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Ecological profiling of bird-mosquito interactions in Central Virginia

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ECOLOGICAL PROFILING OF MOSQUITO-BIRD INTERACTIONS IN

CENTRAL VIRGINIA

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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List of Abbreviations

Ae.	*Aedes*
An.	*Anopheles*
cm	Centimeter
CDC	Centers for Disease Control
Cx.	*Culex*
DEM	Digital Elevation Model
DNA	Deoxyribonucleic Acid
EEE	Eastern Equine Encephalitis
ESRI	Environmental Sciences Research Institute
GIS	Geographic Information Systems
GPS	Global Positioning System
hr.	Hour(s)
km	Kilometers
km^2	Square kilometers
m	Meter
MBP	Mosquito Borne Pathogens
NA	North America
NMT	Nest Mosquito Trap
PCR	Polymerase Chain Reaction
P. citrea	*Protonotaria citrea*
SAS	Statistical Analysis Software
SLEV	St. Louis Encephalitis Virus
U.S.	United States of America
USGS	United States Geological Survey
WNV	West Nile Virus

Abstract

ECOLOGICAL PROFILING OF MOSQUITO-BIRD INTERACTIONS IN CENTRAL VIRGINIA

By Anna Elizabeth Riggan, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2011

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Current methods of mosquito surveillance estimate general population abundances, but fail to represent the relationship of vector abundance to host density important to determining transmission risk of mosquito-borne pathogens (MBP). We sought to address this limitation by creating a novel mosquito trap that directly sampled mosquitoes seeking to feed on nesting birds.

The primary objectives of this study were to (1) assess the efficiency of the Nest Mosquito Trap (NMT) and how this is affected by nest box size. (2) assess whether the NMT affects bird, specifically nest success in Prothonotary Warblers (*Protonotaria citrea)*, and adult behavior. (3) compare our novel trap to existing methods. (4) profile the ecological parameters associated with bird/ mosquito interactions. Our results allow us to conclude that the NMT is not only an effective means of capturing host-seeking mosquitoes and measuring mosquito/ bird interactions, but does not have a deleterious effect on avian nesting success.

Introduction:

Mosquito-borne pathogen (MBP) surveillance involves monitoring the occurrence of pathogens in mosquito vectors, avian amplifying hosts, or accidental equine and human hosts (Newhouse *et al.* 1966; Leemingsawat *et al*. 1988; Cooperband *et al.* 2006). In addition to monitoring pathogen occurrence, understanding interactions among mosquitoes and hosts is critical to defining the risk of pathogen transmission and predicting potential epidemics (Kilpatrick *et al.* 2006; Hamer *et al*. 2009). Current methods of mosquito surveillance estimate general population abundances, an important variable in determining pathogen transmission risk, but fail to represent the relationship of vector abundance to host density (White *et al.* 2009). There are a number of zoonotic pathogens carried by mosquito vectors and amplified in avian hosts (Chevalier, *et al.*, 2008). These include 1) viruses such as, West Nile Virus (WNV), Eastern Equine Encephalitis (EEE), and St. Louis Encephalitis (SLEV) and 2) protozoan paracites such as members of the Plasmodium genus, which are responsible for numerous strains of malaria (Beadell, 2004). One of the central principles involving transmission of MBPs is the Dilution Effect hypothesis. This hypothesis relies largely on the competence of a host to amplify an agent. For the many of the previously listed pathogens (excluding the *Plasmodium* strains that cause malaria in humans), all wild birds are assumed to be competent, albeit to varying degrees. For example, Corvids such as crows and ravens have the greatest capacity for amplifying WNV, but their relative scarcity makes them a low impact host with regards to human infection (Hamer, 2009). Smaller birds exhibit competence for WNV to a lesser degree, but there abundance and proximity to humans makes them more important reservoirs. Mammals, such as humans, horses, and cervids, have been shown to be dead end hosts for the aforementioned pathogens (Kilpatrick, 2006). By that rationale, it is especially important to monitor mosquito abundance, reservoir abundance, and patterns of host/vector interaction.

In an attempt to more accurately document the interaction of mosquito vectors and nesting birds a novel mosquito trap was designed. This trap was designed to capture live mosquitoes as they attempt to feed on nesting birds including incubating adults and nestlings. A well established transmission model shows birds are the definitive hosts of a number of MBPs including West Nile virus (WNV), St. Louis Encephalitis (SLEV), and Eastern Equine Encephalitis virus (EEEV). In this model, the avian host is inoculated with the pathogen via the bite of an infected mosquito. Nesting birds may be especially important to transmission because, in contrast to roosting and foraging birds, there is reduced mobility for both the adults and the offspring (Blackmore *et al*. 1958). Additionally, the nestlings are vulnerable both in their sparse feather cover and immunological competence (Scott *et al*.1990). As a reservoir of the pathogen, many bird species amplify MBPs, regardless of evident disease symptoms (Cooperband *et al.* 2006; Leemingsawat *et al*. 1988; Newhouse *et al.* 1966).

In the summer of 2009, a prototype of a Nest Mosquito Trap (NMT) was tested on a Prothonatary Warbler (*Protonotoria citrea*) population in Central Virginia (Caillouet, *et al*, 2009). The benefit of this trap is that it targets ornithophilic (bird-seeking) mosquitoes in the process of host seeking. The primary objective of the present study is to evaluate the effectiveness of a second-generation redesigned NMT. Secondarily, I sought to understand the ecological interactions of mosquito vectors, avian hosts, and their environment.

While bird-mosquito interactions are central to WNV transmission and amplification, it is the host heterogeneity of *Culex pipiens pipiens* and midsummer shifts in feeding patterns that have been attributed to correspond to the seasonal timing of human WNV infection (Kilpatrick, *et* *al*. 2006; Hamer, *et al*.2009). In a study located in Chicago, Illinois, Hamer, *et al.* (2009) report that in the early summer, more than 80% of bloodmeals are taken from all avian hosts. Kilpatrick, *et al*. (2006) report that in New York City, NY approximately 51% of the total bloodmeals from May to July were from a single avian species, the American Robin (*Turdus migratorius*). In late-summer and early fall the proportion of bloodmeals taken from American Robins drops to approximately 30% as the avian nesting season comes to an end. Correspondingly, the rates at which *Cx. pipiens pipiens* feeds on human hosts increases by 6-fold from early to late summer (Kilpatrick *et al*. 2006). Thus understanding the interaction of mosquito vectors and nesting birds is critical to understanding the transmission dynamics of WNV or any MBP that is amplified by birds. To my knowledge the NMT is the first such live mosquito collection device to document vector-host interactions on nesting birds.

Live mosquito collection devices employ various strategies (usually baits) to selectively target one or more species often at specific life stages (e.g. host-seeking or oviposition seeking). The most often used mosquito collection devices include CO_2-baited CDC Light trap, and the CDC Gravid trap (Slaff, *et al*., 1983; Reiter *et al.* 1986; White *et al.* 2009). While the CDC Light trap underestimates the abundance of *Cx. pipiens pipiens*, the primary vector of WNV, it is the gold standard for collecting a general distribution of host seeking mosquito species (Slaff, *et al*., 1983; White, *et al.*, 2009). The gravid trap is most effective in capturing gravid *Cx. pipiens pipiens* searching for a suitable place to lay their eggs (Reiter, *et al*, 1986). Traditional traps use dry ice, lactic acid, octenol, or live animals to attract mosquitoes (Newhouse, *et al.*, 1966; Leemingsawat, *et al*., 1988; Canyon, *et al.*, 1997).

The capture mechanism of the NMT is a gentle suction created by a rotor fan attached near the entrance of modified nest box. The intent is to draw up ornithophilic mosquitoes seeking to feed on the nest box occupant(s), and collect them intact in a mesh reservoir at the base of the trap. The Nest Mosquito Trap differs from traditional baited traps in that it employs an unrestrained host (Canyon, *et al*., 1997; Darbro, *et al.*, 2006; Griffing,*et al*., 2007; Caillouet, *et al*, 2009). It also preserves the differential feeding patterns between adult birds and nestlings (Blackmore *et al*. 1958; Scott *et al*.1990). Ensuring that the NMT does not alter nesting conditions and behaviors is of primary importance to accurately describing mosquito/nesting bird interaction. As a consequence, avian behavioral monitoring was important in determining the efficacy of the NMT.

Potential effects of NMT on avian behavior

Nesting bird behavior may be disturbed in this study by the same variables as the mosquito vectors, namely sound, changes in airflow, visual, and human interference. The measures of avian behavior change are rates of nest abandonment in the absence of signs of predation.

A study of the effects of research handling, including weighing and banding, on American Robin nesting success did not show a significant change in nest abandonment or chick survival (Ortega, *et al*., 1997). Additionally, a 2003 report from the *Alaska Bird Observatory* studied the effect of military fly-over noise on 28 species of nesting birds and found that nest abandonment rates for the fly-over site were not significantly different from the control site (Rozell, 2003). A study on the effect of urban noise pollution on the amplitude and frequency of bird song did show differences among species with respect to their likelihood to adapt their song (Hu, *et al*., 2010). Though prior reports document a variety of outcomes due to various nest disturbances, this study secondarily aims to determine whether the operation of the NMT has an effect on nesting success.

Taken together the ecological parameters that govern vector/host interactions are responsible for the intensity of MBP transmission. Some of these parameters include local vector and host abundances, spatial aggregation of hosts, and host choice. The effect of local vector and host abundance on transmission intensity has been well studied. Often (but not always) MBP transmission intensity displays a positive linear relationship with vector abundance. Conversely, host abundance may display a negative linear or non-linear relationship with transmission intensity. Less studied is the role of the spatial aggregation of hosts. Many bird species including the American Crow (*Corvus brachyrhynchos*) and European Starlings (*Sturnus vulgaris*) roost in large flocks. Host aggregation has been experimentally shown to dilute the mosquito biting rate thereby potentially dampening MBP transmission intensity (Foppa *et al*. *in press* 2011). Finally, the role of host choice by the vector may affect MBP transmission intensity. Certain bird species, such as the American Crow, are highly susceptible to WNV and their mortality is often used as indicators of WNV presence. Though the American Crow may easily succumb to WNV, due to its short course of fatal infection, it may not be an important amplifying host of the pathogen. Birds such as the American Robin that readily develop WNV infection and sustain high viral titers for longer periods of time are hypothesized to be more important to the transmission of WNV. Consequently the choice of bird species that a mosquito feeds on likely has a significant effect on MBP transmission. If a mosquito displays selectivity in its feeding preferences for a competent host, the result may be efficient local transmission of the pathogen. Likewise a transmission dampening effect may result from selective vector feeding habits. Finally, heterogenous host feeding (non-selectivity) likely reduces transmission intensity, but may spread avian MBP pathogens to other non-definitive hosts including humans. I sought to use Nest

Mosquito Trap as a novel tool to assess previously unstudied parameters of vector-host interactions such as host selectivity, effect of biomass, and timing of nest initiation.

Accordingly, I attempted to meet the following objectives in this study:

Objective 1: Assess the efficiency of the NMT and how this value is affected by nest box size.

Objective 2: Assess whether the NMT affects birds (specifically nest success [in PW] and adult behavior.

Objective 3: Compare the NMT to existing surveillance collection methods.

Objective 4: Using the NMT to assess ecological parameters of bird-mosquito interactions. Specifically, we determined if mosquito burden changed as a function of (1) season (early versus late), (2) bird species, and (3) clutch biomass, and then used this information (along with trap efficiency [objective 1]) to estimate mosquito burden per nestling. (4) Identifying the effect of elevation and slope as a function

Methods:

Trap construction:

The Nest Mosquito Trap is a continuously operated suction device with a collection bag attached on the side and near the top of a nest box (Figure 1). The trap is designed to draw in mosquitoes entering and presumably seeking to feed on the nest box occupant(s), and collect them intact in a mesh reservoir (24 holes per in^2) at the base of the trap. The NMT is composed of opaque, polypropylene box (17.8 x 12.7 cm) with a circular, threaded portal at one end (diameter=10.8cm) for attachment to the nest box and for insertion of drawstring mesh collection bag (13.5 x 11.5 cm). The polypropylene box was painted black to occlude sunlight that may disturb the nest occupants and destroy the insects collected. The trap's suction is provided by a

12.0cm 12 v (5.1 w) direct current fan (Sunon Inc. product number: MEC0381V2-0000-A99) rated for 2600 RPM mounted on the opposite side of the polypropylene box to the collection bag. A sealed gel 12 v 12Ah rechargeable battery (Tempest Inc.) provided power to the fan.

Objective I: Laboratory assessment of NMT efficiency

Colony establishment

We collected *Culex pipiens pipiens* egg rafts from storm-water drop inlets at various locations around Richmond, Virginia. Single egg rafts were put into one gallon of ultrapure filter (Millipore) water until hatching. First instar larval density was then controlled by ensuring only 100 larvae per gallon of ultrapure water. Controlling the larval density ensures even distribution of food and space as well as a consistent size among the emerging adults. The larvae were fed a solution of 3 parts bovine liver powder (Sigma) and 2 parts Brewer's Yeast (Twinlab); adults were fed 10% sucrose, water solution (Vrzal 2010). Mosquitoes were held at 37^{o}C for all stages of development.

NMT laboratory testing parameters

In order to determine the overall capture efficiency of the NMT and the effect of nest box size as laboratory test was performed using the colony raised mosquitoes. Female mosquitoes were tested 24-72 hrs after emergence. Two nest box sizes were used in the test a small box (8 cm x 15 cm x 26 cm) and a large box (11 cm x 15 cm x 26 cm). For each testing replicate, ten female mosquitoes were manually aspirated into a sealed funnel affixed to the entrance of the nest box and allowed to recover before the trap was started. Once the barrier was removed, the trap was allowed to run for 5 minutes. The mosquitoes were allowed to enter naturally (via walking or flying) rather than being blown in order to reflect the natural movement of the vectors and the likelihood of entry in a field setting. The numbers of mosquitoes entering and the number captured were recorded. Test results were not included unless all 10 mosquitoes could be accounted for at the end of the test. The test was repeated 38 times for each box size.

The efficiency of the trap was calculated using the following formula:

$$\text{efficiency:} \quad \frac{\#\text{captured}}{\#\text{mosquitoes entered}}$$

The proportion of mosquitoes entering the nest box that were captured was compared between the two box sizes (α=0.05) via a logistic regression (SAS 9.2, 2009).

General Field Methods:

Site Descriptions

We collected field data at three sites located along the James River in Charles City County, Virginia were used in this study. The first is The Inger and Walter Rice Center for Environmental Life Science (*-77.204117, 37.325558*) (Figure 4), a Virginia Commonwealth University field station located on 494 acres, with a range of habitats, including riparian, wetland, forest, and meadow. The second site (Green) was a private estate located approximately 4 miles west of the VCU Rice Center (*-77.242173, 37.368619*) (Figure 5), and the third site was also a private estate (Wilson), located near the southern boundary of Henrico County, Virginia *(-77.2355701, 37.5288096)* (Figure 6). In addition to these sites, nest apparatuses were also placed at 89 separate locations in collaboration with the Henrico Standing Water Initiative (HSWI) (Figure 7).

Nest apparatus placement

Two nest apparatuses were constructed for this study (platform and box) and modified so that the Nest Mosquito Trap could be attached to one side. Nest platforms were constructed to attract stick nest builders such as American Robins (*Turdus migratorius*). Additionally, two sizes of nest boxes were made to accommodate cavity nesters such as Eastern Bluebirds (*Sialia sialis*) and Prothonotary Warblers (*Protonotoria citrea*). In accordance with the parameters of the ongoing, long term Prothonotary Warbler project initiated by C. and L. Blem in 1986, nest apparatuses were placed ≥20 m apart (Bulluck and Viverette, personal communication). Most nest boxes were installed between 5 and 6 feet off the ground or water on either tree trunks or metal poles. Many nest platforms were also mounted on 6-ft poles set into the dense thicket favored by stick nest builders, but others were installed on the sides of buildings sometimes as high as 10 feet off of the ground (Hoover 2006; Blem and Blem 1994; Blem and Blem 1992). For the Henrico Standing Water Initiative sites, one platform and one small nest box were placed at each location no less than 10 feet apart (Table 1).

During nesting season, all of the nest boxes that had been placed by investigators were surveyed for occupancy, developmental stage and age of offspring, number of offspring, and avian species (Tables 1).

NMT deployment and retrieval:

We deployed NMTs between 1230 and 1730 hours and retrieved the following day between 0930 and 1230 hours and they were operated continuously in the interim. Nest Mosquito Traps were retrieved in the same order they were deployed to ensure approximately equal running time. Any captured mosquitoes were frozen until a morphological ID could be performed (Slaff and Apperson 1989). *Culex pipiens pipiens* and *Cx. restuans* were recorded as a single species due to the difficulty in distinguishing between the morphological characteristics of these closely related species (Jackson *et al.* 2005).

Objective II: Effect of NMT on nesting success and avian behavior

Nesting success

One important consideration for accurate collection of mosquitoes using the Nest Mosquito Trap is to ensure that avian nesting behavior is not adversely affected by the presence and operation of the trap. In order to assess the NMT effect on avian nest success, Prothonotary Warbler nest survey data from the Virginia Commonwealth University Inger and Walter Rice Center *(-77.204117, 37.325558)* and a control site, approximately 4 miles *away (-77.242173, 37.368619)*, were compared to determine if the rate of survival of nestlings to day 5 (D5) was different between the two sites. All subjects nested in boxes provided by the investigators. Nestlings surviving until day 5 were considered a success, regardless of whether they were confirmed to have fledged.

The parameters used to assess nestling success were based on the well documented breeding cycle of the Prothonotary Warbler. Prothonatory Warblers lay one or two clutches of 4-6 eggs over a nesting season (Petit 1999). Once the first egg is laid the subsequent eggs are laid at a rate of one egg per day until the clutch is complete (Petit 1999). The female does not begin incubating the eggs until the last egg is laid and the nestlings typically hatch 12 days later (Petit 1999). Nestlings stay and develop in the nest for 9-10 days (Petit 1999),and Day 5 nestlings were considered a measure of success because they are half way through the development and are at their highest rate of growth, having passed their inflection day for growth (Podlesak and Blem 2002). Nests were considered to have failed if one of the following situations was seen: (1) a female is never documented incubating the eggs, (2) none of the eggs hatch, or (3) boxes with nestlings younger than D5 were found empty.

A Fisher's Exact test (α=0.05) was then performed to determine if the rate of PW nest failure in is significantly different between the NMT treatment site and a nearby control site, Presquile NWR (SAS 9.2, 2009).

Nest Abandonment

In order to determine whether the operation of the NMT had an immediate negative effect on adult nest attendance, surveys of adult attendance were taken at both the deployment and retrieval of the NMT. The abandonment survey accounts for all of the avian species sampled from for this study. The following parameters were used to determine the rate of nest abandonment in the presence of the NMT for all avian species and trap nights (Tables 9 and 10).

Attended nest: A nest is considered attended when the presence of an adult bird was visually confirmed within 24hrs of NMT deployment.

Abandoned nest: A nest is considered abandoned when there is no visual confirmation of the adult's presence within 24 hrs after the removal of the NMT.

Primary sample: A new clutch that is being exposed to a running NMT overnight for the first time.

Secondary samples: Repeated NMT deployments on the same clutch.

Due to the small number of new families (n=29), we were only able to sample 12 nests with eggs and 17 with nestlings during out primary sampling period (Table 9). There is evidence in the literature to suggest that eggs are more likely to be abandoned than nestlings so secondary samples on eggs were not taken (Hoover 2003). As a consequence, we conducted secondary sampling on nestlings only to minimize adverse effects on nest success in the early developmental stages. This allowed a larger sample size with nestlings to maximize our study of mosquito-nestling interaction. Nestling age was recorded relative to their feather cover, nude (no feathers), some feathers (mix of down and feathers), and many feathers (complete coverage, very little down) (Podlesak and Blem 2001). A summary of the proportion of nests abandoned was reported both for primary samples and overall (Tables 9 and 10).

<u>Objective III: Comparison of NMT to existing surveillance collection methods</u>

Proportion of NMT catches to CDC/Light and Gravid traps

One means of assessing the validity of the NMT as an MBP surveillance tool is to compare its catches with that of the traditional CDC light and gravid traps. Specifically, the proportionality of NMT catches to the combined CDC light and gravid trap catches to determine whether this ratio is consistent over the trapping season (Leemingsawat *et al*.1988; Griffing, *et al*, 2007; Caillouet *et al* 2009).

CDC light and gravid traps were set at the VCU Rice Center, Site Green, and Site Wilson on a weekly basis (Table 2). Both traps are suction devices that employ continuously operating 6v fans and mesh collection bags. Approximately 3 pounds of dry ice were used in each of the CDC light traps to draw all host seeking mosquitoes over the course of 15-18 hours. The gravid trap was inoculated with a mixture of 20 liters of water, 250 grams of hay, 250 grams of grass clippings, 30 grams of chicken manure, and 5grams of teaspoon of Brewer's yeast that had been allowed to ferment in a sealed bucket for no less than 24 hours (Cooperband 2008; White 2009). Cooperband (2008) established the attractiveness of chicken feces to gravid *Cx. quinquefasciatus*, while White (2009) effectively used fermented vegetation to attract gravid Culex mosquitoes. The proportions were then modified by the Henrico Standing Water Initiative and the investigator so that all ingredients from both of the aforementioned studies were incorporated. This gravid mixture provided an ideal environment for oviposition by gravid Culex mosquitoes.

The timing of trap deployment and retrieval followed the same schedule as the NMT trap nights, but never on the same night that the NMTs were deployed. CDC light and gravid traps were deployed at each of the three sites between 1230 and 1730 hours and retrieved the following day between 0930 and 1230 hours. The traps were retrieved in the same order they were deployed. Any mosquitoes captured were frozen at -20^{o}C in the laboratory until morphological identifications were performed (Slaff and Apperson 1989).

To assess the consistency of adult mosquito collections the trapping season was divided into 8 one-week sample periods. The number of trap nights for each individual week was recorded by trap type. The weekly mean number of mosquitoes collected was calculated by dividing the total number of Culex spp. mosquitoes captured by the number of trap nights for each trap type. Only trap weeks when both NMTs and CDC light and gravid traps were successfully deployed on the same site were used in the statistical analysis. A multiple proportion, chi-squared analysis was then performed to compare the mean catch composition for Culex spp.of the Nest Mosquito Trap with the combined numbers for the CDC Light and Gravid traps (α=0.05; df=2). The weeks during which at least one mosquito was captured for each of the trap types were examined (Table 13).

<u>Objective IV: Using the NMT to assess ecological parameters of bird-mosquito interactions</u>

Seasonal Effects of Nesting Bird-Mosquito Burden

To determine if the timing of avian nest initiation affects mosquito burden, I investigated mosquito burden across two time periods: Early and Late nesting season. The NMT trapping season began in 21 May 2010 and continued until 22 July 2010. To designate Early and Late trapping season the two month trapping season was divided into even halves. All trap nights between 21 May 2010 and 21 June 2010 were designated as the early season, while all trap nights between 22 June2010 and 22 July 2010 were designated as the late season (Figure 3). Only the sampling at the Rice Center was considered due to small sample size at the other sites. The effect of early (n=20) and late (n=31) season on mosquito burden was assessed using individual, log-transformed Poisson regressions (α=0.05) with *Cx. pipiens pipiens/restuans*, *Cx. salinarius*, *Cx. erraticus*, and Total Culex spp. as the dependent variables (SAS 9.2, 2009).

The Effect of Avian Species on Mosquito Burden

In order to determine whether mosquitoes prefer certain nesting bird species, Nest Mosquito Trap captures for *Cx. pipiens pipiens*, *Cx. salinarius*, *Cx. erraticus* were examined based on the avian host species. Due to the differences in sample sizes among the avian species a robust statistical analysis to determine whether certain avian species have a consistently higher mosquito burden was not possible.

The Effect of Clutch Biomass on Mosquito Burden

In order to further assess the interaction between mosquitoes and nesting birds, the developmental stage of the offspring was examined to determine if it was a factor in mosquito burden as reflected by the Nest Mosquito Trap. No mosquitoes were captured on eggs with an attending adult causing the focus to be turned to nestling age and size as a possible predictor of mosquito burden. Three passerine species were examined, Prothonotary Warblers (*Protonotoria citrea*; n=13), Wren spp. (*Troglodytes spp.*; n=6), and Eastern Bluebirds (*Sialia sialis*; n=10) between the dates of June 22, 2010 and July 22, 2010. Using nest survey data from boxes placed by the investigators, the age and number of nestlings present was determined (Rickefs 1968). The biomass for an individual nestling was then calculated using the following formula developed by Ricklefs 1968:

$(\text{mass (g)}) = A / (1 + e^{(-k*(t50 - to))})$

K=growth rate constant for a given avian species

T_{50}=day after hatching when the inflection point on the growth curve

T_o=Recorded age of nestlings

A=Asymptotic Weight =90% of adult weight

Clutch biomass was then calculated by multiplying the previous results by the number of nestlings present on the day a box was surveyed (Table14).

The effect of clutch biomass on mosquito burden was assessed using individual, log-transformed Poisson regressions (α=0.05) with *Cx. pipiens pipiens/restuans*, *Cx. salinarius*, *Cx. erraticus*, and Total Culex spp. abundances as the dependent variables.

Determination of nestling observed and estimated biting rates

Though total nest- mosquito capture is a convenient way to compare mosquito burden, many avian clutches differ in offspring number. Also, the *individual* bird biting rate is the central parameter in determining the intensity of avian MBP amplification. Since the NMT capture efficiency was establish in a controlled setting, the number of mosquitoes entering the NMT can be estimated from the number of observed mosquitoes. In order to estimate the mosquito biting rate, the per-nestling mosquito burden was calculated. Due to the exclusion of attending adults, the estimated mosquito biting rate refers only to the expected number of bites per nestling. (Tables 6-8; Figure 8). The following formulae were used to calculate the estimated biting rate:

Observed mosquito burden: $\dfrac{\text{\# mosquitoes captured}}{\text{\# nestlings present at time of capture}}$

Estimated mosquito burden: $\dfrac{\text{Observed biting rate}}{\text{Laboratory capture rate (0.383)}}$

A Poisson regression with a log transformation (α=0.05) was performed to determine if the estimated biting rate changed over the trap weeks. This analysis was repeated for with *Cx. pipiens pipiens/restuans*, *Cx. salinarius*, *Cx. erraticus*, and Total Culex spp. (SAS 9.2, 2009).

Spatial Modeling of Rice Center NMT Study

In order to determine spatial influences on bird/ mosquito interactions, topographical attributes such as elevation, slope, and distance to water were assessed for the investigator placed boxes.

First the XY coordinates for each individual box were recorded in the field using a global positioning device (Garmin Nuvi 2200); these coordinates were then loaded into an Arc10, Geographical Information Systems (GIS) for each study site (Figures 4-7).

A database was constructed in Microsoft Excel 2007 using the box coordinates in order to integrate average NMT catches by specific location and by individual mosquito species. This not only allowed visualization of overall mosquito burden by location, but also showed the distribution of these catches between *Cx. pipiens pipiens/restuans*, *Cx. salinarius*, and *Cx. erraticus* (Figure 7).

Use of digital elevation models to identify areas of highest bird-mosquito interaction

Due to the tidal changes associated with our riparian sites, elevation and slope were used as a function of distance from individual boxes to the nearest permanent water body, the James River, but as a means of identifying potential mosquito larval habitat in close proximity to the boxes. Digital elevation models (DEMs) for Charles City County, Virginia and Henrico County, Virginia, 10m resolution, were acquired from the United States Geological Service (USGS).

These DEMs and the shapefiles for box location were then loaded into Arc10 GIS. The slopes of the study sites were then calculated on a per-pixel basis, by first converting the DEM to an elevation raster using the Fill tool located in the Spatial Analyst Hydrology toolbox. The ESRI, elevation raster was then used to calculate the slope via the Slope tool located in the Spatial Analyst toolbox (percent rise; z-value=1). The elevation and slope value for each of the individual boxes was then identified by using the Extract Value to Points tool also located in the Spatial Analyst toolbox.

Once the per pixel values for slope were acquired a multi-ring buffer was placed at 5, 10, 15, and 20 meters around each of the upland boxes at the VCU Rice center. The buffer shapefile was then used to perform at Zonal Statistical analysis (by range) to determine if there were any sudden changes in slope within the 20 meter buffered area. Such changes might indicate a natural cupping of the land around the base of the nest box were water is likely to pool temporarily, forming an ideal larval habitat for *Cx. pipiens pipiens/restuans* . The proximity of such pools to nesting birds may help to predict mosquito-bird interactions (Figure 11).

The general proximity of the James River allowed us to use the elevation values for the investigator-placed nest boxes to estimate the distance to permanent water bodies, including the James River, Harris Creek, and Kimages Creek. A Poisson regression with a log transformation (α=0.05) was performed assess the effect of elevation on abundance of *Cx. pipiens pipiens/restuans*, *Cx. salinarius*, *Cx. erraticus*, and total Culex spp. in occupied boxes (SAS 9.2, 2009).

Results:

Objective I: Laboratory assessment of NMT efficiency

In order to determine the effect of nest box size on the capture efficiency of the NMT a laboratory test was performed using the colony raised mosquitoes. During the 38 replicates a total of 380 female mosquitoes were introduced to each of the two nest boxes. The Nest Mosquito Trap captured a mean of 38.3% (15.7 SE) of mosquitoes that entered the small nest box (n=38) and 32.1% (16.2 SE) of mosquitoes entering the large nest box (n=38) (Figure2). Though the logistic regression was not statistically significant (df=1; test statistic=2.75, P = 0.0974), the effect of nest box size is approaching significance with smaller boxes having a higher capture efficiency.

Objective II: Effect of NMT on avian behavior

Effect of NMT on Avian nesting success

In order to establish if the presence of the Nest Mosquito trap has a deleterious on avian nest success two sites were compared, one with NMTs deployed and a nearby control site, Presquile NWR. The site where NMTs were deployed actually had higher nesting success (Fisher's exact test, t = 4.25, DF=1, p-*value=0.028*), where 12 of the 13 (91.7%) sampled nests were successful compared to only 64.2% (93 of 145) at the control site. However, due to the small sample size (n=13) at the Rice Center it is not possible to say that the NMT has a positive effect on nestling survival.

Nest abandonment resulting from operation of Nest Mosquito Trap

In order to determine the rate of avian nest abandonment resulting from the presence of a continuously operating NMT, surveys of adult attendance were taken with each deployment and retrieval of the NMT. Due to the small number of new families (n=29) there was no secondary sampling on eggs to allow for both primary and secondary sampling on nestlings. Primary samples taken from eggs displayed no instances of abandonment across all of the species examined (Tables 9 and 10). The rate of abandonment for all primary samples taken on eggs was 0% (n=12). For all primary samples on nestlings the abandonment rate was 11.7% (n=17). The rate of abandonment for all secondary samples on nestlings was 5.5% (n=55).

Objective III: Comparison of NMT to existing surveillance methods

Comparing Culex spp. catch composition of NMTs to CDC/Light and Gravid Traps

CDC light and gravid traps were placed at the VCU Rice Center over 8 trap nights and 9 weeks. Of the1700 total mosquitoes captured, 1674 (98.5%) were *Culex* spp.: *Cx. salinarius* (1574; 92.7%), *Cx. erraticus* (58; 3.4%), or *Cx. pipiens pipiens/restuans* (40; 2.4%) (Table 12).

A multiple proportion, chi-squared analysis of the catch composition for Culex spp., was performed for each of 5 trap weeks to compare the Nest Mosquito Trap with the combined numbers for the CDC Light and Gravid traps (SAS 9.2, 2009). The weeks during which at least one mosquito was captured for each of the trap types were examined. Of the 5 trap weeks examined, 3 showed that the Culex spp. catch composition differed significantly between the NMT and the combined CDC light and gravid collections. For the remaining 2, trap weeks there was not a significant difference (Table 13).

Objective IV: Using the NMT to assess ecological parameters of bird-mosquito interactions

During the field collections a total of 154 mosquitoes (2.30 ± 1.40) were collected over 66 trap nights. Mosquito species collected included 111 (72.1%) *Cx. salinarius* (1.67 ± 1.13), 9 (5.8%) *Cx. erraticus* (0.14 ± 0.07), 33 (21.4%) *Cx. pipiens pipiens/restuans* (0.50 ± 0.25), and 2 (1.2%) *Aedes albopictus* (0.049± 0.049) (Table).

Seasonal effects on NMT mosquito abundance

A total of 12 (7.8%) mosquitoes were collected over 35 trap nights (0.35 mean ± 0.18 SE) before June 22, 2010. After June 22, 2010 a total of 142 (92.2%) mosquitoes were collected over 31trap nights (4.58 ± 2.95). A mean of 0.35 ± 0.18 Culex spp. mosquitoes were collected in the Early season while a mean of 4.58 ± 2.95 were collected in the Late portion of the season. The results towards the Late season were significant for the Total Culex spp. (*p-value*$<$0.002) and *Cx. salinarius* (Early=0.15 ± 0.08; Late= 3.39 ± 2.39) (*p-value*=0.024), indicating that there is a higher total mosquito burden in the later season. This trend of higher mosquito burden in the late season was visible for all of the individual species, but was not significant for *Cx. pipiens pipiens/restuans* (Early=0.05 ± 0.05; Late=0.97± 0.53) (*p-value*=0.9410) and *Cx. erraticus* (Early=0.15± 0.15; Late=0.16 ± 0.10) (*p-value*=0.9494) (Table 13).

Avian Species and Mosquito Burden

A survey of NMT catches was performed in order to determine whether certain mosquito species prefer feeding of some avian species over others. Over 66 trapping nights a total of 134 (87.0%) mosquitoes were collected from nest boxes occupied by Eastern Bluebirds, 4 (2.4%) from boxes occupied by Prothonotary Warblers, 5 (3.2%) from boxes occupied by Tree Swallows and 9 (5.8%) from boxes occupied by Wren spp. Though the data is inadequate for statistical comparison, the summary statistics suggest that Eastern Bluebirds (10.31 ± 6.85/mosquitoes per trap night) have a far higher mosquito burden than House Wrens (1.1 ± 0.57/ mosquitoes per trap night), Tree Swallows (1.00 ± 0.63/ mosquitoes per trap night), and Prothonotary Warblers (0.14 ± 0.09/ mosquitoes per trap night). Of the 33 *Cx. pipiens pipiens/restuans* captured with the NMT, 28 were captured on Eastern Bluebirds, 2 were caught on Wren spp., and 1 was caught on Prothonotary Warblers. Of the 114 Cx. salinarius captured, 99 were captured on Eastern

Bluebirds, 6 were caught on House Wrens, 3 were caught on Prothonotary Warblers, and 3 were captured on Tree Swallows (*Tachycineta bicolor*). Of the 9 Cx. erraticus captured, 7 were captured on Eastern Bluebirds, 1 was caught on Wren spp., 0 were caught on Prothonotary Warblers and 1 was captured on Tree Swallows. (Table 5).

Clutch biomass on mosquito abundance

In order to determine if nestling size influences mosquito burden, the total nestling biomass of the nest were calculated for 29 trap nights. This assessment was performed independently of avian species or seasonal influences on mosquito burden. Nestling age was also not a factor when only analyzing biomass because general body size, growth rate, and brooding time differ among avian species. The results were significant for the Total Culex spp. (*p-value*<0.001), *Cx. pipiens pipiens/restuans* (*p-value*=0.018), *Cx. salinarius* (*p-value*=0.002), and *Cx. erraticus* (*p-value*=0.031), indicating that there is a positive relationship between clutch biomass and mosquito burden.

A regression analysis was also performed to determine whether clutch biomass was correlated with trap week. A resulting R-squared value of 0.0338 indicates that these biomass results are independent of the previously reported seasonal influence on mosquito burden.

Nestling biting rate and estimation of total nightly biting rate

In order to account for the underestimation of mosquito burden found in the laboratory efficiency tests for the NMT, the observed field biting rates were corrected by the laboratory efficiency results. This correction retained the proportionality of mosquito burden by trap week, avian species, and season, but showed the estimated mosquito burden to be approximately more than 3X the observed field mosquito burden (Tables 6-8).

Spatial Modeling of Rice Center NMT Study

A Poisson regression was performed in order to see if there is a significant difference in mosquito burden for boxes placed upland and those placed closer to a permanent water body. The difference between the upland and water's edge boxes for the total mosquitoes captured were not significant (df=1; p-value=0.476). The differences between *Cx. pipiens pipiens/restuans* (df=1; p-value=0.601), *Cx. salinarius* (df=1; p-value=0.486), *Cx. erraticus* (df=1; p-value=0.319) were also not significant.

Discussion:

The abundance and infection status of mosquitoes seeking bloodmeals from nesting birds are primary components of determining the intensity of avian pathogens that are vectored by mosquitoes. Prior to the design of the Nest Mosquito Trap these metrics of MBP intensity were unattainable. The evidence provided in this study documents the efficacy of the NMT as a means of monitoring avian MBP transmission and for assessing complex vector-host interactions.

The rationale for targeting nesting birds for this study is that their decreased mobility may make them more susceptible to mosquito parasitism and therefore to MBPs (Caillouet 2009; Griffing 2009; Kilpatrick 2006). Also the timing of annual human WNV transmission appears to coincide with the end of the bird nesting season and a host feeding shift in the primary WNV vector (Kilpatrick 2006). While NMT samples were taken from eggs and nestlings, the complete absence of mosquitoes on the samples collected from eggs suggested that mosquitoes might be drawn to nude and immunologically naïve nestlings rather than incubating adults (Blackmore *et al*. 1958; Burkett-Cadena *et al.* 2010). Conversely, Griffing (2009) states the fraction of mosquitoes landing on nestlings increased as the brooding decreased in adult as the nestlings grew closer to fledging. There is the possibility that the sound, airflow, and visual stimulation caused by the NMT may have caused attending adults to adapt their brooding behavior, though previous research on noise pollution and research handling does not suggest a deleterious effect on passerine nesting behavior (Rozell 2003; Ortega 2009).

A study using infrared video to monitor mosquito biting rate on American Robins reported a far greater per nest biting rate for adults (123.3 ± 32.8) than nestlings (37.26 ± 14.8) (Griffing 2009). While Griffing 2009 recorded the brood size for a given nest, they only calculated their biting rates based as a whole. Accordingly, we adjusted our biting rate calculation to meet the Griffing parameter to allow for comparison with our results. A summary comparison of the mean landing rates per night showed brood mosquito burden to be more than 6-fold higher for platform nesters (37.3 ± 14.8) than cavity nesters (5.66 ± 3.83). These findings are likely explained by the physical barrier provided by the nest box which prevents access of mosquitoes to the cavity nesting hosts such as Eastern Bluebirds, Prothonotary Warblers, and Tree Swallows. Only a small entrance hole provides access to a potential bloodmeal. Conversely, platform and stick-nest building bird, such as American Robins and Eastern Phoebes, have no such physical barriers, providing unrestricted access for mosquitoes to feed.

We were presented with a unique challenge in attempting to restructure this experiment for the cavity nesters that comprised the majority of our avian subjects. The enclosed environment of the nest box not only restricts the access of the camera, but also the mosquitoes. The narrow entrance of the nest box and the lack of space around a brooding, cavity nesting adult may directly hinder mosquito bird interactions relative to the unrestricted access afforded by platform nesters. Due to the fact that adult birds redirect their energy expenditures from incubation of eggs to foraging to feed nestlings, they spend less time on the nest once the chicks have hatched (Pinxton *et al.* 1993). This allows us to focus our analyses on nestling burden though we are not able to conclude that nestlings have a higher mosquito burden than incubating adults. Accordingly, nestling characteristics, such as species and clutch biomass were examined to establish any additional trends in mosquito burden.

<u>I. Laboratory assessment of NMT efficiency</u>

The results for laboratory efficiency show an efficiency of 32.1-38.3 (15.7-16.2 SE). This indicates that the NMT is effective drawing up mosquitoes that enter the box voluntarily. These results also indicate that there is no significant effect of box size on capture rate. The capture rates calculated for the laboratory trials could then be used to account for confounding factors encountered during field trials of the NMT.

There are a number of possible sources of error regarding the efficacy of the NMT in accurately describing natural mosquito-bird interactions. The first of these is the sensitivity of mosquitoes to changes in airflow, which has been shown in the literature to affect flight maneuvering and landing of mosquitoes tested a laboratory wind tube (Cooperband 2006). Cooperband (2006) did, however, show that their laboratory mosquitoes continued to pursue the bait even when presented with wind resistance. Mosquitoes are also sensitive to vibration and sound, making this another deterrent to the mosquitoes coming within suction distance of the NMT (Leemingswat 1988). Mosquitoes in the Leemingswat 1988 study were also not prevented from pursuing bait in the presence of sound deterrents. As a consequence, the absence of bait for our laboratory testing of the NMT may partially account for the perceived underestimated MBP transmission risk resulting for our laboratory test. There is a possibility that using bait for our tests would have provided higher capture numbers, but it might have been difficult to establish a base efficiency given the fluctuations in baiting in the field.

Most of the confounding factors could be controlled for by standardizing the test subjects, trap setup, technique laboratory setting. The bait, however, is central to the rationale of the trap. Laboratory testing also could not account for the effect of trap height in the field. The mounting height of 5 feet is within the optimal range for trap height of bird baited mosquito traps tested by Jansen 2009, however tidal changes in water depth for our nest boxes make it difficult to determine if this optimal placement is consistent over the trapping period.

II. Effect of NMT on avian behavior

General abandonment rates

Our general abandonments rates show that is does not decrease from primary to secondary samples. This allows us to posit that repeated exposure to the NMT is not changing avian abandonment rate. The previous literature on noise pollution and avian behavior displays the resilience of nesting passerines (Rozell 2003). Our results support these findings though our sample size does not allow us to be 100% confident about this.

Effect of NMT of avian nesting success

The percentage of Prothonotary Warbler offspring surviving until D5 was calculated both for the VCU Rice Center, where NMTs were deployed and a control site located approximately 4 miles away. While these results do provide evidence that the NMT does not have an adverse effect on Prothonotary Warbler nesting, there are some limitations to this analysis (Table 11). The first confounding factor is the lack of diversity in the avian species observed. The site data used as the control was a convenience sample, acquired from a long-standing Prothonotary Warbler monitoring study. As a consequence, there was no consensus for nest monitoring criteria established between the NMT and Control sites prior to data collection. Had the monitoring criteria been standardized between the two sites prior to the start of the NMT study, a finer statistical analysis, including more species of birds and success benchmark closer to fledging date, may have been possible.

III. Comparison of NMT to existing surveillance methods

Proportion of NMT catches to CDC/Light and Gravid

There is a negative correlation between the progressive trap weeks and proportionality of the NMT catches to that of combined CDC Light and Gravid traps. Though this relationship is not statistically significant in this study, it does indicate how the current methods of surveillance are an effective means of assessing general abundance, but less reliable in predicting bird-mosquito interactions.

IV. Using the NMT to assess ecological parameters of bird-mosquito interactions

Seasonal Effects on Mosquito Burden

Our evidence documents that birds that are on the nest later in the season experience significantly more contact with mosquitoes than birds sampled in the earlier half of the season. Though three Culex spp. were caught with the NMT, these results of higher mosquito burden in the late season were primarily driven by Cx. *salinarius*. While the positive correlation between late season and mosquito capture numbers was visible for all three of the Culex spp. examined, only for *Cx. salinarius* were these results significant. The results for *Cx. salinarius* could be explained by temporal pulses in emergence, given that this is the most abundant of the three. This is largely due to the brackish conditions around the VCU Rice Center. This trend accounts for the fact that the overall Culex spp. burden is notably more significant than *Cx. salinarius*. These data allow us to posit that results showing a higher mosquito burden may have been statistically significant for *Cx. pipiens pipiens/restuans* and *Cx. erraticus* had the sample size been higher.

Higher mosquito burdens later in the nesting season indicate that there may be higher infection rates for birdsthat hatch later in the nesting season.

The Effect of Avian Species on Mosquito Burden

While the distribution of the samples did not allow for a robust statistical analysis of this effect, the summary statistics presented in Table 6 provides valuable insight. The three avian species examined, the Prothonotary Warbler, the Eastern Bluebird, and House Wren, share many of the same life history characteristics. All three of these bird species are migratory, secondary cavity nesters that lay 2-3 clutches in a breeding season (Petit 1999, Taylor 1983; Pinkowski 1978).

The Eastern Bluebird with an adult length 16–21 cm and a weight of 28-32g is the largest of the bird species examined (Pinkowski 1975). The Prothotary Warbler is the next largest with an adult length 12-13 cm and weight 9-11g (Podlesak and Blem 2001). The House Wren with adult length 11-13cm adult weight 11-12g is the smallest (Styrsky 1999). The summary statistics in Table 5 report that 89.9% of the total mosquitoes were captured on Eastern Bluebirds, while Prothonotary Warblers (2.7%) and House Wrens (5.4%) had a far smaller burden. These observations suggest a positive correlation between body size and mosquito burden, but an experiment would be needed to allow for a statistical analysis and true mechanisms.

The Effect of Clutch Biomass on Mosquito Burden

The absence of mosquitoes captured on eggs and incubating adults allowed us to suspend our examination of factors affecting the mosquito burden on these developmental stages and focus on factors affecting nestlings. Given the similarities among the 3 main avian subjects we were able to establish a positive correlation between clutch biomass and mosquito burden, independently of avian species. Due to the very low capture numbers for the early trapping season (May 21, 2010 to June 21, 2010), only nestlings sampled in the late trapping season (June 22, 2010 to July 22, 2010) were examined for the effect of nestling biomass on mosquito burden. The overall mean mosquito burden observed for all nestlings of the late trapping season was 0.072 ± 0.029 SE mosquitoes captured per gram of biomass.

These findings not only provide information on mosquito burden, but provide insight into the host seeking behavior of the 3 Culex species examined for this study. The biomass and growth rate are different for the 3 bird species, but just as increasing biomass is associated with increasing age so is greater feather cover (Podlesak and Blem 2001; Styrsky 1999; Pinkowski 1975). Mosquitoes find their hosts using chemical cues associated with the host's respiration, lactic acid production, and heat signals (Jansen 2009). The fact that this study showed at strong correlation between biomass and mosquito burden, suggests that increased CO_2 and heat production from larger nestlings is attractive to mosquitoes. Our analysis accounted for total clutch biomass mass which might account for a lesser burden on individual birds. The rate would, in turn, increase as nestlings begin to fledge, leaving fewer occupants in the nest to feed upon.

Spatial Modeling of Rice Center NMT Study

Evidence provided in the current literature indicates that large, permanent bodies of water are less important to oviposition of the *Cx. pipiens pipiens* mosquito than are small, temporary pools of stagnant water (Canyon 2006). This mosquito species, in particular, has evolved to oviposit in water with high organic content because the noxious quality of this environment decreases competition from other mosquito species (White 2009). The Aedes mosquito is an example of a genus that favors small pools of fresh water, and would be unlikely to share a larval habitat environment with the primary West Nile virus vector (Hamer 2009; Kilpatrick 2006). The temporary quality of ephemeral aquatic habitat also allows for *Cx. pipiens pipiens* to safely emerging from the habitat before predators are able to colonize and pose a threat to their larvae (White 2009). It is by this rationale that the spatial analysis was performed.

The riparian sites used in this study provided some unique challenges to identifying optimal larval habitat for *Cx. pipiens pipiens.* Tidal changes made it difficult to consistently determine distance of nest boxes to the permanent water bodies, the James River and Kimages Creek. As a consequence, slope and elevation were used not only as a function of distance to the river bank, but as a means of assessing the topography of the land for possible ephemeral pool formation.

The limitations of this analysis include the lack of visible confirmation of pooling under upland boxes regardless of topography. Also, the rules for box placement dictate that be mounted at least 5 feet off of the ground. This factor and the shifting of box position due to environmental factors such as weather and interference by predators. Our findings indicate that there is no effect of elevation on avian mosquito burden. The effect of slope changes in the 20m buffer surrounding the boxes would need to be visually confirmed in the field to perform a statistical analysis. The GIS methods used in this study will need to be followed up with targeted field observations.

Future studies

While this study has been effective in identifying trends in the interactions between cavity- nesting passerines and mosquitoes in several Central Virginia sites, it will be important to establish a direct link between nesting birds, mosquitoes, and mosquito borne pathogens. Such a study would determine MBP infection rates in mosquitoes collected from nests while also determining infection rates in nesting birds. It will also be interesting to continue this analysis while monitoring fitness parameters that MBP infection may affect such as migration, fecundity, and overall fitness. A more in depth examination of the apparent host selectivity of mosquitoes in this study for Eastern Bluebirds is also warranted.

Determining MBP load in ornithophilic mosquitoes

Several studies in recent years have optimized techniques to determine the avian malaria load in bird blood samples. Waldenstrom (2004) established a nested Polymerase Chain Reaction (PCR) to identify avian malaria parasites to the genus level. This analysis was further refined to the species level using Restriction Fragment Length Polymorphism (RFLP) (Beadell 2004). These results could be further advanced by determining the infectivity and infection status of ornithophilic mosquitoes. These results could then, in turn, be analyzed with avian species, season, nestling biomass, and nest box placement as covariates. This would determine the direct transmission risk for MBP

Summation statement

In summary, this study has established the efficacy and demonstrated the field capacity of a novel tool to assess the interactions of mosquito vectors and nesting avian hosts. The Nest Mosquito Trap may allow for a more in depth understanding of the ecological factors determining mosquito borne pathogen transmission.

Appendix:

Table 1: Summary of investigator placed nest apparatuses by type and site. Summary of occupied nest apparatuses by type and site. Summary of individual bird families by type and site; 3 Upland Rice Boxes and 1 Water Rice Box had 2 separate families.

Box Type/Site	**Small Box**			**Large Box**			**Platforms**			**Total Occupied Apparatuses**		
	Placed Boxes	*Occupied Boxes*	*Individual Families*	*Placed Boxes*	*Occupied Boxes*	*Individual Families*	*Placed Boxes*	*Occupied Boxes*	*Individual Families*	*Placed Boxes*	*Occupied Boxes*	*Individual Families*
Upland Rice	14	5	8	6	0	0	11	0	1	31	5	9
Water Rice	45	19	20	0	0	0	0	0	0	45	19	20
Green	13	4	3	5	1	1	9	0	0	27	5	4
Wilson	15	2	2	5	0	0	15	0	0	35	4	2
HSWI Sites	89	6	6	0	0	0	89	0	0	178	6	6
Total	**176**	**36**	**40**	**16**	**1**	**1**	**124**	**0**	**1**	**316**	**40**	**42**

Table 2: Dates of CDC Light and Gravid Trap Placement and Nest surveys.

Dates	Rice		Green		Wilson	
	CDC Light/ Gravid	*Nest Survey*	*CDC Light/ Gravid*	*Nest Survey*	*CDC Light/ Gravid*	*Nest Survey*
5/19/2010	-------	X	--------	X	---------	X
5/24/10-5/25/10	X	X	X	X	X	-------
5/31/10-6/1/10	X	X	X	X	X	X
6/6/10-6/7/10	X	X	-------	X	-------	X
6/14/10-6/15/10	X	X	X	X	X	X
6/22/10-6/23/10	X	X	X	X	X	X
6/28/10-6/29/10	-------	X	X	X	X	X
7/5/10-7/6/10	X	X	X	X	--------	--------
7/12/10-7/13/10	X	X	X	X	X	X
7/26/10-7/27/10	X	---------	X	-------	X	--------

Table 3: Nest Mosquito Trap (NMT) collection nights by date, site, and developmental stage of offspring.

	Rice		**Green**		**Wilson**		**Henrico**		**Total**	
	Eggs	*Nestlings*	*Eggs*	*Nestlings*	*Eggs*	*Nestlings*	*Eggs*	*Nestlings*	***Eggs***	***Nestlings***
5/21/2010	4	0	2	0	0	1	0	0	**6**	**1**
5/27/2010	3	5	1	0	0	0	1	1	**5**	**6**
6/3/2010	0	0	0	0	0	0	0	1	**0**	**1**
6/9/2010	0	4	0	1	0	0	0	0	**0**	**5**
6/15/2010	0	6	0	1	0	0	0	0	**0**	**7**
6/17/2010	0	5	0	1	0	0	0	0	**0**	**6**
6/22/2010	0	4	0	0	0	0	0	0	**0**	**4**
6/30/2010	0	2	0	0	0	0	1	0	**1**	**2**
7/2/2010	0	1	0	0	0	0	0	0	**0**	**1**
7/6/2010	0	2	0	0	0	1	0	1	**0**	**4**
7/8/2010	0	4	0	0	0	1	0	0	**0**	**5**
7/13/2010	0	3	0	1	0	0	0	1	**0**	**5**
7/15/2010	0	4	0	1	0	0	0	1	**0**	**6**
7/22/2010	0	2	0	0	0	0	0	0	**0**	**2**
TOTAL	**7**	**42**	**3**	**5**	**0**	**3**	**2**	**5**	**12**	**55**

Table 4: Observed host-seeking mosquitoes by trap night.

Mosquito Species	Mean ± SE	95% CI
Cx. salinarius	1.67 ± 1.13	[-0.591, 3.924]
Cx. erraticus	0.14 ± 0.07	[-0.009, 1.009]
Cx. pipiens pipiens/restuans	0.50 ± 0.25	[0.001, 0.272]
Ae. albopictus	0.03 ± 0.02	[-0.012, 0.073]
Total	**2.30 ± 1.40**	**[-0.497, 5.103]**

Table 5: Observed host-seeking mosquitoes (per trap night) by avian species.

Avian Species	n	*Cx. pipiens pipiens/restuans*		*Cx. salinarius*		*Cx. erraticus*		Total	
		Mean ± SE	%	Mean ± SE	%	Mean ± SE	%	Mean ± SE	%
Eastern Bluebird	14	2.15 ± 1.20	90.0	7.62 ± 5.59	86.7	0.54 ± 0.31	77.7	10.31 ± 6.85	88.9
Wren spp.	6	0.20 ± 0.20	6.7	0.60 ± 0.60	5.3	0.10 ± 0.10	11.1	1.10 ± 0.57	5.8
Prothonotary Warbler	28	0.04 ± 0.04	3.3	0.11 ± 0.06	2.6	NONE	0	0.14 ± 0.09	2.5
Tree Swallow	3	NONE	0	0.60 ± 0.40	2.6	0.40 ± 0.24	22.2	1.00 ± 0.63	3.2
All Bird	**51**	**0.61 ± 033**	**21.4**	**2.12 ± 1.46**	**74.0**	**0.16 ± 0.09**	**5.8**	**2.92 ± 1.51**	

Table 6: Observed host-seeking rate (per nestling/trap night) and estimated host-seeking rate for nestlings by bird species for all mosquito species.

		Field Observed		Estimated Host-Seeking Rate	
	n	Mean ± SE	95% CI	Mean ± SE	95% CI
Eastern Bluebird	14	2.89 ± 1.71	[-0.808, 6.582]	7.58 ± 4.49	[-2.121, 17.275]
Prothonotary Warbler	28	0.04 ± 0.02	[-0.008, 0.079]	0.09 ± 0.06	[-0.021,0.208]
Wren spp.	6	0.72 ± 0.48	[-0.518, 1.962]	1.89 ± 1.27	[-1.359, 5.150]
Tree Swallow	3	0.56 ± 0.56	[0.003, 0.095]	1.46 ± 1.46	[0.008, 0.249]
Total	**51**	**0.93 ± 0.49**	**[-0.059, 1.919]**	**2.44 ± 1.29**	**[-0.155, 5.036]**

Table 7: Observed host-seeking rate (per nestling/trap night) and estimated per capita host-seeking rate by mosquito species for all birds over 66 trap nights.

	Field Observed		Estimated per capita host seeking rate	
	Mean ± SE	95% CI	Mean ± SE	95% CI
Cx. salinarius	0.65 ± 0.38	[-0.118, 1.419]	1.71 ± 1.01	[-0.311, 3.743]
Cx. pipiens pipiens/restuans	0.23 ± 0.12	[-0.009, 0.470]	0.61 ± 0.31	[-0.023,1.233]
Cx. erraticus	0.05 ± 0.02	[0.003, 0.095]	0.13 ± 0.06	[0.008, 0.249]
Total	**0.93 ± 0.49**	**[-0.059, 1.919]**	**2.44 ± 1.29**	**[-0.155, 5.036]**

Table 8: Estimated host-seeking rate (per nestling/trap night) by trap week.

Trap Week	Dates	n	*Cx. pipiens pipiens /restuans*		*Cx. salinarius*		*Cx. erraticus*		Total Culex	
			Mean ± SE	%	Mean ± SE	%	Mean ± SE	%	Mean ± SE	%
1	5/21-27/2010	6	0	0	0.11± 0.11	0.91%	0	0	0.11± 0.11	0.66%
2	6/9/2010	5	0.48 ± 0.34	9.1%	0.48 ± 0.34	2.7%	0.18 ± 0.18		1.34 ± 0.85	4.6%
3	6/15-17/2010	12	0	0	0.05 ± 0.05	0.01%	0.16 ± 0.16		0.22 ± 0.17	2.6%
4	6/22/2010	4	0	0	0	0	0	0	0	0
5	6/30-7/2/2010	3	0	0	0	0	0	0	0	0
6	7/6-8/2010	9	0	0	0	0	0.1 ± 0.1	11.1%	0.1 ± 0.1	0.66%
7	7/13-15/2010	9	0.75 ± 0.45	21.1%	2.19 ± 1.35	18.2%	0.1 ± 0.1	11.1%	3.04 ± 1.71	18.4%
8	7/22/2010	2	10.8 ± 2.3	69.7%	31.8 ± 16.1	77.3%	0.99 ± 0.99	33.3%	43.6 ± 14.8	73.0%
Total		**50**		**21.6%**		**72.5%**		**5.9%**		**100%**

Table 9: Adult nest abandonment for primary sampling by avian species for all sites (E=eggs; N=nestlings).

Species		Abandoned	Not Abandoned
Prothonotary Warbler (n=14)			
	E=5	0	5
	N=9	2	7
Wren spp. (n=4)			
	E=1	0	1
	N=3	-------	3
Eastern Bluebird (n=6)			
	E=2	0	2
	N=4	0	4
Carolina Chickadee (n=1)			
	E=1	0	1
	N=0	-------	-------
Tree Swallow (n=3)			
	E=3	0	3
	N=0	-------	--------
American Robin (n=1)			
	E=0	-------	--------
	N=1	0	1
All Species (n=29)			
	E=12	0	12
	N=17	2	15

Table 10: Combined adult nest abandonment for primary and secondary (repeated) sampling by species for all sites (E=eggs; N=nestlings).

Species		Abandoned	Not Abandoned
Prothonatary Warbler (n=33)			
	E=5	0	5
	N=28	2	26
Wren spp.(n=11)			
	E=1	0	1
	N=10	1	9
Eastern Bluebird (n=16)			
	E=2	0	2
	N=13	0	13
Carolina Chickadee (n=1)			
	E=1	0	1
	N=0	0	0
Tree Swallow (n=6)			
	E=3	0	3
	N=3	0	3
American Robin (n=1)			
	E=0	--------	---------
	N=1	0	1
All Species (n=67)			
	E=12	0	12
	N=55	3	52

Table 11: Comparison of Prothonotary Warbler 5 day post-hatch nest success between a site where the NMTs were deployed and a control site approximately 4 miles away.

Nest Success Rates				
Outcome	Control	NMT	Two-sided P-value	Test Statistic
	no./total no. (%)			
Survived until D5	93/145 (64.2)	12/13 (91.7)	*0.0389*	4.247
Did not survive until D5	52/145 (35.8)	1/13 (8.33)		

Table 12: Summary statistics for VCU Rice Center: combined CO_2-baited CDC light and Gravid trap capture numbers.

Species	Mean ± SE	95% CI
Aedes albopictus	0.625 ± 0.419	[-0.368, 1.618]
Aedes triseriatus	0.125 ± 0.125	[-0.171, 0.421]
Aedes vexans	0.750 ± 0.412	[-0.224, 1.724]
Anopheles crucians	0.250 ± 0.164	[-0.137, 0.637]
Anopheles quadrimaculatus	0.500 ± 0.500	[-0.682, 1.682]
Coquillettidia perturbans	0.875 ± 0.441	[-0.167, 1.917]
Culex erraticus	7.250 ± 3.994	[-2.195, 16.695]
Culex pipiens pipiens/restuans	5.000 ± 3.006	[-2.108, 12.108]
Culex salinarius	197.0 ± 78.92	[10.384, 383.62]
Ochlerotatus japonicus	0.125 ± 0.125	[-0.171, 0.421]
Total	**212.50 ± 83.98**	**[13.913, 411.09]**

Table 13: A multiple proportion, chi-squared analysis of the mean catch composition per trap night for Culex spp.,was used to compare the Nest Mosquito Trap with the combined numbers for the CDC Light and Gravid traps. The weeks during which at least one mosquito was captured for each of the trap types were examined. Of the 5 trap weeks examined, 3 showed that the Culex spp. catch composition differed significantly between the NMT and the combined CDC light and Gravid collections. For the remaining 2, trap weeks there was not a significant difference, which might be explained by changing weather patterns or temporal pulses in mosquito emergence.

	Cx. pipiens pipiens/restuans		*Cx. salinarius*		*Cx. erraticus*		Difference in capture composition by week	
Trap Week (Dates)	**CDC Light/Gravid** *no. caught/total*	**NMT** *no.Culex spp. (%)*	**CDC Light/Gravid** *no. caught/total*	**NMT** *no.Culex spp. (%)*	**CDC Light/Gravid** *no. caught/total*	**NMT** *no.Culex spp. (%)*	***P-value***	**Test statistic** df=2
1 (5/21-27/2010)	0.5/48.5 (1.1)	0.5/1.9 (26.5)	47.5/48.5 (97.9)	0.9/1.9 (47.4)	0.5/48.5 (1.1)	0.5/1.9 (26.5)	0.002	12.27
3 (6/5-8/2010)	0.5/6.5 (7.7)	1/2.75 (36.3)	2.5/6.5 (38.5)	1/2.75 (36.3)	3.5/6.5 (53.9)	0.75/2.75 (27.3)	0.527	1.28
6 (7/5-8/2010)	7.5/125.5 (6.0)	0.5/1.65 (30.3)	105.5/125.5 (84.1)	0.5/1.65 (30.3)	12.5/125.5(9.9)	0.65/1.65(39.4)	0.177	3.46
7 (7/12-15/2010)	9.5/213.5 (4.5)	1.5/5.5 (27.2)	203.5/213.5 (95.3)	3.35/5.5 (60.9)	0.5/213.5 (0.23)	0.65/1.5 (2.3)	<0.001	19.89
8 (7/21-26/2010)	24.5/583.5 (4.2)	12/57 (21.1)	546.5/583.5 (93.7)	43/57 (75.4)	12.5/583.5 (2.1)	2/57 (3.5)	<0.001	28.18
All Weeks	**8.5/195.5(4.4)**	**1.8/7.5 (24.0)**	**181.1/195.5 (92.6)**	**4.9/7.5 (65.3)**	**5.9/195.5 (3.0)**	**0.8/7.5 (10.7)**	**0.025**	7.37

Table 14: Summary statistics for mosquito burden by season. A total of 12 mosquitoes were collected over 35trap nights (0.35 mean ± 0.18 SE) before June 22, 2010. From June 22, 2010 a total of 142 mosquitoes were collected over 31trap nights (4.58 ± 2.95). The table displays the data for the trap nights taken from nesting as there were no mosquitoes captured on eggs.

Season		*Cx. pipiens/restuans*		*Cx. salinarius**		*Cx. erraticus*		Total*	
	n	Mean ± SE	%	Mean ± SE	%	Mean ± SE	%	Mean ± SE	%
Early	20	0.05 ± 0.05	0.7	0.15 ± 0.08	2.0	0.15 ± 0.15	2.0	0.35 ± 0.18	4.7
Late	31	0.97 ± 0.53	20.4	3.39 ± 2.39	71.5	0.16 ± 0.10	3.4	4.58 ± 2.95	95.3
Overall	51		21.1		73.5		5.4		100.0

Table 15: Parameters for estimating nestling biomass.

Avian Species	n	K	t_{50}	A	Sources
Prothonotary Warblers	13	0.488	3.5	11.13	(Podlesak and Blem 2002)
Eastern Bluebird	10	0.686	5	27.2	(Pinkowski 1975)
Wren spp.	6	0.513	5	11.9	(Styrsky 1999; Austin 2009)

Table 16: Summary statistics for elevation and mean mosquito abundance. The mean elevation for upland Rice Center boxes was 12.45 ± 1.44 meters while boxes placed at the water's edge had a mean elevation of 1.97 ± 1.17 meters. While a two-tailed t-test shows the elevations to be significantly different between the upland and water's edge boxes (p-value<0.001; df=23; test statistic=9.65), the overlapping confidence intervals indicate this does not significantly affect mosquito burden.

	Upland Boxes (n=9)		**Water's Edge Boxes (n=16)**	
Mosquito Abundance	**Mean ± SE**	**95% CI**	**Mean ± SE**	**95% CI**
Cx. salinarius	0.43 ± 0.27	[-0.198, 1.056]	1.41 ± 1.34	[-0.259, 4.263]
Cx. erraticus	0.02 ± 0.02	[-0.211, 0.073]	0.13 ± 0.08	[-0.033, 0.293]
Cx. pipiens pipiens/restuans	0.25 ± 0.20	[-0.029, 0.714]	0.31 ± 0.27	[-0.259, 0.875]
Total Culex spp.	0.70 ± 0.46	[-0.369, 1.775]	1.84 ± 1.66	[-1.703, 5.391]
Elevation	**12.45 ± 1.44**	**[9.13, 15.78]**	**1.97 ± 0.164**	**[1.62, 2.32]**

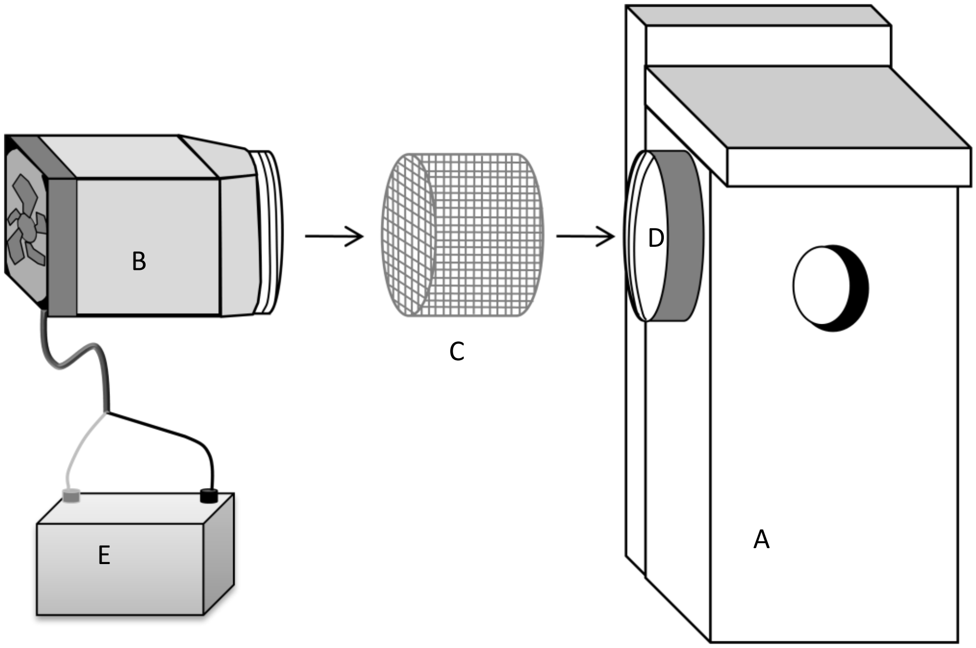

Figure 1: Schematic of Nest Mosquito Trap (NMT) design. A) modified nest box; B) trap body with 12v fan; C) collection bag; D) connection port; E) 12v battery

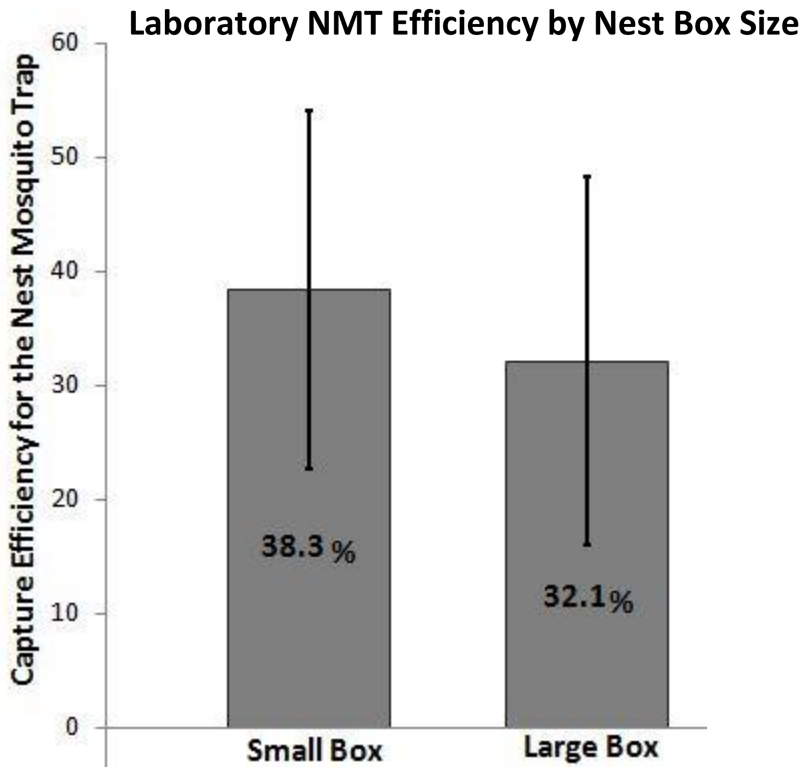

Figure 2: Summary statistics for NMT laboratory efficiency test

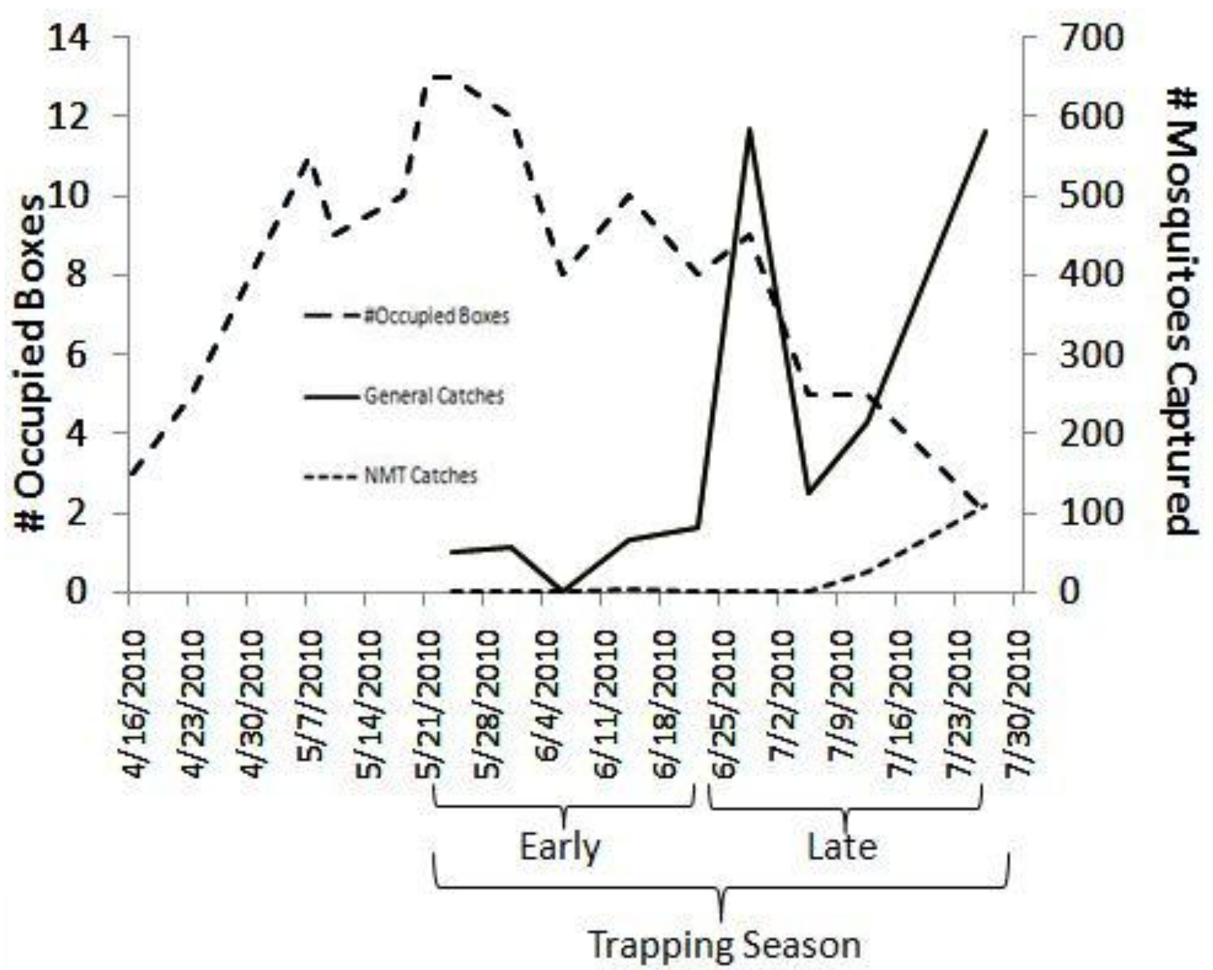

Figure 3: Avian nesting season and NMT trapping season.

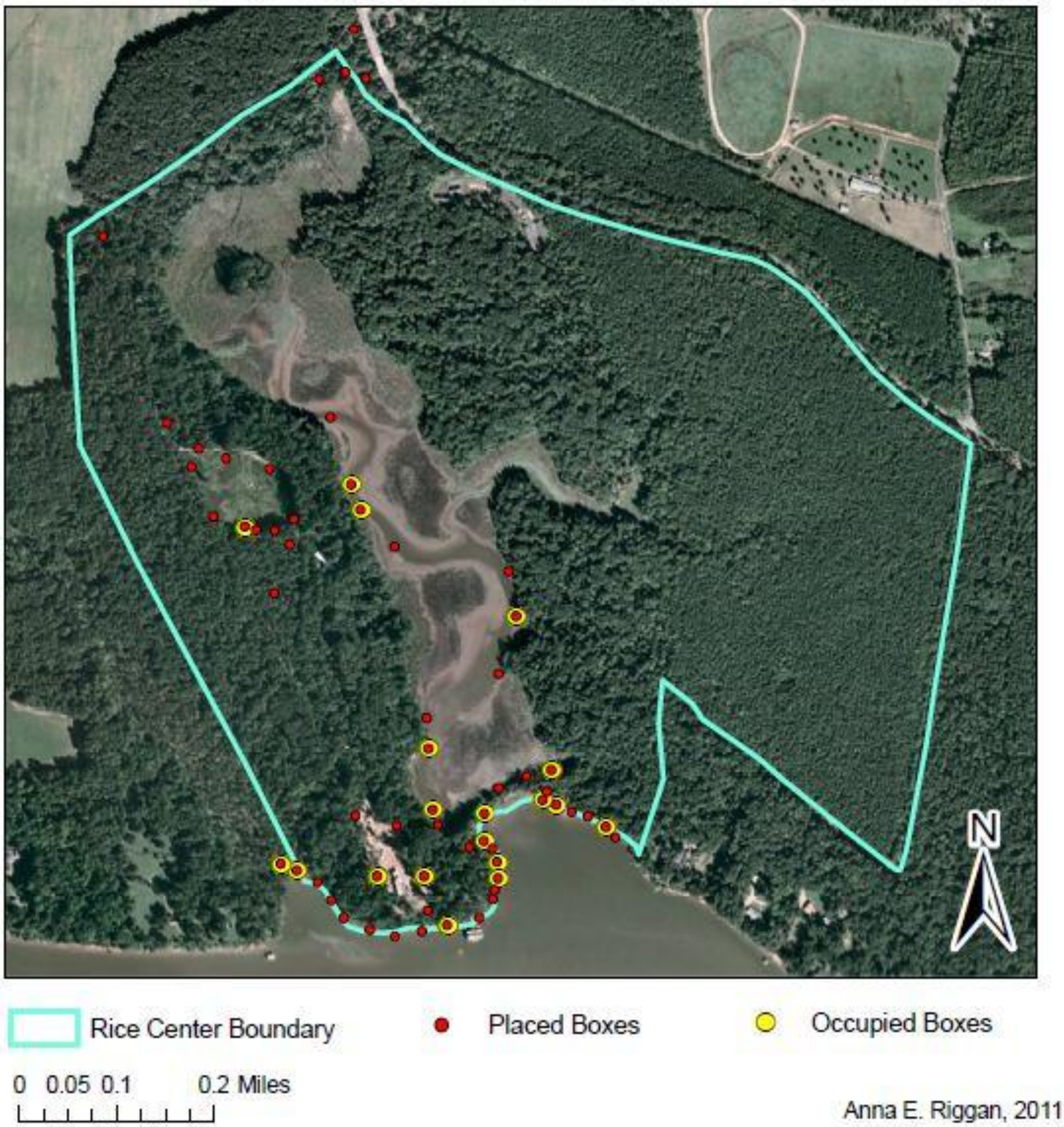

Figure 4: All nest boxes placed by the investigator at the VCU Rice Center.

Figure 5: All nest boxes placed by the investigator at the Site Green.

Figure 6: All nest boxes and platforms placed by the investigator at the Site Wilson.

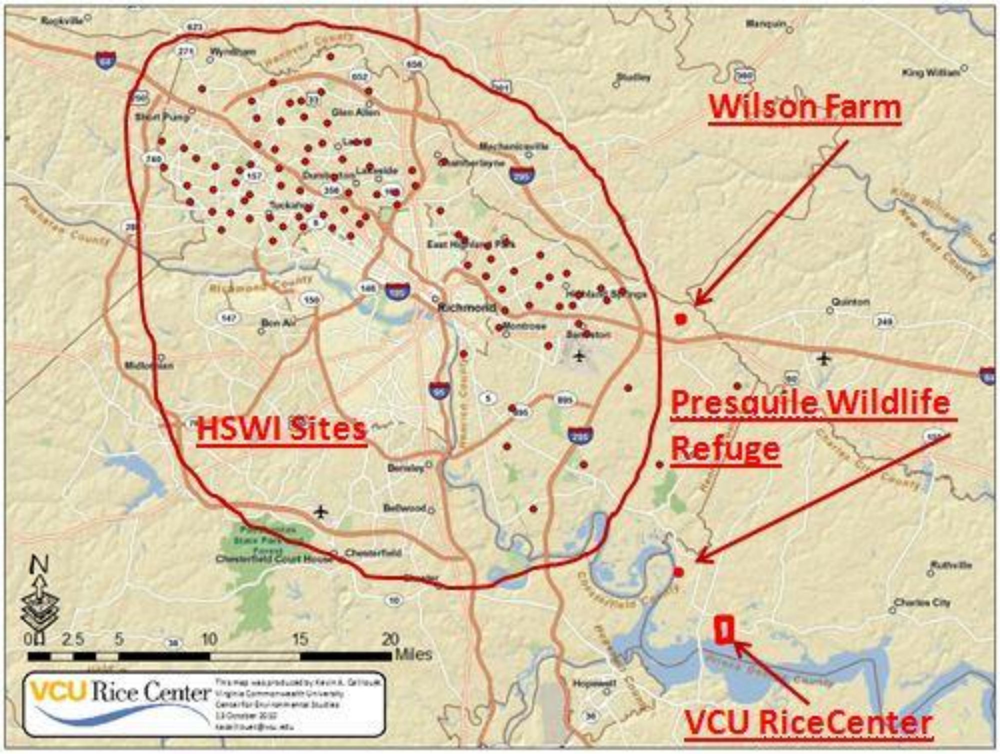

Figure 7 All nest boxes and platforms placed by the investigator at the sites used by the Henrico Standing Water Initiative (HSWI).

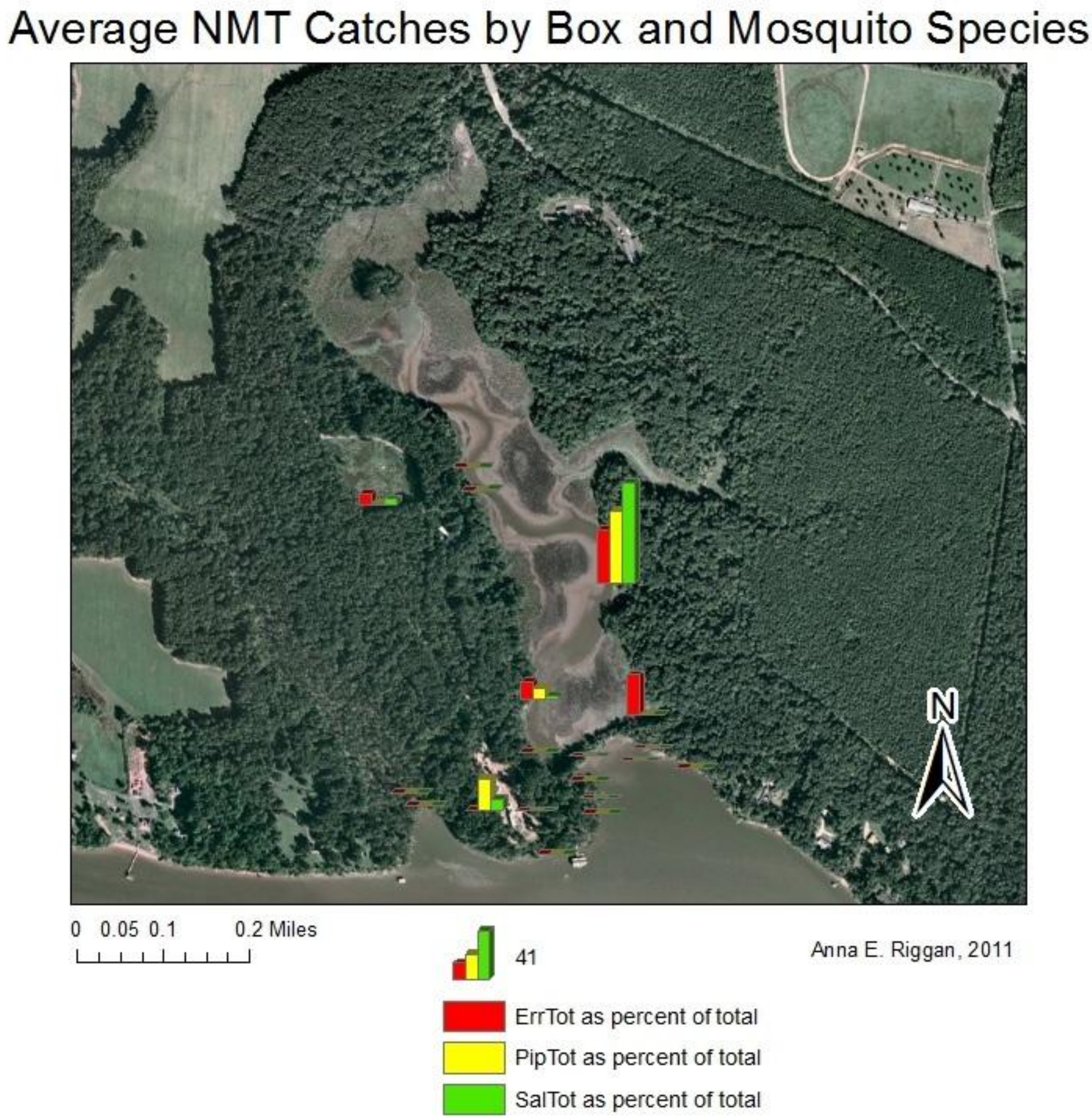

Figure 7: Average NMT catches at the VCU Rice Center by season, nest box, and mosquito species.

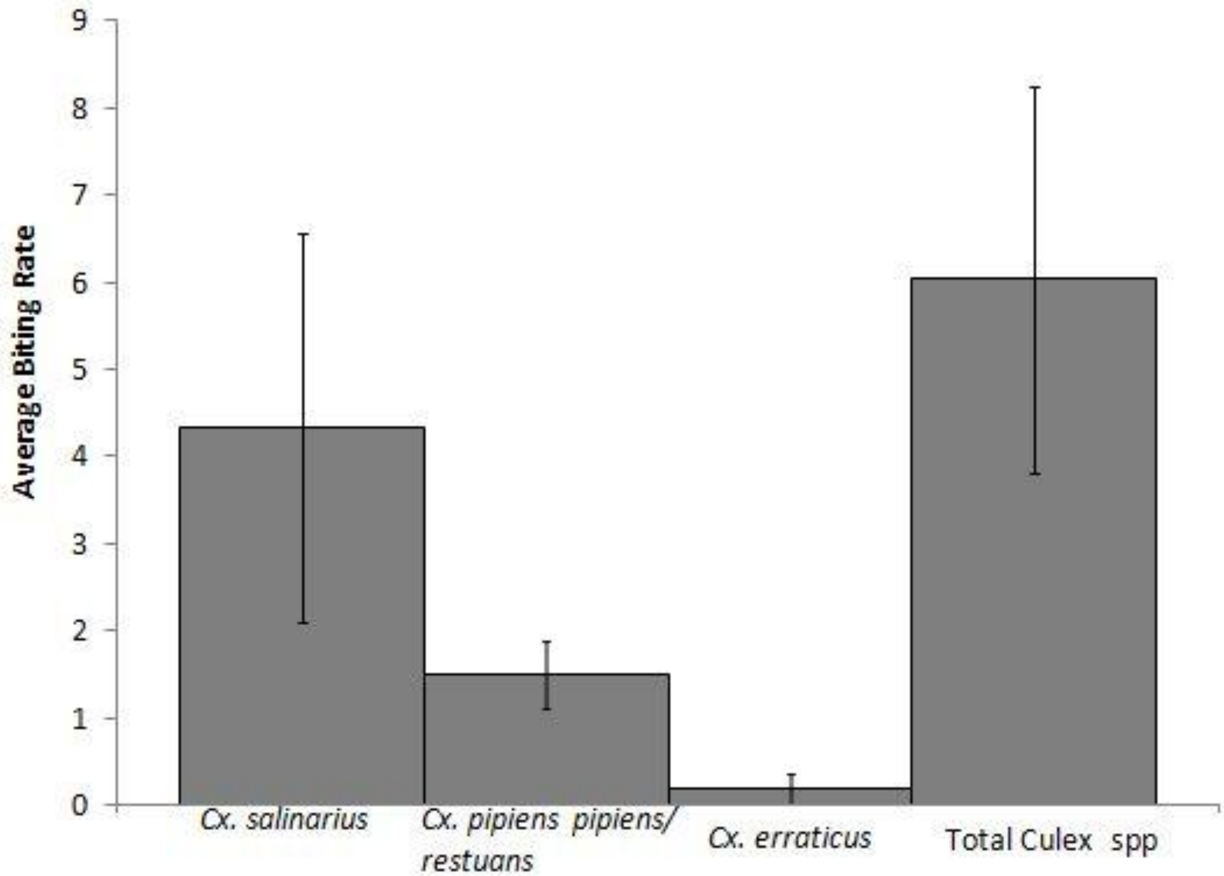

Figure 8: Mean estimated per capita host-seeking rate by mosquito species.

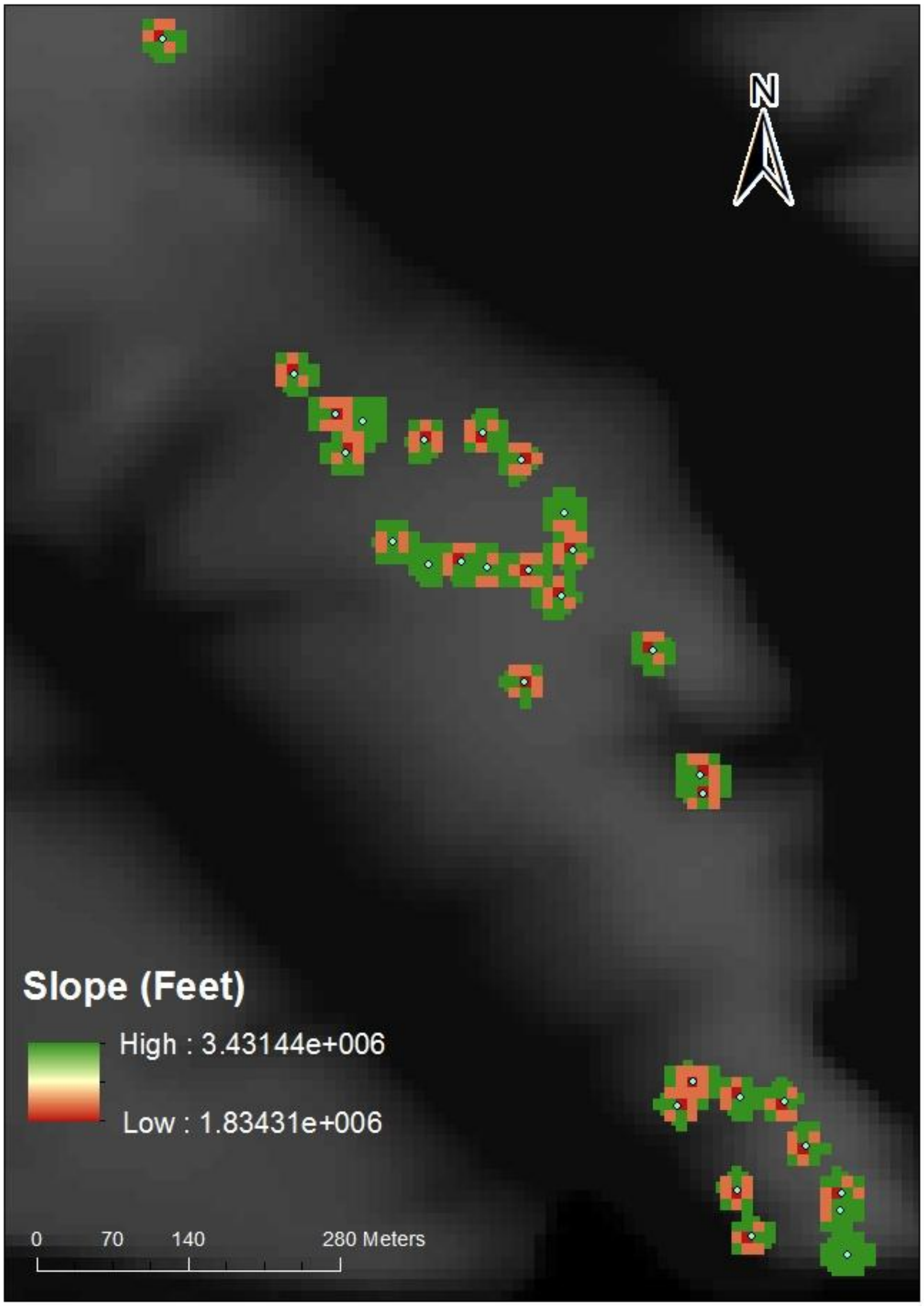

Figure 9: Zonal Statistics of slope around the Upland Rice Center Boxes. Changes in slope within 20-meter buffer indicate areas where water is likely to pool.

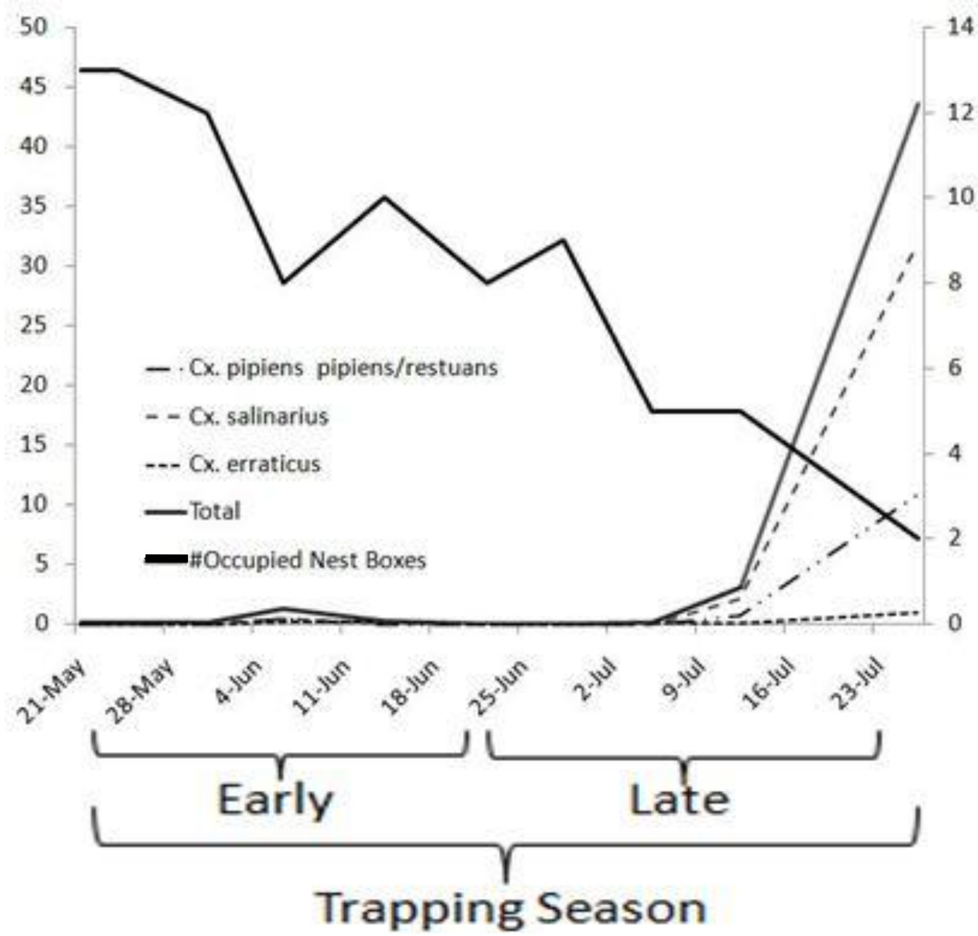

Figure 10: Estimated host-seeking rate over the trapping season.

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