PHYSIOLOGICAL FACTORS AFFECTING THE BACTERICIDAL ACTIVITY OF THE WESTERN FENCE LIZARD (*SCELOPORUS OCCIDENTALIS*) FOR THE LYME DISEASE SPIROCHETE, *BORRELIA BURGDORFERI*

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements of the Degree

Master of Science in Biology

by

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June 2015

COMMITTEE MEMBERSHIP

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ABSTRACT

Physiological Factors Affecting the Bactericidal Activity of the Western Fence Lizard (*Sceloporus occidentalis*) for the Lyme Disease Spirochete, *Borrelia burgdorferi*

Kyle R. Weichert

The Western Fence Lizard (*Sceloporus occidentalis*) is a major host of juvenile stages of the Western Black-legged Tick (*Ixodes pacificus*), which is the vector for the Lyme disease causative spirochete bacterium *Borrelia burgdorferi* in the western United States. Because *S. occidentalis* is reservoir incompetent and capable of eliminating spirochetes from infected ticks, it has been implicated as a major factor in the ecology of Lyme disease in the West. Although complement proteins in lizard blood have been established as the borreliacidal factor, no studies have examined intraspecific variability in host lizard borreliacidal capacity. In Chapter 1 of this thesis, we introduce the complexity of the *Borrelia burgdorferi* transmission cycle and it's implications for transmission risk. In Chapter 2 we tested the hypothesis that host lizard physiological condition impacts their borreliacidal capacity. Blood plasma of lizards in varying physiological conditions was challenged against cultured *B. burgdorferi*, and the complement-mediated inactivation of spirochetes was quantified. Adult lizards had higher bactericidal activity than first-year juveniles, suggesting that complement-mediated inactivation develops with maturity and/or exposure to spirochete antigens. Also, bactericidal activity was positively associated with lizard tick load and body condition. Adult lizard sex did not significantly affect spirochete mortality. Lizards from an inland site with little exposure to ticks had higher bactericidal activity than lizards from a coastal population that is heavily parasitized by ticks.

ACKNOWLEDGMENTS

I would first and foremost like to thank my advisor and committee chair, Dr. Emily Taylor. Without her inspiration, tough love, and guidance this thesis would not have been possible. I would also like to thank my committee members Dr. Larisa Vredevoe and Dr. Candace Winstead for their expertise and support. Also, thanks go to Dr. Steve Rein, Mack Ontiveros, Morgan Rollo, Desiree Mangual, Michelle Chow, Jackie Bates, and Tony Frazier for their knowledge, and physical and mental labor. Also, thank you to Kory Heiken, Endless Mike DeLea, Griffin Capehart, Sam Sampson, George Brusch IV, Dr. Christy Strand, Dr. Michael Black, Natalie Lee, Dana Predeedilok, Gillian Larson, Heather Neldner, and Ryan Baker-Branstetter. And a special thanks to Audrey Weichert for all the loving support. This research was partially funded by the Society for the Study of Reptiles and Amphibians (SSAR), the Chicago Herpetological Society, and the California Polytechnic State University Biological Sciences Department.

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PREFACE

The impact of tick-borne diseases has changed dramatically in North America over the last several decades. Lyme disease, caused by the spirochete bacterium *Borrelia burgdorferi,* has become the most common of these diseases and the frequency of cases is increasing (CDC, 2008). In this thesis, the first of two chapters explores our current understanding of the ecology of the western black-legged tick, *Ixodes pacificus*, the primary vector of *B. burgdorferi* in the western United States. This chapter also reviews the ecology and ability of vertebrate hosts to carry *Borrelia* infections and the implications on the disease ecology of *B. burgdorferi* in the wild including the potential effects of climate change and habitat fragmentation on its distribution and range.

The second chapter reports a series of experiments conducted on the western fence lizard, *Sceloporus occidentalis*, to examine a major deficiency in the understanding of the disease ecology of *B. burgdorferi*: how a host's physiological state can affect its immunological response to *Borrelia* spirochetes. We studied the effects of these physiological factors by using an *in vitro* assay to quantify the spirochete killing potential of *S. occidentalis* plasma. To our knowledge this is the first study that investigates the intraspecific variation in complement immune function against *Borrelia* bacteria. Studying the factors that may affect a host's ability to fight a spirochete infection gives insight into both the immunology and ecology of Lyme disease.

Chapter 1: *Borrelia burgdorferi sensu stricto* in the Western United States

Lyme Disease Ecology

Lyme disease has become the most common arthropod-borne human disease in North America (CDC, 2008). This disease, also known as Lyme borreliosis, occurs primarily in Europe and North America, and is caused by several species of spirochete bacteria belonging to the *Borrelia burgdorferi* sensu lato (*s.l.*) complex. These species (often referred to as "genospecies" within the *B. burgdorferi s.l.* complex) include *B. afzelii*, *B. burgdorferi sensu stricto* (s.s.), *B. garinii*, *B. spielmanii*, *B. bavariensis*, *B. americana*, *B. carolinensis*, *B. californiensis*, *B. kurtenbachii*, and, *B. bissettii* with several more species of uncertain pathogenicity (Kahl et al., 2002; Mannelli et al., 2011; Stanek and Reiter, 2011; Margos et al., 2014). Populations of these *Borrelia* spirochetes are maintained in the wild by a variety of vertebrate hosts and are spread from host to host mainly by ticks of the genus *Ixodes*. The eco-epidemiology of Lyme disease is very complex as each genospecies shows preference for particular hosts and is associated with different clinical symptoms in humans (Gern, 2008; Mannelli et al. 2011). Furthermore, many of the transmission and maintenance mechanisms have yet to be fully studied. The primary tick hosts responsible for the maintenance of *B. burgdorferi s.l.* are *Ixodes persculcatus*, *I. scapularis*, *I. ricinus*, and *I. pacificus*. Of these, *I. pacificus*, the western black-legged tick, has been implicated as a primary vector of Lyme disease in the western United States (Burgdorfer et al., 1985; Lane and Lavoie, 1988; Clover and Lane, 1995). *Ixodes pacificus* is responsible for infecting a variety of vertebrate hosts with *B. burgdorferi* s.s., including humans (Eisen and Lane 2002).

Vector-borne zoonotic diseases are often maintained in nature in complex transmission cycles with multiple vertebrate hosts and their vectors. Disease risk is therefore a function of the ecology of the vertebrate hosts, the vector, and the pathogen. Vertebrate hosts vary in their contributions to pathogen transmission cycles, ranging from reservoir hosts, to amplifying hosts or hosts that support vector populations but do not directly contribute to the spread of the pathogen (Mannelli et al., 2012). An in depth understanding of the roles of vectors and hosts within a community is of crucial importance to effectively control the spread of vector-borne diseases.

The Vector: *Ixodes pacificus*

As with other *Ixodes* ticks, *I. pacificus* goes through three developmental stages after hatching from an egg: larva, nymph, and adult. Each larva, nymph, and adult female consumes a single, large blood meal from a vertebrate host. After this blood meal, nymphs and larvae detach and molt to the next stage, and adult females begin laying eggs (Eisen and Lane, 2002). Adult males may or may not feed, as a blood meal is not required to fertilize eggs (Eisen and Lane, 2002). However, the males may remain on a host for weeks or months seeking females (Oliver, 1989). It typically takes *I. pacificus* about two to three years to complete the developmental cycle from larva to mating and egg-laying (Padgett and Lane, 2001; Eisen and Lane, 2002).

Ixodes pacificus is typically found on the Pacific coast of North America in wooded or scrubby areas with relatively high rainfall. However, isolated populations can also be found in dry inland areas. Populations exist in the Hualapai Mountain County Park in Mojave County, Arizona where high altitude islands of vegetation provide

adequate habitat (Piesman, 2002; Olson et al., 1992) and also in an arid region of southwest Utah where they have observed in scant leaf litter (Piesman, 2002).

Ixodes pacificus is a habitat generalist. Numerous adult and nymphal *I. pacificus* have been collected in habitat characterized as Douglas fir forest, northern coastal scrub, chaparral, and open grassland (Li et al., 2000). Nymphs, however, have been found particularly abundant in habitats where trees such as black oak (*Quercus kelloggii*), Douglas fir (*Pseudotsuga menziesii*), and Pacific madrone (*Arbutus menziesii*) are present with an understory of poison oak (*Toxicodendron diversilobum*) (Clover & Lane, 1995). Tälleklint-Eisen and Lane (1999a) found that areas with high nymphal *I. pacificus* prevalence near Hopland, California also contained redwood (*Sequoia sempervirens*), California bay (*Umbellularia californica*) and bigleaf maple (*Acer macrophyllum*).

Different life stages of *I. pacificus* have varied preferences for questing sites (sites where they actively search for a host). Li et al. (2000) assessed the density and distribution of *I. pacificus* at two parks in northern coastal California in relation to year, different trails, and public use areas by sampling plots of each type of area. They found that adult ticks were typically observed along sun-exposed trails characterized by dense brush and uphill slopes, and nymphal ticks were mostly associated with leaf litter along shaded trails. Furthermore, they showed that adult and nymphal *I. pacificus* densities varied significantly among years and sites, including differences of up to 40 to 50 fold among different areas of the same trail during the same sampling period. Despite this variation, the prevalence of *Borrelia burgdorferi* in the ticks did not differ significantly among years, sites, and tick life stages.

The Hosts: Lizards, Birds, and Mammals

Ixodes pacificus, along with other *Ixodes* ticks, employs an ambush strategy to find and contact hosts. These ticks rarely move more than a few meters while questing (Eisen and Lane, 2002). Hosts are detected by vibrations (from animal movements), odors, body heat, shadows, and carbon dioxide concentrations (Balashov, 1972; Soneshine, 1993; Gherman et al., 2012). They parasitize a wide range of vertebrates including lizards, birds, and mammals (Furman and Loomis, 1984). Rodent reservoirs for *B. burgdorferi* s.s., such as the dusky-footed woodrat (*Neotoma fuscipes*), the California kangaroo rat (*Dipodomys californicus*), and deer mice (*Peromyscus* spp.), are common hosts to larval *I. pacificus* (Lane and Brown, 1991; Brown and Lane, 1992; 1996; Peavey and Lane, 1995), but rarely to nymphs (Lane, 1990a; Lane and Loye, 1991, Wright et al., 2000; Casher et al., 2002). Western fence lizards (*Sceloporus occidentalis*) and alligator lizards (*Elgaria* spp.) typically carry heavy loads of both larval and nymphal *I. pacificus* (Lane and Loye, 1989; Wright et al., 1998; Eisen et al., 2001, Casher et al., 2002).

Eisen et al. (2004) examined the relative importance of lizard versus mammal hosts for the density of *I. pacificus* juveniles (larvae and nymphs) by comparing tick infestation rates in vertebrate hosts in northern coastal California. They found that lizards in oak and fir dominated forest accounted for 93-98 percent of larval tick blood meals and over 99 percent of the nymphal blood meals and lizards in forested areas dominated by redwood and tanoak, accounted for about 31-64 percent of the larval tick blood meals, and 94-100 percent of the nymphal blood meals. For deer mice captured within 10 meters of lizards, lizards carried 36 times more larvae and over 190 times more nymphs than

mice. Furthermore, larval and nymphal ticks were much more abundant on lizards, and in higher proportions in early spring than late spring and early summer.

The role of birds as hosts to *I. pacificus* larvae and nymphs remains uncertain. Manweiler et al. (1990) observed only 5 *I. pacificus* in 138 birds surveyed in Yuba County, California, but Wright et al. (2000) found higher numbers of larval *I. pacificus* in nearby Placer County, with 0.25 larvae and 0.02 nymphs per bird in the 291 birds examined. Furthermore, Slowik and Lane (2001) found 0.06 larvae and 0.09 nymphs per bird in 234 birds in northern coastal California. In Europe, birds have been shown to contribute to the enzootic maintenance and spread of *B. burgdorferi* s.l. by hosting numbers of infected *I. ricinus* (Comstedt et al., 2006; Olsen et al., 1995; Poupon et al., 2006).

Adult *I. pacificus* ticks are closely associated with large mammals such as the Columbian black-tailed deer (*Odocoileus hemionus columbianus*), also known as the "mule deer" (Westrom et al., 1995). In general, *I. pacificus* is abundant in areas where mule deer are abundant (Piesman, 2002). Adults are found in high numbers on these deer, and larvae and nymphs may feed on deer as well, although smaller vertebrates are considered more important (Westrom et al., 1985).

Maintenance of *Borrelia* Spirochetes in Ecosystems

Borrelia spirochetes are maintained in nature by passing between tick vectors and competent vertebrate hosts through horizontal transmission; infections are passed from nymphs to the next cohort of larvae through a vertebrate host (Tsao, 2009). Larvae nearly always hatch from eggs free from infection and only become infected through a blood

meal from an infected vertebrate reservoir host. The infection is conserved in the midgut of the vector tick through each molt into the next life stage (Tsao, 2009). Vertebrate hosts become infected when an infected nymphal or adult tick feeds on them. For a competent host, the infection will likely go systemic, spreading to other tissues of the body. The *Borrelia* infection cycle is then completed when a naïve tick becomes infected by spirochetes when it feeds on a competent host. A vertebrate host is considered a reservoir if it is then able to pass spirochetes obtained from one generation of ticks to the next. It is likely that the majority of spirochete transmissions are from nymphal ticks to larvae via a reservoir host because adult ticks tend to feed on large bodied animals such as deer or ungulates that are poor reservoirs, and larvae are typically born free of infection, and are thereby not capable of transmitting it to an uninfected vertebrate host (Mannelli et al., 2011).

The typical route of infectivity of *Borrelia* spirochetes from reservoir hosts to vector ticks is a systemic infection in the vertebrate reservoir host followed by a relatively long period, up to many months in some cases (Gern et al. 1994), where ticks may be become infected if they bite the host. Under this system, transmission is most likely if infected *Ixodes* nymphs are active in spring to infect the reservoir host, and larvae active in the summer get infected by the newly infected reservoirs. Less commonly, spirochetes can be horizontally transferred to both competent and incompetent hosts (a host that does not carry a sustained spirochete infection) by naïve ticks feeding beside infected ones (Randolph et al., 1996). The close proximity between ticks allows for some spirochetes that enter the host from the infected tick to immediately be taken up by the neighboring tick. This phenomenon has been observed in *Ixodes*

ricinus with *B. burgdorferi s.l.* (Gern and Rais, 1996) and is likely to occur in *I. pacificus* (Wright et al. 2011). Transmission of spirochetes via co-feeding is likely of less ecological significance than systemic transmission, but may still have implications for the contribution to transmission by hosts considered incompetent reservoirs.

Borrelia spirochetes are not usually transferred vertically from adult ticks to offspring, but transovarial transmission of spirochetes from female ticks to larvae has been documented as occurring rarely in *Ixodes ricinus* infected with *B. burgdorferi s.l.* (Bellet-Edimo et al., 2005). This may have implications that affect spirochete maintenance and transmission risk if even just a few infected adult ticks within the tick population are capable of transovarial transmission.

Role of the Vertebrate Host in Transmission

The relative importance of a vertebrate host species in the transmission of *B. burgdorferi* is determined by a combination of: (a) the host's ability to carry a spirochete infection (competence) and subsequent ability to pass it to susceptible ticks (host infectivity), (b) the abundance of the host species, and (c) the population of susceptible ticks and host tick loads (Mather et al., 1989; Brunner and Ostfeld, 2008).

(a) Host competency

Host infectivity is the fraction of uninfected larvae that acquire the infection after feeding on that host species (Mannelli et al., 2011). The extent and duration of infectivity varies among host species, genospecies of *B. burgdorferi s.l.*, the extent of the initial exposure of the host to the infected tick, and the overall tick parasite load (Gern et al., 1994). Many species of small mammals are likely to contribute to the maintenance of

Borrelia burgdorferi in the western United States by acting as competent reservoirs. The primary reservoir hosts of *B. burgdorferi* in the West are the dusky-footed woodrat , western grey squirrel (*Sciurus griseus*), and California kangaroo rat (Brown and Lane, 1992; Lane and Kierans, 1997; Lane et al., 2005; Salkeld and Lane, 2010). Deer mice, specifically *Peromyscus truei* and *P. maniculatus*, have also been implicated as playing a role in spirochete maintenance because they are usually abundant in brushy habitats where ticks are found, they are capable of harboring *Borrelia* spirochetes, and are fed upon by competent bridge vector *I. pacificus*. These *Peromyscus* mice have been shown to infect 7-40 percent of *I. pacificus* larvae fed on them (Peavey and Lane, 1995b; Brown and Lane, 1996).

In contrast to the mammals listed above, the lizards *S. occidentalis* and *Elgaria* spp. are incompetent reservoirs (Lane, 1990a; Lane and Quistad, 1998) despite hosting substantial numbers of juvenile *I. pacificus* ticks (Lane and Loye, 1989; Manweiler et al., 1992). Furthermore, *S. occidentalis* has been shown to cleanse infected ticks of their spirochetal infections (Lane and Quistad, 1998). This may have major implications for the prevalence of Lyme borreliosis in the West. Infected nymphal ticks are the primary stage responsible for infecting naïve reservoir hosts, but if these nymphs feed on *S. occidentalis* or *Elgaria* spp., not only is the lizard not a competent host to the spirochetes, but the potential for that tick to infect another host is removed from the system.

Aside from host competency for spirochete infection, survival, successful feeding of a blood meal, and subsequent molting of the tick from one stage to another can be affected by the host's immune response. Keesing et al. (2009) assessed the immune responses of various vertebrate hosts to *I. scapularis* in New York State. By exposing

field-caught hosts to feeding by larval *I. scapularis*, they found that some host species that are abundantly parasitized in nature were capable of killing many ticks that attempted to attach and feed on them, while others allowed ticks to successfully feed. Squirrels and opossums were among the least competent, killing an average of 83 and 96 percent of ticks that attempted to feed on them, respectively.

The abundance and composition of vertebrate host species varies with location. Some species may be habitat generalists while others may be limited in their habitat preferences, making access to these hosts variable for vector ticks. Also adding to the complexity, host abundance may change over time. For example, the most competent reservoir species may be abundant one year and scarce the next, causing potential tick vectors to find other vertebrate hosts that may have a lower infectivity.

A wide range of vertebrates host *I. pacificus* ticks, but these host species vary in their reservoir competence. As defined by Kahl et al. (2002), a vertebrate reservoir host must be able to: (i) host vector ticks, (ii) acquire *B. burgdorferi* from infected ticks, (iii) allow the bacteria to multiply and persist in its body, and (iv) transmit spirochetes back to feeding vector ticks. Non-reservoir hosts are ecological barriers to *Borrelia* spirochetes. However, these incompetent reservoirs are capable of hosting large numbers of ticks and thereby may contribute indirectly to the transmission of *B. burgdorferi* by augmenting and maintaining the vector population.

(b) Population dynamics of the host species

The ecological transmission system that likely applies to most populations of *B burgdorferi s.s.* in the western United States consists of several host species, including small and medium sized mammals, reptiles, and birds as reservoir hosts (Mannelli et al.,

2011). In this system, small mammals such as mice and woodrats are the primary reservoir hosts. The dynamics of this system are complex and the effects of species composition and host populations on the intensity of *B. burgdorferi s.s.* transmission likely vary with ecological setting, but many studies have tried to characterize overall patterns affecting transmission risk. For example, field studies and subsequent mechanism models from the eastern United States proposed that the abundance of alternate hosts with relatively lower infectivities acts to "dilute" the transmission effects of a host with a high infectivity, termed the "dilution effect" (Schmidt and Ostfeld, 2001; Allan et al., 2003; LoGiudice et al., 2003). Mechanistically, the dilution occurs when a transmission that might have previously infected a susceptible tick from a competent reservoir instead results in no transmission because it occurs on a less competent reservoir (Ogden and Tsao, 2009). Keesing et al. (2006) proposed further "indirect" ways that increased biodiversity could negatively affect transmission, including the idea that reservoir hosts may then be subject to increased competition and predation, thereby lowering the overall population of reservoir hosts with higher infectivities.

Ogden and Tsao (2009) disagree with the dilution effect hypothesis, and propose that increased biodiversity acts to amplify the transmission rather than dilute it. They suggest that the dilution effect is less likely to occur if a pathogen is dominated by density-dependent interactions, as is the case with *B. burgdorferi s.s.*, rather than frequency-dependent interactions. Because each life stage is an obligate parasite, tick abundance is highly dependent on vertebrate host abundance (Wilson et al, 1984; Randolph and Steele, 1985; Daniels and Fish, 1995; Jones et al., 1998; Ostefeld et al., 2001, 2006; Rand et al., 2003, Randolph, 2004), regardless of their role as reservoirs for

B. burgdorferi s.s. It is true that the dilution effect would theoretically lower the Lyme disease risk if it were measured as nymphal tick infection prevalence, the proportion of ticks that are infected. However, if infection risk were measured by the abundance of infected nymphs, an increase in host density would lead to a greater number of ticks and therefore a greater chance of transmission. For example, LoGiudice et al. (2003) found that in New York State, white-footed mice (*Peromyscus leucopus*) infect about 92 percent of feeding *I. scapularis* larvae, and host an average of 28 larvae. Squirrels (*Sciurus* spp.), however, infect about 15 percent of larvae, but host an average of 142 larvae, and therefore contribute to the local population of infected nymphs.

Swei et al. (2011) tested the dilution effect hypothesis by attempting to reverse the dilution. They removed a total of 447 *S. occidentalis* from six 1 ha. plots in Mendocino County, California. They found that the nymphal infection prevalence did not change despite removing an incompetent reservoir from the habitat, but the density of infected nymphs dropped from about 5.5 to 1.2 ticks per plot, thereby lowering the transmission risk.

Despite the density-dependent concept of transmission risk, there is likely a frequency-dependent component to transmission characterized by the effect of prevalence of infection on ticks and hosts alike (Mannelli et al., 2011). To further add to the complexity of this transmission system, the relative importance of each vertebrate host may vary with time. For example, wild fluctuations in population size have been observed in *Peromyscus* mice, and other species may serve to compensate and sustain the transmission cycle (Slajchert et al. 1997). The relationships between interacting host densities and tick loads are complex and easily affected by both host-specific and

ecologically driven factors (Brisson et al. 2008; Brunner and Ostfeld, 2008). For example, Keesing et al. (2009) found that if key hosts, such as opossums and squirrels that kill a high percentage of larval ticks that attempt to feed on them, were removed from their habitats, the risk of transmission would increase greatly due to an increase in vector density. Consequently, the dilution effect, if present, is location specific and the species composition would be critical in assessing disease risk.

(c) Population of vector ticks and tick loads

Tick loads and patterns of infestation can vary widely from host species to host species. Furthermore, there is variation in the distribution of ticks on the host's body and the distribution of tick loads among individuals within a population (Mannelli et al., 2011). Schall et al. (2000) monitored ectoparasite loads on *S. occidentalis* at two sites in California: a southern population in Los Angeles County and a northern population in Mendocino County. Lizards in the southern population had no ticks on them while the northern population had lizards that carried up to 78 ticks. Male lizards had a significantly higher number of ticks, on average. Furthermore, 90 percent of the ticks were found aggregated in the nuchal pouches, folds of skin found on the lizard's neck.

The prevalence of *B. burgdorferi s.s.* infection in questing *I. pacificus* can vary widely over relatively short distances. Tälleklint-Eisen and Lane (1999a) attempted to identify the biotic and abiotic factors that influence the abundance of infected nymphal *I. pacificus* in two regions of Mendocino County and found a 10-fold variation in infection prevalence and a 16-fold variation in infected nymphal density at sites only about 30 kilometers apart. It is likely that the relative abundance of reservoir-competent hosts is an important factor affecting this variation, but the underlying mechanisms have yet to be

fully elucidated. A further factor to consider in the transmission of *B. burgdorferi* is the vector competence of tick species and populations. A study by Estrada-Peña et al. (1998) suggested that different populations of *I. ricinus* may vary in susceptibility to the genospecies *B. afzelii*. *Borrelia* spirochetes must evade the immune functions of the ticks that carry them, and variation in tick physiological state, innate immunity, the density of spirochetes ingested, and the susceptibility of the spirochetes could affect the competence of the tick vector to carry spirochetes.

To add further complexity to the *Borrelia burgdorferi* system, the distribution of ticks on hosts is neither homogeneous nor random. In many host populations, most individuals carry relatively few ticks, and the majority of ticks are aggregated on a small fraction of individuals (Mannelli et al., 2011). It is likely that individuals contribute differently to the maintenance of infectious diseases spread by vectors, and a relatively small proportion of hosts is responsible for the majority of transmission events or feeding a large proportion of vectors (Woodhouse et al., 1997). Several factors may be responsible for this uneven distribution of ticks on hosts. Tick loads can be influenced by season, host age, immune function, sex, and hormonal state, and may further contribute to parasite loads by altering behavior such as movement, home range, and grooming habits (Pollock et al., 2012). Aggregations of ticks may also be affected by questing behavior of the ticks.

The effect of aggregations of larvae and nymphs on the same host at the same time could be a factor in the non-systemic transmission of *B. burgdorferi* and may have implications in the western United States where the reservoir-incompetent *S. occidentalis* is thought to lower disease incidence (Wright et al. 2011). Multiple *I. pacificus*

individuals are commonly found accumulated in the nuchal pouches and around the lizards' eyes (Arnold, 1986; Goldberg and Bursey, 1991; Dunlap and Mathies, 1993; Pollock et al., 2012). Harrison and Bennett (2012) used previously published tick data to parameterize a model to determine the importance of tick aggregations on small mammal hosts in the persistence of various tick-borne diseases. They found that higher levels of aggregation of ticks on hosts led to increased chances of pathogen establishment. In the case of *B. burgdorferi* infections, transmission from tick to a competent host is probable regardless of aggregation. However, even with incompetent reservoirs such as *S. occidentalis*, tick aggregations may still contribute to non-systemic transmission of spirochetes. The possibility of non-systemic transmission blurs the definition of a reservoir host and prompts the question of whether the concept of *B. burgdorferi s.l.* genospecies host specificity is ecologically relevant. Kurtenbach et al. (2002a) probed the question of the ecological relevance of *B. burgdoerferi s.l.* genospecies and provided evidence that *B. burgdorferi s.l.* is structured ecologically into clusters that are host specific and concluded that vertebrate hosts rather than tick species are the key to *Borrelia* spirochete diversity.

Ixodes pacificus is the primary vector transmitting Lyme borreliosis to humans, but other sympatrically occurring tick species are capable of carrying *B burgdorferi* s.s. and transmitting it to other hosts. *Ixodes spinipalpis* has been shown to be an efficient maintenance vector of both *B. burgdorferi* s.s. and, more commonly, *B. bissettii* in Colorado (Maupin et al., 1994, Burkot et al. 2001) and California (Brown and Lane, 1992; Peavey et al., 1997), by transmitting spirochetes between competent hosts. These ticks rarely feed on humans, but *I. pacificus* ticks that feed on these infected hosts may

subsequently infect a human. Peavey et al. (1997) assessed the role of small mammals other than woodrats in the prevalence of *Borrelia burgdorferi* spirochetes in north coastal California in relation to *I. spinipalpis*. They found that the prevalence of infection in mice (18 percent) was much lower than previous studies of infection in woodrats (68 percent) (Lane and Keirans, 1997).

Borrelia Infection in the Vertebrate Host

When an infected nymphal tick finds a host and begins to feed, an increase in spirochete replication occurs in the midgut, accompanied by shifts in regulation of various spirochete genes. Notable shifts include the down-regulation of surface protein *ospA* and the up-regulation of *ospC* (Marconi et al., 1993; Ohnishi et al., 2001; Schwan et al., 1995; Schwan and Piesman, 2000). *OspA* interacts with a protein in the tick gut to facilitate spirochete colonization in tick gut epithelium (Pal et al., 2004) and protects the spirochetes from host-derived bacteriacidal antibodies (Battisti et al., 2008). The up-regulation of *ospC* reduces the effect of the host adaptive immune response to kill the spirochetes in the tick's midgut while feeding, effectively evading the host's immune system and aiding in dissemination; however, the precise mechanism is still unknown (Randolf and Caimano, 2008).

Once *Borrelia* spirochetes gain access to a vertebrate host, they must survive long enough to be transmitted back to uninfected ticks. A crucial line of defense for vertebrate hosts is the complement pathway. Spirochete infections have been shown to activate the hosts' classical and alternative complement pathways (CCP and ACP, respectively), but the efficacy of the complement complexes is host dependent (Kuo et al., 2000). To

combat this, *B. burgdorferi* spirochetes utilize complement regulator-acquiring surface proteins (CRASP) (Kraiczy et al., 2001) and Osp E/F-related proteins (Erp) (Alitalo et al., 2001) to inhibit complement-mediated activity by binding key complement factors. Spirochetes eventually down-regulate *ospC* and up-regulate an antigenic variation of a different outer membrane lipoprotein, variable major protein-like gene (*vlsE*) allowing the spirochetes to persist inside the vertebrate host and evade the immune system (Tsao, 2009).

Despite the abilities of *B. burgdorferi* to evade host immune responses, incompetent hosts such as lizards *S. occidentalis* and *Elgaria* spp. are able to fight off spirochete infections. Blood from these species has been shown to kill 95-98 percent of spirochetes after just 1 hour of in vitro exposure (Kuo et al., 2000). This complement-mediated killing is achieved by a specialized protein factor that physically disrupts the membranes of the spirochetes (Kuo et al., 2000; Lane and Quistad, 1998). It is widely accepted that the host's complement system is a major determinant of the host specificity exhibited in the natural communities of which the hosts are a part (see above) (Kuo et al., 2000; Nelson et al., 2000; Kurtenbach et al., 2002b).

Effect of Climate Change

With predictions of 1.5-2.5 °C global temperature increases over the next few decades (Christensen et al., 2007), tick and host geographic ranges are expected to change accordingly. Gray et al. (2009) and Mannellii et al. (2011) summarized the potential effects of climate change on *I. ricinus* in Europe and Lyme borreliosis risk by proxy. They concluded that because the ticks spend the majority of their time in the

external environment, rather than on a host, climate changes are likely to affect tick survival, development, and reproduction. Furthermore, climate effects on their vertebrate hosts, such as abundance, migration patterns, and diversity, will impact tick abundance and distribution. It is probable that the incidence of Lyme disease will be affected by the climate-induced changes in the complex interactions of tick biology and that of tick hosts. *Ixodes* ticks along with *B. burgdorferi* are predicted to expand their geographic range through the effects of climate change (e.g. Jaenson and Lindgren, 2011; Mannelli et al., 2011). Simon et al. (2014) used a modeling approach that took into account the future distribution of the *P. leucopus* and the *I. pacificus* with respect to climate change and habitat fragmentation to estimate a risk index of *B. burgdorferi*. They predicted a northward range expansion at a rate of 3.5-11 kilometers per year.

A recent study on *I. scapularis* in the United States proposed that climate change may alter the relative proportions of *B. burgdorferi s.s.* strains in a region by influencing tick phenology (Gatewood et al., 2009). *Borrelia burgdorferi s.s.* strains vary in their survival time inside hosts, and some do not persist in reservoir hosts for more than a few weeks. Those short-lived strains would have a greater chance of being passed to receptive larvae if the larvae were feeding at the same time of year as the nymphs. Specifically, the magnitude of the difference between summer and winter daily temperature maxima was positively correlated with the amount of season synchrony between larval and nymphal *I. scapularis*. This may have implications for *I. pacificus* and *B. burgdorferi s.s.* prevalence in the western United States. Changes in *I. pacificus* stage phenology could cause larval and nymphal ticks to more commonly feed side-by-side, increasing transmission chances even within incompetent reservoirs such as *S. occidentalis.*

It is likely that global climate change is already affecting the lifecycles of ticks and their transmission of *Borrelia* spirochetes. However, the complexity of the interacting factors that determine the timing and intensity of tick activity and spirochete transmission makes it difficult to predict likely Lyme disease incidence in future climate change scenarios based on the current understanding of the system. Further studies on tick biology, host abundance, and Lyme borreliosis incidence, specifically in relation to climate change, are required to develop models that may accurately determine the climate conditions suitable for ticks and reservoir hosts.

Conclusions

In the western United States, many studies have been conducted on the ecology of *I. pacificus* and the related rates of transmission of *Borrelia burgdorferi s.s.* through field studies and subsequent laboratory analysis. Nevertheless, conclusions are difficult to draw on key ecological factors such as the role of host species, non-systemic transmission, the dilution effect and the role of non-reservoir hosts. Due to the complexity of this system, new techniques and modeling methods must be developed to untangle the roles of these factors to provide potential mechanisms for lowering the incidence of Lyme borreliosis or to make predictions of how the disease may spread. The elucidation of the effects of increased biodiversity and varying infectivity among hosts is particularly important to help guide land management decisions that will function to decrease Lyme disease risk. Furthermore, modeling the population dynamics of indicator host species in response to climate change would contribute greatly to the evaluation of future climate effects on ticks and tick-borne pathogens.

The majority of studies conducted on the epidemiology and disease risk of Lyme disease has focused on the determining the behavior of tick vectors and the competency of vertebrate hosts. To fully understand how *Borrelia burgdorferi* persists in the wild, it is important to know how the hosts' physiological state, such as hormone levels, body condition, and age, affects its ability to fight the spirochete infections. This information will help to model and make more accurate predictions for how infection risk and prevalence will change with respect to climate change and habitat fragmentation. In Chapter 2 we report a series of experiments that examine this major deficiency our understanding of Lyme disease ecology.

Chapter 2: Physiological Factors Affecting the Bactericidal Activity of the Western Fence Lizard (*Sceloporus occidentalis*) for the Lyme Disease Spirochete *Borrelia burgdorferi*

Introduction

Borrelia burgdorferi sensu stricto (s.s.)[1] is the spirochete bacterium and causative agent of Lyme disease in both humans and wildlife (Stevenson et al., 2002). In the western United States, the Western Black-legged Tick, *Ixodes pacificus*, is the primary vector of *Borrelia burgdorferi* s.s. (Burgdorfer et al., 1985; Lane and Lavoie, 1988; Clover and Lane, 1995). While immature *I. pacificus* have been shown to infest, and subsequently infect, a variety of vertebrate hosts with *B. burgdorferi* s.s. (Bishopp and Trembley, 1945; Lane and Loye, 1991; Lane and Brown, 1991; Apperson et al., 1993; Peavey and Lane, 1995; Durden et al., 2002; Castro and Wright, 2007), not all hosts are competent reservoirs (i.e., host maintains a spirochetal infection and is able to infect other feeding ticks). The Western Fence Lizard, *Sceloporus occidentalis*, is reservoir-incompetent for *B. burgdorferi* (Lane, 1990b; Lane and Quistad, 1998) despite being a major host for juvenile stages of *I. pacificus* (Lane and Loye, 1989; Manweiler et al., 1992). The host-parasite relationship between *S. occidentalis* and *I. pacificus* has been

[1] *Borrelia burgdorferi* sensu lato (s.l.) is a complex of genospecies, of which *Borrelia burgdorferi* s.s. is a member. The *Borrelia burgdorferi* s.l. complex contains six other spirochete genospecies found in North America: *Borrelia andersonii B. americana, B. carolinensis, B. californiensis, B. kurtenbachii,* and, *B. bissettii* (Stanek and Reiter, 2011; Margos et al., 2014)

well-studied, and many factors contribute to tick loads on lizards, including geographic location, host sex, habitat, and host body size, among other variables (Lane and Loye, 1989; Tälleklint-Eisen and Eisen, 1999b; Schall et al., 2000; Eisen et al., 2001; Eisen et al., 2004; Lumbad et al., 2011; Pollock et al., 2012a). Furthermore, *S. occidentalis* has been shown to cleanse previously infected ticks of spirochetal infections (Lane and Quistad, 1998).

Immunological studies have shown that a factor exists in the plasma of *S. occidentalis* that is capable of killing *B. burgdorferi* in less than one hour (Lane and Quistad, 1998; Kuo et al., 2000). The factor responsible for the killing of *B. burgdorferi* are the proteins comprising the alternative complement pathway (ACP), an antibody independent pathway that leads to lysis of *Borrelia* spirochetes when the C5b-9 membrane attack complex (MAC) disrupts their outer membranes (Kochi et al., 1993; Lane and Quistad, 1998; Kuo et al., 2000). The complement-mediated killing of spirochetes was shown to be capable of killing spirochetes independent of antibody action when Cacciapouti et al. (1993) illustrated that bleb (an out-pocketing of cell contents due to membrane disruption) formation was visible on spirochetes even in the absence of antibodies. This type of innate immune function may be important in determining the survival of an animal when it is first exposed to a pathogen, and a successful innate reaction does not require a potentially costly specific response (Lochmiller and Deerenberg, 2000).

While much is known about the importance of this host-parasite relationship in Lyme disease ecology, relatively little is known about the physiological factors that affect the borreliacidal potential of the lizards' blood. Understanding how factors such as sex,

season, and body size affect the ability of *S. occidentalis* to kill *B. burgdorferi* can help in modeling the ecology of Lyme disease and improve the overall understanding of immune function in reptiles. Studying how *S. occidentalis*, a major host to the vector of Lyme borreliosis, responds to *Borrelia* infections can help elucidate the role of the lizard in the maintenance or control of the disease. No studies to date have examined individual variation in the borreliacidal capacity of *S. occidentalis* blood. Our objective in this study was to investigate how host physiological state affects the bactericidal activity of *S. occidentalis* plasma for *B. burgdorferi*. Our specific hypotheses were that borreliacidal capacity is affected by factors that can impact immune function in natural populations, including host sex, season, collection site, and age class. To test these hypotheses, we assessed the borreliacidal capacity of individual lizards via a borreliacidal assay. A culture of *B. burgdorferi* spirochetes was exposed to individual lizards' blood plasma, and the resulting borreliacidal activity was quantified by counting dead spirochetes. Males and females from two different sites were compared across different seasons and age classes. The following paragraphs explain the background and expected effects of each of the independent variables: sex, season, site, and age class.

(1) Host sex affects borreliacidal capacity

Male vertebrates tend to have reduced immune function compared to females (Folstad and Karter, 1992; Schuurs and Verheul, 1990; Tschirren et al., 2003; Zuk and McKean, 1996), and numerous studies have shown that testosterone negatively affects immune function in males, both directly and indirectly (Klein, 2000; Mondal and Rai, 1999, 2002; Pollock et al., 2012b). In lizards, males implanted with testosterone demonstrate immunosuppression, as evidenced by lower lymphocyte counts, testosterone

lymphocyte-mediated immunity, and increased parasite intensity (Cox and John-Alder, 2007; Pollock et al., 2012a). In the wild, male *S. occidentalis* have also been shown to host greater tick loads than females or juveniles (Schall et al., 2000; Casher et al., 2002; Lumbad et al., 2011), and administration of exogenous testosterone increases tick load (Pollock et al., 2012a). Because of the immunosuppressive properties of testosterone, we predicted that female lizards will have higher borreliacidal capacities than males.

(2) Borreliacidal capacity changes with respect to season

Related to hormonal variation, seasonal changes in reproductive state may also result in changed immune function. In lizards, elevated testosterone concentrations during the mating season are responsible for increased energy expenditure and reduced energy acquisition, resulting in a negative energy balance (Cox et al., 2005). Testosterone stimulates males to allocate energy resources towards increased ornamentation to attract mates, increased time patrolling territories, courting activities, and fighting with other males, and away from foraging activities. Furthermore, Pollock et al. (2012b) found that *I. pacificus* exhibited reduced feeding duration when feeding on reproductive female *S. occidentalis*, suggesting that reproductive hosts may have reduced immune function. We predict that the borreliacidal capacity of *S. occidentalis* will be lower in Spring, when the lizards are reproductive.

(3) Host collection site affects borreliacidal capacity

Despite similar habitats, different sites may have drastically different rates of tick parasitism, chances of incurrence of *Borrelia* infection, and genetic diversity. Wetter, coastal sites tend have greater numbers of *I. pacificus* than drier inland sites, and lizards are commonly found with much greater tick loads near the coast (Furman and Loomis,

1984; this study). Our Poly Canyon field site is located near the central California coast and harbors ticks in high densities. Our second field site, Chimineas Ranch, is located about 40 miles inland of Poly Canyon and has not been observed to contain dense populations of ticks. Lizards at Poly Canyon have likely evolved with greater tick parasite pressure and may therefore exhibit stronger responses to tick-borne pathogens than lizards from the relatively tick-free Chimineas Ranch. Furthermore, Poly Canyon lizards may therefore have a greater chance of being exposed to *B. burgdorferi* sensu stricto or sensu lato (s.l.) in their lifetime and may exhibit an amplified immune response based on circulating antibodies for the pathogen or from genetic adaptation. *Borrelia bissettii (*a genospecies of *B. burgdorferi s.l.*) has been documented in rodents from Poly Canyon (Vredevoe et al., 2004; Baker-Branstetter thesis in progress) and lizards' thus have the potential to be exposured to this pathogen is potential. Despite these potentially immune-strengthening effects of sympatric cohabitation with ticks, lizards with larger tick loads may actually be indicative of lowered immune function, and thus reduced borreliacidal capacity. No studies to date have investigated the changes in complement reactivity on subsequent pathogen infections. For these reasons, we predicted that lizards from Poly Canyon would have higher borreliacidal capacities than lizards from Chimineas Ranch. However, within site, during the same season, lizards with higher tick loads would have lower borreliacidal capacities.

(4) Host age class affects borreliacidal capacity

Complement-mediated immune function is innate, but juvenile lizards may not develop full complement-mediated immune function until later in life. Despite many studies done on lizard immune function, little is known about the development of the

ACP with age; however, human infants have been shown to develop ACP function after thirteen months of life (Ferriani et al., 1990). Furthermore, it remains unclear how the borreliacidal capacity of *S. occidentalis*, enacted primarily through the ACP, may be aided by the CCP utilizing antibodies from previous *Borrelia* exposure. Adult lizards have a greater chance of previous exposure to *B. burgdorferi* and may potentially, through assistance from acquired antibodies, exhibit greater borreliacidal capacities. Alternatively, studies have shown that transmission of passive immunity in the form of antibodies may occur from adult reptiles to their offspring, providing the potential for a robust immune reaction in both adults and juveniles (Grindstaff et al., 2003; Schumacher et al., 1999). We predict that adults will have higher borreliacidal capacities than juveniles.

Materials and Methods

Lizard Plasma Collection and Processing

During Fall 2012 and Spring 2013, 58 adult *S. occidentalis* were captured at two field sites (Poly Canyon and Chimineas, see below) by hand-held noose or by hand, and blood samples were collected. Lizards were captured on each of 3 days in September 2012 and 2 days in April 2013 (Table 2) to sample the lizard populations both during and outside of breeding season. At Poly Canyon, 4 males and 8 females were sampled in Fall 2012, and 8 males and 9 females in Spring 2013. At Chimineas Ranch, 9 males and 5 females were sampled in Fall 2012 and 5 males and 9 females in Spring 2013. An additional thirteen juvenile lizards were captured from Poly Canyon on the campus of California Polytechnic State University (Cal Poly) in San Luis Obispo, California, in late

summer 2013. Juveniles were collected in late summer because they are active and most abundant this time of year. Approximately 0.1 to 0.25 mL of blood was collected from each lizard via the retro orbital sinus with a heparinized capillary tube within 10 minutes of capture. Blood samples were stored in microfuge tubes on ice until processing in the lab (usually about 3-5 hours). For each captured lizard, the following data were recorded: sex, snout-to-vent length (SVL), mass, and number of visible ectoparasites, before returning each lizard to the site of capture. Ectoparasites were quantified by counting the number of visible ticks and mites on each lizard. These parasites were typically aggregated around the nuchal pouches and the anterior and posterior armpits. Female lizards were assessed for reproductive condition by palpating for the presence of eggs. For each lizard, body condition, a measure of lizard mass per unit length, was calculated by taking the residuals of the ordinary least squares regression of the SVL and mass, both ln-transformed. Each lizard was released at the site of capture after being marked on the back with white paint to avoid recapture. Each lizard was handled as quickly as possible to prevent stress responses, typically less than five minutes each.

Directly following each field capture session, the samples were taken to the laboratory where they were processed. Each sample was centrifuged at 10,000 RPM for three minutes. The supernatant plasma was then carefully extracted with a Hamilton syringe and placed in a clean microfuge tube and stored at -80°C until assayed. The pellet was discarded.

Field Sites

Poly Canyon – Located on the Cal Poly campus, Poly Canyon is located in San Luis Obispo County in the southwestern foothills of the Santa Lucia mountain range of California. This coastal site is 12 miles from the Pacific Ocean and has an elevation of 150m. The primary habitat consists of rolling hills of annual grassland with interspersed coast live oak woodland and a riparian corridor. Additionally, some areas of dense chaparral occur on the south-facing slopes of some of the higher hills. In Poly Canyon, *I. pacificus* ticks are common and *S. occidentalis* are often heavily infested in spring (Lumbad et al. 2011).

Chimineas Ranch – This site is located 40 miles due east of San Luis Obispo, California, on the western border of the Carrizo Plain. This inland site mostly consists of rolling hills of blue oak woodland and California annual grassland. Vast stands of chaparral are also found near the tops of the rolling hills. To the east are hills of annual grassland with multiple large rocky outcrops. This site was chosen for this study because it has a dense population of lizards that are rarely observed to harbor *I. pacificus* (based on previous years of study), perhaps due to low humidity and the scarcity of appropriate dense woodland habitats that would support larval and nymphal stages that feed on lizards (Eisen et al., 2006).

Bacterial Strain and Culture Conditions

A B31 isolate of *B. burgdorferi* (s.s.), (ATCC 35210) was grown in BSK-H medium (Sigma-Aldrich Co. LLC, St. Louis, MO) at 35°C in 4 mL snap-cap vials. The stock culture was thawed from frozen stock January 2012 and two cultures from the same

isolate were maintained separately for the entirety of the experiment. Each was kept for about 100 passes. Each culture's condition was visibly assessed under darkfield microscopy every 1-2 weeks. After each assessment, cultures were passed by transferring 50 uL to a new 4 mL vial of medium.

Borreliacidal Assay

To determine the borreliacidal capacity of each lizard plasma sample, a biological assay was performed to measure its bactericidal activity. The procedure used in this study was based on the methods of Kuo et al. (2000) in which an assay was performed to assess interspecific borreliacidal capacity. Kuo et al. introduced reptile or mammal plasma to a culture of *B. burgdorferi* and the resulting spirochetal survivability was quantified under dark-field microscopy. For this study, the assay methods were modified in order to discern differences among individual lizards; i.e., to detect *intraspecific variation* in bactericidal activity. Specifically, we compared individual *S. occidentalis*' bactericidal activity across sex, seasons, geographic locations, and age classes.

To perform the borreliacidal assay, 25 μL aliquots of *B. burgdorferi* stock culture were suspended in 110 μL phosphate buffered saline (PBS; pH 7.2, room temperature), an isotonic and biologically neutral medium, in a multi-well assay plate to dilute the culture to a concentration that facilitated counting. The stock culture was between one and two weeks from the last pass when assayed. Directly following, 15 μL samples of thawed and vortexed *S. occidentalis* plasma were mixed with the suspension to give a final volume of 150 μL in each experimental well. The plates were then covered and incubated at room temperature for 30 minutes. After incubation, a 20 μL sample of each

assay well was placed on a clean microscope slide with a coverslip and bactericidal activity was assessed by dark-field microscopy. As a negative control, 25 μL aliquots of spirochetes were cultured in 125 μL PBS to give an identical final concentration.

Counting Spirochetal Density and Determining Borreliacidal Capacity

To assess the borreliacidal capacity of each lizard, both live and dead *B. burgdorferi* spirochetes were counted under 400X total magnification under darkfield microscopy using an Olympus BX51-P microscope to measure bactericidal activity. Spirochetes were considered dead upon the observation of blebs (an outpocketing of cell contents), complete cell lysis, or immotility (no movement for 5 seconds or more). These three criteria are considered essential indicators of *Borrelia* mortality (van Dam et al., 1997) and were considered synonymous with borreliacidal capacity during this study. For each assay sample, spirochete cell counts were recorded in each of nine randomly chosen fields of view. Once the spirochetes observed in these nine fields of view were counted, the ratio of dead spirochetes to the total number of spirochetes counted was calculated to give a "percent mortality" for each sample.

The background percent mortality, or the quantity of dead spirochetes in the *Borrelia* stock culture alone, was calculated each day of data collection. This background percent mortality was determined by performing the assay with no lizard plasma sample at the same *Borrelia* concentration as the samples containing lizard plasma. In a multi-well assay plate, 25 μL of the *B. burgdorferi* culture was suspended in 125 μL PBS, giving the same concentration of culture used in the experimental assay. After a 30-

minute incubation, spirochetes from this assay were counted exactly as with the assay samples containing the lizard plasma. This value functioned as a negative control.

As a positive control, the assay was performed with a pooled lizard plasma sample. This pooled plasma sample was created by combining the plasma of 12 lizards collected in Fall 2012 from Poly Canyon, then aliquotting this mixture into individual sampling tubes for use each day of data collection; none of these lizards were used individually in this study.

Pooled lizard plasma aliquots were stored at -80°C until assayed and used immediately after thawing for each assay. This positive control was designed to correct for possible daily variation in the susceptibility of the *B. burgdorferi* culture. Day to day, the response of the *B. burgdorferi* stock culture to the pooled plasma varied with respect to the culture condition, age, and growth cycle and therefore needed to be standardized.

Using the negative and positive controls to correct for the background percent mortality and variability in culture susceptibility, the borreliacidal capacity of each plasma sample was calculated using the following novel metric:

$$Kc = \frac{(Ks - Kb)}{(Kp - Kb)}$$

Where the K_C (corrected borreliacidal capacity) is determined by subtracting K_b (background percent mortality) from K_S (percent mortality counted in each experimental assay sample). This figure is then divided by K_b minus K_p (percent death in the pooled plasma positive control).

Assay Optimization

The borreliacidal assay was optimized by making serial dilutions of lizard plasma in *B. burgdorferi* stock culture. Each dilution was assessed and compared by counting the spirochetal mortality and comparing across dilutions. The methods used in this study were the results of the dilution that maximized concentration while still sensitive enough to detect intraspecific variation between individual lizards. For plasma concentrations that are too low, samples may contain too many spirochetes to detect any effect of the plasma, or spirochetes may be too numerous to count. Conversely, excessively high plasma concentrations would result in killing nearly all spirochetes in the sample, making intraspecific variation impossible to detect. Plasma samples from ten additional lizards of both sexes from Poly Canyon (not used in this study) were used individually for assay optimization.

Statistical Analysis

All statistical analyses were done with JMP Pro statistical software v. 9.0.2 & 11.2, SAS Institute Inc., Cary, NC, 1989-2007. Generalized Linear Models (GLM) were used to determine the factors that significantly predicted K_C. The first model, consisting of adult lizards only, included site (Poly Canyon or Chimineas Ranch), season (Fall or Spring), visible tick load, body condition (ordinary least squares residuals), and sex (male or female) on K_C (log transformed). The K_C data from all adult lizards did not Fall within a normal distribution, so a logarithmic transformation was used to normalize data. A Tukey-Kramer HSD post-hoc test was run to determine if each experimental group was significantly different from any others. A second Tukey-Kramer HSD post-hoc test was

run to determine if body condition was significantly different between experimental groups. A second GLM was used to determine the effects of interactions between sex, site, and season on K_C. Another GLM was used to determine the effects of sex, season, and site on tick load.

A fourth GLM consisted of both juvenile and adult lizards and included lizard mass (g) and age class (adult or juvenile). Mass was used in this model instead of body condition because body condition indices may differ according to age, making it not an accurate comparison of lizard nutrition. Mass can differ greatly between adult and juvenile lizards. The corrected borreliacidal capacity of all lizards, including the juveniles, was not normally distributed. Also, because some of the juvenile lizards had fewer dead spirochetes than the negative control, their K_C values were negative. To be able to use a log transformation on these data, all figures had to be positive, so 0.1 was added to each number before transformation. A T-test was used to compare the borreliacidal capacity of male and female juveniles.

Results

Site was a significant factor after accounting for the effects of all other factors ($t = 2.81$, $p = 0.01$) with Chimineas averaging higher overall borreliacidal capacities (Figure 1). Mean borreliacidal capacities are shown in Table 3. Tick load was also significantly and positively associated with borreliacidal capacity after accounting for effects of the other factors ($t = 2.22$, $p = 0.03$). Non-significant factors included sex ($t = 1.48$, $p = 0.14$), season ($t = 0.27$, $p = 0.79$), and body condition ($t = 0.33$, $p = 0.74$). Interactions between sex, site, and season were modeled using an alternate GLM, and no interaction between

the three factors were significant: all three factors ($t = 0.37$, $p = 0.71$), interaction between sex and site ($t = 0.12$, $p = 0.91$), interaction between sex and season ($t = -1.32$, $p = 0.19$), interaction between site and season ($t = 1.28$, $p = 0.21$). The Tukey-Kramer post hoc test showed that no experimental group borreliacidal capacities differed significantly from any other (Table 5).

The females from Poly Canyon in fall had significantly lower body condition than females from Chimineas in spring ($p < 0.001$) and males from Chimineas in fall ($p = 0.05$). Females from Chimineas in spring also had significantly higher body condition than males from Poly Canyon in fall ($p = 0.04$) and females from Poly Canyon in spring ($p = 0.035$). All comparison results are shown in Table 6.

There were significantly more ticks parasitizing lizards at Poly Canyon than at Chimineas Ranch after accounting for season and sex ($t = 3.75$, $p < 0.001$). There were also significantly higher tick loads on lizards in Spring vs. Fall after accounting for season and site ($t = 3.41$, $p = 0.001$). Sex did not affect tick load after accounting for site and season ($t = 0.35$, $p = 0.72$).

Juveniles had significantly lower borreliacidal capacities than adults after accounting for differences in lizard mass ($t = 2.69$, $p = 0.01$). Lizards with higher mass within age class had significantly higher borreliacidal capacities ($t = 2.22$, $p = 0.03$). Borreliacidal capacities of male and female juveniles did not differ significantly ($t = -1.05$, $p = 0.31$).

Discussion

Our results show that the borreliacidal action of *S. occidentalis* varies based on host population (site) and age, but not sex or season. Several studies have characterized active complement-mediated immunological responses within reptile species (Koppenheffer, 1987; Sunyer and Lambris, 1998; Sunyer et al., 1998), including alligators (Merchant et al. 2005a,b) and cobras (Vogel and Muller-Eberhand, 1985a,b). However, few studies have evaluated variation among species (Kuo et al., 2000; Merchant et al., 2006), and to date, no studies have compared complement reactivity intraspecifically. The physiological factors affecting the lizards' ability to kill *B. burgdorferi* spirochetes appear to have both environmental and innate components, varying among individuals and with lizard physiological state. Lizard age class had a significant effect on borreliacidal capacity, an effect that is likely independent of environmental conditions. Furthermore, field site location and the number of externally visible ticks each lizard was carrying had significant effects on borreliacidal capacity, indicating environmentally dynamic factors may also affect this ability.

Lizard age-class strongly and significantly affected borreliacidal capacity. First-year juvenile fence lizard plasma showed little to no ability to kill *Borrelia burgdorferi* spirochetes, indicating that either the alternative complement pathway develops sometime before or at the time of maturity, or the CCP plays a larger role in cleansing spirochete infections than previously thought. Juveniles are less likely to have acquired antibodies from previous exposure that would allow the CCP to act in killing *B. burgdorferi*. In fact, many of the assay results for individual juvenile lizards showed a negative borreliacidal capacity. Spirochetes mixed with PBS and the plasma from select juveniles actually

survived better than in the control treatment of PBS alone. This phenomenon leads us to believe that complement mediated immune function develops later in life. Age-related microbial resistance has been shown to occur in vertebrates (Harp et al., 1990), and human neonates have been shown to have lower levels of complement components than normal adults for the first six months of life (Davis et al., 1979). Ferriani et al. (1990) found that the CCP and ACP had differing maturation patterns in humans, with the CCP maturing at one and three months, and the ACP around the thirteen months. Relatively few studies have been done on reptile immune function, and have primarily focused on seasonality of immune function (e.g., Hussein et al., 1979) and immune function related to social interactions, reproduction, temperature, and parasite burdens (Svensson et al., 2001; Uller et al. 2006, Ujavari and Madsen 2006; Madsen et al., 2007; Freedberg et al., 2008; French and Moore, 2008-). More focused studies have been conducted on the immune function of American alligator (Merchant et al., 2005(a); 2005(b); 2006), yet, to our knowledge, no studies to date have shown how reptile complement changes as an individual matures. Furthermore, juvenile *S. occidentalis* are much smaller than adults, and as such, host fewer ticks. They, therefore, face a reduced chance of exposure to *B. burgdorferi.* Juvenile lizards used in the study were not found with any visible ectoparasites. This may suggest that there is a greater contribution of the CCP in *fighting* Borrelia infections than was previously thought. If the ACP of juveniles is functional early in life, potential borreliacidal activity via the CCP would be less likely because previous exposure in juveniles is less likely. The CCP may play a role in the complement mediated killing of *B. burgdorferi*. It is unlikely that all the adults sampled in this study had been previously exposed to the bacterium and this potential difference may account

for the relatively large error in each of the experimental groups. Future studies should try to determine the role that previous exposure plays in the killing of *B. burgdorferi* by testing each plasma sample for anti-spirochete antibodies. The negative borreliacidal capacities observed in many juvenile lizards was unexpected. The spirochetes incubated in the juvenile lizard plasma had fewer dead spirochetes than those incubated in just PBS. It is possible the juvenile plasma was able to nourish the spirochetes during incubation and the biologically neutral PBS allowed for some slight bacterial senescence outside their preferred medium.

Field site had a significant effect on adult lizard borreliacidal capacity. Adult lizards at Chimineas had significantly higher borreliacidal capacities than Poly Canyon lizards. This result was surprising and other factors such as sex and season may still play roles in the differing immune function observed between sites. Male and females had relatively similar borreliacidal activities across both seasons at Chimineas, while Poly Canyon lizards varied both between sexes and season. Whereas some of this variation may also be due to an observed difference in breeding season length, some may be attributed to the effect of tick loads. Perhaps the Poly Canyon lizards have a higher exposure to other pathogens, suppressing their immune systems more than Chimineas lizards. Furthermore, the climate differs greatly between the sites and environmental factors such as available water or mean temperature may explain some of this variation in lizard immune function between sites.

Counter to our prediction, tick load was significantly correlated with *higher* borreliacidal activity in comparable lizards. Increased tick loads are generally considered indicative of reduced immune function and in males, possibly due to higher testosterone

concentrations (Pollock et al., 2012b). Studies have shown increased ectoparasites loads in male free-ranging lizards with experimentally elevated testosterone (Cox and John-Alder, 2007; Klukowski and Nelson, 2001; Olsson et al., 2000; Saino et al., 1995; Salvador et al., 1996), including *S. occidentalis* (Pollock et al., 2012a). However, it remains unclear whether increased parasite loads are due to a testosterone-mediated drop in immune function or behavioral changes. Testosterone stimulates male territorial behavior (Klukowski and Nelson, 1998; Marler and Moore, 1989; Moore, 1986; Sinervo et al., 2000) and movement (Cox et al., 2005; John-Alder et al., 2009; Sinervo et al., 2000) and may cause increased exposure of male lizards to questing ectoparasites. However, the number of ticks feeding on an individual may not be an accurate indication of immune function. This method may lead to results that are inaccurate and misleading because the number of ticks that feed to repletion is difficult to estimate. Many ticks may attach to a lizard, but not all will feed to repletion. The immune systems of tick hosts may actively reject the parasites or cause the host to increase grooming habitat that remove the ticks (Keesing et al., 2009). Because of this, using tick loads to indicate the immune status of the host requires data on the proportion of ticks that feed to repletion.

These results suggest that an increased tick load may indicate a higher borreliacidal capacity in *S. occidentalis,* despite a potentially high level of testosterone. Because testosterone has been shown to reduce complement effectiveness in vertebrates (Grieves et al., 2006; Nissen et al., 1988; Packard and Weiler, 1983), the positive effect of tick load on borreliacidal capacity may indicate study lizards have been previously exposed to *B. burgdorferi*. Previously exposed lizards would have developed specific antibodies to help combat a spirochete infection via the classical complement pathway

(CCP; Kochi et al., 1993). Furthermore, lizards that carry higher tick loads have a greater chance of being exposed to the infection by chance alone. *In vivo*, a greater tick load may serve to initiate a more robust immune response by greater stimulation. Additionally, many spirochetes may be killed inside the ticks from the influx of blood before they are released into the lizards' bodies.

Lizards showing higher tick counts have a greater chance of exposure to *B. burgdorferi* and it is possible complement-mediated bacteriolysis of infectious agents involves at least two immunological mechanisms. The CCP utilizes antibodies to start a cascade of activation events that ultimately leads to the activation of the protease C4b2a, further leading to the activation of the complement C5b-9 membrane attack complex (MAC) that induces lysis of invading bacteria by puncturing their outer membranes (Kochi et al., 1993). The alternative complement pathway (ACP) is antibody-independent and uses the protease C3bBb to start a cascade of activation events that ultimately leads to the activation of the MAC (Whaley and North, 1997). *Borrelia burgdorferi* is capable of eliciting an immune response by activating both the CCP and the ACP (Kochi and Johnson, 1988) but the effectiveness of each pathway is host specific. Kuo et al. (2000) showed that in *S. occidentalis*, complement-mediated killing of *B. burgdorferi* was accomplished primarily via the ACP. However, it is possible that antibodies acquired from prior exposure to the pathogen may act along with ACP, via the CCP, to cleanse the body of spirochetes more effectively. This possibility is supported by our data showing an increase in borreliacidal activity with increased tick load. However, it is possible that no lizards in this study had been previously exposed. The status of *I. pacificus* tick infections with *B. burgdorferi* in San Luis Obispo County is unresolved. Several rodent

hosts for these ticks routinely harbor *B. bissettii* and *B. burgdorferi*, but field collections of adult *I. pacificus* at various sites have largely yielded negative results for infection (Vredevoe et al, 2004, Baker-Branstetter (thesis in progress). This does not preclude the idea that *Borrelia* may be present in tick populations but infection levels are kept at a low rate by feedings on *S. occidentalis* as primary hosts for this tick. *In vivo*, both antibodies and C3bBb are opsonins and promote phagocytosis of antigens. This *in vitro* assay did not account for the phagocytic killing of *Borrelia* that may occur in the bodies of *S. occidentalis.*

Further studies could be done to distinguish the contribution of antibodies to the killing of *Borrelia* by *S. occidentalis*. By heat-treating lizard plasma samples, complement protein would be destroyed and the heat-labile antibodies would remain. If the heat-treated plasma was added to the heat-treated plasma of a confirmed non-exposed lizard, antibody mediated lysis would occur in the combined sample, but not in the non-exposed plasma sample alone.

Lizard tick loads not only correlated with higher borreliacidal capacities, but were also significantly different between sites, across both seasons. Poly Canyon lizards had substantially more ticks, on average, than Chimineas lizards in both Fall and Spring. This was expected based on previous knowledge of the field sites. Spring tick loads were much greater than in Fall at Poly Canyon, and only slightly higher than Fall at Chimineas. Seasonal variations in parasite loads have been observed in a wide range of animal taxa such as insects (Zuk, 1987), fish (Mitchell, 1989), birds (Teel et al., 1989), sheep (Theodoropoulos et al., 1998), and lizards (Eisen and Eisen, 1999; Schall et al., 2000; Eisen et al., 2001; Lumbad et al., 2011, Pollock et al., 2012a). Host sex did not

significantly affect lizard tick load, despite many studies showing that male animals across diverse taxa typically exhibit higher parasite loads than females (Poulin, 1996; Zuk and McKean, 1996; Anthony et al., 1994; Aubret et al., 2005; Klukowski and Nelson, 2001; Moore and Wilson, 2002; Morand et al., 2004; Folstad et al., 1989; Zuk, 1990; Tschirren et al., 2003), including lizards (Salkeld and Schwarzkopf, 2005; Schall and Marghoob, 1995; Schall et al., 2000). Testosterone is believed to be the primary reason for this increase in parasite loads in males; it has been shown that male lizards with experimentally elevated testosterone have higher parasite loads than control males (Cox and Alder, 2007; Hughes and Randolph, 2001; Klukowski and Nelson, 2001; Olsson et al., 2000; Roberts et al., 2004; Saino et al., 1995; Salvador et al., 1996), however the mechanism by which this happens is unknown.

Tick loads on lizards significantly predicted higher borreliacidal capacities, however further studies need to be conducted to determine the mechanism behind this phenomenon. Besides being major hosts to *I. pacificus*, *S. occidentalis* can also host substantial numbers of mites of the genera *Eutrombicula* and *Geckobiella* (Allred and Beck, 1962; Klukowski, 2004; Schall et al., 2000; Schall and Smith, 2006). These ectoparasites commonly congregate in the lizards' nuchal pouch, a fold of skin near the tympanum, but will also attach to other areas of exposed skin such as around the eyes and between scales (Arnold, 1986; Goldberg and Bursey, 1991; Dunlap and Mathies, 1993; Pollock et al., 2012a). Because tick loads in this study were determined by counting visible ticks only, it is possible that more ectoparasites were hosted by study lizards without our knowledge in obscured places such as between scales. This may have led to an underestimation of tick loads on the lizards in this study and may have skewed the

results. Additionally, life stage of ticks observed on lizards was not determined and may have implications to an individual lizard's borreliacidal capacity. Transmission of spirochetes to vertebrate hosts is primarily accomplished by nymphal ticks (Mannelli et al. 2011). Larvae are typically born free of spirochetes and adults tend to host on large-bodied vertebrates that make poor reservoirs. To fully elucidate the effect of tick load on borreliacidal capacity, future studies should accurately quantify the number of ectoparasites on each lizard (not just visible individuals), determine the life stage of each parasite, and determine what proportion of ectoparasites feed until repletion.

We hypothesized that host sex would affect borreliacidal capacity and predicted female lizards would have higher borreliacidal capacities than males. Females generally have better immune function than males, often due to the inhibitory effects of testosterone (Folstad and Karter, 1992; Klein, 2000; Mondal and Rai, 1999, 2002; Pollock et al., 2012(b); Tschirren et al., 2003; Zuk and McKean, 1996). Testosterone has been implicated as being immunosuppressive in many vertebrates (Klein, 2000; Mondal and Rai, 1999, 2002) including *S. occidentalis* (Pollock et al., 2012(a), Pollock et al., 2012b). For example, Grossman (1985) showed that the mass of organs involved with immune function increased in male rats after castration, and furthermore, immunoglobulin production was overall higher in females. In this study, male and female lizards did not have significantly different borreliacidal capacities, causing us to reject the hypothesis that host sex affects borreliacidal capacity in *S. occidentalis.* The effect of sex on lizard borreliacidal capacity did, however, change with season at both sites, although not significantly. Borreliacidal capacity differed only slightly between the sexes and seasons at Chimineas, with both sexes maintaining a relatively high borreliacidal capacity

over Fall and Spring. Conversely, at Poly Canyon, males and females had lower capacity in Fall and higher in Spring. On average, sampled populations of females from Poly Canyon, which showed low borreliacidal capacities in the Fall, had relatively elevated capacities in Spring. Male lizards maintained slightly higher borreliacidal capacities than females in the Fall, decreasing slightly in Spring and averaging much lower than females. This observed decrease in borreliacidal capacity in males from Fall to Spring is likely due to the immunosuppressive effects of testosterone. In *S. occidentalis*, natural fluctuations in testosterone occur throughout the year, with peak levels in males occurring during the breeding season, typically spring (Taylor et al., unpublished data). Because of this seasonality in testosterone levels and its known immunosuppressive effects, we predicted male and female lizards would show greater immunosuppression, and thus, lower borreliacidal capacities in Spring. Many studies have illustrated changes in immune function in correlation with breeding season (Saad and Elridi, 1984; Kortet et al., 2003; Lozano and Lank, 2003).

Sampling season did not, however, significantly predict lizard borreliacidal capacity. Male and female lizards from Chimineas showed only a slight decrease in borreliacidal capacity from Fall to Spring, and Poly Canyon males and females showed an unexpected *increase*. A site-related difference observed in breeding condition may be responsible for the unexpected and differing seasonal effects between sites. In Fall of 2012, two of the eight Poly Canyon females were palpably gravid at the time of capture whereas no females were observed reproductive at Chimineas. This extremely late breeding pulse is not typically observed in *Sceloporus* species. Numerous studies have shown that increased reproductive effort leads to decreased immune function (Nordling et

al., 1998; Cichon et al., 2001; Ardia, 2005) including in female tree lizards (French et al., 2007(a), 2007b). It is possible that female lizards have an energetic cost due to offspring investment that negatively influences immune function. This would lead to a decrease in body condition. The late season breeding pulse we observed was accompanied by a diminished body condition in Poly Canyon lizards in fall. Both males and females from Poly Canyon in the fall had lower average body conditions than lizards sampled in spring and Chimineas in fall, significantly in some cases (see Table 6). The potentially prolonged breeding season observed at Poly Canyon may have been responsible, in part, for the relatively low borreliacidal capacities observed in both female and male lizards during Fall 2012 by lowering body condition.

Successive and late season reproductive bouts from 2012 may represent increased reproductive effort for this breeding season, and may be responsible for decreased immune function in late season breeders. The higher borreliacidal capacities observed in Spring 2013 Poly Canyon lizards were recorded near the beginning of the breeding season when their immune system may not have been as depressed as at the end of the breeding season after potentially laying many clutches, each increasing the overall reproductive effort. Males from Chimineas did show a decrease, on average, in borreliacidal capacity from Fall to Spring, but not significantly. The gravid females from Poly Canyon in Fall 2012 did not show reduced borreliacidal capacities compared to the other females. However, palpating for gravidity may not always detect a female in reproductive condition. Other females may have been gravid without being detected. Future studies could conduct a radioimmunoassay to determine level of reproductive

hormones to determine if reproductive condition affects borreliacidal capacity in *S. occidentalis*.

Individual lizard body condition, as measured by the ordinary least squares residual (OLSR) method, did not have a significant effect on borreliacidal capacity. We expected lizards with higher body condition to have stronger immune responses because body condition is closely related to an animal's health and vigor and may be an indication of an individual's fitness (Peig and Green, 2009). Immune function has been shown to decrease as body condition decreases across many taxa (eg. Chandra and Newberne, 1977;), including pythons (Ujvari and Madsen, 2005), snapping turtles (Borysenko and Lewis, 1979), and birds (Navarro et al., 2003). Nutrition is important for immune function and malnutrition causes lowered function of neutrophils, macrophages, and natural killer cells (Stephenson, 2001). Theoretically, lizards that are able to obtain more food and nutrients have a correspondingly higher mass per unit length. In this study, variation in lizard body condition was relatively low and may not show much of the range of body condition in *S. occidentalis.* With a larger sample size, we could have sampled lizards of much lower and higher body conditions, and possibly clarify the role of body condition in borreliacidal capacity. Recently, calculating body condition accurately in vertebrates has come into debate with many methods being described (Stevenson and Woods, 2006; Murphy et al., 1991; LeCren, 1951; Schulte-Hostedde et al., 2001, Garcia-Berthou, 2001). Peig and Green (2010) reviewed and provided critical comments on these methods, suggesting the use of a scaled mass index method because it accounts for the changing relationship between mass and length as body size changes and growth occurs. However, Schulte-Hostedde et al. (2005) investigated the OLSR method

in relation to the fat content and lean dry mass of small mammals and found the OLSR method to be an accurate metric of body condition. We chose to use the OSLR method because it is simple and has been shown to accurately estimate body condition. Furthermore, *S. occidentalis* is a relatively small vertebrate and using a scaled mass index to account for body size changes during growth would have little effect on body condition calculations.

The assay used in this study to measure the borreliacidal capacity of each lizard was similar to the methods used by Kuo et al. (2000), but was modified to be sensitive enough to compare individuals of the same species. This technique was difficult to optimize and may be prone to some variability. For example, the *Borrelia* stock culture had varying results to the same lizard's plasma when assayed at different times. Much of this variation may be attributed to the growth cycles of *Borrelia* bacteria en vitro. Because of this variation, negative and positive controls were used as standards, and measured each assay session. The possibility of variation in the stock culture and optimization of the assay may be part of the reason for variation in borreliacidal capacity within experimental groups and may partially explain the relatively large standard errors.

Our results suggest that both developmental traits (age-class) and environmental variability (field site and tick load) affect borreliacidal capacity in *S. occidentalis*. However, each lizard was sampled only once and not tracked through the different seasons of the study. These results give no indication of how the borreliacidal capacity of each individual lizard varies over time, seasonally or over its lifetime. A mark-recapture or controlled laboratory study should be conducted to elucidate the effects of

environmentally variable factors versus variation in the borreliacidal capacity of individual *S. occidentalis*.

Due to their capability of complement-mediated killing of *B. burgdorferi,* and the cleansing of infected *I. pacificus* that feed on them, *S. occidentalis* is generally thought to be a factor in controlling Lyme disease prevalence in the western United States (Eisen et al., 2004; Salkeld and Lane, 2010). In the western United States, *B. burgdorferi* is transmitted by *I. pacificus* (Burgdorfer et al., 1985) and is maintained by reservoir hosts including the dusky-footed woodrat (*Neotoma fuscipes*), western grey squirrel (*Sciurus griseus*), California kangaroo rat (*Dipodomys californicus*), and deer mouse (*Peromyscus maniculatus*) (Lane and Brown, 1991; Brown and Lane, 1992; Lane et al., 2005; Salkeld and Lane, 2010). Despite the many mammalian reservoir hosts, lizards host the largest proportion of larval and nymphal *I. pacificus*, around 90 percent (Casher et al., 2002). Additionally, the blood of western fence lizard and southern alligator lizard (*Elgaria* multicarinata) actively kills the *Borrelia* spirochetes (Kuo et al., 2000). However, the lizards' role in the risk of human transmission is not fully understood. Swei et al. (2011) observed that when lizards were removed from a field site, the density of infected ticks was reduced because of a lack of hosts for larval and nymphal stages, and thereby reducing the risk of *Borrelia* exposure to humans. Although the lizards are incompetent reservoirs, they amplify the tick populations leading to potentially greater exposure to competent reservoir hosts as well as humans. Further investigation is necessary to elucidate the western fence lizard's role in Lyme disease transmission risk. Understanding the physiological factors that affect the borreliacidal capacity of *S. occidentalis* can help efforts to map the spread of Lyme disease, understand its disease

ecology, and predict how regimes may change with climate change and habitat fragmentation.

In this study we found that lizard host age class, field site, and visible tick load significantly affect their ability to kill *Borrelia burgdorferi*. However, the reasons for these observed effects are not fully understood. Future studies should work to determine the mechanisms by which these observed effects create this effect. Lizard mass, field site, and tick load may vary along with one another and teasing apart the effects of each may be difficult. Greater sample sizes, accurate ectoparasite counts, and modeling techniques may help elucidate the effects of these factors.

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APPENDICES

Appendix A: Tables

Table 1: The known vertebrate hosts of *Ixodes pacificus* adults (A), nymphs (N), and larvae (L) (compiled from Castro and Wright, 2007. Hosts marked with an asterisk (*) are compiled from Newman et al. 2015 with no stage data available. Each hosts' competency to *Borrelia burgdorferi* sensu stricto is noted if known. Competency to *Borrelia burgdorferi* sensu lato (BSSL) if known and the source of which is provided.

Host Scientific Name	Host Common Name	Tick Stage	*Borrelia* Competency	Source
Class Reptilia				
Aspidoscelis tigris	Western whiptail	N, L		
Elgaria caerulea	Northern alligator lizard	N, L		
Elgaria multicarinata	Southern alligator lizard	N, L		
Elgaria sp.	Alligator lizard	A, N, L	Competent	Kuo et al. 2000
Eumeces gilberti	Gilbert's skink	N, L	Possibly Competent	Levin et al. 1996
Eumeces skiltonianus	Western skink	N, L	Possibly Competent	Levin et al. 1996
Sceloporus graciosus	Sagebrush lizard	N, L	Incompetent	
Sceloporus occidentalis	Western fence lizard	N, L	Incompetent	Kuo et al. 2000
Uta stansburiana	Common side-blotched lizard	N, L		
Class Aves				
Agelaius phoeniceus	Red-winged blackbird	*		
Aimophila ruficeps	Rufous-crowned sparrow	N, L		
Aphelocoma californica	Western scrub jay	N, L		
Baeolophus inornatus	Oak titmouse	N, L	Competent	Newman et al. 2015
Callipepla californica	California quail	N, L		
Carduelis psaltria	Lesser goldfinch	L	Competent	Newman et al. 2015

Host-Scientific Name	Host Common Name	Tick Stage	Borrelia Competency	Source
Carpodacus mexicanus	House finch	L		
Carpodacus purpureus	Purple finch	N, L		
Catharus ustulatus	Swainson's thrush	N, L		
Certhia americana	Brown creeper	N, L		
Chamaea fasciata	Wrentit	L		
Chondestes grammacus	Lark sparrow	N, L		
Colaptes auratus	Northern flicker	N, L		
Contopus sordidulus	Western wood-pewee	*		
Corvus brachyrhynchos	American crow	N, L		
Cyanocitta stelleri	Steller's jay	N, L		
Dendroica nigrescens	Black-throated gray warbler	L	Competent	Newman et al. 2015
Empidonax difficilis	Pacific-slope flycatcher	*		
Euphagus cyanocephalus	Brewer's blackbird	L		
Icterus bullockii	Bullock's oriole	N, L		
Junco hyemalis	Dark-eyed junco	A, N, L	Competent	Newman et al. 2015
Melanerpes formicivorus	Acorn woodpecker	N, L		
Meleagris gallopavo	Wild turkey	N		
Melospiza melodia	Song sparrow	N, L		
Myiarchus cinerascens	Ash-throated flycatcher	L		
Otus kennicottii	Western screech owl	N, L		
Passerella iliaca	Fox sparrow	N, L		
Passerina amoena	Lazuli bunting	N, L		
Pheucticus melanocephalus	Black-headed grosbeak	N, L	Competent	Newman et al. 2015
Picoides nuttallii	Nuttall's woodpeckers	L		
Pipilo crissalis	California towhee	N, L		
Pipilo maculatus	Spotted towhee	N, L		

Host-Scientific Name	Host Common Name	Tick Stage	Borrelia Competency	Source
Piranga ludoviciana	Western tanager	*		
Psaltriparus minimus	Bushtit	L		
Sialia mexicana	Western bluebird	N	Competent	Newman et al. 2015
Sitta carolinensis	White-breasted nuthatch	N, L		
Spizella passerina	Chipping sparrow	N, L		
Sturnus vulgaris	European starling	N, L		
Thryomanes bewickii	Bewick's wren	A, N, L	Competent	Newman et al. 2015
Toxostoma redivivum	California thrasher	N, L		
Troglodytes aedon	House wren	N, L		
Troglodytes troglodytes	Winter wren	N		
Turdus migratorius	American robin	N, L		
Vermivora celata	Orange-crowned warbler	L	Competent	Newman et al. 2015
Vermivora ruficapilla	Nashville warbler	L		
Vireo cassinii	Cassin's vireo	N	Competent	Newman et al. 2015
Vireo gilvus	Warbling vireo	N, L		
Vireo huttonii	Hutton's vireo	N, L		
Wilsonia pusilla	Wilson's warbler	N		
Zonotrichia atricapilla	Golden-crowned sparrow	N, L	Competent	Newman et al. 2015
Zonotrichia leucophrys	White-crowned sparrow	N, L		
Class Mammalia				
Axis axis	Axis deer	A		
Bos taurus	Cow	A	BBSL	Anderson, 1988
Canis familiaris	Dog	A, N	BBSL	Anderson, 1988
Canis latrans	Coyote	A		
Capra hircus	Goat	A		
Cervus elapus nannodes	Tule elk	A		

Host-Scientific Name	Host Common Name	Tick Stage	Borrelia Competency	Source
Chaetodipus californicus	California pocket mouse	A, N, L		
Chaetodipus fallax	San Diego pocket mouse	L		
Chaetodipus penicillatus	Desert pocket mouse	N		
Chaetodipus spinatus	Spiny pocket mouse	L		
Dama dama	Fallow deer	A		
Didelphis virginiana	Virginia opossum	N, L	BBSL	Anderson, 1988
Dipodomys agilis	Pacific kangaroo rat	L		
Dipodomys californicus	California kangaroo rat	N, L		
Dipodomys deserti	Desert kangaroo rat	A, N		
Dipodomys venustus	Narrow-faced kangaroo rat	N		
Equus caballus	Horse	A	BBSL	Anderson, 1988
Equus hybrid	Mule	A		
Felis catus	Cat	A, N	BBSL	Anderson, 1988
Glaucomys sabrinus	Northern flying squirrel	L		
Homo sapiens	Human	A, N, L		
Lepus californicus	Black-tailed jackrabbit	A, N, L		
Lynx rufus	Bobcat	A		
Microtus californicus	California vole	A, N, L		
Microtus townsendii	Townsend's vole	L		
Mus musculus	House mouse	L		
Mustela frenata	Long-tailed weasel	A		
Neotoma fuscipes	Dusky-footed woodrat	A, N, L		
Neotoma lepida	Desert woodrat	A, N, L		
Odocoileus virginianus	White-tailed deer		BBSL	Anderson, 1988
Odocoileus h. columbianus	Columbian black-tailed deer	A, N, L		
Peromyscus boylii	Brush mouse	N, L		

Host-Scientific Name	Host Common Name	Tick Stage	Borrelia Competency	Source
Peromyscus californicus	California mouse	N, L		
Peromyscus eremicus	Cactus mouse	N, L		
Peromyscus maniculatus	Deer mouse	N, L		
Peromyscus truei	Pinyon mouse	N, L		
Procyon lotor	Raccoon	A, N	BBSL	Anderson, 1988
Puma concolor	Mountain lion	A, N		
Rattus rattus	Black rat	N, L		
Reithrodontomys megalotis	Western harvest mouse	A, N, L		
Scapanus latimanus	Broad-footed mole	L		
Sciurus griseus	Western gray squirrel	N, L		
Sorex vagrans	Vagrant shrew	L		
Spermophilus beecheyi	California ground squirrel	A, N, L		
Sus scrofa	Wild pig	A		
Sylivagus audubonii	*Audubon's cottontail*	*A*		
Sylivagus bachmani	*Brush rabbit*	*N, L*		
Tamias quadrimaculatus	Long-eared chipmunk	N		
Tamias senex/ochrogenys	Allen's/Yellow-cheeked	N, L		
Tamias sonomae	Sonoma chipmunk	N, L		
Taxidea taxus	American badger	A		
Urocyon cinereoargenteus	Gray fox	A		
Urocyon littoralis	Island gray fox	A, N		
Ursus americanus	Black bear	A		
Peromyscus leucopus	White-footed mouse		BBSL	Anderson, 1988
Tamias striatus	Eastern chipmunk		BBSL	Anderson, 1988
Ammotragus lervia	Wild sheep		BBSL	Anderson, 1988
Sciurus carolinensis	Gray squirrel		BBSL	Anderson, 1988

Table 2: Collection dates, locations, sample sizes, and age class of *Sceloporus occidentalis* from which blood samples were taken. A subset of these lizards was used for the borreliacidal assay.

Date	Site	# of Males	# of Females
Adults			
9/15/2012	Poly	17	24
9/16/2012	Poly	6	2
9/22/2012	Chimineas	30	20
4/13/2013	Poly	34	19
4/20/2013	Chimineas	20	24
Juveniles			
8/15/2013	Poly	1	1
8/19/2013	Poly	8	3

Table 3: Sample sizes and mean corrected percent mortality ($\mathbf{K_C}$) ± 1 SEM of adult *Sceloporus occidentalis* across both sites and seasons. $\mathbf{K_C}$ is a metric for the borreliacidal capacity of *S. occidentalis.*

		Fall 2012		**Spring 2013**	
Site	**Sex**	**n**	**K_C ± SE**	**n**	**K_C ± SE**
Poly Canyon	Male	4	24.7 ± 12.3	8	39.3 ± 11.4
	Female	8	19.5 ± 4.5	9	60.6 ± 14.4
Chimineas	Male	9	61.8 ± 15.2	5	40.4 ± 19.0
	Female	5	62.3 ± 15.7	9	56.0 ± 11.7

Table 4: Sample sizes and mean corrected percent mortality ($\mathbf{K_C}$) ± 1 SEM of all juvenile *Sceloporus occidentalis* from Poly Canyon in Fall 2013.

Sex	**n**	**K_C ± 1 SEM**
Males	9	3.4 ± 3.7
Females	4	-1.8 ± 2.4

Table 5: Statistical values from the Tukey-Kramer HSD post-hoc test on the borreliacidal capacity of adult *Sceloporus occidentalis* experimental groups.

^MPF = Males from Poly Canyon in Fall 2012; FPF = Females from Poly Canyon in Fall 2012, MCF = Males from Chimineas in Fall 2012; FCF = Females from Chimineas in Fall 2012; MPS = Males from Poly Canyon in Spring 2013; FPS = Females from Poly Canyon in Spring 2013; MCS = Males from Chimineas in Spring 2013; FCS = Female from Chimineas in Spring 2014.

Comparison	To	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FCF	FPF	1.188632	0.5079409	-0.42067	2.797938	0.2941
FPS	FPF	1.137353	0.4329420	-0.23433	2.509040	0.1716
FCS	FPF	1.075559	0.4329420	-0.29613	2.447246	0.2268
FCF	MPF	1.064834	0.5976924	-0.82883	2.958499	0.6351
FPS	MPF	1.013556	0.5354167	-0.68280	2.709913	0.5622
MCF	FPF	1.012695	0.4329420	-0.35899	2.384382	0.2946
FCF	MPS	0.961375	0.5217087	-0.69155	2.614301	0.5952
FCS	MPF	0.951762	0.5354167	-0.74460	2.648119	0.6376
FPS	MPS	0.910096	0.4490154	-0.51252	2.332708	0.4754
MCF	MPF	0.888897	0.5354167	-0.80746	2.585254	0.7118
FCS	MPS	0.848302	0.4490154	-0.57431	2.270914	0.5647
MCF	MPS	0.785438	0.4490154	-0.63717	2.208050	0.6559
FCF	MCS	0.692608	0.5635098	-1.09276	2.477972	0.9191
FPS	MCS	0.641330	0.4969690	-0.93321	2.215873	0.8979
FCS	MCS	0.579535	0.4969690	-0.99501	2.154079	0.9376
MCF	MCS	0.516671	0.4969690	-1.05787	2.091214	0.9657
MCS	FPF	0.496024	0.5079409	-1.11328	2.105329	0.9757
MCS	MPF	0.372226	0.5976924	-1.52144	2.265891	0.9984
MCS	MPS	0.268767	0.5217087	-1.38416	1.921693	0.9995
MPS	FPF	0.227257	0.4611297	-1.23374	1.688251	0.9996
FCF	MCF	0.175937	0.4969690	-1.39861	1.750480	1.0000
FPS	MCF	0.124658	0.4200154	-1.20607	1.455390	1.0000
MPF	FPF	0.123798	0.5456161	-1.60487	1.852469	1.0000
FCF	FCS	0.113073	0.4969690	-1.46147	1.687616	1.0000
MPS	MPF	0.103460	0.5584558	-1.66589	1.872811	1.0000
FCS	MCF	0.062864	0.4200154	-1.26787	1.393596	1.0000
FPS	FCS	0.061794	0.4200154	-1.26894	1.392526	1.0000
FCF	FPS	0.051279	0.4969690	-1.52326	1.625822	1.0000

Table 6: Statistical values from the Tukey-Kramer HSD post-hoc test on the body condition of adult *Sceloporus occidentalis* experimental groups.

^MPF = Males from Poly Canyon in Fall 2012; FPF = Females from Poly Canyon in Fall 2012, MCF = Males from Chimineas in Fall 2012; FCF = Females from Chimineas in Fall 2012; MPS = Males from Poly Canyon in Spring 2013; FPS = Females from Poly Canyon in Spring 2013; MCS = Males from Chimineas in Spring 2013; FCS = Female from Chimineas in Spring 2014.

Comparison	To	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FCS	FPF	3.556289	0.6988112	1.34225	5.770328	0.0002*
FCS	MPF	2.805385	0.8642155	0.06730	5.543473	0.0410*
MPS	FPF	2.299135	0.7443089	-0.05905	4.657323	0.0608
MCF	FPF	2.243119	0.6988112	0.02908	4.457158	0.0450*
FCS	FPS	2.242677	0.6779464	0.09474	4.390610	0.0350*
MCS	FPF	1.944106	0.8198668	-0.65347	4.541684	0.2786
FCF	FPF	1.812966	0.8198668	-0.78461	4.410544	0.3635
FCS	FCF	1.743323	0.8021570	-0.79814	4.284792	0.3855
FCS	MCS	1.612184	0.8021570	-0.92928	4.153652	0.4863
MPS	MPF	1.548230	0.9014029	-1.30768	4.404139	0.6761
MCF	MPF	1.492215	0.8642155	-1.24587	4.230302	0.6703
FPS	FPF	1.313612	0.6988112	-0.90043	3.527651	0.5709
FCS	MCF	1.313170	0.6779464	-0.83476	3.461103	0.5334
FCS	MPS	1.257155	0.7247552	-1.03908	3.553392	0.6653
MCS	MPF	1.193201	0.9647347	-1.86336	4.249763	0.9166
FCF	MPF	1.062061	0.9647347	-1.99450	4.118623	0.9535
MPS	FPS	0.985522	0.7247552	-1.31071	3.281759	0.8705
MCF	FPS	0.929507	0.6779464	-1.21843	3.077440	0.8656
MPF	FPF	0.750904	0.8806782	-2.03934	3.541151	0.9888
MCS	FPS	0.630494	0.8021570	-1.91097	3.171962	0.9931
FPS	MPF	0.562708	0.8642155	-2.17538	3.300796	0.9978
FCF	FPS	0.499354	0.8021570	-2.04211	3.040822	0.9984
MPS	FCF	0.486169	0.8420894	-2.18182	3.154155	0.9990
MCF	FCF	0.430153	0.8021570	-2.11132	2.971622	0.9994
MPS	MCS	0.355029	0.8420894	-2.31296	3.023015	0.9999
MCF	MCS	0.299013	0.8021570	-2.24246	2.840482	0.9999
MCS	FCF	0.131140	0.9095606	-2.75061	3.012894	1.0000
MPS	MCF	0.056016	0.7247552	-2.24022	2.352252	1.0000

Appendix B: Figures

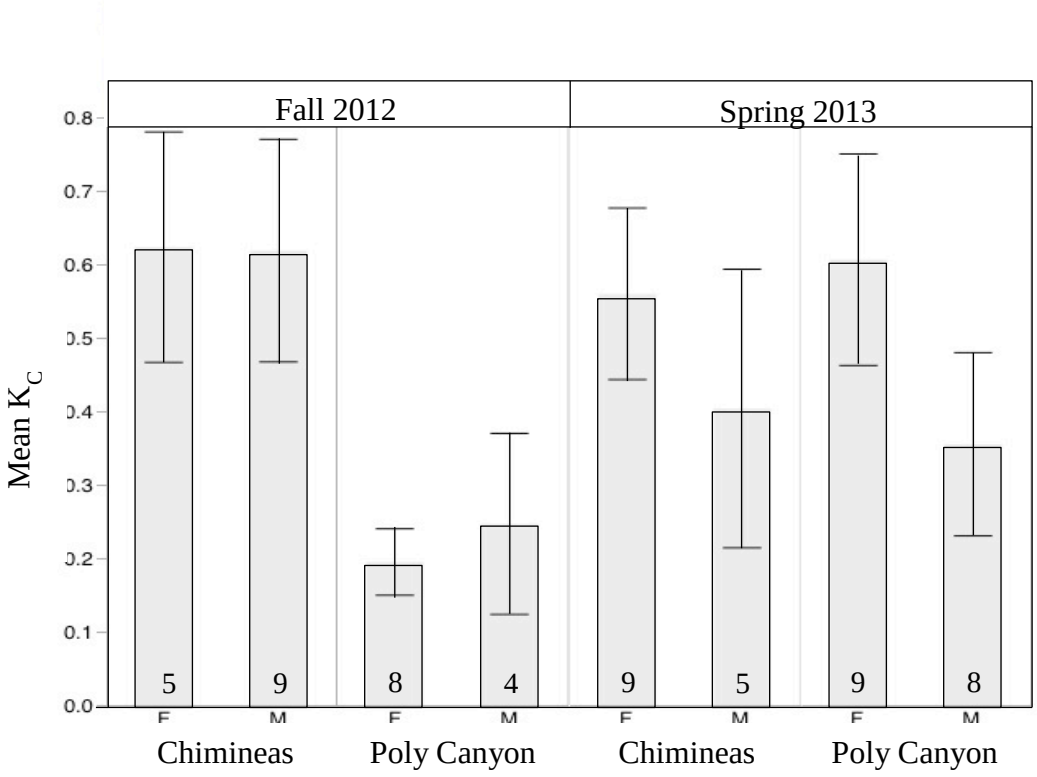

Figure 1: Mean borreliacidal capacity (K_C) for male (M) and female (F) *Sceloporus occidentalis* from Poly Canyon and Chimineas for Fall 2012 and Spring 2013 showed a significant effect of site, but non-significant effects of season and sex. Numbers within the bars represent sample size (N). Data backtransformed to original values. Error bars represent 1 SEM.

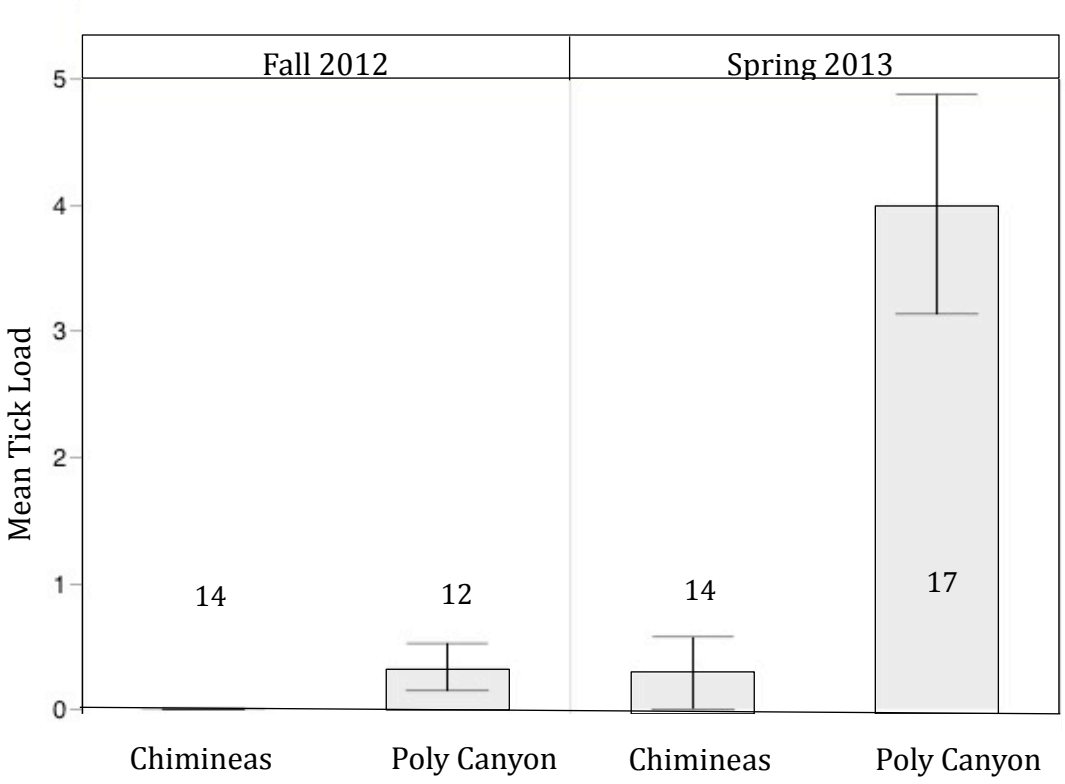

Figure 2: Adult *S. occidentalis* had a significantly higher mean tick loads at Poly Canyon than at Chimineas Ranch in spring but remained low at both sites in fall. Tick load represents the mean number of subadult ticks on individual lizards. Numbers within or above the bars represent sample size (N). Error bars represent 1 SEM.

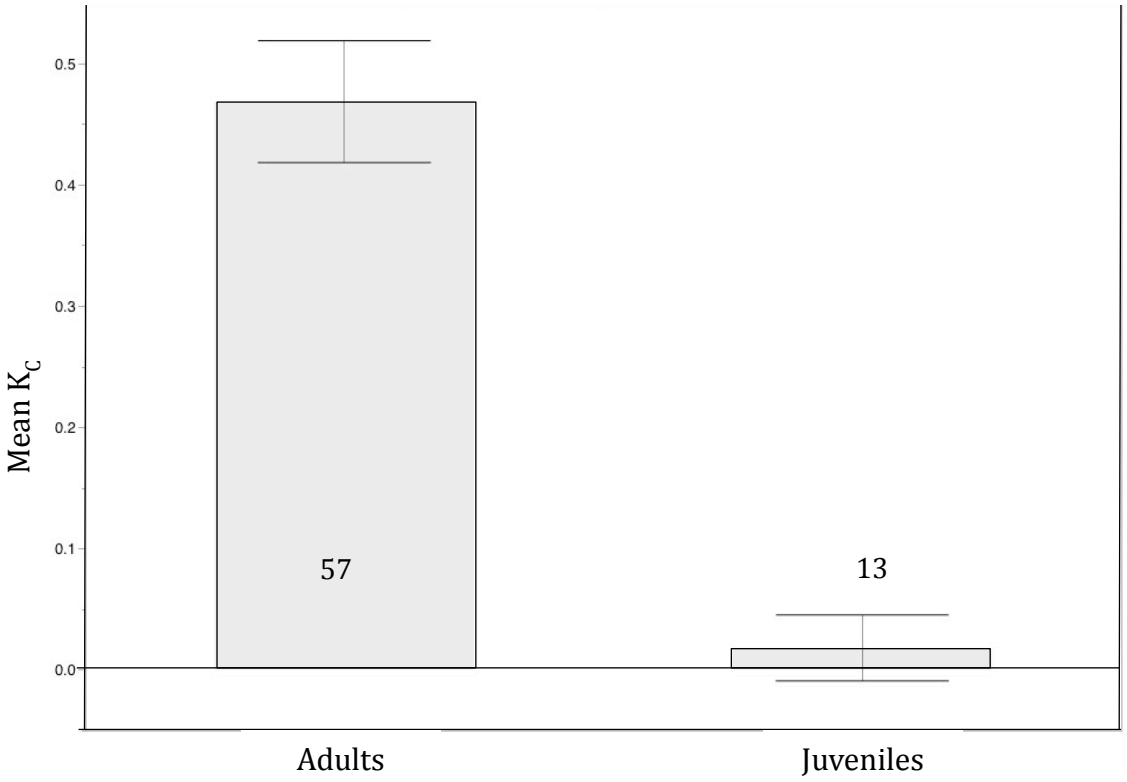

Figure 3: Adult *S. occidentalis* had significantly higher mean borreliaciadal capacity (K_C) than juveniles. Numbers within or above the bars represent sample size (N). Error bars represent 1 SEM.