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# Survival of Off-Host Rhipicephalus (Boophilus) annulatus (Acari: Ixodidae) Larvae in Study Arenas in Relation to Climatic Factors and Habitats in South Texas, USA

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# SURVIVAL OF OFF-HOST RHIPICEPHALUS (BOOPHILUS) ANNULATUS (ACARI: IXODIDAE) LARVAE IN STUDY ARENAS IN RELATION TO CLIMATIC FACTORS AND HABITATS IN SOUTH TEXAS, USA.

A Thesis

by

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## ABSTRACT

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The cattle fever tick, *Rhipicephalus* (*Boophilus*) *annulatus* (Say), is an economically destructive arthropod because of its ability to vector bovine babesiosis. Cattle fever ticks can spend more than 90% of their life cycle as questing larvae, but the effect of climatic factors on their off-host behavior and survival is unclear. The goal of this study was to measure the effects of specific ecological factors on off-host larvae in nature. The study was conducted in a south Texas pasture over a 20-mo period, during which time larval populations were surveyed and ambient weather variables - relative humidity and temperatures – were recorded. Oviposition success and larval survival varied between cattle fever tick cohorts and was affected by relative humidity and canopied (with tree cover) versus exposed habitat. The results show that relative humidity and the interaction of relative humidity and inhabiting canopied habitats play a key role in oviposition success. Additionally, canopied habitats have a positive influence on off-host larval survival in the spring and summer.

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# CHAPTER I

## INTRODUCTION

Ixodid ticks belonging to the subgenus *Boophilus* are known for vectoring bovine babesiosis or "cattle tick fever."(Awad et al. 2011). Babesiosis is caused by a hemolytic protozoan of the genus *Babesia,* which is transferred transovarially from adult female tick to her larvae. The larvae then transmit *Babesia* spp. and thus babesiosis to the host animal (Howell et al. 2007). Babesiosis was widespread throughout the US during the nineteenth century until a nationwide eradication program was implemented in 1906. This resulted in eradication of babesiosis in the US by 1946 and the establishment of a permanent quarantine "buffer" zone along the Rio Grande between Texas and Mexico. While measures are being taken to avoid the reintroduction of ticks that vector the disease into the US, there are still incidences of infestation along the Texas-Mexico border (Graham and Hourrigan 1977). This coupled with the difficulties of monitoring free-roaming hosts such as nilgai and white-tailed deer complicates eradication efforts.

Most of the *Boophilus* tick's life cycle is off host (not feeding). Larvae pose the greatest threat to infecting cattle and potentially spreading disease. It is the larval stage that is responsible for the transmission of *Babesia bovis* (Howell et al. 2007). However, most eradication efforts focus on pasture vacation or treatment such as vaccination and dipping of cattle in acaricides. Currently the only off-host strategy is closing a pasture when ticks are found and removing the cattle (the blood meal host) that occupy it. This does not account for free-roaming host nor does

it provide any direct suppression of larval ticks. In ideal conditions larval ticks can survive up to 8 months, but in most cases being off-host subjects them to increased environmental stress, limits hydration and provides no food source. Therefore, a better understanding of the ecological conditions that affect larval survival would be beneficial for developing novel off-host management strategies.

To date, most ecological studies exploring the effects of climate and habitat on *Boophilus* larvae have been conducted on *Rhipicephalus (Boophilus) australis* (*R. (B.) australis*) and *Rhipicephalus (Boophilus) microplus (R. (B.) microplus*) , leaving a paucity of literature on *Rhipicephalus (Boophilus) annulatus (R. (B.) annulatus).* Therefore, the goal of this study is to strengthen our understanding of climatic factors that affect the development and survival of off-host *R. annulatus* larvae. Specifically, ecological factors in south Texas, an area located in the quarantine zone and the gateway to potentially infesting the rest of the US. This work will be utilized to create a clearer foundation for future ecological studies to help enhance off-host eradication efforts in south Texas.

I hypothesize that *R. annulatus* larvae will have a greater survival in canopied habitats during times of high humidity and mild temperatures. Larvae may have a higher chance of survival if they are not in direct sunlight, and shielded underneath tree canopies. Additionally, temperature and humidity are the two most influential factors on tick larvae survival (Davey et al. 1991).

# CHAPTER II

## REVIEW OF LITERATURE

Ticks are described as hematophagous, obligate ectoparasites of terrestrial vertebrates. They fall under the arthropod Class Arachnida, order Acari, which shares the suborders Parasitiformes (mites) and Ixodida (ticks). Ixodida are unique among the Acari in that they possess a large body size (2-30 mm) and have specialized mouthparts (Black and Piesmant 1994). Ixodida are further divided into three families: Ixodidae (hard ticks), Argasidae (soft ticks), and Nuttaliellidae (containing a monotypic genus that shares characteristics of both Ixodidae and Argasidae).

Ixodidae are referred to as "hard ticks" due to the possession of a hard shield called the "scutum" that Argasidae lack (Storer et al. 2003). While both hard and soft ticks are responsible for vectoring disease in humans and animals, Ixodidae are of greater medical significance because they are more common and more difficult to remove than soft ticks, resulting in higher chances of disease transmission (Storer et al. 2003, Pitches 2006).

Ixodidae also have important economic and agricultural implications. In particular, species of the subgenus *Boophilus* vector bovine babesiosis or "cattle tick fever". Bovine babesiosis is a disease caused by an intraerythrocytic apicomplexan protozoan parasites of the genus *Babesia*, and is mostly found in tropical and sub-tropical areas (Alonso et al. 1992, Awad et al. 2011, Rios-Tobon et al. 2014). *Babesia* spp. are ranked as the most economically important

arthropod-transmitted pathogen of livestock worldwide (Bock et al. 2004). Clinical signs of infected cattle include fever, depression, hemoglobinuria, jaundice, anemia, abortion, diarrhea, muscle wasting, tremor, convulsions, and coma (Vos and Waal 2004).

*Boophilus* ticks are responsible for vectoring babesiosis in cattle specifically in southern Europe, southern USA, Australia, and Latin America (Bock et al. 2004, Ravindran et al. 2006). Babesiosis took a toll on the American cattle industry and economy in the nineteenth century which eventually led to the implementation of an eradication program in 1906. The cattle fever tick, *R.(B.) annulatus*, as well as the related *R.(B.) microplus*, were almost entirely eradicated from the United States by 1946 with the exception of a permanent quarantine "buffer" zone along the Rio Grande in south Texas (Graham and Hourrigan 1977). While measures are being taken to prevent the reintroduction of these cattle fever ticks, there are periodically recurring infestations along the south Texas-Mexico border (Davey 1986, Lohmeyer et al. 2011). Difficulty in controlling free-roaming ungulate hosts, such as nilgai and white-tailed deer, along with the fact that most of the cattle fever tick life cycle is off-host complicates eradication efforts (Nuñez et al. 1985).

Most tick species undergo a four-part life cycle that includes the egg, larval, nymph, and adult stages. Ixodidae ticks undergo either one-host, two-host, or three-host life cycles. *Boophilus* ticks are one-host ticks, spending 80-90% of their lifecycle off-host as questing larvae and commencing through the larval, nymphal, and adult stages of their life cycle on-host (Fig. 1) (Needham and Teel 1991, Randolph 2004). Gravid females lay their eggs off-host. These eggs hatch into six-legged larvae which then seek hosts by questing. Questing describes a behavior in which larvae seek hosts by climbing up vegetation and form clusters with other larvae. Forming these clusters not only helps the larvae conserve water and moisture, it aids in ensuring greater

numbers of larvae will attach to a host. The larvae will then extend their legs and wait for a host to pass by (Davey et al. 1991, Yoder and Knapp 2009). Larvae will cling onto a passing host and hoist the cluster onto the host to blood feed and continue their lifecycle on-host (Fig. 2) (Nicholson et al. 2019).

In addition to the majority of the *Boophilus* life-cycle consisting of the larval off-host stage, larvae and nymphs are responsible for the transmission of *Babesia* protozoans to their hosts, causing the host to contract babesiosis. *Babesia* spp. reproduce within the gut of adult female *Boophilus* ticks during acquisition feeding on an infected bovine host, then its kinete stage parasites invade tick ovaries and infect the eggs (Howell et al. 2007). The adult female does not directly transmit *Babesia* spp. to cattle but rather, passes the kinetes transovarially to larvae. Infectious sporozoites develop within the salivary glands of larvae and is transmitted to a host as larvae and nymphs blood feed (Howell et al. 2007). Thus, the off-host larval stage is the key to controlling the spread of *Babesia* spp.

Being off-host, engorged females, larvae and eggs are at the mercy of their environment. Questing larvae and unhatched eggs are subject to harsh ecological conditions, water and energy loss, and starvation (Randolph 2004). Temperature, humidity, precipitation and habitat are some ecological factors that affect the survivability and questing activity of off-host tick stages with temperature and humidity being the most influential (Table 1) (Davey et al. 1991).

Ambient temperatures affect the survivability and questing activity of larval ticks as well as the hatchability of eggs. Exposure to high temperatures within 40-45 ˚C causes ticks to lose their capacity to retain moisture (Londt and Whitehead 1972). Temperature is also influential on the timing and survivability of incubating eggs, thus determining the number of larvae born (Branagan 1973, Davey et al. 1980, 1982, Ouhelli et al. 1982, Chilton and Bull 1994). Davey

(1986) reported that the egg incubation time for *R. annulatus* is 52 days at 20 ˚C and 16 days at 35 ˚C. The temperature at which incubating eggs are held has also been shown to influence the life expectancy of hatched larvae. Additionally, Hitchcock (1955) reported that eggs of *R.(B.) australis* had a greater hatching success when temperatures ranged between 21-37 ˚C. In contrast, *R.(B.) annulatus* larvae did not hatch from eggs held at 32-35˚C (Strey et al. 1991). Cold temperatures also influence the survivability of larvae. Gothe (1967) showed that R. microplus only survived 72 hours when exposed to a temperature of 0˚C and died when exposed to lower temperatures. Optimal temperatures for *R.(B.) australis* development has been found to be 28˚C with thresholds of 12˚C to 40˚C (Sutherst and Maywald 1985), while *R.(B.) microplus* larvae survival had a positive correlation with ambient temperatures under canopied conditions in south Texas pastures (Leal et al. 2018). Larvae of *R.(B.) microplus* were observed to have the longest survival at 20˚C (Davey et al. 1991).

At all stages, it is crucial that ticks maintain optimal balance between wetness and dryness (Daniel and Dubabek 1994). Low humidity levels have been shown to be detrimental to larvae as it causes them to dehydrate (Lees 1946, Yoder et al. 2006). Larvae have a high surface area to volume ratio, causing them to lose moisture rapidly through evaporation (Randolph and Storey 1999). Studies have shown that larvae tend to have greater survival rates at higher humidity levels (Knülle and Devine 1972, Tukahirwa 1976, Davey et al. 1991). Larvae of R. australis have demonstrated high survival rates at 90-95% relative humidity (RH) (Roberts 1971), while larvae of *R.(B.) microplus* and *R.(B.) annulatus* did not survive a relative humidity of ≤66-67% regardless of temperature (Davey et al. 1991). Additionally, low relative humidity has been shown to shorten larval questing time, and in extremely dry conditions larvae may not commence in questing at all (Randolph and Storey 1999). One of the major threats to successful

oviposition is desiccation. Teel (1984) found that drier conditions led to a decrease in egg hatching in R. microplus and R. annulatus. Decline in hatching rate has also been shown to correlate with a decline in larval survivability (Sutherst and Bourne 2006).

The influence of rainfall on off-host larval survivability and successful oviposition is highly dependent on the distribution and amount of rainfall. Increased rainfall can result in optimal humidity, milder temperatures and increases in vegetation leading to lower morality rates (Kaiser et al. 1988, Garcia et al. 2011). Abundance of larvae, nymphs, and adult ticks was shown to be positively correlated with monthly rainfall (Mooring et al. 1994). Additionally, Rhipicephalus larvae have been shown to predominately hatch at the end of rainy seasons (Yeoman 1966). However, when conditions are excessively wet, populations of R. microplus larvae have been shown to diminish (Keesing et al. 2017). Similarly, Leal et al. (2018) observed a decrease in oviposition success when rainfall was high, presumably due to the drowning and washing away of unhatched eggs.

Larvae habitat is dictated by where the female oviposits her eggs. Because of this and the movement of free-roaming hosts, larvae are subjected to inhabiting optimal and suboptimal habitats that influence survival and questing success. *Boophilus* larvae have increased survivability and longevity in canopied habitats versus exposed habitats (Garris and Popham 1990, Garris et al. 1990, Teel et al. 1997, Corson et al. 2001, Leal et al. 2018). In open pastures, larvae of *R. (B.) australis* have been observed concealing themselves under grass blades, leaf axils, and seed heads in an attempt to shield themselves from direct sunlight (Wilkinson 1953). Additionally, studies using other species of Ixodidae have also found that their larvae have higher survival rates in areas with vegetation shade (Norval 1977, Mooring et al. 1994).

To date, most studies exploring the effect of climate and ecological factors on the survivability of *Boophilus* larvae has been conducted on *R. (B.) australis*. Early literature mistakenly classified *R. (B.)australis* from Australia as *R. (B.) microplus*. Complicating matters, these studies were conducted in Australia, a climate and ecosystem different from that of the quarantine zone in south Texas, USA. While morphologically similar, *R. (B.) australis*, *R. (B.) microplus*, and *R. (B.) annulatus* have been proven to be different species through cross mating and genetic studies (Estrada Peña et al. 2012, Burger et al. 2014, Low et al. 2015, Ali et al. 2016). The cattle fever ticks, *R. (B.) microplus* and *R. (B.) annulatus* have a parapatric distribution in Texas along the north side of the Rio Grande Valley (Lohmeyer et al. 2011). While an ecological study has been done on *R. (B.) microplus* larval survivability in south Texas (Leal et al. 2018), there is a paucity of literature on the ecological effects of south Texas on *R. (B.) annulatus* larvae survivability. Therefore, the goal of this study is to strengthen the understanding of the effects of a south Texas climate on the development and survival of off-host *R. (B.) annulatus* larvae, to create a clearer foundation for future ecological studies to help enhance off-host eradication efforts in south Texas.

# CHAPTER III

# METHODOLOGY AND FINDINGS

## Study Site

This study was conducted in a pasture at Moore Air Base located near Edinburg, TX, USA (26.3871◦ N, 98.3376◦ W; elevation 66 m) at the United States Department of Agriculture (USDA)-Agricultural Research Service, Cattle Fever Tick Research Laboratory. The lower Rio Grande Valley is a semi-arid, subtropical region with ambient temperatures averaging between lows of 8 °C in the winter and highs of 36 °C in the summer. Annual rainfall ranges between 380-750 mm and is highly erratic both seasonally and annually. The experimental pasture contains vegetation characterized as Tamaulipan scrub brushland (Correll and Johnston 1970). The soil is a shallow calcareous clay with caliche near the surface. Vegetative cover within the pasture is around 90%, with a canopy cover of around 20%. The dominant tree species is honey mesquite, *Prosopis glandulosa* (Torr.), with shrubby acacias, *Vachellia farnesiana* (L.) Willd., *Vachellia rigidula* (Benth.), and spiny hackberry, *Celtis ehrenbergiana* (Klotzsch). Typical of pastureland of south Texas, the dominant understory plant is buffelgrass, *Pennisetum ciliare* (L.), with the common forbs silverleaf nightshade, *Solanum elaeginifolium* (Cav.) and cowpen daisy, *Verbesiana encelioides* (Cav.). Plant names follow the USDA Plants Database (United States Department of Agriculture 2006).

## Rearing of Ticks

Ticks were reared as described previously (Leal et al. 2017). Briefly, larval ticks were placed on stanchioned cattle and allowed to develop until females were engorged and dropped from the host. These females were held in petri dishes (at 27 ± 1◦C, 80 ± 5% relative humidity (RH)) for oviposition. Experimental colonies of *R. (B.) annulatus* were maintained under optimal conditions in a climate-controlled room (Davey, 1986; Davey et al., 1984). The strain designated as "Klein Grass" was used to infest study arenas as described below.

## Study Arenas

As in the earlier study with *R. (B.) microplus* (Leal et al. 2018), female *R. (B.) annulatus* were released into study arenas. These study arenas consisted of 18 individual metal tubs (American Metalcraft, Franklin Park, IL, USA) with the dimensions measuring 60 cm in diameter and 30 cm in height. Tubs were filled 22 cm deep with soil from the surrounding pasture and a selected grown plant was transferred to each tub to establish a study arena (Fig.3A). Each was planted with one of three common south Texas pasture plants: buffelgrass, silverleaf nightshade, or cowpen daisy. These plants were ideal for the local conditions because they thrive in semiarid pastures. Buffelgrass is an invasive dominant pasture grass in south Texas and northern Mexico, native to Africa (Arriaga et al. 2004). Silverleaf nightshade is a plant native to south Texas that contains spines with a sticky texture (Mekki 2007). Cowpen daisy or yellow-top, is also native to south Texas. It grows throughout the year in regions with mild winters (Grichar and Sestak 1998). If a plant died, it was replaced between tick cohort introductions. A total of 18 study arenas (14 for cohort 1) were scattered throughout the eight-hectare pasture (Fig. 4). The study arenas were arrayed so that some (n = 8) were situated under the canopy of a large mesquite tree (Fig. 3B) with others (n = 10) placed in exposed situations

away from the trees. In cohort 1, the corresponding numbers were eight and six study arenas. It is important to note that we did not measure or observe a difference in biotic factors in the arenas as compared to open ground.

## Data Collection

At the beginning of each cohort all study arenas were infested by releasing one engorged female at the center of each tub near the stem of the plant. Females would most often seek a hiding place in the root mass or less often, would dig into the soil. Consequently, they were not generally observable after the first day and in any case, once placed, these females were not disturbed during oviposition (typically a 3- to 4-wk period). No adult ticks were observed completing oviposition near the rim of the study arenas, nor were any larvae observed crawling over the rim of the study arenas. Presumably some female cattle fever ticks suffered depredation, but the cause of mortality was not directly determined. Starting at week 3, larval ticks were sampled using the standard flag method as previously described (Leal et al. 2018). A white flannel cloth (dimension, 25 × 20 cm) was placed directly over the plant then dragged in opposite directions to represent a potential passing host (collection time of approximately 40 s). Each flannel cloth was then placed in a numbered zip-lock bag corresponding to each tub. Larvae attached to the cloth were collected with clear adhesive tape then mounted directly on a data sheet following the recording methods of Wilkinson (1961). Thus, the means of determining population response to environmental variables was a destructive sampling method which provided numbers captured as a proxy for larval abundance. Twelve censuses were taken per month, with 2 to 3 d between each census. All arenas were sampled at each collection date. Sampling clock-times were varied to include all periods of day and night. Data were collected continuously over a 20-mo period. Abiotic factors were measured by a HOBO Pro model V2

micro weather station (Onset Computer Corporation, Bourne, MA, USA) to record ambient temperatures and percent relative humidity. A rain gauge was installed at each end of the experimental pasture and read after each rain event.

## Cohorts

There was a total of 9 cohorts in this study. The cohorts typically extended over more than one season so for the purposes of this study cohorts were defined by the date on which engorged females were introduced into the study arenas, in accordance with the parallel study of *R. (B.) microplus* (Leal et al. 2018). A new cohort would begin as the previous one ended (day range is stated in the results). To ensure no larvae remained from a previous cohort, sampling continued in positive arenas approximately 2 weeks after the last larvae was collected. If a couple of arenas still had larvae, counting would continue and a new set of arenas would be filled, planted and used to start the next cohort. Each cohort consisted of a full set of 14-18 study arenas that were infested at the same time by releasing individual engorged females into each arena and the resulting populations were monitored as described above. For analyzing potential seasonal effects each cohort was assigned to the season that corresponded to the month when the cohort was initiated, i.e, winter (November-February), spring (March-May), summer (June-August), and fall (September-October). For each study arena in each cohort we measured the time interval from the introduction of females to the first positive larval sample (incubation stage), and from the first to the last positive larval sample (larval stage).

## Statistical Analysis

Parameters measured for each cohort were as follows: total numbers of larval ticks per individual arena, total larvae per each positive arena, mean larvae per arena in all, canopied, and

exposed habitats and percentage of arenas positive for larvae. A “positive” arena was one in which larvae were detected, indicating survival and reproductive success by the released gravid female. Difference between mean numbers of larvae per cohort, mean numbers of larvae per canopied and exposed habitats within and between cohorts, mean relative humidity between cohorts, mean maximum and mean minimum relative humidity within and among cohorts, mean temperature between cohorts, mean maximum and mean minimum temperature within and among cohorts, duration of the incubation stage by cohort, and duration of the larval stage by cohort were conducted by one-way analysis of variance (ANOVA) with Student-Newman Keul’s multiple comparison posttest. For analyzing potential seasonal effects, mean numbers of larvae by season and percentage of arenas positive for larvae by season, and by habitat were done by pair-wise t-test assuming unequal variance. Linear regression was used to measure correlation between mean larval numbers by cohort and corresponding weather variables. The aforementioned analyses were performed using Graphpad Instat (Graphpad Software Inc., 2009).

Overall survival, oviposition success, and post-incubation survival were analyzed using multifactorial ANOVAs with temperature, relative humidity, precipitation and habitat (input categorically) as main factors. These multifactorial ANOVAs were performed using JMP Pro 13 (SAS Institute Inc., 2016). Stepwise reduction of all full models was used, removing highest-order interactions and always the least significant effect first (Crawley 1993). Factors were removed when $p > .05$. Model reduction stopped when no further interactions could be removed. No non-significant main effects were removed. Interactions or differences were considered significant when $p < .05$.

## Results

Mean (± SD) larval tick numbers were analyzed by cohort, by season, and by habitat, including all study arenas, and separately for positive study arenas only (Table 2 and Table 3). The mean numbers of larvae counted from arenas containing one of the three plant species included in this study were not consistent. Cowpen daisy arenas had the highest numbers ($242.7 \pm 367.5$, $n = 17$); silver-leaf nightshade the lowest ($71.8 \pm 220.1$, $n = 17$); with buffelgrass intermediate ($158.3 \pm 261.4$, $n = 78$). These differences, though large, were not statistically significant ($p = 0.14$) because of the high variation within and among cohorts. Similar results were reported in our parallel study of *R. (B.) microplus* (Leal et al. 2018). Therefore, data from arenas with different plant species were combined in the further analysis.

The mean number of larvae per cohort were markedly and significantly higher in cohorts 3 (spring), 5 (fall), and 7 (spring) (Table 2). Mean larval numbers in cohorts 3, 5, and 7 were not significantly different from each other. Not surprisingly oviposition success (positive study arenas) was high in these three cohorts (94.4%, 100%, 100% respectively). In contrast, cohorts 2 (winter) and 8 (summer) essentially failed (Table 2), the lack of larvae indicating a failure of the females to lay eggs or for those eggs to hatch, and therefore these two cohorts were excluded from the further analysis. In comparison, cohort 1 (winter) and cohorts 4 and 9, both summer cohorts, had low to moderate levels (21-70%) of reproductive success, and low to moderate numbers of larvae.

Considering the results by season, the data in Table 3 shows significantly higher numbers of larval ticks in the spring and fall cohorts compared to winter and summer, with the lowest survival in summer. Overall, the summer cohorts had the lowest mean numbers, 41.1 larval ticks recovered per study arena. Among these, cohort 4 had a mean of 68.7 and cohort 9 had a mean of 13.2 larvae recovered per study arena (n.s. at $p = 0.05$). Notably cohort 4 had relatively strong

reproductive success (72.2%) compared to cohort 9 with only 38.9% positive arenas. The numbers in the winter cohorts 1 (mean = 0.38) and 6 (mean = 126.4) were very different from one another ($p < 0.01$) and this was in large part due to a much greater oviposition success (100%) in cohort 6 compared to cohort 1 (only 21.4%). Overall spring and fall cohorts had significantly higher means (325.1 and 257.7 larvae recovered) than winter (mean = 71.3) and summer cohorts (mean = 41.1) (Table 3).

Multifactorial analysis confirmed that habitat significantly influenced tick survivability. Fig. 5 shows mean numbers of larvae per cohort collected from canopied habitats and exposed habitats (total numbers per cohort shown in Table 2). With the exception of the winter cohorts, canopied habitats had markedly higher mean numbers of ticks per study arena compared to exposed habitats. Specifically, spring cohort 7 had fourfold higher mean numbers of larvae in canopied habitats than in its corresponding exposed habitats. In the fall cohort canopied arenas had threefold higher numbers than in the corresponding exposed arenas. Similarly, the summer cohorts had four to fivefold mean numbers of larvae in the canopied study arenas than in their respective exposed study arenas. In contrast, for the winter cohorts there was no significant difference in mean numbers between the exposed and canopied study arenas.

Habitat significantly affected the mean larval numbers by cohort (Table 4). In the multifactorial analysis, relative humidity and temperature showed no detectable effect on total larval survivability (Table 4). However, the multifactorial analysis did indicate that habitat and RH had an effect on oviposition success (Table 5). But during the larval phase only habitat had a detectable effect on survivability (Table 6). This was an interesting result given the variability in RH (Fig. 6) and a similar variability trend in temperature among the different cohorts (Fig. 7). Specifically, spring cohort 3 and fall cohort 5 recorded significantly higher mean RH (72.7% and

71.6% respectively) compared to the rest of the cohorts. In contrast, cohorts 1 (winter), 7 (spring) and 9 (summer) exhibited relatively similar mean RHs which were significantly ($p < 0.001$) lower (61.7 – 63.7%) than the other cohorts. Although winter cohort 1 did have a mean-max RH similar to the other cohorts, the mean-min RH during cohort 1 was significantly lower than all other cohorts (Fig. 6). The other winter cohort (cohort 6), had significantly higher mean RH and mean-min RH compared to cohort 1 (Fig. 6). Winter cohort 6 was among the larger larval populations compared to the other cohorts (Table 2). The mean-max RH was similar between cohorts that included winter, fall, summer and spring (cohorts 1, 3-6). However, these cohorts had significantly higher mean-max RH levels compared to spring cohort 7 and summer cohort 9.

Ambient temperature showed a more predictable pattern than RH, with summer cohorts 4 and 9 having the highest mean temperatures (cohort 4 with a mean temperature of 30.6 °C, and cohort 9 with a mean temperature of 30.5 °C) whereas winter/fall cohorts had the lowest (winter cohorts with a mean temperature of 18.1 °C for cohort 1 and 21.9 °C for cohort 6, and fall cohort with a mean temperature of 19.4 °C). Not surprisingly, cohort 1 (winter) recorded the lowest overall mean ambient temperature and mean-min temperature (Fig. 7) compared to all other cohorts. However, cohort 1 did have a mean-max temperature similar to fall cohort 5 and cohort 6 (the other winter cohort). As expected, summer cohorts 4 and 9 had significantly higher mean ambient temperatures as well as mean-min and mean-max temperatures (Fig. 7). However, mean-min temperatures in the summer cohorts were similar to those of the spring cohorts (3 and 7) and fall cohort 5 (Fig. 7). These similarities are not unexpected given the overlapping months among several of the cohorts (Table 2).

Rainfall by cohort is shown in Fig. 8. Spring cohort 3 had the most precipitation. There was no measurable precipitation in winter cohort 1 and virtually no precipitation in cohorts 7 and

9. Cohorts with meaningful amounts of rainfall generally had large mean numbers of larvae compared to the dry cohorts. The clear exception was cohort 7 which had virtually no precipitation but large mean numbers of larvae. Consequently, the multifactorial analysis failed to detect an influence of precipitation (Table 4, Table 5, Table 6). This result seems counterintuitive and even contradictory considering that there was a detectable effect from relative humidity (Table 5). A simple regression analysis of RH against precipitation gave a very significant ($p = 0.007$, $r2 = 0.79$) correlation (Fig. 9). Based on that result we ran a regression analysis of precipitation against larval numbers and found a correlation between rainfall and the larval phase but only in exposed habitats (Fig. 10).

Five of the nine cohorts started in one season and finished in another (Table 2). So, we looked at the possibility that habitat, relative humidity, temperature and precipitation might have preferentially affected different stages of the tick life cycle (female incubation-oviposition success vs. = larval survival). Fig. 11 shows the differences in the mean number of days cohorts spent in the two different life cycle stages. Cohort 6, one of the cohorts that started in winter and finished in spring, had a significantly ($p < 0.01$) longer mean incubation stage indicated by the time females were placed in study arenas until the first larvae was found (Fig. 11). This cohort had 100% positive study arenas and one of the highest larval populations among the cohorts (Table 2). The other winter cohort (cohort 1) also had a slightly longer incubation period compared to the other non-winter cohorts (Fig. 11). However, cohort 1 had only 21.4% positive study arenas, a low larval population and was shorter in duration compared to cohort 6 (Table 2). The exception was cohort 3, which started in spring and finished in summer and had a significantly ($p < 0.01$) longer mean larval stage (post-incubation) compared to cohorts 4, 6, 7, 9 and 1 (Fig. 11). The length of the larval stage was not significantly different between the majority of cohorts (1, 4, 6-9). Yet, the

mean number of larval ticks collected during this time was significantly different between some of these cohorts (Table 2). Indicating that the destructive sampling method used minimally affected tick survivability. As expected, multifactor analysis showed that mean RH significantly influenced oviposition success (Table 5), while only habitat significantly influenced larval survivability (Table 6).

# CHAPTER IV

# DISCUSSION AND CONCLUSIONS

## Discussion

Ecological studies on tick population dynamics almost uniformly report a strong effect of humidity on off-host survival (Needham and Teel 1991). This was true in the present study as well. The contrastingly large difference in larval numbers between the two winter cohorts was traceable to rainfall during the incubation phase of cohort 6, resulting in 100% oviposition success among study arenas vs only 21% success in cohort 1 which had no measurable precipitation. The multifactorial analysis detected a strong interaction between RH and oviposition success. The incubation mean RH for cohort 1 was 60% while the mean RH during incubation of cohort 6 was 68%, and this difference in RH was statistically significant. Not surprisingly, the regression analysis showed a significant correlation between rainfall and relative humidity. In contrast to the results of the parallel study with *R. (B.) microplus* in which there was an inverse relationship between larval survival and rainfall (Leal et al. 2018), the data in this study with *R. (B.) annulatus* showed a positive correlation. The cause of the difference in result may be the fact that there was no rain during any of the incubation phases of the cohorts in this study. Heavy rains dispersing the egg masses was suspected as the cause of the negative interaction in the *R. (B.) microplus* study.

We found no significant effect of temperature on larval numbers in this study. Strey et al. (1991) reported data from lab experiments that a range of temperatures from 17-36 °C are

optimal for egg viability and Davey et al. (1991) found a similar range was optimal for the larval stage. During this field study the temperatures experienced were generally within this optimal range and hence no detrimental effect would be expected. During winter cohort 6 the only freeze dates during the experiment occurred (7-8 January 2017) just four days after release of the female and it is likely that eggs had not yet been laid, hence this extreme temperature had no measurable effect. The summer cohorts had significantly fewer larval numbers than the spring and fall cohorts which might have been in part due to high temperatures, but the analysis could not separate this parameter from relative humidity. Davey et al. (1991) likewise found no effect of temperature on *R. (B.) annulatus* larvae in the lab except when RH was below optimal.

In the parallel study arena study by Leal et al. (2018), *R. (B.) microplus* was found to have highest numbers in the spring and lowest in the fall. In the present study with *R. (B.) annulatus* the highest numbers were in spring and fall with lowest in summer and winter. In the multifactorial analysis much of this difference was explained as a habitat interaction. Survival was significantly better in spring and summer in canopied habitats, whereas there was no difference between exposed and canopied habitats in terms of survival in fall and winter. Contrastingly, with *R. (B.) microplus*, canopied habitats were better in summer, but exposed habitats had better survival in winter (Leal et al. 2018). These results are in accord with the general observation that *R. (B.) microplus* is a tropical tick, finding optimal conditions in hot, humid regions, whereas *R. (B.) annulatus* is a temperate zone tick adapted to cooler, drier regions (Estrada Peña and Venzal 2006).

Future studies could possibly look at the microclimate and its effect on tick larvae survival. While our study looked at ambient ecological factors, another study can be

implemented to measure the microclimate of canopied and exposed habitats and the effect they have on larvae survival.

## Conclusions

This study supports previous literature that identify a relationship between habitat, relative humidity, and larval cattle fever tick survival. We showed that successful oviposition and egg hatching is dependent on relative humidity and habitat, while larval survival is dependent mainly on habitat. These results are particularly relevant for infestations in south Texas. Our study also suggests the environment of south Texas has become a favorable area for *R. (B.) annulatus* to inhabit, as suggested by the model by Teel (1991) and our weather data. With this new information on *R. (B.) annulatus* larvae, better off-host control can be implemented. This study can provide program managers and the scientific community with knowledge about how larval population dynamics respond to the interaction between seasonality and habitat. For example, it will inform researchers where and when the larvae will be most abundant – in canopied habitats and when relative humidity is high. This study also provides results based on natural conditions validating model predictions based on laboratory studies done previously on *R. (B.) annulatus*. This information, in return, can provide the foundation for future ecological studies on *R. (B.) annulatus* larvae.

| Abiotic/Biotic Factors | Optimal for Larval Questing | Description |
|---|---|---|
| **Humidity** | ≥ 80-85% | Larvae restrict questing activity to optimal relative humidity levels. Dry conditions will cause larvae to dehydrate. |
| **Temperature** | 3-38°C | Ticks adapted to cold temperatures, such as *I. ricinus*, have been shown to commence in questing at temperatures as low as 3°C. While questing activity has been shown to be curtailed at ≥ 38°C. |
| **Precipitation** | Precipitation in late spring/early summer | Precipitation contributes to higher levels of larval questing by providing optimal humidity, milder temperatures, and lower saturation deficit. |
| **Clustering** | Larger clusters | Larger clusters reduce moisture loss and maximizes host attachment. |
| **Photoperiod** | Presence of light during appropriate photoperiod | Ticks commence in questing depending on the specific photoperiod of the species of tick. |
| **Seasonality** | Species dependent relationship between adaptive physiology and ecology | Fluctuation in climate, for a given region, dictate pronounce deviations in developmental maturation which create seasonal periods of questing. |
| **Habitat** | Sheltered site with optimal microclimate | Optimal habitats produce prime microclimate conditions for questing architecture. |
| **Predation and Natural Enemies** | Low predation | Predatory mites, ants, spiders, and carabid beetles. |

Table 1. Factors that affect tick larvae questing behavior.

| Habitat | Winter | Spring | Summer | Fall |
|---|---|---|---|---|
| Canopied | 63.9 ± 173.7 $^{B}$ | 485.9 ± 429.8 $^{A}$ | 60.9 ± 151.8 $^{B}$ | 346 ± 337.1 $^{AB}$ |
| Exposed | 80.7 ± 184.5 $^{B}$ | 124.0 ± 151.6 $^{B}$ | 14.2 ± 41.7 $^{B}$ | 147.5 ± 197.5 $^{B}$ |
| All | 71.3 ± 175.8 $^{B}$ | 325.1 ± 378.7 $^{AB}$ | 41.1 ± 102.8 $^{AB}$ | 257.7 ± 294.1 $^{AB}$ |
| Positive Gardens Only | 108.6 ± 208.8 $^{B}$ | 334.4 ± 380.0 $^{AB}$ | 67.8 ± 148.8 $^{B}$ | 257.7 ± 294.1 $^{AB}$ |

Table 2. Larval tick numbers in relation to season and habitat. Collective (over 20 months) results comparing the mean ± standard deviation seasonal numbers by habitat. Statistical comparison of means was performed by ANOVA using Student-Newman-Keuls multiple comparisons post test. Means followed by the same letter are not significantly different at p = .05. Means followed by different letters are significantly different from one another. Cohorts were assigned to a season based on when engorged female ticks were dropped into the arenas.

| Cohorts | Season Started (Duration) | N Gardens | Positive Gardens | | Total Larvae | Mean ±SD Larvae | Mean ±SD Larvae Canopied | Mean ±SD Larvae Exposed |
|---|---|---|---|---|---|---|---|---|
| | | | N | (%) | | | | |
| 1 | Winter-Spring (Jan 18 - Mar 7) | 14 | 3 | 21.4 | 7 | 0.38 ± 1.6 | 0 | 1.2 ± 1.6 |
| 2 | Winter-Spring (Feb 10 – Mar 21) | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | Spring-Summer (Mar 3 - Jul 11) | 18 | 17 | 94.4 | 4998 | 277.7 ± 343.9 | 403.1 ± 404.0 | 120.9 ± 163.9 |
| 4 | Summer (Jun 21 - Sep 6) | 18 | 13 | 72.2 | 1254 | 69.7 ± 160.5 | 107.5 ± 206.2 | 22.4 ± 56.9 |
| 5 | Fall-Winter (Sept 19 - Feb 7) | 18 | 18 | 100 | 4640 | 257.8 ± 343.9 | 346 ± 337.2 | 147.5 ± 197.5 |
| 6 | Winter-Spring (Jan 3 - May 8) | 18 | 18 | 100 | 2276 | 126.4 ± 221.3 | 115.3 ± 224.5 | 140.4 ± 231.9 |
| 7 | Spring-Summer (April 24-Jul 17) | 18 | 18 | 100 | 6706 | 372.5 ± 415.0 | 568.8 ± 460.1 | 127.2 ± 149.7 |
| 8 | Summer (Jun 11 – Jul 20) | 17 | 1 | 5.9 | 2 | 0.11 | 0.2 ± .67 | 0 |
| 9 | Summer (Jun 11 - Aug 7) | 18 | 7 | 38.9 | 237 | 13.2 ± 47.5 | 18.6 ± 60.8 | 4.6 ± 10.0 |

Table 3. Survivability of total and mean ( ± SD) number of tick larvae collected in canopied and exposed habitats. Oviposition success indicated by the percentage of gardens (% positive) that had at least 1 larvae tick collected. N= the number of arenas/one engorged female per arena.

| Tick Larvae Survival- All Tick Arenas | | | | |
|---|---|---|---|---|
| **Source** | **Nparm** | **DF** | **F Ratio** | **Prob > F** |
| Mean Temp | 1 | 1 | 0.1363 | 0.6406 |
| Mean RH | 1 | 1 | 6.2127 | 0.0205* |
| Habitat | 1 | 1 | 8.8129 | 0.0037* |

Table 4. JMP multifactor ANOVA results comparing the effects of mean temperature, mean relative humidity, and habitat on the number of tick larvae collected in each cohort. Habitat was input in the analysis as either being canopied or exposed. Both mean RH and habitat significantly affected tick numbers. * indicates statistical significance between the independent variable and larval numbers.

| Oviposition Success- Number of Positive Arenas | | | | |
|---|---|---|---|---|
| **Source** | **Nparm** | **DF** | **F Ratio** | **Prob > F** |
| Mean Incubation Temp | 1 | 1 | 2.0999 | 0.1500 |
| Mean Incubation RH | 1 | 1 | 40.6334 | <0.0001* |
| Habitat (+) | 1 | 1 | 1.4206 | 0.2357 |
| Habitat * Mean Incubation RH | 1 | 1 | 4.0174 | 0.0473* |

Table 5. JMP multifactor ANOVA results comparing the effects of mean temperature, mean relative humidity, and habitat on oviposition success per cohort. Habitat was input in the analysis as either being canopied or exposed. RH and interaction of RH and habitat significantly affected oviposition. * indicates statistical significance between the independent variable and larval numbers.

**Post-oviposition Survival-Positive Tick Arenas Only**

| Source | Nparm | DF | F Ratio | Prob > F |
|---|---|---|---|---|
| Mean Larval Stage Temp | 1 | 1 | 0.4154 | 0.5208 |
| Mean Larval Stage RH | 1 | 1 | 0.7748 | 0.3810 |
| Habitat (+) | 1 | 1 | 11.2175 | 0.0012* |

Table 6. JMP multifactor ANOVA results comparing the effects of mean temperature, mean relative humidity (RH), and habitat on tick larvae survival post-oviposition per cohort. Habitat was input in the analysis as either being canopied or exposed. Only the habitat significantly affected tick larvae survival. * indicates statistical significance between the independent variable and larval numbers. (+) = positive only tick arenas.

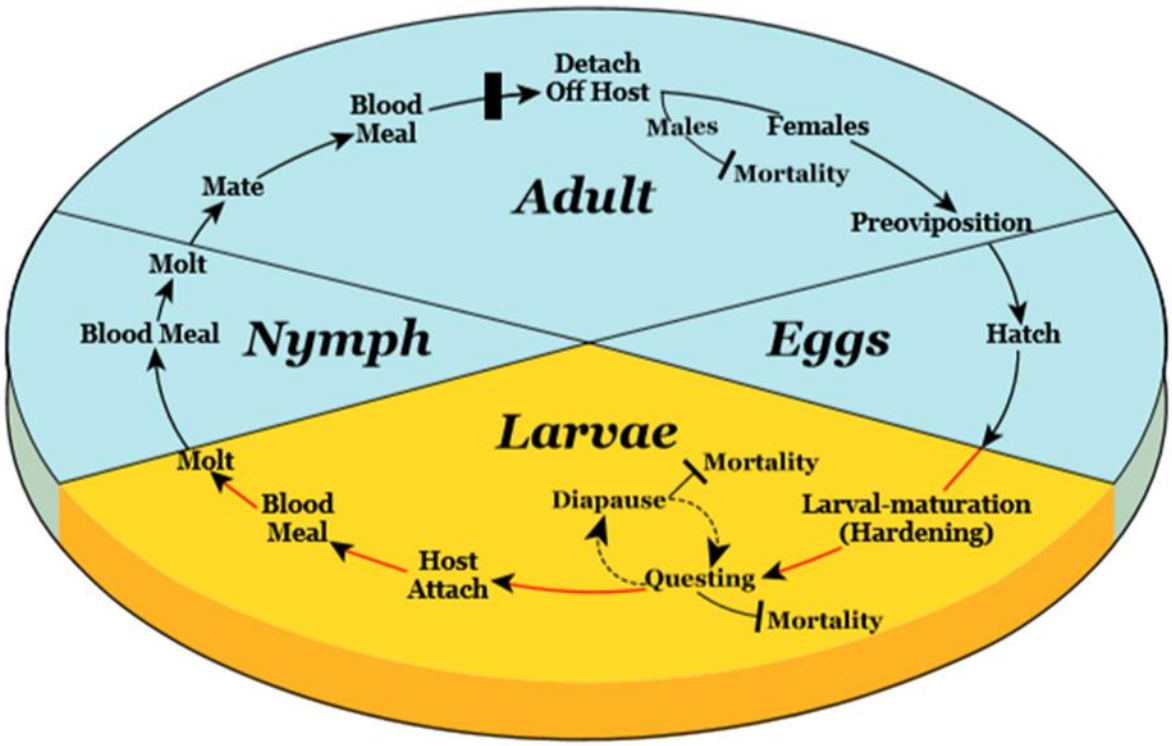


Figure 1. Life cycle of the *Boophlius* tick.


Fig 2. *Boophilus* larvae forming clusters.

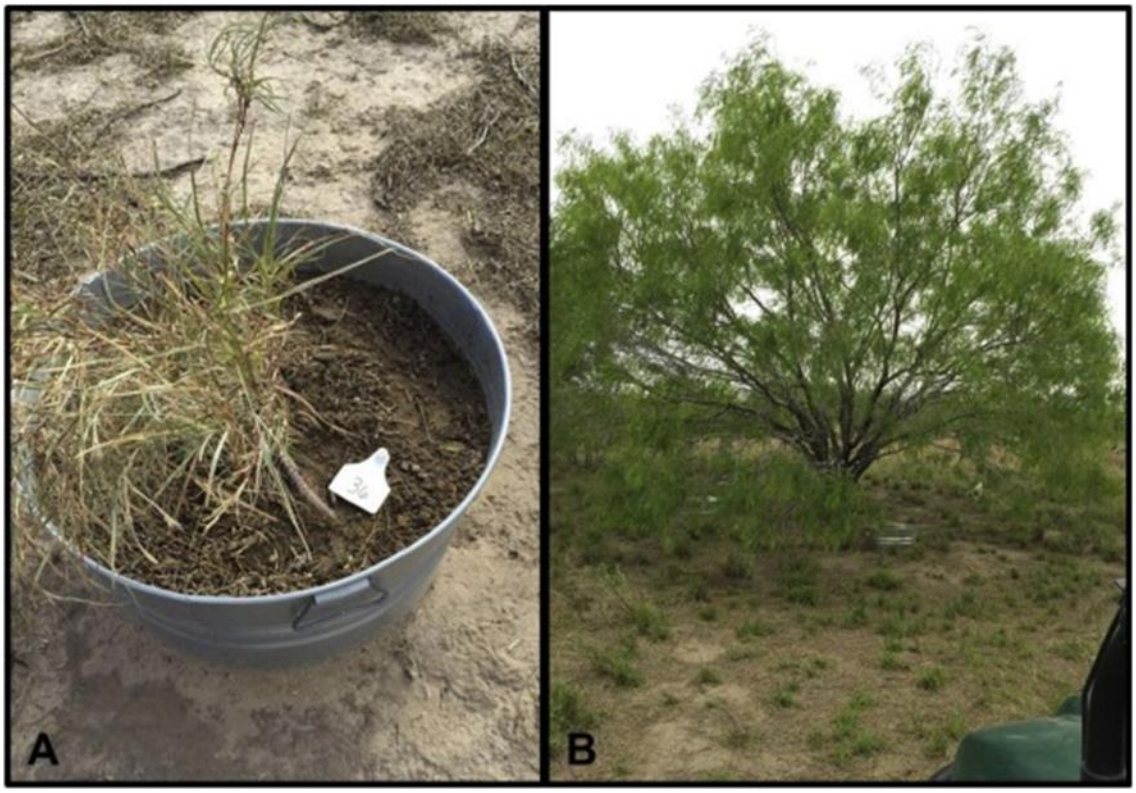


Figure 3. Study arena and habitat in south Texas pasture. A.) A representative image of a study arena planted with *Pennisetum ciliare*. B.) A representative image of a study arena under a canopied habitat.

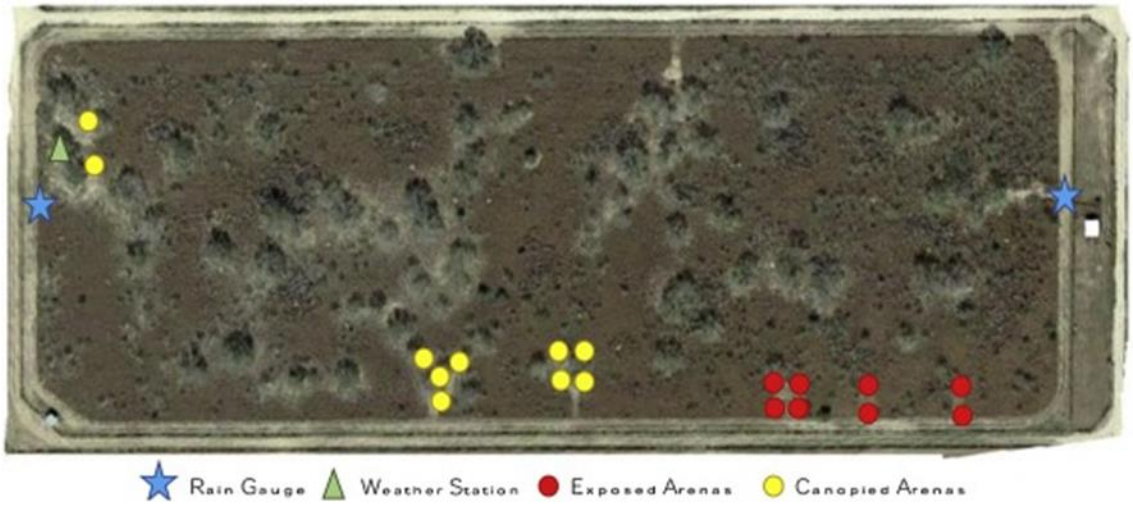


Figure 4. Satellite photo of the eight-hectare study pasture showing the locations of canopied and exposed study arenas.

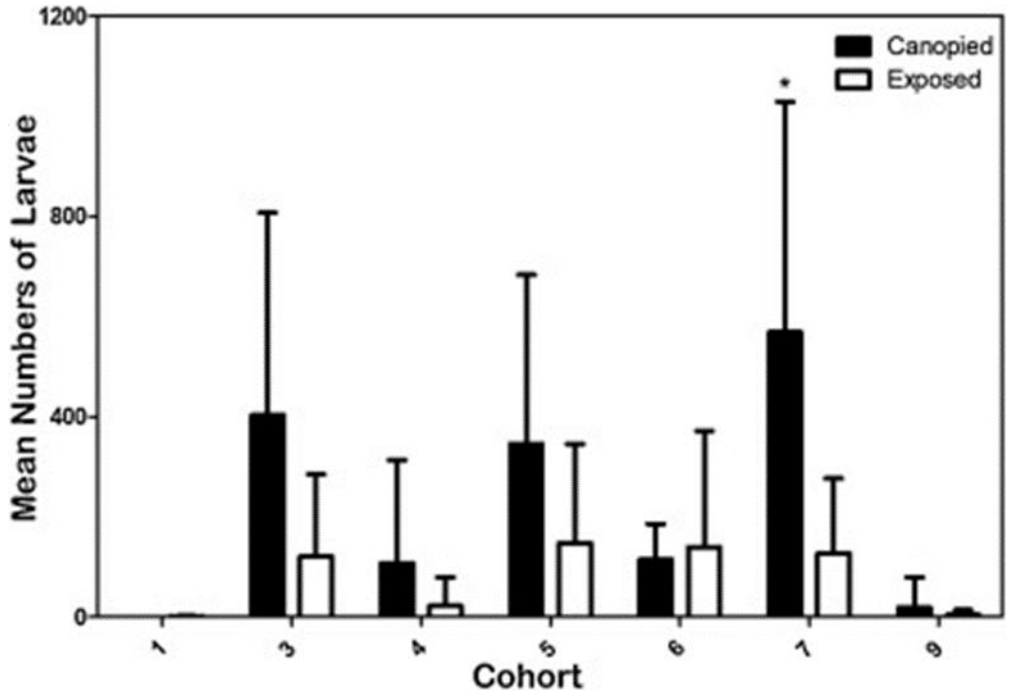


Figure 5. The number (mean + SD) of *Rhipicephalus (Boophilus) annulatus* larvae collected from canopied (black bars) and exposed (white bars) habitats per cohort. No larvae were found in study arenas from the canopied habitat in cohort 1. * indicates a statistical difference between canopied and exposed study arenas within a cohort ($p < 0.01$). Number of ticks collected from each habitat per cohort presented in Table 3. Cohorts 2 and 8 excluded from analysis

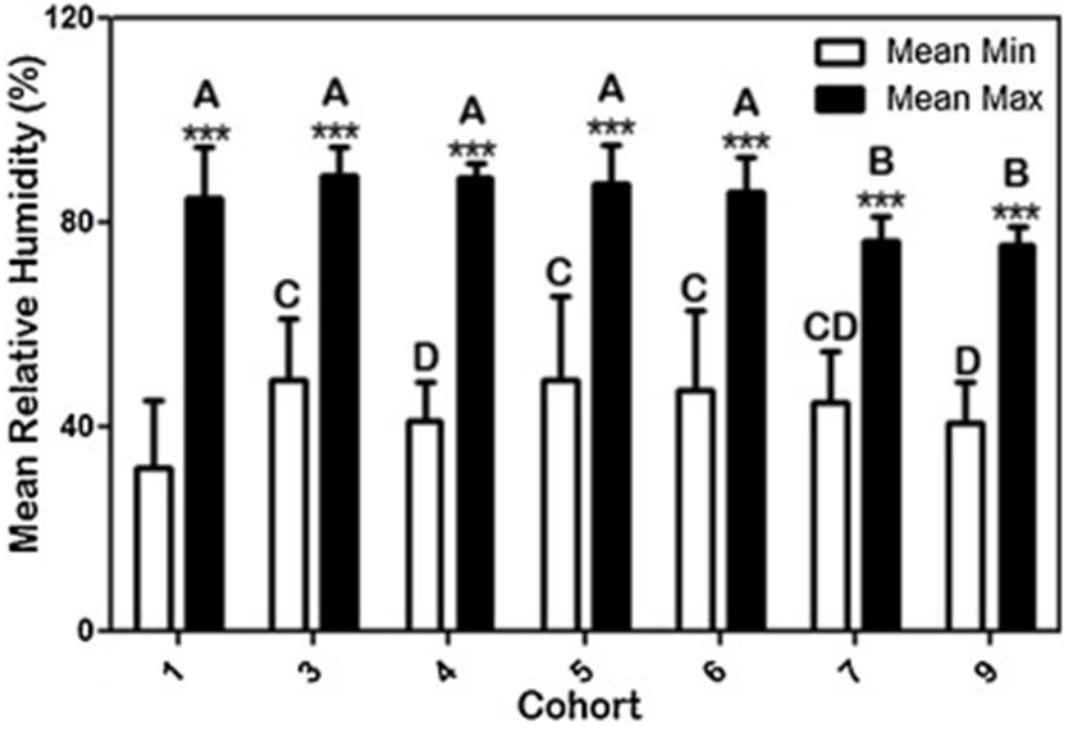


Figure 6. The mean minimum + SD (white bars) and maximum (black bars) percent relative humidity (%RH) in each cohort during *Rhipicephalus (Boophilus) annulatus* larval collections for the duration of each cohort. Different letters indicate $p < 0.001$ comparing %RH between cohorts; *** = $p < 0.001$ comparing max and min %RH within cohorts.

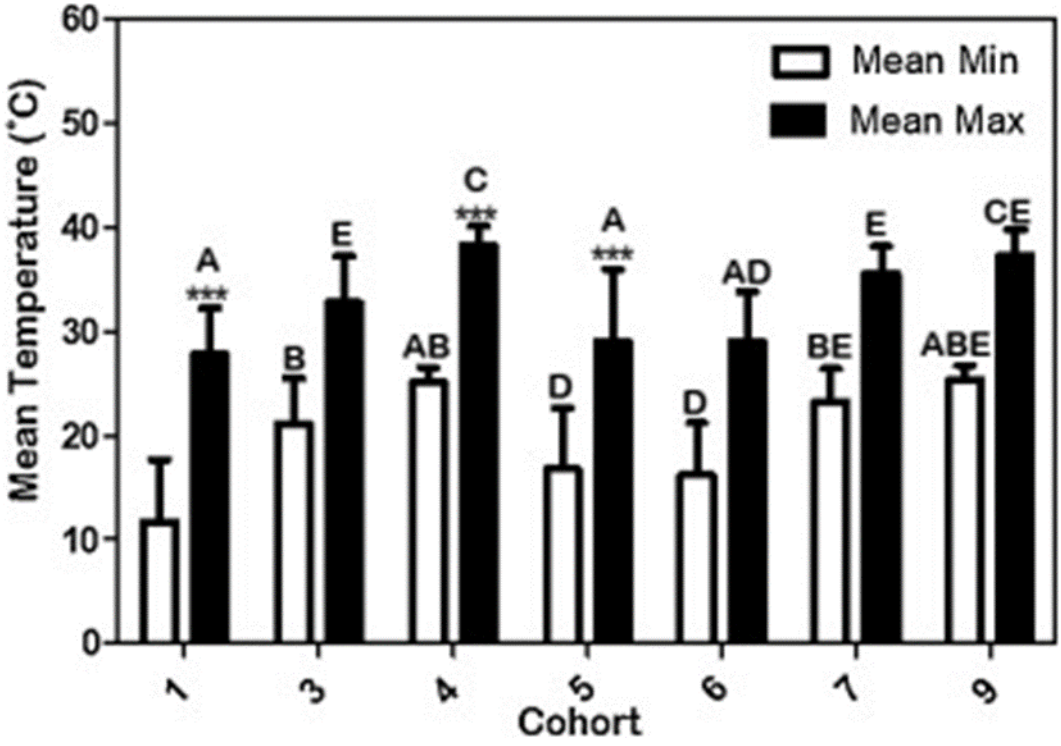


Figure 7. The mean minimum + SD (white bars) and mean maximum (black bars) temperature in degrees Celsius during *Rhipicephalus (Boophilus) annulatus* larval collections for the duration of each cohort. Different letters indicate $p < 0.05$ comparing temperature between cohorts; *** = $p < 0.001$ comparing max and min temp within cohorts. Cohorts 2 and 8 excluded from analysis.

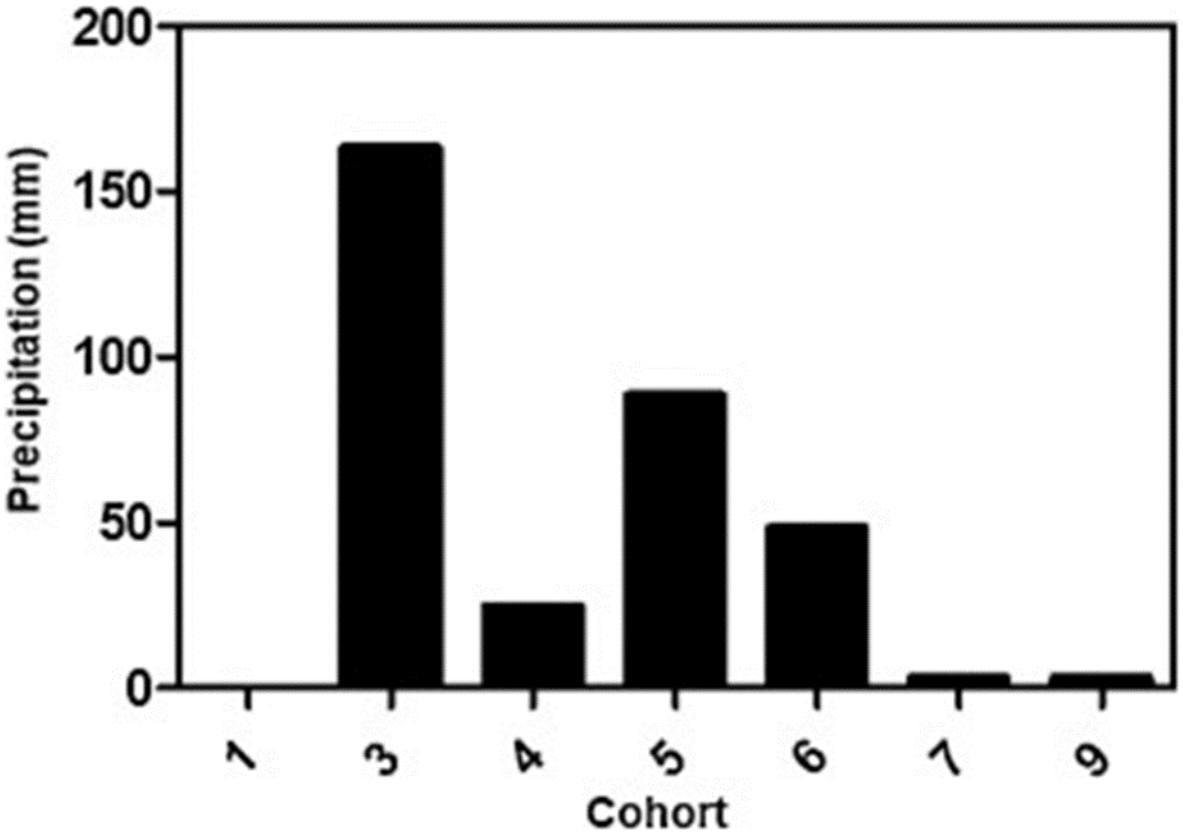


Figure 8. Total millimeters of precipitation measured per cohort.

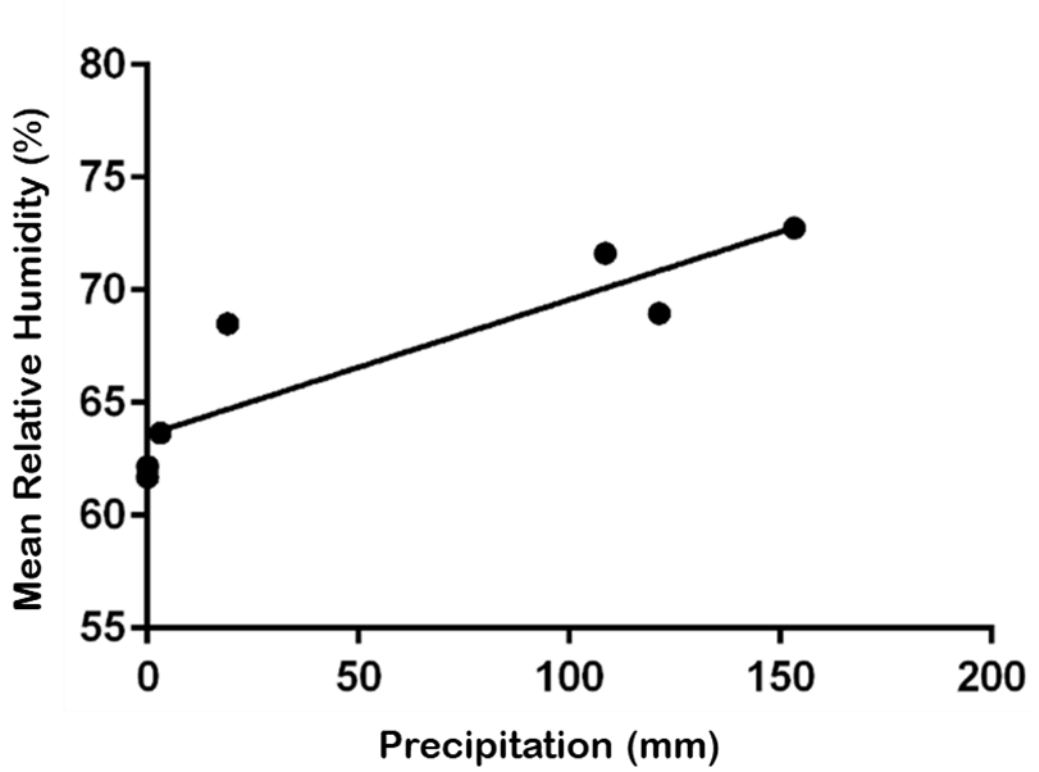


Figure 9. Regression graph representing a correlation between mean relative humidity and precipitation throughout the study. ($p = 0.007$, $r^2 = 0.79$).

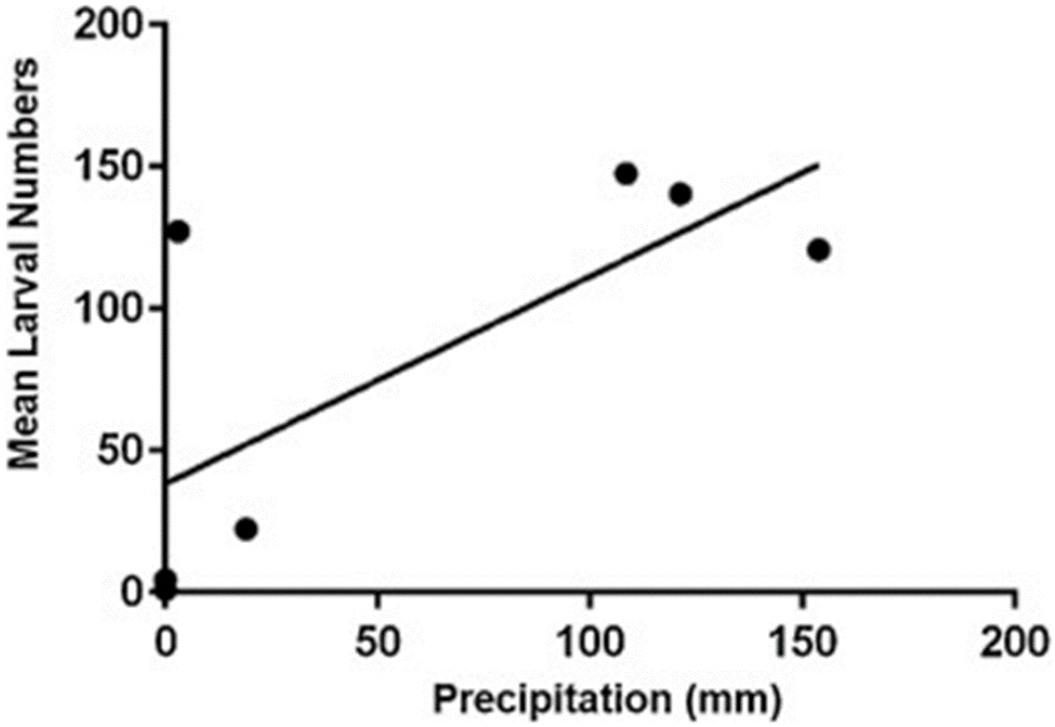


Figure 10. Regression graph suggesting a correlation between mean numbers *of Rhipicephalus (Boophilus) annulatus* larvae collected from exposed habitats and precipitation in millimeters ($p = 0.06$, $r^2 = 0.52$).

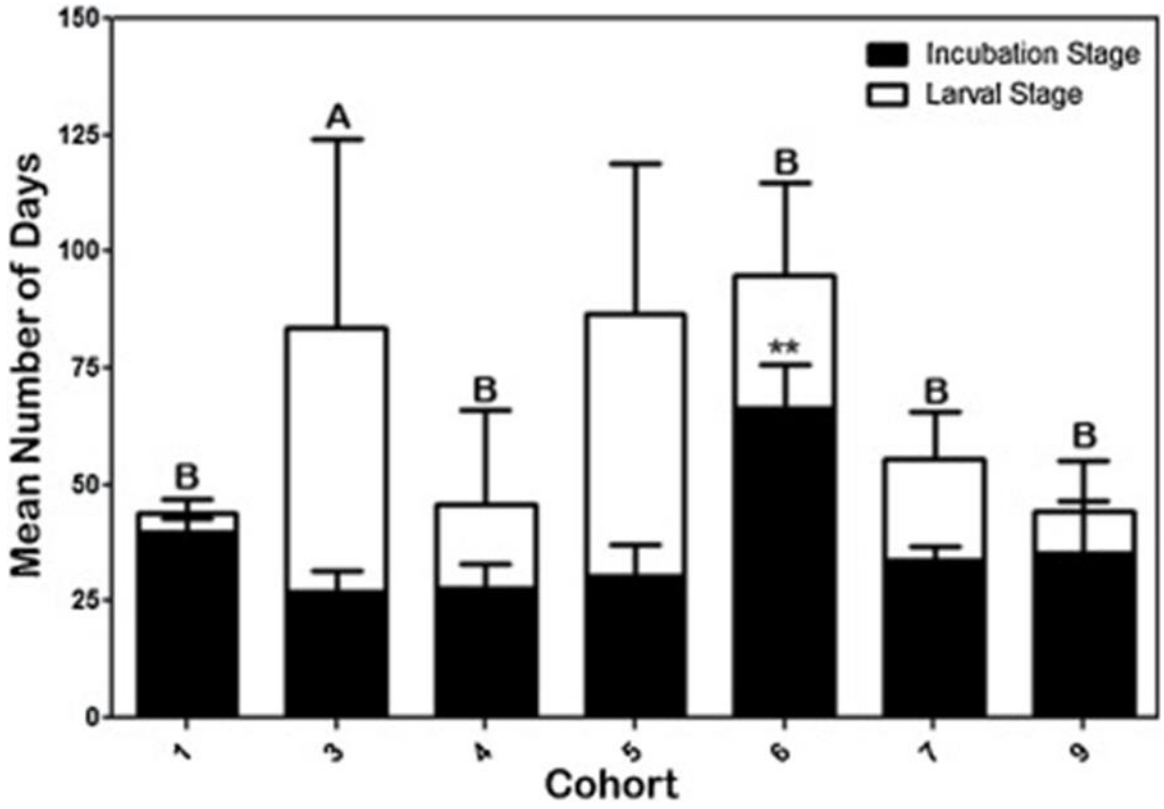


Figure 11. Mean (+ SD) number of days of *Rhipicephalus (Boophilus) annulatus* egg incubation (black bars) and larval (white bars) stages in only positive study arenas from each cohort. The duration of the egg incubation period was defined as the day an engorged female was placed in a study arena until the day the first larvae were collected in each study arena. Larval stage was defined by the day the first larvae was collected in each study arena until the last day larvae was collected from the same study arena. ** = $p < 0.01$ = incubation stage cohort 6 vs incubation stage cohort 3, 4, 7, 9; A vs. B = $p < 0.01$ in larval stage cohort 3 vs. larval stage 1, 4, 6, 7, and 9. Number of ticks collected and number of positive study arenas per cohort presented in Table 2. Cohorts 2 and 8 excluded from analysis.

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