Ecological modeling over seven years to describe the number of host-seeking *Amblyomma americanum* in each life stage in northeast Missouri

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ABSTRACT: *Amblyomma americanum* (L.), the lone star tick, is a vector of pathogens in humans and other animals throughout the United States. Our objective was to characterize how environmental factors influence patterns of *A. americanum* activity throughout its life cycle by creating statistical models that describe the number of active off-host larvae, nymphs, and adults in northeast Missouri from 2007 to 2013. Ticks were collected every other week from a permanent sampling grid in a second-growth forest and in an old field habitat. Each of the three life stage models considered six meteorological variables and one biotic variable. Regression modeling was used to make candidate models which were evaluated with eight selection criteria. Best-selected models were useful in describing seasonality and magnitude of *A. americanum* activity for larvae, nymphs, and adults. While distinct subsets of environmental variables were optimal in each life stage, all three models incorporated cumulative degree days, habitat, and number of ticks in the previous life stage. These models further elucidate how environmental and demographic factors influence patterns of host-seeking activity throughout the *A. americanum* life cycle, providing insight into how changing climate may impact risk of tick-borne pathogen transmission. *Journal of Vector Ecology* **43 (2): 271-284. 2018.**

Keyword Index: Amblyomma americanum, lone star tick, environmental variables, regression modeling, off-host activity, life cycle.

INTRODUCTION

Amblyomma americanum (L.), the lone star tick, is a three-host ixodid tick and vector of pathogens in humans and other animals. Importance of *A. americanum* in pathogen transmission has become especially evident over recent decades as its reported distribution has grown northward (Keirans and Lacombe 1998, Springer et al. 2014) and westward (Cortinas and Spomer 2013) from the southeast and central United States. Although the species is associated with secondary forest and dense undergrowth (Hair and Howell 1970), modeling suggests that average vapor pressure in July is the most important determinant of suitable habitat (Springer et al. 2015). While known to transmit Heartland virus (Savage et al. 2013, Godsey et al. 2016) and pathogens that cause tularemia and southern tick-associated rash illness, increases in *Amblyomma*-associated human ehrlichiosis (Childs and Paddock 2003), caused by the bacteria *Ehrlichia chaffeensis* and *E. ewingii,* have been studied most intensively in recent years.

As an aggressive and non-specific feeder throughout all three life stages (Koch 1981, Kollars et al. 2000), *A. americanum* interacts with many potential reservoirs of infectious pathogens. *Ehrlichia chaffeensis*, the agent which causes human monocytic ehrlichiosis, does not exhibit transovarial transmission (Long et al. 2003), so ticks must be inoculated as either larvae or nymphs by feeding on an infected host. White-tailed deer (*Odocoileus virginianus*), an important host for all life stages of *A. americanum*, often exhibit persistent or recrudescent infection by *E. chaffeensis* (Davidson et al. 2001) and therefore act as a competent reservoir, though raccoons,

opossums (Lockhart et al. 1997), and coyotes (Kocan et al. 2000) have also been implicated. While adult ticks are generally infected with *E. chaffeensis* at higher rates (Steiert and Gilfoy 2002, Mixson et al. 2004), nymphs are more prevalent and less conspicuous than adults, and therefore may pose a greater risk in pathogen transmission. Understanding phenology of ticks throughout their life cycle is essential in characterizing seasonal patterns of tick-borne diseases (Randolph et al. 1999, Kurtenbach et al. 2006). *Amblyomma americanum* have three physiologically distinct life stages: larvae, nymphs, and adults. Ticks must search for and acquire a blood meal to advance through development, after which they drop off the host and molt into the subsequent life stage. Following adult on-host mating, engorged females produce a single clutch containing thousands of eggs and die soon afterwards. Development can be completed in less than 22 weeks under laboratory conditions (Troughton and Levin 2007), but usually requires multiple years in the wild (Hair and Howell 1970, Bouzek et al. 2013). Throughout their distribution in the United States, *A. americanum* seem to complete its life cycle in three active seasons, hatching and feeding as larvae in summer of year one, feeding as nymphs primarily in the spring of year two, and feeding and mating as adults in the spring of year three before dropping off-host to oviposit.

Because ticks spend the large majority of their life off-host, seasonal and daily trends in activity are heavily influenced by environmental conditions. In ticks and mites, inactivity can be attributed to quiescence, an immediate and reversible response to adverse conditions, or to diapause, an inherent response to anticipated unfavorable conditions (Belozerov 2008, 2009). Seasonality of *A. americanum*

activity seems to be largely regulated by temperature and photoperiod. Photoperiod diapause has been demonstrated in *A. americanum* (Pound et al. 1993). Day length has been associated with changes in the timing of nymph feeding (Barnard et al. 1985) and molting time is shorter in regimes where photoperiod is increasing (Pound and George 1988). Patrick and Hair (1977) observed that adult females repress repletion while feeding until May and postulated that this may be a photoperiod response that allows for a narrow window of larval hatching. It was suggested that similar repression of repletion in nymphs until June maximizes survivorship during the hottest and driest part of the summer (Patrick and Hair 1977), after which newly-molted adults generally do not resume questing until the following spring (Semtner et al. 1973, Robertson et al. 1975). Short day exposure reduces water loss apparently to prepare for winter water conservation but was demonstrated to be quickly reversible (Yoder et al. 2016). Activity ceases on average at temperatures less than 7° C (Clark 1995), and studies suggest that winter conditions do not trigger behavioral diapause (Stewart et al. 1998). Nymphs and adults overwinter with relatively high success, but unfed larvae rarely survive (Sonenshine and Levy 1971, Koch 1984, Foré and Kim, unpublished data). All three life stages exhibit faster development under high temperatures (Koch 1984). These field and lab results are consistent with recent simulation modeling to examine the diapause in each life stage (Ludwig et al. 2016).

Daily questing activity is governed by a trade-off between energy conservation, host-seeking activity, and maintenance of water balance. To survive such long periods of time without feeding, observed to span up to two years and nine months in *A. americanum* adults (Semtner and Hair 1976), ticks must tightly regulate energy expenditure. While known to hunt actively over horizontal distances in the presence of a host (Hair and Howell 1970), *A. americanum* quests primarily as a sit-and-wait strategist, waiting on vegetation for passing animals. Nymphs and adults migrate vertically in vegetation to maximize questing success, but this often exposes ticks to more desiccating conditions. Once dehydration becomes intolerable, ticks must therefore descend to microenvironments of higher relative humidity where they can absorb water vapor through both active (Sigal et al. 1999) and passive mechanisms (Needham and Teel 1991). Despite this risk, nymph and adult host-seeking activity is more prevalent under conditions of high saturation deficit (Schulze et al. 2002, Schulze and Jordan 2003, Schulze and Jordan 2005). Eggs and larvae, alternatively, cope poorly with low humidity and high temperature (Patrick and Hair 1979, Koch and Dunn 1980), possibly due to their small size.

In this study, we created broad-scale regression models that describe the number of active off-host *A. americanum* larvae, nymphs, and adults in two different habitats in northeast Missouri from 2007 to 2013. Each of these three models determines how the number of questing ticks is associated with seven environmental factors. In a previous study, Kaizer et al. (2015) found that the number of questing larvae was most associated with cumulative degree days, number of adults prior to sampling, and habitat. Developing

models of activity for each life stage should provide a more comprehensive characterization of *A. americanum* activity in response to environmental conditions, which is useful in assessing potential for pathogen transmission.

MATERIALS AND METHODS

Tick collection

Ticks for this study were collected as part of a long-term monitoring study that began in 2007. The location and methodology is fully described in Petry et al. (2010). Ticks were collected from two permanent 70x120 m sampling grids approximately 300 m apart in Adair County, MO, one representing an old field habitat of primarily non-native grasses and the other a second-growth forest dominated by hickory. A total of 141 sampling sessions were conducted every other week in each habitat (n = 282) between February 22, 2007 and December 2, 2013, each of which occurred contiguously across sites between 10:00 and 15:00. Capture success for *A. americanum* larvae, nymphs, and adults differs across sampling methods and habitats (Petry et al. 2010). To ensure capture of all life stages, eight drag transects and eight dry ice baits were used at regular intervals in each grid. In drag sampling, each 1 m² flannel cloth, cut into strips for ease of movement through vegetation, was pinned to a wooden dowel and dragged along a 30 m transect. For each dry ice bait, approximately 200 g of dry ice was placed on a 1 m² flannel cloth and allowed to sublimate for 1 h to coincide with the timing of drag sampling. Cloths were sealed in bags and transported to the lab, where ticks were picked from cloths and preserved in 95% ethanol for later identification by species and life stage under a dissecting microscope. The number of ticks collected was summed across drag and bait cloths for each site for each sampling date. No sampling occurred when ground was covered by snow and ice or when predicted high temperatures were below 7° C.

Environmental variables

Environmental variables that are appropriate for our modeling environment are those that can be collected on a continuous basis consistent with our sampling regime. Meteorological variables fit this modeling environment well. Five broad-scale, ambient meteorological variables were used: cumulative degree days, cumulative precipitation, saturation deficit, daily average wind speed, and day length. As vegetation can modify these ambient conditions, habitat was included as a variable and was defined as a binary variable with 0 and 1 representing the field and forest sites, respectively. The model also included one biotic variable, the number of ticks in the previous life stage, as the number of ticks collected over an interval for one life stage can be used to predict future number of active ticks in the subsequent stage. (Bouzek et al. 2013). One limitation of our modeling environment is that data that assess variation on a broader time scale, such as yearly host availability estimates, does not fit.

All meteorological data, recorded at the Kirksville Regional Airport in Adair County, MO (approximately 10

km from the study area), were obtained from the National Weather Service (www.noaa.gov). Saturation deficit was calculated according to Schulze et al. (2001), using average hourly temperature and relative humidity for the 4-h period spanning from 3 h prior to 1 h after sampling start time. Precipitation and degree days were each considered over a selected duration. Cumulative precipitation was calculated by summing total precipitation of each day within an *n*-day period prior to sampling. Cumulative degree days, as described in Bouzek et al. (2013), was calculated as the average of the maximum and minimum temperatures for each day minus a threshold temperature of 0° C, summed over an *n*-day period prior to sampling.

A time lag (lag) was used to access the number of ticks in the previous life stage based on Bouzek et al. (2013). Lag for the field (Bouzek et al. unpublished) and forest (Bouzek et al. 2013) habitats, respectively, in larvae (a), nymphs (b), and adults (c) was calculated as the average number of (a) adults collected 12-22 and 10-20 weeks prior to sampling, (b) larvae collected 38-46 and 34-46 weeks prior to sampling, and (c) nymphs collected 40-52 and 44-52 weeks prior to sampling.

Statistical analyses

We used count regression modeling in this study to develop one broad model for each life stage to explain the total number of ticks collected in each sampling session. All modeling was conducted in R v3.3.1 using 'GAMLSS' v5.0-1 (Rigby and Stasinopoulos 2005) and 'pscl' v1.4.9 (Zeileis et al. 2008, Jackman 2017) packages. Durations over which precipitation and degree days best describe activity in each life stage were selected from 10, 30, and 60 days prior to sampling. The ten-day interval was considered to capture the accumulated change in conditions between sampling sessions; 30- and 60-day intervals were arbitrarily considered to assess changes from the adult to larval activity periods in a season. As in Kaizer et al. (2015), one-variable negative binomial II models that describe the number of active ticks were created for each duration, and durations with the lowest Akaike Information Criterion (AIC) value (Akaike 1973, 1974) were chosen for subsequent modeling. In larvae and adults, the smallest AIC values were observed for models incorporating total precipitation over ten days and cumulative degree days over 60 days prior to sampling. Nymphs displayed the smallest AIC values for models incorporating total precipitation over 30 days and cumulative degree days over ten days prior to sampling.

Global models containing all seven variables were fit to data using Poisson (POI), negative binomial I (NEGBIN1), negative binomial II (NEGBIN2), zero-inflated Poisson (ZIP), and zero-inflated negative binomial (ZINB) regressions. Zero-inflated regression types employ an additional logit model which represents the probability that a tick is ineligible to be part of the questing population. The count and logit components of ZIP and ZINB regressions work in opposition, where higher count model coefficients result in a larger number of expected ticks and higher logit model coefficients result in a smaller number of expected ticks. Variables displaying coefficients with the same sign in the count and

logit models therefore are discordant in outcome, while variables displaying coefficients with the opposite sign in the count and logit models are concordant in outcome. For each life stage, the regression type with the lowest AIC value and variance inflation factor ($\hat{c}$) (Cox and Snell 1989, Burnham and Anderson 2002) closest to one was chosen for subsequent analysis. AIC tends to be biased towards more complicated models (Hurvich and Tsai 1989). For this reason, zero-inflated models were disregarded if a less complex regression type (i.e., NEGBIN1, NEGBIN2, POI) exhibited similar AIC and $\hat{c}$ values. Regression types where $\hat{c}$ was much less than one or much greater than four were usually excluded, as these situations indicate underdispersion and overdispersion, respectively (Burnham and Anderson 2002).

Selected regression types were used to generate 127 candidate models for each life stage, incorporating all possible combinations of the seven variables. Models within each life stage were compared using eight model selection criteria: AIC, Kullback information criterion (KIC) (Cavanaugh et al. 2003), "corrected" versions of AIC (AICc) (Sugiura 1978, Hurvich and Tsai 1989) and KIC (KICc) (Cavanaugh et al. 2003), and "quasi" versions of the four previous criteria which adjust for overdispersion (Lebreton et al. 1992, Hurvich and Tsai 1995, Burnham and Anderson 2002, Kim et al. 2014). The best selected models were validated with residual plots.

Relative importance of variables in explaining the activity of each life stage was quantified using averaged composite weights $w_+(j)$ (Burnham and Anderson 2002). The Akaike weights (w_i), reflecting the optimality of each individual model, were calculated across all 127 models as described by Burnham and Anderson (2002). The composite weight $w_+(j)$ for a given variable was then calculated by summing Akaike weights of all models which included that variable. After repeating this procedure across all eight selection criteria, the resulting composite weights for each variable were averaged. The averaged composite weights closest to one indicate the greatest relative importance of a variable in explaining activity for a given life stage.

RESULTS

From February, 2007 to December, 2013, more ticks were collected in the forest site (1,426 adults, 4,876 nymphs and 29,945 larvae) than the field site (368 adults, 1,020 nymphs and 9,219 larvae), though total field counts surpassed forest counts for nymphs in 2009 and for larvae in 2010 and 2011 (Figure 1). The average number of adult, nymphal, and larval ticks per year was 203.7 (183.9 sd), 696.6 (657.7 sd), and 4,277.9 (4,432.0 sd) in the forest site, and 52.6 (21.1 sd), 145.7 (71.6 sd) and 1,317 (1,307.8 sd) in the field site, respectively.

Adult and nymph activity generally began concurrently in March or early April. Adults, however, usually became inactive before the end of July, while nymphs continued questing at marginal rates into September. Nymphal activity often displayed a distinct, albeit small, peak in August or early September. While most larval activity occurred between early July and October, small numbers of larvae questing before June accounted for 0.12% and 0.62% of total larvae observed

in field and forest sites, respectively. Cumulative degree days (over 60 days) were generally at a maximum during peak larval activity and near a minimum during peak adult activity (Figure 1). Cumulative degree days over ten days, the selected duration for nymphs, alternatively ranged from low values at the onset of nymph activity in spring to high values at the end of nymph activity in early autumn (Figure 1).

Selected regression types were NEGBIN2 in larvae (AIC = 1554.42; $ĉ$ = 1.43), NEGBIN1 in nymphs (AIC = 1306.53; $ĉ$ = 0.81), and ZIP in adults (AIC = 1177.20; $ĉ$ = 3.81). No models yielded $ĉ$ values between one and four in nymphs, so the nearest regression type, NEGBIN1, was selected. AIC and $ĉ$ values for NEGBIN2 were close to those of ZINB in larval modeling, so NEGBIN2 was selected due to its relative simplicity. ZIP was selected in adults, as no other models displayed $ĉ$ values between one and four.

As the eight selection criteria generally displayed similar rankings of candidate models, one model was designated as optimal in each life stage. For larvae, the same four-variable NEGBIN2 model was selected as best by all criteria except QKIC and QKICc, for which it was second-best by a very small margin (ΔQKIC = 0.31; ΔQKICc = 0.42). A six-variable nymph NEGBIN1 model similarly was best across seven criteria and second best according to QAIC (ΔQAIC = 0.14). In adults, criteria unanimously selected the ZIP model which included all seven variables.

For the explanatory variables included in each life stage model (Table 1), positive coefficients indicate that increases in environmental variables are associated with a larger number of active ticks (natural log of the mean expected count), while negative coefficients indicate the opposite. Average composite weights, which represent the relative importance of each variable in explaining tick activity, differed across larvae, nymphs, and adults (Table 1). Cumulative degree days, habitat, and lag from the previous life stage, however, were incorporated into all three best-fit models. Adult and nymph count equations exhibited qualitatively similar relationships with all variables except precipitation, which

Table 1. Beta coefficient estimates and average composite weights for variables included in the best-selected larval, nymph, and adult regression models. Larger average composite weights indicate greater importance of a variable relative to others across all candidate models for a given life stage.

	Variable	Coefficient estimate		Avg. composite weight
		Count	Zeroes[a]	
Larva	Intercept	-2.8476	-	-
	Cumulative degree days (60 days)	0.0041	-	1.00
	Cumulative precipitation (10 days)	-0.1276	-	0.81
	Saturation deficit	-	-	0.27
	Average wind speed	-	-	0.35
	Day length	-	-	0.29
	Lag from previous stage	0.0374	-	0.84
	Habitat	0.6910	-	0.67
Nymph	Intercept	-8.8271	-	-
	Cumulative degree days (10 days)	-0.0037	-	0.93
	Cumulative precipitation (30 days)	-	-	0.38
	Saturation Deficit	0.0447	-	0.88
	Average wind speed	0.0306	-	0.78
	Day length	0.0135	-	1.00
	Lag from previous stage	0.0014	-	1.00
	Habitat	0.4724	-	0.98
Adult	Intercept	-11.6337	13.0796	-
	Cumulative degree days (60 days)	-0.0021	0.0003	1.00
	Cumulative precipitation (10 days)	0.0447	0.2116	0.97
	Saturation deficit	0.0956	0.1448	1.00
	Average wind speed	0.0305	-0.1116	0.92
	Day length	0.0161	-0.0158	1.00
	Lag from previous stage	0.0070	-0.3411	1.00
	Habitat	0.6631	-0.5984	1.00

[a]Zero-inflated regression was not used in larval and nymph modeling.

was not incorporated in the nymph model. The count and logit models of adult ZIP regression displayed concordance (coefficients with opposite signs) in all variables except saturation deficit and cumulative precipitation (Table 1). Rising saturation deficit and cumulative precipitation are associated with a higher expected number of active adults according to the count model, but are also associated with a higher probability that ticks are not questing according to the logit model (Table 1). Seasonal trends of predicted activity were similar to that of observed activity in all three life stages (Figure 2, 3, 4). Additionally, model predictions generally captured the observed differences in magnitude of activity between field (Figure 2a, 3a, 4a) and forest (Figure 2b, 3b, 4b) habitats. Model residuals (Figure 2c-d, 3c-d, 4c-d) were usually minimal during periods of inactivity and modest during tick active season but were occasionally extreme when models mischaracterized the magnitude of a spike in tick activity. Specifically, a large underestimation of larval activity occurred for the field site in 2011 (Figure 2a, 2c) and for both habitats in 2013 (Figure 2), while a large overestimation of larval activity occurred in 2008 for the forest site. The nymph model notably underestimated forest activity in 2012 (Figure 3b, 3d), a year characterized by dramatic and distinct spikes in observed nymph activity throughout the season.

DISCUSSION

Our models demonstrate that broad-scale environmental variables are effective in describing patterns of off-host activity for *Amblyomma americanum* larvae, nymphs, and adults in northeast Missouri. Best-selected models generally performed well in tracking seasonal timing and magnitude of activity in both forest and field habitats. Furthermore, a distinct subset of variables was optimal for each life stage, providing insight into what physiological and demographic constraints most influence tick behavior throughout the *A. americanum* life cycle.

Seasonal patterns of *A. americanum* activity varied slightly across years and between habitats for each life stage but were similar to those described in southern Missouri (Kollars et al. 2000), Oklahoma (Hair and Howell 1970), central (Jackson et al. 1996) and southwest Mississippi (Goddard 2007), Georgia (Davidson et al. 1994), Kentucky, and Tennessee (Mount et al. 1993). Adult activity generally ranged from early March to late July and peaked by mid-May, while nymph activity ranged from March to early October and was most concentrated from May through July. As recorded in Mississippi (Jackson et al. 1996, Goddard 2007), Oklahoma (Hair and Howell 1970), and Kentucky and Tennessee (Mount et al. 1993), nymphs often displayed a secondary peak in activity during August or September, though the relative magnitude of this peak varies across years in Missouri. It is unknown whether this activity represents a resurgence of aged nymphs, or initial questing of newly molted nymphs which arose from summer larvae. However, low larval molting success (7.8%) during hot and dry July conditions in Oklahoma (Koch 1983) suggests that newly molted nymphs would not be prevalent until temperatures

cool. Larvae generally emerged in late July and early August, two to four weeks later than in southeastern states (Hair and Howell 1970, Mount et al. 1993, Davidson et al. 1994, Jackson et al. 1996, Goddard 2007). Very small numbers of larvae, however, were collected in March and April, an observation also made in Georgia (Davidson et al. 1994) and southwest Mississippi (Goddard 2007). Because development from female attachment to larval hatching requires approximately 84 days under laboratory conditions at 22-24° C (Troughton and Levin 2007) and longer under colder temperatures *in situ* (Patrick and Hair 1979, Koch 1983), it is unlikely that March and April larvae are progeny of adults which fed and mated in the same year. Therefore, we suspect that this activity instead represents larvae that have successfully overwintered.

These relatively consistent and widespread patterns in seasonality likely arise from the effects of winter temperatures on tick behavior and phenology. *Amblyomma americanum* is freeze-intolerant (Burks et al. 1996), especially in immature stages (Needham et al. 1996). While not shown to exhibit behavioral diapause in response to winter conditions (Stewart et al. 1998), *A. americanum* ceases all activity at temperatures below approximately 7° C (Clark 1995). Winter therefore limits the active season of nymphs and adults while killing almost all unfed larvae (Sonenshine and Levy 1971, Koch 1984, Foré and Kim unpublished data). This inability of larvae to overwinter allows for introduction of only one generation per year. As few nymphs that molt into adults resume host-seeking before winter quiescence (Semtner et al. 1973, Robertson et al. 1975), and low second-winter survivorship of adults (30%) and nymphs (2%) (Koch 1984) decreases the likelihood that unfed ticks join a younger cohort, distinct generations rarely coexist in the same developmental stage. It therefore seems probable that *A. americanum* progresses through its life cycle in relatively discrete cohorts. Maintenance of this cohort structure from year to year likely explains why the number of ticks collected over lag intervals proposed by Bouzek et al. (2013) was a relatively important predictor of activity in all three of our life stage models.

Cumulative degree days and day length were also useful in tracking seasonal patterns of *A. americanum* activity, but our models suggest that the effects of these variables differ across life stages. Temperature and photoperiod are known to directly influence tick behavior and developmental rates (Koch and Dunn 1980, Barnard et al. 1985, Pound and George 1988). Simulation studies suggest that climate-independent diapause plays a role in some but not all life stage transitions and may interact with climate-dependent tick activity and development (Ludwig et al. 2016). Because *A. americanum* does not seem to exhibit winter diapause (Stewart et al. 1998), adults and nymphs can begin questing immediately at microenvironmental temperatures above their minimum coordinated activity threshold – approximately 9.1, 10.2, and 12.3° C for females, males, and nymphs, respectively (Clark 1995). Warming temperatures therefore allow nymphs and adults to become active in spring when day length is high and cumulative degree days are low. Alternatively, larvae, due to poor overwintering success and long egg incubation period, do not hatch and become active until mid-late summer when

Figure 1. Environmental variables plotted over field (dotted) and forest (solid) *A. americanum* observed counts for larvae, nymphs, and adults from 2007 to 2013. Cumulative degree days was calculated over ten days (CumDD10) prior to sampling for nymphs and over 60 days prior for larvae and adults (CumDD60). Cumulative precipitation was calculated over 30 days prior to sampling for nymphs (CumPrecip30) and over ten days prior for larvae and adults (CumPrecip10). *Note: Scale of y-axis differs in each panel.*
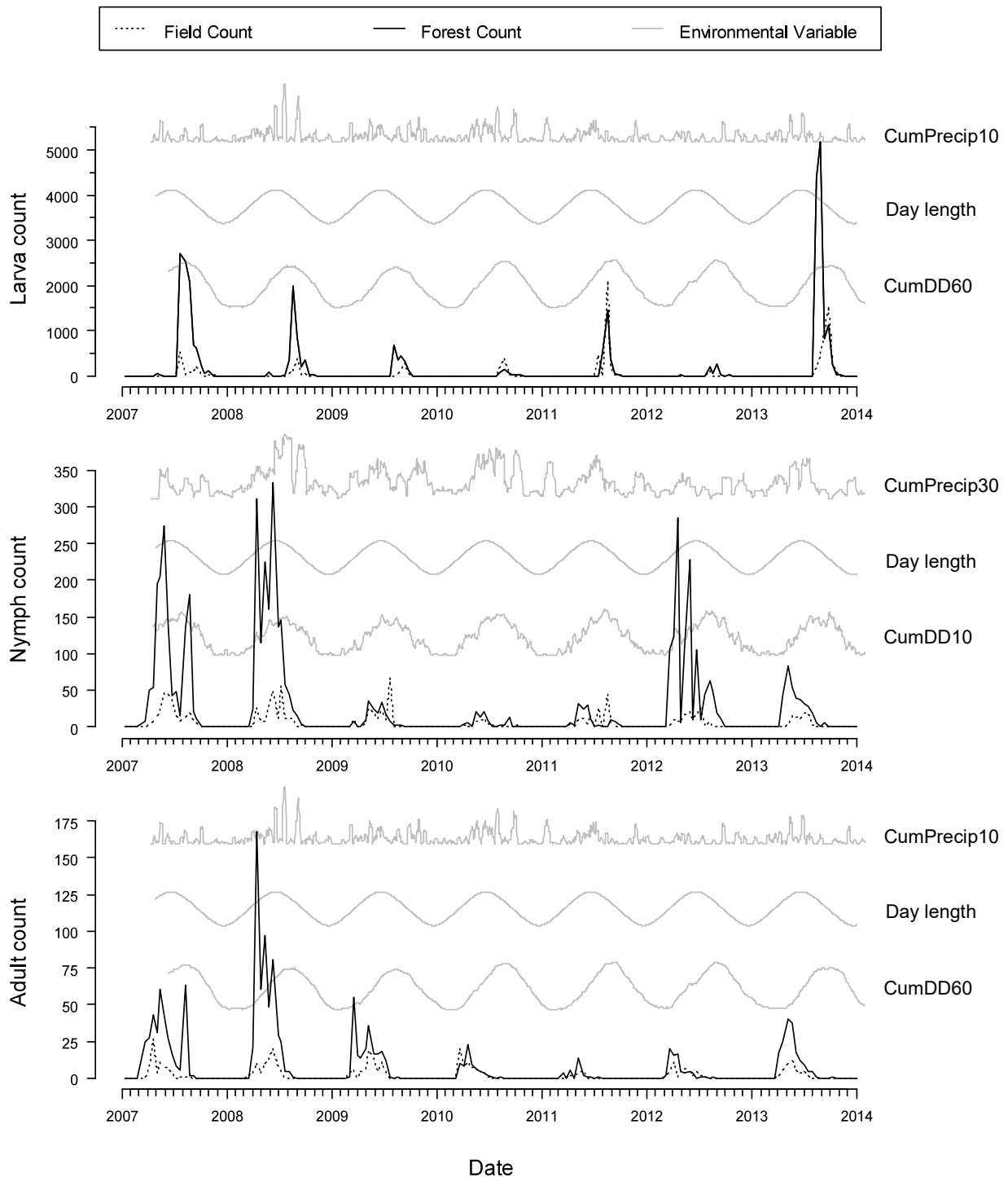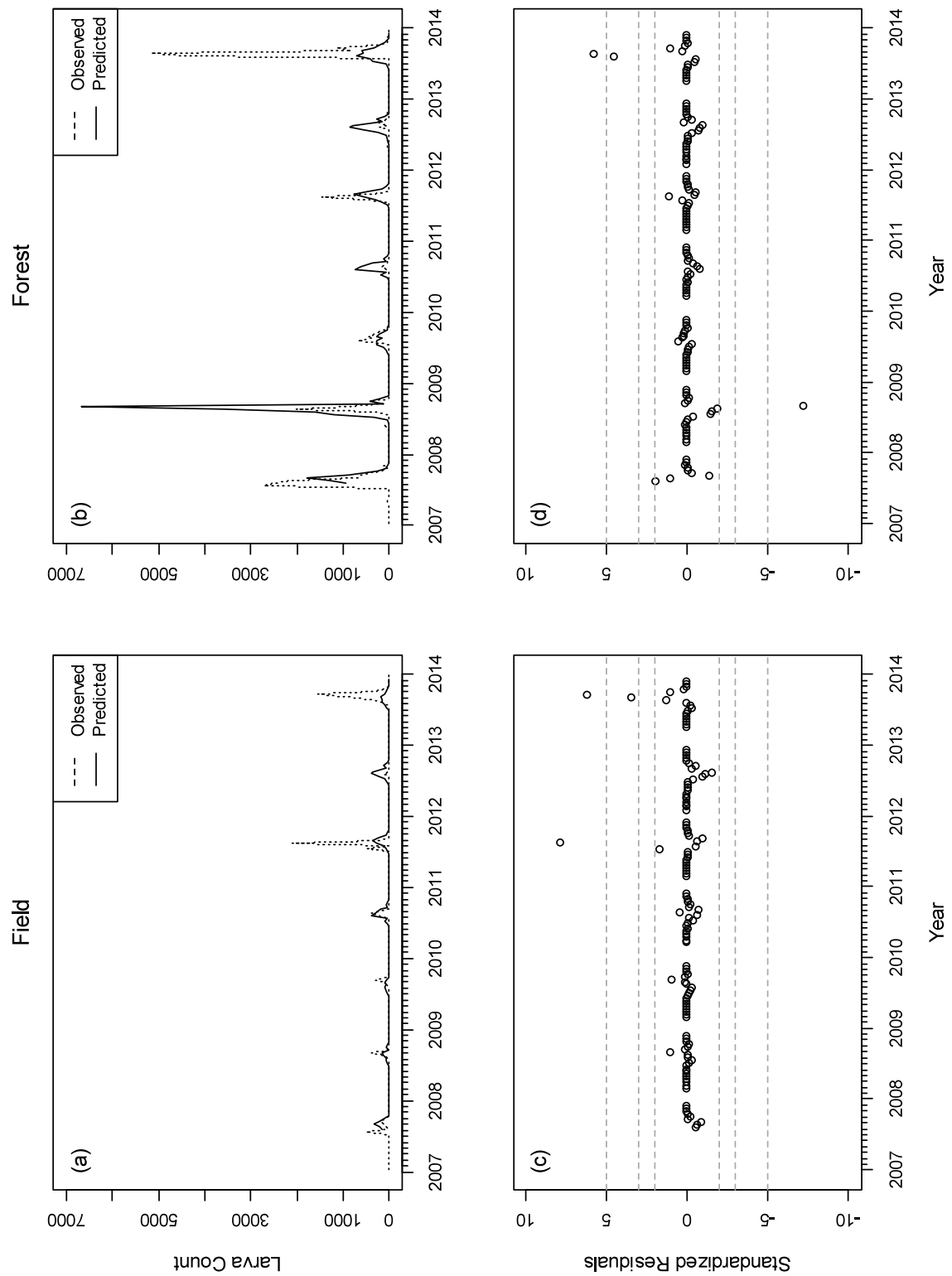
Figure 2. *Amblyomma americanum* larvae observed counts (dotted) and mean expected values (solid) for the field (a) and forest (b) habitats according to the best selected model. Field (c) and forest (d) model standardized residuals were calculated such that positive residuals represent model underestimation of activity and negative residuals represent model overestimation of activity. For reference, dashed lines are included at two, three, and five standard residuals from zero.
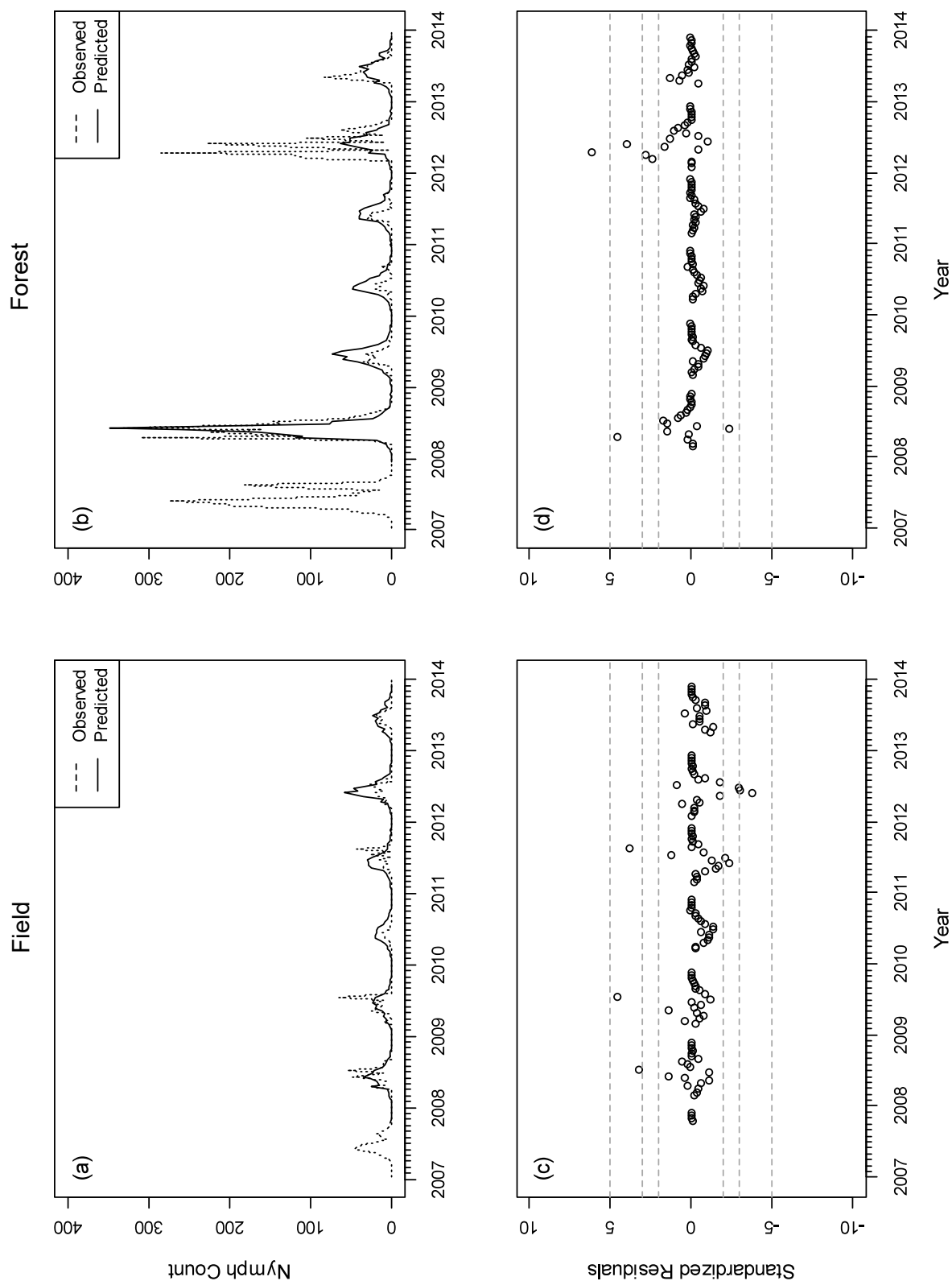
Figure 3. *Amblyomma americanum* nymph observed counts (dotted) and mean expected values (solid) for the field (a) and forest (b) habitats according to the best selected model. Field (c) and forest (d) model standardized residuals were calculated such that positive residuals represent model underestimation of activity and negative residuals represent model overestimation of activity. For reference, dashed lines are included at two, three, and five standard residuals from zero.
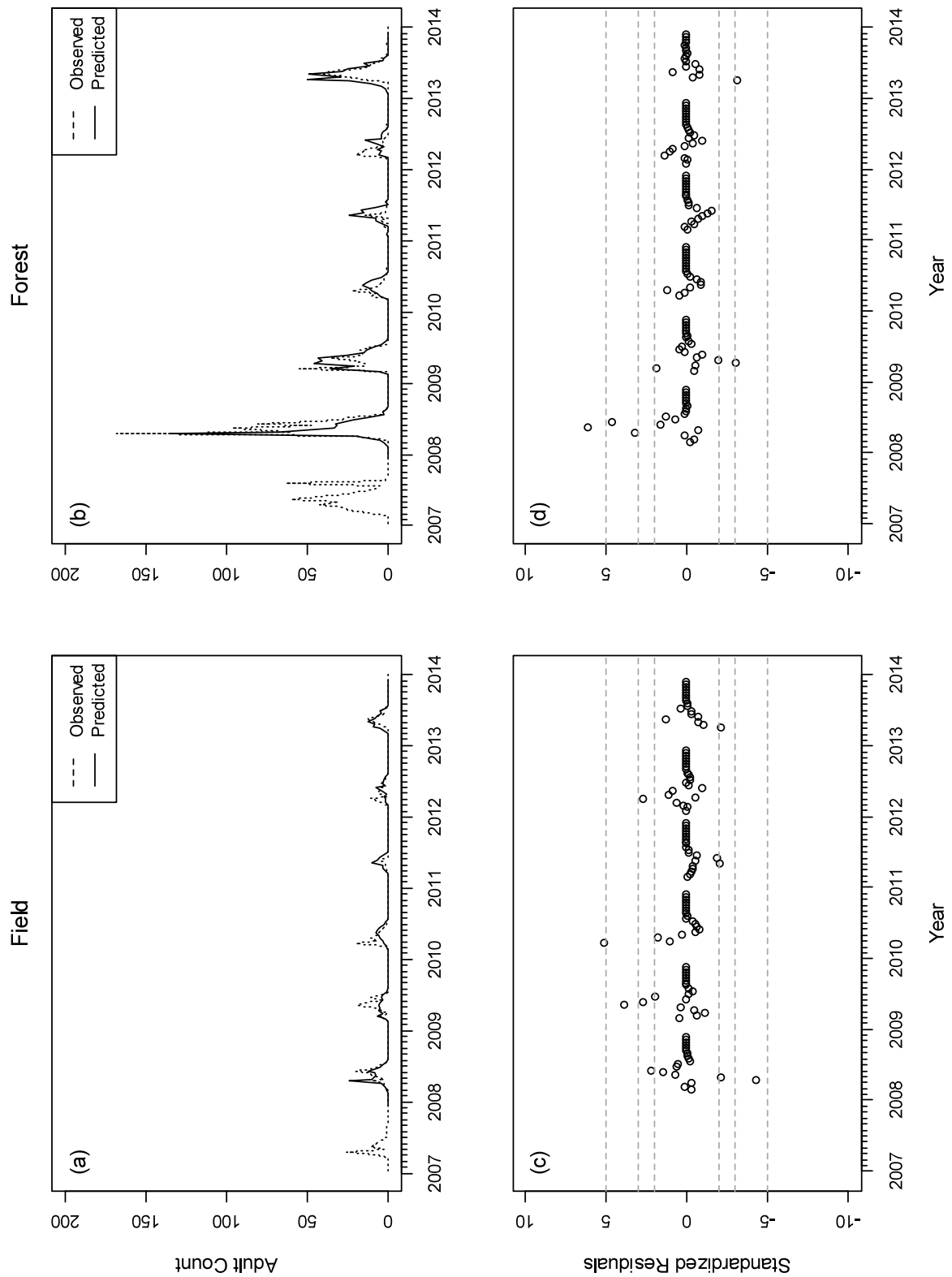
Figure 4. *Amblyomma americanum* adult observed counts (dotted) and mean expected values (solid) for the field (a) and forest (b) habitats according to the best selected model. Field (c) and forest (d) model standardized residuals were calculated such that positive residuals represent model underestimation of activity and negative residuals represent model overestimation of activity. For reference, dashed lines are included at two, three, and five standard residuals from zero.

cumulative degree days is high and day length is intermediate. Simulations (Ludwig et al. 2016) suggested that some engorged larvae may experience morphogenetic diapause and others show no diapause.

While seasonality of activity is largely shaped by low winter temperatures and day length, daily patterns in tick questing behavior are heavily influenced by the effects of microenvironment on water balance (Knülle and Rudolph 1982). When ticks climb vegetation to seek hosts, they often expose themselves to relative humidities below their critical equilibrium humidity (Knülle and Wharton 1964), meaning they exhibit a net efflux of moisture to the environment. Ticks must therefore quest strategically in response to microhabitat conditions in order to conserve water. While *A. americanum* exhibits a high adult critical equilibrium humidity, ranging from 80% to 88% (Sauer and Hair 1971, Hair et al. 1975, Jaworski et al. 1984), and larger whole-body permeability than some other *Amblyomma* species (Needham and Teel 1991), *A. americanum* adults can tolerate loss of almost 70% of their initial water pool before reaching a non-ambulatory state (M.D. Sigal 1990. PhD dissertation. The Ohio State University, Columbus). Though nymphs are more susceptible to dehydration (Yoder and Benoit 2003) than adults, both life stages quest at higher frequencies during dry midday hours (Schulze and Jordan 2003) and in shrub layer microhabitats with increased saturation deficit (Schulze and Jordan 2005). Our models reflect this pattern of questing in desiccating environments, as they indicate that nymph and adult activities tend to increase with higher wind speed and saturation deficit, both of which contribute to drying conditions. In contrast, however, Semtner and Hair (1973) observed considerable decreases in *A. americanum* activity during abnormally hot and dry mid-summer days. This observation, in addition to the discordance of saturation deficit in our zero-inflated adult model, suggests that adult and nymph activities only increase with saturation deficit up to a certain threshold, after which dehydrating conditions become too extreme for maintenance of host-seeking behavior.

Relative to nymphs and adults, larvae are very susceptible to desiccation (Koch 1983). While one would therefore expect precipitation to increase relative humidity and allow for more larval activity, high precipitation resulted in fewer active larvae and more active adults according to our models. The over-prediction of larvae by our model in 2009 in the forest site could likely be attributed to very heavy rains experienced in that year. Eggs (Yoder et al. 2012) and larvae (Koch 1986) can survive for many days while submerged in water, so flooding is unlikely to be lethal. Heavy rains and strong winds, however, can wash away over 90% of ticks in a larval mass (Hair and Howell 1970). As replete females show capacity to select oviposition sites which limit egg exposure to desiccating conditions (Patrick and Hair 1979), displacement to less favorable microhabitats could additionally reduce survivorship and questing success. Furthermore, as *Ixodes ricinus* exhibits significant aversion to movement over wet surfaces (Krober and Guerin 1999), persistent moisture on vegetation due to rainfall could restrict mobility and reduce host-seeking activity.

Habitat was an important variable in all three life-stage models, presumably because habitat moderates broad-scale meteorological variables to determine the quality and abundance of microenvironments available to questing ticks. Forest habitats have demonstrated higher levels of *A. americanum* activity (Sonenshine and Levy 1971, Semtner and Hair 1973, Bouzek et al. 2013, Kaizer et al. 2015), survivorship (Semtner et al. 1971, Patrick and Hair 1979, Koch 1984), and hatching success (Patrick and Hair 1979) than open meadows. Accordingly, our models indicated that larvae, nymphs, and adults were more active in the forest site than in the field site. Meadows generally display higher temperatures and lower humidities than forests (Semtner et al. 1971, Robertson et al. 1975), placing ticks at greater risk of desiccation. Lower observed questing heights in *Ixodes scapularis* (Vail and Smith 2002) and *I. ricinus* (Randolph and Storey 1999) nymphs under drying humidities suggest that ticks adjust questing strategy in direct response to these stressful microhabitat conditions. Furthermore, as lipid content of *I. ricinus* nymphs in dry questing arenas decreased at twice the rate of nymphs in wet arenas (Randolph and Storey 1999), heightened desiccation stress in field habitats may accelerate *A. americanum* energy consumption, ultimately reducing activity and longevity.

While models in all three life stages performed well in capturing timing of *A. americanum* active seasons, magnitude of activity was sometimes mischaracterized, likely due to stochastic events which were not accounted for by our selected environmental variables. For example, our model notably underestimated forest nymph activity in 2012. This year was characterized by an extreme drought which resulted in considerable declines of small mammal populations (Foré, unpublished), as well as an outbreak of hemorrhagic disease in white-tailed deer throughout Missouri (Stevens et al. 2015). Widespread declines in keystone host populations may have decreased questing success, ultimately inflating the number of host-seeking nymphs that we collected in our sampling grids. Abundance data for white-tailed deer and other important hosts, however, were not available at a temporal scale compatible with our models. Another extreme residual occurred in 2008 when, following the largest spike in forest adults observed throughout our data set, our larval model overestimated forest activity by a margin of thousands. This markedly low larval activity, relative to that of spring adults, occurred immediately after the most extreme period of rainfall observed throughout our data set. Beyond causing displacement of larvae and eggs, extreme rainfall may have increased mortality by bolstering growth of entomopathogenic fungi, some of which are known to decrease hatching success (Yoder et al. 2012) and disrupt water balance (Yoder et al. 2006) in *A. americanum*. In general, the larval model displayed large residuals more often than in nymphs and adults. Larvae exhibit extreme spatial aggregation *in situ*, often questing in groups of hundreds or thousands on a single piece of vegetation. While unconfirmed by genetic studies, the limited dispersal ability of larvae (Hair and Howell 1970) suggests that these groups hatched from a single clutch of eggs before migrating vertically to quest.

This aggregation adds considerable variation to our observed larval counts and likely reduces model precision.

By modeling all three life stages of *A. americanum*, we gain a more comprehensive understanding of how changing climate could influence population dynamics to affect vector potential in the future. Climate modeling predicts that the range of suitable *A. americanum* habitat will move northward as temperatures increase (Springer et al. 2015). Larvae in Georgia were observed during April and May (Davidson et al. 1994) in larger numbers relative to summer months than in our study, suggesting that larval overwintering success may be higher at warmer, southern latitudes. If overwintering success increases throughout the range of *A. americanum* due to changing climate, population dynamics could shift and ultimately affect pathogen transmission. For example, as larvae can molt quickly and with high success under mild June temperatures (Koch 1983), those which overwinter and feed on infected hosts in spring would emerge as molted, pathogenic nymphs well before environmental cues curtail nymph activity. This could increase pathogen transmission throughout summer and early autumn, effectively extending the disease season. Future studies should quantify overwinter survivorship and seasonality of activity for all three life stages of *A. americanum* across latitudinal clines to better understand how changing climate could affect risk of pathogen transmission.

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