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Title: Modeling the Dynamics of Vector–host Interactions in Avian Communities: Eastern Equine Encephalitis Virus as a Model System.

Abstract approved:

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Understanding the dynamics of seasonal epizootics of vector-borne pathogens infecting multiple host species presents several challenges. The transmission potential of competent hosts depends on factors influencing the contact rate between hosts and vectors. Feeding preferences of vectors can determine which host species drive the prevalence of infection throughout the overall population, although that species might be a tiny fraction of the competent population. To explore this, we developed a mathematical model for Eastern equine encephalitis (EEE) virus in bird-feeding mosquitos (Culiseta melanura) and perching birds in freshwater hardwood swamps in the Northeastern U.S. We examined the impact that vector feeding preferences, host community structure, and seasonal changes in host populations have on pathogen transmission. We measured strong feeding preference by Cs. melanura for wood thrush (Hylocichla mustelina) and common grackle (Quiscalus quiscula). We show that these feeding preferences result in increased prevalence of EEE in mosquitos and thus risk of spillover throughout the course of the season.

Modeling the Dynamics of Vector–host Interactions in Avian Communities: Eastern
Equine Encephalitis Virus as a Model System

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Timothy Muller, Author

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Introduction

Zoonotic pathogens, infectious diseases of animals that can be transmitted to humans, are a significant threat to human health throughout the world, making up an estimated 60% of known infectious diseases and 75% of emerging infectious diseases [1]. Predicting epizootics can be challenging due to the expense of data collection and the complex dynamics characterizing these systems [2]. Mosquito-borne infections cause millions of deaths each year and over 50% of the population of the world lives in areas with mosquito species that are known to transmit zoonotic pathogens [3].

Changes in host populations over the course of a season can lead to changes in vector feeding preference, which in turn may lead to a rise in spillover infections in humans [4]. In the case of West Nile Virus (WNV), increased vector feeding preference on certain preferred hosts is associated with an intensification of human cases of WNV [5]. Biodiversity among host species may also lead to a reduction in pathogen prevalence over the course of the season in certain systems [6]. In addition, there are numerous potential mechanisms through which host diversity can increase or decrease risk of infection in the hosts and spillover to humans. [7].

To examine the dynamics of a zoonotic pathogen in a complex host population, we analyzed a system where one vector species takes blood meals from multiple host species that are competent for transmission. As a prototypical example, we considered Eastern equine encephalitis (EEE) virus (Togaviridae, *Alphavirus*), a highly pathogenic mosquito-borne zoonosis that is responsible for occasional outbreaks of severe disease in humans and horses, resulting in high mortality and neurological impairment in most survivors [8]. In the Northeastern U.S., EEE virus is maintained in an enzootic cycle involving the bird-feeding mosquito *Culiseta melanura* and perching birds (Passeriformes) in freshwater hardwood swamps. However, the identity of key avian species that serve as principal virus reservoir, i.e. hosts that frequently harbor the virus, and amplification hosts, i.e. hosts in which the virus multiplies, has not been established [9–12]. Without knowing these key species, we instead examined the impact of the potential hosts present in the system.

One potential outcome of increased species diversity in a model system is known as the dilution effect. The dilution effect occurs when high host diversity dilutes the

impact of the principal virus reservoir, reducing vector–host interactions and subsequent transmission risk. This premise has been applied to tick-borne Lyme disease, but its application and relevance to other vector-borne pathogens has been questioned [13]. EEE virus occurs in freshwater swamps where host species diversity is relatively high, e.g. 99 avian species were encountered in one of our study sites. As a result of this high diversity of host species, we would expect to see low prevalence in mosquitos of EEE and thus low risk of spillover infections over the course of a year [7].

We built a mathematical model with many host species to examine the impact on pathogen transmission of vector feeding preferences, host community structure, and seasonal changes in host populations. We parameterized this model with data collected from field studies conducted in four historic EEE virus foci in Connecticut [14]. Using this model, we found that changes in vector feeding preference over the course of the season, namely the increase in preference for feeding on wood thrush, leads to increased virus prevalence in mosquitos. This leads to an increase in virus prevalence in all of the hosts, including larger increases in the hosts with higher feeding preferences. Most importantly, increased prevalence in mosquitos increases the risk of spillover infections to humans and horses.

Methods

Researchers at the Connecticut Agricultural Experiment Station collected female *Cs. melanura* mosquitos and analyzed their blood meals over the course of two years in four historic EEE virus foci in Connecticut, in the Northeastern U.S (Table 1) [14]. At the same sites and during the same time span, bird point-count surveys were performed to determine the composition of the host community.

Bird species	Count surveys	Blood meals
American robin	517	154
Chipping sparrow	200	61
Common grackle	462	77
Northern cardinal	253	59
Tufted titmouse	759	148
Wood thrush	62	206
Other species	3499	422

Table 1. Total bird counts and mosquito blood meals by bird species observed over 2 years at 4 study sites in Connecticut, U.S. [14]. Over 99 avian species were identified: species with minimal blood-meal data or count surveys were combined to form the "other species" class.

To estimate the size of the bird populations from April to September, we used bootstrap resampling and smoothing splines fitted to the bird-count data (Supplemental Material). We similarly used resampling and smoothing splines to estimate the number of blood meals taken by mosquitos from each bird species over time. These techniques provide sample estimates of bird counts and blood meals, allowing us to estimate their average behavior and variation around that average. Our transmission model simulates the dynamics of EEE virus in hardwood swamps by representing the epidemiological status of host bird species and mosquito vectors. Six avian host species (j = 1, 2, …, 6) were selected for high abundance in bird counts or as sources of blood meals: wood thrush, American robin, tufted titmouse, common grackle, chipping sparrow, and northern cardinal. The remaining bird species were combined into a seventh category (j = 7) of other birds. For each of the bird classes, we used a standard SIR sub-model [15], with each class's population divided into the Susceptible (S_j), infectious (I_j), and recovered (R_j). This yields 21 differential equations for the 7 bird classes: for j = 1, 2, …, 7,

$$\frac{dS_j}{dt} = \chi_j(t)(1 - \epsilon) - \lambda_b S_j - F_j(t)\frac{S_j}{N_j}$$

$$\frac{dI_j}{dt} = \chi_j(t)\epsilon + \lambda_b S_j - \gamma_b I_j - F_j(t)\frac{I_j}{N_j}$$

$$\frac{dR_j}{dt} = \gamma_b I_j - F_j(t)\frac{S_j}{N_j}$$

The χ_j and F_j terms represents the increases and decreases in host population i observed from the spline estimates(Supplemental Material). To minimize fadeouts

and the effect on pathogen dynamics due to the timing of the introduction of the pathogen, we assumed a constant small proportion of birds entering the population are infectious (ε). Hosts recover from infection at a rate of γ_b and the proportion recovered decreases due to the influx of new susceptible or infectious birds. Mosquitos enter the system by birth (b_v) and leave by death (d_v): we took these rates to be equal so that the mosquito population size is constant over the season. For the sake of simplicity, we assumed that vectors do not recover from infection. The mosquito population is divided into susceptible (S_v) and infectious (I_v) using an SI sub-model:

$$\frac{\mathrm{d}S_v}{\mathrm{d}t} = b_v N_v - \lambda_v S_v - d_v S_v$$
$$\frac{\mathrm{d}I_v}{\mathrm{d}t} = \lambda_v S_v - d_v I_v$$

Transmission of EEE from bird to vector is defined by the vector force of infection (λ_v) and occurs when a susceptible mosquito takes a blood meal from an infected bird. Transmission of EEE from vector to bird species j is defined by the host force of infection (λ_j) and occurs when an infected mosquito takes a blood meal from a susceptible bird. The force of infection is defined as the per-capita rate per unit time, or hazard, at which susceptible hosts or vectors become infected. Differences in vector feeding preference give rise to species-specific host forces of infection. The number of female mosquitos, their biting rate, the feeding preference, and bird abundances determine the rate of mosquito bites on each bird species. The proportion of female mosquitos that are infectious, the rate at which those mosquitos feed (ν), and the vector-to-host transmission rate (β_b) provide the force of infection for the bird species. Similarly, the proportions of each bird class that are infectious, the rate at which female mosquitos bite these infected birds, and the host-to-vector transmission rate (β_v) give the mosquito force of infection. Due to lack of comprehensive data we assumed that all selected bird species had the same host-to-vector and vector-to-host transmission rates. Thus, the forces of infection are

$$\lambda_j = \frac{\nu \beta_b i_v \alpha_j}{\sum_{k=1}^{n} \alpha_k N_k}$$
$$\lambda_v = \frac{\beta_v \sum_{j=1}^{n} \alpha_j \frac{I_j}{N_j}}{\sum_{k=1}^{n} \alpha_k N_k}$$

The feeding index α_j assesses the proportion of blood meals from host species j relative to the abundance of that species in the host community. In other words, the feeding index measures the likelihood of a blood meal on a given bird species per bird of that species in the host community [2,16]. To calculate the feeding indexes, the proportion of the blood meals that are on bird species j is

$$\alpha_j = \frac{f_j / \sum_k f_k}{N_j / \sum_k N_k},$$

where N_j is the number of birds of species j in the population and f_j is the number of blood meals on bird species j.

Using serological samples from a number of host species in upstate NY from prior EEE outbreaks [17], we estimated the final serologically positive fractions of the host populations over the course of a season. We found the value of the vector-to-host transmission parameter that gave the best model fit to these serological estimates.

Results

Over the course of a season, the population of tufted titmouse increased gradually; wood thrush, common grackle, and chipping sparrow populations fluctuate; and little change was observed in the American robin and northern cardinal populations (Figure 1). In particular, wood thrush was infrequently observed compared to the other species.
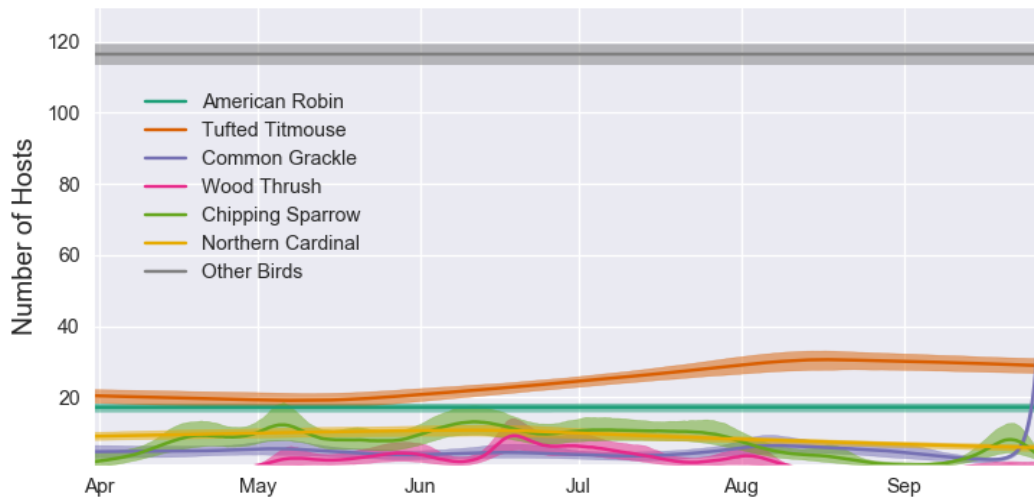

Figure 1. Estimated bird population sizes from April through September based on point-count surveys. Smoothing splines were used to connect the data at different times. Bootstrap resampling was used to estimate the variation in the data, which generated variation in the splines. The median values (solid lines) and the 95% confidence intervals (shaded regions) are shown.

The vector feeding preference is the proportion of feedings on given host species relative to that host's abundance in the host population. The feeding preference for each species fluctuates slightly during the early part of the season, with slight increases in preference in common grackle and slight decreases observed for tufted titmouse (Figure 2). In particular, wood thrush has a large increase in feeding preference late in the season despite having a very small population size.
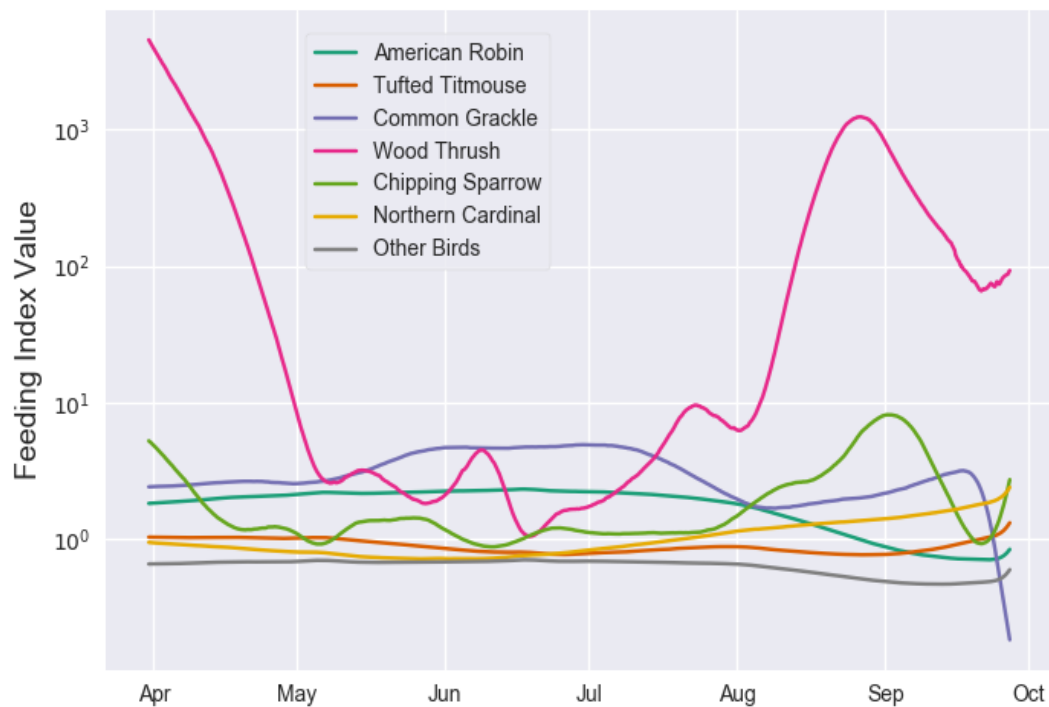

Figure 2: Feeding index for each selected host species over the course of the season.
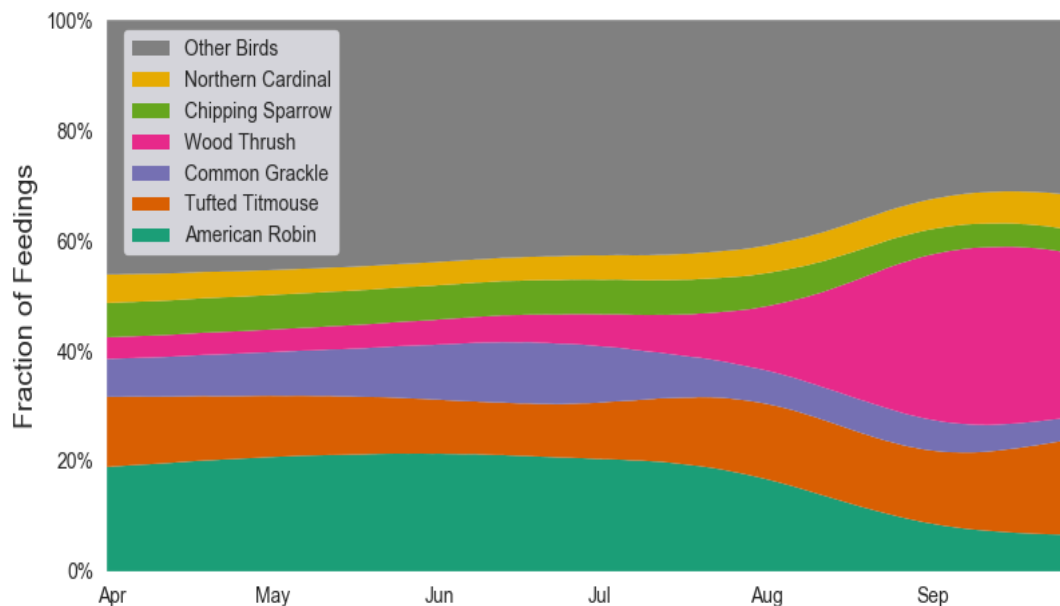
Figure 3. Fraction of feedings of *Cs. melanura* mosquitos on each host species over the course of the season.

Simulations of pathogen transmission show an initially substantial proportion of wood thrush infectious, which is due in part to the extremely small observed population at the start of the season (Figure 3). With the exception of an early spike in Wood Thrush, prevalence is near zero in each species until an increase in infections in wood thrushes begins in late July. Once this occurs, prevalence increases in the other species, most notably in common grackle, chipping sparrow, and northern cardinal. The resurgence of infections in wood thrush in late July occurs due to an increase in both the abundance of wood thrush and in vector feeding preference for wood thrush.
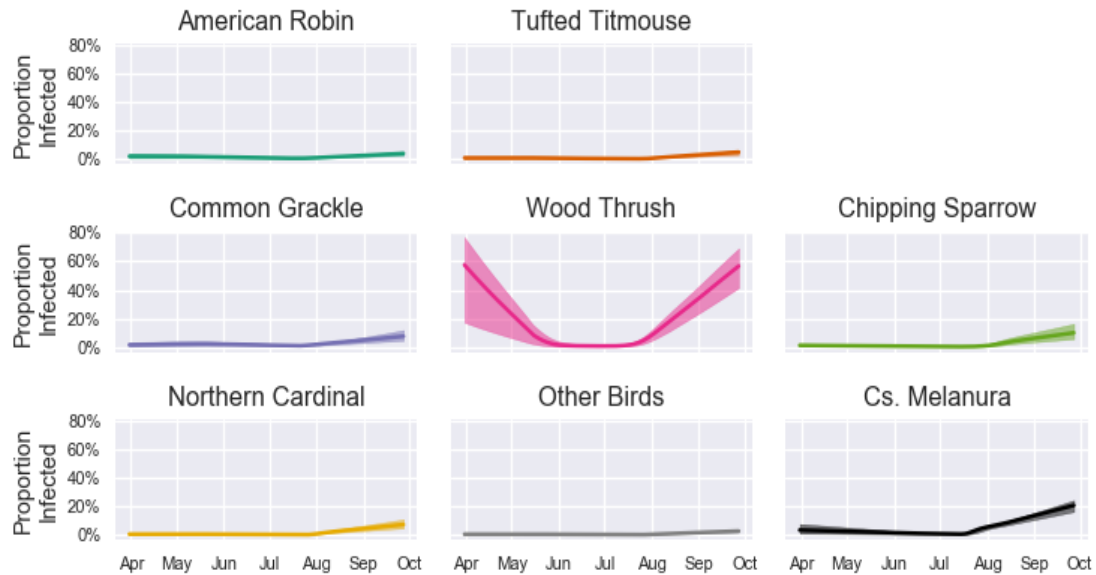
Figure 4. Simulated EEE infection dynamics in hosts and vectors over the course of the season.

We examined the impact of each selected host species by simulating EEE transmission with each host species removed. Removing wood thrush from the simulated bird community leads to no sustained transmission over the course of the season (Figure 4). Removing common grackle or American robin from the bird community caused small reductions in prevalence below that with all species present, while there was little to no change in prevalence when removing each of the remaining species.
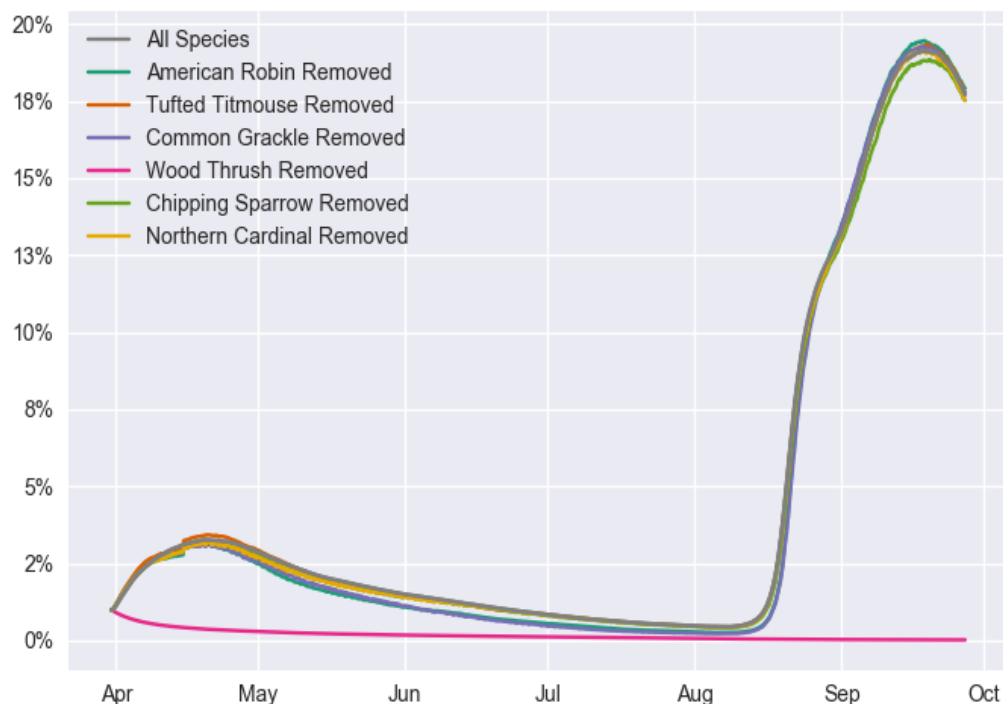
Figure 5. EEE prevalence in mosquitos over the course of the season. Included is the simulated prevalence for the model with all species present ("All Species") and with each host species removed.

Discussion

We examined the impact on EEE virus dynamics of temporal changes in the sizes of bird populations and in the feeding preferences of the mosquito *Cs. melanura* on those birds. Our transmission model demonstrated how feeding preferences for host species with small populations can lead to an amplification effect across all host species. We found that wood thrush, in particular, may effectively serve as a principal reservoir host that serves to amplify EEE virus toward the end of the summer and into early fall. Adult wood thrush have a molt period that extends from July through early October [18]. During this period they lose flight feathers, and some individuals drop primary feathers over a few days. This extensive molting impairs flight efficiency and makes them cautious and difficult to observe [18]. This molting period overlaps our observed increase in wood thrush feeding preference, suggesting that the wood thrush may be more susceptible to being fed upon during this period.

The resulting role that the small population of wood thrush play in the transmission dynamics of our model have broader implications for understanding the dissemination of vector-borne pathogens in species-rich host communities. The concept of the dilution effect suggests that increases in host diversity may lead to a reduction in disease risk due to the dilution of competent host species [6,14]. Our model demonstrates that EEE virus may readily amplify in areas with high host-species diversity due to preferential feeding on a few competent host species. Comprehensive data on the competence for EEE infection, replication, and transmission in various passerine bird species hosts is not available. One study suggests that the American robin is a competent host [19], but we found no data for the other host species in our study. Without this data, we assumed equal competence of all host species in order to determine which hosts might be driving forces of amplification. Comprehensive data on differential competence between host species could alter the outcomes of the model. Moreover, verification that wood thrush is indeed a competent host is needed to confirm its role as an amplification host.

In conclusion, we found that the preferential feeding of *Cs. melanura* on wood thrush drives the prevalence of EEE throughout the course of the season. The results of our transmission model suggest that despite wood thrush having a small observed population, vector feeding preferences can cause wood thrush to play a vital role in amplifying EEE virus. More broadly, these results show that changes in host abundance and in vector feeding preference can drive an increase in disease prevalence and spillover risk to humans and other incidental species.

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Supplemental Material

Our EEE transmission model was parameterized with data from avian point-count surveys and from the analysis of mosquito blood meals (Molaeli, 2016). For each of two types of data, we developed a probabilistic model for the data using elementary processes so that we could use likelihood-based methods to interpolate to times between samples and to use sampling to generate alternative datasets to estimate the uncertainty in our results. We used cubic smoothing splines for interpolation in time to generate a continuous-time estimates of bird species abundance and mosquito feeding preference that were then used in our transmission model. We explain in greater detail below these methods.

The avian point count surveys were conducted weekly from April to October, giving counts by bird species at each of 30 different times. We assumed that the avian counts followed species-independent Poisson processes. As a result, samples at each time for each species were generated from a Poisson process with mean equal to the corresponding count from the data. Mosquito blood meals were collected monthly from May to October and analyzed to determine the bird hosts fed upon. A total of 1120 blood meals were collected and analyzed. We assumed the mosquitos' choices of hosts on which to feed are independent from each other, and that at each given feeding event only one host is fed upon. As a result of these assumptions, we chose a multinomial distribution to model the number of blood meals on each bird species in a month. With this distribution we generated sample the blood meal counts at each time point with probabilities corresponding to the fraction of blood meals from each host observed at that time point.

We used constructed cubic smoothing splines to interpolate continuous curves in time for the bird counts and mosquito blood-meal counts. Let $\{t_i, Y_i : i = 1, \ldots, n\}$ be a set of observations, modeled by the relation $Y_i = f(t) + \epsilon_i$, where the ϵ_i are independent, zero mean random variables. For smoothing coefficient λ, the smoothing spline is the minimizer over spline parameters θ of the log likelihood of the probabilistic model for the data plus a term that penalizes rapid oscillations:

$$\hat{f}(t,\lambda) = arg\ \min_{\theta} nl(f(t,\theta) \mid d) + \lambda \int_0^1 |f''(t,\theta)| dt.$$

At the interior knots, the spline and its first and second derivatives are constrained to be continuous. We assumed that the splines were periodic with period 1 year to mimic the annual seasonality in bird populations.

We used cross-validation to determine the optimal smoothing parameter λ. Once we found the optimal smoothing parameter value, we sampled each distribution 1000 times, and for each of these samples we created a new corresponding spline with the new data and the original optimal smoothing parameter. With this collection of 1000 sampled splines, we generated median and confidence intervals for the distribution of these splines in time over the course of the season.

We then used each sampled spline to parameterize the host populations in our transmission model, giving us 1000 simulations of EEE transmission in the bird community. In particular we now had continuous in time population curves for each of the host species, and were able to calculate the rate of increase or decrease in time accordingly. These values would be used as the χ and F terms in our ODE models, respectively.

Using serological samples from a number of host species in upstate NY from prior EEE outbreaks, we estimated the final serologically positive fractions of the host populations over the course of a season. We found the value of the vector-to-host transmission parameter that gave the best model fit to these serological estimates.

The system of ordinary differential equations were solved numerically using with the SciPy optimize package in Python. The applicable files can be found at https://github.com/mullert613/Thesis.