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Determining environmental predictors of *Amblyomma americanum* (Lone Star Tick) population densities in central Georgia, United States: 2019 and 2021

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Bachelor of Science
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Thesis Committee Chair: Dr. Gonzalo Vazquez-Prokopec

An abstract of
a thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
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Abstract

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Vector-borne diseases pose a significant public health threat due to the number of diseases and pathogens these vectors can transmit to humans. Specifically, tick-borne pathogens, like *Borrelia*, the pathogen that causes Lyme Disease, and *Rickettsia*, the pathogen that causes Rocky Mountain Spotted Fever, have traditionally been of greatest concern. Recently however, a new disease has emerged in the southern continental United States. Vectored by the Lone Star Tick, *Amblyomma americanum,* Heartland virus is a relatively novel pathogen that was recently confirmed to be carried by *Amblyomma* in Georgia. Population sizes for this tick have been on the rise in recent years, and a deeper understanding of when population sizes peak within a given field season was needed to determine risks. For this study, we collected tick abundance and density values from two field sites in Putnam and Jones counties on a weekly basis during the spring, summer, and early fall of 2019 and 2021. Weather factors, like rainfall, temperature, and humidity, were abstracted from an online source and analyzed together with collected density values to determine biologically plausible environmental drivers. It was determined through cross-correlations and predictive modeling that the strongest predictors of population sizes were the maximum daily temperature, cumulative rainfall, and relative humidity 15 days prior to a collection event. When determining safety guidelines and recommendations around tick-borne infections in the future, these values should be considered for their relationship with population size, as increases in population sizes increase the risk of pathogen transmission.

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Introduction

Vector-borne diseases are a major public health concern and comprise a substantial portion of the total global burden of disease (1, 2). The WHO estimates that 80% of the global population is at risk for vector borne diseases, which can cause both severe health complications as well as economic loss (1). Ticks comprise a prominent section of the vector-borne disease emergency, transmitting the greatest diversity of all arthropod-borne pathogens, being responsible for the most cases of all vector borne diseases, and contributing over 90% of all vector-borne pathogens in the continental United States (3, 4). The number of tick-borne disease cases doubled in the continental United States between 2004 and 2016, and in 2017, about 54,000 vector-borne disease cases were linked to ticks. We have seen in recent years an overall increase in the number of ticks and reported cases of their associated diseases as increases in temperature have been an important factor for extending the habitable range and active seasons of many tick species (5), but the impacts on overall population sizes and the seasonality are less well studied.

Of all pathogens transmitted by ticks, *Borrelia burgdoferi*, the pathogen responsible for Lyme disease and primarily vectored by *Ixodes* ticks, is currently the most commonly reported in the continental United States (6), while globally, around 500,000 cases of Lyme disease are diagnosed or treated each year (7). The burden for Lyme falls primarily on New England, New York, and the Midwest. While Ixodes species are found in the western United States as well, the primary species in the area, *Ixodes pacificus,* is a much less efficient vector compared to *I. scapularis*, and the burden of the disease in states like California and Washington is much lower compared to the east and Midwest. However, as climate change continues to impact the range of all ticks, including Ixodes species, the typical burden may change as well.

An equally apparent and concerning tick-borne pathogen is *Rickettsia ricketsii*, the pathogen responsible for Rocky Mountain Spotted Fever (8). This is cited as the most lethal of all diseases transmitted by ticks and covers an expansive range across the continental United States, reaching every state outside of Maine and Vermont, though the primary burden falls on the southeastern and midwestern United States. *Rickettsia* is often overlooked as a major contributor to morbidity and mortality, even though it is the most common emerging or re-emerging vector-borne pathogen globally (9). The main vector for this pathogen is the *Dermacentor variabilis*, showing that the public health emergency surrounding tick and other vector-borne pathogens is not isolated to a single vector or single disease.

Four the purposes of this study, we focused on the lone star tick, *Amblyomma americanum*, which is a common vector for numerous arboviruses in humans, including southern tick-associated rash illness (STARI), Ehrlichia, and alpha gal syndrome (10, 11). This species makes up the vast majority of all ticks in Georgia, with abundance and density numbers substantially greater than those seen for *Ixodes* or *Dermacentor* species. Population sizes for the *Amblyomma* have been on the rise in recent years, leading to an increased incidence of pathogen transmission (12), coupled with an expanded geographic distribution due to changes in environmental factors (3). This species is currently predominant along the east coast and upper Midwest, with recent identifications in previously uninhabited areas, pointing to range expansions further north and west as climate change continues (13). Recent collections have documented Amblyomma's appearance in states such as Kansas, Oklahoma, and Nebraska (13) with increased occurrences in Michigan, Wisonsin, and Minnesota as early warnings their expected northward expansion has already begun.

One of the primary causes of concern for the rise in *Amblyomma americanum* population sizes lies in their recently discovered ability to transmit Heartland Virus (14). Heartland virus (HRTV) is a relatively novel pathogen compared to others like Lyme and Rickettsia, and is an RNA virus known to cause severe fever with thrombocyte syndrome virus, with all reported cases so far being severe or fatal. There has been documented infection in Lone Star Ticks, confirming the presence and circulation of the virus in Georgia and other states where this vector is prominent (14). The virus was first documented in humans in 2009, with cases of severely decreased white blood cell and platelet counts, but only around 35 cases have been confirmed so far (15). Positive samples have been previously identified in Georgia populations as early in the season as mid-April and no later than mid-June (14). The official case definition for HRTV includes febrile illness, headache, and fever, with low blood cell and platelet counts distinguishing it from other common tick-borne infections (15). The geographic burden of HRTV closely matches that of *Amblyomma* ticks, with the peak seasons of HRTV reports coinciding with previously established *Amblyomma* questing seasons (15). A better understanding of this emerging pathogen and the vector that carries it is needed to limit the number of cases and improve diagnostic procedures.

Previous studies have developed a well-defined timing for the occurrence of population peaks for *Amblyomma americanum* ticks in central Georgia, with adult populations peaking in April through July and nymphs having a bimodal peak, first in March to June and again later in August and September (16). Other studies looking into the seasonality of *Amblyomma americanum* have noted an absence of ticks or tick activity during collections that occurred in November through February, with two main nymphal peaks in early June and late August, and adults displaying a single peal in late May (17). This seasonality was again upheld by further research conducted in Georgia showing nymphs were most numerous from April through June before declining throughout August and into September, with adults being collected at their highest numbers from March to May before declining through the rest of the summer (18). Nymphs were further shown to first appear in March before reaching their peaks in May, while adults had an active peak slightly earlier and were not seen after July. In addition to this, other research has concluded that adults were active from April to late August, with populations peaking in June, while nymphs in some cases were found as early as February and as late as October, with populations peaking in May and June (19).

While environmental factors are widely accepted to play a key role in determining the transmission patterns of vector-borne pathogens into humans, the specifics on how environmental cues like rainfall, relative humidity, and maximum temperature drive population peaks during tick's primary questing season is less well known. Global climate change, along with its impacts on environmental factors like temperature and rainfall, together with effects on hosts and habitat, are likely to cause prominent changes in the risk of infection for many tick- and vector-borne pathogens (20, 21). Previous research has also document how tick questing activity, abundance, and population density are all influenced by temperature (20, 22), with changing climate producing a mixed bag of influences, facilitating both an increase in the number and duration of droughts, having a negative impact on tick abundance (21), and more years with warmer winters followed by dryer springs, positively impacting tick abundance (22). Humidity has also been documented in previous research as the most influential environmental parameter impacting tick activity and relative abundances throughout a field season (17), with the availability of preferred habitats and vegetation playing a significant role as well (5). In addition to this, previous studies have shown a drastic change in yearly behavior, and even slight alterations in overall seasonality, due to environmental factors like temperature and relative humidity (18).

For the current study, tick collection events in Putnam and Jones County Georgia were carried out on a weekly basis for the 2019 and 2021 field seasons to further understand the impacts and importance of different environmental factors on tick populations. Environmental factors collected from the Cedar Creek weather station (23), including daily average, maximum, and minimum temperature, daily average relative humidity, and cumulative rainfall were considered for their influence on collected tick density.

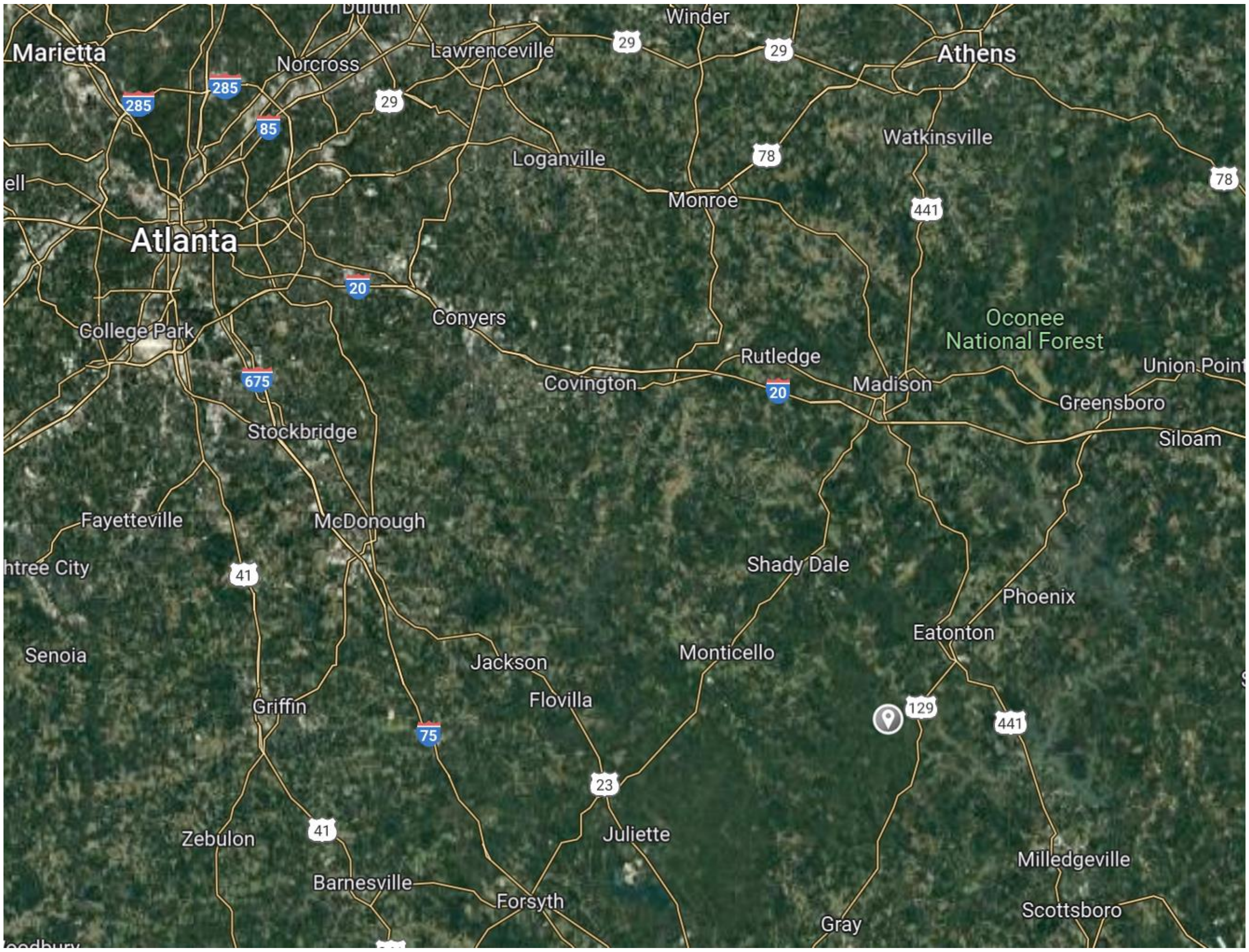
Key to this study was the identification of biologically meaningful lag times between low and high extreme values for the environmental factors and the number of ticks collected on a given day per unit of effort, a measurement used as a proxy for the population size in the sampled areas. We hypothesized there to be a positive relationship between environmental factors like temperature and humidity on tick density with a negative relationship with total rainfall. We also expected there to be between a seven and fifteen day lag for these interactions.

A better understanding of why the populations peak when they do could improve safety guidelines related to tick-borne diseases and reduce the incidence of positive cases in humans. As ticks can pose a prominent public health problem, assessing the risk of human infection throughout the primary questing season by developing a better understanding of the factors impacting population density is necessary to keep people safe and informed. We expect to see alterations in both host abundance and availability due to climate and environmental impacts on vector seasonality, as well as expansion of suitable ranges and the duration of primary questing or active periods as climate change continues to worsen (5). The results of this study can be used during future tick seasons to reduce the risk of tick to human transmission of pathogens.

Methods

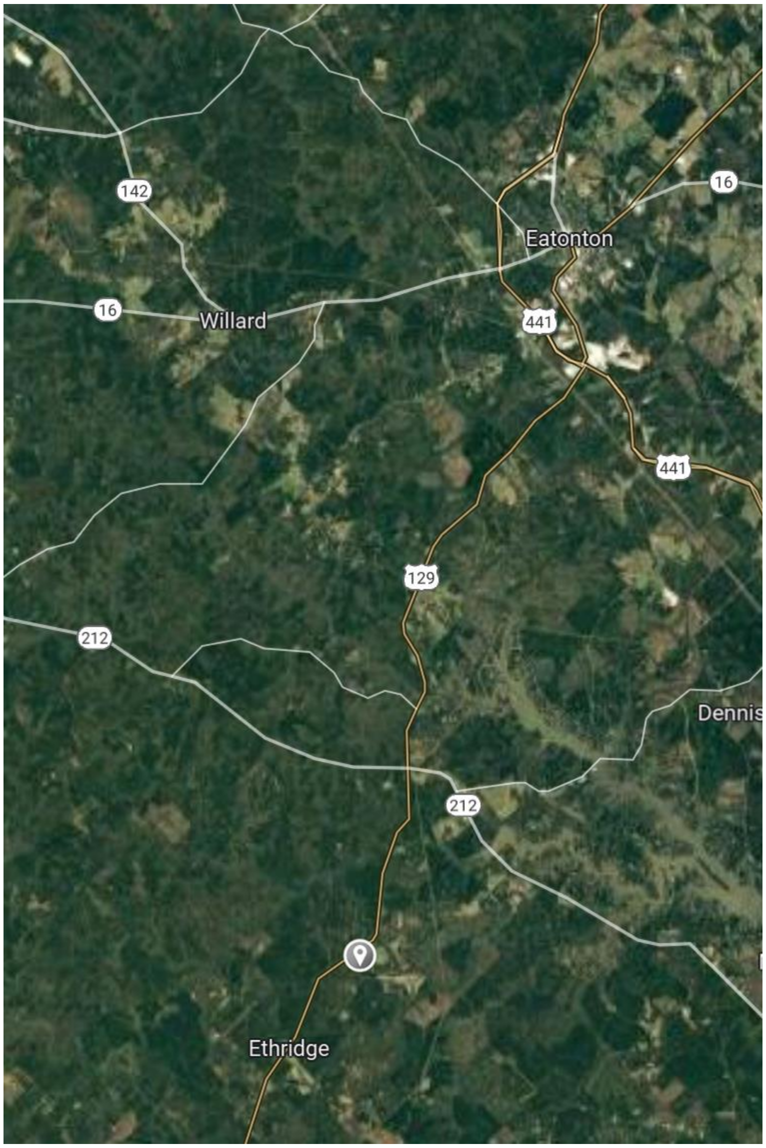
Study Sites

Collection sites were chosen following exploratory data analysis conducted in 2018 for Putnam and Jones County Georgia to determine sites with high densities of ticks (14). The two sites sampled in 2019 and 2021 were the sites with the highest number of ticks collected during this initial analysis. Site A was located on a hunting ground, with heavily wooded sections containing the understory vegetation often associated with *Amblyomma* ticks and with a high potential for an adequate number of primary hosts such as white-tailed deer and small mammals. This site was located on Kinderhook Road just South of Eatonton Georgia in Putnam County and is part of the Chattahoochee-Oconee National Forest, coordinates 33.201865, -83.443216. Site B was located across from a farm between two roads, containing a similar composition to the other site in terms of understory vegetation. This site was located off Stallings Road north of Haddock Georgia in Jones County, coordinates 33.154951, - 83.450885. Both sites contained deciduous woodlands and were located in a humid subtropical climate, making these areas ideal for the development of ticks with open access to favorable fauna and grass cover (14) and abundant shrubby vegetation ideal for tick questing (17). In previous studies, *Amblyomma* ticks have been heavily reported in woodland habitats containing dense undergrowth (19) with some preference towards pine, mixed-pine, and pine-regeneration forests as well (15). The sites collected at for the current study both fell within the Piedmont ecoregion of Georgia, specifically the Southern Outer Piedmont subregion characterized by relatively low precipitation and elevation and an abundance of Loblolly-shortleaf pine forests (24). These sites again showed promise for high densities of ticks due to dense shrub layers containing a variety of woody plants and vines along with a canopy dominated by hardwood trees and an understory rich in hawbrushes (25).



Location of sites relative to Atlanta.

Location of Site A relative to Eatonton.



Location of Site B relative to Eatonton

Data Collection

Amblyomma americanum abundance values were collected on a weekly basis during the primary questing seasons in 2019 and 2021, approximately May through August, with collections extending into October during the 2021 season. Ticks were collected via flagging by brushing the understory of collection sites with cloth flags (26). When ticks were questing in flagged areas, they would stick to the cloth material and could be placed in field vials for later analysis. Once transported back to the lab, samples were cleaned and separated by species, gender (male vs female), and life stage (nymph vs adult). Species identification was confirmed using a dichotomous key, and gender for *Amblyomma americanum* ticks was determined through sexual dimorphism, with the females often being much larger and displaying a prominent white spot on their scutums, the “lone star” that gives them their common name. Values for the number of researchers who participated in a collection event, the total number of ticks collected from each site in a given day, as well as total numbers for each species and the number of nymphs and adults, were all recorded.

Weather values were obtained from Weather Underground, an online site recording daily data on precipitation, humidity, temperature, and other values from various weather stations across the country (23). The station selected for this study was the Cedar Creek station, the closest available for both of our sites. Values for average, maximum, and minimum temperature, along with average humidity, and cumulative rainfall were taken directly from the provided tables and entered into excel to be further analyzed in RStudio.

Data Analysis

Values pertaining to the total collection effort, abundance of ticks, and species composition were broken down by field season. We also noted the number of samples that tested positive for HRTV as well as the difference in tick abundance between the two sites sampled from. Values for the total number of ticks collected on each day, the abundances, were standardized considering the collection effort to estimate the catch per unit effort as a proxy for population density. This value was obtained by dividing the total number of ticks by the number of people who collected on a given day. On days when we were unable to collect ticks at both primary sites, the initial density was multiplied by 2. Time spent and area covered in a given day and at a given site remained relatively constant throughout the study period, and minor variations in those values were not adjusted for when calculating the density. For data analysis, density and abundance values from the individual sites were pooled together both because they were relatively independent, the number of ticks collected at one site did not impact the number of ticks collected at the other site, and values were fairly similar, it was rare to have one site with a relatively high number and one site with a relatively low number on the same collection day.

A plot for tick density during the course of the field seasons was produced in RStudio using the ggplot2 package. Values were separated by the year a collection event took place, either 2019 or 2021, and broken down by total density, nymphal density, and adult *Amblyomma americanum* density, with stars to indicate when a sample that tested positive for heartland virus was collected. A similar analysis was conducted to show the visual relationships between density, total rainfall in the seven days prior to a collection event, and average maximum relative humidity the seven days before a collection event during the primary questing season.

To further determine the timescale of the interaction between temperature, rainfall, humidity, and tick density, cross-correlation plots were produced for the 2019 and 2021 seasons. This analysis was broken down by nymphs and all *Amblyomma americanum*. These plots showed both the strength of and the lag on which the relationships in question were acting to inform biologically meaningful variables to include in potential predictive models.

A predictive model from this data was constructed to determine an expected number of ticks on a given day from known values of the predictor variables. Variables of interest for the model included the environmental factors of total rainfall, maximum temperature, and relative humidity on lag times of 0, 7, and 15 days prior to collection events as well as the year and week of collection. The variable "year" represented the year when a collection event occurred, either 1 for 2019 or 2 for 2021. The "year-week," referred to in the remainder of the analyses as just "week," is a chronological number assigned to each week, 1-52, within a given year. Potential models including different sets of the variables of interest as well as three different model types, linear regression, generalized linear model regression, and generalized linear mixed model regression, were considered for fit and predictive power based on their AIC (Akaike Information Criterion) values. Models with interaction terms between the environmental factors along with generalized linear mixed models considering both year or week as random effect variables were also constructed and considered for fit based on their AIC values. Once a final model was selected, predictive and random effect plots were also produced to test the model's ability to accurately predict expected values of abundance based on the included variables.

All data analysis was conducted in RStudio using the lme4, glmmTMB, ggplot2, and sjPlot packages.

Results

The total number of ticks collected during each season were predominantly, and almost exclusively, comprised of nymph and adult *Amblyomma americanum*, with adult *Ixodes scapularis*, *Dermacentor variabilis*, and *Amblyomma maculatum* totaling to less than 2% of all ticks collected. Total collection effort in the 2021 season was slightly higher than in the 2019 season, at 101 and 77 respectively, due to tick collection events continuing much later into the fall school semester, resulting also in a greater number of ticks collected during the 2021 season, 7484 compared to 6175. Between the two sites visited for tick collecting, Site A on Kinderhook Road produced a slightly greater number of ticks in the 2019 season, 3534 compared to site B on Stallings Road, which produced 2641 ticks, though in the 2021 season, collections from site B resulted in a greater number of ticks, 4272 versus 3212 respectively. In total, the two sites were highly similar, with 6746 or 49.4% of the total ticks collected for this study coming from site A. Overall, there was little difference in terms of species composition (75% and 74% nymphs, 24% and 26% adult *Amblyomma americanum*, 1.2% and 1.1% adult *Dermacentor variabilis*, 0.049% and 0.027% *Ixodes scapularis*, and 0.16% and 0.25% Amblyomma maculatum) between the 2019 and 2021 seasons. Combined between the two years, a total of 13,659 ticks were collected from a total collection effort of 178 units.

In general, as the number of ticks collected at one site increased, the number of ticks collected at the other site increased as well. The slope for this line was positive, at 0.138, with an r squared value of 0.035. Values between the two were similar on any given collection day. For this analysis, tick abundance was used instead of density. Because of the similarities between the numbers collected at each site, abundance and density values were pooled together to give a total on a single collection day for future analyses.

At every collection event, the density of nymphs exceeded the density of adults, though this difference and the total tick density varied significantly throughout the field seasons. Tick collection events began earlier in the field season in 2019, at week 18, but went much longer into the Fall during the 2021 season, with the last collection event taking place at week 40. Positive heartland virus samples did not appear after week 25 during either season, and no samples tested positive from collection events where the total density was below 150 ticks collected per unit of effort.

Tick density varied greatly throughout each field season, while relative humidity remained constant. There did not appear to be a clear visual trend between the humidity seen on any given week and the tick density. Variations in tick density also did not express a clear visual relationship with weekly rainfall, as the variation in one factor did not appear to cause or be responsible for the variation in the other. Variations in both did not follow a clear trend; as the value of one variable increased between weeks, the value for the other variable did not consistently increase or decrease.

Seven of the twelve relationships between tick density and proposed environmental drivers do not appear to have a significantly strong relationship based on the lagged cross-correlation. The bars for this analysis often did not extend far enough in the positive or negative direction to be considered biologically meaningful. The strongest relationship appeared with rainfall in the 2019 season and both groupings of ticks (just nymphs and all ticks), with greater cumulative rainfall approximately four days before a collection event having a negative impact on the number of ticks collected; the greater the amount of rainfall four days prior, the fewer ticks expected during a collection event. Maximum temperature had a positive relationship with both groupings of ticks in the 2019 season as well, with higher temperatures approximately three days before collection having a positive impact on the number of ticks collected: the higher the maximum temperature seen three days before a collection event, the greater the expected tick density during that collection event. Lastly, a relatively weak relationship appeared for all ticks in 2021 with relative humidity, with higher values of relative humidity between 5 and 7 days before a collection event having a positive impact on the number of ticks collected.

The lowest AIC values were seen in the GLMMs when looking at models with the week as a random effect. However, week of collection was ultimately determined to not exhibit a strong random effect, and moving forward in analyses only models controlling for week were considered. Among these models, the lowest AIC value was reported from a model controlling for maximum temperature, relative humidity, and total rainfall on a fifteen-day lag time, as well as the collection week. This model also included the interaction between rainfall and humidity as an effect along with the random effect of the year a collection event occurred.

From this, the final recommended model for predicting tick abundance on a given day is as follows**:**

Abundance = (-0.05)*Maximum Temperature 15 + (-1.81)*Cumulative Rainfall 15 + (-0.13)*Relative Humidity 15 + 0.002*Week + (0.023)*(Humidity*Rainfall)

The predictive plots generated from this model show that as the average maximum temperature and average relative humidity over the fifteen days prior to a tick collection event increase, the predicted number of ticks decreases. As the total rainfall in the previous 15 days increases, the expected number of ticks also increases. The week number when a collection event occurred has relatively little influence on the predicted number of ticks when accounting for the environmental predictors of rainfall, temperature, and humidity. Predictions made from this value alone had relatively low certainty.

Table 1

Table 1	2019	2021	Total
Total Effort	77	101	178
Total Ticks	6175	7484	13659
N AMA (% total)	1458 (23.6)	1970 (26.3)	3428 (25.1)
N D.Var (% total)	74 (1.2)	84 (1.12)	158 (1.16)
N I.Scap (% total)	3 (0.049)	2 (0.027)	5 (0.037)
N A.Mac (% total)	10 (0.16)	19 (0.25)	29 (0.21)
N nymphs (% total)	4630 (75.0)	5525 (73.8)	10155 (74.3)
QPCR Positive HRTV Samples	3	1	4
Total Ticks Site A - KH (% total)	3534 (57.2)	3212 (42.9)	6746 (49.4)
Total From Site B - ST	2641	4272	6913

Table 1: Breakdown of ticks collected by species and by site. Collection results from 2019 are shown in the first column, with 2021 in the second column and the total from the two seasons in the final column. Ticks were broken down into adult *Amblyomma americanum*, adult *Dermacentor variabilis*, adult *Ixodes scapularis*, adult *Amblyomma maculatum*, and nymphs. Nymphs were not speciated, but assumed to be *Amblyomma americanum*, and adults for this table were not further broken down by sex. The number of positive Heartland Virus (HRTV) samples was also included. The values in the parentheses represent the percent of the total ticks each species and site comprised.

Figure 1

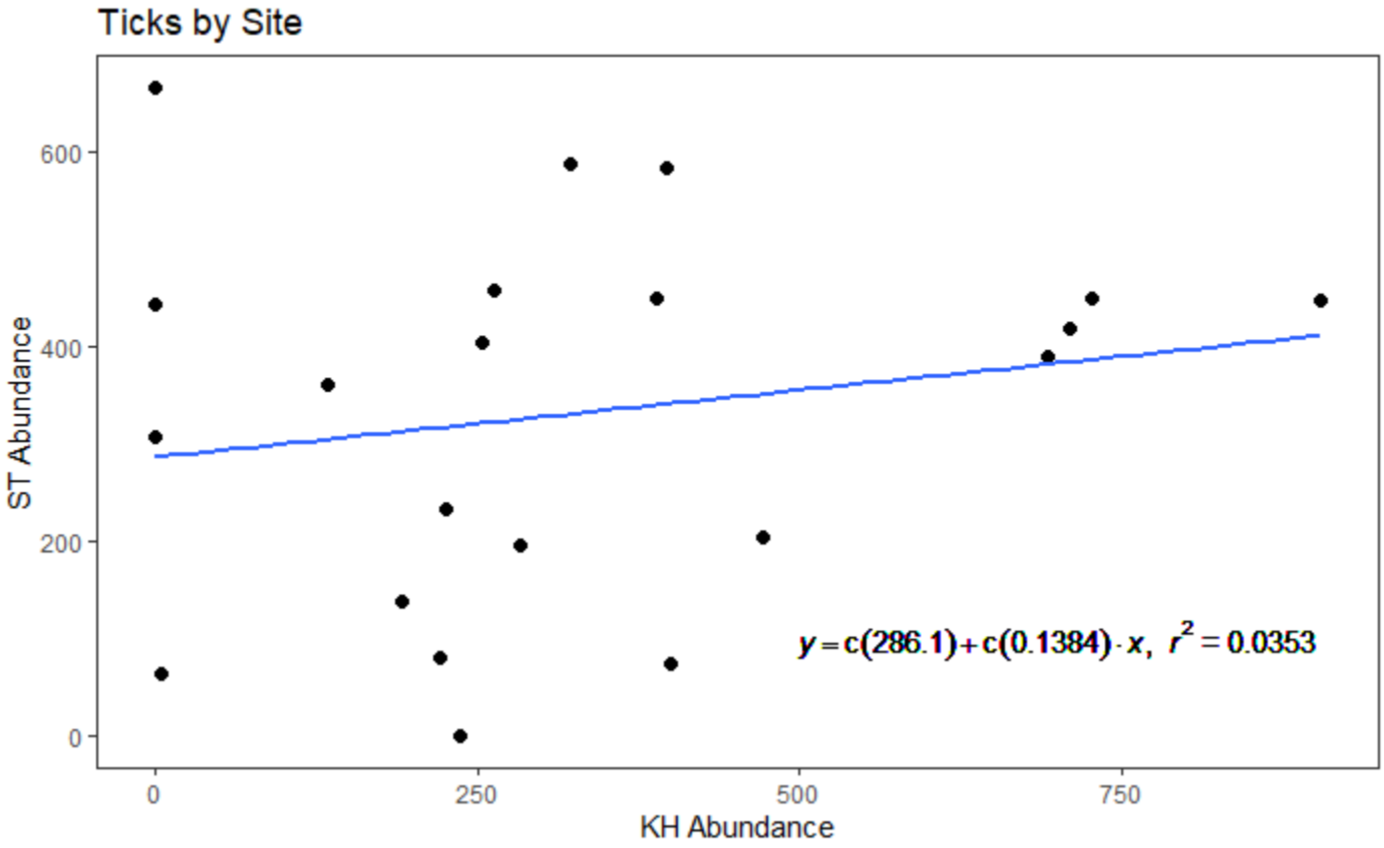


Figure 1: Relationship between tick abundances collected at each site. X-axis represents the number of ticks collected on a given day at the Kinderhook Road site (KH) with the Y-axis representing the number of ticks collected on that same day for the site on Stallings Road (ST). The blue line shows the slope of the regression with the equation of this line in the bottom right corner. For this line, the intercept was 286.1 with a slope of 0.1384 and a r squared value of 0.0353.

Figure 2

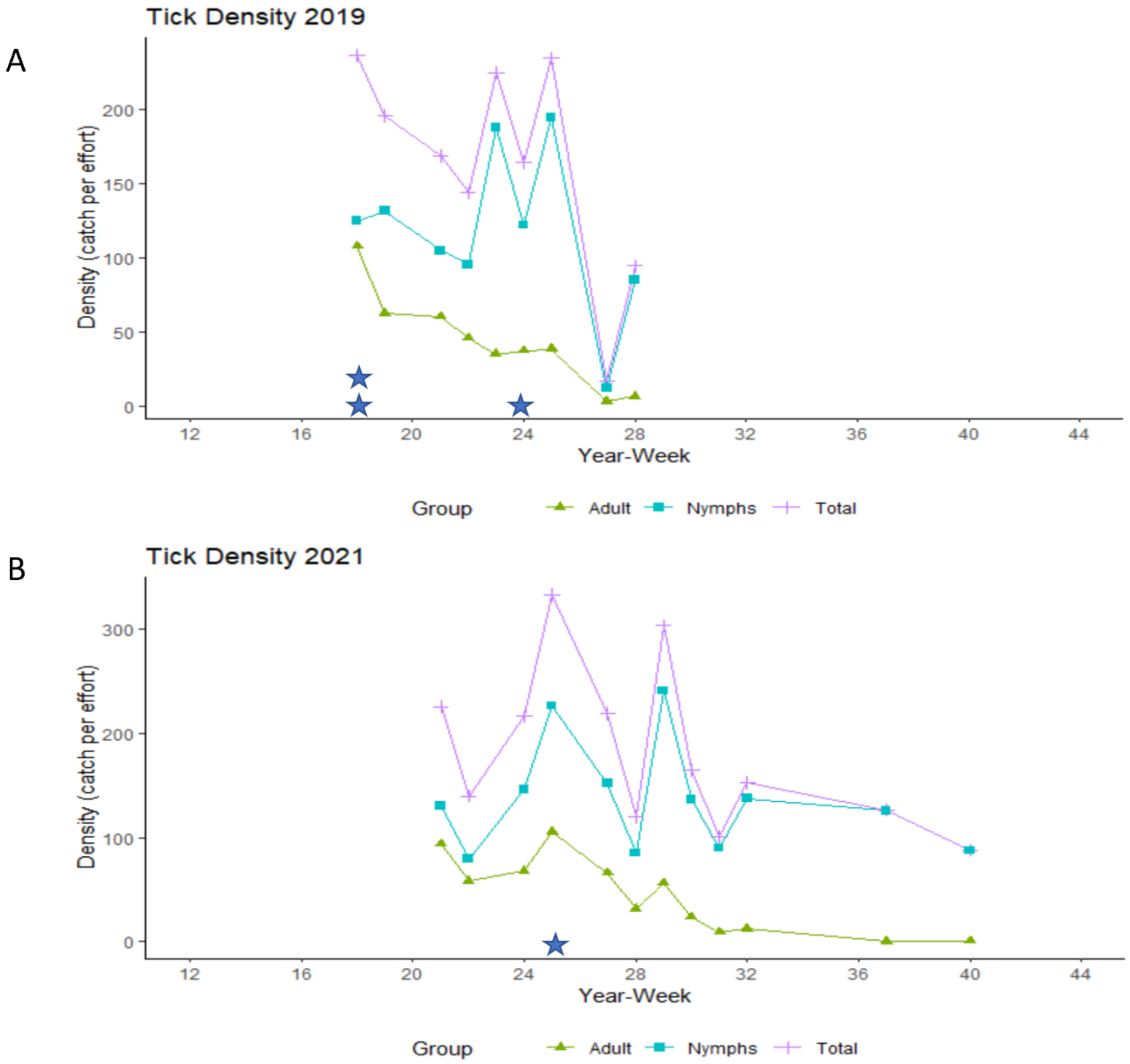


Figure 2: Temporal trend in tick density throughout the (A) 2019 and (B) 2021 field seasons. The X-axis is broken down by week, which assigns a number, 1-52, to each week in a given year. The X-axis is separated into weeks of four to represent the collection events and trends from a single month. The Y-axis represents the tick density, measured as the number of ticks caught per unit effort. A single unit of effort corresponds to a single person performing a collection at one site on a single occasion. Green lines represent the density of adult *Amblyomma Americanum* ticks, both males and females. Blue lines represent the density of nymphs, with purple lines representing the total tick density. Stars were placed near the X-axis above weeks when a positive HRTV sample was collected.

Figure 3

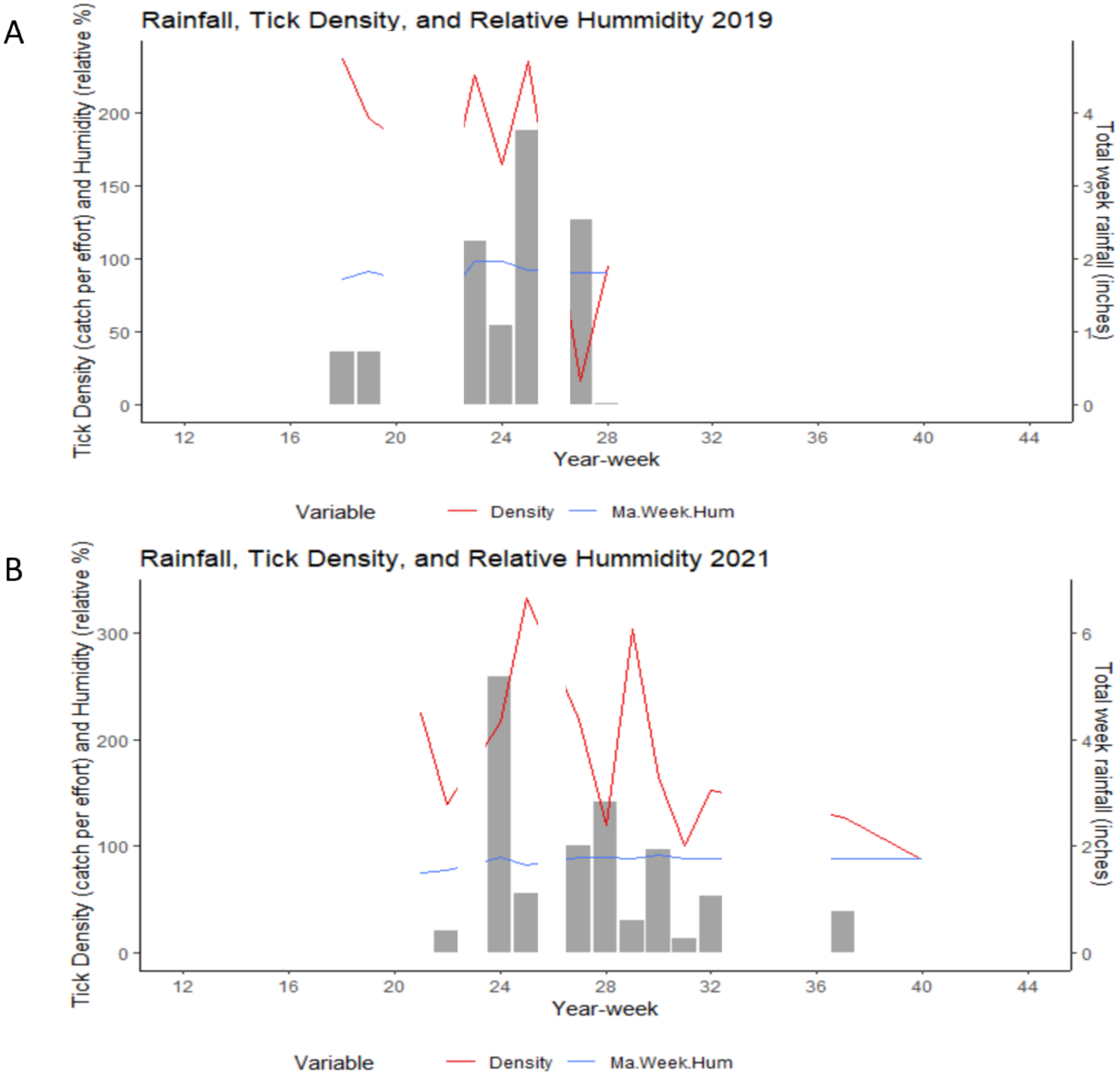


Figure 3: Interrupted time series for the relationship between tick density, relative humidity in a given week, and total rainfall in a given week for the (A) 2019 and (B) 2021 field seasons. The X-axis represents the year week of the collection year, marked 1-52. The primary Y-axis on the left represents the values for tick density, on the red line in units of catch per effort, and the relative humidity, on the blue line as the relative percent. The secondary Y-axis represents the total rainfall in a given week, shown on the graph in the gray bars.

Figure 4

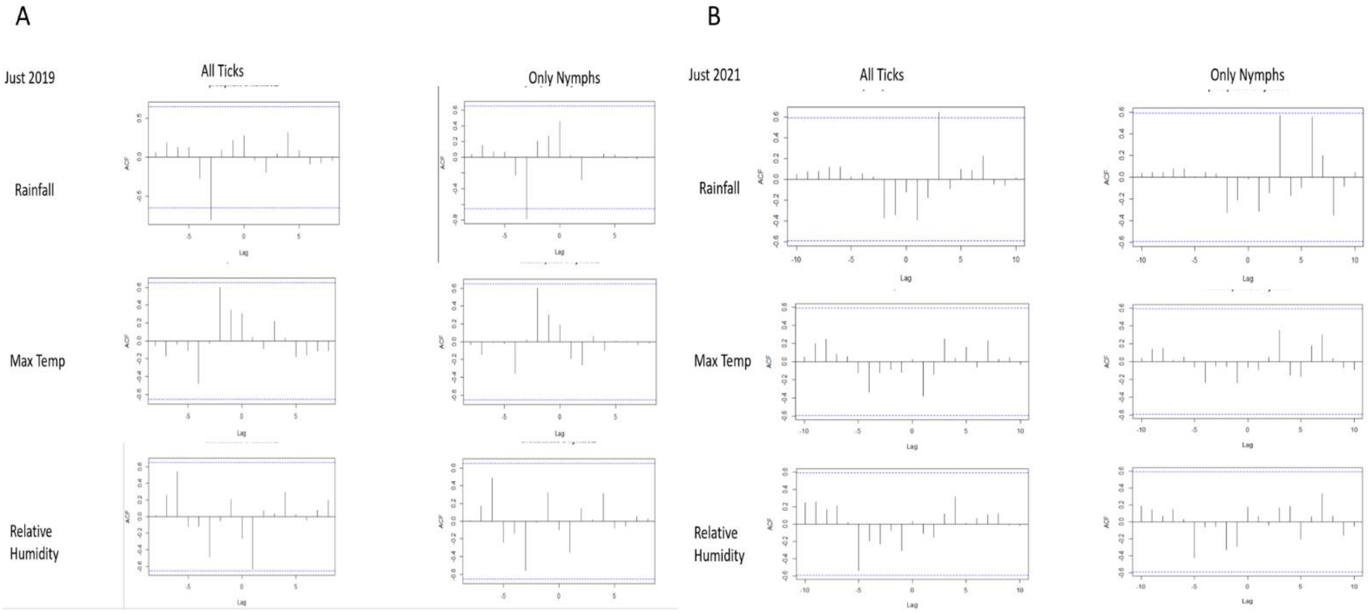
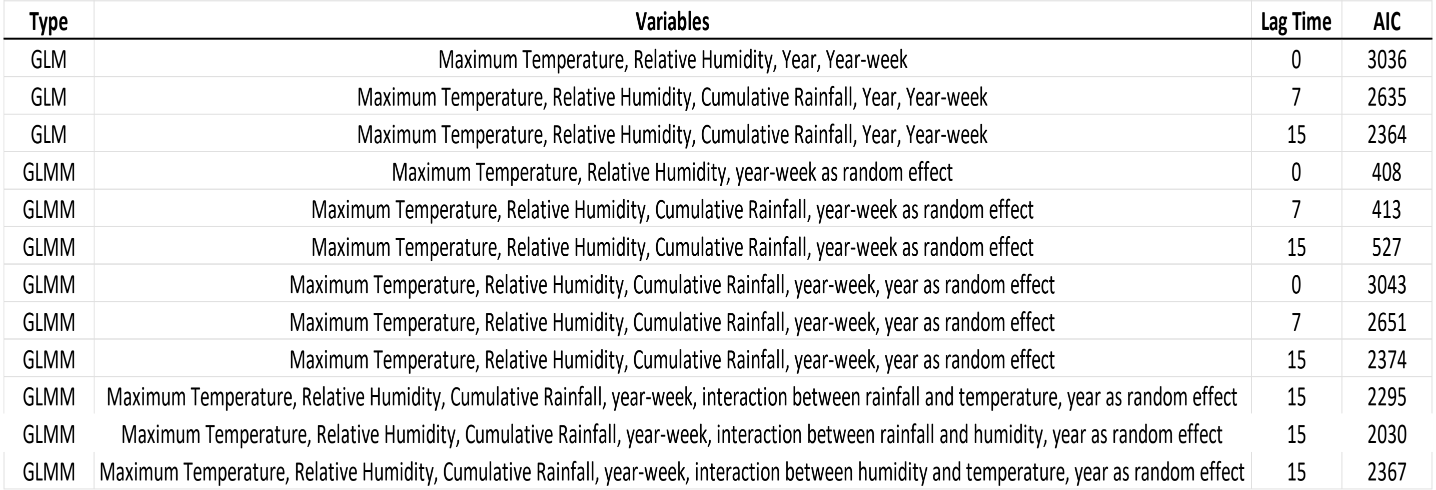


Figure 4: Lagged cross-correlations between all ticks and just nymphs with the three biologically plausible environmental drivers of density; rainfall, maximum temperature, and relative humidity. Section A displays the results for the 2019 season, with the first column expressing the lagged correlation between each proposed driver and all ticks. Column 2 shows these same relationships with just the nymphal densities. The top row shows the lagged relationship with rainfall, the middle row shows the relationship with maximum temperature, and the bottom row shows the relationship with relative humidity. Section B shows the same relationships in the same layout for the 2021 season. A negative value on the X-axis suggests a negative lag time; the values of the environmental variable has an impact on the tick density in the future. The strength of the association is represented by how far up or down on the Y-axis the bars extend, with stronger associations represented by larger magnitudes. The dotted lines at the top and bottom of the frames in each panel represent the threshold for a strong or meaningful relationship.

Table 2



Type	Variables	Lag Time	AIC
GLM	Maximum Temperature, Relative Humidity, Year, Year-week	0	3036
GLM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, Year, Year-week	7	2635
GLM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, Year, Year-week	15	2364
GLMM	Maximum Temperature, Relative Humidity, year-week as random effect	0	408
GLMM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, year-week as random effect	7	413
GLMM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, year-week as random effect	15	527
GLMM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, year-week, year as random effect	0	3043
GLMM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, year-week, year as random effect	7	2651
GLMM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, year-week, year as random effect	15	2374
GLMM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, year-week, interaction between rainfall and temperature, year as random effect	15	2295
GLMM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, year-week, interaction between rainfall and humidity, year as random effect	15	2030
GLMM	Maximum Temperature, Relative Humidity, Cumulative Rainfall, year-week, interaction between humidity and temperature, year as random effect	15	2367

Table 2: Tested predictive models. Different models were tested based on the type of model, either generalized linear or mixed effects, the predictor variables included from rainfall, maximum temperature, relative humidity, year, and collection week, as well as the lag of those variables, either 0, 7, or 15 days before a collection event. The model's ability to predict the number of ticks we would expect to collect was determined by analyzing the Akaike Information Criterion, or AIC, value.

Figure 5

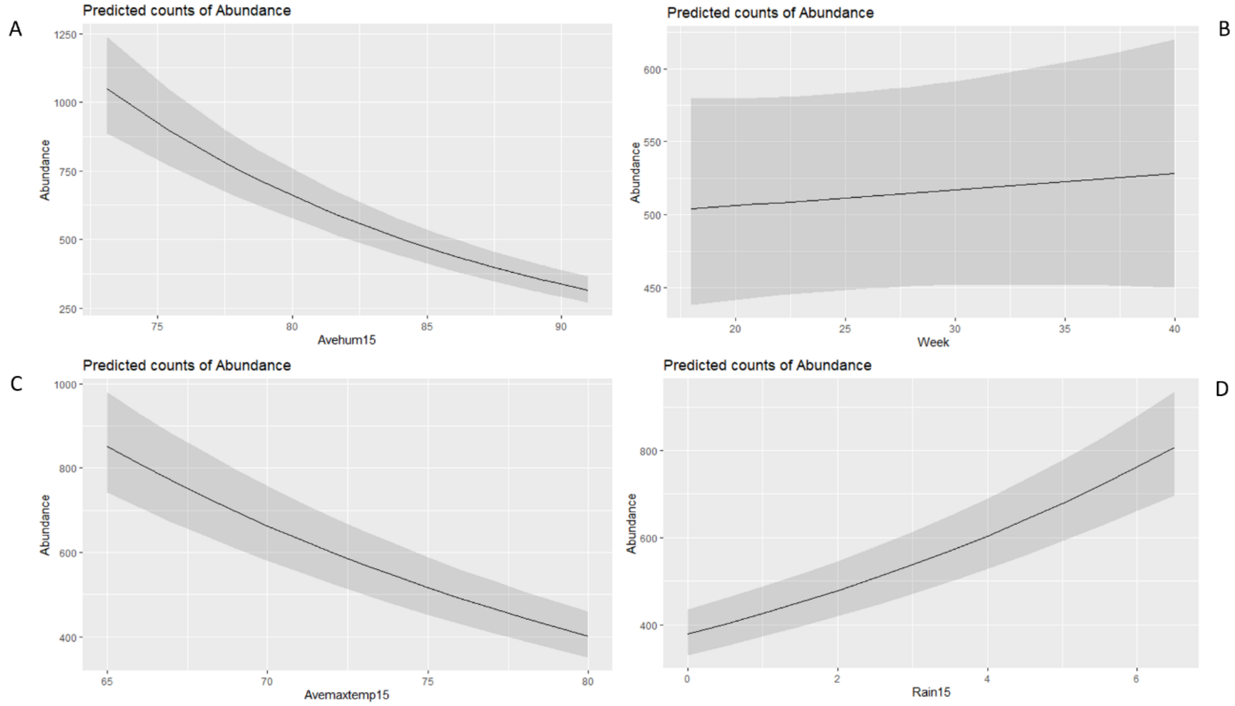


Figure 5: Predictive plots for the final recommended model. For all sections, the X-axis represents the value for that variable, either in degrees Fahrenheit for temperature, week number for week, relative percent for humidity, and total inches for rainfall. The Y-axis represents the expected abundance of ticks in total count. The gray surrounding each line represents the uncertainty in a given prediction. The wider the range of this gray section, the greater the uncertainty in the prediction. Panel A represents the predicted abundance in response to the relative humidity seen in the fifteen days leading up to a collection event. Panel B represents this same relationship in terms of the week number of the collection year, with panels C and D representing the maximum temperature and total rainfall respectively.

Discussion

This study provides further knowledge towards improving our understanding of the seasonality portrayed by *Amblyomma americanum* ticks in central Georgia. Overall results from collection efforts during the 2019 and 2021 seasons confirmed that *Amblyomma* were the primary species of ticks, with adults and nymphs of this species comprising over 97% of the total ticks collected in each of those two years. This suggests that the pathogens of greatest concern in Georgia are those associated with the lone star tick, especially when considering the newly documented emergence of heartland virus, along with other well-established pathogens like alpha-gal, STARI, and Bourbon virus. While the total number of ticks collected in the 2021 season was greater than in 2019, conclusions about the overall population sizes in the area are more difficult to determine, even when controlling for collection effort, as other variables could have been in play, such as the variation not only in the number of researchers in the field but which researchers were available on a given week for a collection event. It would be difficult to say definitively that previously documentations of increasing tick population sizes were upheld or supported by the results of the current study.

Very few samples tested positive for heartland virus. With only four combined positive pools over the two seasons, it was difficult to draw conclusions pertaining to relative infection risk throughout the tick's primary active season. However, two potential implications related to the timing of these positive samples as well as other characteristics about those specific collection events can be drawn. First, there appears to be a threshold density for positive samples, as no positive samples were collected on days where the tick density was below 150 ticks per effort. Second, no positive samples were collected beyond week 25, suggesting, as one might expect, that the risk for heartland virus transmission is highest during the first half of the field season, when questing activity for these ticks is at its highest.

Total tick density peaked around the same time each year in early June as expected. Collection events during the later end of the field season, starting at about week 30 in late July to early August, consisted almost exclusively of nymphs, especially for the 2021 field season, again as expected for the second of two peaks commonly seen for nymphs in Georgia. The collections from this study align well with previously established reports of *Amblyomma* seasonality in terms of both the overall timing of population peaks and the nature of those peaks, with adults displaying only a single peak while nymphs appeared to be more bimodal (18, 19). Previous research showed adult tick populations peaking in May, with nymphs peaking in Juna and August (16), with additional studies confirming this seasonality by noting a drastic decline in populations leading up to November and almost no ticks identified again until early March (17). This established seasonality was again well captured during the collections conducted in the 2019 and 2021 field seasons.

Conclusions and Recommendations

Vector-borne diseases pose a significant public health risk due to the variety of deadly pathogens these vectors, including ticks, can transmit. A better understanding of when population densities of these ticks are highest during their primary questing seasons was needed to determine when the risk of these pathogens is of the greatest concern to human health. Though in general the timing of population peaks for the *Amblyomma americanum* adult ticks and nymphs are well understood, knowledge around the factors influencing this timing and the ability to predict an expected abundance on a given day are lacking. The results of this study can be used during future questing seasons to assess the dangers to human health based on measurable environmental factors. When determining the risk of tick bites throughout the season, public health officials should take into account the amount of rainfall, as well as the average maximum temperature and relative humidity, seen in the past fifteen days, as this offers the most reliable prediction of an expected number of ticks on a given day.

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Appendix

Site A: Kinderhook Road

Walsh 24

Walsh 25

Walsh 26

Site B: Stallings Road