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Abiotic Factors Contributing to the Survival of Three Tick Species in Southeastern Virginia, *Amblyomma Americanum* (Lone Star Tick), *Dermacentor Variabilis* (American Dog Tick), and *Amblyomma Maculatum* (Gulf Coast Tick)

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ABIOTIC FACTORS CONTRIBUTING TO THE SURVIVAL OF THREE TICK SPECIES IN SOUTHEASTERN VIRGINIA, *AMBLYOMMA AMERICANUM* (LONE STAR TICK), *DERMACENTOR VARIABILIS* (AMERICAN DOG TICK), AND *AMBLYOMMA MACULATUM* (GULF COAST TICK)

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ABSTRACT

ABIOTIC FACTORS CONTRIBUTING TO THE SURVIVAL OF THREE TICK SPECIES IN SOUTHEASTERN VIRGINIA, *AMBLYOMMA AMERICANUM* (LONE STAR TICK), *DERMACENTOR VARIABILIS* (AMERICAN DOG TICK), AND *AMBLYOMMA MACULATUM* (GULF COAST TICK)

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Amblyomma americanum, *Amblyomma maculatum*, and *Dermacentor variabilis* are hard-bodied ticks in the Hampton Roads area of southeastern Virginia. This study consisted of two field projects focused on these tick species. To estimate the off-host survival of local tick species, a capture-mark-recapture (CMR) study was performed. An environmental survival study was performed to quantify the ability of these three tick species to survive in situ. Four field sites were used in the Hampton Roads region covering a variety of habitat types and vegetation; specifically two drier, upland field sites and two flood-prone sites. CMR was conducted from May through September at two field sites in 2014 (one dry, one wet), then all four sites in 2015. The environmental survival study was conducted May through September of 2015 at all four sites.

CMR ticks were captured on flags, marked with fingernail polish, and returned to the location of capture. *Amblyomma americanum* was the dominant species collected (95% in 2014, 87% in 2015) when compared to the other tick species collected: *D. variabilis*, *A. maculatum*, and *Ixodes* spp. In 2014, 1 *D. variabilis* female and 32 *A. americanum* ticks were recaptured. One *A. americanum* nymph and 1 *D. variabilis* female were recaptured an additional time. For *A.*

americanum, the average time-to-recapture was 30 days with a maximum of 71 and a minimum of 8 days. Only 1 male *A. americanum* tick was recaptured in 2015, 27days post initial marking.

In the environmental survival study, *A. americanum*, *D. variabilis,* and *A. maculatum* ticks were placed inside environmental containers in situ over four months. The containers were checked at fixed intervals to quantify survival. A Cox Regression survival analysis indicated there is a significant difference in survival between species across all field sites. There is a 50.5-times higher risk of mortality for *A. maculatum* compared to *A. americanum*, a 4.3-times higher risk of mortality for *A. maculatum* compared to *D. variabilis*, and an 11.9-times higher risk of mortality for *D. variabilis* compared to *A. americanum.* There is also significantly higher mortality in field sites prone to flooding than in drier, upland field sites.

This thesis is dedicated to my husband Butters for his everlasting support and unconditional love.

You are my inspiration, I love you!

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

INTRODUCTION

Ticks are highly specialized, hematophagous, arachnid ectoparasites with over 870 known species found all over the world (Anderson and Magnarelli, 2008). They infest every class of terrestrial vertebrates, parasitizing mainly mammals (including humans), birds, and reptiles. Ticks are vectors of a greater variety of disease-causing agents (protozoa, bacteria, virus, and fungus) than any other arthropod (Sonenshine and Roe, 2014; Estrada-Peña and Jongejan, 1999; Oliver, 1989; Sonenshine, 1993). They are a major transmitter of pathogens to humans (second only to mosquitoes), livestock, and wildlife (Anderson, 2002; Sonenshine, 1993; Sonenshine and Roe, 2014). While obtaining a blood meal from its host, a tick concentrates the proteins, returning water and ions back into the host through saliva secretions. During this process, ticks may acquire pathogenic organisms from infected hosts then transmit these agents to other hosts during subsequent blood meals (Anderson and Magnarelli, 2008; Needham and Teel, 1991). Some tick species have host preferences, specializing in feeding on a specific group of vertebrates or a specific species, while others are more opportunistic feeders (Anderson, 2002; Anderson and Magnarelli, 2008; Childs and Paddock, 2003; Oliver, 1989; Sonenshine and Roe, 2014). Contact between tick and host is achieved through different questing behaviors including hunting and ambushing (Crooks and Randolph, 2006; Apanaskevich and Oliver, 2014). Ticks spend over 90% of their time off-host in the environment where they quest for a host, seek shelter in the leaf litter or substrate, or lay dormant (diapause) and molt between life stages. Questing for a suitable blood meal places large demands on the tick's osmoregulation (Anderson, 2002; Needham and Teel, 1991). Abiotic factors such as temperature, relative humidity (RH), and atmospheric pressure will have effects on the rates of water absorption and water loss while ticks are off-host (Kahl and Alidousti, 1997; Sigal, 1990). Similarly, the overall condition of the tick including species, life stage, sex, and age may affect its dehydration vulnerability (Needham and Teel, 1991). Biotic factors such as behavioral responses, active water absorption from the air (Knülle and Rudolph, 1982), and cuticular waterproofing processes (Sigal, 1990) aid in maintaining proper water balance while off-host.

IXODIDAE

Ixodidae (hard-body ticks) are the dominant tick family with more than 700 identified species (Oliver, 1989; Teel et al., 2010) including ticks from the genera *Amblyomma* and *Dermacentor*, which were the focus of this study. The ticks in the Ixodidae family are of the most medical and veterinary importance (Oliver, 1989) because of the variety of potential diseases caused by pathogens transmittable to humans, livestock, and domestic animals (Sonenshine and Roe, 2014). Ixodidae ticks have a punctuated life history with four developmental stages: egg, six-legged larva, eight-legged nymph, and eight-legged adult. Although hard-bodied ticks are able to survive long periods of time without sustenance, the latter three parasitic stages depend on vertebrate hosts to provide a suitable blood meal. This blood meal is necessary for larvae and nymphs to molt into their next life stage, and for adult females to successfully reproduce (Hooker et al., 1912). Life cycles of hard-bodied ticks are classified according to the number of times they change hosts. They are described as one-host, two-host, or three-host parasites (Anderson and Magnarelli, 2008; Apanaskevich and Oliver, 2014). One-host ticks spend the majority of their life cycle on one host, whereas two- or three-host species utilize a succession of two or three hosts, respectively (Anderson and Magnarelli, 2008; Apanaskevich and Oliver, 2014; Oliver, 1989). Though some Ixodidae species molt between life stages while on host (one- or two-host tick species), the majority of Ixodidae ticks are three-host tick species that molt off-host (Sigal, 1990). All three tick species used in this study, namely *Amblyomma maculatum*, *Amblyomma americanum*, and *Dermacentor variabilis*, are three-host ticks that molt off-host (Apanaskevich and Oliver, 2014; Sonenshine, 1991; Troughton and Levin, 2007).

Once on the host, ticks insert their hypostome into the flesh through a wound produced from cutting the flesh with its chelicerae. The hypostome, containing denticles (teeth) along with a cement-like secretion, allows for secure attachment to the host (Anderson and Magnarelli, 2008). Over a few days to multiple weeks, ticks ingest blood, lymph, and lysed tissue along with water and ions from the host (Anderson, 2002). Ticks secrete saliva that contains compounds for preventing blood clotting, dilating capillaries in the skin, and suppressing inflammatory responses in their hosts. Through saliva secretion, ticks may transmit pathogenic organisms such as *Rickettsia rickettsii* (Rocky Mounted spotted fever, RMSF), *Ehrlichia canis* (ehrlichiosis), *Borrelia burgdorferi* (Lyme disease), and *Rickettsia parkeri* (Tidewater spotted fever) (Anderson and Magnarelli, 2008; Sonenshine and Roe, 2014; Wright et al., 2011).

Generally, Ixodidae ticks have a two-year life cycle (Sonenshine and Roe, 2014). There is much variety in the time needed for feeding and molting in hard-bodied ticks depending on the tick species, the host species, the environment type, and seasonal factors including temperature, relative humidity, and photoperiod. Egg incubation can range from two days to a couple of months. Once hatched, larvae typically remain in a mass close to the hatch location and begin questing for a blood meal one to three weeks after hatching. Larvae feed to repletion anywhere from three to 30 days depending on species and conditions (Apanaskevich and Oliver, 2014; Loomis, 1961). Loomis (1961) found *A. americanum* eggs needed 24 to 31 days of incubation in order to hatch in laboratory conditions (21-26 °C, 45-60% RH). These hatched larvae needed an interval of one week to 14 days for cuticle hardening before they began to feed for three to seven days. Troughton and Levin (2007) found *A. americanum* egg incubation to last 56 days on average in laboratory conditions and larval feeding to occur between four to nine days (22-24 °C, >90% RH). Troughton and Levin also found *D. variabilis* eggs needed five to eight weeks of incubation in laboratory conditions in order to hatch, with the hatched larva feeding for two to eight days. *Amblyomma maculatum* egg incubation time, compiled from laboratory and field studies by Teel et al. (2010), ranged from 19 to 142 days with hatched larva feeding for three to ten days. Fully fed larval ticks will detach from the host, fall into the environment, and molt into a nymph one week to multiple months later depending on species and conditions. Larval *D. variabilis* were observed dropping from their host after four days on average in laboratory conditions, then molting into the nymphal stage within two to three weeks (Troughton and Levin, 2007). *Amblyomma americanum* larva were observed molting three to four weeks post engorgement (Troughton and Levin, 2007), whereas *A. maculatum* ranged from one week to over four months in field conditions (16-30°C) (Hooker et al., 1912). Nymphal ticks seek out another host and can remain attached for three to 25 days until full engorgement (Apanaskevich and Oliver, 2014; Loomis, 1961; Troughton and Levin, 2007). Once engorged, they detach from the host and molt into adults one week to ten months post feeding (Anderson and Magnarelli, 2008; Apanaskevich and Oliver, 2014; Troughton and Levin, 2007). Troughton and Levin (2007) found nymphal *A. americanum* fed for three to eight days in laboratory conditions, then molted into adults five to six weeks after engorgement. Whereas *D. variabilis* nymphs fed for five to 11 days, molting to adults within three to five weeks (Troughton and Levin, 2007). Nymphal *A. maculatum* were observed to feed for five to 11 days in field conditions with nymphal molting occurring from 17 days to 2.5 months (Hooker et al., 1912). As adults, mated females feed to engorgement for one week to 30 days, increasing body mass up to 100 times, then lay clusters of eggs in crevices or leaf litter prior to death (Anderson and Magnarelli, 2008; Apanaskevich and Oliver, 2014; Oliver, 1989). Adult female *D. variabilis* ticks were shown to feed for eight days on average in laboratory conditions whereas adult female *A. americanum* fed for an average of 12 days (Troughton and Levin, 2007). Adult female *A. maculatum* were reported feeding from eight to 21 days from compiled field and laboratory studies (Teel et al., 2010). The number of produced eggs can range from a few thousand to over 30,000 depending on the species and the level of engorgement with the maximum on record of 36,206 eggs from *A. variegatum* (Dipeolu and Ogunji, 1980). Hooker et al. (1912) monitored seven engorged *A. maculatum* females and reported between 4,560 and 11,265 eggs deposited per female with an average of 8,282. Laboratory studies (27°C, 45-95% RH) by Sonenshine and Tigner (1969) found *D. variabilis* egg clutches contained 4,097-6,713 eggs with an average of 5,379 and *A. americanum* clutches ranged from 4,056-8,188 eggs with an average of 6,436. Males of some species are not capable of feeding, but many will find hosts and feed intermittently, remaining on the host for weeks or months in order to mate with a feeding female (Oliver, 1989). Both male and female of the study species seek blood meals as adults (Hooker et al., 1912; Troughton and Levin, 2007; Teel et al., 2010).

QUESTING

Different tick species exhibit different questing behaviors. Most non-nidicolous (exophilic) Ixodidae ticks such as *Ixodes scapularis* and *D. variabilis* quest passively by waiting on the edges of vegetation to ambush their host as it brushes by. Others like *H. dromedarii* in the

Middle East, quest by actively hunting, periodically attacking from ground cover (Apanaskevich and Oliver, 2014; Crooks and Randolph, 2006; Sonenshine, 1993). *Amblyomma americanum* and *A. maculatum* use a combination of both questing methods (Goddard et al., 2011; Sonenshine, 1993). Nidicolous ticks (nest-dwelling), such as *I. crenulatus*, spend most of their life in the nests and burrows of their hosts, and rarely or never quest outside the hosts' residence. (Apanaskevich and Oliver, 2014; Oliver, 1989; Randolph, 2014).

Ambush questing tick species climb from the substrate, typically leaf litter, to the edge of grasses and brush-like vegetation. The back pairs of legs on the tick grasp the grass blade or stem while the front two forelegs are extended and waved back and forth in attempts to locate hosts using sensory cues (Fig. 1). Hunter questing tick species will leave their shelter in the leaf litter or substrate and crawl or run towards sensory cues to obtain a host. Some ticks have been observed traveling as far as 21 meters when attracted by host odors and body temperature (Sonenshine, 1993; Randolph, 2014). Odor sensory cues such as host exhaled carbon dioxide and ammonia or pheromones in host urine (Sonenshine, 2006) are sensed by ticks through an aggregate of receptors positioned in the Haller's organ. This organ is located on the tarsus of the front pair of legs and is present in all active life stages, providing olfactory senses to locate potential hosts (Klompen and Oliver, 1993; Sonenshine, 1993). Additionally, the Haller's organ anterior pit senses humidity levels in the environment (Foelix and Axtell, 1972; Lees, 1948) to aid in water balance while questing.

Ambush and hunting quest rhythms vary according to species. The main factors determining questing patterns are moisture and temperature (Apanaskevich and Oliver, 2014; Sonenshine, 1993, 2005). During host seeking activity, the tick climbs up vegetation or exits shelters typically into areas where temperatures are higher and the air is less saturated with water, leaving the tick more vulnerable to desiccation. Returning to sheltered microhabitats near the soil between questing sessions to maintain homeostasis is critical (Knülle and Rudolph, 1982) since tick water loss rates increase with higher temperatures and lower humidity (Sigal, 1990). Additionally, vertical distribution in the vegetation during ambush questing varies according to desiccation tolerance associated with life stage (Knülle and Rudolph, 1982; Sonenshine, 2005). Immature life stages of ticks remain closer to the ground or climb to lower heights than adult ticks in part because those life stages are more susceptible to desiccation (Knülle and Rudolph, 1982; Yoder and Benoit, 2003). This vertical distribution also aids in determinant of host specificity at each life stage. *Ixodes ricinus* larval numbers were found by Mejlon and Jaenson (1997) to be greatest closer to the ground, between 0 and 29 cm, in both high and low vegetation types observed. Additionally, the nymphal ticks in their study were most abundant between the height of 50 and 59 cm on vegetation, with adults found between 60 and 79 cm. Such vertical distributions allow contact with their preferred host size. Shrews and rodents (*Sorex* spp., *Clethrionomys glareolus*, and *Microtus agrestis*), which forage at ground level, tend to be parasitized with *I. ricinus* larva, whereas larger and taller mammals like roe deer and hares (*Capreolus capreolus* and *Lepus* spp.) are parasitized by the nymphal and adult stages of *I. ricinus* (Mejlon and Jaenson, 1997). *Amblyomma maculatum* adults were observed in the field by Goddard et al. (2011) questing to heights from 20 to 75 cm and positioning themselves on the tips of the observed vegetation. Adult *A. maculatum* feed on large mammals such as cattle and deer, so the vertical distribution observed by Goddard et al. (2011) would aid in locating their preferred host. *Amblyomma maculatum* larvae and nymphs quest near the base of the vegetation, which enables them to contact small mammals such as the cotton rat (*Sigmodon hispidus hispidus*) and ground-dwelling birds (Bishopp and Trembley, 1945; Clark et al., 2001;

Teel et al., 2010). If host questing is continuously unsuccessful or environmental conditions are considered unfavorable for questing, Ixodidae ticks can enter diapause; a state of low metabolic activity and reduced behavioral activity that aids in survival during environmental extremes (Randolph, 2014; Sonenshine, 1993). Ticks exhibiting diapause can remain in the leaf litter until conditions and/or host availability improves.

Fig. 1. Adult female, *D. variabilis* in the ambush questing position on a marker flag at the Stephens Tract field site; photograph taken by Lindsey A. Bidder on June 7, 2015.

WATER BALANCE IN HARD-BODIED TICKS

The challenges associated with osmoregulation vary between the stages of on-host versus off-host. When a tick is on a host, the microhabitat is relatively stable and the blood meal provides adequate levels of water (Sigal, 1990). Proper water balance to prevent over hydration while ticks feed is maintained by returning water to the host through salivation (Anderson, 2002). Ixodidae ticks spend over 90% of their time off-host, for example *D. variabilis* was observed off-host as much as 98% of the time by Anderson and Magnarelli (2008). When a tick is off-host, large demands are placed on its ability to maintain homeostasis (Anderson, 2002). Abiotic factors such as temperature, RH, and atmospheric pressure will have effects on the rates of their water gain and loss (Sigal, 1990). Similarly, biotic factors such as behavioral responses, active water absorption from the air (Knülle and Rudolph, 1982), and cuticular waterproofing processes (Sigal, 1990) are used to minimize dehydration.

Osmoregulation while off-host in routinely unstable environments relies on the behavioral and physiological ecology of tick species (Sigal, 1990). Ticks, compared to other arthropods, survive longer without food or water than any other group. This longevity depends mainly on abiotic conditions in the soil and vegetation, the climatic conditions in the habitats where ticks quest for hosts, diapause, and a tick’s ability to reduce water loss through transpiration (Sigal, 1990; Sonenshine, 1993). When ticks quest for a host, there is an increased loss of water (Crooks and Randolph, 2006); therefore, ticks will move to microclimates in the leaf litter to absorb water directly from the atmosphere (Anderson, 2002). Microclimate conditions have been observed influencing activity and abundance of tick species (Bertrand and Wilson, 1996; Rynkiewicz and Clay, 2014) with environmental restrictions affecting survival (Garrett and Sonenshine, 1979). Low relative humidity and extreme temperatures are harmful to tick survival (Carroll, 2003; Stafford, 1994; Vandyk et al., 1996) as seen with *I. scapularis*, where adverse moisture events were negatively related to total seasonal nymphal tick densities (Berger et al., 2014). Additionally, interspecific biotic variation in ticks may promote habitat preferences for species in order to maintain proper water balance. *Dermacentor variabilis* is typically found in humid habitats whereas *D. andersoni* is found in areas where the ground is prone to being very dry in the summer months, such as rocky shrub-covered slopes. *Hyalomma asiaticum* can successfully live in the dessert (Knülle and Rudolph, 1982), a habitat that would promote desiccation and inevitable mortality for other tick species.

Off-host tick water balance is maintained by the reduction of water loss to the atmosphere and the uptake of water vapor from the atmosphere. Ixodidae ticks lose water by evaporation through the integument and the spiracles (Knülle and Rudolph, 1982). The large integument surface consists of a cuticle covered by a thin epicuticle (Knülle and Rudolph, 1982; Pugh et al., 1988). There is interspecific variation in the level of waxy lipids in the epicuticle, which provides a type of waterproofing (Knülle and Rudolph, 1982; Lees, 1946). *Amblyomma americanum* has a lower water loss rate than *A. maculatum* and tends to be more “waterproofed”. This could be due to the cuticle of *A. americanum* containing more waxy lipids (Sigal, 1990). Cuticle water permeability increases as temperatures increase. There is a transition temperature at which the structure of the cuticular lipid molecules changes. Once the transition temperature is reached, the water permeability of the cuticle increases even more, exposing the tick to an even higher desiccation risk (Daniel and Dusbábek, 1994). Ixodidae adult and nymphal ticks have a tracheal opening at a pair of spiracles located laterally behind the last pair of legs. The tracheal system is used for gaseous oxygen and carbon dioxide exchanges, through which water is also lost. The mechanics of the spiracles (phases of open, closed, or fluttering) may reduce water vapor transpiration from the tracheal system by only opening during gaseous exchange, otherwise remaining closed (Fielden and Duncan, 2014; Knülle and Rudolph, 1982; Lees, 1946; Pugh et al., 1988). The variation in the frequency of the phases and morphology of the spiracles could affect water balance in tick species. The larval life stage lacks a tracheal system, relying instead on diffusion across the integument for gaseous exchanges (Knülle and Rudolph, 1982).

Ticks do not drink water and have never been reported probing moist objects with their mouth-parts (Lees, 1946). However, ticks do have the ability to directly absorb vapor water from the atmosphere (Kahl and Alidousti, 1997; Knülle and Rudolph, 1982; Lees, 1946; Sigal, 1990). By blocking adult *A. variegatum* mouthparts with paraffin wax, Knülle and Rudolph (1982) found ticks were unable to absorb atmospheric water and continuously lost water. They confirmed the mouth region as the site of active water uptake, not the cuticle as once presumed by Lees (1946). During extreme dehydration, ticks secrete oral fluids containing salts (Na^+, K^+, and Cl^-) that are hyperosmotic to hemolymph (Knülle and Rudolph, 1982; Sigal, 1990; Sigal et al., 1991). This fluid accumulates between the mouthparts and palps, drying to a crystalline form which takes up atmospheric water. The now hydrated saliva is presumed to be "swallowed and passed into the midgut" (Knülle and Rudolph, 1982). *Dermacentor variabilis*, *A. americanum*, and *A. maculatum* adults have been observed demonstrating this method of active water uptake to maintain proper hydration (Sigal, 1990).

The critical equilibrium humidity (CEH) is a minimal humidity threshold needed for tick survival (Knülle and Rudolph, 1982). Though there is much debate on how this threshold is monitored and interpreted, the CEH for the majority of Ixodidae tick species ranges from 75% to 95% RH. If at any point, a tick is in an environment below this critical equilibrium, the tick will continuously lose water (Sigal, 1990). Above this range they are able to maintain their water level by atmosphere uptake (Knülle and Rudolph, 1982). *Amblyomma americanum* ticks have been observed producing the hyperosmotic fluid capable of water sorption in the CEH range of 80%-90%, preventing mortality (Sigal, 1990). More specifically, Knülle and Rudolph (1982) reported a collection of CEH thresholds stating: *A. americanum* adults require a RH of 80%-82%, *A. maculatum* adults 88%-93%, and *D. variabilis* adults 80%-87% (Hair et al., 1975; Knülle and Rudolph, 1982). Tick age was not reported to alter the CEH range in unfed adult *A. americanum* by Jaworski et al. (1984); but, Knülle and Rudolph (1982) found the activity level of the tick can alter the range of CEH. Ticks that had been active for less than 2 weeks, had a CEH of approximately 92% RH but those that had been active for closer to a month could obtain equilibrium only in saturated air (Knülle and Rudolph, 1982). Once a tick falls below the minimum amount of water necessary for normal behavior, the tick "loses its ability to behaviorally extend its legs to walk, or physiologically recover from the effect of continued desiccation" (Sigal, 1990) and will perish. Some tick species exposed to a RH below CEH die of dehydration quickly (*I. ricinus*), others seem to lose water at a slower rate and survive longer. An unfed adult female *D. variabilis* was able to survive up to 27 days in 0% RH and 25°C (Knülle and Rudolph, 1982; Lees, 1946).

The variations in morphology and physiology of both individual ticks and tick species demonstrate the evolution of adaptions to maintain proper water balance (Sigal, 1990). These evolved biotic tools could aid in the ticks' ability to adjust to changing climates and establish populations in various habitats. Understanding how water loss rates in ticks may regulate their habitat requirements could help determine suitable habitats and future habitats for certain tick species (Anderson, 2002; Benoit and Denlinger, 2010). The focus of this study was to gather more information about the relationship between abiotic and biotic factors restricting some species to more moist habitats in the Hampton Roads area of Virginia while others can persist in more dry habitats.

TICK SPECIES STUDIED

There are 16 documented hard-bodied tick species in the southeastern area of Virginia (VA) (Sonenshine, 1979; Nadolny et al., 2014). The dominant tick species in the Hampton Roads area of southeastern VA is *A. americanum*, the lone star tick (Nadolny et al., 2014). There are also two newly established species, *A. maculatum* (Gulf Coast tick) and *I. affinis* which currently has no common name (Nadolny et al., 2011; Wright et al., 2011). Five *A. maculatum* ticks were found in the 1960s by Sonenshine et al. (1965), but established populations were only recently discovered. *Ixodes affinis* ticks have also only recently become established in the state (Nadolny et al., 2011, 2014; Sonenshine et al., 1965; Wright et al., 2011). *Dermacentor variabilis* (American dog tick) is one of the most widely distributed ticks in the United States (US) (Bishopp and Trembley, 1945) and is commonly collected in surveillance studies in the Hampton Roads area. *Dermacentor variabilis* is also the primary vector of the agent associated with Rocky Mounted Spotted fever (RMSF) in the US (Anderson and Magnarelli, 2008; Bishopp and Trembley, 1945; Nadolny et al., 2014; Sonenshine et al., 1965; Thorner et al., 1998). The following species established in the Hampton Roads area of Virginia will be the focus of this study: *Amblyomma americanum*, *Amblyomma maculatum*, and *Dermacentor variabilis*.

Amblyomma americanum, commonly known as the lone star tick, is a three-host, non-nidicolous tick that quests for hosts by ambush and hunting methods (Sonenshine, 1993). The distribution of *A. americanum* covers much of the southeastern and mid-Atlantic portion of United States as well as parts of Central and South America (Estrada-Peña and Jongejan, 1999). Its range includes west-central Texas, north through Iowa, and eastward in a broad belt along the Atlantic Coast extending as far north as New York, Connecticut, Rhode Island, and Maine (Fig. 2) (CDC, 2013; Childs and Paddock, 2003; Ginsberg et al., 2002; Ijdo et al., 2000; Keirans and

Lacombe, 1998). *Amblyomma americanum* are found in habitats that are well suited for white-tail deer (*Odocoileus virginianus*), a preferred host for all life stages of this species. Predominately, *A. americanum* are found in woodland habitats with dense underbrush (Childs and Paddock, 2003), near edge habitat along woody vegetation, and in secondary successional fields (Semtner et al., 1971). *Amblyomma americanum* constitutes 97% (N=66,590) of the ticks collected on flags during surveillance studies in the Hampton Roads area of Virginia (Nadolny et al., 2014). In Tennessee, Fryxell et al. (2015) found 97% (N=5050) of field collected ticks were also *A. americanum.*

All three life stages of *A. americanum* feed predominantly on medium to large sized mammals, mainly white-tailed deer. Larvae and nymphs are also found feeding on various ground-feeding birds such as quail (*Colinus virginianus*) and wild turkey (*Meleagris gallopavo*) (Bishopp and Trembley, 1945; Childs and Paddock, 2003). *Amblyomma americanum* is the primary human-biting tick in the southeastern area of the US (Nadolny et al., 2014; Stromdahl and Hickling, 2012) with all three active life stages observed biting humans (Childs and Paddock, 2003). Of 913 ticks collected from humans in Georgia and South Carolina (1990 through 1995), 83% were *A. americanum* and represented all active life stages (Felz et al., 1996). Similarly, 70-95% of the tick species found on humans during the DOD Human Tick Test Kit Program were *A. americanum*, as reported from 2004 to 2010 in New Jersey, Maryland, Virginia, Kentucky, and South Carolina (Stromdahl and Hickling, 2012).

Amblyomma americanum is a vector for many disease-causing pathogens afflicting a wide variety of vertebrates including humans. It is a known vector of *Ehrlichia chaffeensis* and *E. ewingii*, the causative agents of ehrlichiosis in humans and animals (Anderson and Magnarelli, 2008; Childs and Paddock, 2003; Stromdahl and Hickling, 2012). *Francisella tularensis*, the agent of tularemia in humans and animals, is also vectored by this species (Ogden et al., 2014; Stromdahl and Hickling, 2012). Field collected *A. americanum* have been shown to harbor *Rickettsia rickettsii*, the causative agent of RMSF, although transmission is extremely low (Childs and Paddock, 2003; Stromdahl and Hickling, 2012). In one study, *Borrelia lonestari* was considered the causative agent for Southern Tick-Associated Rash Illness (STARI) associated with *A. americanum* and producing Lyme disease-like symptoms (Childs and Paddock, 2003). In another study, Wormser et al. (2005) concluded neither *B. lonestari* nor *B. burgdorferi* (agent of Lyme disease) is the causative agent for STARI. *Amblyomma americanum* is also a vector of *R. parkeri*, the agent of the disease *R. parkeri* rickettsiosis (Goddard, 2003). *Rickettsia parkeri* can be acquired by *A. americanum* by co-feeding alongside infected *A. maculatum* (known carriers of *R. parkeri*). In a laboratory study using guinea pigs, Wright et al. (2015) reported *A. americanum* nymphs co-feeding alongside *R. parkeri*-infected *A. maculatum* adults acquired *R. parkeri*. Additionally, Goddard (2003) reported transstadial and transovarial transmission of *R. parkeri* in *A. americanum* ticks, with 53% of the 150 *R. parkeri*-inoculated nymphal *A. americanum* testing positive as adults. *Amblyomma americanum* is also known to harbor *Rickettsia amblyommii*, currently considered nonpathogenic bacteria in the spotted fever group rickettsiae (SFGR) (Childs and Paddock, 2003; Nadolny et al., 2014).

Fig. 2. Distribution of the lone star tick, *Amblyomma americanum*, in the United States. Figure adapted from CDC website (http://www.cdc.gov/ticks/geographic_distribution.html, accessed November 7th, 2015).

Dermacentor variabilis, commonly known as the American dog tick, is a three-host, non-nidicolous species that quests for hosts via ambush (Sonenshine, 1993). Though its abundance varies greatly in different localities, *D. variabilis* ranges as far south as Mexico and north into Canada (Bishopp and Trembley, 1945; Hooker et al., 1912). In the US, it is more common throughout California on the west coast, and in the eastern two-thirds of the US (Fig. 3) (Bishopp and Trembley, 1945; CDC, 2013; Hooker et al., 1912; Thorner et al., 1998).

Dermacentor variabilis prefers a more humid environment, often found in mesic areas along old field-forest edges, trails, roadsides, and in second-growth forests (Anderson, 2002; Sonenshine, 1972; Sonenshine, 1979). Adults feed predominantly on medium to large sized mammals including raccoons, dogs, foxes, and humans (Sonenshine, 1979). Larva and nymphal ticks prefer smaller mammals such as mice and voles (Anderson and Magnarelli, 2008; Bishopp and Trembley, 1945; Clark et al., 2001). In Virginia, Sonenshine (1979) reported 74% of *D. variabilis* nymphs and larva collected were found on the white-footed mouse (*Peromyscus leucopus*) and 69% of adults were found on two species: raccoon (*Procyon* spp.) and opossum (*Didelphis* spp.). *Dermacentor variabilis* along with *I. scapularis* are the primary human-biting ticks in the northeastern area of the US (Merten and Durden, 2000; Nadolny et al., 2014; Stromdahl and Hickling, 2012). In Georgia and South Carolina, Felz et al. (1996) reported 11.4% of the ticks found on humans were *D. variabilis.* In the Hampton Roads area of Virginia, *D. variabilis* is the second prevalent species constituting 6% (N=66,590) of the ticks collected on flags during surveillance studies (Nadolny et al., 2014). Additionally, in Tennessee, Fryxell et al. (2015) found 3% (N=5050) of field collected ticks were *D. variabilis*, second only to *A. americanum*.

Dermacentor variabilis is the primary vector of *R. rickettsii* in the eastern parts of the US (Anderson and Magnarelli, 2008; Nadolny et al., 2014; Sonenshine, 1965; Thorner et al., 1998). *Rickettsia rickettsii*, the causative agent of RMSF, is a treatable but potentially lethal pathogen and is one of the most commonly reported tick-borne rickettsial diseases in the US (McQuiston, 2012). Recently however, molecular tests of *D. variabilis* in areas reporting RMSF have found an absence of *R. rickettsii*, and instead have detected *R. amblyommii* and *R. montanensis* (Stromdahl and Hickling, 2012). *Rickettsia montanensis* vectored by *D. variabilis* is generally considered nonpathogenic, although recently it has been associated with afebrile rash illness (McQuiston, 2012; Nadolny et al., 2014). *Dermacentor variabilis* has also been reported as a vector of *F. tularensis* (Estrada-Peña and Jongejan, 1999; Ogden et al., 2014).

Fig. 3. Distribution of the American dog tick, *Dermacentor variabilis*, in the United States. Figure adapted from CDC website (http://www.cdc.gov/ticks/geographic_distribution.html, accessed November 7th, 2015).

Amblyomma maculatum is a three-host, non-nidicolous tick that quests for hosts via ambush and hunting methods (Sonenshine, 1993). It is native to Central and South America including Mexico, Jamaica, Belize, West Indies, Columbia, Venezuela, and Peru (Sumner et al., 2007; Teel et al., 2010). In the US, *A. maculatum* has been found bordering the Gulf Coast and Coastal South Atlantic, continuing through Florida, and north to Kentucky and Mississippi, continuing up the coast through Virginia, including remote sections of Virginia's barrier islands (Gaff, unpublished). *Amblyomma maculatum* have an inland US range expanding to include Kansas, Oklahoma, and Texas (Fig. 4) (Bishopp and Trembley, 1945; CDC, 2013; Merten and Durden, 2000; Semtner and Hair, 1973; Sumner et al., 2007). Incidental collections of *A. maculatum* have been reported in Iowa and Maine, but no permanent populations have been reported (Teel et al., 2010). In the Hampton Roads area of Virginia, Wright et al. (2011) recently confirmed established populations in the area with Nadolny et al. (2014) reporting *A. maculatum* constitutes 2% (N=66,590) of the ticks collected on flags during surveillance studies from 2010-2012. Additionally, in Tennessee, Fryxell et al. (2015), found 7 *A. maculatum* ticks (N=5050) during field collections.

Amblyomma maculatum ticks are associated with tall-grass prairies and coastal uplands including areas with low shrub patches and edge habitat of prairies bordered by wooded uplands (Semtner and Hair, 1973; Teel et al., 2010). Adults feed predominantly on medium to large sized mammals including humans, and are major pests for livestock; whose movements are a possible cause of species range expansion (Bishopp and Trembley, 1945; Teel et al., 2010). Larva and nymphal ticks prefer smaller mammals including cotton rats (*Sigmodon hispidus hispidus*) and ground-dwelling birds (Bishopp and Trembley, 1945; Clark et al., 2001; Teel et al., 2010).

In the US, *A. maculatum* is the main vector of *Rickettsia parkeri*, the agent of the disease *R. parkeri* rickettsiosis, and more commonly named American Boutonneuse fever or Tidewater spotted fever (Fryxell et al., 2015; Nadolny et al., 2014; Wright et al., 2011). In southeastern

Virginia there were two confirmed cases of *R. parkeri* rickettsiosis, including the index case in 2002 (Whitman et al., 2007). Additionally, 20 *R. parkeri* infections were reported from mainly southern states in the US: Alabama, Florida, Kentucky, Maryland, Mississippi, North Carolina, South Carolina, and Texas (Paddock et al., 2010; Wright et al., 2011). *Amblyomma maculatum* is also the principal vector of *Hepatozoon americanum*, the causative agent of American canine hepatozoonosis in US coyotes and domestic dogs, as well as *Leptospira pomona*, the agent of leptospirosis in livestock. This tick species also vectors the agent associated with Panola Mountain *Ehrlichia* which infects people and domestic animals, and heartwater disease from the agent *Ehrlichia ruminantum*, infecting ruminant livestock (Teel et al., 2010). Although *A. maculatum* ticks are currently reported to bite humans less frequently than other tick species, they have historically been recognized as an aggressive human-biting tick (Hunter and Bishopp, 1911; Paddock et al., 2010). Felz et al. (1996) reported 9 (N=913) *A. maculatum* ticks were found on humans in Georgia and South Carolina. The increase in the *A. maculatum* tick range and the pathogens it harbors makes this species a concern for human and animal health.

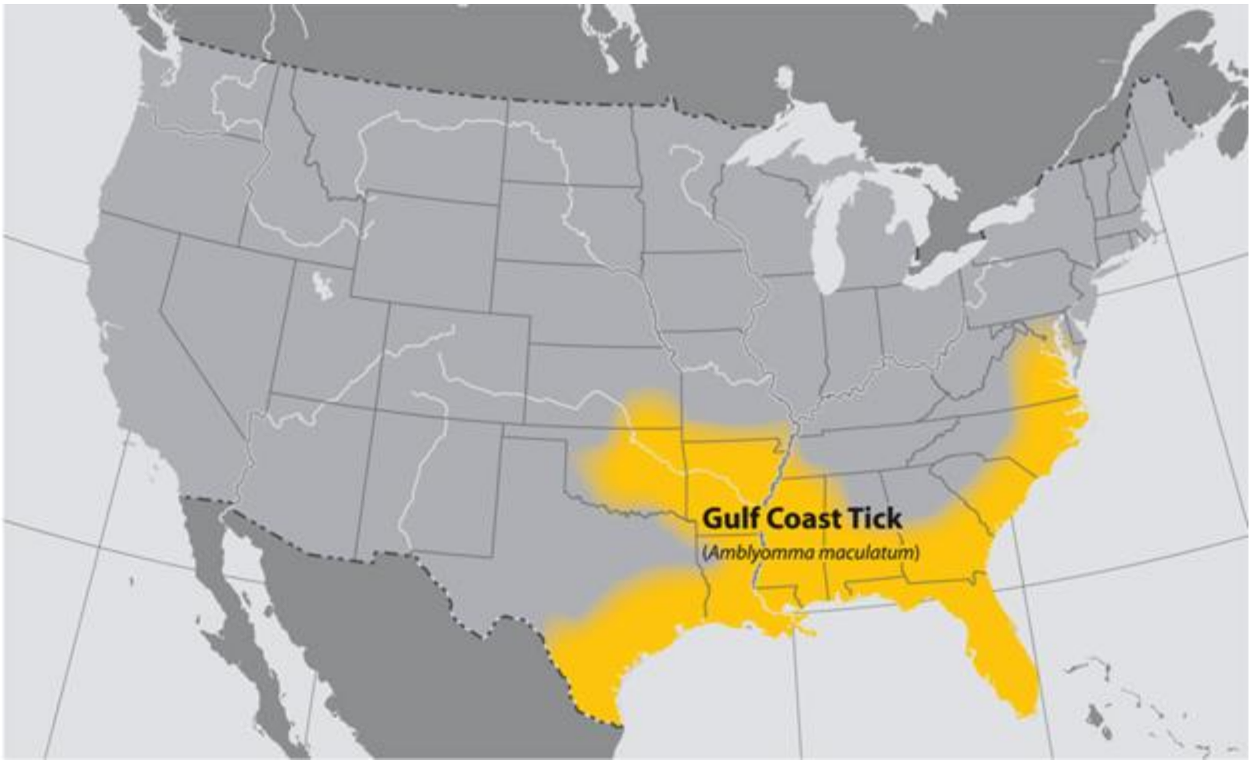

Fig. 4. Distribution of the Gulf Coast tick, *Amblyomma maculatum*, in the United States. Figure adapted from CDC website (http://www.cdc.gov/ticks/geographic_distribution.html, accessed November 7th, 2015).

EXPERIMENT 1: ENVIRONMENTAL SURVIVAL STUDY

STUDY GOALS

An environmental survival study was performed to obtain data on the abiotic factors contributing to the survival of unfed tick species in four sites in southeastern Virginia, two drier-upland habitats and two sites prone to flooding. A known quantity of unfed, adult ticks of three local species were housed inside environmental containers and placed in situ over a four month period. The containers were checked at fixed intervals to count the number of surviving ticks in order to quantify the ability of each tick species to survive specifically with regards to naturally occurring temperatures, humidity, soil saturation, and inundation. Prior work has been done with

I. scapularis in four habitats in Maryland (Carroll, 2003) and in two locations in Connecticut (Bertrand and Wilson, 1996). Locally, *A. americanum* and *D. variabilis* were studied by Garrett and Sonenshine (1979). Ongoing tick surveillance studies in the Hampton Roads area of Virginia suggest there has been a change in the biodiversity of the tick species' populations over the last 40 years (Childs and Paddock, 2003; Garrett and Sonenshine, 1979; Nadolny et al., 2014). Observations from tick surveillance studies and tick survival by Garrett and Sonenshine during the 1970s will be compared to the findings of this environmental survival study. These studies are important because they provide information about how tick morphology and physiology may contribute to the temporal and spatial variability of three tick species in southeastern Virginia. They also give insight into the success of ticks actively questing for a host, aiding in the ongoing tick surveillance studies in the Hampton Roads area.

During the environmental survival study the following hypotheses were tested: 1) there is a significant difference between tick survival by field site; 2) there is a significant difference between tick survival by species; 3) there is a significant difference between tick survival by sex. These hypotheses were generated based on reviews from prior studies using *A. americanum* and *D. variabilis* by Garrett and Sonenshine (1979). Lower tick survival was reported from environmental survival studies in the Great Dismal Swamp (Chesapeake, VA) when compared to a more upland forest site in Newport News, VA. Additionally, surveillance studies support habitat preference for different tick species (personal communication: Gaff). This research was also supported by a pilot study performed from May through September of 2014 which tested the study methods and application. The pilot study demonstrated the use of in situ environmental containers as a successful way of testing tick survival.

METHODS AND MATERIALS

The environmental survival study was completed at four field plots in the Hampton Roads region covering a variety of habitat types, vegetation, and levels of soil saturation. Field sites were: Newport News Park (Newport News, VA; wooded edge habitat with low level undergrowth, public access), Hampton (Hampton, VA; wooded edge habitat with low level undergrowth, restricted access), Stephens Tract (Chesapeake, VA; closed canopy, late secondary successional wooded habitat prone to flooding, limited access), and Jacobson Tract (Chesapeake, VA; early secondary successional habitat dominated by grasses prone to flooding, limited access). Permission to use each field site was granted by the owner of that location.

Microcosms called tickaria (singular tickarium, description adapted from Yunik et al., 2015), were used in the environmental survival study to house *A. americanum*, *D. variabilis*, and *A. maculatum* ticks at each field site over a four month period. Following the housing design by Garrett and Sonenshine (1979), cylindrical, metal canisters protecting a chiffon cloth bag containing soil, leaf litter, a structural support, and ticks were submerged into the ground at each of the four field plots. Male and female adult unfed ticks were obtained from Oklahoma State University (OSU) raised colonies. The experimental ticks did not harbor disease-causing microorganisms and standard laboratory safety procedures were followed for each experimental tickarium and whenever handling the ticks.

The tickaria consisted of cylindrical metal containers approximately 16.5 cm in height and 15 cm in diameter. Holes were placed into the bottom of the containers to allow for precipitation drainage. Chiffon cloth bags were sewn to fit the internal dimensions of the containers. Approximately 2.5 cm of soil and 2.5 cm of leaf litter from the field site was placed inside the chiffon bag along with 25 adult ticks of a single species (approximately half male, half female). To keep the cloth bag from collapsing on the leaf litter and ticks, and to provide artificial structure for questing behavior, a plastic berry basket was placed inside the bag on top of the leaf litter. The chiffon bag was closed with fabric tape and sealed into the metal tickarium with aluminum screening secured to the top of the container with rubber bands, in hopes to deter wildlife interaction (Fig. 5 and Fig. 6). Two replicates of four designated tickaria were placed at each field site per individual tick species, totaling eight tickaria per species per site. The 24 total tickaria representing all three tick species were randomly placed within each respective field plot. They were submerged into the ground approximately 5 cm, for a total of 96 tickaria across all four sites (Fig. 6). The submerged depth was chosen to allow the sediment and leaf litter inside the tickarium to be level with the surrounding environment (Fig. 5). Marker flags were used to label individual treatment groups. From May through September 2015, two replicate tickaria were removed every four weeks per species at each field site and taken to the laboratory. In the laboratory, the chiffon bags were opened and surviving ticks were recorded. Temperature, current weather condition, relative humidity (at ground level), and soil moisture were recorded at each transect every two weeks. Soil moisture was determined through the collection of a sediment core; the difference between "wet" and "dry" weight was recorded (Fig. 7).

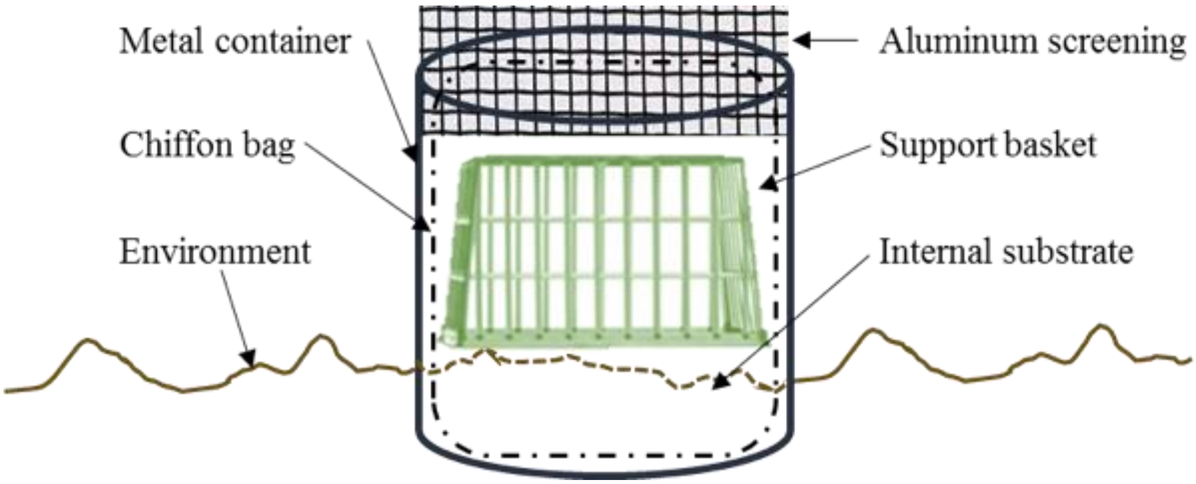

Fig. 5. Tickarium schematic.

Fig. 6. Tickarium design empty (left), cloth bags, support baskets, and metal screen lids (middle), in situ tickaria (right); photographs taken by Lindsey A. Bidder on April 26, 2015 and May 2, 2015.

Fig. 7. Sediment core, temperature, and humidity being collected at a field site (left), sediment core (middle), weight measurement of sediment core (right); photographs taken by Lindsey A. Bidder on August 2 and 25, 2015.

Over the course of this study a total of 2,508 male and female adult unfed ticks were observed (2,374 experimental ticks and 134 incubator ticks). The 2,374 experimental ticks consisted of: 791 *A. americanum* (392 male, 399 female), 791 *A. maculatum* (394 male, 397 female), and 792 *D. variabilis* (387 male, 405 female) (Table 1). During the initial setup of the tickaria, there were a total 2,400 experimental ticks (800 per species) divided amongst the 96 tickaria. Although, a total of 26 ticks were not recovered from the tickaria when removed from the field to check for survival (9 *A. americanum,* 9 *A. maculatum,* and 8 *D. variabilis*). These ticks were presumed "missing" and were not used in analysis. There were a total of 134 spare ticks that were placed in a laboratory incubator (Table 13), they were used as a comparative study group: 60 *A. americanum* (31 male, 29 female), 37 *A. maculatum* (18 male, 19 female), and 37 *D. variabilis* (all female). There were no available male *D. variabilis* to place with the laboratory incubator tick group. This was because a sudden male *D. variabilis* die-off occurred while preparing the tickaria in the field which exhausted the male *D. variabilis* supply. During the initial setup of the study; spare female *D. variabilis* were used to ensure that proper tick numbers in the *D. variabilis* tickaria were met.

FIELD AND LABORATORY WORK

Forty-eight tickaria total were submerged in the field on May 2, 2015; 24 tickaria at the Hampton site and 24 tickaria at the Newport News site. Forty-eight tickaria total were submerged in the field on May 9, 2015; 24 tickaria at the Jacobson Tract and 24 tickaria at the Stephens Tract. Field measurements and a sediment core sample was collected at each site on the setup date. Each site was visited bi-weekly to collect additional field measurements and to monitor the tickaria for disturbance. Every four weeks (once a month) over a four month study period, six tickaria were removed from each site (24 total), representing two replicates for each species. The study concluded on August 25, 2015 at the Hampton and Newport News sites and on August 30, 2015 for the Jacobson Tract and Stephens Tract field sites. Tick survival determination was performed in a laboratory and standard laboratory safety procedures were followed. All tickaria were opened and ticks checked for survival 24 to 48 hours after removal from the field. Immediately upon opening a tickarium in the laboratory, questing and ambulatory ticks were collected from the cloth bag and leaf litter, placed on painter's tape for counting purposes (Fig. 8), documented as alive per sex and species, then placed in holding vials. Once all living ticks were removed, the sediment and leaf litter was placed on a metal tray in order to find any remaining ticks. Ticks were carefully removed from the leaf litter and sediment as they were located. A sediment sieve was used if all the ticks were not accounted for after thoroughly searching through the substrate by hand. Ticks showing obvious mortality (i.e. missing limbs or having severe body damage) (Fig. 9) were placed in a holding vial and documented as dead per sex and species. Ticks that looked intact but were not showing initial signs of activity were placed into a petri dish. Warm breath was used in attempts to stimulate the ticks for 3 minutes. If no activity was observed the ticks were recorded as dead. If movement was observed ticks were recorded as alive (Needham et al., 1996; Scifres et al., 1988).

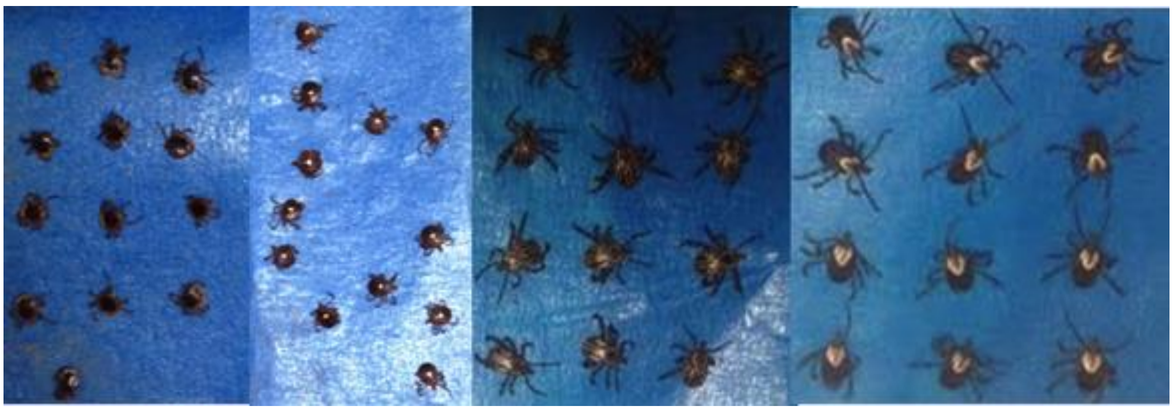

Fig. 8. Living ticks placed on painter's tape separated by species and sex for survival counting purposes; from the left: male *A. americanum*, female *A. americanum*, male *A. maculatum*, and female *A. maculatum*, photographs taken by Lindsey A. Bidder on September 2, 2014 and June 9, 2015.

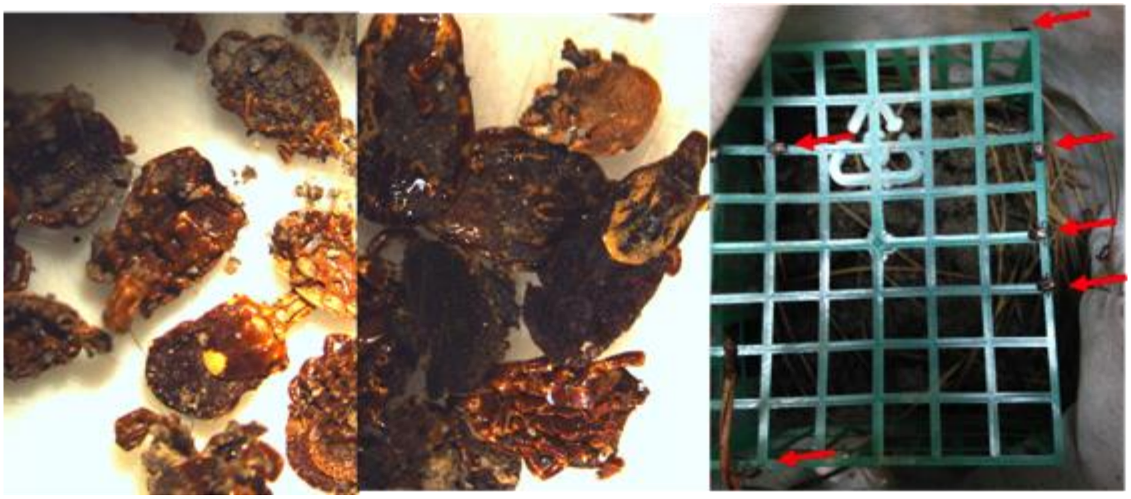

Fig. 9. Ticks showing obvious mortality (left and middle), questing and ambulatory ticks (right); photographs taken by Lindsey A. Bidder on September 2, 2014 and June 2, 2015.

STATISTICAL ANALYSIS

A Priori Power analysis was conducted using G*Power3 software (Faul et al., 2007). The data used for the power analysis in the environmental survival study was from four field sites with three tick species including two sexes using a 0.05 alpha, 0.25 effect size F and a power target of 0.95. This returned a total sample size required of 252 for the three tick species consisting of two sexes and comparing the four field sites. The experimental sample size used in the study was 2,374 ticks total.

A Kaplan-Meier and Cox Regression survival analysis was run using the IBM® SPSS® version 22 program (IBM Corporation 1989, 2013) on the experimental groups. The data used for the analysis included the number of *A. americanum*, *A. maculatum*, and *D. variabilis* ticks by sex and field site censored by mortality, over the four month study period. For the Kaplan-Meier analysis the following tests were run: Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon), and Tarone-Ware; a survival plot was also produced. For the Cox Regression analysis, an

Omnibus test of model coefficients was run and the following plots were produced: survival and log-minus-log. The Cox Regression results are reported in the results section and the Kaplan-Meier results are reported in the appendix section, except in specific analyses. In analyses where a comparative factor had zero mortality, it is inappropriate to run a Cox Regression. In those cases, the Kaplan-Meier results are reported in the results section and the Cox Regression results are reported in the appendix section.

RESULTS

The environmental survival study ran from May 2 to August 25, 2015 for the Hampton and Newport News sites, and from May 9 to August 30, 2015 for the Jacobson and Stephens Tracts (Table 1 and Table 47). The experimental study group consisted of 2,374 male and female adult ticks: 791 *A. americanum* (392 male, 399 female), 791 *A. maculatum* (394 male, 397 female), and 792 *D. variabilis* (387 male, 405 female). Of the intended 600 ticks placed at each field site, 596 male and female ticks were recovered from tickaria at the Hampton field site encompassing 200 *A. americanum* (100 male, 100 female), 197 *A. maculatum* (100 male, 97 female), and 199 *D. variabilis* (100 male, 99 female). At the Jacobson Tract field site 595 total ticks were recovered encompassing 196 *A. americanum* (97 male, 99 female), 201 *A. maculatum* (98 male, 103 female), and 198 *D. variabilis* (98 male, 100 female). At the Newport News field site 588 total ticks were recovered encompassing 196 *A. americanum* (97 male, 99 female), 195 *A. maculatum* (98 male, 97 female), and 197 *D. variabilis* (98 male, 99 female). At the Stephens Tract field site 595 total ticks were recovered encompassing 199 *A. americanum* (98 male, 101 female), 198 *A. maculatum* (98 male, 100 female), and 198 *D. variabilis* (91 male, 107 female) (Table 1 and Table 2).

Temperature, current weather condition, relative humidity (at ground level), and soil moisture were recorded at each plot every two weeks over the study period. The environmental and weather data was collected from May 2 to September 22, 2015 at the Hampton and Newport News sites, and from May 9 to September 16, 2015 at the Stephens and Jacobson Tracts (Table 46). Over the four month study period, the average precipitation ranged from 1.9 cm to 7.0 cm with the Stephens Tract having the highest average precipitation (7.0 cm) and the Hampton site having the least average recorded (1.9 cm). The average RH ranged from 55.3% to 64.7% with the Stephens Track having the highest average RH (64.7%) and both Newport News and Jacobson Tract having the lower averages (55.3% and 55.8% respectively). Temperature averages ranged from 28.0°C to 34.7°C, with the Jacobson Tract being the warmest and the Stephens Tract being relatively the coolest. Average soil saturation ranged from 15.6% to 20.0% saturation with the Hampton site being the most saturated (20.0%) and the other three field sites having similar saturation levels of approximately 16% (Table 3).

At the conclusion of the four month period: 786 (99%) *A. americanum* survived (389 male, 397 female) with 5 (<1%) ticks confirmed dead (3 male, 2 female). Of the *A. maculatum* ticks, 540 (68%) survived (277 male, 263 female) with 251 (32%) ticks confirmed dead (117 male, 134 female). Of the *D. variabilis* ticks, 733 (93%) survived (370 male, 363 female) with 59 (7%) ticks confirmed dead (17 male, 42 female) (Table 4 and Table 5). At the Hampton site, 556 (93%) ticks survived (282 male, 274 female) with 40 (7%) ticks confirmed dead (18 male, 22 female) at the conclusion of the four month study period. At the Jacobson Tract, 464 (78%) ticks survived (227 male, 237 female) with 131 (22%) ticks confirmed dead (66 male, 65 female). At the Newport News site, 525 (89%) ticks survived (265 male, 260 female) with 63 (11%) confirmed dead (28 male, 35 female). At the Stephens Tract, 514 (86%) ticks survived

(262 male, 252 female) with 81 (14%) ticks confirmed dead (25 male, 56 female) (Table 5). Of the cumulative 315 ticks confirmed dead from all three species, 16 (5%) were of ticks from the tickaria removed after one month in situ, 29 (9%) after two months in situ, 110 (35%) after three months in situ, and 160 (51%) after four months in situ (Table 6 and Fig. 14).

Kaplan-Meier and Cox Regression survival analyses were performed to test the following hypotheses: 1) there is a significant difference between tick survival by species; 2) there is a significant difference between tick survival by field site; 3) there is a significant difference in tick survival by sexes.

1) The results of the Cox Regression survival analysis indicates that there is a significant difference ($p<0.0005$) in survival among species across all field sites over the four month study period (Table 7). There is a 50.5-times ($p<0.0005$) higher risk of mortality for *A. maculatum* when compared to *A. americanum*, a 4.3-times ($p<0.0005$) higher risk of mortality for *A. maculatum* when compared to *D. variabilis*, and an 11.9-times ($p<0.0005$) higher risk of mortality for *D. variabilis* when compared to *A. americanum* (Table 8, Fig. 15, and Fig. 16).

2) The results of the Cox Regression survival analysis indicates there is a significant difference ($p<0.0005$) in tick survival between the field sites prone to flooding (Stephens and Jacobson) and the more dry, upland field sites (Hampton and Newport News) over the four month study period. There is a 2.0-times ($p<0.0005$) higher risk of mortality for ticks at the wet sites than the dry sites (Table 9, Fig. 17, and Fig. 18). There is also an overall significant difference ($p<0.0005$) between tick survival for each individual field site over the four month study period (Table 10). Although, there is no significant difference ($p=0.155$) between tick survival at the Newport News site when compared to the Stephens site but all other site comparisons are significant (Table 11, Fig. 19, and Fig. 20).

3) The results of the Cox Regression survival analysis indicates there is no significant difference ($p=0.058$) in survival between sexes (Table 12, Fig. 21, and Fig. 22).

Of the 5 *A. americanum* mortalities, 2 female ticks died at the Stephens Tract and 1 male tick died at each of the other three sites: Hampton, Newport News, and Jacobson Tract (Table 5). The Cox Regression survival analysis indicates there was no significant difference ($p=0.899$) in *A. americanum* survival across all four of the field sites (Table 30, Table 31, Fig. 34, and Fig. 35).

Of the 59 *D. variabilis* mortalities, 32 (54%, 12 male, 20 female) died at the Jacobson Tract site, 1 female (2%) died at the Newport News site, and 26 (44%, 5 male, 21 female) died at the Stephens Tract. No *D. variabilis* mortality was observed from the Hampton Site (Table 5). The results of the Kaplan-Meier survival analysis indicates there is a significant difference ($p<0.0005$) between tick survival for each individual field site over the four month study period (Table 34 and Fig. 39). Although, a Kaplan-Meier pairwise comparison survival analyses indicates there is no significant difference ($p=0.425$) in *D. variabilis* survival between the Stephens Tract and the Jacobson Tract sites. There is also no significant difference ($p=0.317$) in *D. variabilis* survival between the Hampton and Newport News sites (Table 35). A Cox Regression survival analysis indicates there is a significant difference ($p<0.0005$) in *D. variabilis* survival between the collective field sites prone to flooding (Stephens and Jacobson) and the more dry, upland field sites (Hampton and Newport News) over the four month study period. There is a 57.9-times ($p<0.0005$) higher risk of mortality for *D. variabilis* when the species is containerized at the more wet sites than the more dry sites (Table 33, Fig. 37, and Fig. 38). Additionally, there is a 1.2-times ($p<0.0005$) higher risk of *D. variabilis* mortality at the Jacobson Site than the Stephens site (Table 37, Fig. 40, and Fig. 41).

Of the 251 *A. maculatum* mortalities, 39 (16%, 17 male, 22 female) died at the Hampton site, 61 (24%, 27 male, 34 female) died at the Newport News site, 98 (39%, 53 male, 45 female) died at the Jacobson Tract, and 53 (21%, 20 male, 33 female) died at the Stephens Tract (Table 5). The results of the Cox Regression survival analysis indicates there is a significant difference ($p<0.0005$) between tick survival for each individual field sites over the four month study period (Table 42). Although, the survival analyses indicates there is no significant difference between *A. maculatum* survival when comparing the Stephens Tract to the Hampton and the Newport News sites ($p=0.178$ and $p=0.349$ respectively); but, there is a significant difference ($p<0.05$) in survival between all other site comparisons (Table 43). Additionally, there is a significant difference ($p<0.0005$) in *A. maculatum* survival between the collective field sites prone to flooding (Stephens and Jacobson) and the more dry, upland field sites (Hampton and Newport News) over the four month study period. There is a 1.5-times ($p=0.003$) higher risk of mortality for *A. maculatum* when the species is containerized at the more wet sites than the more dry sites (Table 39 and Fig. 43). Additionally, there is a 1.8-times ($p<0.0005$) higher risk of *A. maculatum* mortality at the Jacobson Tract than the Stephens Tract (Table 43, Fig. 46, and Fig. 47).

Table 1. Initial number of experimental ticks by field site per species and sex in the environmental survival study, and the start and end dates of the study per field site in 2015.

Field Site / Date	Species	Num. Male Ticks	Num. Female Ticks	Total Ticks
Hampton	*A. americanum*	100	100	200
May 2 – Aug. 25	*A. maculatum*	100	97	197
	D. variabilis	100	99	199
Newport News	*A. americanum*	97	99	196
May 2 – Aug. 25	*A. maculatum*	98	97	195
	D. variabilis	98	99	197
Jacobson Tract	*A. americanum*	97	99	196
May 9 – Aug. 30	*A. maculatum*	98	103	201
	D. variabilis	98	100	198
Stephens Tract	*A. americanum*	98	101	199
May 9 – Aug. 30	*A. maculatum*	98	100	198
	D. variabilis	91	107	198
Totals		**1173**	**1201**	**2374**

Table 2. Initial number of experimental ticks by tickaria removal month per species and sex in the environmental survival study.

Month	Species	Num. Male Ticks	Num. Female Ticks	Total Ticks
1	*A. americanum*	100	100	200
	A. maculatum	99	100	199
	D. variabilis	97	100	197
2	*A. americanum*	96	100	196
	A. maculatum	100	97	197
	D. variabilis	100	100	200
3	*A. americanum*	97	99	196
	A. maculatum	98	100	198
	D. variabilis	98	100	198
4	*A. americanum*	99	100	199
	A. maculatum	97	100	197
	D. variabilis	92	105	197
Totals		**1173**	**1201**	**2374**

Table 3. Maximum (Max), minimum (Min), and average (Avg) environmental and weather data recorded over the four month study period per field site; Wet Sites = collective data from Jacobson and Stephens Tract, Dry Sites = collective data from Hampton and Newport News.

	Relative Humidity (%)			**Temperature (°C)**			**Precipitation (cm)**			**Soil Saturation (%)**		
Site	**Max**	**Min**	**Avg**	**Max**	**Min**	**Avg**	**Max**	**Min**	**Avg**	**Max**	**Min**	**Avg**
Hampton	84.0	39.0	57.3	40.0	19.0	29.5	6.1	0.0	1.9	20.6	10.4	20.0
Newport News	81.0	37.0	55.3	41.0	20.0	30.5	12.7	0.5	5.1	33.4	13.1	16.3
Jacobson Tract	76.0	41.0	55.8	44.0	27.0	34.7	12.7	0.0	4.8	21.0	11.5	16.0
Stephens Tract	76.0	52.0	64.7	34.0	22.0	28.0	12.7	1.3	7.0	21.7	7.5	15.6
Wet Sites	76.0	41.0	61.7	44.0	22.0	30.2	12.7	0.0	5.8	21.7	7.5	15.7
Dry Sites	84.0	37.0	56.3	41.0	19.0	30.0	12.7	0.0	3.6	29.7	10.4	17.6

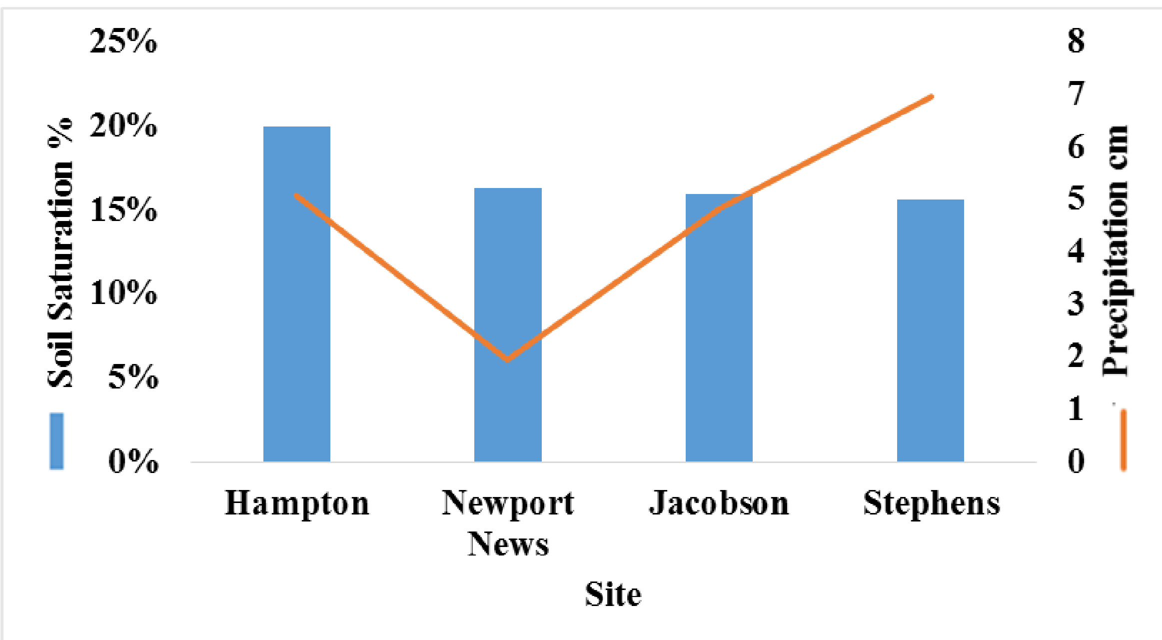

Fig. 10. Average soil saturation (%) and precipitation (cm) recorded over the four month study period per field site.

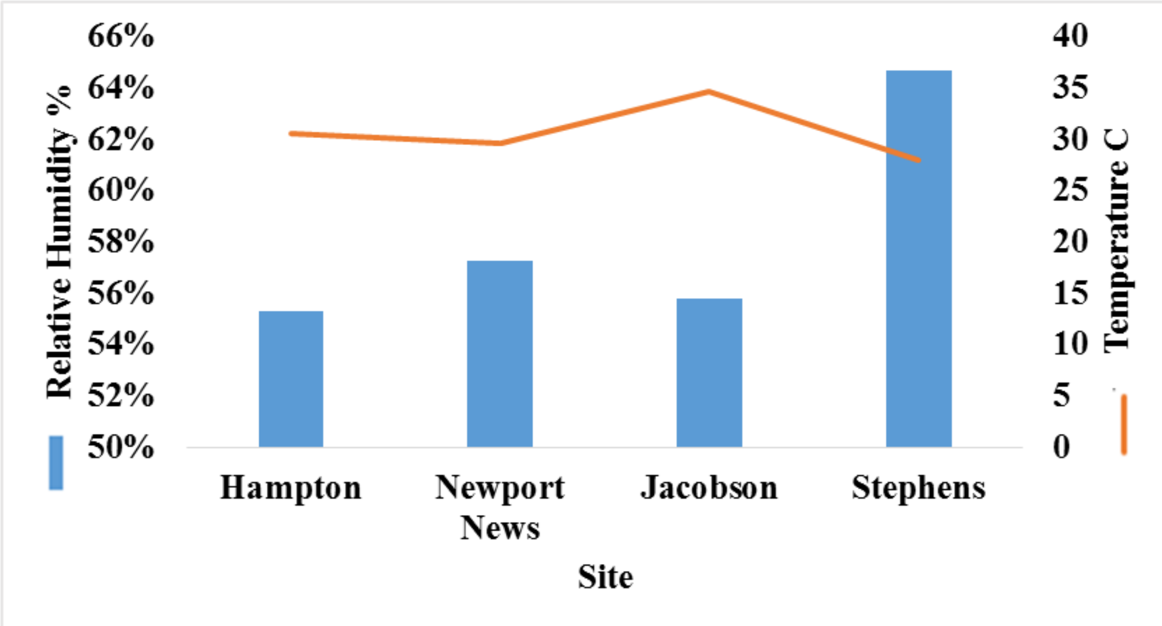

Fig. 11. Average relative humidity (%) and temperature (°C) recorded over the four month study period per field site.

Table 4. Total number of tick survival and mortality by species in the environmental survival study.

Species	N	Total Survival	Total Mortality
A. americanum	791	786	5
A. maculatum	791	540	251
D. variabilis	792	733	59

Table 5. Total number of male and female tick survival and mortality by field site per species and sex in the environmental survival study.

		Survival		Mortality		Totals	
Field Site	**Species**	**Male**	**Female**	**Male**	**Female**	**Survival**	**Mortality**
Hampton	*A. americanum*	99	100	1	0	199	1
	A. maculatum	83	75	17	22	158	39
	D. variabilis	100	99	0	0	199	0
Newport News	*A. americanum*	96	99	1	0	195	1
	A. maculatum	71	63	27	34	134	61
	D. variabilis	98	98	0	1	196	1
Jacobson Tract	*A. americanum*	96	99	1	0	195	1
	A. maculatum	45	58	53	45	103	98
	D. variabilis	86	80	12	20	166	32
Stephens Tract	*A. americanum*	98	99	0	2	197	2
	A. maculatum	78	67	20	33	145	53
	D. variabilis	86	86	5	21	172	26
Totals		**1036**	**1023**	**137**	**178**	**2059**	**315**

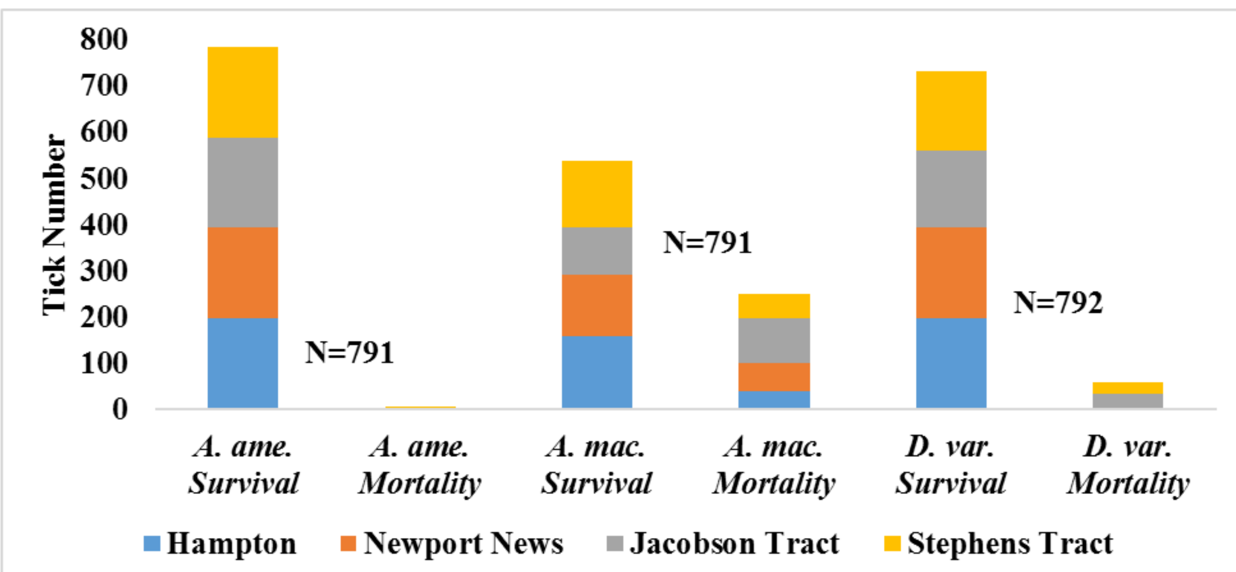

Fig. 12. Total number of tick survival and mortality in the experimental ticks over the four month study period per field site. *A. ame.* = *A. americanum* (N=791), *A. mac.* = *A. maculatum* (N=791), *D. var.* = *D. variabilis* (N=792).

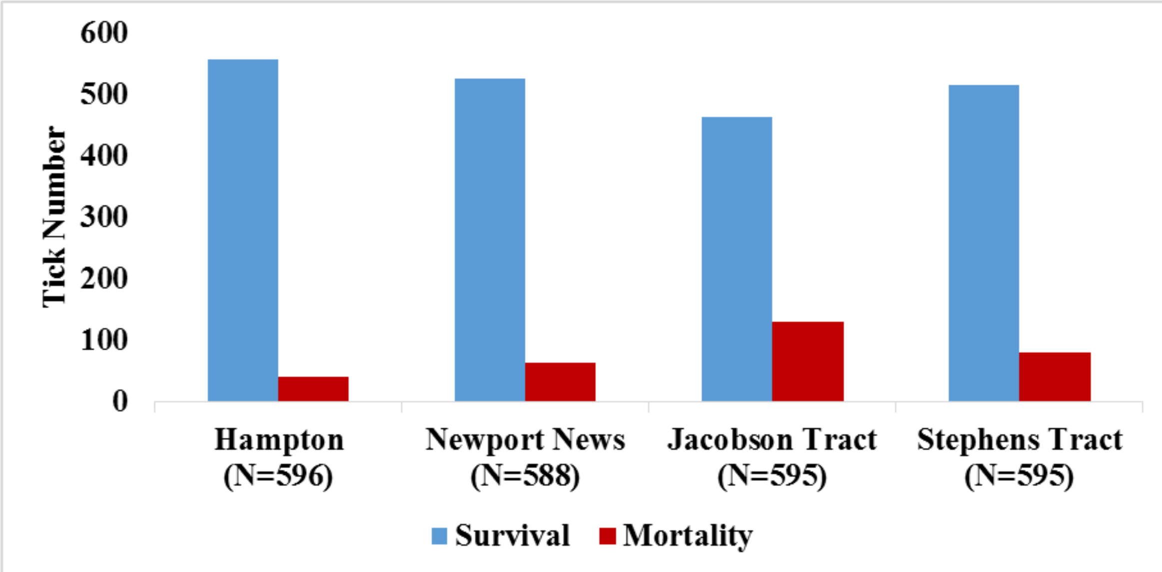

Fig. 13. Total number of tick survival and mortality in the experimental ticks over the four month study period per field site.

Table 6. Total number of male and female tick survival and mortality by month per species and sex in the environmental survival study.

		Survival		**Mortality**		**Totals**	
Month	**Species**	**Male**	**Female**	**Male**	**Female**	**Survival**	**Mortality**
1	*A. americanum*	99	100	1	0	199	1
	A. maculatum	93	97	6	3	190	9
	D. variabilis	95	96	2	4	191	6
2	*A. americanum*	95	100	1	0	195	1
	A. maculatum	88	85	12	12	173	24
	D. variabilis	100	96	0	4	196	4
3	*A. americanum*	96	98	1	1	194	2
	A. maculatum	65	50	33	50	115	83
	D. variabilis	89	84	9	16	173	25
4	*A. americanum*	99	99	0	1	198	1
	A. maculatum	31	31	66	69	62	135
	D. variabilis	86	87	6	18	173	24
Totals		**1036**	**1023**	**137**	**178**	**2059**	**315**

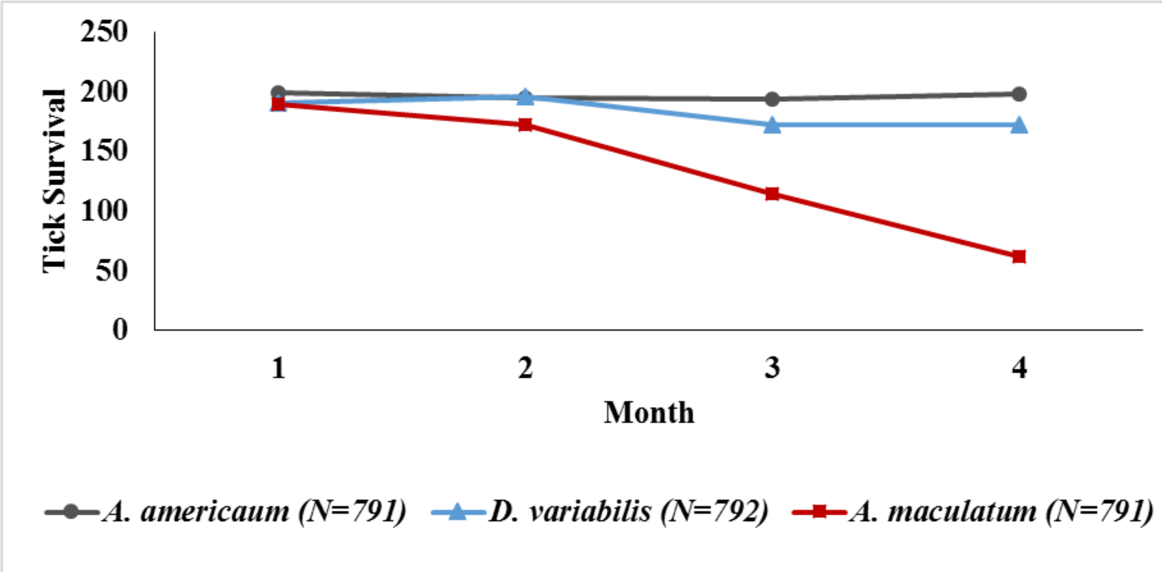

Fig. 14. Total number of tick survival in the experimental ticks over the four month study period by species.

Table 7. Cox Regression Omnibus tests of model coefficients table for the experimental ticks comparing species.

χ^2	df	p-value
319.548	2	<0.0005

Table 8. Cox survival analysis table for the experimental ticks comparing species. *A. ame.* = *A. americanum*, *A. mac.* = *A. maculatum*, *D. var.* = *D. variabilis*.

	A. americanum					***D. variabilis***				
Species	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
A. ame.	-	165.611	-	-	-	-2.473	28.180	1	<0.0005	0.084
A. mac.	3.921	75.370	1	<0.0005	50.451	1.448	100.223	1	<0.0005	4.256
D. var.	2.473	28.180	1	<0.0005	11.853	-	165.611	-	-	-

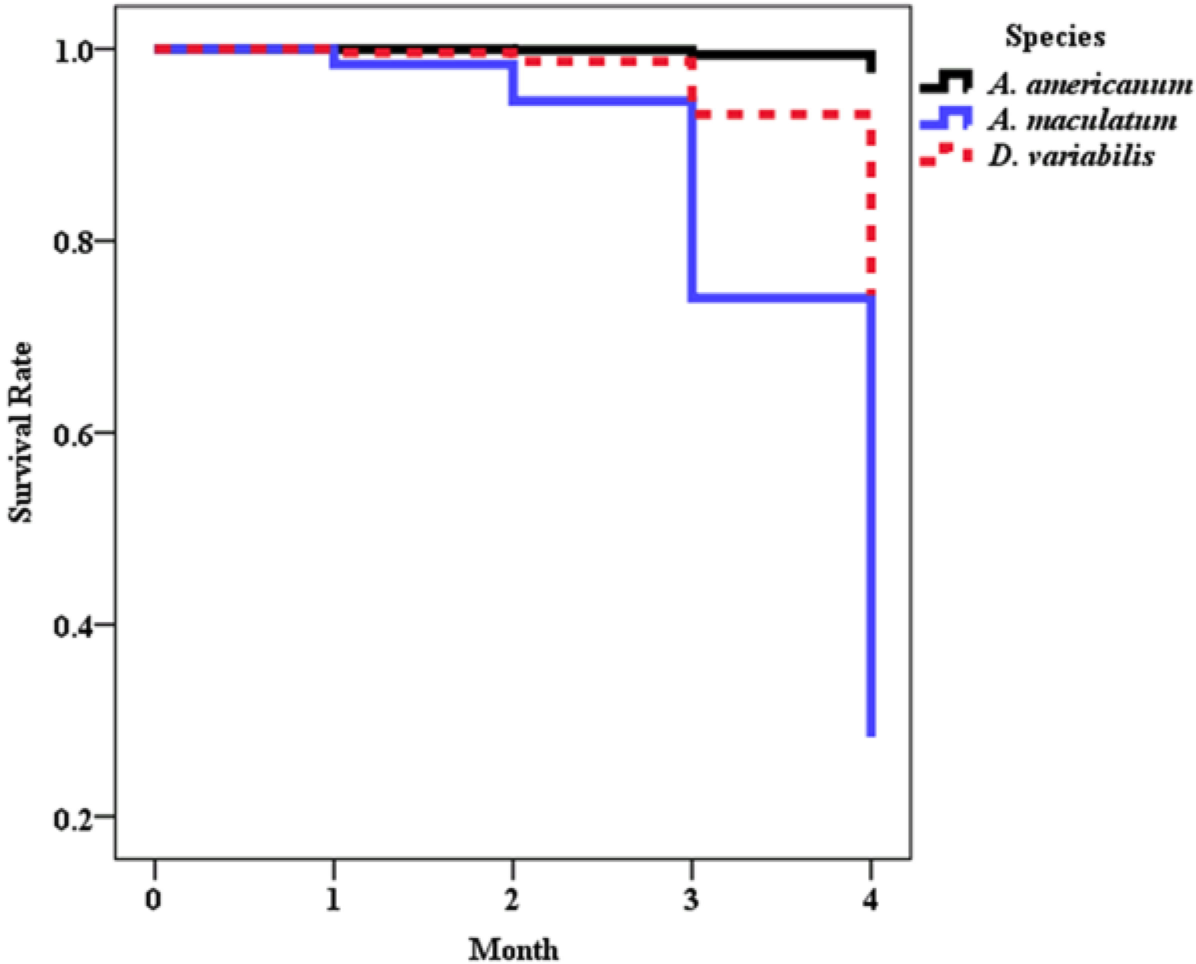

Fig. 15. Cox Regression survival rate for the experimental ticks by month per species.

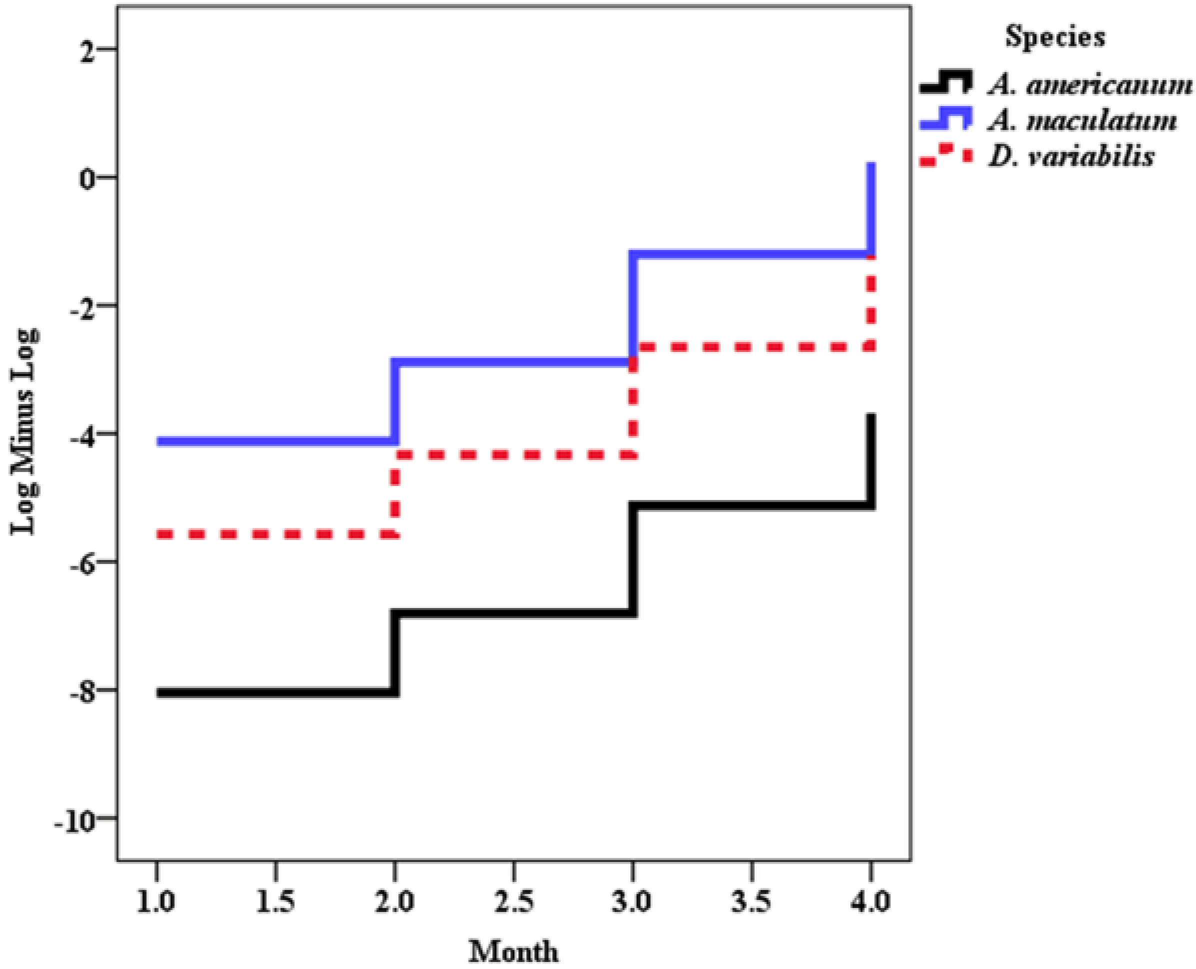

Fig. 16. Cox Log Minus Log for the experimental ticks by month per species.

Table 9. Cox survival analysis table for the experimental ticks comparing wet sites (Stephens and Jacobson) to dry sites (Hampton and Newport News).

ß	**χ^2**	**df**	**p-value**	**Exp(ß)**
0.713	35.230	1	<0.0005	2.040

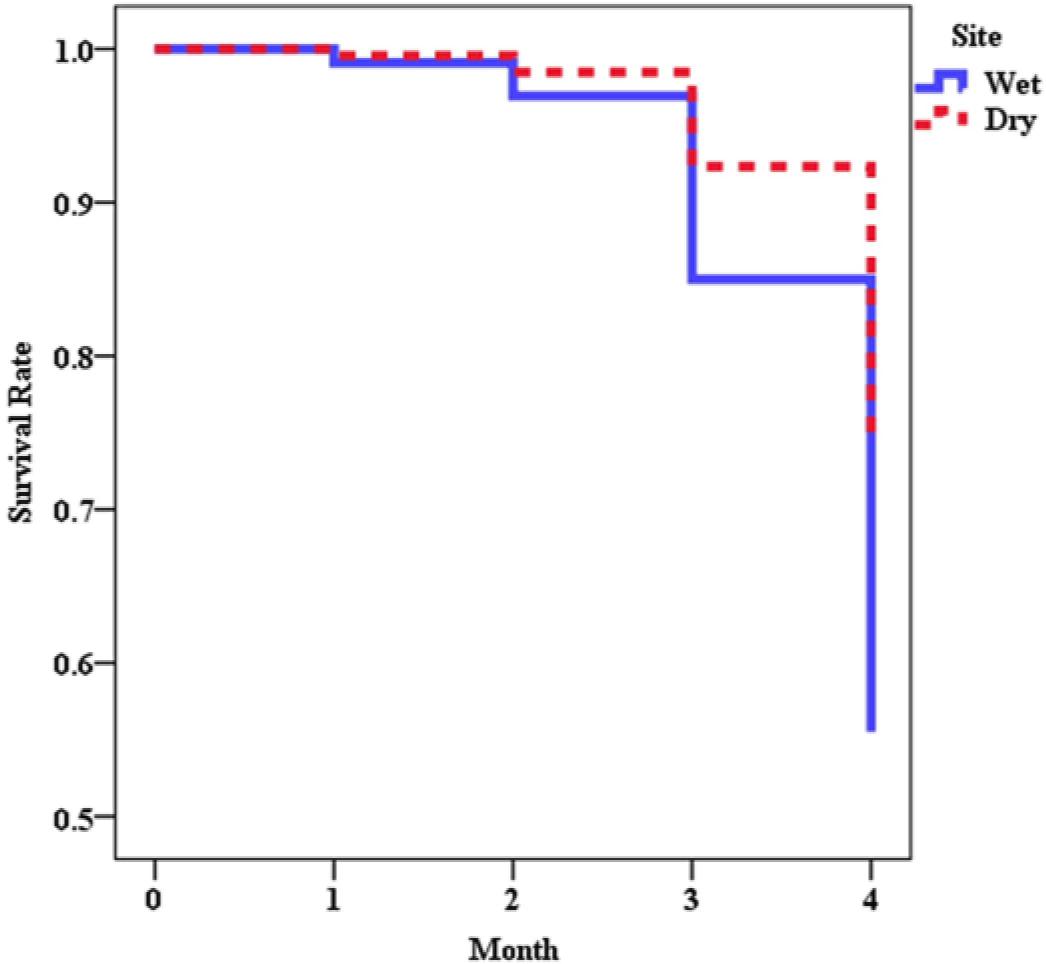

Fig. 17. Cox Regression survival rate for the experimental ticks by month per wet (Stephens and Jacobson) and dry (Hampton and Newport News) sites.

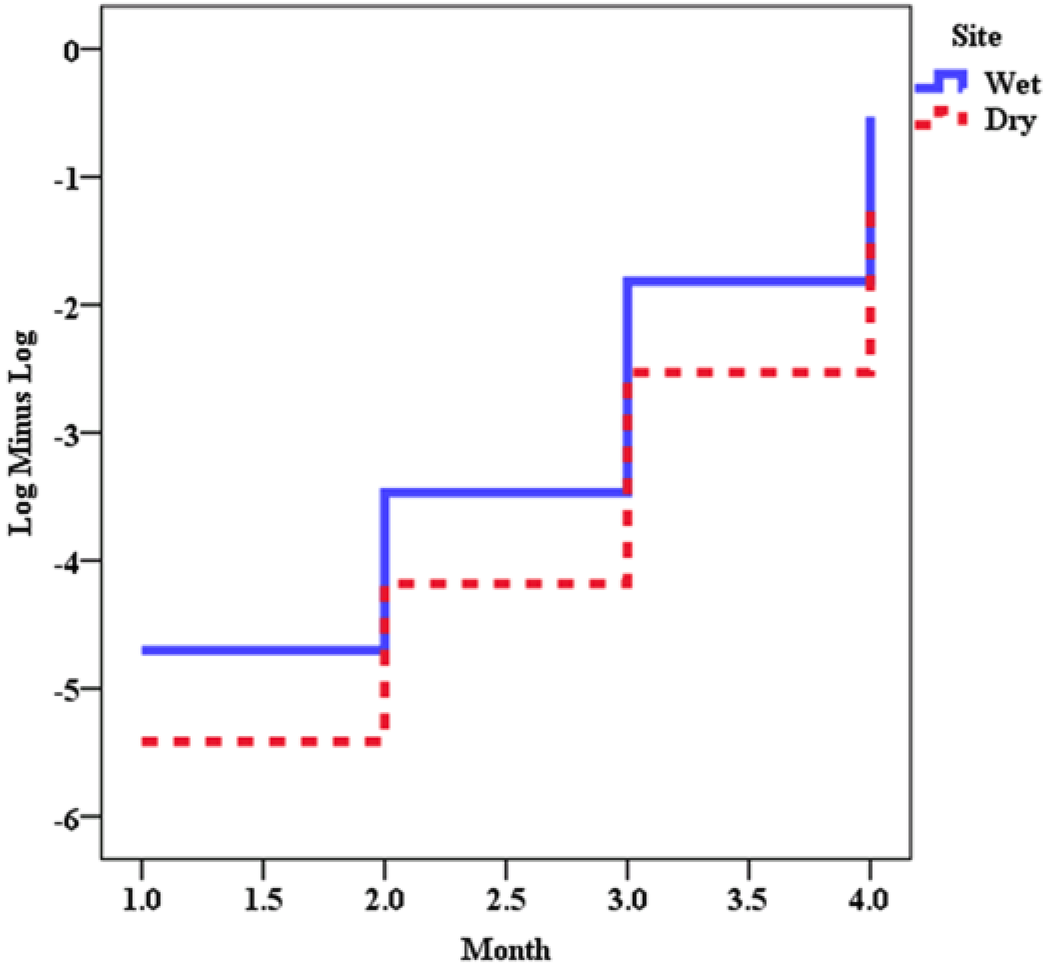

Fig. 18. Cox Log Minus Log for the experimental ticks by month per wet (Stephens and Jacobson) and dry (Hampton and Newport News) sites.

Table 10. Cox Regression Omnibus tests of model coefficients table for the experimental ticks comparing individual sites.

χ^2	**df**	**p-value**
55.963	3	<0.0005

Table 11. Cox survival analysis table for the experimental ticks comparing individual sites. Sites: NN = Newport News, HA = Hampton, JC = Jacobson Tract, ST = Stephens Tract.

	ST					JC				
Site	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
NN	-0.239	2.025	1	0.155	0.787	-0.719	21.970	1	<0.0005	0.487
HA	-0.701	13.160	1	<0.0005	0.496	-1.181	42.713	1	<0.0005	0.307
JC	0.480	11.512	1	0.001	1.615	-	-	-	-	-
ST	-	-	-	-	-	-0.480	11.512	1	0.001	0.619

	NN					HA				
Site	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
JC	0.719	21.970	1	<0.0005	2.052	1.181	42.713	1	<0.0005	3.256
HA	-0.462	5.222	1	0.022	0.630	-	-	-	-	-
ST	0.239	2.025	1	0.155	1.270	0.701	13.160	1	<0.0005	2.016
NN	-	-	-	-	-	0.462	5.222	1	0.022	1.587

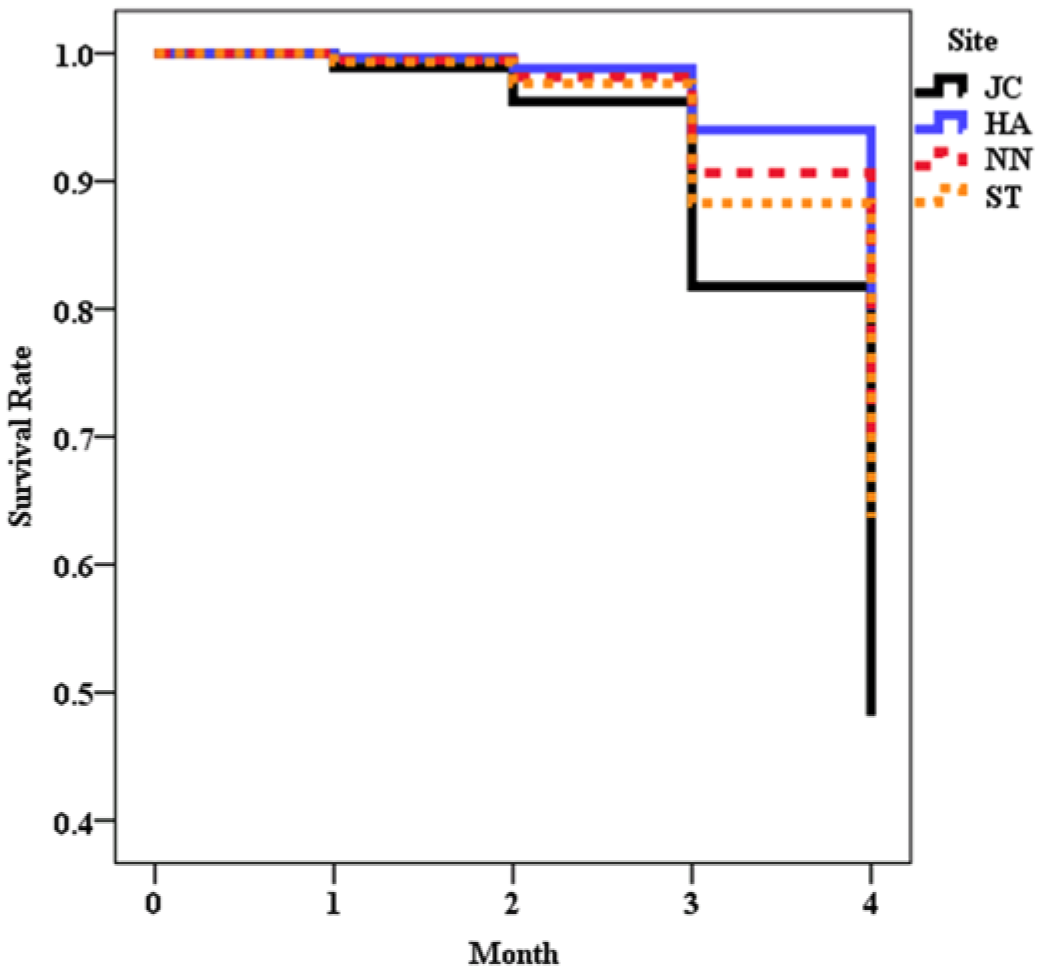

Fig. 19. Cox Regression survival rate for the experimental ticks by month per site. Sites: JC = Jacobson Tract, HA = Hampton, NN = Newport News, ST = Stephens Tract.

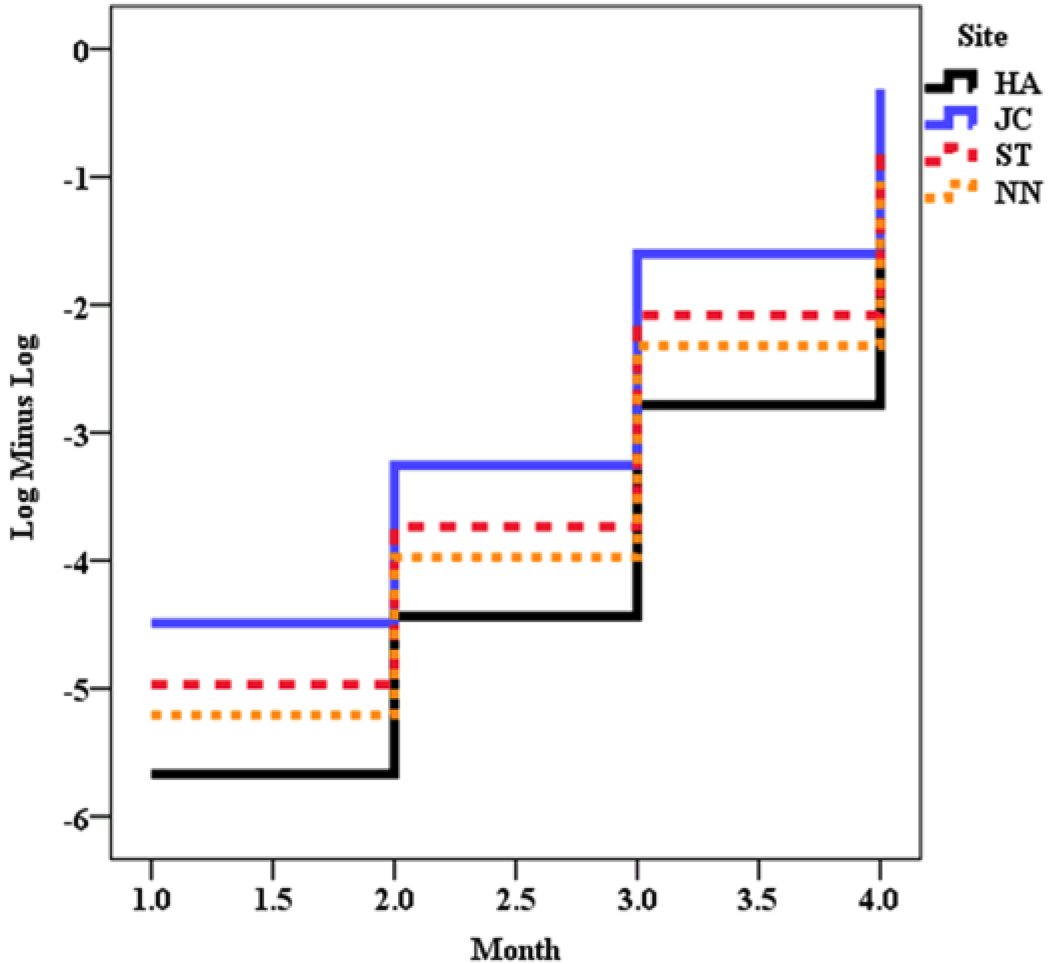

Fig. 20. Cox Log Minus Log for the experimental ticks by month per site. Sites: HA = Hampton, JC = Jacobson Tract, ST = Stephens Tract, NN = Newport News.

Table 12. Cox Regression survival analysis table for the experimental ticks comparing sex.

ß	**χ^2**	**df**	**p-value**	**Exp(ß)**
-0.215	3.593	1	0.058	0.806

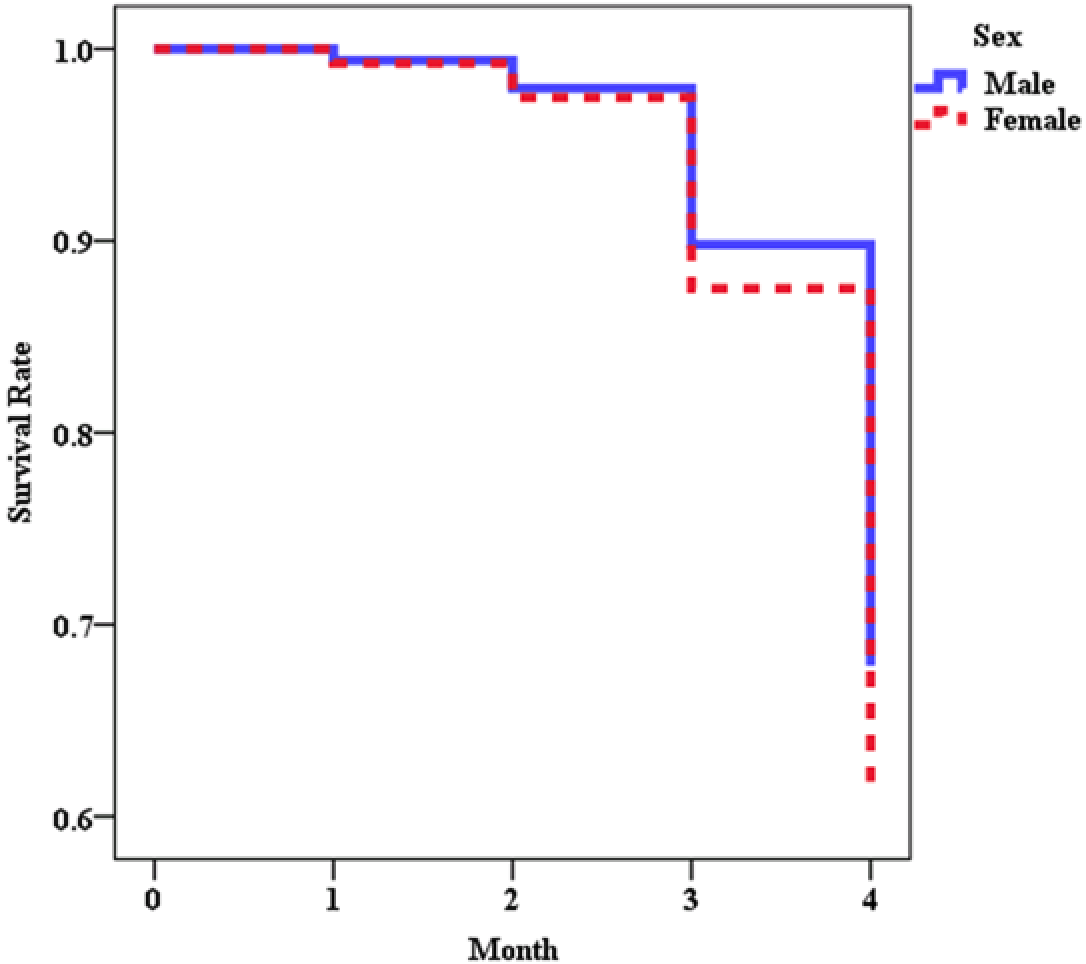

Fig. 21. Cox Regression survival rate for the experimental ticks by month per sex.

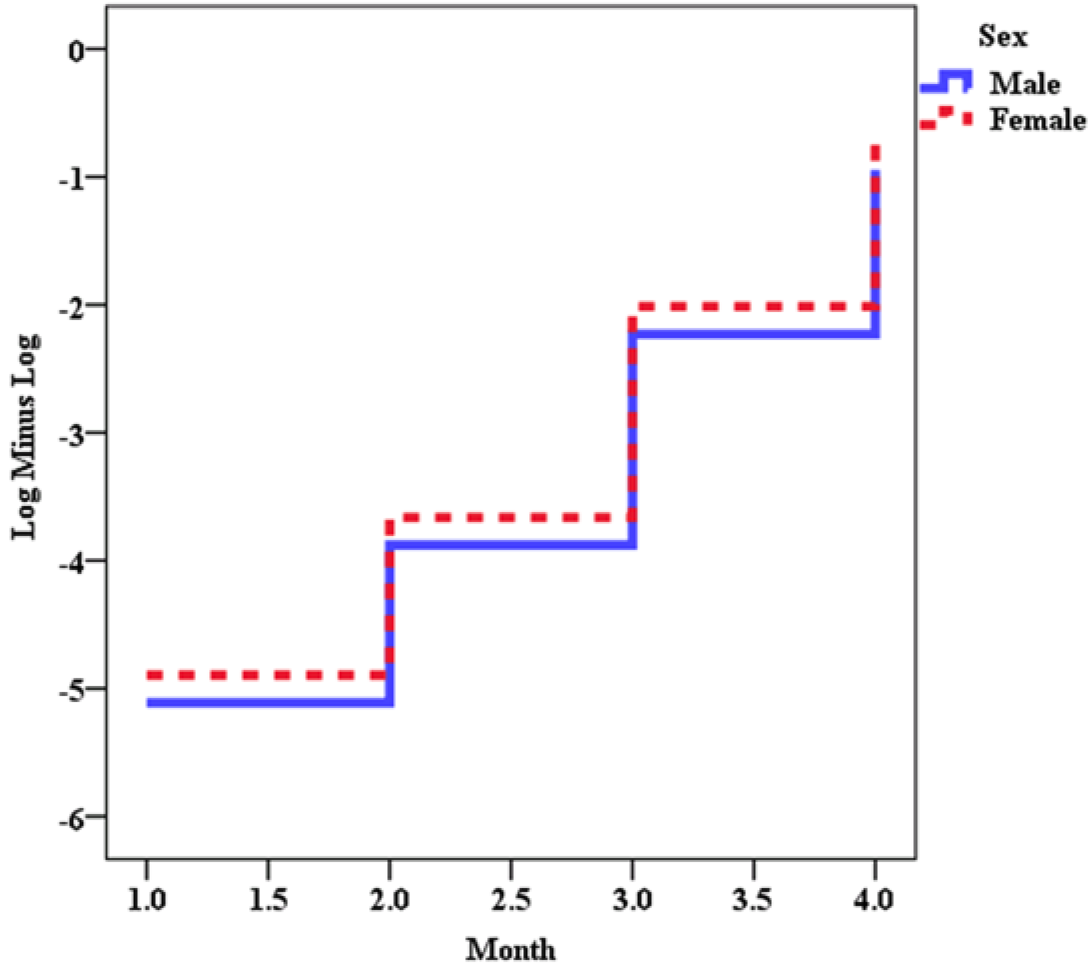

Fig. 22. Cox Log Minus Log for the experimental ticks by month per sex.

LABORATORY TICKS

A total of 134 ticks were placed in a laboratory incubator at Old Dominion University (ODU) in order to observe any naturally occurring mortality and behavioral differences between species and sexes in ideal conditions. The incubator ticks consisted of 60 *A. americanum* (31 male, 29 female), 37 *A. maculatum* (18 male, 19 female), and 37 *D. variabilis* (all female). The incubator was maintained at 26°C and 93% RH with a photo period of 14 light hours to 10 dark.

These conditions are compatible with the Oklahoma State University (OSU) tick rearing facility's optimal conditions for artificial housing of ticks, 23°C and 90% RH with a photo period of 15 light hours and 9 dark (personal communication: OSU). The incubator ticks were housed in vials, separated by species and sex, and placed in the incubator on May 2, 2015. Every four weeks for a total of four months, the vials were removed from the incubator and checked for mortality. The same aforementioned protocols for verifying survival or mortality were conducted.

Survival estimates are reported for the total sampling event incorporating tick mortality for each species over the four month study period. The purpose of this study was to monitor the ability of each tick species to survive in optimal conditions; therefore, data are reported for observational applications but not statistically analyzed.

After the four months, all *A. americanum* laboratory ticks survived. Of the *A. maculatum* species, 17 males and 18 females were recorded as dead with only 1 tick for each sex (2 total) surviving at the conclusion of the study. Of the *D. variabilis* ticks, 35 females were recorded as dead with only 2 female ticks surviving (Table 13). Though *D. variabilis* and *A. maculatum* are relatively less likely to survive in laboratory conditions than *A. americanum*, at the conclusion of the study, ticks of each species did survive.

Table 13. Survival of laboratory ticks by species and sex over the four month study period. *Date the ticks were initially placed in the incubator. There were no laboratory *D. variabilis* male ticks.

		Survival		Mortality	
Species	**Date**	**Male**	**Female**	**Male**	**Female**
A. americanum	***5/2/2015**	31	29	-	-
	6/2/2015	31	29	0	0
	6/28/2015	31	29	0	0
	7/28/2015	31	29	0	0
	8/28/2015	31	29	0	0
	Totals	**31**	**29**	**0**	**0**
A. maculatum	***5/2/2015**	18	19	-	-
	6/2/2015	18	17	0	2
	6/28/2015	16	16	2	1
	7/28/2015	13	10	3	6
	8/28/2015	1	1	12	9
	Totals	**1**	**1**	**17**	**18**
D. variabilis	***5/2/2015**	-	37	-	-
	6/2/2015	-	35	-	2
	6/28/2015	-	32	-	3
	7/28/2015	-	9	-	23
	8/28/2015	-	2	-	7
	Totals		**2**		**35**

DISCUSSION

The purpose of the environmental survival study was to obtain data on the abiotic factors contributing to the survival of unfed tick species across four sites in southeastern Virginia. These sites represent two generalized habitats: upland, drier landscapes, and wet landscapes prone to flooding. The three hypotheses tested provided valuable insight into the ability of each tick species to survive specifically with regards to naturally occurring temperatures, humidity, soil saturation, precipitation, and inundation. The results of this study show that *A. maculatum,* exhibited the poorest survival across all four field sites. Additionally, there was more *A. maculatum* mortality observed at the two wet sites, Stephens Tract and Jacobson Tract, than the drier more upland Hampton and Newport News field sites (60% vs 40%, $p<0.0005$). There was limited mortality (93% survival) in the *D. variabilis* species across all four sites, with higher survival at the drier sites. Of the 59 *D. variabilis* mortalities, all but 1 tick death was observed at the wet field sites. According to this study, *A. americanum* is best suited for survival across all four habitat types with the least mortality of only 5 individuals. Additionally, the observed limited mortality of *A. americanum* was consistent across all four months in situ, suggesting the amount of time containerized in the environment had no effect on their susceptibility to mortality. Similarly, *D variabilis* mortality was consistently lower the first two months in situ (6 and 4 total mortalities respectively) with increased mortality numbers for the last two months (25 and 24 respectively). *Amblyomma maculatum* mortality increased substantially over the four month study period, suggesting the longer this species is off-host and exposed to these habitats, the higher the mortality risk (Table 6).

The overall success of both *A. americanum* and *D. variabilis* survival at the four field sites in this study is not surprising since these two tick species have been documented in Virginia throughout the last century (Childs and Paddock, 2003; Hunter and Bishopp, 1911; Sonenshine, 1979). This study supports that *A. americanum* and *D. variabilis* remain well suited to survive in the variable landscapes and habitats across southeastern Virginia. Additionally, the environmental survival study supports locally collected abundance and dispersal data. Garrett and Sonenshine (1979) collected these two tick species on flags during surveillance studies conducted from June through July of 1971 and in July of 1972; reporting 3,334 adult *D. variabilis* and 183 *A. americanum* adult and nymphal ticks from transects at the Great Dismal Swamp. Garrett and Sonenshine (1979) also reported 10 adult *D. variabilis* and 2,822 *A. americanum* adults and nymphal ticks from transects in the Newport News Park (Table 14).

More recently, the tick surveillance study by the ODU Biological Sciences department report collecting these two species at all 13 surveyed sites from 2009-2016 (personal communication, Gaff). These sites represent 8 counties and cities across southeastern Virginia, including a mix of landscapes, habitats, and degrees of human disturbance (Nadolny et al., 2014). *Amblyomma americanum* and *D. variabilis* compose the two dominant tick species in the Hampton Roads area with *A. americanum* representing 97% (N=66,590) of the ticks collected from 2010-2012 and *D. variabilis* constituting 6%. Alternatively, the relatively lower survival of *A. maculatum* at the four field sites across this study was rather surprising. As a newly established species in the southeastern area of Virginia, this study suggests *A. maculatum* are not well suited to survive long periods of time off-host exposed to the local environment. Garrett and Sonenshine (1979) did not report collecting *A. maculatum* ticks during the flagging studies in 1971-1972. Recently, adult *A. maculatum* ticks have been collected at 29 cities and counties across Virginia, with 20 of those locations having established populations, consisting of 6 or more adult *A. maculatum* (Wright et al., 2015). Of the 251 *A. maculatum* mortalities recorded in this environmental study, the lowest mortality was recorded at the Hampton site (39) where only a small number of *A. maculatum* have been collected in recent ODU survey studies (personal communication, Gaff). The majority of the mortalities (98) was at the Jacobson Tract site, adjacent to the Great Dismal Swamp, where the ODU survey studies have recently found established populations (personal communication, Gaff). This proposes the question of why *A. maculatum* seem to be more suited to survive in upland, drier habitats but tend to be established in the areas prone to flooding.

The design of the environmental survival study was configured after a study by Mary Keith Garrett and Dr. Daniel Sonenshine (1979). Garrett and Sonenshine’s environmental survival study incorporated laboratory reared and wild-caught *A. americanum* and laboratory reared *D. variabilis* tick species. These ticks were held in environmental containers submerged in situ at three field sites (two in the Great Dismal Swamp, one in Newport News Park) over a four month study period from May through September of 1972. Garrett and Sonenshine (1979) reported environmental restrictions on tick survival for *A. americanum* and *D. variabilis* at the field sites in the Great Dismal Swamp. At the conclusion of their four month study and collectively across the two swamp sites, Garrett and Sonenshine observed 75% tick survival for the laboratory reared *A. americanum* ticks, 22% tick survival for the wild-caught *A. americanum*, and 66% survival for the laboratory reared *D. variabilis* ticks. The ticks held in situ at the Newport News Park site had 83%, 78%, and 90% survival respectively (Table 15). Garrett and Sonenshine (1979) observed flooding in the environmental containers held at the two swamp sites. The inundation occurred when host-seeking activity had ceased and the ticks were in a quiescent state in the leaf litter. Garrett and Sonenshine stated that these ticks did not move from the inundated leaf litter and died within 14 days. At the conclusion of their studies, they determined inundation, temperature, humidity variations, soil pH, and predation were important ecological limiting factors affecting tick survival. They further stated that inundation is the "most significant single environmental factor limiting tick survival" (Garrett and Sonenshine, 1979). The results of my study also suggest that the habitats adjacent to the Great Dismal Swamp are relatively poor for certain tick species, specifically *D. variabilis* and *A. maculatum*. Collectively, there was significantly ($p<0.0005$) more mortality across all three tick species at the two field sites adjacent to the swamp when compared to the more upland sites. These results support those reported by Garrett and Sonenshine (1979), although in this study, inundation is believed to be one of multiple factors for the increased mortality at the wet sites, not the main factor. Ticks can survive being submerged in water for an extended period of time. Unfed nymphal *A.*

americanum have been reported surviving up to 19 weeks submerged in 20°C water in laboratory conditions and adults surviving up to three weeks (Koch, 1986). Barrett et al., (2009) found mortality would occur in nymphal *A. americanum* ticks when water temperatures were greater than 51°C. Additionally, Fielden et al. (2011) confirmed the use of plastron respiration for underwater survival in *D. variabilis* ticks. The spiracular plate serves as a plastron and through the use of hydrophobic hairs or other cuticular projections, a thin layer of air can be trapped and oxygen absorbed. Over the four month study period in 2015, only two observations were made where the tickaria were inundated. On June 7, 2015 and July 6, 2015 observations were made at the Stephens Tract plot where some of the tickaria were partly under water. On both occasions, half of the tickaria were saturated with water i.e. the soil in the canisters was very wet, but the tickaria were not under water. The other half of the tickaria were approximately 50% under water where the top portion of the tickaria, including the berry basket, was observed out of the water (Fig. 23). Additionally, on June 7, 2015 the very tall vegetation (over waist-high) at the Jacobson Tract was completely laid down as if a flood event leveled the vegetation (Fig. 24). No flooding was observed at the Jacobson Tract, and no tickaria were found inundated. When both flooding events were observed at the Stephens Tract, tickaria were being removed from the field to be processed at the ODU laboratory. Month one tickaria were removed from the field on June 7 and month two tickaria were removed on July 6, 2015. At this site, tick mortality for month one and two was minimal for *A. maculatum* (7 total) and *D. variabilis* (1 total), suggesting inundation was not the reason for the observed mortality. Although, there was higher recorded mortality during month three and four tickaria removals when inundation was not noted. There was 16 observed *A. maculatum* mortality during the month three tickaria removals and 30 from month four. There was 9 observed *D. variabilis* mortality during month three and 16 during month four removals. Furthermore, there was a difference in mortality between the tickaria at the Stephens Tract that were only saturated with precipitation compared to the tickaria that were truly inundated. When looking at *D. variabilis* mortality in month three, there were 8 individuals observed in the inundated tickaria with only 1 mortality in the saturated tickaria. Similarly, there were 16 recorded mortalities for *D. variabilis* in the inundated tickaria compared to 0 in the saturated ones during month four removals. During month three there was 7 to 9 *A. maculatum* mortalities when comparing saturation to inundation, and 5 to 25 mortalities respectively in month four. This suggests, the fluctuation of inundation over time is more stressful to ticks than just inundation itself, and this variation could be a means for mortality. Additionally, the wet environment could provide conditions favorable to fungal or bacterial pathogens that may be harmful to ticks over time (Carroll, 2003).

Table 14. Total number of *A. americanum* and *D. variabilis* ticks collected by Garrett and Sonenshine from June through July of 1971 and in July of 1972 compared to the number of ticks collected from May through September of 2014 and 2015, per general field site. “Wet” represents the collective sites in or adjacent to the Great Dismal Swamp; “Dry” represents the drier, upland sites.

		Site	
Year	**Species / life stage**	**Wet**	**Dry**
***1971-1972**	***A. americanum* adult**	158	1016
	***A. americanum* nymph**	25	1806
	***D. variabilis* adult**	3334	10
2014-2015	***A. americanum* adult**	52	46
	***A. americanum* nymph**	104	317
	***D. variabilis* adult**	7	2

*Adapted from Garrett and Sonenshine (1979)

Table 15. Percent tick survival of ticks containerized in situ by Garrett and Sonenshine in 1972 compared to the percent tick survival in 2015, per species by general field site habitat. Wet = the collective sites in or adjacent to the Great Dismal Swamp; Dry = the drier, upland sites. All 2015 tick species were lab raised.

		Survival (%)	
Year	**Species**	**Wet**	**Dry**
***1972**	***A. americanum* (lab raised)**	75%	83%
	***A. americanum* (wild caught)**	22%	78%
	***D. variabilis* (lab raised)**	66%	90%
2015	***A. americanum***	99%	99%
	D. variabilis	86%	99%
	A. maculatum	62%	75%

*Adapted from Garrett and Sonenshine (1979)

Fig. 23. Stephens Tract tickaria that were partially inundated (top two photos), arrows pointing to standing water. Empty submersion holes from where tickaria were removed (bottom two photos) some with standing water, some without; photographs taken by Lindsey A. Bidder June 7, 2015.

Fig. 24. Jacobson Tract field site with normal vegetation appearance (left, photo taken on May 26, 2015) compared to the flattened vegetation from possible flooding (right, photo taken on June 7, 2015); photographs taken by Lindsey A. Bidder.

Low relative humidity and extreme temperatures have been shown to be detrimental to tick development and survival. The critical equilibrium humidity for the majority of tick species ranges from 75% to 95% RH (Knülle and Rudolph, 1982). The average relative humidity observations at all four sites during this study were well below the critical equilibrium (Table 3). Though only one RH measurement was taken bi-weekly, the low recorded RH values would suggest the experimental ticks were continuously losing water while containerized in situ during those low relative humidity periods. If the relative humidity reached above the critical equilibrium range (such as during flooding events), the ticks would have then been able to maintain their water level by atmosphere uptake (Knülle and Rudolph, 1982). Of the 52 measurements of RH, only four values were within the proposed critical equilibrium humidity range. This suggests either the ticks were continuously stressed in regards to maintaining homeostasis or the RH measurements were not an adequate representation of the true environment. The average RH for the combined wet sites was 62% and 56% for the dry sites. The maximum RH of 84% was recorded at the Hampton site and the minimum of 37% was recorded at the Newport News site. Though not observed in his study, if one considers the idea that the RH values fluctuated throughout the day above and below the critical equilibrium range, it is possible that *A. americanum* is more suited to handle this stressor, with *A. maculatum* being less suited. This suggests the higher mortality observed with *A. maculatum* in situ may relate to interspecific biotic variations aiding or hindering tick survival when exposed to the environment off-host.

The average combined temperature at the wet sites was similar to the dry sites (30°C) but the maximum temperature recorded at the wet sites (44°C) was higher than the maximum at the dry sites (41°C). With significantly ($p<0.0005$) more mortality at the wet sites compared to the dry sites, the higher temperatures may have affected tick survival for the same reason of interspecific biotic variations being a benefit or hindrance. On average, there was approximately 2 cm more precipitation recorded at the wet sites than the dry. The average soil saturation was higher at the combined dry sites (Hampton and Newport News) with 18% saturation. The maximum collective soil saturation recorded (30%) was also observed at the combined dry sites. Though it may seem counter-intuitive, the lower soil saturation values recorded for the two wet sites adjacent to the swamp (Stephens Tract and Jacobson Tract) confirm the description of those sites as flood zone areas. Instead of the soil absorbing the precipitation, the water pools on the surface at those locations, promoting inundation of the landscape. The process or processes responsible for the lower survival at the wet sites may be attributed to species specific biotic factors that help the ticks adjust to temperature and humidity variations over time. A tick's inability to handle the continuous variations in the aforementioned factors is believed to be the main limitation to survival in the habitats of southeastern Virginia.

Climate change is believed to have an impact on the geographic range of ticks, their hosts, and the transmission of tick-borne diseases (Childs and Paddock, 2003; Gilbert et al., 2014; Ogden et al., 2008). These studies gathered more information about the factors potentially promoting or restricting the increasing geographic ranges of some tick species in both the flood zones and upland habitats in southeastern Virginia. Over the last century the average temperature in southeastern Virginia has increased by 1.2°F and precipitation has increased by 3.22 inches (NOAA, 2015). There has also been an increase in heavy rainfall events over the last three to five decades. Downpours are heavier and more frequent, increasing flood events across the US including areas in the southeastern region (Walsh et al., 2014). Understanding how tick species survive in their habitat while fasting off-host could be key in determining tick dispersal, survival, and activity (Anderson, 2002). The environmental survival study suggests the combination of relative humidity, temperature, and inundation may limit tick survival for certain species, specifically *A. maculatum* and less severely *D. variabilis*. If the climatic conditions of southeastern Virginia follow the aforementioned trend of increased precipitation and temperature, certain tick species may not be able to survive. Tick species susceptible to mortality in the flood-zone landscapes may become more concentrated on the edges of the forests and fields, avoiding the internal sections of the landscapes to promote survival. Additionally, the continuous construction and urbanization of the Hampton Roads area could create additional areas of suitable habitat for tick species. The increase in urban structures, roads, and manicured parks could provide suitable ecotones for tick survival along forest and wetland edges that would have otherwise been less optimal. Further studies focused on the habitat and climate conditions that may influence the distribution of tick and tick host populations is needed.

The biggest limitation to this study was proper recording of the internal microclimate of each tickarium. Though environmental measurements were collected outside the tickaria, an internal, continuous data logger would have been a more accurate representation of the true conditions inside. Microclimate conditions have been observed influencing activity and abundance of tick species (Bertrand and Wilson, 1996; Rynkiewicz and Clay, 2014) with environmental restrictions affecting survival (Garrett and Sonenshine, 1979). Without accurately recording the internal microclimate of each tickarium a detrimental low relative humidity and/or extreme temperature affecting survival could not be properly recorded (Carroll, 2003; Stafford, 1994; Vandyk et al., 1996). Internal data loggers should be used if possible for future environmental studies. Additionally, berry baskets were used to provide internal support in the tickarium, preventing the cloth bag from collapsing on the leaf litter and ticks. The baskets also represented artificial vegetation and provided a questing platform. Real vegetation would provide a more natural habitat for the ticks and maybe more realistic for questing behavior. A modified tickarium with living vegetation should be considered in future designs.

EXPERIMENT 2: CAPTURE-MARK-RECAPTURE STUDY

STUDY GOALS

A capture-mark-recapture (CMR) study was performed to obtain off-host tick survival estimates at four habitats in southeastern Virginia. In the CMR study, adults and nymphs of all tick species collected via standard flagging methods was used to estimate their respective survival over a four month period (May through September). Prior work has been done using colored enamel paint in *D. variabilis* (Smith et al., 1946), radioisotope tagging in *D. variabilis* (Sonenshine et al., 1968), fluorescent powder in *I. scapularis* (Daniels et al., 2000), DecoColor paint pens in *I. pacificus* (Kramer et al., 1993) and fingernail polish for multiple tick species (Gaff et al., 2015).

METHODS AND MATERIALS

The CMR study incorporated transects at a total of four different sites in the Hampton Roads region: Newport News Park (Newport News, VA; public access), Hampton (Hampton, VA; restricted access), Stephens Tract (Chesapeake, VA; limited public access), and Jacobson Tract (Chesapeake, VA; limited public access). The sites cover a variety of landscapes, vegetation, and levels of soil saturation. The 486 meter Newport News transect is part of the Newport News Park and includes wooded edge habitat with low level undergrowth. The 168 meter Hampton transect is located in Hampton and also consists of wooded edge habitat with low level undergrowth. The 200 meter Stephens Tract transect in Chesapeake is adjacent to the Great Dismal Swamp National Wildlife Refuge. It is a closed canopy, late secondary successional, wooded habitat, and prone to flooding (Nadolny et al., 2014; personal communication: Gaff). The 200 meter Jacobson Tract transect in Chesapeake is also adjacent to the Great Dismal Swamp. It is an early secondary successional habitat dominated by grasses, also prone to flooding. ESRI Geographic Information System (GIS) Arc 10.2.2 software was used to calculate the length (meters) of each transect sampled via Global Positioning System (GPS) coordinates collected at each site (Table 44). Permission to use each field site was granted by the owner of that location.

In order to estimate the respective abundance of host-seeking ticks, standard flagging tick collection methods (Ginsberg and Ewing, 1989; Nadolny et al., 2014) were performed bi-weekly at the selected field sites. Flagging was performed from May through September during 2014 and 2015. In 2014, only two of the sites were used in order to test the CMR method (Stephens Tract and Newport News), all four field sites were used in 2015. The flag consists of a 1-m^2 sheet of white denim attached to a 122-cm dowel rod. Questing ticks grasp the flag as it sweeps through vegetation, leaf litter, and on the ground. Every few meters, the flag is checked for ticks. Adults and nymphs, of all tick species, were carefully removed from the flag with forceps and placed on painter's tape (legs down) to minimize movement. A fine tipped pin held in the eraser of a standard pencil was used to paint the tick with finger nail polish (Fig. 25). Once marked, the ticks were returned to the approximate location of their capture. A different color of polish was used for every sampling event. If a tick was recaptured, a second blot of colored polish was painted on the tick alongside the previously painted color, then rereleased. Temperature, current weather condition, relative humidity (at ground level), and soil moisture were recorded at each transect every two weeks. Soil moisture was determined through the collection of a sediment core; the difference between "wet" and "dry" weight was recorded.

Because of the low probability of adequately painting, releasing, and recapturing larval ticks and the large numbers of larvae collected while sampling, specifically *A. americanum* larva, CMR techniques were not performed on that life stage. Therefore, survival estimates for larval ticks were not studied via CMR methods.

Fig. 25. Capture-mark-recapture tick painting (left), one-color painted ticks (middle), two-colored painted ticks, red and green, for a second recaptured tick (right); photos taken by Lindsey A. Bidder May 2014.

ANALYSIS

Abundance estimates of nymphal and adult life stages were compiled for the total sampling event incorporating the numbers of marked and unmarked ticks per field site over the four month study period. Due to the low recaptures during the CMR study, data are reported for observational applications but not statistically analyzed.

Four assumptions were made for this CMR study: 1) the population was open (i.e., mortality, immigration, and emigration were expected to occur); 2) “all individuals were equally likely to be captured (i.e., marked and unmarked ticks behave the same); 3) marked individuals retained their marks throughout the study; 4) sampling time was negligible.” (Daniels et al., 2000). Population dynamics including immigration (example ticks becoming active after dormant periods) and mortality (including predation) have been reported with life stage and species specific abundances increasing and decreasing throughout the year (Childs and Paddock, 2003; Sonenshine et al., 1966; Sonenshine, 1979); validating the first assumption of an open population. Additionally, marked ticks that were successful in finding a host would be attached to their host and removed from the CMR sampling area. Tick emigration via host removal also validates the first assumption of an open population. For assumption two, enamel marking of ticks does not injure them or change their behavior (Smith et al., 1946). Smith et al. (1946) captured adult *D. variabilis* ticks and kept them in an outdoor breeding cage. The ticks were placed into one of two groups, marked with colored enamel or not marked. The marked ticks outlived the ones that were unmarked, surviving from June 1940 to August of 1941. Additionally, Sonenshine et al. (1966) observed marked adult *D. variabilis* climbing vertical surfaces in a laboratory setting, validating assumption two that marking ticks does not change their behavior. Assumption three was confirmed while conducting CMR in the 2014 study; marked ticks retained their fingernail polish weeks after being painted. Confirming assumption four, CMR was performed bi-weekly during the summer months when adult and nymphal ticks are active in southeastern Virginia (Childs and Paddock, 2003; Sonenshine et al., 1966; Sonenshine, 1979). On average, the CMR events were conducted over 30 minutes with a two person team in the afternoon (approximately four o'clock pm). This timeframe is considered consistent and short enough to preclude any changes in tick population.

RESULTS

During the 2014 CMR study, 419 total nymphal and adult ticks were marked encompassing four tick species. There were 87 ticks marked at the Stephens Tract transect and 332 marked at the Newport News transect (Table 16). Of those marked, 398 (95%) were *A. americanum* (343 nymphs, 55 adults), 7 (2%) were *D. variabilis* adults, 1 (<1%) was an *A. maculatum* adult, and 13 (3%) were *Ixodes* spp. (1 nymph, 12 adults). *Amblyomma americanum* was the dominant tick species captured at both field sites with the majority, 322 individuals, captured at the Newport News transect and 76 at the Stephens Tract transect. Of the *D. variabilis* ticks captured, the majority (6) were from the Stephens Tract transect with only 1 individual captured at the Newport News transect. The only *A. maculatum* captured was at the Stephens Tract. Of the *Ixodes* spp. captured, 9 were from the Newport News transect and 4 were from the Stephens Tract transect.

Of the ticks marked, 33 total were recaptured, 28 at the Newport News transect and 5 at the Stephens Tract transect. The total recaptures consisted of 1 *D. variabilis* female and 32 *A. americanum* (25 nymphs, 7 adults). Two ticks were recaptured an additional time, 1 *A. americanum* nymph at the Newport News transect and 1 *D. variabilis* female at the Stephens Tract transect (Table 16). The only recovered *D. variabilis* was recaptured 29 days post initial marking, then recaptured an additional time 42 days post initial marking (Table 18). Across both sites, the average time between the date an *A. americanum* tick was initially marked then recaptured was 30 days with a minimum of 8 days. The longest recorded time frame between mark and recapture was 71 days from an *A. americanum* nymph at the Stephens Tract transect (Table 19).

During the 2015 CMR study, 158 total nymphal and adult ticks were marked encompassing three tick species. There were 46 (29%) ticks marked at the Newport News transect, 26 (16%) marked at the Hampton transect, 60 (38%) marked at the Jacobson Tract transect, and 27 (17%) marked ticks at the Stephens Tract transect (Table 20). Of those marked, 138 (87%) were *A. americanum* (87 nymphs, 51 adults), 2 (1%) were *D. variabilis* adults, 2 (1%) were *A. maculatum* adults, and 16 (10%) were *Ixodes* spp. (1 nymph, 15 adults). *Amblyomma americanum* was the dominant tick species captured across all field sites with the majority, 54 individuals, captured at the Jacobson Tract. Additionally, all 27 marked ticks at the Stephens

Tract transect were *A. americanum.* The 2 *D. variabilis* ticks were captured at two different sites, 1 individual at the Newport News transect and 1 at the Jacobson Tract transect. The 2 *A. maculatum* captured were at the Jacobson Tract transect. The *Ixodes* spp. were captured at every site except the Stephens Tract transect (Table 20). There was only one tick recapture in 2015, a male *A. americanum* at the Newport News site, 27 days post the original marking (Table 20).

Table 16. Capture-mark-recapture results for 2014 per species by field site.

Site	**Species**	**Life Stage/Sex**	**Originally Marked**	**Recaptured**	**2nd Recapture**
Newport News	*A. americanum*	Male	13	2	
	A. americanum	Female	19	2	
	A. americanum	Nymph	290	24	1
	D. variabilis	Male	1		
	Ixodes. spp.	Male	6		
	Ixodes. spp.	Female	3		
Stephens Tract	*A. americanum*	Male	9	2	
	A. americanum	Female	14	1	
	A. americanum	Nymph	53	1	
	D. variabilis	Male	2		
	D. variabilis	Female	4	1	1
	A. maculatum	Male	1		
	Ixodes. spp.	Male	3		
	Ixodes. spp.	Nymph	1		
2014 Total			**419**	**33**	**2**

Table 17. *Amblyomma americanum* recapture results at the Newport News field site from 5/12/2014 to 9/22/2014. Dates of originally marked ticks represent the initial capture and marking of the tick, days post initial release represent the number of days that passed from the initial capture/marked date until the date when recaptured.

Life stage / Sex	Number of ticks	Date Originally Marked	Date Recaptured	Days Post Initial Release	Date 2nd Recapture	Days Post Initial Release
Female	1	May-12	May-20	8		
Nymph	2	May-12	May-20	8		
Nymph	6	May-12	Jun-2	21		
Female	1	May-12	Jun-17	36		
Nymph	3	May-12	Jun-30	49		
Nymph	1	May-20	Jun-2	13		
Male	2	May-20	Jun-2	13		
Nymph	1	May-20	Jun-17	28		
Nymph	2	May-20	Jun-30	41		
Nymph	1	May-20	Jul-28	69		
Nymph	1	Jun-2	Jun-30	28	Aug-12	71
Nymph	3	Jun-30	Jul-14	14		
Nymph	1	Jun-2	Jul-14	42		
Nymph	2	Jun-30	Jul-28	28		
Nymph	1	Jul-14	Aug-12	29		

Table 18. Recapture results at the Stephens Tract field site from 5/9/2014 to 9/26/2014. Dates of originally marked ticks represent the initial capture and marking of the tick, days post initial release represent the number of days that passed from the initial capture/marked date until the date when recaptured; *A. ame.* = *Amblyomma americanum*, *D. var*. = *Dermacentor variabilis*.

Species	Life stage / sex	Number of ticks	Date Originally Marked	Date Recaptured	Days Post Initial Release	Date 2nd Recapture	Days Post Initial Release
D. var.	Female	1	Jun-30	Jul-29	29	Aug-11	42
A. ame.	Male	1	May-19	Jun-16	28		
	Nymph	1	May-19	Jul-29	41		
	Male	1	Jun-16	Jun-30	14		
	Female	1	Jul-29	Aug-11	12		

Table 19. 2014 *Amblyomma americanum* recapture rates from date initially marked/released to recapture date per field site.

Site	Average	Maximum	Minimum
Newport News	31	71	8
Stephens Tract	24	41	12
Both field sites	30	71	8

Table 20. Capture-mark-recapture study results for 2015. The only recapture occurred 27 days post initial release (originally marked 5/19/2015 recaptured 6/15/2015).

Site	Species	Life Stage	Originally Marked	Recaptured
Newport News	*A. americanum*	Male	8	1
	A. americanum	Female	6	
	A. americanum	Nymph	27	
	D. variabilis	Female	1	
	Ixodes. spp.	Male	1	
	Ixodes. spp.	Female	3	
Hampton	*A. americanum*	Male	5	
	A. americanum	Female	3	
	A. americanum	Nymph	9	
	Ixodes. spp.	Male	5	
	Ixodes. spp.	Female	3	
	Ixodes. spp.	Nymph	1	
Jacobson Tract	*A. americanum*	Male	7	
	A. americanum	Female	6	
	A. americanum	Nymph	41	
	D. variabilis	Male	1	
	A. maculatum	Female	2	
	Ixodes. spp.	Male	2	
	Ixodes. spp.	Female	1	
Stephens Tract	*A. americanum*	Male	9	
	A. americanum	Female	7	
	A. americanum	Nymph	10	
2015 Total			**158**	**1**

DISCUSSION

The purpose of the CMR study was to obtain survival estimates for tick species across four sites in southeastern Virginia. These sites represent two generalized habitats: upland, drier landscapes, and wet landscapes prone to flooding. The results of this study indicate *A. americanum* is the dominant species collected across all four sites. During both summers, *A. americanum* constituted 95% of the tick species collected in 2014 and 87% in 2015. The 2014 CMR study suggests adult *A. americanum* are more abundant at the flood-prone Stephens Tract site (32 adults) when compared to the more upland Newport News site (23 adults). Although, *A. americanum* nymphs are more abundant at the Newport News site (290 nymphs) when compared to the Stephens Tract (53 nymphs). It is interesting to note that this only partially supports the findings of Garrett and Sonenshine (1979) on which this study was based. Garrett and Sonenshine (1979) reported *D. variabilis* was the dominant tick species collected at multiple transects in the Great Dismal Swamp from 1971-1972. They reported 3,334 *D. variabilis* adults collected versus 158 *A. americanum* adults and 25 *A. americanum* nymphs (183 total). In the 2014 and 2015 CMR study at the two field sites adjacent to the Dismal Swamp, *A. americanum* was the dominant species collected. In this study a total of 7 *D. variabilis* adult ticks were captured versus 52 adult and 104 nymphal (156 total) *A. americanum* (Table 14). This suggests there has been a biodiversity shift in the dominant tick species over the last 40 years at the Great Dismal Swamp. *Amblyomma americanum* is now more dominant than *D. variabilis* at these locations and was the overall dominant species collected in the 2014-2015 CMR study. Additionally, Garrett and Sonenshine (1979) reported 10 *D. variabilis* adult ticks collected at the field site in the Newport News park from 1971-1972, and 1016 *A. americanum* adults and 1806 nymphs (2822 total). For the 2014-2015 CMR study, 46 *A. americanum* adults were collected and 317 nymphs (363 total) at the Newport News Park transect with only 2 *D. variabilis* adults collected. The 2014-2015 CMR results support the findings of Garrett and Sonenshine (1979) observing the dominant tick species at the Newport News Park field site remains *A. americanum* (Table 14).

Four tick species were collected by Garrett and Sonenshine (1979) through standard flagging methods from June through July of 1971 and in July of 1972 across multiple sites in the Great Dismal Swamp and Newport News Park: *A. americanum*, *D. variabilis, I. scapularis*, and *Haemaphysalis eporispalustris*. The 2014-2015 CMR study collected *A. americanum*, *D. variabilis*, *A. maculatum*, and *Ixodes* spp. from similar locations from May through September. It is interesting to note, Garrett and Sonenshine (1979) did not report collecting any *A. maculatum* at their field sites, whereas 3 were collected from the sites adjacent to the Great Dismal Swamp during the 2014-2015 CMR study. Further supporting the notion of a tick species biodiversity change over the last 40 years, as well as validating the reports of *A. maculatum* recently becoming an established species in the Hampton Roads area (Wright et al., 2011).

Though CMR was proven to be a successful method of off-host survival estimates for *A. americanum*, low sample sizes for other species was a limitation. Additionally, overall low sample numbers in the 2015 CMR study hindered any analysis for that data set. The continued application of CMR over multiple years would be best to properly assess the off-host tick survival and estimated populations for tick species.

CONCLUSION

The average recapture rate for *A. americanum* during the 2014 CMR study was 30 days post initial marking with a maximum of 71 days and a minimum of 8 days observed. This suggests ticks are exposed to environmental factors on average for one month while searching for a host. Extrapolating this rate to other local tick species, one could conclude certain biotic differences among tick species could hinder or help with survival in the environment over that time. Likewise, biotic factors could promote or hinder some tick species from becoming established in certain habitats. As reported in the 2015 environmental study, there is a significant difference ($p<0.0005$) in survival between the three tick species studied across all four sites over a four month period. *Amblyomma americanum* had the least mortality (<1%) when compared to *A. maculatum* and *D. variabilis*. This suggests *A. americanum* could successfully remain in the environment searching for a host for the one month average. Likewise, *A. americanum* could survive not only 71 days off-host in the environment (as seen in the 2014 CMR study) but up to four months in the environment, as reported in the environmental study. *Amblyomma maculatum* had the highest mortality (32%) in the environmental survival study. Mortality increased the longer *A. maculatum* was kept in situ and exposed the environmental elements. Even after one month in situ, more *A. maculatum* ticks died (9) than the total mortality recorded for *A. americanum* (5). After two months in situ, 24 mortalities were observed which constituted 12% of the total ticks for that month's study group. By month four, 135 (69%) of the month's *A. maculatum* ticks were dead. The recent establishment of *A. maculatum* locally, coupled with the unsuccessful survival of this species in the environment (mortality in as little as one month), raises questions on how it is able to survive in Hampton Roads. One can speculate this species could be very successful at acquiring a host, therefore temporarily removing it from the elements. Additionally, *A. maculatum* has been reported producing from 8,000 to over 18,000 eggs in laboratory and field studies (Hooker et al., 1912; Teel et al., 2010.) suggesting this species' individual mortality is possibly offset by high egg yields.

Reviewing shifts in local climate data could provide some insight into the change in species biodiversity in these habitats and others in the Hampton Roads area. The biotic and abiotic factors affecting distribution of ticks has a direct impact on the transmission dynamics of many human pathogens vectored. The ability to model the population dynamics of these tick species would aid in preventing human and tick encounters, ultimately reducing tick-borne diseases.

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APPENDICES

EXPERIMENTAL TICKS

Table 21. Kaplan-Meier survival analysis test of equality table for the experimental ticks comparing species.

Test	χ^2	df	p-value
Log Rank	385.158	2	<0.0005
Breslow	250.270	2	<0.0005
Tarone-Ware	327.737	2	<0.0005

Table 22. Kaplan-Meier survival analysis pairwise comparisons table for the experimental ticks comparing species.

		A. americanum		***A. maculatum***		***D. variabilis***	
Test	**Species**	χ^2	**p-value**	χ^2	**p-value**	χ^2	**p-value**
Log Rank	***A. americanum***	-	-	304.368	<0.0005	47.563	<0.0005
	A. maculatum	304.368	<0.0005	-	-	159.954	<0.0005
	D. variabilis	47.563	<0.0005	159.954	<0.0005	-	-
Breslow	***A. americanum***	-	-	204.819	<0.0005	33.814	<0.0005
	A. maculatum	204.819	<0.0005	-	-	93.782	<0.0005
	D. variabilis	33.814	<0.0005	93.782	<0.0005	-	-
Tarone-Ware	***A. americanum***	-	-	262.494	<0.0005	42.303	<0.0005
	A. maculatum	262.494	<0.0005	-	-	129.010	<0.0005
	D. variabilis	42.303	<0.0005	129.101	<0.0005	-	-

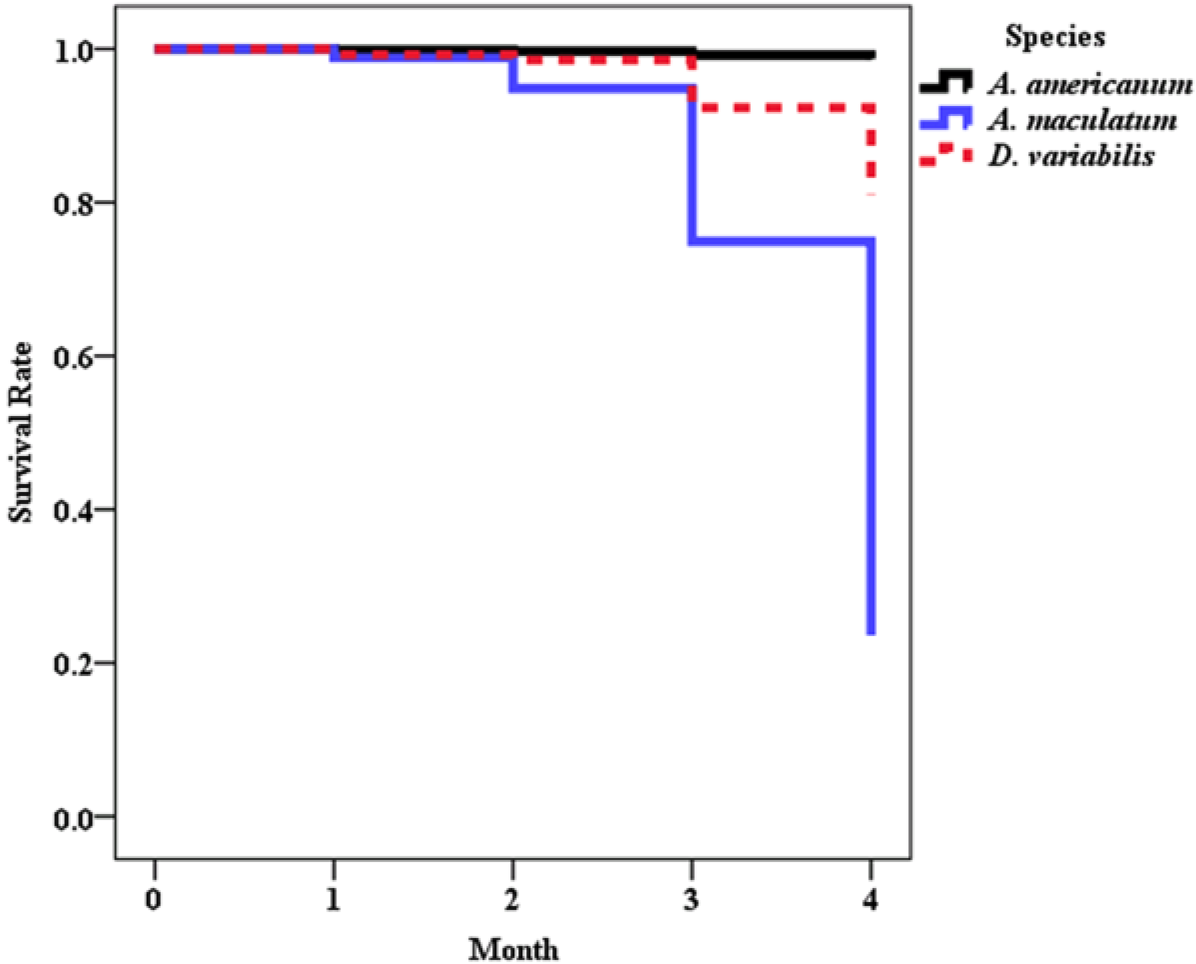

Fig. 26. Kaplan-Meier survival rate of experimental ticks by month per species.

Table 23. Kaplan-Meier survival analysis test of equality table for the experimental ticks comparing wet sites (Stephens and Jacobson) to dry sites (Hampton and Newport News).

Test	χ^2	df	p-value
Log Rank	44.292	1	<0.0005
Breslow	57.366	1	<0.0005
Tarone-Ware	53.249	1	<0.0005

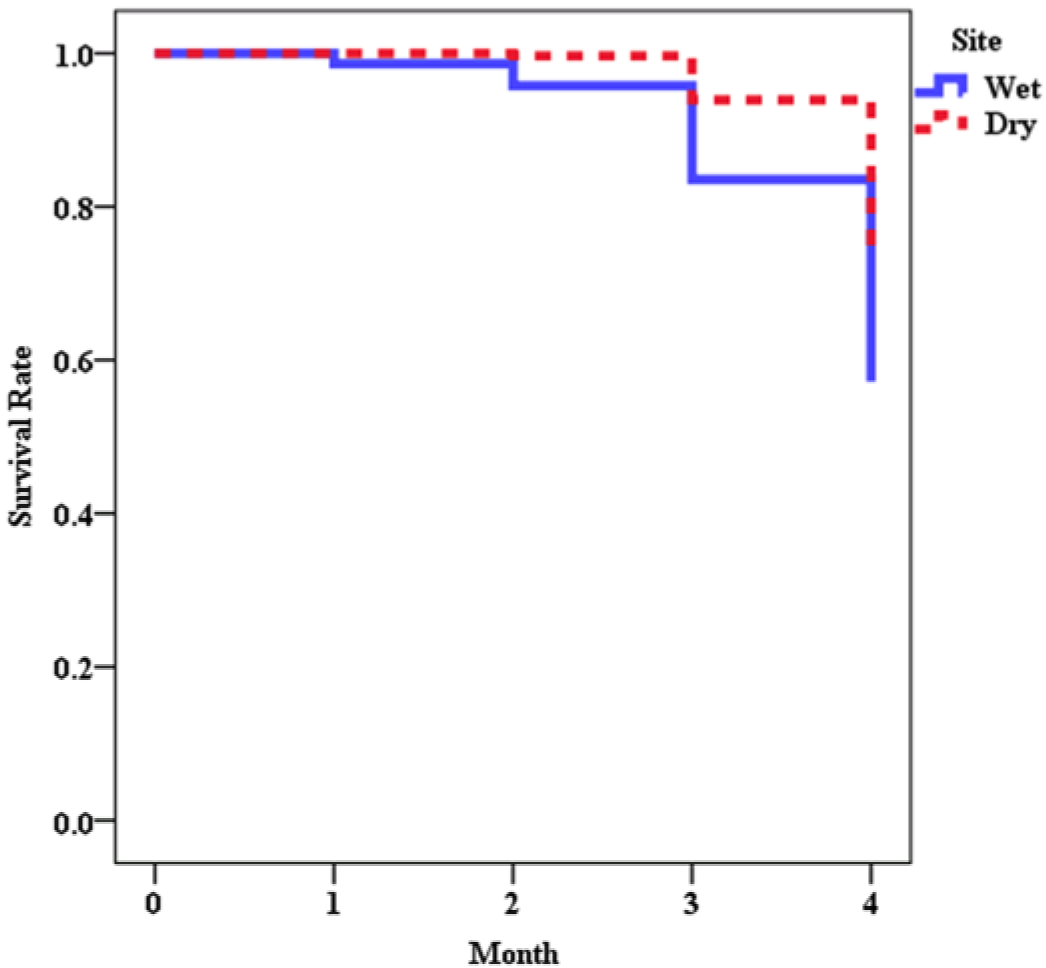

Fig. 27. Kaplan-Meier survival rate for the experimental ticks by month comparing wet sites (Stephens and Jacobson) to dry sites (Hampton and Newport News).

Table 24. Kaplan-Meier survival analysis test of equality table for the experimental ticks comparing sites.

Test	**χ^2**	**df**	**p-value**
Log Rank	67.450	3	<0.0005
Breslow	97.415	3	<0.0005
Tarone-Ware	85.108	3	<0.0005

Table 25. Kaplan-Meier survival analysis pairwise comparisons table for the experimental ticks comparing individual sites. Sites: NN = Newport News, HA = Hampton, JC = Jacobson, ST = Stephens.

		NN		HA		JC		ST	
Test	**Site**	**χ^2**	**p-value**	**χ^2**	**p-value**	**χ^2**	**p-value**	**χ^2**	**p-value**
Log Rank	**NN**	-	-	6.309	0.012	28.209	<0.0005	2.596	0.107
	HA	6.309	0.012	-	-	55.656	<0.0005	16.185	<0.0005
	JC	28.209	<0.0005	55.656	<0.0005	-	-	14.458	<0.0005
	ST	2.596	0.107	16.185	<0.0005	14.458	<0.0005	-	-
Breslow	**NN**	-	-	2.675	0.102	44.387	<0.0005	4.855	0.028
	HA	2.675	0.102	-	-	58.470	<0.0005	13.260	<0.0005
	JC	44.387	<0.0005	58.470	<0.0005	-	-	24.788	<0.0005
	ST	4.855	0.028	13.260	<0.0005	24.788	<0.0005	-	-
Tarone-Ware	**NN**	-	-	4.367	0.037	38.574	<0.0005	3.788	0.052
	HA	4.367	0.037	-	-	59.777	<0.0005	14.987	<0.0005
	JC	38.574	<0.0005	59.777	<0.0005	-	-	20.514	<0.0005
	ST	3.788	0.052	14.987	<0.0005	20.514	<0.0005	-	-

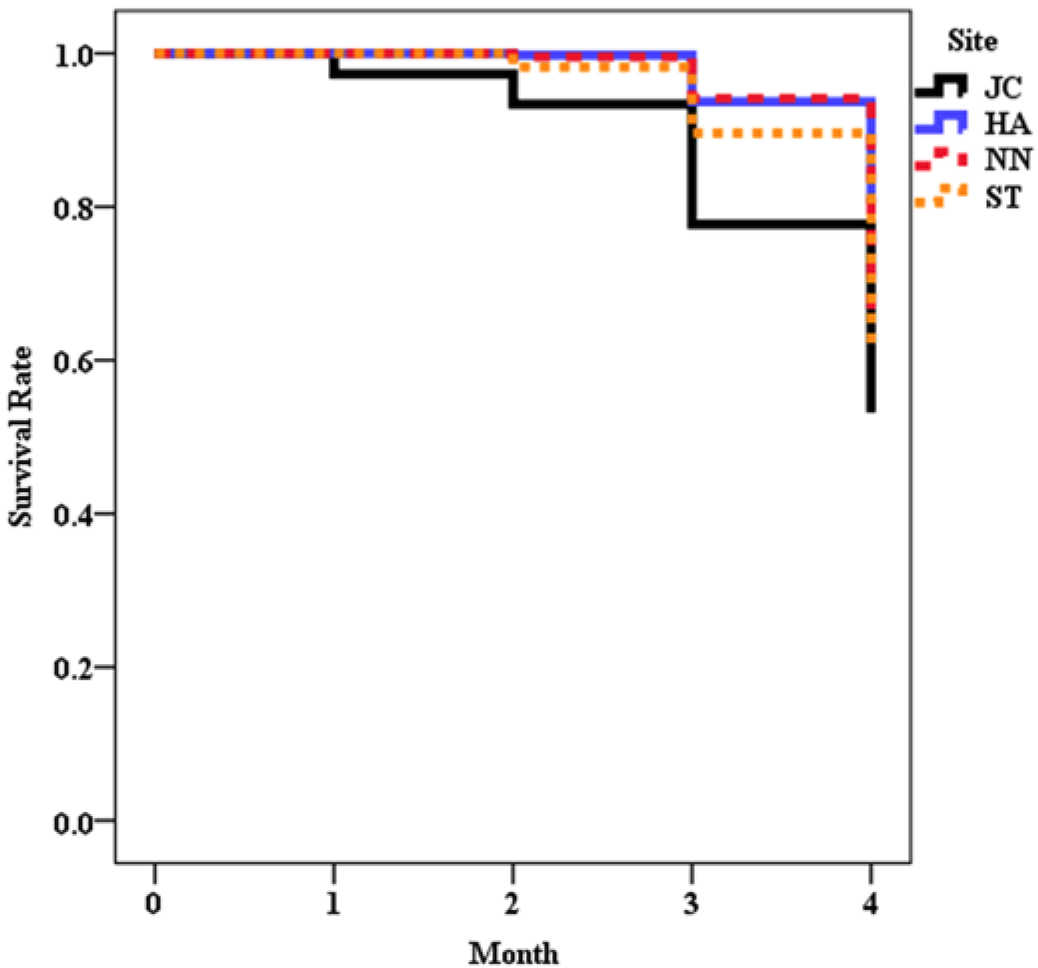

Fig. 28. Kaplan-Meier survival rate of the experimental ticks by month per site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

Table 26. Kaplan-Meier survival analysis test of equality table for the experimental ticks comparing sex.

Test	**χ^2**	**df**	**p-value**
Log Rank	4.347	1	0.037
Breslow	2.857	1	0.091
Tarone-Ware	3.872	1	0.049

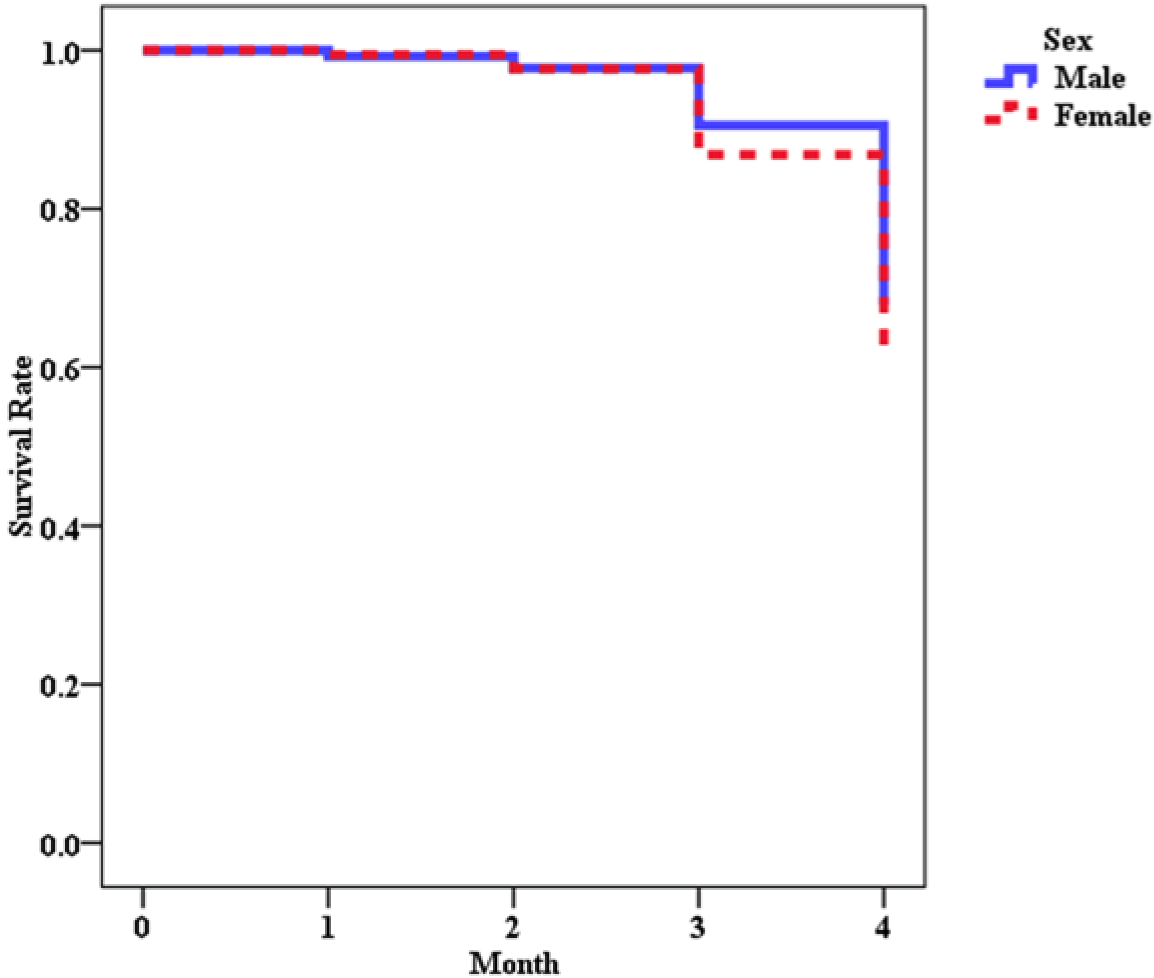

Fig. 29. Kaplan-Meier survival rate for the experimental ticks by month per sex.

SPECIES SPECIFIC

AMBLYOMMA AMERICANUM

Table 27. Kaplan-Meier survival analysis test of equality table for experimental *A. americanum* comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Test	χ^2	**df**	**p-value**
Log Rank	0.205	1	0.650
Breslow	0.123	1	0.726
Tarone-Ware	0.139	1	0.709

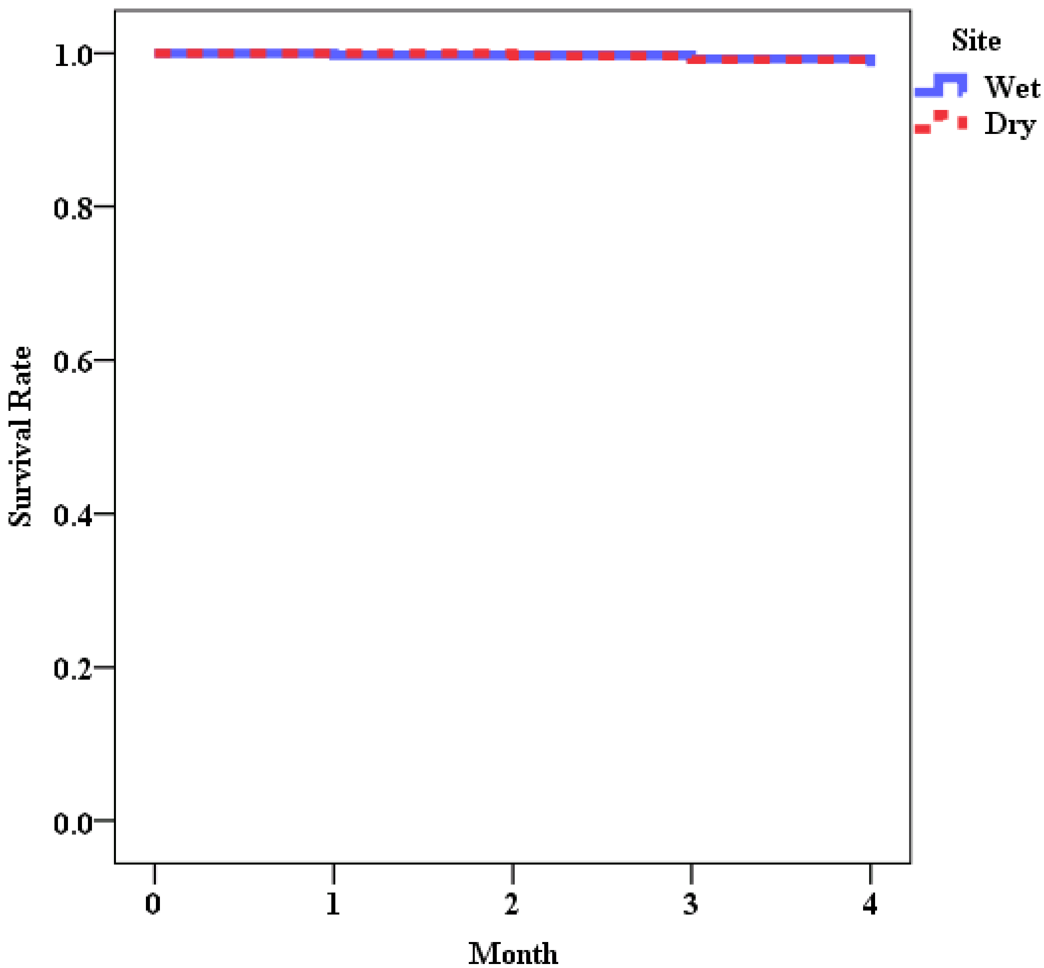

Fig. 30. Kaplan-Meier survival rate for experimental *A. americanum* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Table 28. Cox regression survival analysis table for experimental *A. americanum* comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

ß	χ^2	**df**	**p-value**	**Exp(ß)**
0.411	0.202	1	0.653	1.508

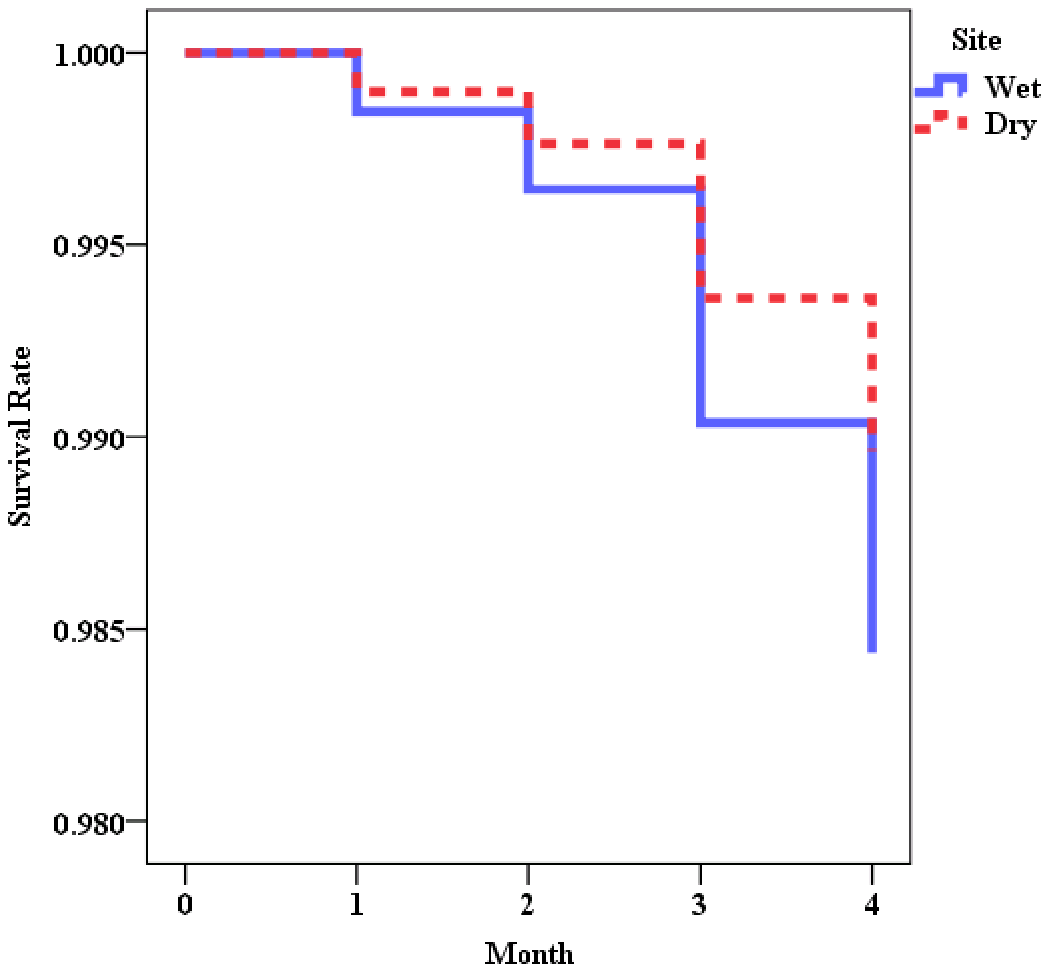

Fig. 31. Cox Regression survival rate of experimental *A. americanum* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

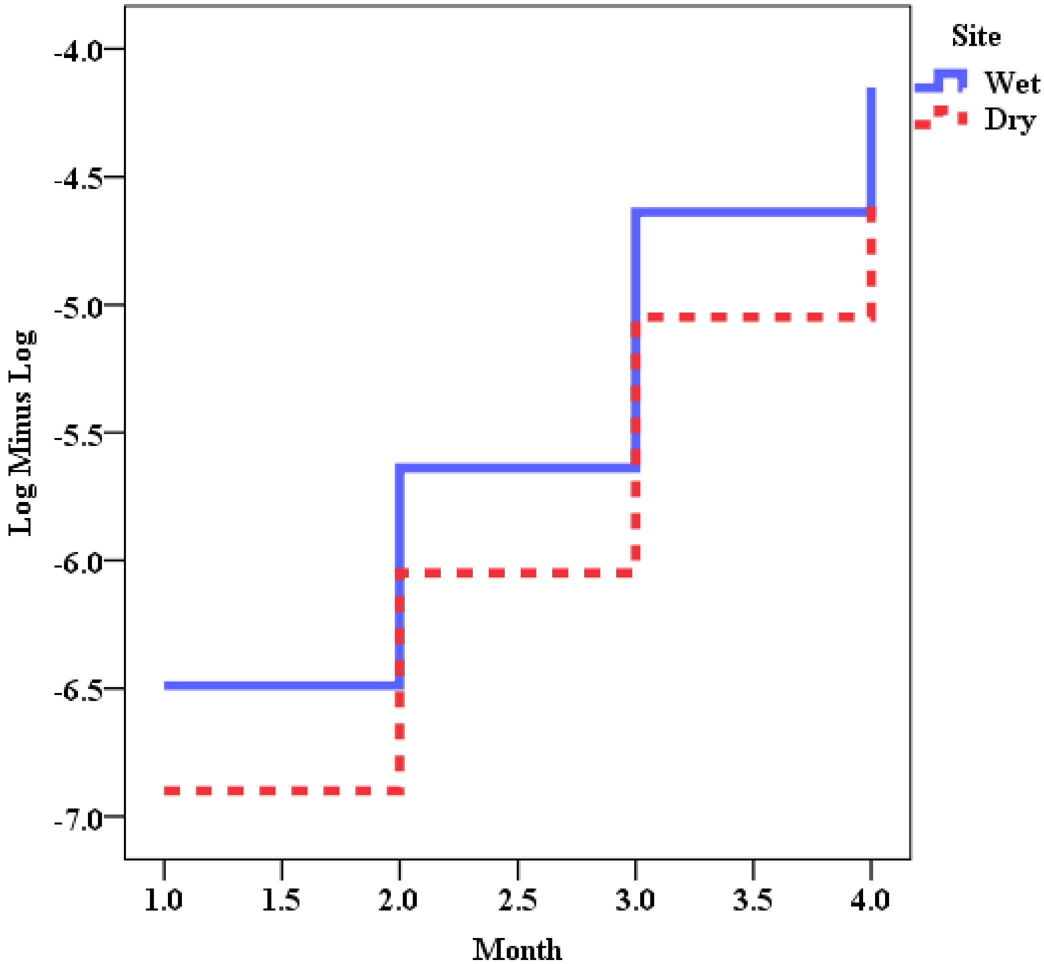

Fig. 32. Cox Log Minus Log for experimental *A. americanum* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Table 29. Kaplan-Meier survival analysis test of equality table for experimental *A. americanum* comparing individual field sites.

Test	χ^2	**df**	**p-value**
Log Rank	1.518	1	0.218
Breslow	1.287	1	0.257
Tarone-Ware	1.447	1	0.229

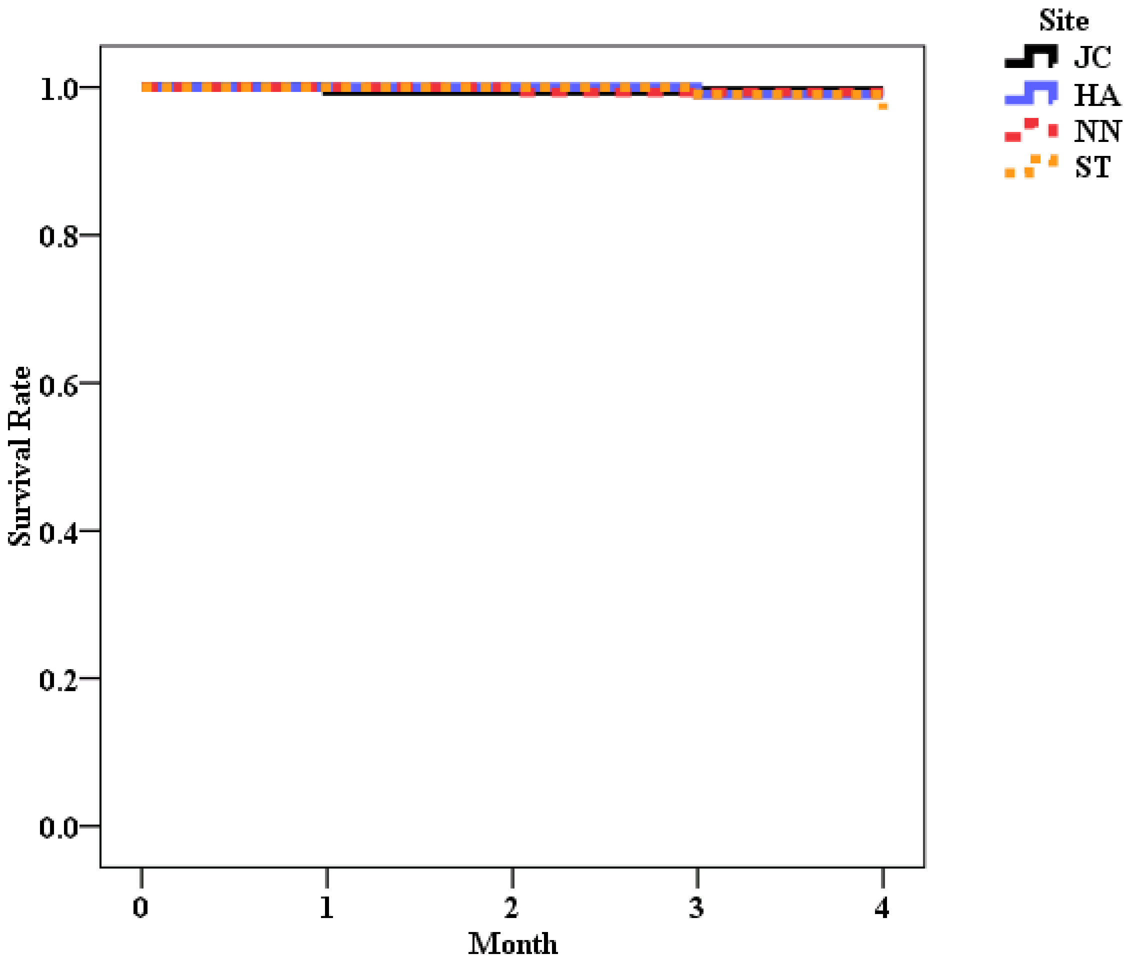

Fig. 33. Kaplan-Meier survival rate for experimental *A. americanum* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

Table 30. Cox Regression Omnibus tests of model coefficients table for experimental *A. americanum* ticks comparing individual field sites.

χ^2	df	p-value
0.588	3	0.899

Table 31. Cox Regression survival analysis table for experimental *A. americanum* ticks comparing individual field sites. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

	JC					ST				
Site	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
HA	-0.022	0.000	1	0.988	0.979	-0.700	0.326	1	0.568	0.497
NN	-0.004	0.000	1	0.998	0.996	-0.682	0.310	1	0.578	0.506
ST	0.678	0.306	1	0.580	1.970	-	-	-	-	-
JC	-	-	-	-	-	-0.678	0.306	1	0.580	0.508

	HA					NN				
Site	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
HA	-	-	-	-	-	-0.018	0.000	1	0.990	0.983
NN	0.018	0.000	1	0.990	1.018	-	-	-	-	-
ST	0.700	0.326	1	0.568	2.013	0.682	0.310	1	0.578	1.978
JC	0.022	0.000	1	0.988	1.022	0.004	0.000	1	0.998	1.004

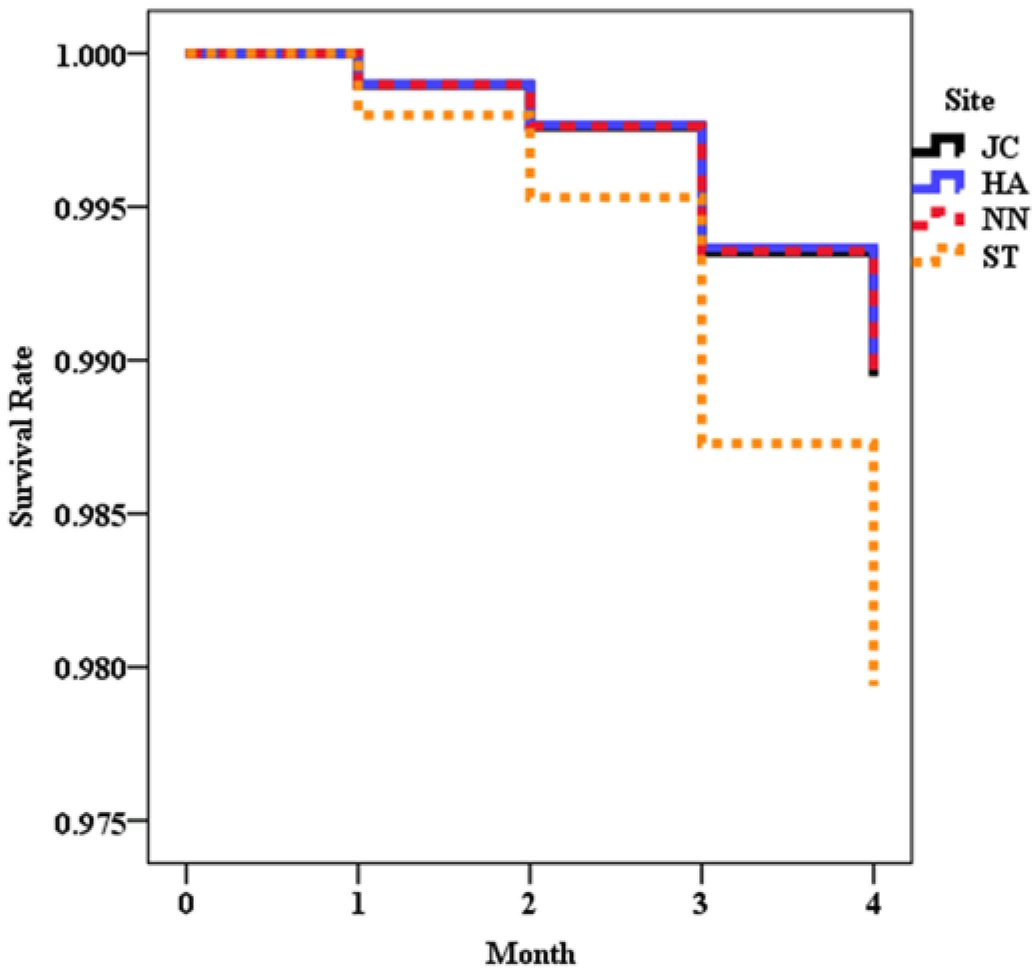

Fig. 34. Cox Regression survival rate of experimental *A. americanum* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

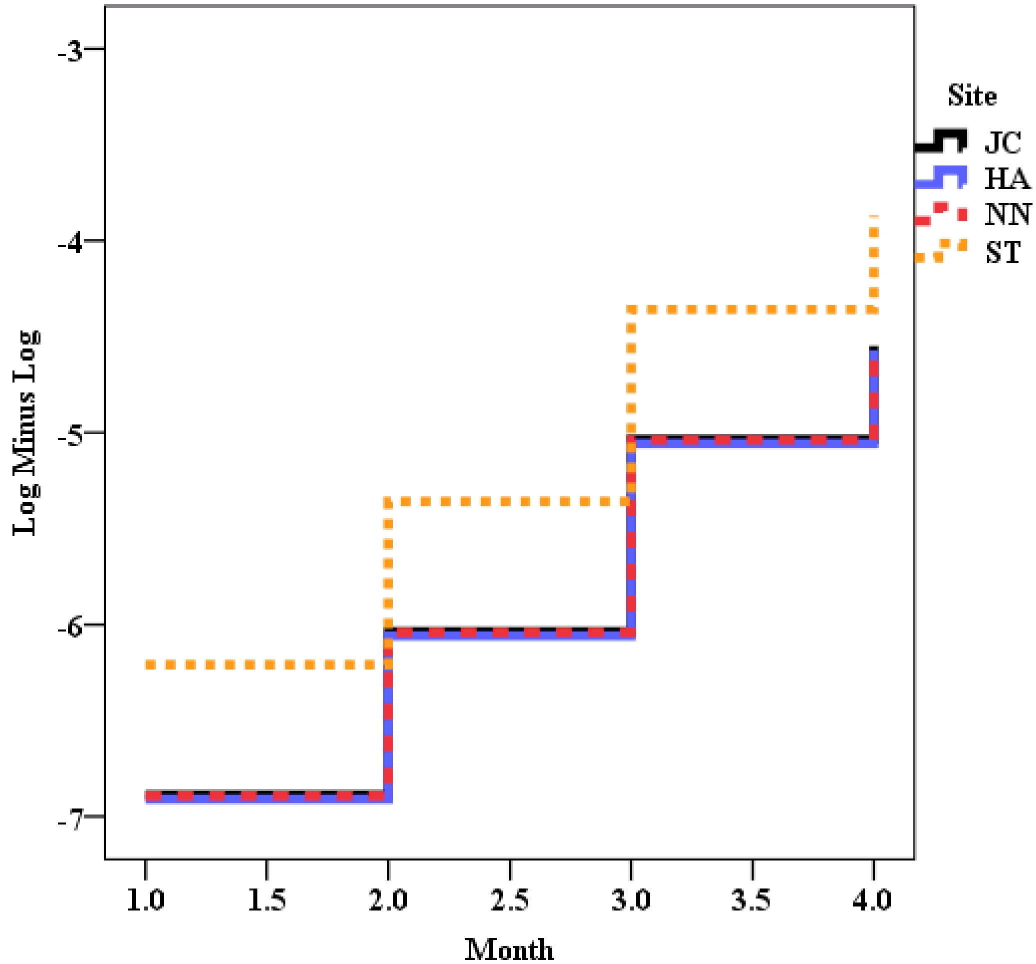

Fig. 35. Cox Log Minus Log for experimental *A. americanum* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

DERMACENTOR VARIABILIS

Table 32. Kaplan-Meier survival analysis test of equality table for experimental *D. variabilis* comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Test	χ^2	**df**	**p-value**
Log Rank	59.395	1	<0.0005
Breslow	47.297	1	<0.0005
Tarone-Ware	55.936	1	<0.0005

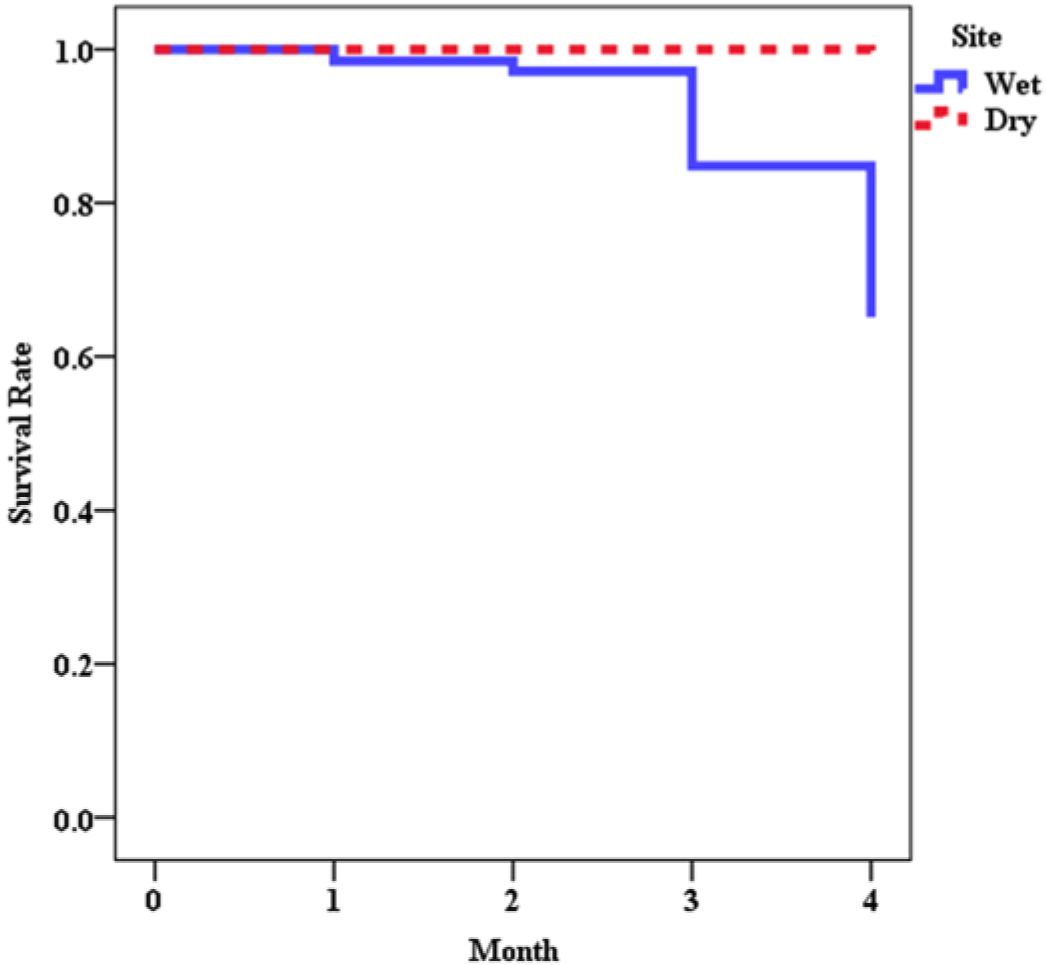

Fig. 36. Kaplan-Meier survival rate for experimental *D. variabilis* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Table 33. Cox regression survival analysis table for experimental *D. variabilis* comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

ß	χ^2	df	p-value	Exp(ß)
4.059	16.194	1	<0.0005	57.899

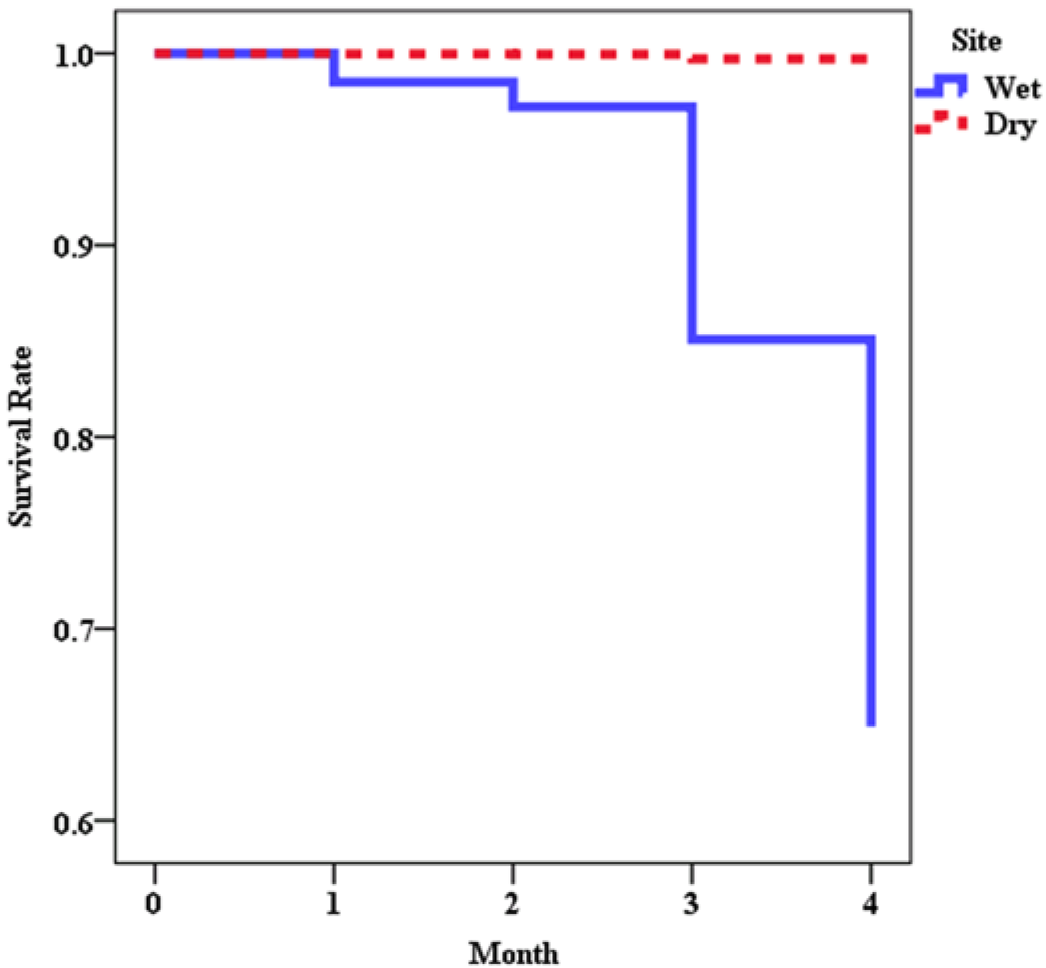

Fig. 37. Cox Regression survival rate of experimental *D. variabilis* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

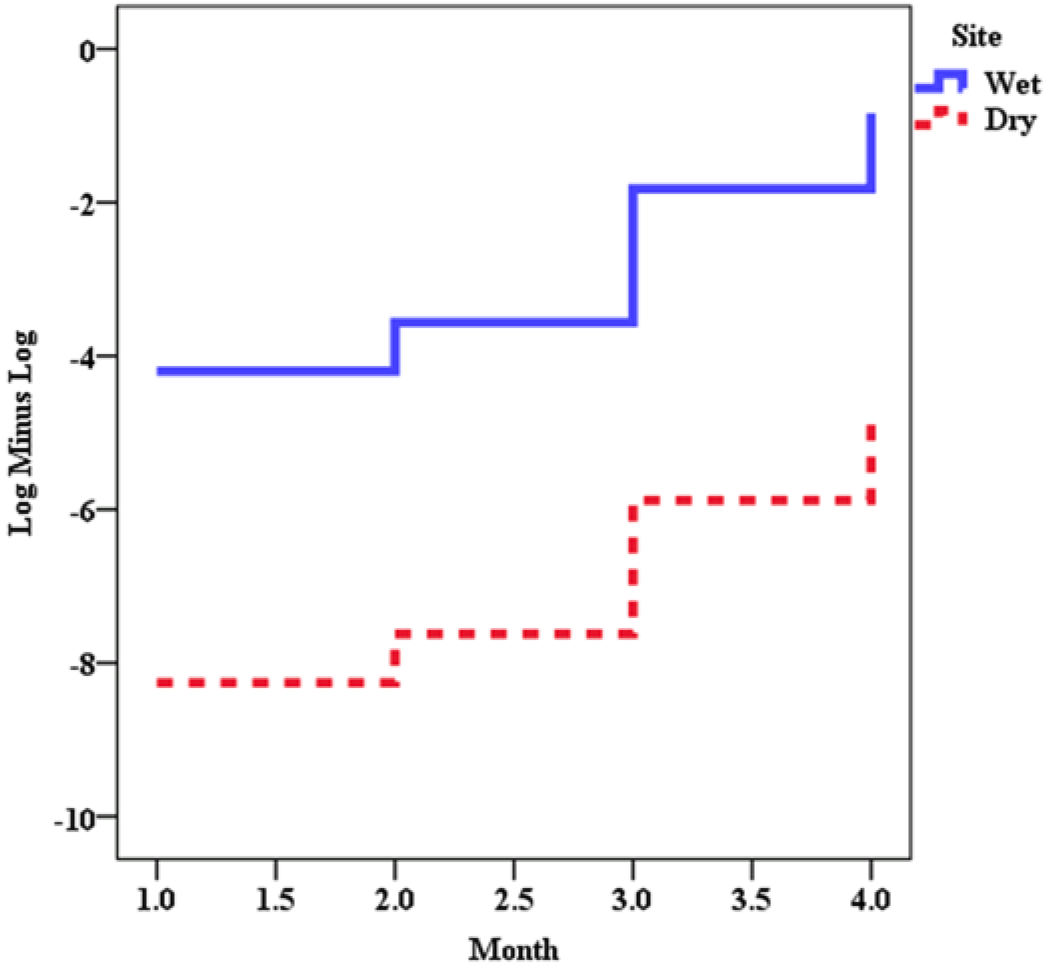

Fig. 38. Cox Log Minus Log for experimental *D. variabilis* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Table 34. Kaplan-Meier survival analysis test of equality table for experimental *D. variabilis* comparing individual field sites.

Test	χ^2	**df**	**p-value**
Log Rank	60.591	3	<0.0005
Breslow	57.042	3	<0.0005
Tarone-Ware	60.877	3	<0.0005

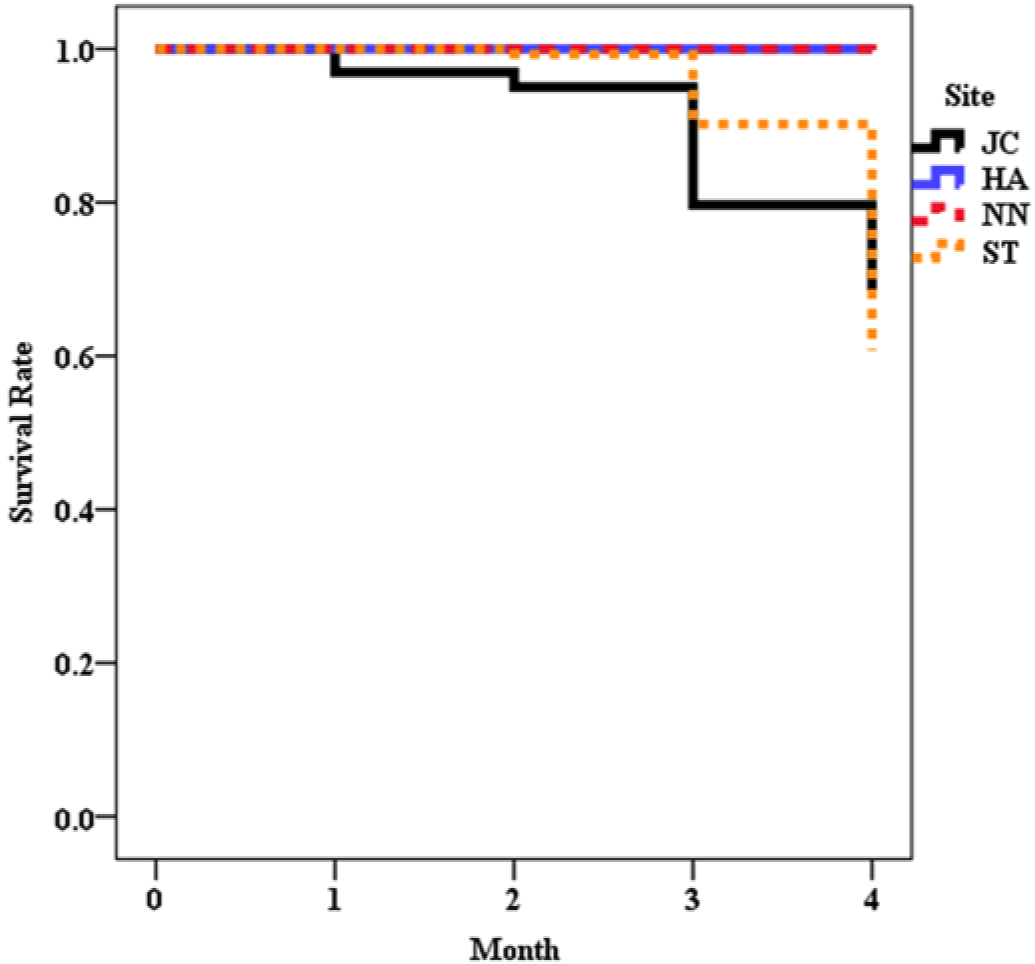

Fig. 39. Kaplan-Meier survival rate for experimental *D. variabilis* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

Table 35. Kaplan-Meier survival analysis pairwise comparisons table for experimental *D. variabilis* ticks comparing individual field sites. Sites: JC = Jacobson, ST = Stephens, HA = Hampton, NN = Newport News.

		JC		ST		HA		NN	
Test	**Site**	**χ^2**	**p-value**	**χ^2**	**p-value**	**χ^2**	**p-value**	**χ^2**	**p-value**
Log Rank	**JC**	-	-	0.638	0.425	33.726	<0.0005	30.696	<0.0005
	ST	0.638	0.425	-	-	29.301	<0.0005	26.332	<0.0005
	HA	33.726	<0.0005	29.301	<0.0005	-	-	1.000	0.317
	NN	30.696	<0.0005	26.332	<0.0005	1.000	0.317	-	-
Breslow	**JC**	-	-	5.034	0.025	27.642	<0.0005	26.705	<0.0005
	ST	5.034	0.025	-	-	24.059	<0.0005	22.522	<0.0005
	HA	27.642	<0.0005	24.059	<0.0005	-	-	1.000	0.317
	NN	26.705	<0.0005	22.522	<0.0005	1.000	0.317	-	-
Tarone-Ware	**JC**	-	-	2.606	0.106	31.817	<0.0005	30.017	<0.0005
	ST	2.606	0.106	-	-	27.563	<0.0005	25.289	<0.0005
	HA	31.817	<0.0005	27.563	<0.0005	-	-	1.000	0.317
	NN	30.017	<0.0005	25.289	<0.0005	1.000	0.317	-	-

Table 36. Cox Regression Omnibus tests of model coefficients table for experimental *D. variabilis* ticks comparing individual field sites.

χ^2	**df**	**p-value**
56.073	3	<0.0005

Table 37. Cox Regression survival analysis table for experimental *D. variabilis* ticks comparing individual field sites. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

	JC					ST				
Site	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
HA	-13.253	0.010	1	0.921	0.000	-13.058	0.010	1	0.922	0.000
NN	-3.457	11.589	1	0.001	0.032	-3.262	10.249	1	0.001	0.038
ST	-0.195	0.544	1	0.461	0.823	-	-	-	-	-
JC	-	-	-	-	-	0.195	0.544	1	0.461	1.215

	HA					NN				
Site	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
HA	-	-	-	-	-	-9.796	0.005	1	0.942	0.000
NN	7.796	0.025	1	0.874	2430.621	-	-	-	-	-
ST	11.058	0.50	1	0.822	63466.127	3.262	10.249	1	0.001	26.111
JC	11.253	0.052	1	0.819	77105.219	3.457	11.589	1	0.001	31.722

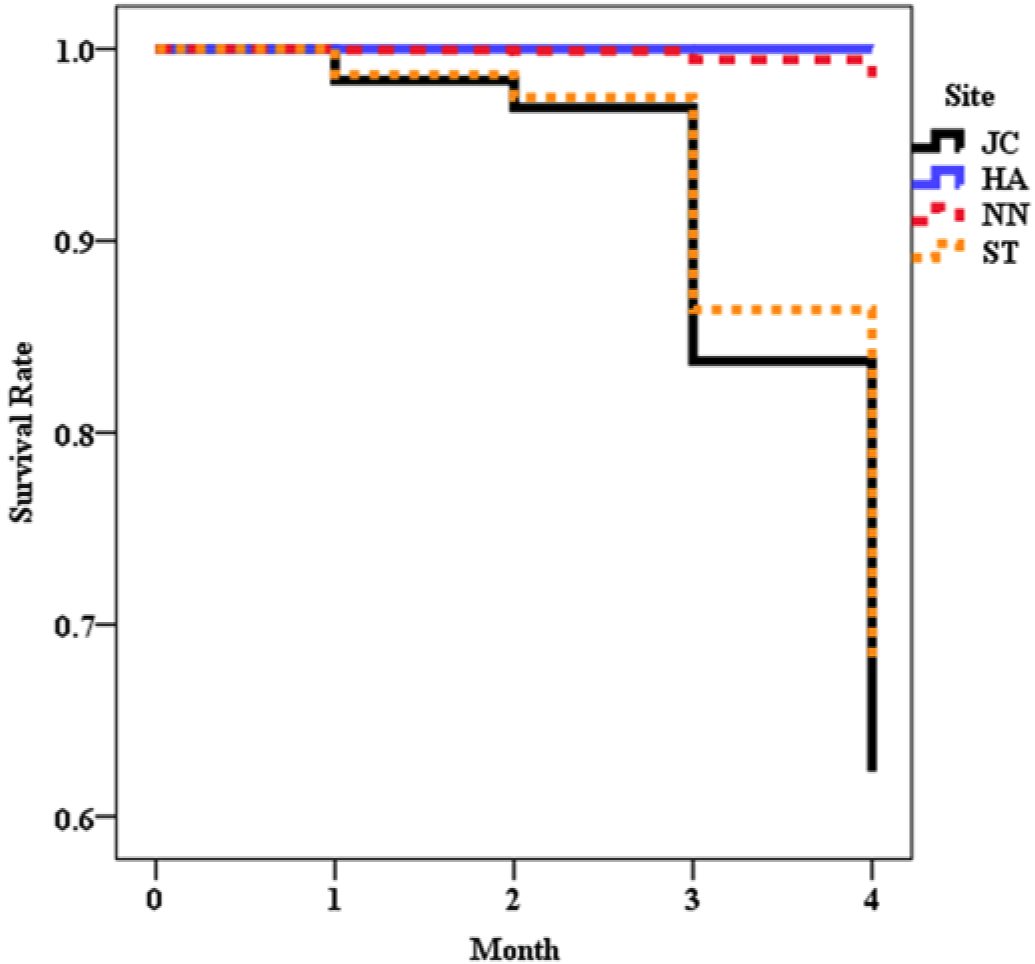

Fig. 40. Cox Regression survival rate of experimental *D. variabilis* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

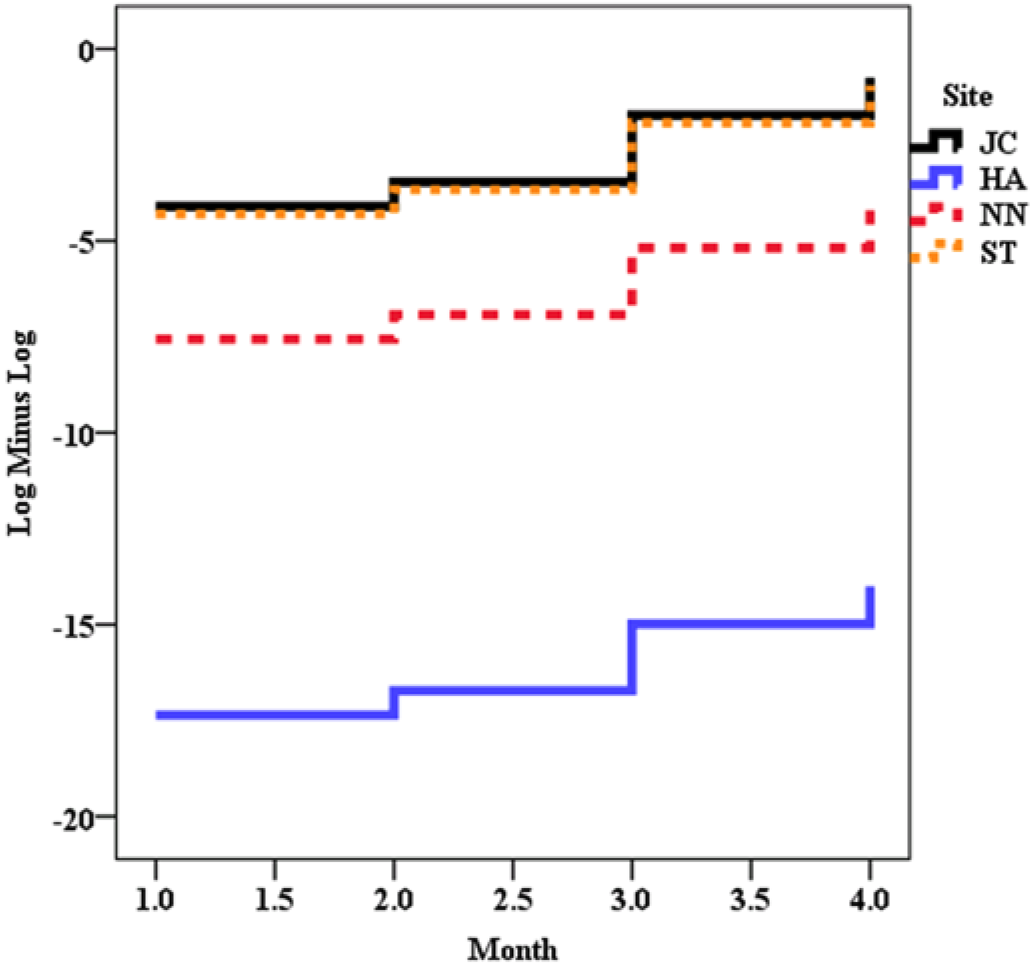

Fig. 41. Cox Log Minus Log for experimental *D. variabilis* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

AMBLYOMMA MACULATUM

Table 38. Kaplan-Meier survival analysis test of equality table for experimental *A. maculatum* comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Test	χ^2	df	p-value
Log Rank	15.791	1	<0.0005
Breslow	25.286	1	<0.0005
Tarone-Ware	21.176	1	<0.0005

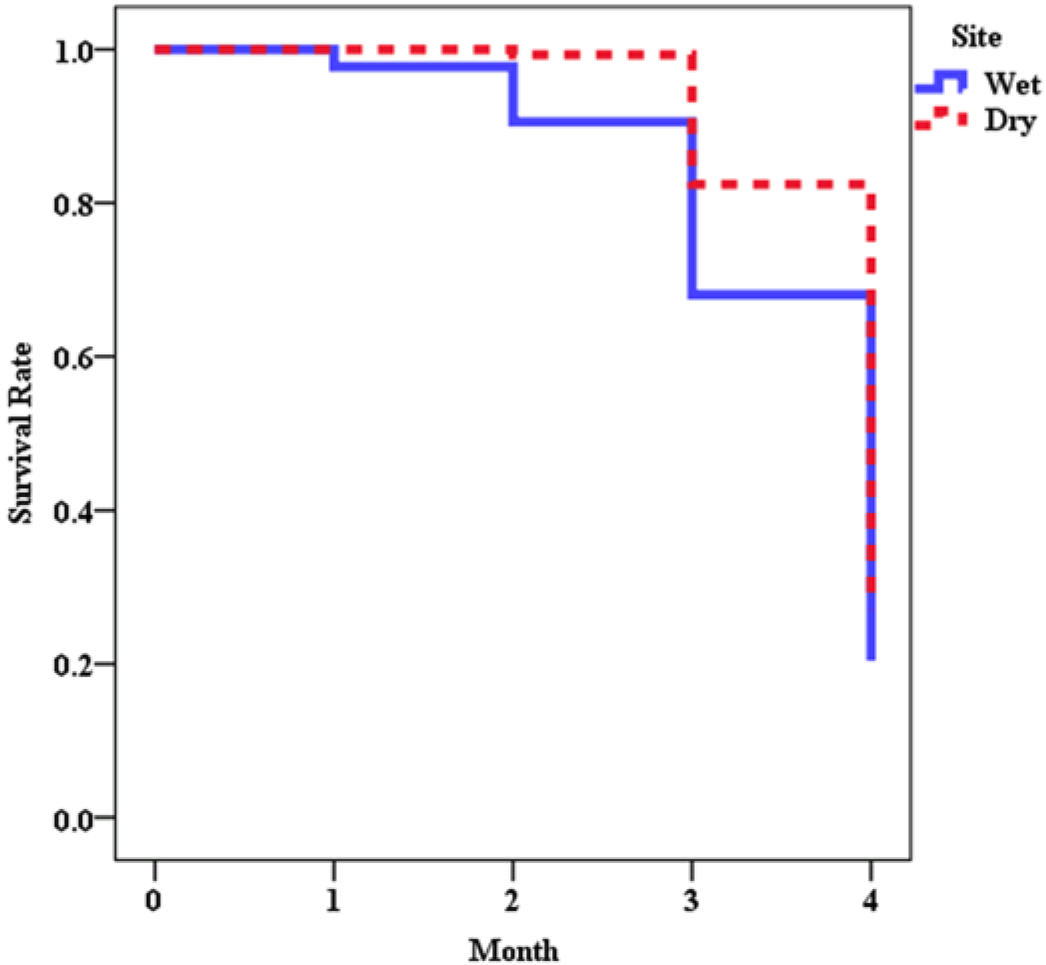

Fig. 42. Kaplan-Meier survival rate for experimental *A. maculatum* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Table 39. Cox regression survival analysis table for experimental *A. maculatum* comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

ß	**χ^2**	**df**	**p-value**	**Exp(ß)**
0.381	8.727	1	0.003	1.464

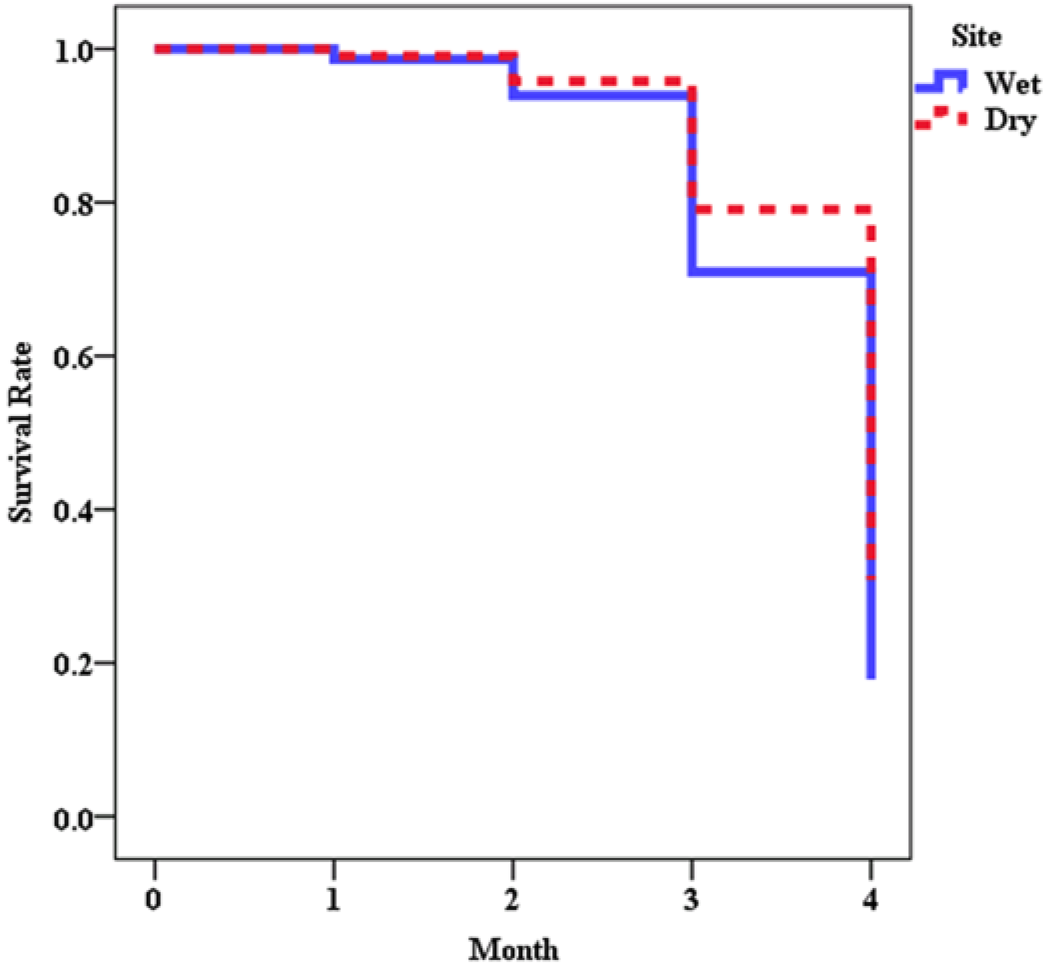

Fig. 43. Cox Regression survival rate of experimental *A. maculatum* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

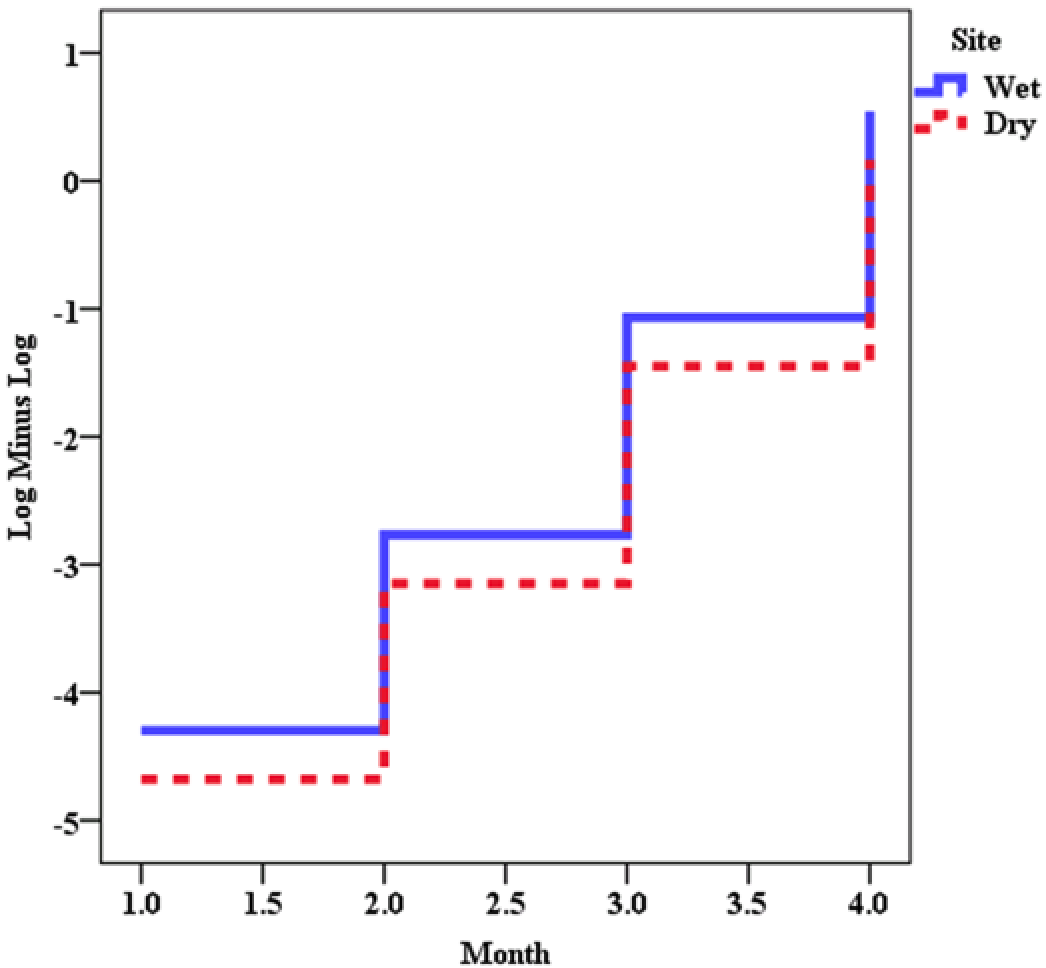

Fig. 44. Cox Log Minus Log for experimental *A. maculatum* by month comparing wet (Stephens and Jacobson) and dry (Hampton and Newport News) field sites.

Table 40. Kaplan-Meier survival analysis test of equality table for experimental *A. maculatum* comparing individual field sites.

Test	χ^2	**df**	**p-value**
Log Rank	51.104	3	<0.0005
Breslow	59.787	3	<0.0005
Tarone-Ware	56.125	3	<0.0005

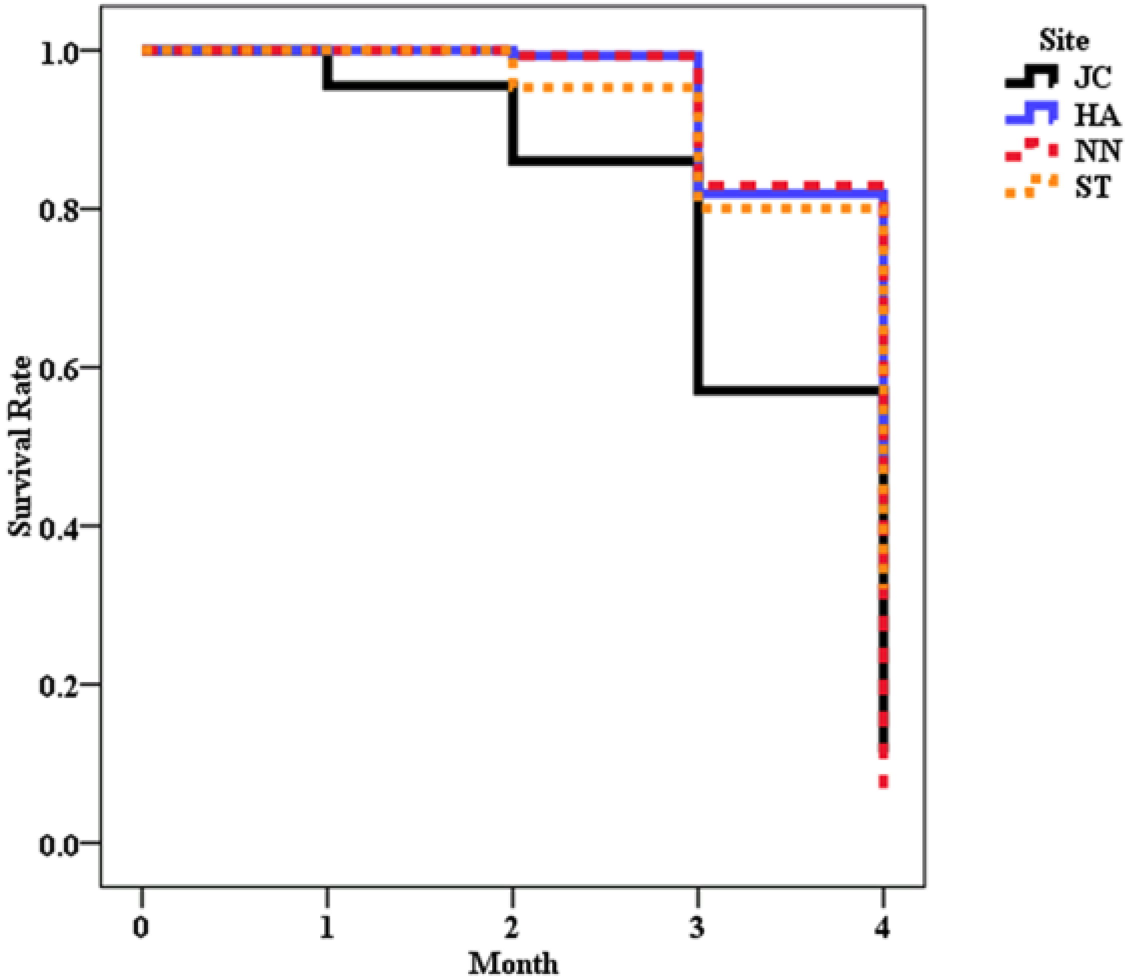

Fig. 45. Kaplan-Meier survival rate for experimental *A. maculatum* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

Table 41. Kaplan-Meier survival analysis pairwise comparisons table for experimental *A. maculatum* ticks comparing individual field sites. Sites: JC = Jacobson, ST = Stephens, HA = Hampton, NN = Newport News.

		JC		**ST**		**HA**		**NN**	
Test	**Site**	**χ^2**	**p-value**	**χ^2**	**p-value**	**χ^2**	**p-value**	**χ^2**	**p-value**
Log Rank	**JC**	-	-	22.480	<0.0005	97.977	<0.0005	15.523	<0.0005
	ST	22.480	<0.0005	-	-	2.768	0.096	1.926	0.165
	HA	37.977	<0.0005	2.768	0.096	-	-	10.053	0.002
	NN	15.523	<0.0005	1.926	0.165	10.053	0.002	-	-
Breslow	**JC**	-	-	22.079	<0.0005	35.730	<0.0005	24.965	<0.0005
	ST	22.079	<0.0005	-	-	2.654	0.103	0.007	0.933
	HA	35.730	<0.0005	2.654	0.103	-	-	3.146	0.076
	NN	24.965	<0.0005	0.007	0.933	3.146	0.076	-	-
Tarone-Ware	**JC**	-	-	22.915	<0.0005	37.852	<0.0005	21.259	<0.0005
	ST	22.915	<0.0005	-	-	2.684	0.101	0.375	0.540
	HA	37.852	<0.0005	2.684	0.101	-	-	6.001	0.014
	NN	21.259	<0.0005	0.375	0.540	6.001	0.014	-	-

Table 42. Cox Regression Omnibus tests of model coefficients table for experimental *A. maculatum* ticks comparing individual field sites.

χ^2	**df**	**p-value**
28.594	3	<0.0005

Table 43. Cox Regression survival analysis table for experimental *A. maculatum* ticks comparing individual field sites. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

	JC					ST				
Site	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
HA	-0.894	22.292	1	<0.0005	0.409	-0.284	1.816	1	0.178	0.753
NN	-0.434	7.076	1	0.008	0.648	0.176	0.876	1	0.349	1.192
ST	-0.610	12.781	1	<0.0005	0.544	-	-	-	-	-
JC	-	-	-	-	-	0.610	12.781	1	<0.0005	1.840

	HA					NN				
Site	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**	**ß**	**χ^2**	**df**	**p-value**	**Exp(ß)**
HA	-	-	-	-	-	-0.460	5.036	1	0.025	0.631
NN	0.460	5.036	1	0.025	1.584	-	-	-	-	-
ST	0.254	1.816	1	0.178	1.329	-0.176	0.876	1	0.349	0.839
JC	0.894	22.292	1	<0.0005	2.445	0.434	7.076	1	0.008	1.543

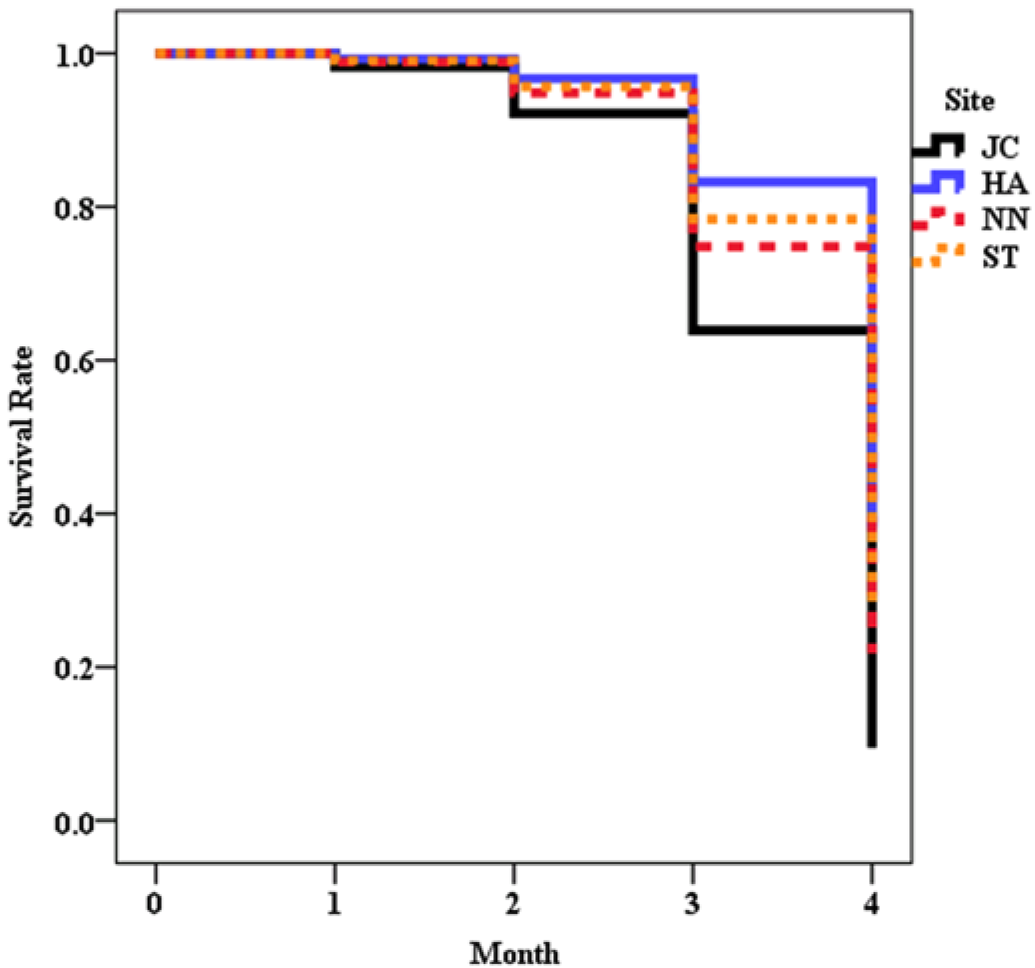

Fig. 46. Cox Regression survival rate of experimental *A. maculatum* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

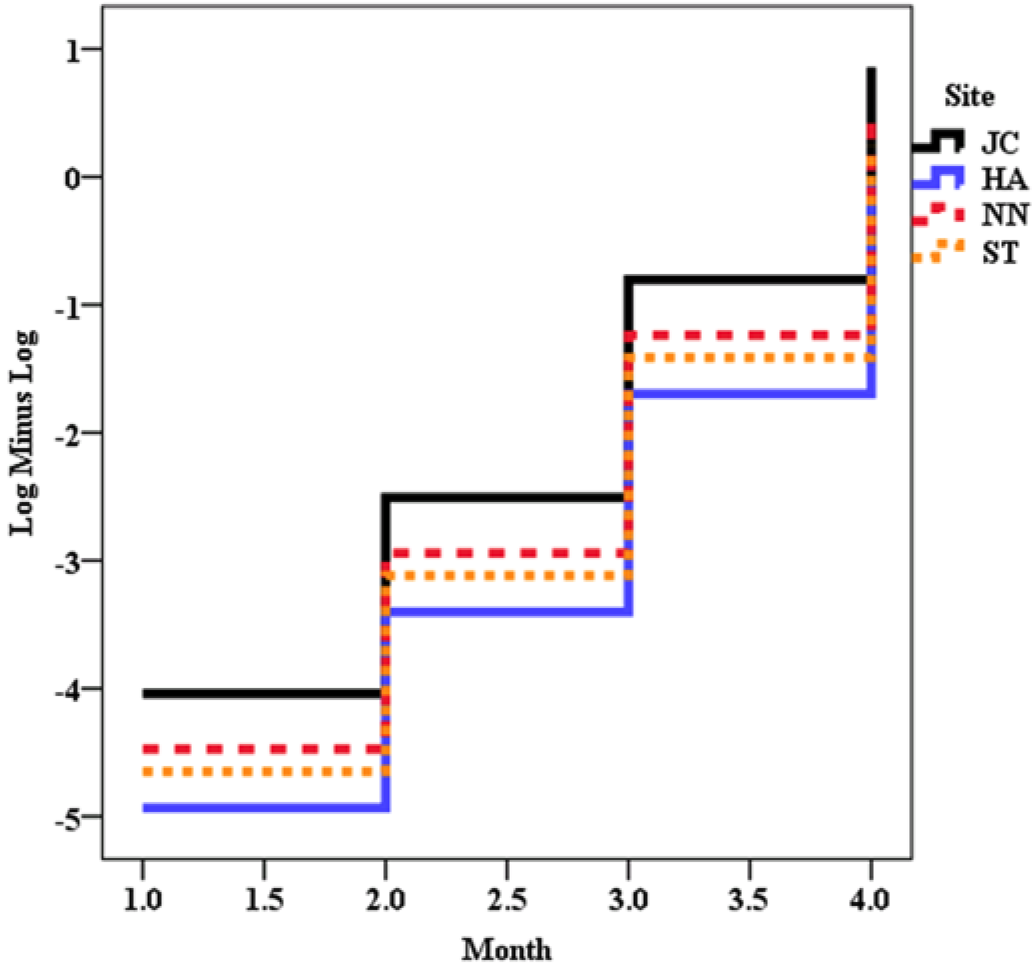

Fig. 47. Cox Log Minus Log for experimental *A. maculatum* by month per individual field site. Sites: JC = Jacobson, HA = Hampton, NN = Newport News, ST = Stephens.

ENVIRONEMNTAL DATA

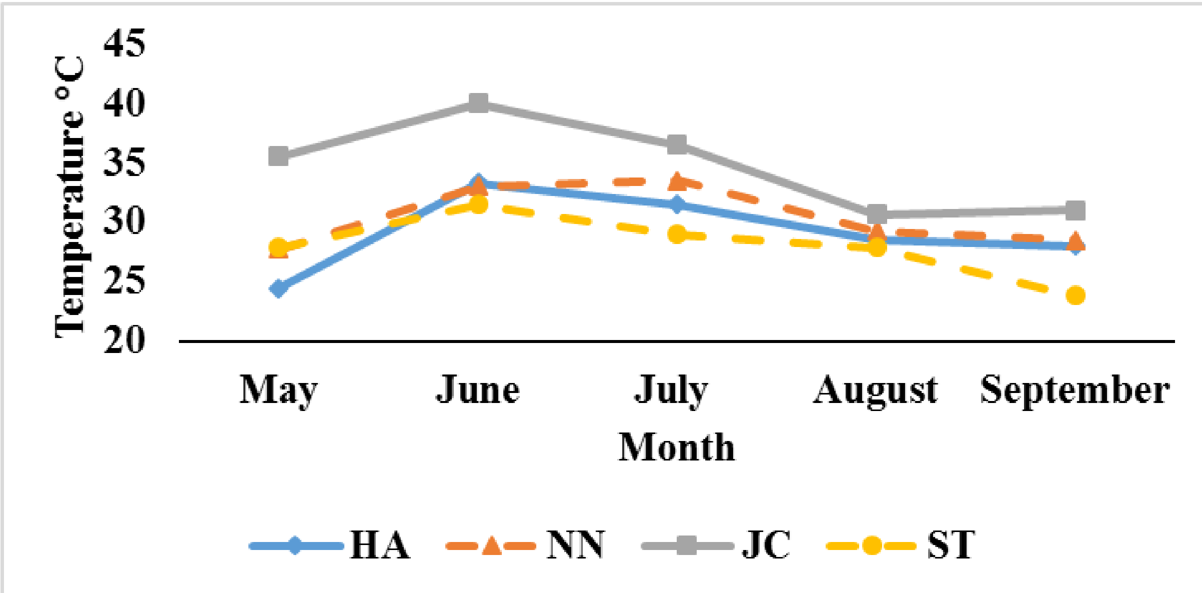

Fig. 48. Average temperatures (°C) over the four month study period by field site. Sites: HA = Hampton, NN = Newport News, JC = Jacobson, ST = Stephens.

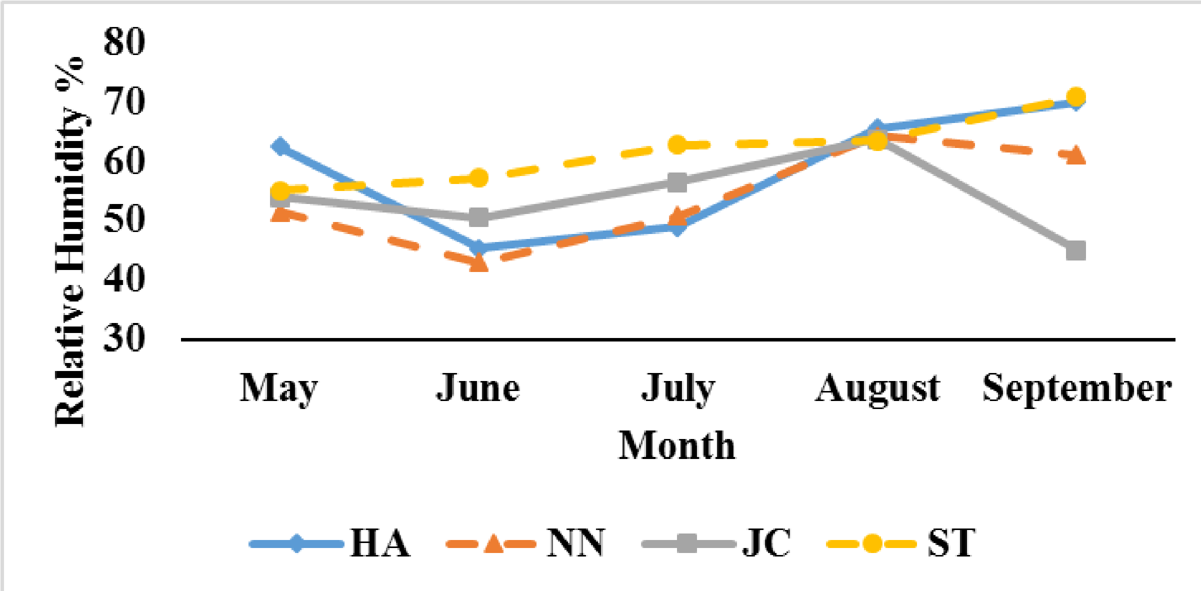

Fig. 49. Average relative humidity (%) over the four month study period by field site. Sites: HA = Hampton, NN = Newport News, JC = Jacobson, ST = Stephens.

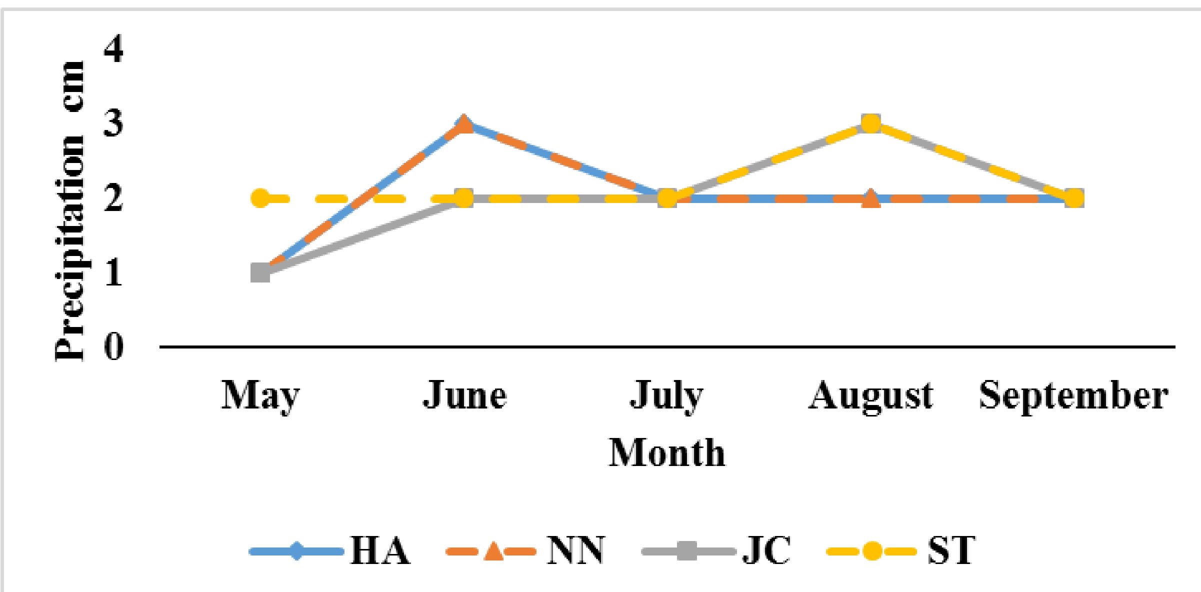

Fig. 50. Average precipitation (cm) over the four month study period by field site. Sites: HA = Hampton, NN = Newport News, JC = Jacobson, ST = Stephens.

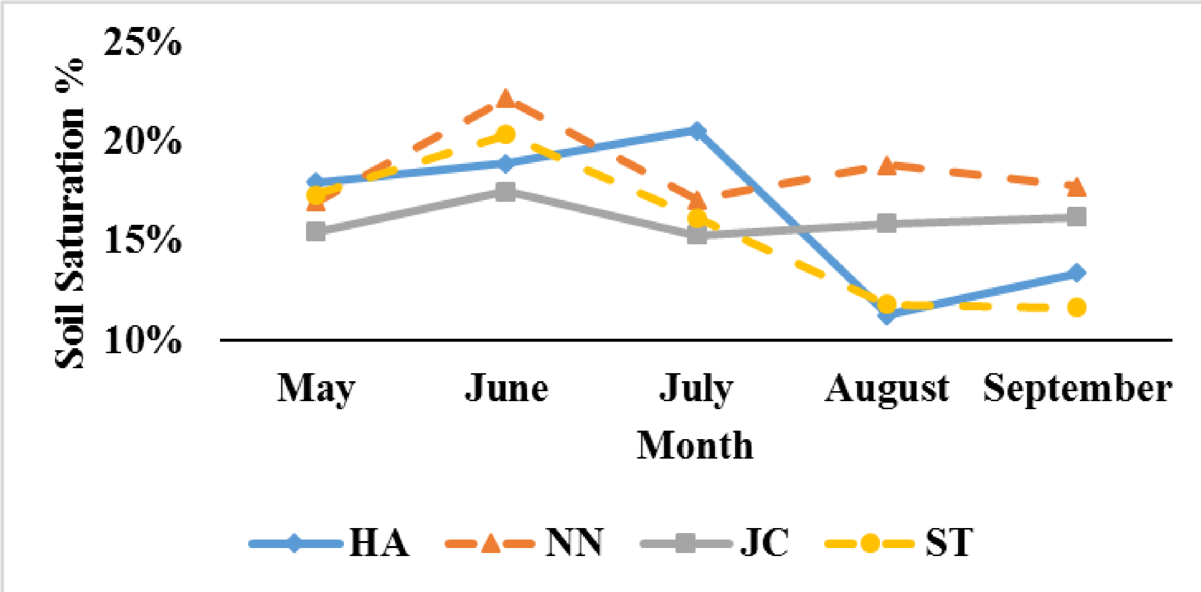

Fig. 51. Average soil saturation (%) over the four month study period by field site. Sites: HA = Hampton, NN = Newport News, JC = Jacobson, ST = Stephens.

Table 44. Experimental field site locations, transect descriptions, and lengths.

Site Location	**Transect**	**Description**	**Total length (m)**
Newport News	Newport News	Dirt road edge habitat, low level undergrowth, aged wooded forest ecotone	486
Hampton	Hampton	Wooded edge habitat, low level undergrowth	168
Chesapeake	Jacobson Tract	Early secondary successional habitat, dominated by grasses, prone to flooding	200
Chesapeake	Stephens Tract	Closed canopy, late secondary successional, dense wooded habitat, prone to flooding	200

Table 45. Average percent relative humidity (RH %), temperature (degrees Celsius), and precipitation (centimeters) collected bi-monthly from May 2015 – September 2015 by field site and month. * Precipitation was not collected at the Stephens Tract field site in May.

Site / Month	**RH %**	**Temp. °C**	**Prec. cm**
Newport News			
May	59.00	25.00	1.27
June	42.00	36.00	8.81
July	49.50	32.00	7.11
August	66.00	28.00	3.56
September	66.50	29.00	0.89
Stephens Tract			
May	54.75	29.25	*
June	61.75	29.50	10.92
July	71.25	29.00	9.53
August	67.83	27.33	3.64
September	67.50	22.00	3.81
Hampton			
May	62.50	24.50	0.51
June	45.33	33.33	3.98
July	49.00	31.50	2.16
August	65.50	28.50	0.38
September	70.00	28.00	0.00
Jacobson Tract			
May	54.00	35.50	2.29
June	50.50	40.00	7.75
July	56.50	36.50	9.65
August	63.67	30.67	0.42
September	45.00	31.00	5.08

FIELD DATA COLLECTED

Table 46. Environmental data collected bi-monthly in May – September 2014 and 2015. Relative humidity (RH %) and temperature (degree Celsius) was collected both years, precipitation (centimeters) was only collected in 2015. * Precipitation was not collected at this time due to rain gauge tampering. **Only one precipitation reading was collected for the two transects at Stephens Tract.

Date	Site	Transect	RH %	Temp C	Prec. cm (2015)
5/9/2014	Stephens tract	6	53	31	
5/9/2014	Stephens tract	2	51	29	
5/12/2014	Newport News	R4	36	34	
5/19/2014	Stephens tract	6	59	23	
5/19/2014	Stephens tract	2	59	23	
5/20/2014	Newport News	R4	52	27	
6/2/2014	Newport News	R4	27	31	
6/3/2014	Stephens tract	6	28	46	
6/3/2014	Stephens tract	2	36	31	
6/16/2014	Stephens tract	2	65	30	
6/16/2014	Stephens tract	6	70	29	
6/17/2014	Newport News	R4	48	34	
6/30/2014	Newport News	R4	57	25	
6/30/2014	Stephens tract	2	58	31	
6/30/2014	Stephens tract	6	67	30	
7/14/2014	Newport News	R4	54	32	
7/17/2014	Stephens tract	2	64	26	
7/17/2014	Stephens tract	6	69	26	
7/28/2014	Newport News	R4	50	38	
7/29/2014	Stephens tract	2	45	31	
7/29/2014	Stephens tract	6	39	33	
8/11/2014	Stephens tract	2	62	29	
8/11/2014	Stephens tract	6	63	29	
8/12/2014	Newport News	R4	73	32	
8/25/2014	Stephens tract	6	50	29	
8/25/2014	Stephens tract	2	52	28	
8/27/2014	Newport News	R4	52	29	
9/10/2014	Stephens tract	6	84	24	
9/10/2014	Stephens tract	2	86	24	
9/11/2014	Newport News	R4	69	31	
9/22/2014	Newport News	R4	42	25	

Table 46 continued.

Date	**Site**	**Transect**	**RH %**	**Temp C**	**Prec. cm (2015)**
9/26/2014	Stephens tract	6	58	27	
9/26/2014	Stephens tract	2	62	24	
5/2/2015	Newport News	R4	64	20	Rain Gauge Deployed
5/2/2015	Hampton	PW	65	19	Rain Gauge Deployed
5/9/2015	Stephens tract	6	58	29	Rain Gauge Deployed
5/9/2015	Stephens tract	2	52	31	Rain Gauge Deployed
5/9/2015	Jacobson Tract	0	67	27	Rain Gauge Deployed
5/19/2015	Newport News	R4	54	30	1.27
5/19/2015	Hampton	PW	60	30	0.51
5/26/2015	Jacobson Tract	0	41	44	2.29
5/26/2015	Stephens tract	6	54	29	*
5/26/2015	Stephens tract	2	55	28	*
6/1/2015	Newport News	R4	40	35	2.03
6/1/2015	Hampton	PW	45	31	0.76
6/7/2015	Jacobson Tract	0	48	40	12.70
6/7/2015	Stephens tract	6	65	26	11.68
6/7/2015	Stephens tract	2	65	26	**
6/15/2015	Newport News	R4	37	41	11.68
6/15/2015	Hampton	PW	39	40	5.08
6/22/2015	Jacobson Tract	0	53	40	2.79
6/22/2015	Stephens tract	6	55	34	10.16
6/22/2015	Stephens tract	2	62	32	**
6/28/2015	Newport News	R4	49	32	12.7
6/28/2015	Hampton	PW	52	29	6.10
7/6/2015	Jacobson Tract	0	48	38	>12.70
7/6/2015	Stephens tract	6	69	28	>12.70
7/6/2015	Stephens tract	2	76	28	**
7/12/2015	Newport News	R4	58	32	11.18
7/12/2015	Hampton	PW	59	31	3.30
7/21/2015	Jacobson Tract	0	65	35	6.60
7/21/2015	Stephens tract	6	70	30	6.35
7/21/2015	Stephens tract	2	70	30	**
7/26/2015	Newport News	R4	41	32	3.05
7/26/2015	Hampton	0	39	32	1.02
8/2/2015	Jacobson Tract	0	56	30	0.76
8/2/2015	Stephens tract	6	70	30	6.60
8/2/2015	Stephens tract	2	71	29	**
8/9/2015	Newport News	R4	81	26	5.59

Table 46 continued.

Date	**Site**	**Transect**	**RH %**	**Temp C**	**Prec. cm (2015)**
8/9/2015	Hampton	PW	84	24	0.51
8/17/2015	Jacobson Tract	0	59	31	0.51
8/17/2015	Stephens tract	6	63	29	3.05
8/17/2015	Stephens tract	2	65	29	**
8/25/2015	Hampton	PW	47	33	0.25
8/25/2015	Newport News	R4	51	30	1.52
8/30/2015	Stephens tract	6	68	24	1.27
8/30/2015	Stephens tract	2	70	23	**
8/30/2015	Jacobson Tract	0	76	31	0.00
9/8/2015	Newport News	R4	60	34	0.51
9/8/2015	Hampton	PW	69	31	*
9/15/2015	Stephens tract	6	67	22	3.81
9/15/2015	Stephens tract	2	68	22	**
9/16/2015	Jacobson Tract	0	45	31	5.08
9/22/2015	Newport News	R4	73	24	1.27
9/22/2015	Hampton	PW	71	25	0

Table 47. Environmental survival study data by date tickarium were submerged (Sub) and removed (Rem) from the field, treatment group (Trt) and replicate (Rep), field site (Site), species (Spec), and recorded mortality (Dead) or survival (Alive) in relation to the initial number of ticks placed in situ (Start); M = male, F = female. Site NN = Newport News, HA = Hampton, ST = Stephens, JC = Jacobson. Tick species: Aa = *A. americanum*, Am = *A. maculatum*, Dv = *D. variabilis*. 1a = tickaria removed from field after one month, replicate a, etc.

Date Sub	**Date Rem**	**Trt/ Rep**	**Site**	**Spec**	**M Start**	**F Start**	**M Alive**	**M Dead**	**F Alive**	**F Dead**	**Total Alive**	**Total Dead**
5/2/15	6/1/15	1a	NN	Aa	13	12	13	0	12	0	25	0
		1b	NN	Aa	12	13	12	0	13	0	25	0
		1a	NN	Am	13	12	13	0	12	0	25	0
		1b	NN	Am	12	13	12	0	13	0	25	0
		1a	NN	Dv	13	12	12	0	12	0	24	0
		1b	NN	Dv	12	13	11	0	13	0	24	0
		1a	HA	Aa	13	12	13	0	12	0	25	0
		1b	HA	Aa	12	13	12	0	13	0	25	0
		1a	HA	Am	13	12	13	0	12	0	25	0
		1b	HA	Am	12	13	12	0	13	0	25	0
		1a	HA	Dv	13	12	13	0	12	0	25	0
		1b	HA	Dv	12	13	12	0	14	0	26	0
5/9/15	6/7/15	1a	ST	Aa	13	12	13	0	12	0	25	0
		1b	ST	Aa	12	13	11	0	14	0	25	0
		1a	ST	Am	13	12	12	0	12	0	24	0
		1b	ST	Am	12	13	12	0	13	0	25	0
		1a	ST	Dv	13	12	13	0	12	0	25	0
		1b	ST	Dv	12	13	12	0	13	0	25	0
		1a	JC	Aa	13	12	13	1	11	0	24	1
		1b	JC	Aa	12	13	12	0	13	0	25	0
		1a	JC	Am	13	12	11	2	10	2	21	4
		1b	JC	Am	12	13	8	4	12	1	20	5
		1a	JC	Dv	13	12	10	2	9	3	19	5
		1b	JC	Dv	12	13	12	0	12	1	24	1

Table 47 continued.

Date Sub	Date Rem	Trt/ Rep	Site	Spec	M Start	F Start	M Alive	M Dead	F Alive	F Dead	Total Alive	Total Dead
5/2/15	6/28/15	2a	NN	Aa	13	12	11	0	12	0	23	0
		2b	NN	Aa	12	13	11	1	13	0	24	1
		2a	NN	Am	13	12	13	0	11	0	24	0
		2b	NN	Am	12	13	12	0	11	1	23	1
		2a	NN	Dv	13	12	13	0	12	0	25	0
		2b	NN	Dv	12	13	12	0	13	0	25	0
		2a	HA	Aa	13	12	13	0	12	0	25	0
		2b	HA	Aa	12	13	12	0	13	0	25	0
		2a	HA	Am	13	12	12	1	12	0	24	1
		2b	HA	Am	12	13	12	0	13	0	25	0
		2a	HA	Dv	13	12	13	0	12	0	25	0
		2b	HA	Dv	12	13	12	0	13	0	25	0
5/9/15	7/6/15	2a	ST	Aa	13	12	13	0	12	0	25	0
		2b	ST	Aa	12	13	12	0	13	0	25	0
		2a	ST	Am	13	12	11	2	10	1	21	3
		2b	ST	Am	12	13	11	1	10	3	21	4
		2a	ST	Dv	13	12	13	0	11	1	24	1
		2b	ST	Dv	12	13	12	0	13	0	25	0
		2a	JC	Aa	13	12	12	0	12	0	24	0
		2b	JC	Aa	12	13	11	0	13	0	24	0
		2a	JC	Am	13	12	12	1	12	0	24	1
		2b	JC	Am	12	13	5	7	6	7	11	14
		2a	JC	Dv	13	12	13	0	11	1	24	1
		2b	JC	Dv	12	13	12	0	11	2	23	2

Table 47 continued.

Date Sub	Date Rem	Trt/ Rep	Site	Spec	M Start	F Start	M Alive	M Dead	F Alive	F Dead	Total Alive	Total Dead
5/2/15	7/26/15	3a	NN	Aa	13	12	12	0	11	0	23	0
		3b	NN	Aa	12	13	12	0	13	0	25	0
		3a	NN	Am	13	12	12	1	9	3	21	4
		3b	NN	Am	12	13	8	3	4	9	12	12
		3a	NN	Dv	13	12	13	0	12	0	25	0
		3b	NN	Dv	12	13	12	0	13	0	25	0
		3a	HA	Aa	13	12	12	1	12	0	24	1
		3b	HA	Aa	12	13	12	0	13	0	25	0
		3a	HA	Am	13	12	5	8	2	9	7	17
		3b	HA	Am	12	13	12	0	12	0	24	0
		3a	HA	Dv	13	12	13	0	12	0	25	0
		3b	HA	Dv	12	13	12	0	13	0	25	0
5/9/15	8/2/15	3a	ST	Aa	13	12	12	0	12	0	24	0
		3b	ST	Aa	12	13	12	0	12	1	24	1
		3a	ST	Am	13	12	12	1	6	6	18	7
		3b	ST	Am	12	13	9	3	7	6	16	9
		3a	ST	Dv	13	12	10	2	6	6	16	8
		3b	ST	Dv	12	13	11	1	13	0	24	1
		3a	JC	Aa	13	12	12	0	12	0	24	0
		3b	JC	Aa	12	13	12	0	13	0	25	0
		3a	JC	Am	13	12	5	8	5	7	10	15
		3b	JC	Am	12	13	2	9	5	10	7	19
		3a	JC	Dv	13	12	7	6	4	8	11	14
		3b	JC	Dv	12	13	11	0	11	2	22	2

Table 47 continued.

Date Sub	Date Rem	Trt/ Rep	Site	Spec	M Start	F Start	M Alive	M Dead	F Alive	F Dead	Total Alive	Total Dead
5/2/15	8/25/15	4a	NN	Aa	13	12	12	0	13	0	25	0
		4b	NN	Aa	12	13	13	0	12	0	25	0
		4a	NN	Am	13	12	0	12	0	11	0	23
		4b	NN	Am	12	13	1	11	3	10	4	21
		4a	NN	Dv	13	12	13	0	11	0	24	0
		4b	NN	Dv	12	13	12	0	12	1	24	1
		4a	HA	Aa	13	12	13	0	12	0	25	0
		4b	HA	Aa	12	13	12	0	13	0	25	0
		4a	HA	Am	13	12	13	0	10	1	23	1
		4b	HA	Am	12	13	4	8	1	12	5	20
		4a	HA	Dv	13	12	13	0	12	0	25	0
		4b	HA	Dv	12	13	12	0	12	0	24	0
5/9/15	8/30/15	4a	ST	Aa	13	12	13	0	11	1	24	1
		4b	ST	Aa	12	13	12	0	13	0	25	0
		4a	ST	Am	13	12	0	12	0	13	0	25
		4b	ST	Am	12	13	11	1	9	4	20	5
		4a	ST	Dv	6	19	3	2	5	14	8	16
		4b	ST	Dv	12	13	12	0	13	0	25	0
		4a	JC	Aa	13	12	13	0	12	0	25	0
		4b	JC	Aa	12	13	11	0	13	0	24	0
		4a	JC	Am	13	12	1	11	4	9	5	20
		4b	JC	Am	12	13	1	11	4	9	5	20
		4a	JC	Dv	13	12	10	3	12	0	22	3
		4b	JC	Dv	12	13	11	1	10	3	21	4

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EDUCATION

- Master of Science, Biology, Old Dominion University, Norfolk, VA, December 2016
- Bachelor of Science, Biology, The College of William and Mary, Williamsburg, VA, August 2005
- Associates of Science, Biology, Richard Bland College of The College of William and Mary, Petersburg, VA, May 2003

PRESENTATIONS

- *Amblyomma americanum* in Southeastern Virginia 2010 to 2012, Biology Graduate Student Organization Spring Symposium, March 2012, Old Dominion University, Norfolk, Virginia
- Temporal and Spatial Variability of *Amblyomma americanum* in Southeastern Virginia, International Symposium on Biomathematics and Ecology Education and Research, Marymount University, October 2013
- My planned research: Abiotic Factors Contributing to the Survival of Three Tick Species in Southeastern Virginia, *Amblyomma americanum*, *Dermacentor variabilis*, and *Amblyomma maculatum*, Biology Graduate Student Organization Spring Symposium, March 2014, Old Dominion University, Norfolk, Virginia
- Abiotic Factors Contributing to the Survival of Three Tick Species in Southeastern Virginia, Virginia Mosquito Control Association meeting January 2016, Portsmouth, Virginia
- An Introduction to your Local Ticks, the Pathogens They Carry, and your Local Tick Research Team, Virginia Master Naturalists, Hampton, VA, February 2016
- Abiotic Factors Contributing to the Survival of Three Tick Species in Southeastern Virginia, *Amblyomma americanum*, *Dermacentor variabilis*, and *Amblyomma maculatum*, Biology Graduate Student Organization Spring Symposium, April 2016, Old Dominion University, Norfolk, Virginia

PROFESSIONAL EXPERIENCES

- Water Quality Technician, Pretreatment and Pollution Prevention, Hampton Roads Sanitation District, Virginia Beach, VA, April 2011 – Present
- Fishery Biologist I, Dockside Sampling Program, Alaska Department of Fish and Game, Kodiak, AK, January 2009 – August 2010.