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The Assessment of Predictor Variables for Hard Tick Abundance in Southwestern Missouri

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THE ASSESSMENT OF PREDICTOR VARIABLES FOR HARD TICK ABUNDANCE IN SOUTHWESTERN MISSOURI

A Master's Thesis

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In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Casey L. Adkins

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ABSTRACT

The risk of tick-borne infection is increasing across the United States, and in Missouri, ticks are expanding into novel regions due to climate change, habitat fragmentation, and biodiversity loss. Regions in which ticks are encroaching experience novel vectors for lineage associated pathogens. Novel tick detection can be low due to sampling practices targeting known ticks, which can lead to unreliable distribution maps and poor predictive distribution models. Such models should account for biotic factors, abiotic factors, and their interactions to provide a dynamic view of their impact on tick abundance and identify variables that can serve as indicators. Further, a simple comparison of sampling methods in different habitats for tick abundance, diversity, and life stage allows for the determination of the most effective sampling technique to gain a holistic view of tick communities. I completed a set of surveys to account for biotic factors, abiotic factors, and sampling design in tick distribution in Southwest Missouri. I used tick drags and sampled the following biotic and abiotic factors: small mammals, ants, ambient temperature, relative humidity, litter depth, and canopy cover. Factors were tested directly on tick abundance using generalized linear models, and indirect relationships, like the effect of location, were analyzed using a linear mixed effect model. To test method efficiency, I executed drags and carbon-dioxide traps in two different habitat types, forest and grassland, and compared captures in terms of abundance, species, and life stages. Indirect relationships and location explained tick abundance more clearly than direct relationship and two methods of sampling resulted in more effective analysis of tick communities. Understanding tick communities and the driving forces behind the movement of tick populations is needed to increase the awareness of public health programs of tick-borne diseases in the region.

KEYWORDS: tick, abiotic, biotic, ants, abundance, sampling methods, generalized linear model, linear mixed effect model, Missouri

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Approved:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

OVERVIEW

Ticks are parasitic arthropods that feed on vertebrates by attaching to their hosts and taking blood meals at each life-stage. The level of host specificity for a tick species depend the tick life stage or host availability and seasonality [1,2]. The two families of ticks, Ixodidae and Argasidae, are known as the 'hard ticks' and the latter as the 'soft ticks', and this taxonomic separations is based on differences in physiology, morphology, and life cycles [3]. The first life stage of a hard tick's life cycle is the egg. The second stage is the larval stage, in which it has six legs. The larva must obtain a blood meal, generally from a small mammal or bird, to molt into the eight-legged nymph stage [2]. A blood meal is also required for the final molt into the adult stage of the life cycle as well as after for reproduction. Adult hard ticks display a questing behavior by climbing on the vegetation, the tick extends its forelegs from the leafy vegetation to grasp an unsuspecting host as they brush by the vegetation [4]. Ticks transfer pathogens to their host during the feeding process by salivary gland secretions that are cement-like substances that aid in the attachment of the tick; the saliva may also serve to avert the host immune response [4].

The Centers for Disease Control and Prevention (CDC) recognizes five tick species in Missouri that carry a number of zoonotic diseases. *Rhipicephalus sanguineus*, the brown dog tick, is distributed across the United States and is known for transmitting the bacteria that causes Rocky Mountain spotted fever, which occurs in Mexico and the southwestern U.S. in humans [5,6]. *Dermacentor variabilis*, the American dog tick, distributed across the eastern U.S. and Midwest, is a vector for tularemia and Rocky Mountain spotted fever [6–8]. The CDC estimates the distribution of *Ixodes scapularis*, the blacklegged tick, to include Missouri but abundance to be low. *I. scapularis* is known as a vector for pathogens that cause the following diseases:

anaplasmosis, *Borrelia miyamotoi* disease in the form of relapsing fever, Lyme disease, ehrlichiosis, babesiosis, and Powassan virus [9–12]. In southern Missouri, *Amblyomma maculatum*, the gulf coast tick, occurs and is a vector for rickettsiosis in the form of spotted fever. *Amblyomma americanum*, the lone star tick, is distributed throughout Missouri and is a vector for pathogens that cause the following diseases: ehrlichiosis, southern Lyme disease, Heartland virus, tularemia, and carries meat allergy-causing agents [8,13–16].

Most of these diseases are uncommonly diagnosed in humans, but the rate of tick-borne infection is rising in the U.S. and infections are being diagnosed outside of the vectors distributional ranges [17]. The increase and spread of tick species are hypothesized to be the result of three factors: climate change, habitat fragmentation, and loss of biodiversity [10,18–21]. As the climate warms and seasonal patterns change, the ideal habitat for ticks and their hosts are shifting [10]. Habitat fragmentation can cause a reduction in biodiversity because habitat specialist hosts are losing habitat due to fragmentation while more generalist species or edge-specialist remain stable or increase in abundance [18,22,23]. Hosts are traveling further distances to gain needed resources between patches while carrying ticks with them. These changes have been observed as distributional shifting factors for common tick hosts, such as mammals [10,18,19,23]. Such a scenario could lead to an increase in tick abundance because changing climate patterns may create ideal microhabitats and increased density of generalist host species.

My research analyzed tick abundance in southwest Missouri to assess predictor variables and the most effective sampling methodology. The first chapter tested abiotic and biotic factors as predictor variables for tick abundances, by using abiotic variables such as ambient temperature, relative humidity, litter depth, and canopy cover, and biotic variables such as small mammal abundance and ant abundance to tick abundance. The second chapter tested the

efficiency of two common sampling methods: drags and carbon-dioxide traps. This provides future researchers with a better understanding of what abiotic and biotic factors can be used to predict tick abundance, and which sampling methods should be used to obtain representative data that best reflect tick abundance.

EVALUATION OF BIOTIC AND ABIOTIC VARIABLES INFLUENCING TICK ABUNDANCE

Introduction

Ticks are a common vector for zoonotic pathogens, including *Anaplasma phagocytophilum* (the causative agent of human granulocytic), *Babesia microti* (the causative agent of human Babesiosis), and *B. burgdorferi* the causative agent of Lyme disease)[1–3]. Importantly, the geographic ranges of pathogens and vectors are not always coincident, but the expansion of the pathogen range might rely on the presence of a suitable vector [4]. Some ticks expand their range after introduction, while others take advantage of changes in the abundance of suitable hosts [5]. These undetected populations in novel regions can present previously uncommon diseases, and early detection of the vector is key in preserving public health [6]. And yet, local level detection efforts rates are inadequate in many regions. This is a public health concern because local health agencies rely on these detection data to bring awareness of vectors to the public [7]. To maintain a working distribution of tick populations a combination of biotic and abiotic factors should be considered to increase accuracy in the variables that are used to create these maps (Figure 1).

The most relevant biotic factor for ticks are the hosts they use as food sources because ticks must obtain a blood meal before being able to morph into the next life stage and females must feed before eggs can be laid [2]. Small mammals and birds serve as important hosts to ticks because of their relative abundance and ecological habits [4]. High densities of hosts could increase the rate of tick transmission due to the close proximity of hosts [8]. Increased numbers of small mammals will have a direct positive impact on tick abundance (Figure 1). This could

allow for pathogens to rapidly spread from one host to another, which increases the risk of human infection [5]. The second biotic factor to consider are ants because they have direct and indirect effects on ticks, wherein indirect effects are the result of ant interactions with small mammals. Ants have been reported to predate small mammals and avoidance of ant nests has been observed in small mammal communities [9,10]. Ants are terrestrial omnivores that occupy myriad habitats, including many of the same habitats as small mammals in deciduous forests [10,11]. Further, ants can act as important competitors for seeds and other vegetation, thus increasing the density of rodents with the absence of ants [12]. This interspecific interaction can lead to lower small mammal abundance and diversity in habitats where ants are present [9,12], thus decreasing host availability and tick abundance (Figure 1). Directly, ants have been found to have a negative impact on tick abundance due to predation. This has been demonstrated with preliminary data showing ants harvesting engorged ticks [10]. Some ant genera are expected to have a negative impact on tick abundance while others will not [11,13; Figure 1]. A third variable that is linked to ambient temperature is canopy cover; there is a negative relationship between canopy cover and ambient temperature because the shade created by the canopy cools the ambient temperature (Figure 1). Canopy cover has a direct negative effect on tick abundance, with a positive direct effect on litter depth (Figure 1). Litter provides a more stable relative humidity and temperature microenvironment in which ticks can gain refuge from harsher conditions that can result in freezing or desiccation as well as protection from predators [14,15]. These relationships lead to the expected direct unimodal effect of litter depth on tick abundance, due to the refuge litter creates for ticks (Figure 1).

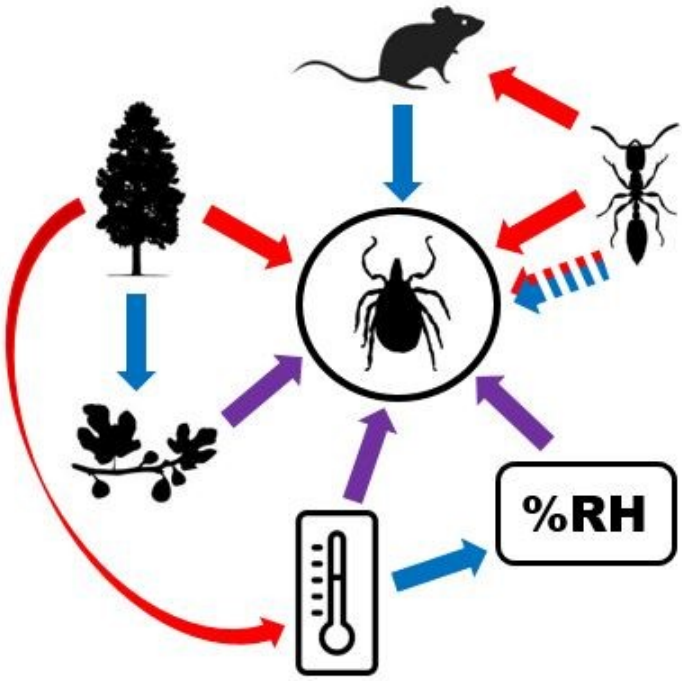

Figure 1) A conceptual figure to display the positive and negative relationships between biotic and abiotic variables on tick abundances. Each image represents one of the variables measured, reading clockwise from the top; mouse (small mammal abundance), ant (ant abundance), %RH (relative humidity), thermometer (ambient temperature), tree branch (litter depth), and tree (canopy cover). The arrows represent the effect one variable has on tick abundance or another variable within the community, blue arrows represent a positive relationship, red arrows represent a negative relationship, and purple arrows represent a unimodal relationship. The dashed arrow represents the potential random effect of ant communities, a positive or negative relationship dependent on the sampling location.

Tick abundance and distribution are also mediated by abiotic factors that impact their physiology and behavior. Ambient temperature and relative humidity are commonly used in predictive modeling for tick species across the United States in a global climate context [16–18]. Studies have demonstrated the importance of temperature and relative humidity on the questing behavior of all life-stages [18,19]. Additionally, at less than 10 ˚C, little to no development may occur [15,20]. Ambient temperature is expected to have a positive relationship with tick abundance until reaching a threshold temperature due to the risk of desiccation at hotter temperatures or risk of mobility loss at colder temperatures [19,20; Figure 1]. The abundance of

unfed ticks is predicted to decrease with relative humidity because tick activity and questing behavior and risk of desiccation is reduced [19; Figure 1]. Ambient temperature and relative humidity are closely linked because warmer air holds more moisture than cooler air [22]. This leads to a positive relationship between temperature and relative humidity (Figure 1).

The goal of this study is to determine if these biotic and abiotic variables serve as reasonable predictor variables for tick abundance in southwest Missouri. Using a series of statistical models, I compared the support for each of these predictions on their own and in concert. The results expand the knowledge of Missouri ticks and the habitats they occupy and should further the understanding of fluctuations of tick densities and the health risks they pose.

Methods

I sampled five sites in four Missouri counties: Taney, Dallas, Christian, and Douglas. Data collection occurred in June – July 2018 (Figure 2). Christian County was sampled at two different locations and will be denoted as 'Christian A' and 'Christian B'. The sites included Missouri conservation areas, private lands, and Missouri State University research land. These locations were selected for sampling because of the similarity within the mature hardwood forest habitat with a lower level of habitat fragmentation. At each site, I completed two 200m sampling transects in forested habitats. Tick drags were completed along the transects, using a white 1m × 1m flannel cloth fastened to a wooden dowel on one side to keep the cloth spread across the ground [23]. All ticks on the drag and on researchers were collected every 5m and stored in 95% ethanol. In the lab, collected ticks were identified to species and life stage. All ticks collected underwent the same statistical measures, yet due to the small sample sizes of other species, only *Amblyomma americanum* was analyzed as an individual species. All three life stages, larva,

nymph, and adult, of *A. americanum* were analyzed. Approval for this project was obtained from the Missouri State University Institutional Animal Care and Use Committee (IACUC) and Missouri Department of Conservation (MDC) prior to collection (IACUC, ID #18-029.0; MDC #17723).

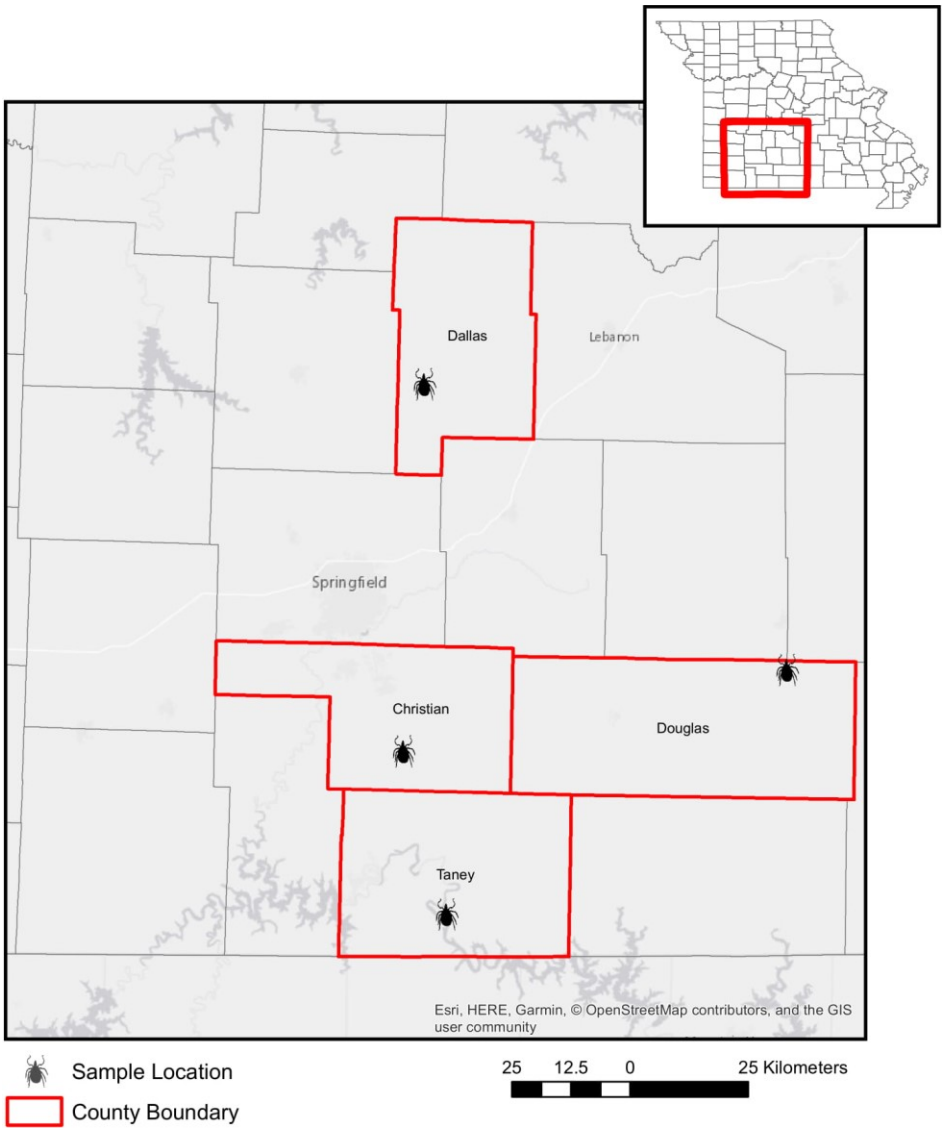

Figure 2) Map of the five sampling sites in southwestern Missouri, which included two nearby sites in Christian County.

Biotic Variables. Small mammals were trapped along the 200m transect with trap stations every five meters. Two Sherman live-traps were used at each station, alternating between two small traps (7.62 × 8.89 × 22.86 cm) and one small and one large trap (10.16 × 11.43 × 38.10 cm) at each trap station to maximize capture efficiency. Ants were trapped using a protein bait, which was ~4g of wet cat food, every 5m. The protein bait was available for two hours in the afternoon, then all ants at the bait were collected and stored in 95% ethanol [11,24]. In the lab, ants were identified to genus according to Fisher and Cover [2007]. To assess the effect of ants on ticks, I used a series of generalized linear models (GLMs) using the glm function in R [26]. For models using ant abundance data as the response variable, I used a Poisson regression with log transformed predictor variable. The GLM accessed the predicted direct negative effect of ant abundance on ticks. Litter depth and canopy cover also were recorded using a standard metric ruler and a spherical densitometer model-C, and tested under the same modeling framework.

Abiotic Variables. Environmental data were obtained at the 5m collection stations where ant baits were placed in the afternoon. I measured relative humidity and ambient temperature at the ground, 0.5m, and 1m from the ground using Hygrometer PCE-555 version 2.0. A simple analysis of correlations between abiotic variables was completed, resulting in strong correlations between the three relative humidity measurements and the three ambient temperature measurements (Appendix A1 – A2). Therefore, I used only the 1m measure of relative humidity and ambient temperature for the GLM. Poisson regression GLMs were used to test the predicted direct effects of relative humidity and predicted direct effects of ambient temperature on ticks [25].

Biotic and Abiotic Variables. Biotic and abiotic effects were then tested for a combined effect on tick abundance using the same Poisson regression GLM. To compare goodness-of-fit of models of varying complexity of the model, I used a version of Akaike Information Criteria (AIC) that controls for small sample sizes AICc; [27] and penalizes for additional parameters [28,29]. Because I had no *a priori* hypotheses of the appropriate parameter combinations, I used the MuMIn package in R [30] to evaluate all predictor combinations. The resulting output for all parameter combinations was then used to build the variables in a linear mixed effect model.

In the case that an unmeasured confounding variable based on location influenced the relationship between ticks and ant abundance, I used a linear mixed effect model [47; Table 1]. The basic principle of the location serving as the correlated random effect variable was used to build the formula under the lmer4 package with the glmer function within R. The most supported linear mixed effect model was compared to the null model without location as the random effects using an analysis of variance (ANOVA). Additionally, the relationship between tick and ant abundance with the effect of location was analyzed without any abiotic effects using the same modeling structure. This model results in the effect of location on tick abundance strictly due to ant abundance.

Results

I collected 4,063 ticks representing four species: *A. americanum*, *A. maculatum*, *Dermacentor variabilis*, and *Ixodes scapularis*. However, *A. americanum* represented all but 9 individuals (Table 1).

Biotic Variables. Twelve total small mammals were collected, eleven *Peromyscus leucopus* and one *Neotoma floridana* with a one percent collection rate. Because of this limited

sample size, I did not analyze small mammals as a biotic variable. A total of 4,791 ants were collected, representing nine genera: *Camponotous*, *Myrmica*, *Tapinoma*, *Lasius*, *Crematogaster*, *Formica*, *Monomorium*, *Pheidole*, and *Temnothorax* (Table 2). The GLM of total tick abundance and total ant abundance showed a significant relationship ($\beta = -0.554$; $P < 0.001$; $R^2 = 0.00531$; Figure 3) with a negative trend. I did not find a relationship for any life stages of *A. americanum* (larva, $\beta = -5.05$; $P = 0.0755$; $R^2 = 0.0106$; nymph $\beta = 1.50$; $P = 0.152$; $R^2 = 0.0069$; adult $\beta = 0.106$; $P = 0.210$; $R^2 = 0.0053$, respectively). I found a significant relationship between the following ant genera and tick abundance, *Camponotous* ($\beta = -0.509$; $P < 0.001$; $R^2 = 0.00279$), *Myrmica* ($\beta = -1.03$; $P < 0.001$; $R^2 = 0.0391$; Figure 4), *Tapinoma* ($\beta = -0.586$; $P < 0.001$; $R^2 = 0.0122$; Figure 5), *Crematogaster* ($\beta = -0.285$; $P< 0.001$; $R^2 = 0.0094$). No significant relationship of tick abundance and the following ant genera were found, *Lasius* ($\beta = 0.0971$; $P = 0.802$; $R^2 = 0.0037$), and *Monomorium* ($\beta = 0.0695$; $P = 0.419$; $R^2 = 0.0020$).

Table 1) Tick abundances collected at sampled counties in southwestern Missouri.

County	All Species	*A. americanum* Adults	*A. americanum* Nymph	*A. Americanum* Larva	*A. maculatum* Nymph	*D. variabilis* Adult
Taney	1682	101	1579	0	1	1
Christian A	665	18	646	0	0	1
Christian B	503	8	238	256	0	1
Douglas	1130	20	344	763	0	3
Dallas	83	0	39	42	1	1

Litter Depth. Litter depth did not have a statistically significant effect on total tick abundance ($\beta = -0.663$; $P = 0.213$; $R^2 = 0.004$; Figure 6) or with any life stage of *A. americanum* and litter depth (adult; $\beta = 0.010$; $P = 0.550$; $R^2 = -0.0004$, nymph; $\beta = -0.279$; $P = 0.471$; $R^2 = 0.007$, larva; $\beta = -0.389$; $P = 0.287$; $R^2 = -0.002$).

Canopy Cover. A statistically significant and negative effect was found between tick abundance and canopy cover ($\beta = -0.771$; $P < 0.001$; $R^2 = 0.0007$). Canopy cover had a significant and negative effect on adult and nymph *A. americanum* abundance (adult, $\beta = -2.83$; $P < 0.001$; $R^2 = 0.0095$; nymph, $\beta = -1.91$; $P < 0.001$; $R^2 = 0.003$). Canopy cover had a significant and positive effect on larva abundance ($\beta = 9.21$; $P < 0.001$; $R^2 = 0.0041$).

Table 2) Ant abundances collected at sampled counties in southwestern Missouri.

County	All genera	*Camponotous*	*Myrmica*	*Tapinoma*	*Lasius*	*Crematogaster*	*Formica*	*Monomorium*	*Pheidole*	*Temnothorax*
Taney	1815	24	106	1	0	1206	2	462	4	32
Christian A	531	187	53	14	39	211	2	24	0	1
Christian B	1168	177	428	15	5	911	18	0	0	0
Douglas	1086	120	18	740	34	156	4	14	0	0
Dallas	191	70	105	16	0	0	0	0	0	0

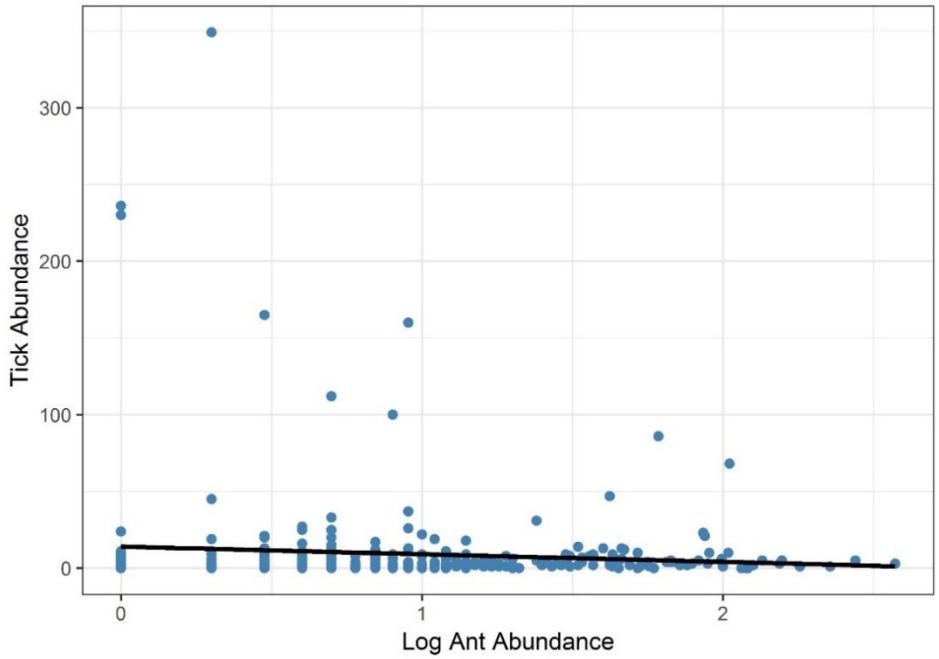

Figure 3) Tick abundance plotted as a function of log ant abundance ($\beta = -0.554$; $P < 0.001$; $R^2 = 0.00531$).

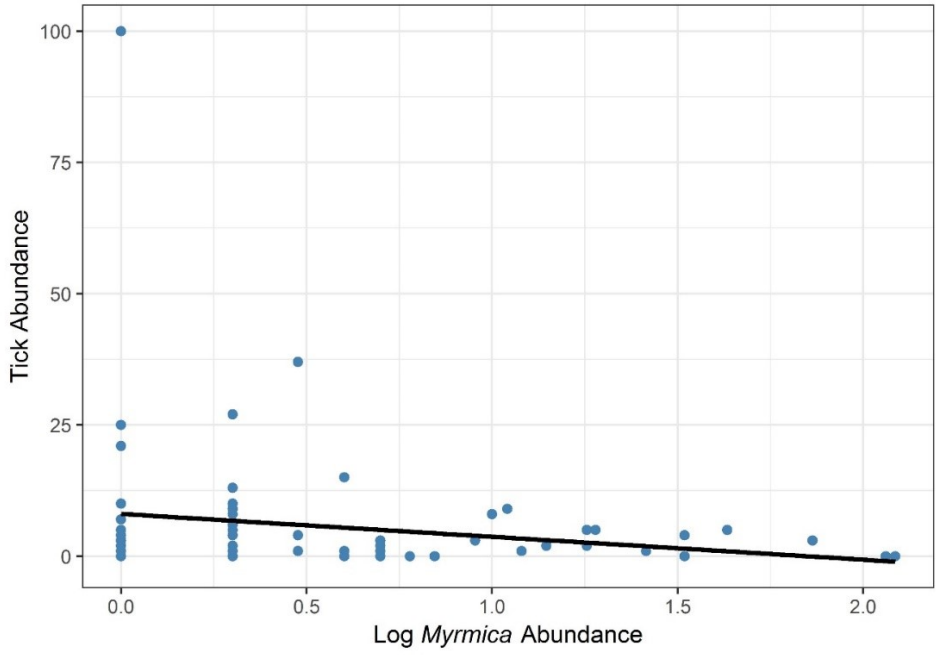

Figure 4) Tick abundance plotted as a function of log ant genera *Myrmica* spp. abundance ($\beta = -1.03$; $P < 0.001$; $R^2 = 0.0391$).

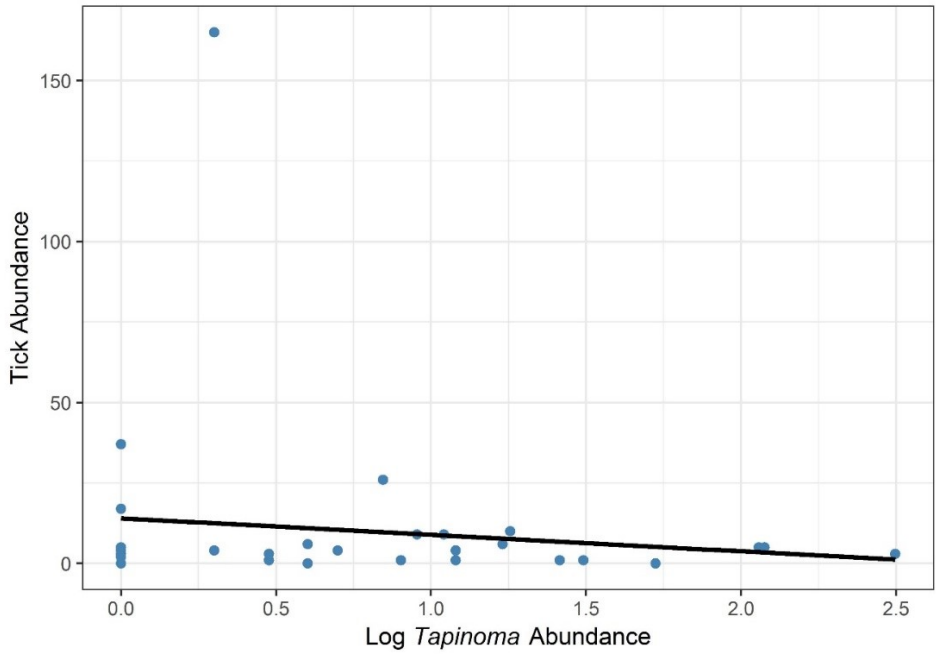

Figure 5) Tick abundance plotted as a function of log ant genera *Tapinoma* spp. abundance (β = -0.586; $P < 0.001$; $R^2 = 0.0122$).

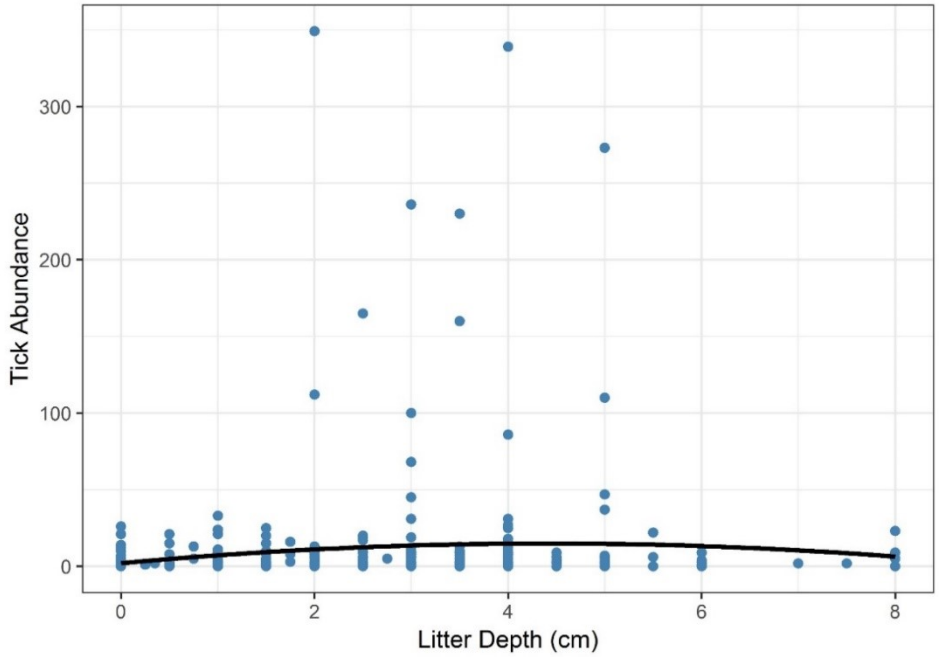

Figure 6) Total tick abundance plotted as a function of litter depth (β = -0.663; $P = 0.213$; $R^2 = 0.004$).

Abiotic Variables. The abiotic, environmental variables, including relative humidity and ambient temperature, were analyzed with respect to the total tick abundance and the three life stages of *A. americanum*.

Relative Humidity. There was no significant relationship between total tick abundance and relative humidity ($\beta = -224.24$; $P = 0.0367$; $R^2 = 0.0367$; Figure 7). The abundance of adult and nymph *A. americanum* had a significant and negative relationship with relative humidity (adult; $\beta = -48.79$; $P < 0.0488$; $R^2 = 0.0209$; Figure 8). The nymph and larva life stage of *A. americanum* and relative humidity showed no statistically significant relationship (nymph; $\beta = -101.92$; $P = 0.188$; $R^2 = 0.0421$, larva; $\beta = -120.51$; $P = 0.105$; $R^2 = 0.0019$).

Ambient Temperature. There was not a significant relationship between tick abundance and ambient temperature ($\beta = -0.2602$; $P = 0.0636$; $R^2 = 0.0235$; Figure 9). For all life-stages of *A. americanum* there was not a detectable relationship with ambient temperature (adult, $\beta = -0.0019$; $P = 0.655$; $R^2 = 0.066$; nymph, $\beta = -0.119$, $P = 0.243$; $R^2 = 0.0249$; Figure 10; larva, $\beta = -0.139$; $P = 0.152$; $R^2 = 0.0005$).

Abiotic and Biotic Variables. Then GLM assessing the relationships between biotic and abiotic variables on tick density resulted in direct and indirect relationships, represented by interaction terms ($R^2 = 0.498$; Table 3). The best-supported multivariate model included ant abundance, relative humidity, canopy cover, ambient temperature, litter depth and interactions with ant abundance and canopy cover, ant abundance and relative humidity, and canopy cover and relative humidity. All direct relationships resulted in a positive effect on tick abundance; canopy cover ($\beta = 23.4$), relative humidity ($\beta = 21.4$) and ambient temperature ($\beta = 5.20$) with a negative interaction between canopy and relative humidity ($\beta = -15.7$; Table 3). Ant abundance resulted in a positive effect on tick abundance ($\beta = 7.60$) while the interactions between ant and

canopy (β = -4.96) and between ant and relative humidity (β = -0.715) negatively affected tick abundance (Table 3).

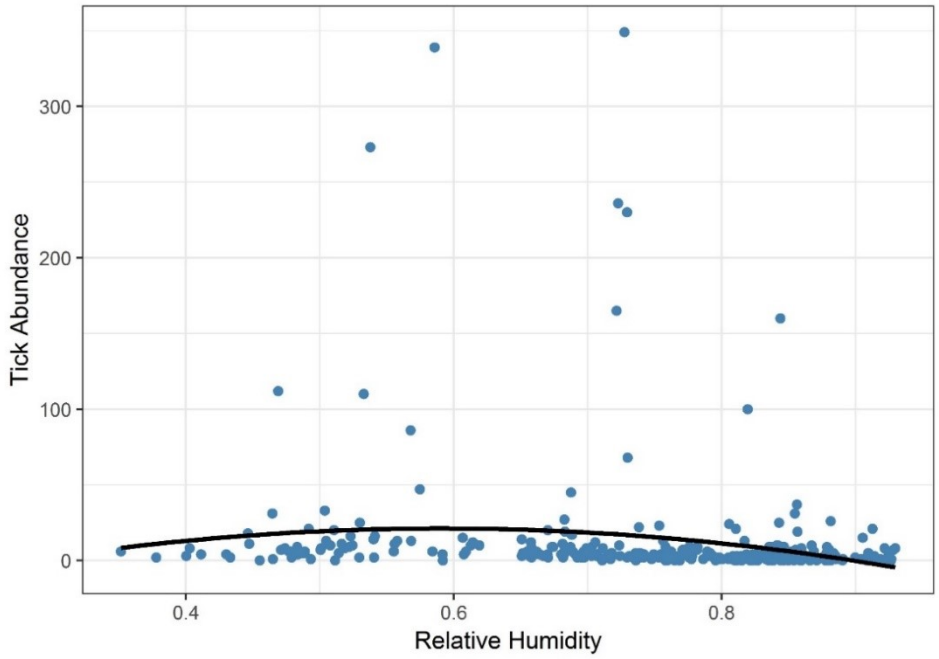

Figure 7) Tick abundance plotted as a function of relative humidity (β = -224.24; P = 0.0367; R^2 = 0.0367).

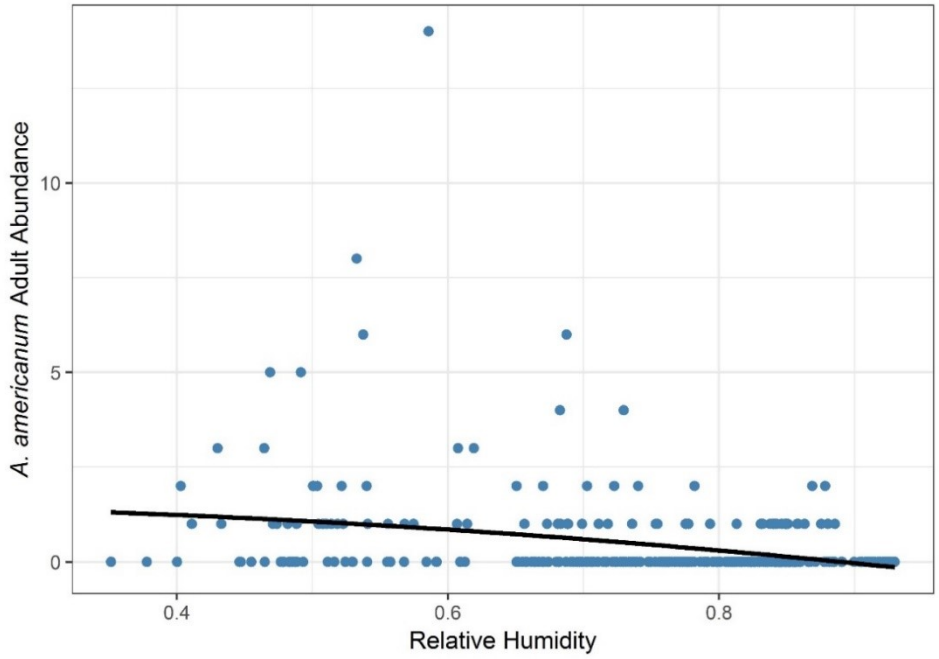

Figure 8) Adult *A. americanum* abundance plotted as a function of relative humidity (β = -48.79; P < 0.0488; R^2 = 0.0209).

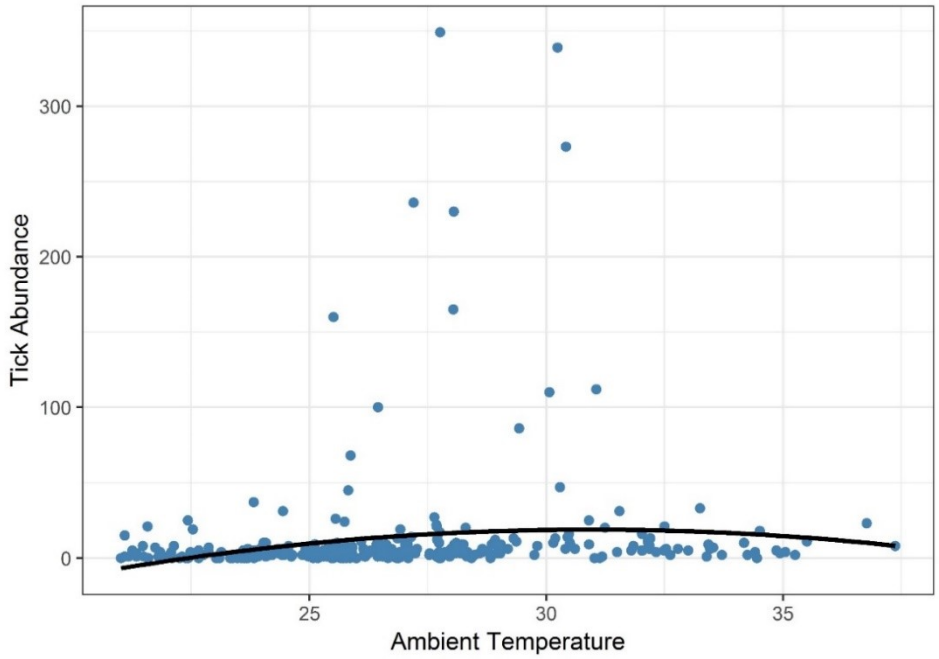

Figure 9) Tick abundance plotted as a function of ambient temperature (β = -0.2602; *P* = 0.0636; R^2 = 0.0235).

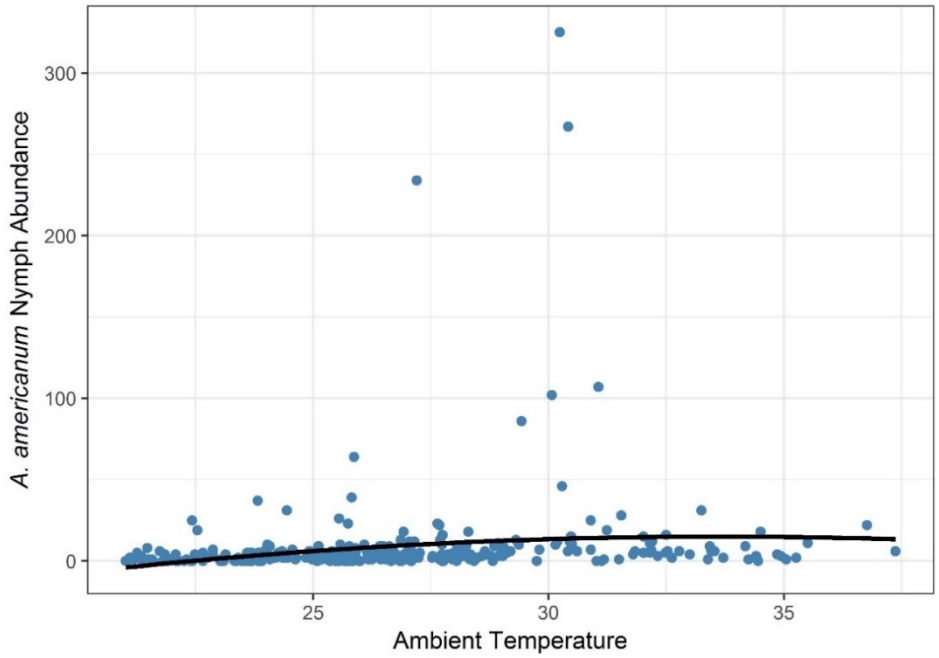

Figure 10) Nymph *A. americanum* abundance plotted as a function of ambient temperature (β = -0.119, *P* = 0.243; R^2 = 0.0249).

Table 3) AIC table of GLM analysis with abundances, log transformed litter depth, log transformed ambient temperature, and arcsine squared relative humidity and canopy cover.

Ant	Canopy	Litter	RH	Temp.	Ant*Canopy	Ant*RH	Canopy*RH	df	logLik	AICc	ΔAICc
7.60	23.4	NA	21.4	5.20	-4.96	-0.715	-15.7	8	-4042.8	8102.1	0
7.61	23.4	0.003	21.4	5.20	-4.96	-0.715	-15.7	9	-4042.8	8104.2	2.118
7.37	22.7	NA	19.2	4.68	-5.27	NA	-14.7	7	-4046.7	8107.8	5.645

The model then used in the linear mixed effect model determined the fixed effects of relative humidity, litter depth, ambient temperature, canopy cover, with interactions of ant abundance and canopy, interactions of ant abundance and relative humidity, and interactions of canopy and relative humidity with the random effect of location, which best explains the relationship between tick abundances and ant abundances ($R^2_{GLMM\ (m)} = 0.335$; $R^2_{GLMM\ (c)} = 0.898$; Figure 11). At Christian A and Dallas sites, ant abundance had a negative effect on tick abundance while Christian B, Douglas, and Taney sites demonstrated ant abundance had a positive effect on tick abundance. The ANOVA to compare the top model with the null model provides that location significantly affects tick abundance ($\chi^2 = 986.58$; $P < 0.001$).

When comparing the effect of location on the relationship between tick abundance and ant abundance with abiotic fixed effect, variables are removed from the linear mixed effect model. The effect of location increased for Douglas and Dallas counties (Figure 12). Taney, Christian A, and Christian B sites showed a reduction in location effect without the fixed abiotic variables ($R^2_{GLMM\ (c)} = 0.915$; Figure 12).

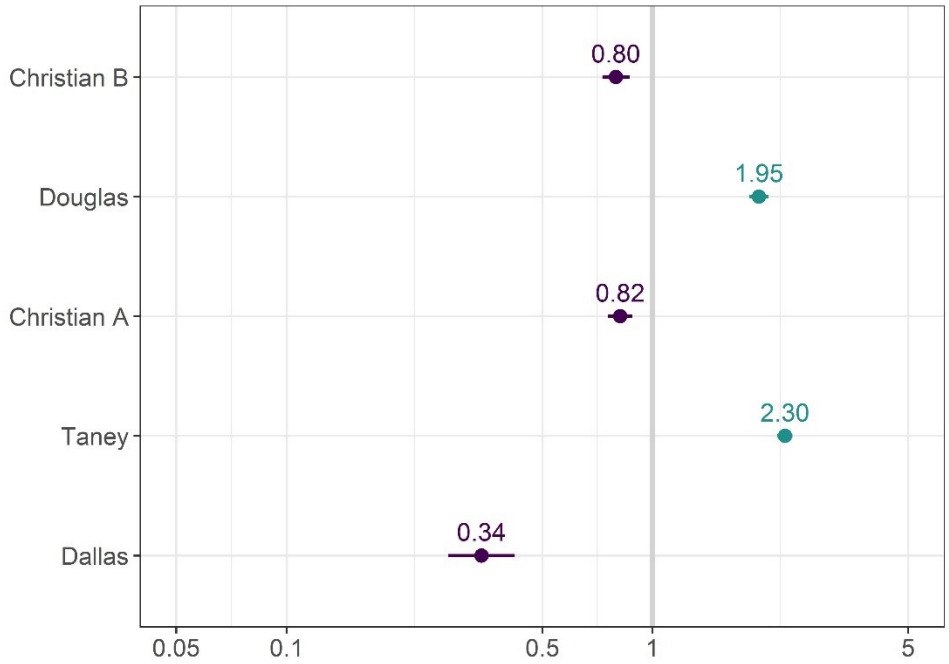

Figure 11) Random effect of location between tick abundances and ant abundances with fixed abiotic effects (χ^2 = 989.58; $P < 0.001$; $R^2_{GLMM\ (m)}$ = 0.335; $R^2_{GLMM\ (c)}$ = 0.898).

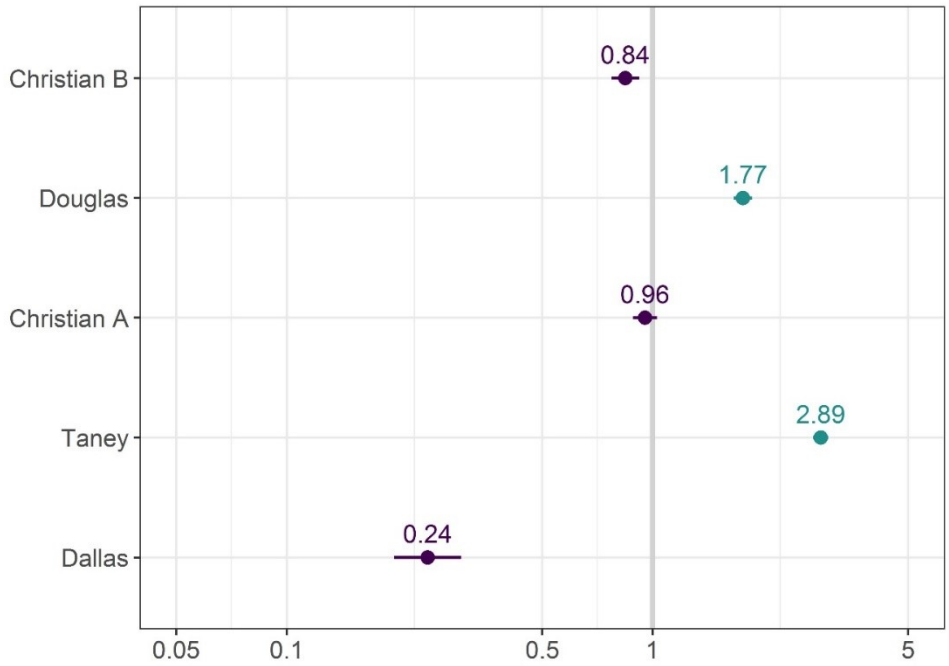

Figure 12) Random effect of location between tick abundances and ant abundances without fixed abiotic effects ($R^2_{GLMM\ (c)}$ = 0.915).

Discussion

Similar to the native wood ants in Europe, this study shows Missouri native ant populations have an effect on tick abundance [10]. There is a negative effect of ant abundance on tick abundance, as predicted in figure one, but the direct effect explains little variation in the data. Ant genera *Myrmica* and *Tapinoma* display a negative effect on tick abundance while explaining a reasonable amount of variation in the data, these two genera are the dominant genera in Douglas County and Dallas County. The lack of explanation from the direct ant abundance on tick abundance may be due to the effect of location found in the linear mixed effect model.

The linear mixed effect model determined there was an effect of location on ant abundance impacting tick abundance, whether the effect is positive or negative independent of location (Figure 13). From the models, we can infer there is a series of interactions creating a dynamic network of variables influencing tick abundance. At two locations, Christian A and Dallas counties, ant abundance has a negative impact on the tick abundance, whereas, at the remaining sites, Christian A, Douglas, and Taney, ant abundance had a positive effect on tick abundance (Figure 11). When fixed abiotic effects are removed from the model the random effects shift (Figure 12), this shift was thought to be due to sampling seasons, but sites were sampled within days of each other so effect of location is not likely the result of sampling date. Ant populations may be the source of location affecting tick abundance.

Dominant ant species may influence the effect of locations; Christians A, Christian B, and Taney counties all experienced a reduction of location effect while having the same most abundant ant genus, *Crematogaster* (Table 2). *Crematogaster* species are known to be aggressive and often predate larger organisms through group cooperation [32]. The presence or absence of

these more dominant species may cause the effect of location to decrease with *Crematogaster* ants present because they have a greater effect on tick abundance than location itself. With certain ant species in greater abundance, the abundance of ticks could decrease, thus causing the risk of tick-borne disease to decrease.

As biotic variables, small mammals were predicted to have a positive effect on tick abundance (Figure 1), but for this study, small mammal abundance could not be analyzed for its effect on tick abundance. Missouri has been experiencing a decline in small mammal occupancy over the last few years yet the tick abundance is still high. The stable abundance of ticks may be due to other host availability regardless of the decreased small mammal abundance. Southwestern Missouri has a high white-tail deer population, with more than 40,000 deer harvested in 2015–2016, which may be facilitating the tick populations [33]. Investigating other possible hosts that could be influencing tick abundance would be beneficial to understanding the host dependency of ticks (Figure 13).

Relative humidity exhibited a negative relationship with tick abundance and all life stages for *A. americanum.* Relative humidity could serve as a strong predictor variable for adult and nymph *A. americanum*. This result likely is due to the predicted behavioral and physiological factors that, relative humidity can have on ticks (Figure 13). Additionally, there was a consistent positive relationship across total abundance and *A. americanum* life stages with ambient temperature as predicted. Ambient temperature is the best at predicting adult and nymph life stages of *A. americanum*. The direct relationships between abiotic variables, litter and canopy cover, show significance yet does minimal in explaining variation in the data. I believe significance was found in the GLMs of the direct relationships because if the large sample size weighting the standard error in the regression. The GLM yields a negative relationship between

tick abundance and canopy cover such that the increase of canopy cover causes a decrease in adult and nymph *A. americanum* life stages, yet an increase in larva. The increase of canopy cover likely would increase the depth of litter because sites that were mature forest resulted in greater leaf litter. The depth of forest floor litter had a positive impact on tick abundances, yet the significant GLM of the nymph life stage displayed a unimodal distribution with the peak around three centimeters of litter depth (Figure 13). An appropriate accumulation of litter depth might be ideal for ticks due to need for protection from predators or desiccation, yet an overabundance of litter can decrease their ability to move and participate in questing behavior.

Evaluating all environmental factors together with interactions represented the variation in the data most clearly and definitively. There is more complexity within the system of variables impacting tick abundance than just the variables direct impact (Figure 13). This relationship between location and ant abundance with their impacts on tick abundance explains the relationship more than the direct biotic and abiotic effects. Interacting variables have more influence, positive or negative, on tick abundance and probably represent the natural structure of the system to a greater degree. Obtaining a better understanding of the current ant and tick community dynamics in southwestern Missouri is beneficial because with a changing climate, habitat structure, and the predicted invasion of red fire ants in Missouri the dynamics will be shifting rapidly [34]. These altered communities could have an impact on tick species presence and abundance that are common vectors for zoonotic diseases.

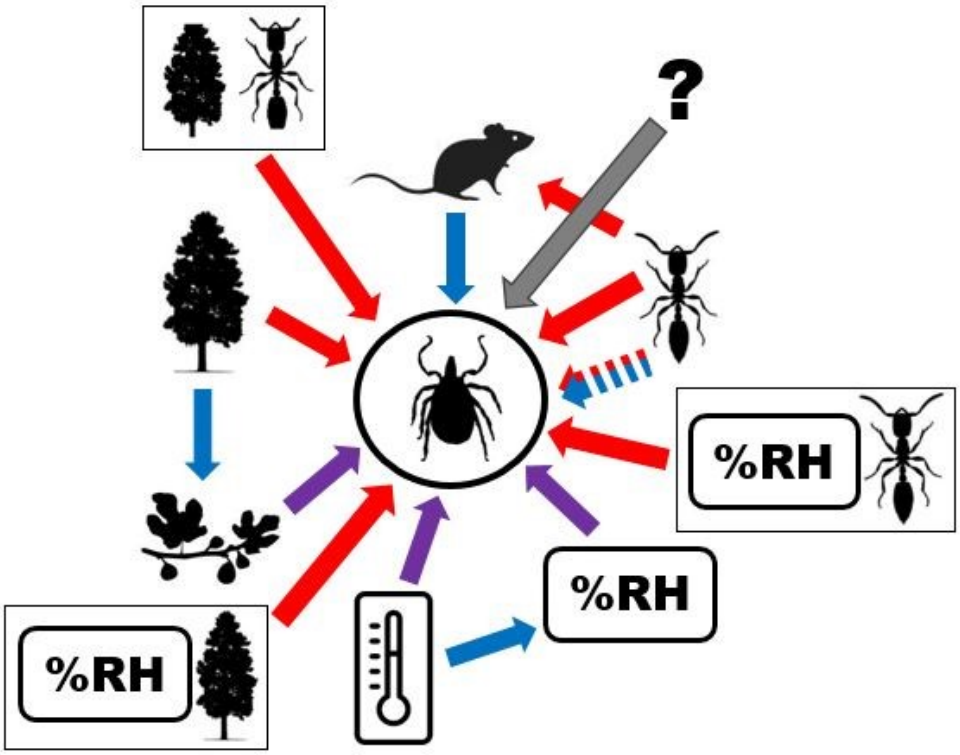

Figure 13) A conceptual figure to display the positive and negative interactions between biotic, abiotic, and tick abundances. Each image represents one of the variables measured, reading clockwise from the top; mouse (small mammal abundance), ant (ant abundance), %RH (relative humidity), thermometer (ambient temperature), tree branch (litter depth), and tree (canopy cover). The arrows represent the effect one variable has on tick abundance or another variable within the community, blue arrows represent a positive interaction, red arrows represent a negative interaction, and purple arrows represent unimodal interaction. The gray arrow represents the unmeasured variables that also impact tick abundance. The dashed arrow represents the potential random effect of location, a positive or negative interaction dependent on the sampling location.

In 1945, the Ozarks of Missouri was known to have a high abundance of *A. americanum* [35], and in this study, it was by far the most abundant species. With *A. americanum* as the most dominant species in southwestern Missouri, the public health risk of Lyme disease is low because *A. americanum* is unlikely to serve as a vector for the Lyme disease bacteria (*B. burgdorferi*). However, it is competent for a similar zoonotic spirochete, which is thought to be, *B. lonestari*, which has the infectious prevalence of 5.6% in southeast Missouri and associated disease may be more of a public health concern for southwest Missouri than Lyme disease [36].

A. americanum is also thought to be a carrier of the galactose-alpha-1,3-galactose (alpha-gal) sugar that can trigger an immune response in humans to a protein found in red meat, which is known as alpha-gal syndrome [37,38]. Alpha-gal syndrome has been diagnosed in Missouri, while the highest prevalence rate in ticks of ~46% has been found in the neighboring states Arkansas and Tennessee [38].

As the threat of tick-borne illness increases due to numerous environmental factors, data regarding tick abundance, distribution, and species richness are needed for public health programs and management decisions [17,39,40]. Biotic and abiotic variables can be used as predictors for presence, absence, or abundance of zoonotic disease vectors. Known predictors will expand the understanding of influencing factors for vector populations and their invasions into novel geographic regions [16,18]. The challenge is deriving and measuring variables that can reliably represent such biotic effects, like ant abundance, across broad mosaics.

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EVALUATION OF SAMPLING METHODOLOGY ON TICK ABUNDANCE AND DIVERSITY

Introduction

As the climate changes and habitats become more fragmented, scientists are called to develop predictive distributional maps for taxa [1,2]. For species of public health concern, such as ticks, these estimates are important tools for management and risk-estimate development [1,3]. The species distribution models use a combination of occurrence data and environmental predictors, and both must be reliable to yield estimates that are precise [4,5].

For ticks, publicly available datasets yield sparse data in some regions [5,6] and investigations require substantial field work to address this uncertainty. Two widely accepted collection methods drags and traps, are used to target specific local tick species and life stages, but using one sampling methods could result in lower detection rates of novel species and this can lead to bias occurrence records [7,8]. The dragging method consists of flannel fabric secured to a wooden dowel rod being pulled through or on top of vegetation or ground to collect questing ticks. The fabric mimics a host passing through the vegetation and allows the tick to grasp it. The carbon dioxide trap method utilizes the carbon dioxide released by dry-ice to attract ticks to a specific area for collection [9].

Previous research has shown that generally, adults are more likely to be trapped in carbon dioxide traps than drags while nymphs are more likely to be collected in drags than a carbon dioxide traps [8]. The common Missouri species*, A. americanum*, primarily inhabits forest and is most active in April–June. Adult *A. americanum* have been the most abundantly collected in forested habitat by a carbon dioxide trap [10,11]. *A. americanum* nymphs are more commonly

found in forest habitat by carbon dioxide traps, and little knowledge of collection efficiency for the larva life stage [10,11]. Thus, research in determining sampling bias and effective methodology for a variety of tick species, life stages, and different habitat types are necessary.

The goal of this study was to help determine habitats and methodology that will reduce sampling bias in Missouri tick species, so an accurate population assessment can be completed within the rapidly changing environment. This knowledge will help public health risk decisions made on local education and outreach for tick-borne diseases [2]. I expected forest habitats to have the greatest total abundance and the greatest abundance of adults compared to the grassland habitats. Carbon dioxide traps are expected to collect the most adult ticks and drags are expected to collect the most nymph and larval ticks in both habitat types. I predicted for there to be a relationship between sampling method efficiency and habitat type.

Methods

Tick collection occurred in five counties in south-central Missouri: Taney, Ozark, Christian, Greene, and Barry in May 2018 (Figure 14). Within each county, two habitats were selected and sampled, forest and grassland, and the two methods of tick collection were implemented, carbon dioxide baiting and dragging. Approval for this project was obtained from the Missouri State University Institutional Animal Care and Use Committee (IACUC) and Missouri Department of Conservation (MDC) prior to collection (IACUC, ID #18-029.0; MDC #17723).

The carbon dioxide traps were made of plastic food containers with puncture holes added to the sides and top of the container to allow the carbon dioxide to escape and dissipate into the environment. Inside the food container, I placed ~0.5k of dry ice, then the container sat at the

center of a 1m^2 white flannel on the ground [9]. Carbon dioxide traps were placed at each site for two hours in the afternoon and the trapped ticks were then collected off the flannel cloth and preserved in ethanol.

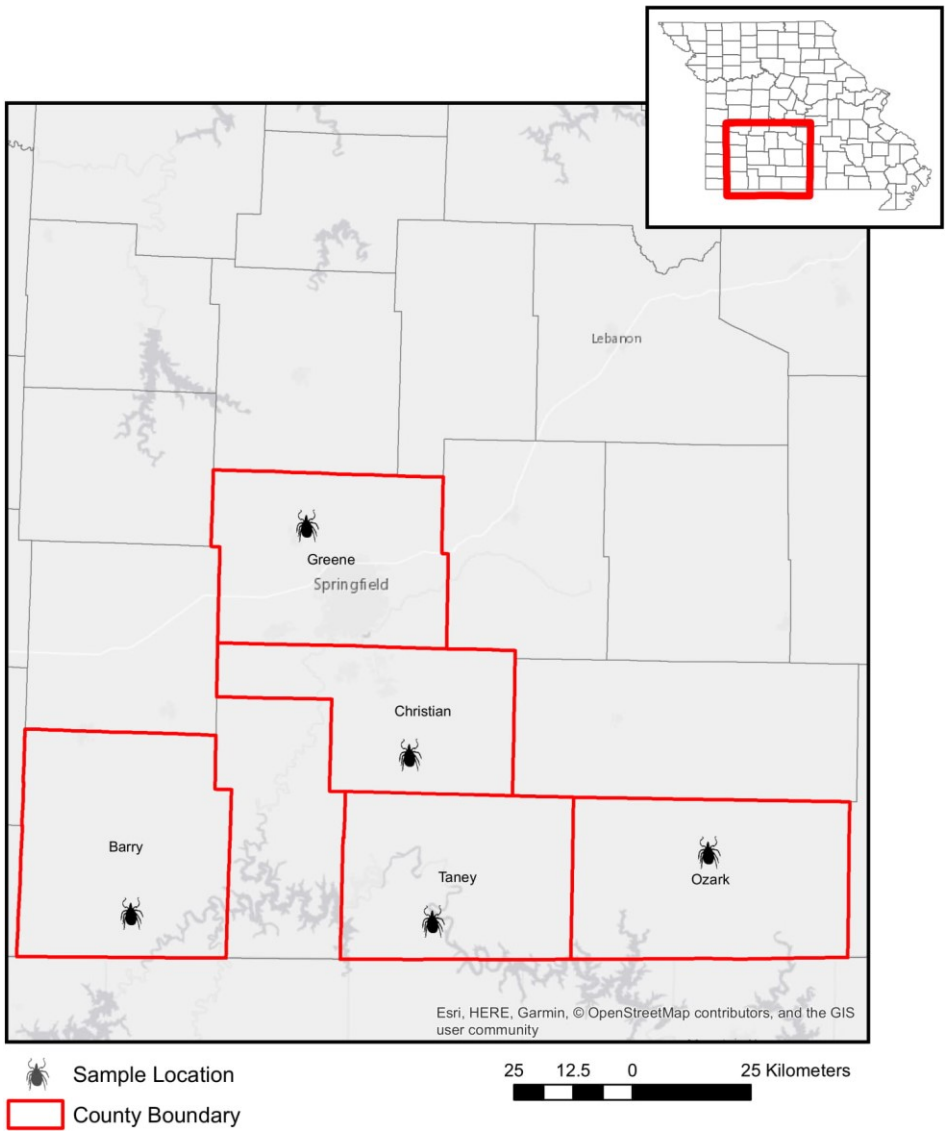

Figure 14) Map of sampling sites in southwestern Missouri.

The drag sampling technique consisted of four 100m transects, each following a cardinal direction, with a 1m^2 white flannel fabric attached to a wooden rod to help maintain an even spread of the fabric [8,12]. To ensure no ticks were lost throughout the drag, I stopped every 20m of the 100m to collect ticks from the drag and preserved them in ethanol.

In the lab, ticks from both sampling methods and habitat types are identified to species and life stage. To determine the possible difference in abundance between sampling technique in a forested habitat or grassland, I used a Wilcoxon Signed Rank. Due to sample size limitations amongst species, only *A. americanum* life stages were analyzed individually.

Results

I collected a total of 2,734 individuals from the five counties. In the forest habitat, a total of 2,094 were collected, 1,108 from a carbon dioxide trap and 986 from a drag. In the grassland habitat a total of 640 individuals were collected, 260 from a carbon dioxide trap and 380 from a drag. Taney County had the greatest total abundance in the forest habitat, while Ozark County had the greatest total abundance in the grassland habitat (Table 4). Barry County had the lowest total tick abundance for both habitat types (Table 4).

I detected three species: *D. variabilis*, *I. scapularis*, and *A. americanum*. A total of seven *D. variabilis* adults were collected within both habitat types and only in Greene County (Table 4). Within the forested site, five were collected, three by drags and two by carbon dioxide traps (Table 4). In the grassland habitat, two individuals were collected, one by each sampling method (Table 4). One adult *I. scapularis* was collected in the forest habitat in Christian County by drag and another adult was collected by carbon dioxide trap in grassland habitat in Christian County (Table 4). In the forest habitat, two nymph *I. scapularis* were collected with both sampling techniques and one *I. scapularis* nymph was collected by drag in grassland habitat (Table 4). The most abundant species for both habitat types and all life stages was *A. americanum* with nymphs being the most commonly collected life stage of the species (Table 4).

Table 4) Tick abundances in forested or grassland habitat types with drag or carbon dioxide collection method in all counties in southwestern Missouri.

		A. americanum			*D. variabilis*	*I. scapularis*	
County	All Species	Adults	Nymph	Larva	Adult	Adult	Nymph
Forest							
Taney	1457	158	1299	0	0	0	0
Drag	472	20	452	0	0	0	0
CO_2	985	138	847	0	0	0	0
Ozark	145	31	102	12	0	0	0
Drag	128	20	96	12	0	0	0
CO_2	17	11	6	0	0	0	0
Christian	146	20	125	0	0	1	0
Drag	101	10	91	0	0	0	0
CO_2	45	10	34	0	0	1	0
Greene	281	67	200	7	5	0	2
Drag	231	47	174	6	3	0	1
CO_2	50	20	26	1	2	0	1
Barry	65	8	57	0	0	0	0
Drag	54	5	49	0	0	0	0
CO_2	11	3	8	0	0	0	0
Grassland							
Taney	47	20	18	8	0	0	1
Drag	34	11	14	8	0	0	1
CO_2	13	9	4	0	0	0	0
Ozark	403	115	288	0	0	0	0
Drag	210	21	189	0	0	0	0
CO2	193	94	99	0	0	0	0
Christian	87	33	54	0	0	0	0
Drag	73	22	51	0	0	0	0
CO2	14	11	3	0	0	0	0
Greene	84	22	59	0	2	1	0
Drag	47	11	25	0	1	1	0
CO2	37	11	34	0	1	0	0
Barry	19	7	12	0	0	0	0
Drag	16	5	11	0	0	0	0
CO2	3	2	1	0	0	0	0

There was significance between sampling method and nymphs but no significant difference in habitat or sampling method on *A. americanum* adult and larva life stages (Table 5). More *A. americanum* nymphs were collected by the drag method regardless of habitat type. Although there was no significant difference between habitat and sampling method for *A.*

americanum, carbon dioxide traps collected more individuals than drags (Figure 15). Nymph life stages of *A. americanum* were most commonly collected in forest habitats than grassland regardless of sampling technique, yet a similar abundance is collected in grassland habitats between methods (Figure 16). The larval *A. americanum* was the least abundant of all life stages, the greatest abundance was collected in a forested habitat with a dragging method (Figure 17).

Table 5) A Wilcoxon Signed Rank test comparing habitat type and sampling method on the abundance of *A. americanum* at different life stages.

	A. americanum		
	Adult	Nymph	Larva
Habitat	$P = 0.819$ W = 53.5	$P = 0.131$ W = 70.5	$P = 0.331$ W = 59.5
Sampling Method	$P = 0.5931$ W = 42.5	$P = 0.049$ W = 23.5	$P = 0.234$ W = 38.5

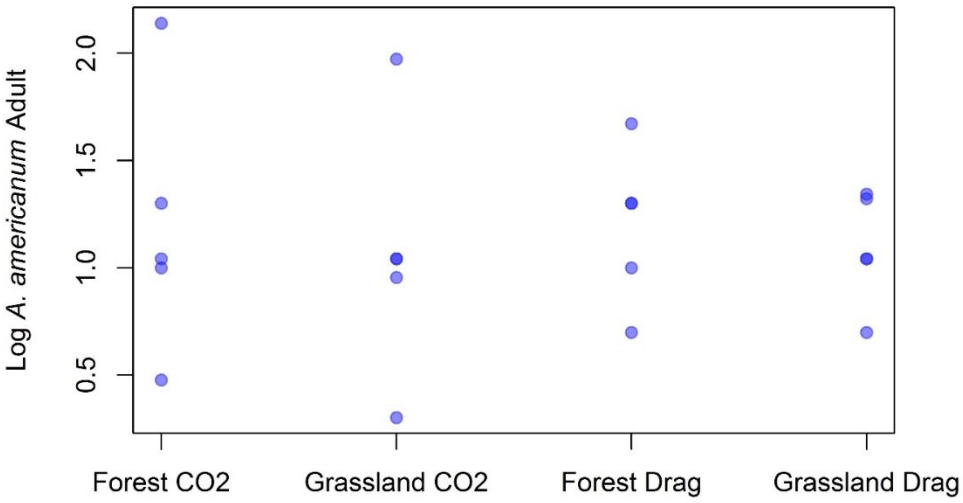

Figure 15) Log transformed *A. americanum* adult abundance at different habitat type by drag or carbon dioxide sampling.

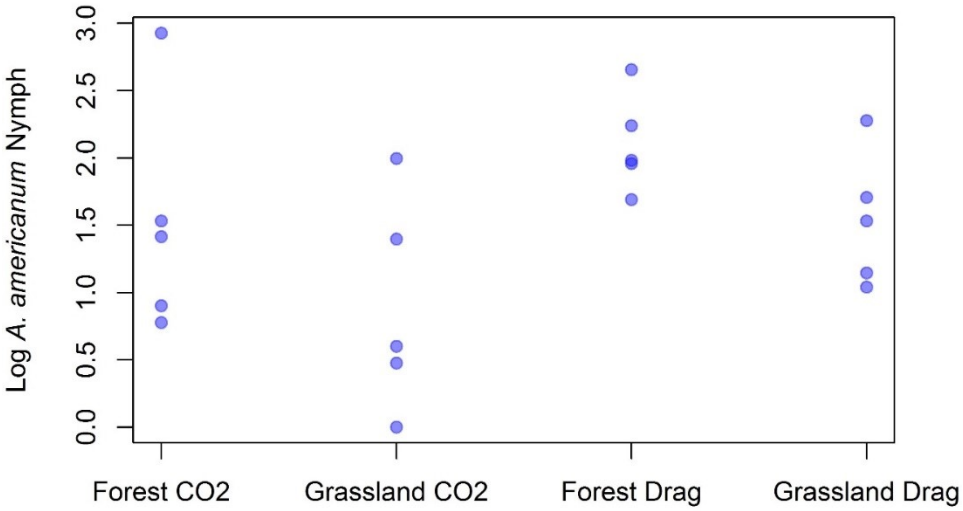

Figure 16) Log transformed *A. americanum* nymph abundance at different habitat type by drag or carbon dioxide sampling.

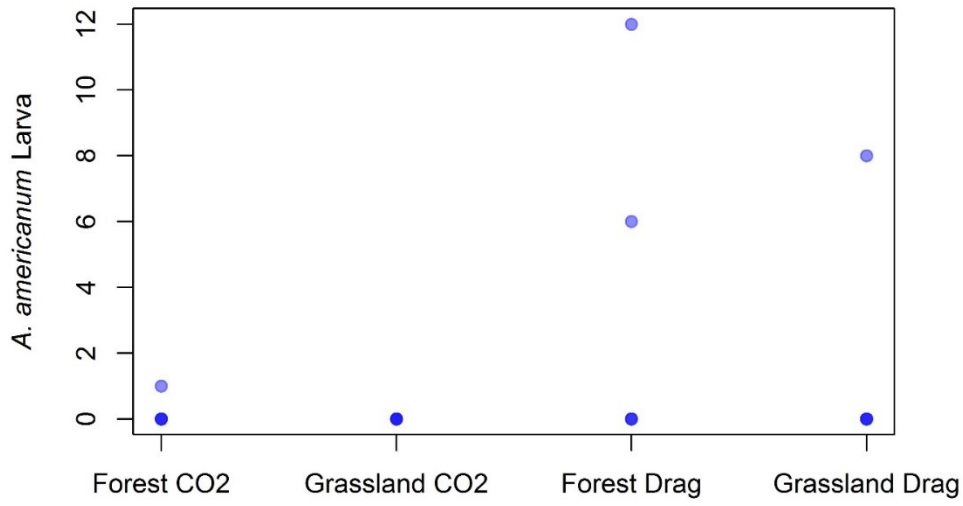

Figure 17) *A. americanum* larval abundance at different habitat type by drag or carbon dioxide sampling.

Discussion

As expected, tick abundance was highest in forest habitat regardless of sampling technique which may be due to microenvironmental differences that facilitate growth, as well as

available hosts. Overall, carbon dioxide traps were more efficient at collecting ticks in a forest than drags. Carbon dioxide traps were predicted to collect more adults than drags, which was found in the raw abundances, but nymphs were collected more commonly in a carbon dioxide trap than a drag method and this may be due to that adult and nymph ticks are more mobile and at less of a risk of desiccation than larva [13]. In grasslands, the drag sampling technique had the greatest collection abundance for *A. americanum* nymph and *A. americanum* larva. This result may be due to grassland sites having lower litter depths compared to forested sites which may allow for more questing behavior in the less mobile life stages, meaning they are more likely to be collected by a drag [9,14].

With the need for the monitoring of tick distribution shifts due to climate change, habitat fragmentation, and loss of biodiversity, unbiased sampling techniques and predictive modeling accuracy are needed in Missouri. Currently, tick species may be moving into novel regions of Missouri and appropriate tick surveys are needed to understand the movement of these populations. For instance, the currently accepted distribution for *A. maculatum* is along the southern border of Missouri but with a changing climate and habitat structure, this species could move into novel regions of the state. A similar situation is occurring with *I. scapularis*, the vector for Lyme disease, which has a native range of the eastern and northeastern portion of the U.S. but has been spreading westward. Its current accepted distribution is across the state of Missouri [15,16], yet this study shows the abundance of *I. scapularis* is very low. Regional surveys are necessary to gather an understanding of locally abundant species and their distributions. These regional surveys can then be used to create predictive distribution models at the regional level and beyond to monitor the movement of ticks.

In conclusion, there was no significant difference between habitat types or sampling methods impacting ticks collected abundance, but there are marginal differences in collections. Using both forms of sampling methods should increase the species diversity in the collections, and the research will gain a better understanding if the tick community in either habitat type. Additionally, a two method collection will help reduce sampling bias when looking for a species within a novel region. With the reduction of sampling bias and the standardization of tick sampling methods, collections can be used in population and community comparisons [8]. Additionally, unbiased sampling occurrence records are beneficial for predictive distribution modeling, so human health risk for diseases ticks serve as a vector for can be predicted and evaluated [15,17].

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SUMMARY

The results in chapter one demonstrate that only comparing direct relationships between ticks and biotic or abiotic variables is not the most effective method in explaining the system. This is because the interactions between abiotic and biotic variables explain the dynamic relationships between tick abundance and its environment. Biotic and abiotic factors do have influence with respect to location, and ant abundance and tick abundance are highly influenced by the environments at the sampling sites, and especially the dominant ant genera present. Having a clear understanding of biotic and abiotic interactions that influence tick abundance will allow modelers to select appropriate variables that will yield more accurate estimates of current tick populations and how they may be moving through space.

Testing the efficiency of sampling methods in chapter two reveals the importance of implementing two methods of sampling to gain a full understanding of tick communities in a region. Drag sampling method resulted in the greatest number of *A. americanum* larva in both forest and grassland habitats. In the grassland habitat, dragging collected the greatest number of individuals from all species and the greatest number of *A. americanum* nymphs. Carbon-dioxide sampling collected the greatest number of all species and the greatest number of *A. americanum* adults and nymphs in the forest habitat. In the grassland habitat, carbon-dioxide sampling collected the greatest number of adult *A. americanum*. These results demonstrate the importance of using two sampling methods or using a method that is most efficient in the habitat being sampled.

With the increasing risk of tick-borne disease for humans, it is important to have a clear understanding of the tick populations in local regions so public health efforts are utilized correctly [24]. Local tick surveys should use multiple sampling methods for detection of novel

tick species and measure appropriate biotic and abiotic variables. These efforts will allow for predictive modeling of tick distributions and human disease risk to become more accurate. Insight of the biotic and abiotic factors and efficiency of sampling methods influence the perception of this dynamic system which is necessary to understanding the movement and emergence of ticks and their pathogens in novel regions. This insight will strengthen public health programs to educate people on tick-borne disease risk so prevention, diagnosis, and treatment of these diseases become more effective in reducing human illness.

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APPENDIX

Part A) Relative humidity (RH) correlation values of the three measurements at the 0.0m (ground), 0.5m, and 1.0m.

	RH 0.0m	RH 0.5m	RH 1.0m
RH 0.0m	1.00	0.985	0.966
RH 0.5m	0.985	1.00	0.965
RH 1.0m	0.966	0.965	1.00

Part B) Ambient temperature (Temp.) correlation values of the three measurements at the 0.0m (ground), 0.5m, and 1.0m.

	Temp. 0.0m	Temp. 0.5m	Temp. 1.0m
Temp. 0.0m	1.00	0.927	0.974
Temp. 0.5m	0.927	1.00	0.919
Temp. 1.0m	0.974	0.919	1.00

Part C) Means and data range (from low to high) of relative humidity (RH) per the three measurements at the 0.0m (ground), 0.5m, and 1.0m from each site within the counties collected.

	RH 0.0m		RH 0.5m		RH 1.0m	
	Mean	Range (low-high)	Mean	Range (low-high)	Mean	Range (low-high)
Taney						
Site A	0.594	0.374-0.759	0.579	0.352-0.712	0.578	0.378-0.729
Site B	0.532	0.427-0.615	0.522	0.422-0.610	0.515	0.400-0.614
Christian A						
Site A	0.827	0.748-0.858	0.825	0.720-0.877	0.821	0.739-0.857
Site B	0.704	0.637- 0.749	0.697	0.603-0.746	0.696	0.613-0.749
Christian B						
Site A	0.847	0.754-0.867	0.845	0.752-0.868	0.834	0.352-0.866
Site B	0.819	0.737-0.854	0.818	0.751-0.859	0.816	0.742-0.857
Dallas						
Site A	0.911	0.864-0.974	0.909	0.847-0.930	0.901	0.811-0.930
Douglas						
Site A	0.762	0.674-0.919	0.748	0.396-0.793	0.754	0.658-0.813
Site B	0.855	0.766-0.889	0.854	0.788-0.894	0.854	0.799-0.886

Part D) Means and data range (from low to high) of ambient temperature per the three measurements at the 0.0m (ground), 0.5m, and 1.0m from each site within the counties collected.

	Temperature 0.0m (˚C)		Temperature 0.5m (˚C)		Temperature 1.0m (˚C)	
	Mean	Range (low-high)	Mean	Range (low-high)	Mean	Range (low-high)
Taney						
Site A	29.80	25.25-38.19	29.74	25.40-36.83	29.49	25.50-35.26
Site B	32.21	28.91-37.77	32.01	28.90-35.78	32.09	28.92-37.37
Christian A						
Site A	24.34	22.44-27.13	24.83	22.45-22.55	24.23	22.08-27.02
Site B	28.11	26.61-30.79	27.97	26.37-30.18	27.92	26.33-30.5
Christian B						
Site A	24.38	23.40-25.71	24.43	23.49-25.73	24.43	22.87-25.80
Site B	26.28	24.93-28.59	26.44	25.01-29.93	26.40	25.08-28.82
Dallas						
Site A	22.53	21.22-25.81	22.48	20.40-25.86	22.56	21.02-26.00
Douglas						
Site A	26.543	24.87-29.03	26.58	24.88-29.04	26.85	24.92-36.77
Site B	25.746	23.26-28.13	25.64	23.28-28.25	25.78	23.33-28.33

Part E) Means and data range (from low to high) of litter depth and canopy cover from each site within the counties collected.

	Litter Depth (cm)		Canopy Cover	
	Mean	Range (low-high)	Mean	Range (low-high)
Taney				
Site A	1.87	0.00-5.00	0.883	0.636-0.987
Site B	3.03	0.50-6.00	0.967	0.886-1.00
Christian A				
Site A	3.35	0.50-7.50	0.991	0.949-1.00
Site B	3.40	1.00-8.00	0.995	0.982-1.00
Christian B				
Site A	3.25	1.00-7.00	1.00	0.92-1.00
Site B	3.71	1.50-8.00	0.99	0.92-1.00
Dallas				
Site A	1.86	0.00-6.00	0.989	0.901-1.00
Douglas				
Site A	3.09	0.50-8.00	1.00	1.00-1.00
Site B	0.963	0.00-6.00	0.998	0.960-1.00