infected with Malaria: Physiological and Behavioral Consequences. *Science, 217,* 1057-1059.

Schall, J.J., & Dearing, M. D. (1987). Malarial Parasitism and Male Competition for Mates in the Western Fence Lizard, *Sceloporus occidentalis*. *Oecologia, 73,* 389-392.

Schall, J. J., & Houle, P. R. (1992). Malarial Parasitism and Home Range and Social Status of Male Western Fence Lizards, *Sceloporus occidentalis*. *Journal of Herpetology, 26(1),* 74-76.

Schall, J. J., Prendeville, H. R., & Hanley, K. A. (2000). Prevalence of the Tick, *Ixodes pacificus*, on Western Fence Lizards, *Sceloporus occidentalis*: Trends by Gender, Size, Season, Site, and Mite Infestation. *Journal of Herpetology, 34(1),* 160 – 163.

Schall, J. J., & Sarni, G. A. (1987). Malarial Parasitism and the Behavior of the Lizard, *Sceloporus occidentalis*. *Copeia, 1987(1),* 84-93.

Schindelin, J., Aganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji – An Open Source Platform for Biological Image Analysis. *Nature Methods, 9(7),* 676-682.

Schiöth, H. B., Tatjana, H., Ling, M. K., Ringholm, A., Fredriksson, R., Cerdá-Reverter, J. M., & Klovins, J. (2005). Evolutionary Conservation of the Structural, Pharmacological, and

Genomic Characteristics of the Melanocortin Receptor Subtypes. *Peptides, 26,* 1886-1900.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 Years of Image Analysis. *Nature Methods, 9(7),* 671-675.

Schoeler, G. B., & Lane, R. S. Efficiency of Transovarial Transmission of the Lyme Disease Spirochete, *Borrelia burgdorferi,* in the Western Blacklegged Tick, *Ixodes pacificus* (Acari: Ixodidae). *Journal of Medical Entomology, 30(1),* 80-86.

Schoeler, G. B., & Wikel, S. (2001). Modulation of Host Immunity by Haematophagous Arthropods. *Annals of Tropical Medicine & Parasitology, 95(8),* 755-771.

Scott, J. D., Anderson, F. F., Durden, L. A., Smith, M. L., Manrod, J. M., & Clark, K. L. (2016). Ticks Parasitizing Gallinaceous Birds in Canada and First Record of *Borrelia burgdorferi*-infected *Ixodes pacificus* (Acari: Ixodidae) from California Quail. *Systematic and Applied Acarology, 21(1),* 1-12.

Seddon, R. J., & Hews, D. K. (2016a). Phenotypic Correlates of Melanization in two *Sceloporus occidentalis* (Phrynosomatidae) populations: Behavior, Androgens, Stress Reactivity, and Ectoparasites. *Physiology & Behavior, 163,* 70-80.

Seddon, R. J., & Hews, D. K. (2016b). Populations of the Lizard, *Sceloporus occidentalis,* that Differ in Melanization have Different Rates of Wound Healing. *Journal of Experimental Biology, 325A,* 491-500.

Sheldahl, L. A., & Martins, E. P. (2000) The Territorial Behavior of the Western Fence Lizard, *Sceloporus occidentalis*. *Herpetelogica, 56(4),* 469-479.

Smith, L. C., & John-Alder, H. B. (1999). Seasonal Specificity of Hormonal, Behavioral, and Coloration Responses to Within- and Between-Sex Encounters in Male Lizards (*Sceloporus undulatus*). *Hormones and Behavior, 36,* 39-52.

Sousa, W. (1992). Interspecific Interactions among Larval Trematode Parasites of Freshwater and Marine Snails. *American Zoologist, 32(4),* 583-592.

Sousa, W. P., & Grosholz, E. D. (1991). The Influence of Habitat Structure on the Transmission of Parasites. In *Habitat Structure: The Physical Arrangement of Objects in Space.* (ed. S. S. Bell, E. D. McCoy, and H. R. Mushinsky), pp. 300-318. Suffolk: St Edmundsbury Press.

Spence, A. R., Durso, A. M., Smith, G. D., Skinner, H. M., & French, S. S. (2017). Physiological Correlates of Multiple Parasitic Infections in Side-Blotched Lizards. *Physiological and Biochemical Zoology, 90(3),* 321-327.

Stebbins, R. C. (2003). *A Field Guide to Western Reptiles and Amphibians,* 3rd. ed. pp. 288-290,486. New York: Houghton Mifflin.

Steverding, D. (2008). The History of African Trypanosomiasis. *Parasites & Vectors, 1(3).*

Stuart-Fox, D. M., & Johnston, G. R. (2005). Experience Overrides Colour in Lizard Contests. *Behaviour, 142,* 329-350.

Swei, A., Ostfeld, R. S., Lane, R. S., and Briggs, C. J. (2011). Impact of the Experimental Removal of Lizards on Lyme Disease Risk. *Proceedings of the Royal Society B, 278,* 2970-2978.

Swierk, L., & Langkilde, T. (2013). Sizing-up the Competition: Factors Modulating Male Display Behavior During Mate Competition. *Ethology, 119,* 948-959.

Swierk, L., Ridgway, M., & Langkilde, T. (2012). Female Lizards Discriminate between Potential Reproductive Partners Using Multiple Male Traits when Territory Cues are Absent. *Behavioral Ecology and Sociobiology, 66(7),* 1033-1043.

Tälleklint-Eisen, L., & Eisen, R. J. (1999). Abundance of Ticks (Acari: Ixodidae) Infesting the Western Fence Lizard, *Sceloporus occidentalis*, in Relation to Environmental Factors. *Experimental and Applied Acarology, 23,* 731-740.

Taylor, P. W., & Elwood, R. W. (2003). The Mismeasure of Animal Contests. *Animal Behaviour, 65,* 1195-1202.

Thamm, S., & Wells, K. (2009). Ectoparasite Infestations of Hedgehogs (*Erinaceus europaeus*) are Associated with Small-Scale Landscape Structures in an Urban-Suburban Environment. *EcoHealth, 6(3),* 404-413.

Thomas, F., Adamo, S., & Moore, J. (2005). Parasitic Manipulation: Where are We and Where Should We Go? *Behavioural Processes, 68,* 185-199.

Thomas, F., Schmidt-Rhaesa, A., Martin, G., Manu, C., Durand, P., & Renaud, F. (2002). Do Hairworms (Nematomorpha) Manipulate the Water Seeking Behaviour of Their Terrestrial Hosts? *Journal of Evolutionary Biology, 15,* 356-361.

Thompson, J. T., Bissell, A. N., & Martins, E. P. (2008). Inhibitory Interactions between Multimodal Behavioural Responses May Influence the Evolution of Complex Signals. *Animal Behaviour, 76,* 113-121.

Torio, A. J. (1992). Effect of Parasitic Infection on Male Color Pattern and Female Choice in Guppies. *Behavioral Ecology, 3(4),* 346-351.

Tsuji, J. S. (1988). Seasonal Profiles of Standard Metabolic Rate of Lizards (*Sceloporus occidentalis*) in Relation to Latitude. *Physiological Zoology, 61(3),* 230-240.

Utzinger, J., Müller, I., Vounatsou, P., Singer, B. H, N'Goran, E. K., & Tanner, M. (2003). Random Spatial Distribution of *Schistosoma mansoni* and Hookworm Infections among School Children within a Single Village. *Journal of Parasitology, 89(4),* 686-692.

Vardo, A. M., Wargo, A. R., & Schall, J. J. (2005). PCR Detection of Lizard Malaria Parasites: Prevalence of *Plasmodium* Infections with Low-Level Parasitemia Differs by Site and Season. *Journal of Parasitology, 91(6),* 1509-1511.

Veiga, J. P., Salvador, A., Merino, S., & Puerta, M. (1998). Reproductive Effort Affects Immune Response and Parasite Infection in a Lizard: A Phenotypic Manipulation Using Testosterone. *Oikos, 82(2),* 313-318.

Verhulst, S., Dieleman, S. J., & Parmentier, H. K. (1999). A Tradeoff Between Immunocompetence and Sexual Ornamentation in Domestic Fowl. *Proceedings of the National Academy of Sciences of the United States of America, 96,* 4478-4481.

Viana, L. A., Winck, G. R., Almeida-Santos, M., da Silva Telles, F. B., Gazêta, G. S., & Rocha, C. F. D. (2012). New Host Records for *Amblyomma rotundatum* (Acari: Ixodidae) from Grussaí Restinga, Rio de Janeiro, Brazil. *Revista Brasilieira de Parasitologia Veterinaria, 21(3),* 319-322.

Waladde, S. M., & Rice, M. J. (1982). The Sensory Basis of Tick Feeding Behavior. In *Physiology of Ticks* (ed. Obenchain, F. D., & Galun, R.), pp. 71-118. Oxford: Pergamon Press.

Wesołowska, W., & Wesołowski, T. (2013). Do *Leucochloridium* Sporocysts Manipulate the Behaviour of Their Snail Hosts? *Journal of Zoology, 292,* 151-155.

Wiens, J. J. (2000). Decoupled Evolution of Display Morphology and Display Behaviour in Phrynosomatid Lizards. *Biological Journal of the Linnean Society, 70,* 597-612.

Yang, X., & Cox-Foster, D. L. (2005). Impact of an Ectoparasite on the Immunity and Pathology of an Invertebrate: Evidence for Host Immunosuppression and Viral Amplification. *Proceedings of the National Academy of Sciences,* 102(21), 7470-7475.

Zahavi, A. (1975). Mate Selection – A Selection for a Handicap. *Journal of Theoretical Biology, 53(1),* 205-214.

Zamora-Camacho, F. J., Reguera, S., Rubiño-Hispán, & Moreno-Rueda. (2015). Eliciting an Immune Response Reduces Sprint Speed in a Lizard. *Behavioral Ecology, 26(1),* 115-120.

Zuk, M., and McKean, K. A. (1996). Sex Differences in Parasite Infections: Patterns and Processes. *International Journal for Parasitology, 26(10),* 1009-1024.