

SLEEPLESS AND RESTLESS: EFFECTS OF TRAFFIC NOISE AND MOSQUITO
INCURSIONS ON EASTERN BLUEBIRD (*Sialia sialis*) INCUBATION BEHAVIORS

By

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TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS	3
LIST OF TABLES	6
LIST OF FIGURES	7
ABSTRACT.....	8
CHAPTER	
1 INTRODUCTION	10
Noise Pollution, Anthropogenic Expansion, and Impacts on Wildlife.....	10
Noise Pollution and Breeding Ecology of Avian Species	11
Mosquitoes as a Source of Disturbance	11
Study Objectives	12
Objective 1: Noise and Mosquito Effects on Behavior	12
Objective 2: Noise, Mosquito and Behavior Effects on Hatching Success	13
2 METHODS	15
Study System	15
Study Species.....	15
Study Sites	15
General Field Methods	16
Video Recording Protocol	18
Defining Incubation Behavior	18
Preen	19
Settle.....	19
Agitation.....	19
Egg Turn.....	19
Time Off Eggs	20
3 DATA ANALYSIS	23
Data Reduction	23
Behavior PCA.....	23
PC_ACT	23
PC_CHL	24
PC_AG	24
Data Structure and Variable Management	24
Temperature	24
Noise.....	25
Hatching Success.....	25

Hypothesis Testing	26
H1: Noise and Mosquito Effects on Behavior.....	26
H2: Noise, Mosquito and Behavior Effects on Hatching Success	27
4 RESULTS	30
H1: Noise and Mosquito Effects on Behavior.....	31
Model 1.1: PC_ACT.....	31
Model 1.2: PC_CHL.....	32
Model 1.3: PC_AG.....	32
H2: Noise, Mosquito and Behavior Effects on Hatching Success.....	33
5 DISCUSSION.....	51
What is “Good” Incubation Behavior?	51
Mosquitos as a Natural Nuisance to Incubating Females	52
Interactive Effects of Disturbances.....	54
Limitations and Future Directions	55
Noise Measurements	55
Personality	55
Conclusion.....	56
LIST OF REFERENCES.....	58
BIOGRAPHICAL SKETCH	63

LIST OF TABLES

<u>Table</u>		<u>page</u>
2-1	Description of raw variables collected from the field and from video recordings.	22
3-1	Component loadings for the behavior PCA.	28
3-2	Variables used in the mixed models and their role in each hypothesis tested.	29
4-1	Model output for H1, model 1.1: activity level.	35
4-2	Model output for H1, model 1.2: relaxed incubation.	41
4-3	Model output for H1, model 1.3: agitated behavior.	44
4-4	Model output for H2: hatching success.	47

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
1-1 Conceptual diagram of the study.	14
2-1 Map of the three field sites.....	21
4-1 Average noise level and noise range across nest boxes by location.	35
4-2 Mosquito level by location.....	36
4-3 (H1, Model 1.1) Marginal predicted values of female activity (PC_ACT) in relation to environmental temperature.	38
4-4 (H1, Model 1.1) Marginal predicted values of female activity (PC_ACT) as a function of mosquito level.	39
4-5 (H1, Model 1.1) Marginal predicted values of female activity (PC_ACT) as a function of the interaction between average noise level and mosquito level	40
4-6 (H1, Model 1.2) Marginal predicted mean values of relaxed incubation behaviors (PC_CHL) as a function of mosquito level.....	42
4-7 (H1, Model 1.2) Marginal predicted mean values of relaxed incubation behaviors (PC_CHL) as a function of the interaction between average noise and mosquito level.....	43
4-8 (H1, Model 1.3) Marginal predicted mean values of agitated behaviors (PC_AG) as a function of mosquito level	45
4-9 (H1, Model 1.3) Plot of marginal predicted mean values of agitated behaviors (PC_AG) as a function of the interaction between noise range and mosquito level	46
4-10 (H2) Marginal predicted mean values of transformed hatching success (THS) as a function of relaxed incubation behaviors (PC_CHL)	48
4-11 (H2) Marginal predicted mean values of transformed hatching success (THS) as a function of agitated behaviors (PC_AG)	49
4-12 (H2) Marginal predicted mean values of transformed hatching success (THS) as a function of the interaction between average noise level, noise range, and mosquito level.....	50

Abstract of Thesis Presented to the Graduate School
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Incubation in avian species requires the maintenance of egg temperature at optimal temperatures for development, and as a result, incubating individuals must adjust their behavior to accommodate changes in environmental factors, and may be more sensitive to potential disturbances. Amidst rapid human urbanization, increasing noise levels, in addition to natural sources of disturbance such as insect harassment, may alter regular incubation behaviors during this critical period and result in reduced reproductive success. In order to assess the potential impacts these combined disturbances have on incubation behavior and hatching success, I deployed small cameras in Eastern Bluebird (*Sialia sialis*) nest boxes and recorded videos of nocturnal incubation behavior. I hypothesized high levels of noise and mosquitos would increase agitated behavior and decrease normal incubation behaviors, ultimately leading to reduced hatching success. Results demonstrated mosquito level was the most important predictor of incubation behavior; high levels of mosquitos were associated with increased agitation and overall activity levels but decreased relaxed incubation behaviors. Noise had no direct impacts on behaviors but had significant interactions with mosquito level. Frequency of both agitations and relaxed incubation behaviors increased hatching success, suggesting behavioral adjustments in response to mosquito incursions have no reproductive consequences. Quiet nests had the

highest hatching success while chronically noisy nests had the lowest hatching success, but only when mosquito levels were elevated; in the absence of mosquitos, there was no significant difference in hatching success based on noise—pointing to the importance of studying interactive effects between anthropogenic and biotic disturbances.

CHAPTER 1 INTRODUCTION

In avian species, the incubation period is vital to the proper development of young. In most avian species, nest attentiveness is necessary for maintaining the optimal incubation temperature for development, and as a result those incubating individuals may be particularly susceptible to disturbances during this period. Disturbances come in many forms, but this study focuses on two; anthropogenic disturbance in the form of noise, and biotic disturbance in the form of mosquito incursions.

Noise Pollution, Anthropogenic Expansion, and Impacts on Wildlife

Noise pollution is defined as “unwanted sound”, or more specifically as sound that is harmful to physiological or psychological health in frequency, intensity and/or duration (Oguntunde et al., 2019). In human-dominated landscapes, noise pollution comes from a variety of sources including air, land and sea transportation, construction, traffic, and residential areas (Schomer et al., 2001; Singh & Davar, 2004). Noise pollution is pervasive throughout both human-dominated and human-adjacent matrices and is considered to be a growing global public health issue. In humans, there are direct links between exposure to noise and adverse health effects ranging from hearing loss, high blood pressure, sleep disturbance and cognitive impairment (WHO, 2011). Beyond being a human health issue, noise pollution has been shown to have many detrimental impacts on wildlife. Its scope is extensive, and there are documented impacts on multiple levels from species level (Ru et al., 2018), to community (Chen & Koprowski, 2015) and ecosystem-wide effects (Munro, 2018). Noise pollution can impact multiple facets of ecology, from foraging (Siemers et al., 2011; Luo et al., 2015), reproduction (Lagadère, 1982), migration (Ware et al., 2015) and communication (Rossi-Santos, 2015; Grade & Sieving, 2016).

Noise Pollution and Breeding Ecology of Avian Species

Despite the extensive body of literature on the impacts of environmental noise on avian communication, little research has been done to date on the impacts of noise on reproduction. In recent years, a handful of studies have demonstrated considerable effects of noise on the breeding ecology of wildlife species. Ortiz-Urbina et al. (2020) showed a strong correlation between road traffic noise and the distribution of Cinereous Vultures (*Aegypius monachus*), with a threshold level of noise beyond which the vultures would not breed in what were otherwise suitable breeding habitats. Although considered to be a disturbance-tolerant species (Kight, 2005), Eastern Bluebirds (*Sialia sialis*) in territories with higher levels of environmental noise were associated with reductions in productivity and smaller brood sizes (Kight et al., 2012). Further research on Eastern Bluebirds in Florida found similar results, and also suggested that environmental noise disrupted the females' incubation behavior (Liu, 2020). Incubation behavior is normally regulated by ambient temperature, but noise appeared to significantly alter the incubation behaviors of females, resulting in lower hatching success. These studies point to a clear gap in our knowledge on the impacts of noise on reproductive success and highlight the need for additional studies on the relationship between noise and female incubation behaviors.

Mosquitoes as a Source of Disturbance

Mosquitos represent another potentially major, yet vastly understudied, source of disruption to female incubation. A majority of the current literature on the relationships between mosquitos and their hosts, including studies on Eastern Bluebirds, focus on the role of mosquitos as a vector for disease, particularly the West Nile virus (Hill et al., 2010; Caillouët et al., 2013), with little information on the effects of mosquitoes as a nuisance and disturbance. Beyond transmitting disease, mosquitos have been shown to have a more general negative impact on hosts. In Barn Owls (*Tyto alba*), mosquito bites negatively impacted the overall health and

condition of nestlings through mechanisms including blood loss and the energetic costs of regeneration, leading to reduced nestling survival in nest boxes not treated with insecticide (Efstathion et al., 2019). Mosquito parasitism has also been associated with egg loss and adult mortality in Brünnich's Guillemot (*Uria lomvia*, Gaston et al., 2002). In the Waved Albatross (*Phoebastria irrorata*), elevated mosquito levels have been linked to egg neglect and desertion (Anderson & Fortner, 1988). Mosquitos have been associated with alterations in behavior in other taxa as well (e.g. in reindeer *Rangifer tarandus tarandus*, Valente et al., 2020), and present themselves as a possible source of disturbance to incubating females.

Study Objectives

The goals of this study were to explore the potential impacts of both anthropogenic noise and the presence of mosquitoes on the breeding ecology of Eastern Bluebirds, looking specifically at nocturnal female incubation behaviors. The objectives were twofold; to elucidate the role of mosquito and noise disturbances in modulating female incubation behavior, and how these disturbances, in combination with incubation behaviors, impact hatching success (see Figure 1-1).

Objective 1: Noise and Mosquito Effects on Behavior

My first objective was to compare the patterns of nocturnal incubation behaviors of female Eastern Bluebirds across naturally occurring noisy and quiet locations, and to determine how female incubation behaviors may be modulated by the presence of mosquitoes and anthropogenic noise. I hypothesized that anthropogenic noise and mosquito levels would hinder optimal incubation behaviors of Eastern Bluebirds. I predicted that high noise and high mosquito levels would decrease the frequency of relaxed incubation behaviors while increasing the frequency of agitated behaviors exhibited by the female compared to consistently low noise levels. Additionally, females exposed to a large range of noise levels (i.e. intermittent or acute

noise) will be the most sensitive to disturbances, and exhibit higher levels of agitation compared to females inhabiting nest boxes with more consistent noise levels (whether high or low).

Objective 2: Noise, Mosquito and Behavior Effects on Hatching Success

The second objective is to disentangle the effects of noise, mosquitos, and behaviors on hatching success, and to assess whether noise and mosquitos have a direct impact on hatching success. Should behaviors have a significant effect on hatching success, my prior results from the first objective will inform whether noise and mosquito levels may indirectly impact hatching success by influencing behavior. I hypothesized that incubation behavior, noise level, and mosquito level all have direct impacts on hatching success. I predicted that elevated levels of agitation would negatively affect hatching success, while a high frequency of relaxed incubation behaviors would increase hatching success. I predict that as mosquito presence increases, hatching success will decrease. Quiet nests in low noise areas are predicted to have the highest hatching success, with lower hatching success in chronically noisy nest boxes. Finally, I predict that females exposed to intermittent noise, and experiencing a wide range of noise levels, will have the lowest hatching success.

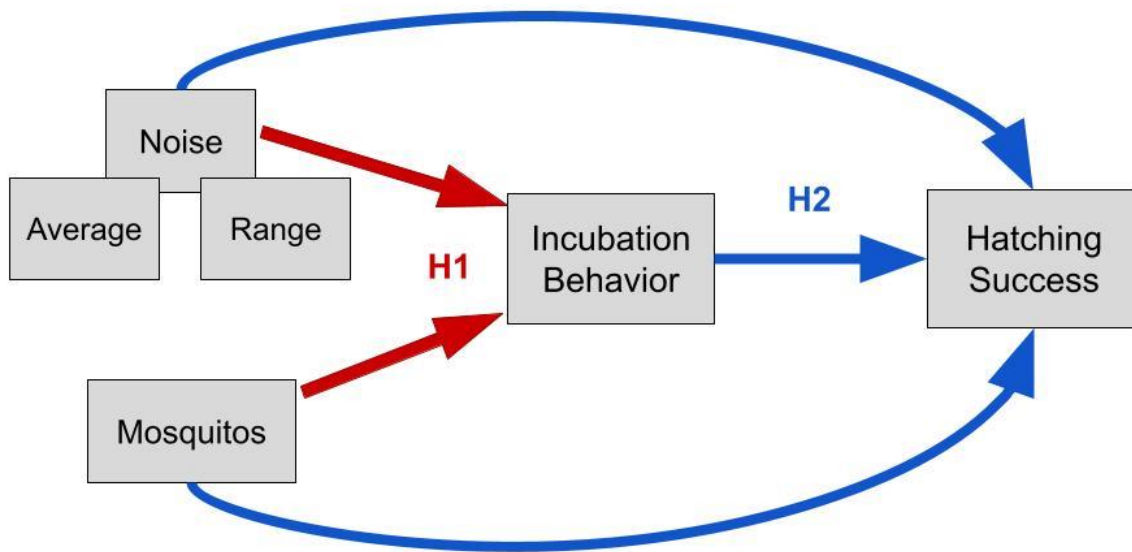


Figure 1-1. Conceptual diagram, including all major variables of interest. Red arrows denote the first hypothesis, that mosquitos and both types of noise impact incubation behavior. The second hypothesis stipulates that noise and mosquitos effect hatching success directly, as well as through the modulation of behavior, and is represented by the blue arrows.

CHAPTER 2 METHODS

Study System

Study Species

The Eastern Bluebird is a common backyard bird found throughout the central and eastern United States. They are found as far south as Nicaragua with a range that extends into southern Canada during the breeding season. Its extensive range and commonality made it an ideal study species to attribute a large scope of relevance to my research project. Eastern Bluebirds typically nest in cavities and have been known to favor the use of artificial nest boxes when available. Once a breeding pair has selected a nesting site, several days are dedicated to gathering nesting material and building the nest cup. The female begins laying once the building of the nest is complete, and usually lays only one egg per day, with the final clutch size generally ranging from three to seven eggs. Incubating is done exclusively by the females and begins once she has laid the last or the penultimate egg (Cooper et al., 2005). Incubation duration varies slightly with latitude, but generally lasts about 11-19 days, and a duration of about 14 days is the most common (Gowaty & Plissner, 1998).

Study Sites

This study took place across three field sites; on the main campus of the University of Florida in Gainesville, Florida (29.64833°N, 82.34944°W), at the Alachua Conservation Trust's Prairie Creek Lodge (29.5858391°N, 82.2408443°W), and at the Ordway-Swisher Biological Station (29.7196951°N, 82.0090584°W). All of the study sites contained large open grass patches with scattered trees, which is ideal bluebird nesting habitat (Pinkowski, 1979). These sites represent varying levels of urbanization, providing a range of noise exposure levels for comparison (see Figure 2-1). The University of Florida campus (UF Campus) had an overall

average noise level of 54.4 dBA, with nest boxes ranging from an average of 46.9 dBA up to an average of 60.9 dBA. Prairie Creek had the lowest range among the field sites, ranging from 44.4 to 52.4 dBA, with an average of 48.2 dBA. At the Ordway-Swisher Biological Station (OSBS), noise levels varied between 38.5 dBA and 59.2 dBA, and the overall average noise level was 46.0 dBA. To control for potential site-based effects, nest boxes were placed in relatively quiet and relatively noisy locations within each field site. On the UF Campus, the golf course represented the quieter location and averaged 53.8 dBA, while the south Lake Alice area was considered the noisier area and had an average noise level of 55.0 dBA. At Prairie Creek, the nest boxes alongside county road 234 were considered noisy compared to the nest boxes placed within the conservation area; note that none of these “noisier” road boxes were occupied by bluebirds, and therefore Prairie Creek is considered as a single location throughout this study. In OSBS, the northern section bordered by state route 26 was the noisier area, averaging 52.2 dBA, while the south-western area in the center of the field station was considered quiet, with an average of 39.8 dBA.

General Field Methods

Observations were conducted during the breeding season (February-July) in 2021. In January and February, 79 Gilbertson-style bluebird nest boxes (www.NABS.org) were placed across the three field sites. Within each field site, nest boxes were placed either in locations where bluebirds have been known to previously utilize nest boxes, or in locations that were deemed suitable habitat for bluebirds. Boxes were placed in a variety of sites, including along a golf course, next to a commuter parking lot, near nature trails, by buildings, on protected land and by a state road.

Nest boxes were placed at a minimum of approximately 100 m from each other. Nest box setups consisted of a bluebird box mounted on a stainless steel pipe. An approximately 1.2 m

piece of rebar, hammered halfway into the ground, provided support for the steel pipe. All nest boxes were placed approximately 1.2 m above the ground.

Each nest box was checked at least once a week. Ambient noise levels were measured periodically at all nest boxes using a mobile phone software (Decibel X). For ambient noise measurements, the mobile software was run for approximately two minutes (average duration = 124 seconds), with the microphone of the mobile device pointed in the same direction as the entrance to the nest box to more closely simulate the noise intensity the female experiences when in the nest box. The minimum, average, and maximum noise level as well as the range in noise (maximum - minimum) were derived from these recordings for each nest box and recorded in decibels, A scale (dBA). Once a nest had eggs, it was considered “active” and was monitored with greater frequency. Two temperature monitors (iButton DS1921G-F5) were placed at each active nest; one inside of the nest cup, nestled under the eggs to provide an estimate of egg temperature (used as an indicator for incubation temperature), and one placed just outside of the nest box to provide a representation of the microclimate used by the female to adjust her incubation efforts.

Once the clutch was complete and the female began incubating, a small spy camera (HZTCAM Mini HD Camera) equipped with an infrared lens was placed inside of the nest box to record nocturnal incubation behavior. Active nests were each filmed twice; once about halfway through the incubation period (approx. day 7 after clutch was completed), and once at the very end of incubation near hatching time (approx. day 14 after clutch was completed). Resulting videos were scored for particular behaviors, including agitations and shifting of the eggs. Based on video data, mosquito level was added as a variable causing disturbance.

Video Recording Protocol

Incubation videos were recorded using a small, infrared spy camera set up on the inside of the lid to the nest box, with the lens of the camera facing down at the female. To provide a longer battery life and extend recording time, the camera was rigged to an external battery pack (Charmast Portable Charger) secured with a zip tie to the stainless steel pole elevating the nest box. The cameras were not equipped to programming specific start and end times, therefore cameras recorded video continuously until the battery was depleted or the camera was manually turned off when collected. Each camera had a 32 GB microSD with the capacity to hold approximately 6 hours of film, split into individual videos each about ten minutes in length. Due to limited storage, once the microSD reached capacity and for as long as the camera continued to record, the oldest file would be replaced to make space for each new file. This video rollover was necessary to maximize the number of hours of nocturnal incubation recorded, as recording equipment was often set up at least two to three hours before sunset. Cameras were deployed with enough time before dusk to allow time for habituation and to ensure a female would return to the nest even if the camera placement had flushed her. Nest videos were later analyzed manually using VLC video viewer (<https://www.videolan.org/vlc/>), with all target behaviors scored and resulting data entered into a master spreadsheet of raw variables (see Table 2.1 for raw variables).

Defining Incubation Behavior

Four distinct, recurring behaviors were defined while processing the incubation videos; preen, egg turn, agitation and settle. As opposed to daytime incubation, the female does not leave the nest during the night, meaning there are no separate incubation bouts, and the female is almost constantly in contact with the eggs to warm them. To account for the short amounts of time in which the female was not sitting on the eggs, we created an additional variable referred to

as “off eggs”. Due to both the angle of view and proximity of the camera to the focal female, we were unable to determine when females were sleeping or resting versus when they were awake, therefore no variables relating to rest are included.

Preen

Preens were defined as standard self-maintenance behaviors in which the female used her beak (or more rarely, her feet/claws) to comb through her feathers to clean them. Females occasionally fully stood up while preening to better reach their chest and belly.

Settle

A “settle” was when a female appeared to shift the eggs beneath her, or adjust her own positioning, using a vigorous side to side, “shimmy”-like motion. This was done without standing up nor exposing the eggs, as the female remained in the nest cup for the duration of the behavior.

Agitation

We defined agitations as non-incubation related behaviors, in which females were clearly disturbed by an external force, usually either a loud noise (e.g. from a truck driving by) or a mosquito landing on her. Agitations included sharp head turns and flicks, often done in the direction of the disturbance, as well as instances in which the female was abruptly awoken from a resting state. Those females intently harassed by multiple mosquitos also occasionally lunged and snapped their beak them.

Egg Turn

Egg turns consisted of a female fully standing up off the eggs in order to readjust by the eggs and herself. The female stands, sometimes moving herself back to perch on the edge of the nest cup, then bends over and uses her beak to move the eggs around. She then sits back down onto the eggs while moving her body in a side-to-side motion, and settles deeply in the nest cup.

Time Off Eggs

Due to the fact that both the egg turns and, occasionally, the preen behavior resulted in the female fully standing up and exposing the eggs, we chose to include a separate variable to measure the total amount of time the female spent off the eggs over the course of each ten minute video file.

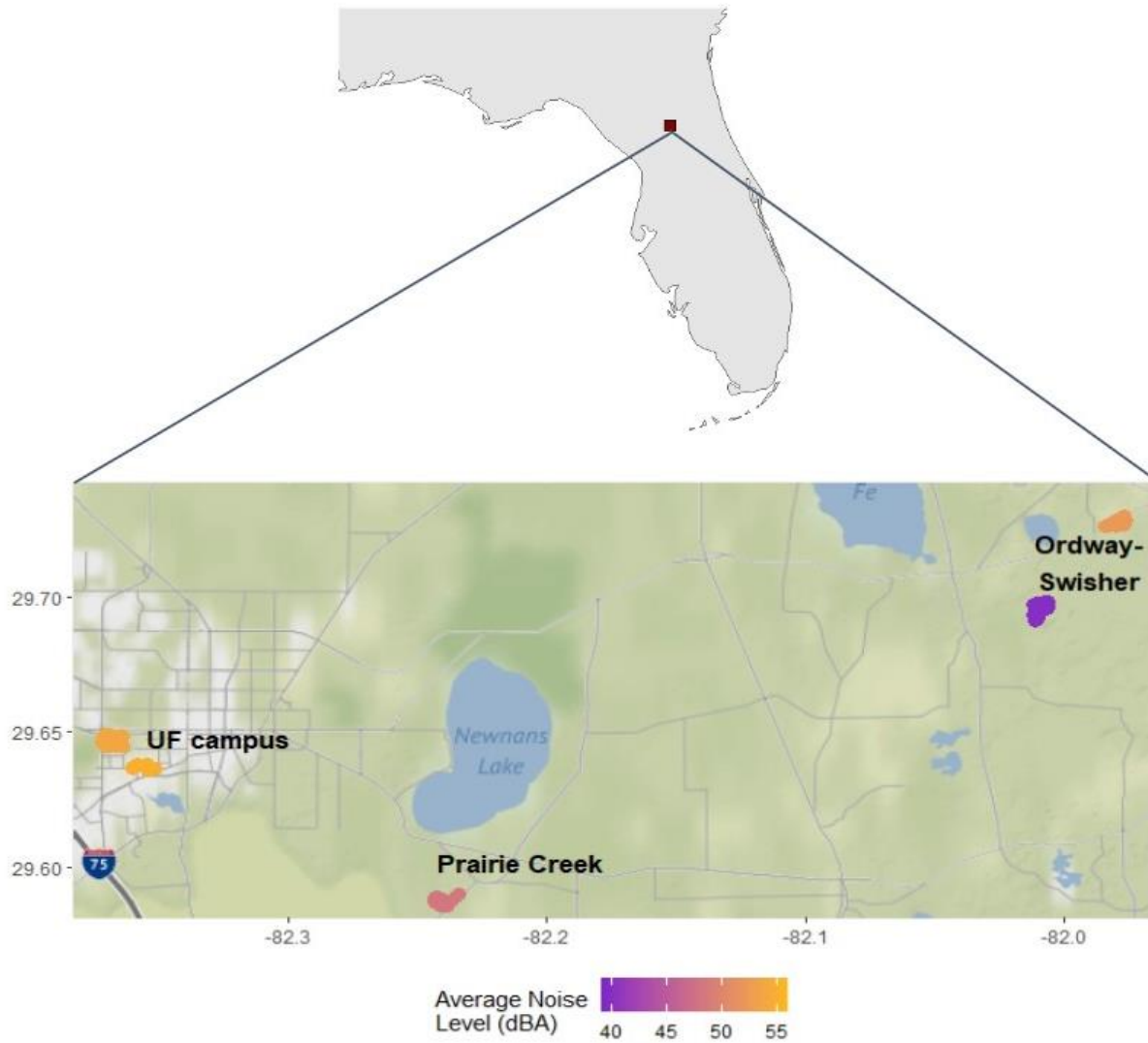


Figure 2-1. Map of the three field sites, color-coded by average noise level for each area. Overall average noise per site: UF Campus, 54.4 dBA; Prairie Creek, 48.2 dBA; OSBS, 46.0 dBA.

Table 2-1. Description of raw variables collected from the field and from video recordings.

Variable Name	Description	Units
Ambient Noise	(1) Average minimum ambient noise level at nest box; (2) Average mean ambient noise level at nest box; (3) Average maximum ambient noise level at nest box; (4) Average range in ambient noise level (average maximum - average minimum)	dBA
Environmental Temperature	Environmental temperature taken just outside the nest box using iButton temperature logger	°C
Nest Temperature	Proxy for incubation temperature; temperature inside nest box using iButton temperature logger placed in the nest cup	°C
Mosquitoes	Number of mosquitoes in the nest box during filming; indication of level of mosquito disturbance. Capped at 5 due to perception limitations.	Count (0 - 5)
Agitation	Sharp head movements and flicks, or other instances in which a female is suddenly roused from a resting position.	Count
Preen	Self-maintenance behavior in which the female runs her beak through her feathers to clean them.	Count
Settle	Incubation behavior involving the female shifting the eggs beneath her in a side-to-side, “shimmy”-like movement; done while further settling down on the eggs, without standing.	Count
Egg turn	Incubation behavior in which the female fully stands up and uses her bill to move the eggs around, before settling back down deeply on the eggs using a “shimmy” motion similar to a “settle”.	Count
Off-eggs	Duration of time the female spent off the eggs but still inside the nest during a ten-minute period.	Seconds (s)
Hatch Success	Number of eggs in clutch divided by number of chicks hatched	Proportion

CHAPTER 3 DATA ANALYSIS

All data reduction and statistical analyses were conducted using Stata (version 17.0).

Data Reduction

A principal components analyses (PCA) was used to combine correlated measures and create uncorrelated composite variables for my analyses, effectively reducing the number of variables needed. Both the Kaiser-Meyer-Olkin measure of sampling adequacy and the Bartlett test of sphericity were used to determine the suitability of each set of candidate variables for PCA. Hair et al. (2006) suggest that values of above 0.5 for the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy are acceptable, and a significant p-value (< 0.05) for the Bartlett test of sphericity indicates the data are suitable for data reduction techniques such as a PCA.

Behavior PCA

Three biologically significant principal components (PC_ACT: Eigenvalue = 1.96, explaining 39.21 % of variation, scores ranging from -1.80 to 5.09; PC_CHL: Eigenvalue = 1.27, explaining 25.40 % of variation, scores ranging from -3.62 to 10.20; PC_AG: Eigenvalue = 0.82, explaining 16.40 % of variation, scores ranging from -3.57 to 3.68; total of 81.01% variance explained) were derived from preen, settling, egg turn and agitation behaviors (in counts per ten minute video) as well as time spent off the eggs (in seconds, per ten minute video). The data were deemed suitable for use in a PCA (KMO > 0.5 ; Bartlett test of sphericity significant, $p < 0.001$).

PC_ACT

All the behaviors (preens, settles, egg turns, and agitations) loaded highly and positively on PC_ACT (Table 2-1). I defined PC_ACT as a measure of activity level, with high scores of

PC_ACT corresponding to highly active females, and low scores representing unmoving females that were likely sleeping or resting.

PC_CHL

Agitations, preens and settling behaviors loaded negatively, while egg turning and time off eggs loaded positively on PC_CHL (Table 2-1). I interpreted PC_CHL as a metric for typical incubation behavior. High scores correlate to a higher frequency of relaxed (“chill”), typical incubation behaviors, whereas low scores correspond to disturbed females that did not display typical incubation behaviors as frequently or consistently.

PC_AG

Agitations (consisting mainly of head turns) were the only behavior to load very strongly and positively on PC_AG, while preening behaviors loaded strongly and negatively (Table 2-1). PC_AG was defined as a measure of agitation and disturbance; high scores of PC_AG are interpreted as highly disturbed females whose agitation took precedence over all other behaviors, while low scores correspond to very relaxed females who partook mainly in self-maintenance behaviors.

Data Structure and Variable Management

In total, there were eleven variables used in the models; five predictor variables, two random effects, and four response variables (see Table 3-2).

Temperature

The raw data included two variables for temperature: environmental temperature and nest temperature. Including both these variables in any model would be inappropriate due to a high correlation between them, therefore for both hypotheses, I chose to retain only environmental temperature as a predictor. With each hypothesis, the focus is on the impacts of noise and

mosquitos on behaviors—since female incubation behavior should mainly be informed by the environmental temperature, this is the variable that was included.

Noise

The raw data contained four separate measures of noise for each nest: maximum noise, average noise, minimum noise and noise range (defined the difference, in dBA, between the maximum and minimum noise level). These raw noise variables were deemed unsuitable for use in a PCA ($KMO < 0.5$; Bartlett test of sphericity significant, $p < 0.001$). To reduce the number of correlated variables used in my analyses, I chose to use the average noise and the noise range, as these were the most informative variables in relation to the focus of the study. For ease of modelling, the raw continuous variables were converted into categorical variables, each with two levels (high and low). The raw average noise variable ranged in values from 38.5 to 60.9 dBA; nests with average an average noise level up to 50 dBA were classified as “low”, and nests with an average noise level above 50 dBA were deemed “high”. Noise range varied between 11.2 and 33.3 dBA; nests with a range of noise lower than 22 dBA were grouped in the “low” category, and nests with ranges in noise greater than 22 dBA were classified as “high”.

Hatching Success

To render the raw hatching success variable viable for use in the models, I employed an angular transformation, also referred to as the arcsine transformation. This transformation has been similarly used for hatching and nest success data in previously published studies across various taxa (birds, Briskie & Mackintosh, 2004; toads, Sadinski et al., 2020; turtles, Horrocks & Scott, 1991). This transformation yielded a new variable, THS, where $THS = \text{ASIN}(\text{SQRT}(\text{Hatching Success}))$.

Hypothesis Testing

Both hypotheses were tested using generalized linear mixed models (GLMMs), and model selection was conducted using the same protocol for all hypotheses. Using stepwise backwards selection, I started with the full (saturated) model, then removed the least significant interactions one at a time. If a term was removed that did not improve model, then it was replaced and a different term was removed. No main effects terms were removed because all were hypothesized *a priori* to influence the response variables, or were already known to influence avian incubation behaviors (nuisance variables: temperature). This removal process was continued until the model AIC could no longer be reduced by a significant amount, which I defined a drop of two or more AIC points. All models contained varying combinations of main effects and interaction effects from a set of variables (see Table 3-1 for variable distribution across hypotheses).

H1: Noise and Mosquito Effects on Behavior

To test the effects of noise and mosquitos on behavior, the full dataset with all observations from each nest was used ($n = 678$ observations across 33 nests). The first hypothesis was divided into three separate models, one for each of the three new variables (PC_ACT, PC_CHL, and PC_AG) resulting from the PCA on the raw behavioral measures. Other than changing the response variable to correspond to each behavior variable in turn, all three full starting models were identical. The full models included four main effects and four interactions terms of interest. The main effects included were environmental temperature, mosquito level, noise range and average noise. The four interaction terms included all possible two- and three-way interactions between the mosquito level, average noise, and noise range variables. These models also included both nest box ID and unique nest code as random effects,

with nest code nested within box ID, to account for differing sampling effort across nests and for reuse of nest boxes across the nesting season.

H2: Noise, Mosquito and Behavior Effects on Hatching Success

For the second hypothesis, I used a condensed dataset in which the values for all variables were averaged across the multiple recordings for each nest, resulting in a dataset where each row represented a unique nest ($n = 33$). The full starting model contained six main effects and a single interaction term. The main effects included mosquito level, average noise, noise range, and the three behavior variables: PC_ACT, PC_CHL, and PC_AG. Environmental temperature was not included as a main effect, both to avoid confounding with mosquito level (as the two variables are highly correlated) and to allow me to focus the model to test the effects of interest. Additionally, I felt it unnecessary to include as the literature already contains a plethora of studies linking environmental temperature to reproductive success (Pipoly et al., 2013; Wegge & Rolstad, 2017; Coppes et al., 2021). In order to reduce the number of terms used and avoid overfitting the model, I also chose to drop the two-way interactions included in the H1 models, and retain only the three-way interaction between mosquito level, average noise and noise range. Keeping only the three-way interaction successfully reduced the number of terms yet still allowed me to test the interactive effects of all three main disturbance variables of interest.

Table 3-1. Component loadings for the three behavior variables, PC_ACT, PC_CHL, and PC_AG, resulting from the behavior PCA.

Variable	PC_ACT	PC_CHL	PC_AG
Agitation	0.4637	-0.2413	0.6987
Preen	0.4641	-0.2258	-0.7121
Egg Turn	0.4641	0.4902	-0.0406
Settle	0.5331	-0.3744	0.0224
Off Eggs	0.2643	0.7143	0.0506

Table 3-2. Variables used in the mixed models and their role in each hypothesis tested. P = predictor variable, R = response variable, RE = random effect.

Term	Variable Characteristic		Role		Description
	Type	Number of levels	H1	H2	
Average Noise	categorical	2	P	P	Average noise level at each nest box; either high (H) or low (L)
Noise Range	categorical	2	P	P	Range in noise levels at nest; either high (H) or low (L)
Environmental Temperature	continuous	NA	P		Environmental temperature taken just outside the nest box using iButton temperature logger
Mosquitoes	continuous	NA	P	P	Number of mosquitoes in the nest box during filming
PC_ACT	continuous	NA	R	P	Behavioral PC coding for high activity levels
PC_CHL	continuous	NA	R	P	Behavioral PC coding for relaxed incubation behavior
PC_AG	continuous	NA	R	P	Behavioral PC coding for disturbed, agitated behavior
Transformed Hatch Success	continuous	NA		R	Proxy for hatching success; hatching success data transformed using an angular (arcsine) transformation
Nest Code	categorical	NA	RE		The unique code given to each active nest
Box ID	categorical	NA	RE	RE	The ID of each nest box

CHAPTER 4 RESULTS

Out of a total of 79 nest boxes deployed, 35 were occupied by nesting birds, spanning a total of 54 nesting attempts. Among these nesting attempts, 48 reached the laying stage (had at least one egg) and were considered active; 46 of these were by Eastern Bluebirds, one was by Carolina chickadee, and one was by great-crested flycatcher. Video recording equipment was deployed at 40 out of the 46 active bluebird nests throughout the field season, 30 of which were filmed twice during the incubation/brooding periods for a total of 70 filming attempts. Only the successful filming sessions from the incubation period are included in the analyses ($n = 33$); film sessions from the brooding stage ($n = 35$) and empty nests/failed recording attempts ($n = 3$ and $n = 2$ respectively) were excluded from analyses.

From the 33 successful incubation recording sessions, I collected 1,118 videos totaling approximately 186 hours of filming. Due to the nature of the recording equipment and the lack of ability to set the recording hours, many of the cameras recorded a mixture of both day and night. For the purposes of this study, I have only analyzed videos recorded at night, defined as videos recorded before the female first left the nest for the day, usually around sunrise. This put the total number of usable footages at 678 videos, amounting to approximately 113 video hours split among 33 separate nesting attempts.

Noise. The quietest nest averaged 38.5 dBA and was located in the southern portion of OSBS. The noisiest nest box was located on the golf course of the UF Campus and averaged 60.9 dBA over the course of the breeding season. Overall the UF Campus locations were the noisiest, with the southern OSBS being the quietest (Figure 4-1, A). The golf course (UF Campus) had the largest variability in noise range, while Prairie Creek and the southern portion

of the OSBS had the lowest ranges in noise (Figure 4-1, B). Mosquito levels were generally variable and did not appear to be significantly different between field sites (Figure 4-2).

H1: Noise and Mosquito Effects on Behavior

Separate models were fitted for each of the composite variables resulting from the PCA on the raw behavior measures. My predictions were that high average noise levels and high ranges in noise would increase agitated behaviors while decreasing regular incubation behaviors, and that high mosquito level would have similarly disruptive effects on behavior.

Model 1.1: PC_ACT

The final model predicting highly active female behavior included three significant terms; two main effects, environmental temperature (Enviro_temp), and mosquito level (Mozz), in addition to one interaction term between average noise level and mosquitoes (Avg_noise * Mozz; see table 4-1). At environmental temperatures of 30°C or above, females were significantly more active than at lower temperatures (Figure 4-3). An increase in activity with warmer temperatures is expected, as the warmer the environment, the less closely a female needs to incubate in order to maintain the eggs at an appropriate temperature for development. Considering the videos were taken at night, a rise in environmental temperature is also likely to coincide with the sunrise, when females are more likely to increase their overall activity as they awaken and prepare to leave the nest for the first time that day. Females also experienced significantly higher activity in nests with high mosquito levels, and there was a steady increase in PC_ACT values as the mosquito level increased (Figure 4-4).

Although average noise level as a main effect had no significant effect on PC_ACT, the interaction term with mosquito level was significant. The predicted marginal means showed no difference in estimated values of PC_ACT between high and low average noise levels at any level of mosquito incursions (see Figure 4-5). However, the predicted marginal means plot does

show a noticeable difference in the slope of the two lines, indicating a trend in which the activity levels of a female exposed to increasing levels of mosquitos rises at a higher rate when she inhabits a nest box with a higher average noise level.

Model 1.2: PC_CHL

The best model, using relaxed incubation behavior as the response variable, included two significant main effects and one significant interaction term: mosquito level (Mozz), average noise level (Avg_noise), and the interaction between mosquitos and average noise level (Avg_noise * Mozz; see Table 4-2). Mosquitos had a strong negative effect on incubation behavior; the greater the mosquito presence, the lower the frequency of relaxed incubation behaviors (Figure 4.6). Average noise levels were significant in the model, but the marginal means plot showed no significant difference in PC_CHL scores between females in high and low average noise nest boxes. Similar to the results from the models on PC_ACT, the role of average noise is made clearer in the interaction with mosquito level (Figure 4-7). Both females in noisy and quiet nest boxes experience a decrease in relaxed incubation behaviors as mosquito levels increase, indicating that mosquitos are the main force modulating the females' behavior. In the absence of mosquitos, females in high average noise nest boxes appear to exhibit incubation behaviors more frequently than their low noise average counterparts, however the decline in incubation behaviors as mosquito level increases is much sharper. Paralleling the results from the model on activity level, this model suggests that females in areas of higher noise levels are more susceptible to changes in behavior as a result of the interaction between noise and mosquito disturbances.

Model 1.3: PC_AG

The final model for highly agitated behaviors included two significant terms: mosquito level (Mozz), and the interaction between mosquito level and noise range level (Range_noise *

Mozz; see table 4-3). In line with my prediction, mosquito level was the main driver of agitated behavior; as mosquito levels increased, agitated behaviors sharply increased (Figure 4-8). Noise range did not have a significant effect by itself, but the marginal predicted means plot for the interaction between noise range and mosquito level suggests a difference in the intensity of the effect of mosquito based on noise range (Figure 4-9). Contrarily to my prediction, females in nest boxes with lower ranges in noise levels appear to exhibit greater agitation in response to mosquitos as compared to their high range counterparts.

H2: Noise, Mosquito and Behavior Effects on Hatching Success

The best model for the transformed hatching success variable (THS) included three significant main effects and one significant interaction term (see Table 4-4). Significant main effects included mosquito level (Mozz), frequency of incubation behavior (PC_CHL), and agitation level (PC_AG). The significant interaction was a three-way interaction term between mosquito level, noise range, and average noise (Avg_noise * Range_noise * Mozz). Although mosquitos had a positive and significant effect on hatching success in the model, the marginal predicted means yielded large confidence intervals and implied that the effect of mosquitos was in fact negligible. As predicted, higher scores of PC_CHL, meaning a higher frequency of relaxed incubation behaviors, had significantly higher rates of hatching success compared to lower scores of PC_CHL (Figure 4-10). This supports my hypothesis that these particular behaviors, especially egg turns, play an important role in hatching success. In contradiction to my predictions, PC_AG had a significantly positive effect on hatching success; as the frequency of agitated behaviors increased, hatching success increased (Figure 4-11).

The final significant term for the hatching success model was the three-way interaction between mosquito level and the two noise variables (Figure 4-12). In the absence of mosquitos, there was no statistically significant difference in hatching success between nests, regardless of

noise average or range. As mosquito level increased, the interactive effects of the two noise types were revealed; the quietest nest, those with low average noise combined with a low range of noise, had significantly higher hatching success than the chronically noisy nests—those with high noise averages and a low range in noise level. Females residing in nest boxes that experienced intermittent noise (high noise range), regardless of the average noise level, had similar hatching success across mosquito levels.

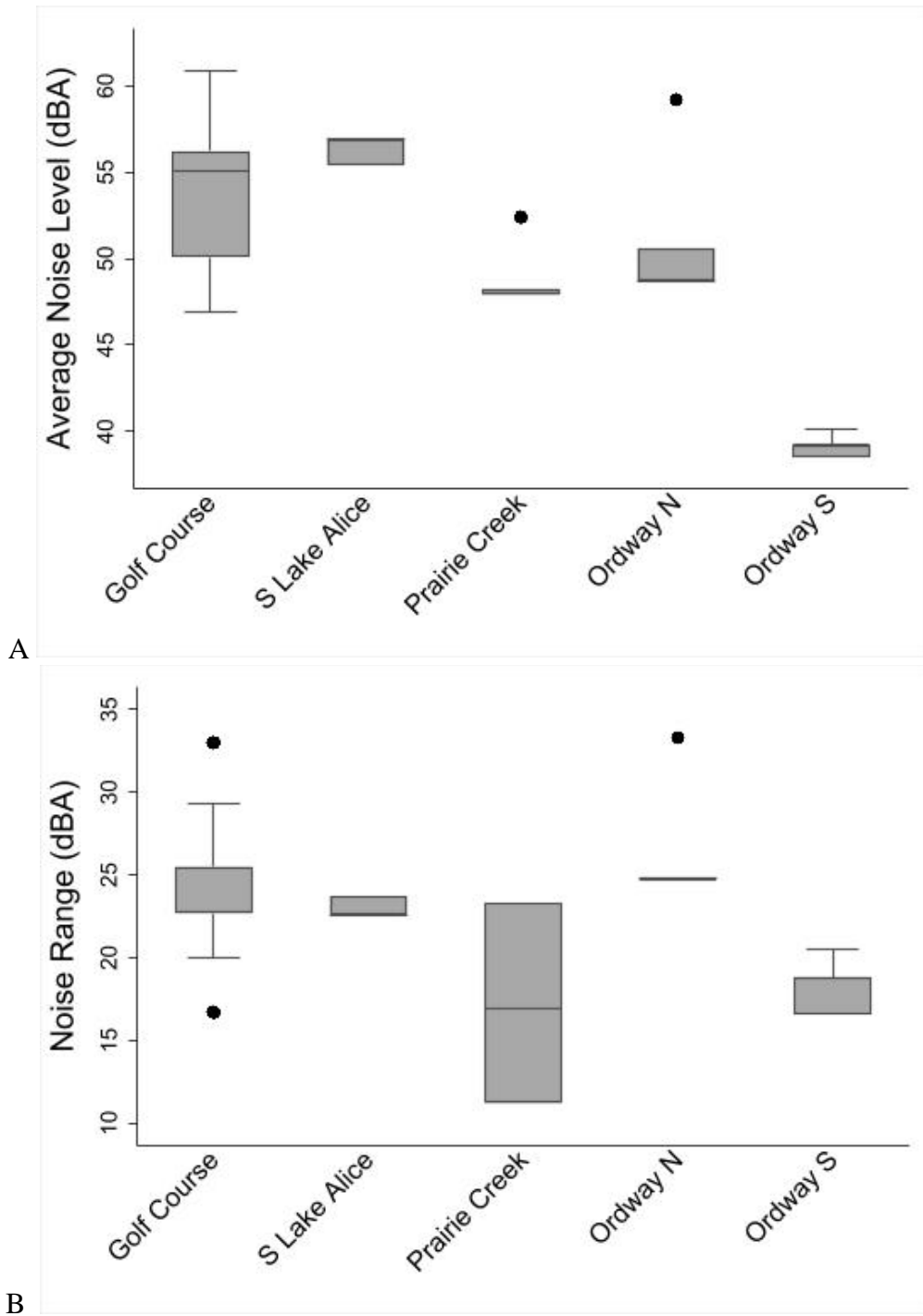


Figure 4-1. Descriptive plots depicting the spread of A) average noise level and B) noise range across nest boxes by location. The Golf Course and S Lake Alice make up the two UF Campus areas, and the Ordway N and S are part of the OSBS.

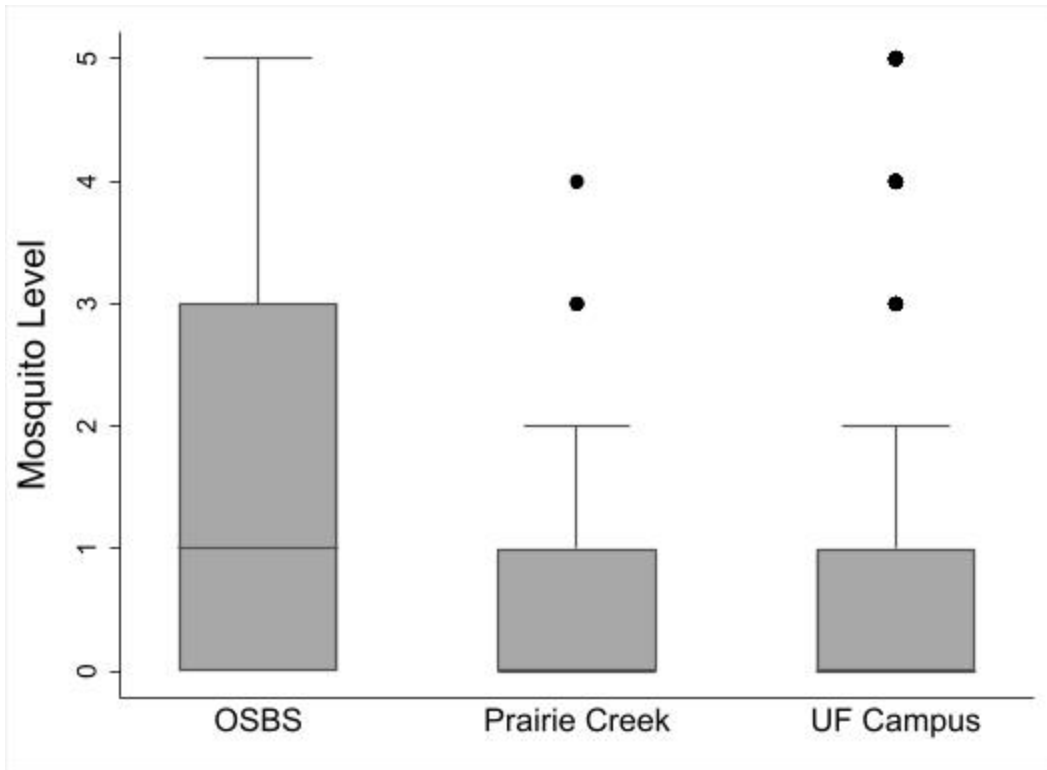


Figure 4-2. Descriptive plot; mosquito level by location. Mosquito level was variable but did not significantly vary by site.

Table 4-1. Output for Model 1.1, activity level, with PC_ACT as the response variable. In terms that include a categorical variable (Avg_noise or Range_noise), each level of the variable is compared to the reference level (i.e. high average noise is compared to low average noise).

Terms	Coef	Std.Err	z	P > z
Enviro_temp	0.043	0.018	2.38	0.017
Mozz	0.346	0.067	5.17	0.000
Avg_noise	-0.141	0.426	-0.33	0.741
Range_noise	-0.492	0.419	-1.17	0.240
Avg_noise * Mozz	-0.261	0.096	-2.73	0.006
Range_noise * Mozz	0.108	0.213	0.51	0.613
Avg_noise * Range_noise * Mozz	0.386	0.288	1.34	0.179

Model Chi-square = 57.57, N = 678 observations, P < 0.001, Log likelihood = -908.657

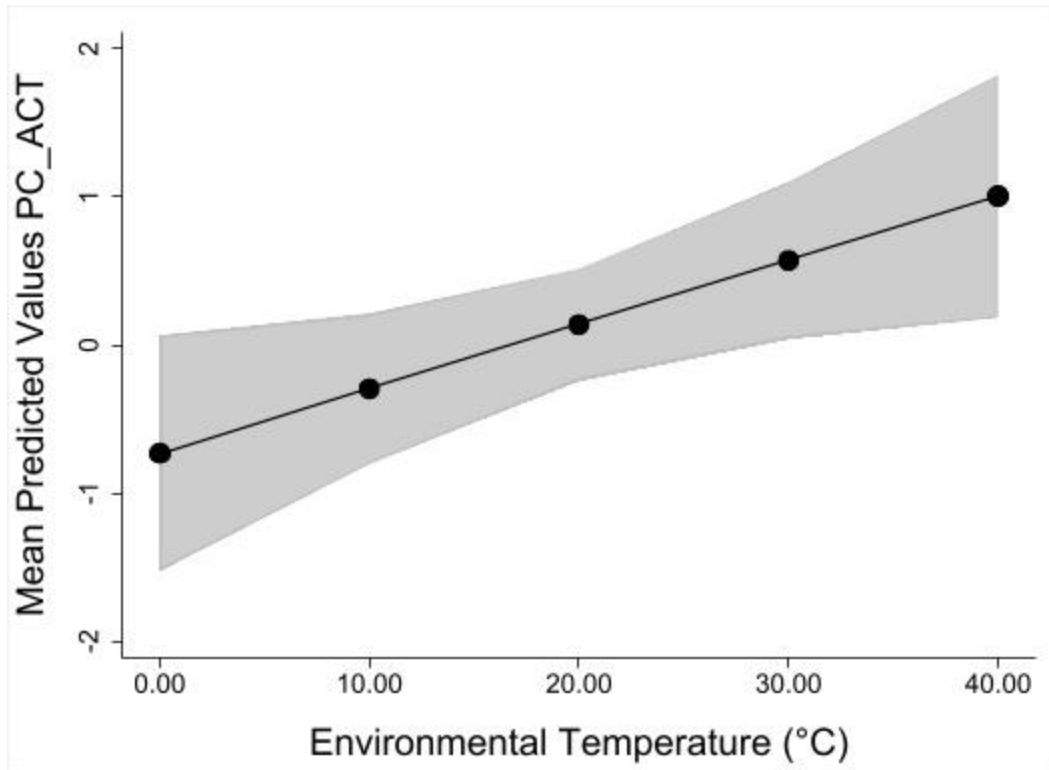


Figure 4-3. (H1, Model 1.1) Plot of marginal predicted values of female activity (PC_ACT) in relation to environmental temperature. With increasing environmental temperature, females display more behaviors and are generally more active. Shaded area represents 95% confidence interval.

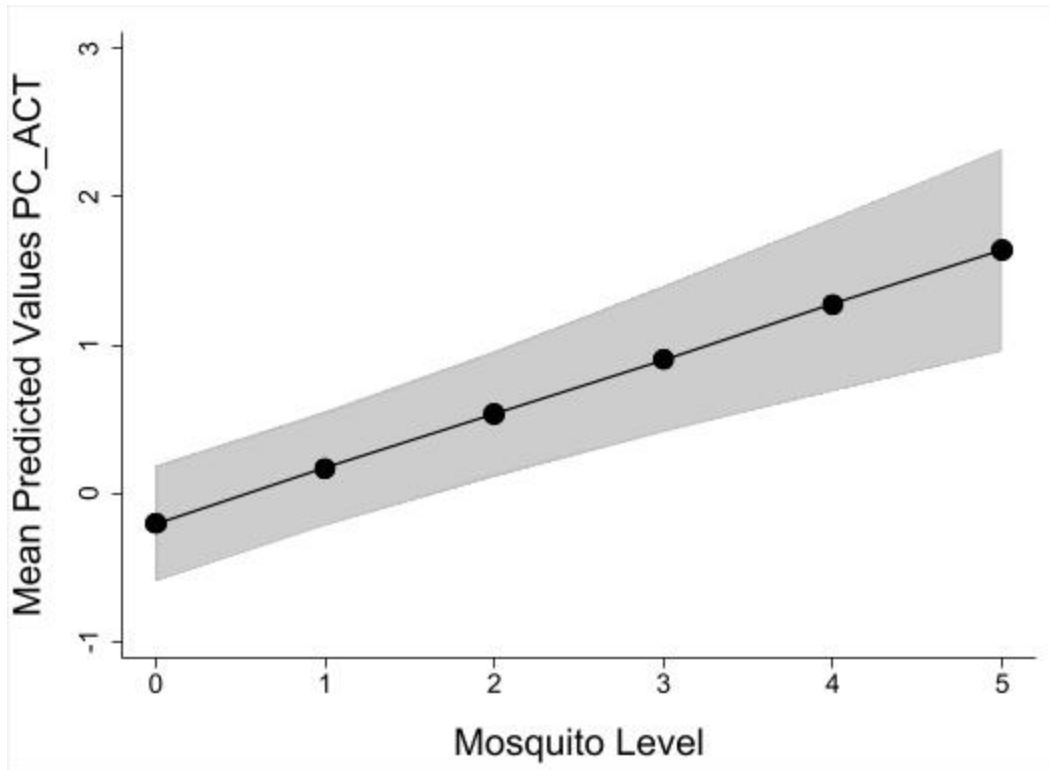


Figure 4-4. (H1, Model 1.1) Plot of marginal predicted values of female activity (PC_ACT) as a function of mosquito level. The greater the number of mosquitos in the nest box, the higher the values of PC_ACT, and the more active the female was. Shaded area represents 95% confidence interval.

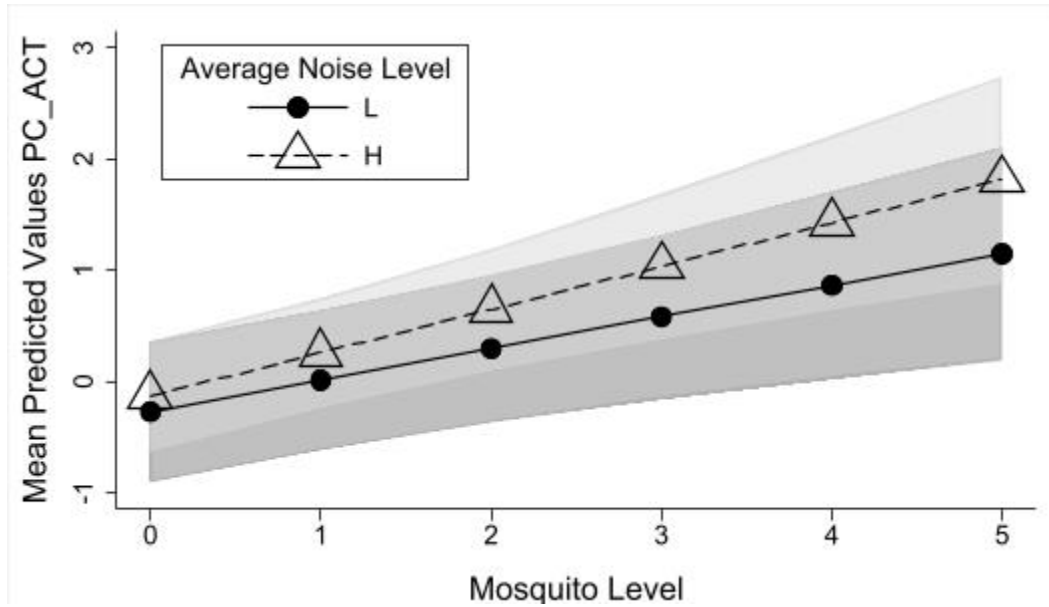


Figure 4-5. (H1, Model 1.1) Plot of marginal predicted values of female activity (PC_ACT) as a function of the interaction between average noise level and mosquito level. There is no significant difference between activity levels of females in high versus low noise nest boxes, but the slope of the increase in activity is steeper for females inhabiting areas of higher average noise levels. Shaded areas represent 95% confidence intervals.

Table 4-2. Output for Model 1.2, relaxed incubation behavior, with PC_CHL as the response variable. In terms that include a categorical variable (Avg_noise or Range_noise), each level of the variable is compared to the reference level (i.e. high average noise is compared to low average noise).

Terms	Coef	Std.Err	z	P > z
Enviro_temp	0.028	0.016	1.77	0.077
Mozz	-0.324	0.060	-5.41	0.000
Avg_noise	-0.496	0.214	-2.32	0.020
Range_noise	0.016	0.202	0.08	0.936
Avg_noise * Mozz	0.191	0.087	2.19	0.028

Model Chi-square = 37.49, N = 678 observations, P < 0.001, Log likelihood = -994.265

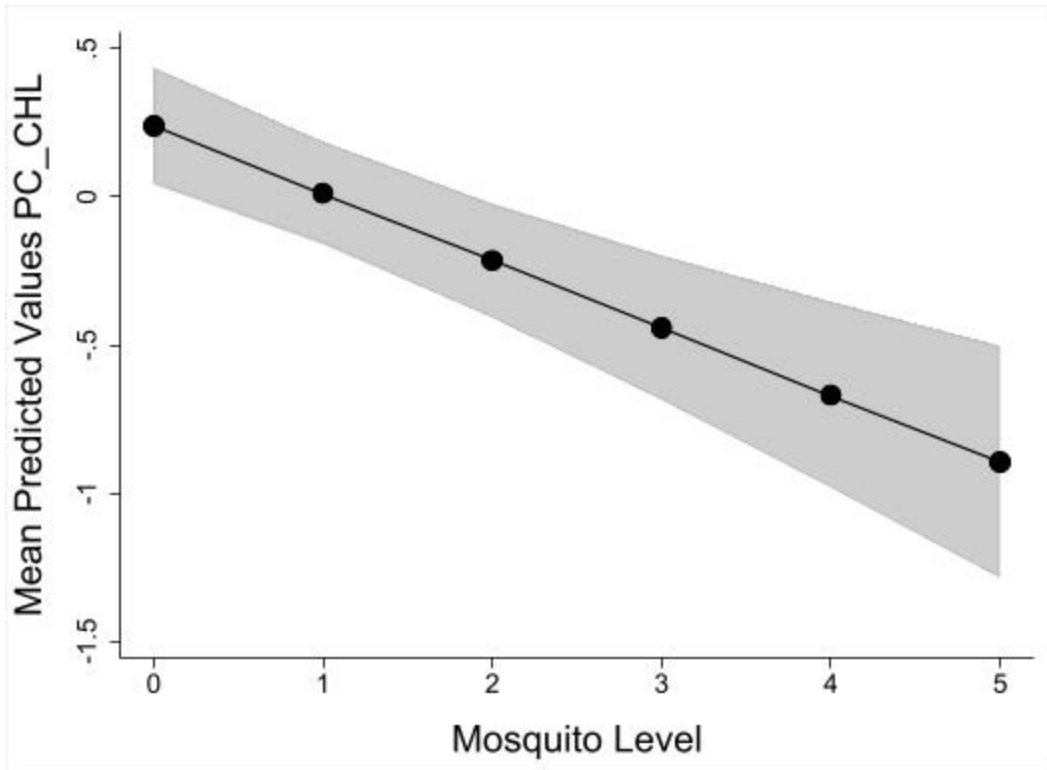


Figure 4-6. (H1, Model 1.2) Marginal predicted mean values of relaxed incubation behaviors (PC_CHL) as a function of mosquito level. There is a clear negative relationship; the greater the mosquito presence, the less likely the females were to display relaxed incubation behaviors. Shaded area represents 95% confidence interval.

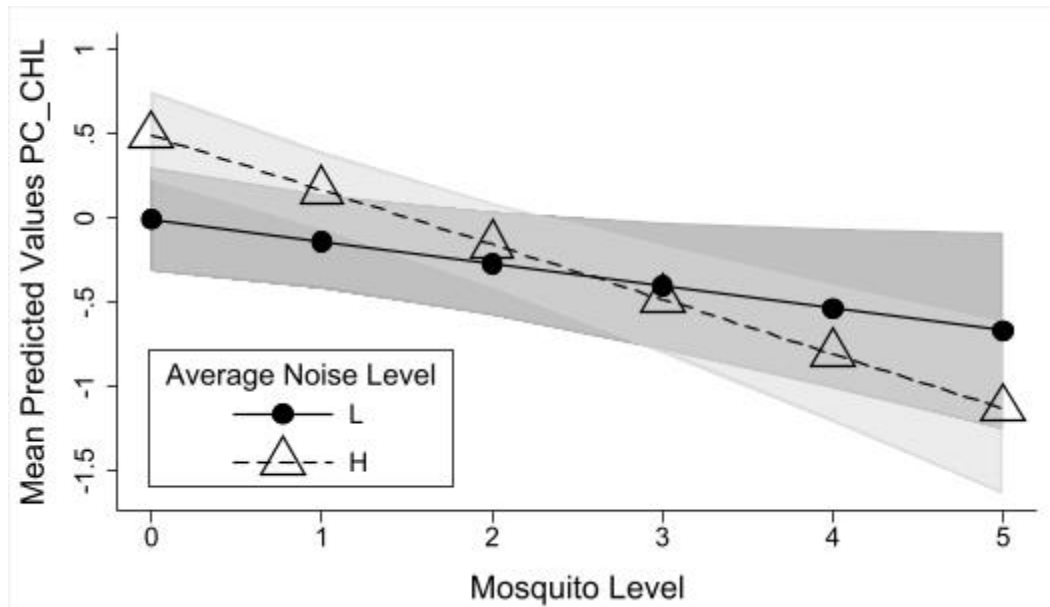


Figure 4-7. (H1, Model 1.2) Plot of marginal predicted mean values of relaxed incubation behaviors (PC_CHL) as a function of the interaction between average noise and mosquito level. The crossover between lines indicates a significant slope. At the highest levels of mosquitos, females in noisy nest boxes are significantly less likely than their quiet counterparts to display relaxed incubation behaviors. Shaded areas represent 95% confidence intervals.

Table 4-3. Output for Model 1.3, agitated behavior, with PC_AG as the response variable. In terms that include a categorical variable (Avg_noise or Range_noise), each level of the variable is compared to the reference level (i.e. high average noise is compared to low average noise).

Terms	Coef	Std.Err	z	P > z
Enviro_temp	-0.009	0.014	-0.67	0.500
Mozz	0.114	0.051	2.24	0.025
Avg_noise	0.209	0.297	0.70	0.482
Range_noise	0.132	0.279	0.47	0.637
Avg_noise * Mozz	0.029	0.073	0.40	0.692
Range_noise * Mozz	0.322	0.159	2.03	0.043
Avg_noise * Range_noise	-0.402	0.420	-0.96	0.339
Avg_noise * Range_noise * Mozz	-0.314	0.213	-1.48	0.139

Model Chi-square = 24.89, N = 678 observations, P = 0.0016, Log likelihood = -709.875

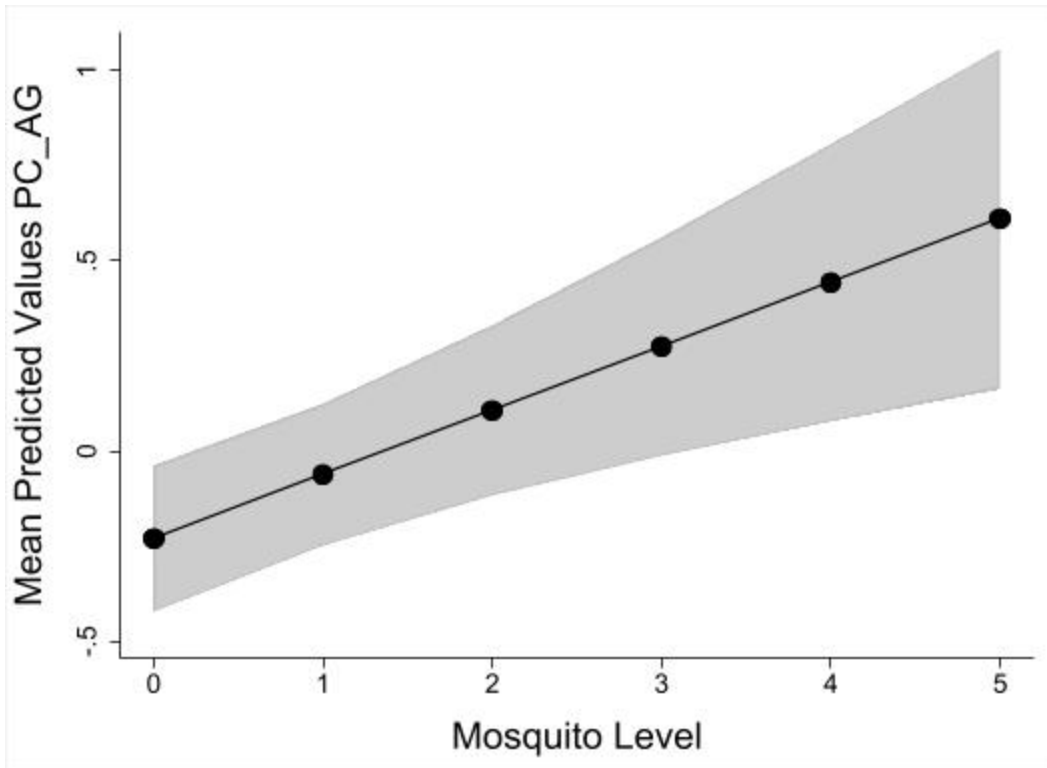


Figure 4-8. (H1, Model 1.3) Marginal predicted mean values of agitated behaviors (PC_AG) as a function of mosquito level. There is a significant and strong positive relationship; the higher the mosquito level, the more agitated the female is. Shaded area represents 95% confidence interval.

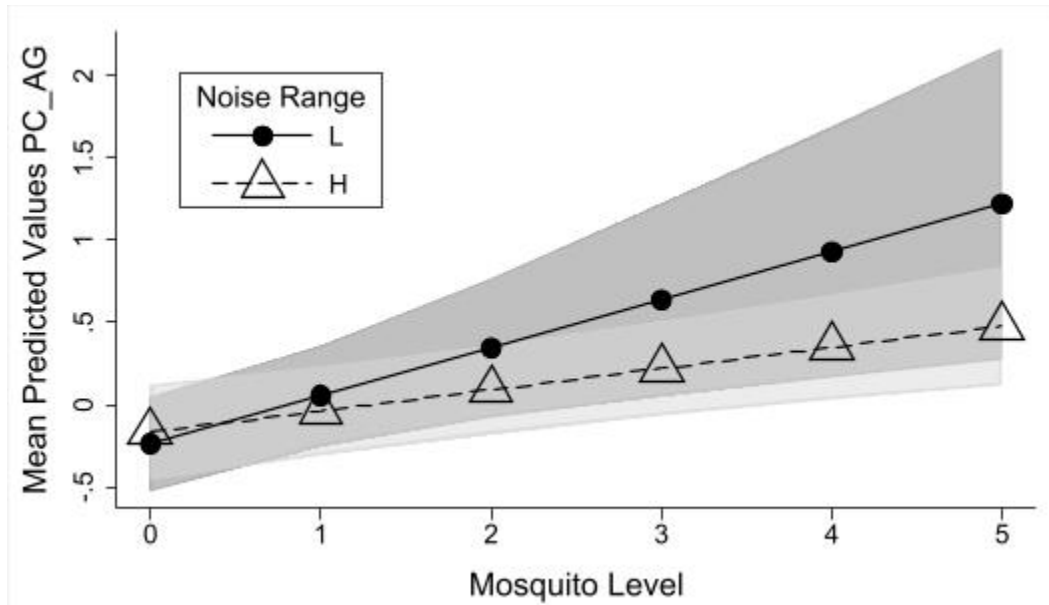


Figure 4-9. (H1, Model 1.3) Plot of marginal predicted mean values of agitated behaviors (PC_AG) as a function of the interaction between noise range and mosquito level. The rate at which agitations increased was higher for females residing in nest boxes with a low range of noise. Shaded areas represent 95% confidence intervals.

Table 4-4. Model output for H2, hatching success, using THS as the response variable. In terms that include a categorical variable (Avg_noise or Range_noise), each level of the variable is compared to the reference level (i.e. low average noise is compared to high average noise).

Terms	Coef	Std.Err	z	P > z
Mozz	0.704	0.356	1.98	0.048
Avg_noise	0.309	0.239	1.29	0.197
Range_noise	-0.332	0.234	-1.42	0.156
PC_ACT	0.087	0.087	1.01	0.314
PC_CHL	0.468	0.131	3.57	0.000
PC_AG	0.426	0.139	3.07	0.002
Avg_noise * Range_noise * Mozz	-0.932	0.369	-2.53	0.011

Model Chi-square = 37.44, N = 33 observations, P = 0.000, Log likelihood = -13.134

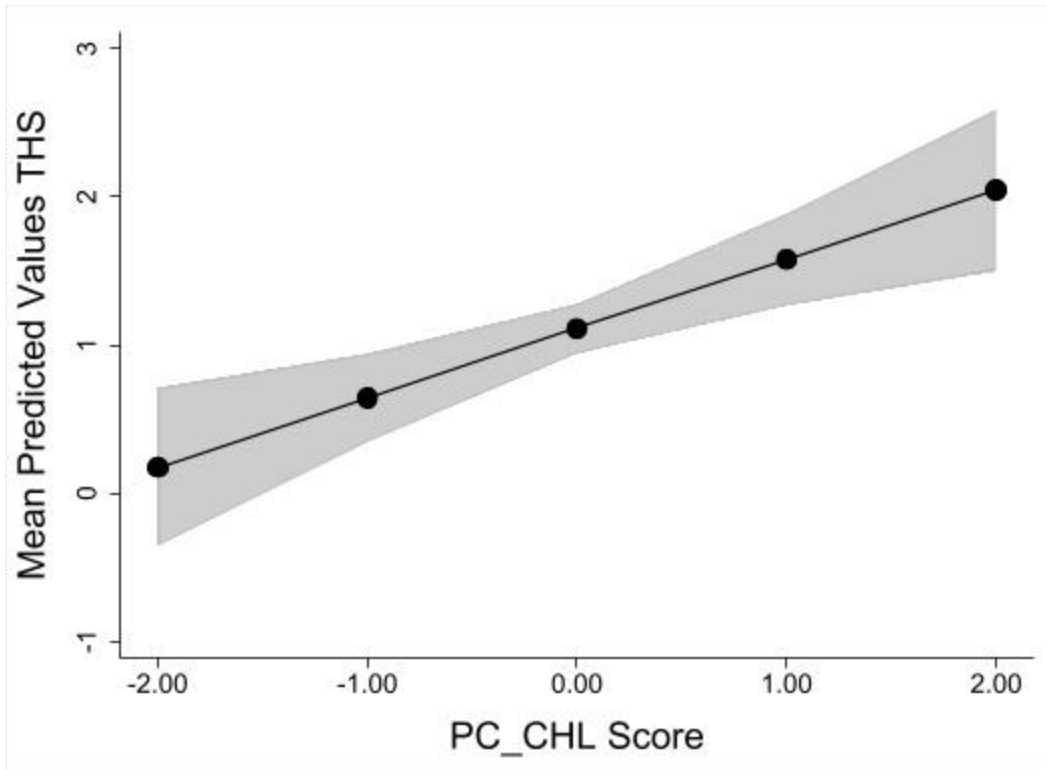


Figure 4-10. (H2) Marginal predicted mean values of transformed hatching success (THS) as a function of relaxed incubation behaviors (PC_CHL). The greater the frequency of relaxed incubation behaviors, the higher the hatching success. Shaded areas represent 95% confidence intervals.

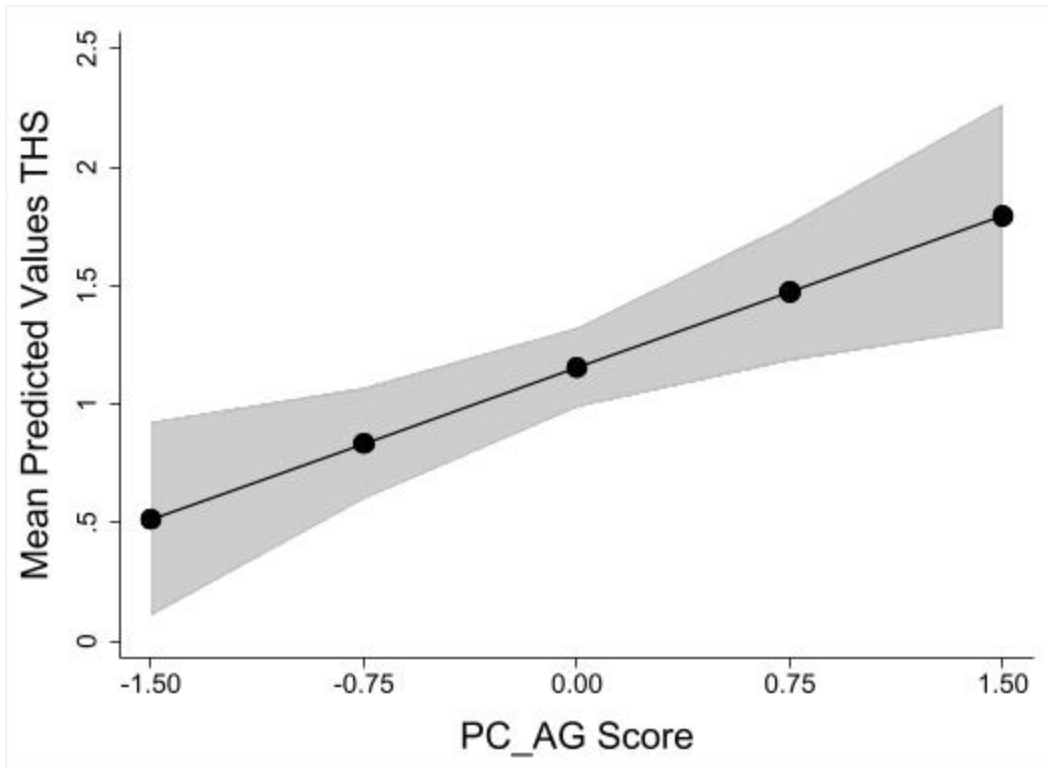


Figure 4-11. (H2) Marginal predicted mean values of transformed hatching success (THS) as a function of agitated behaviors (PC_AG). As the frequency of agitated behaviors increased, hatching success increased. Shaded areas represent 95% confidence intervals.

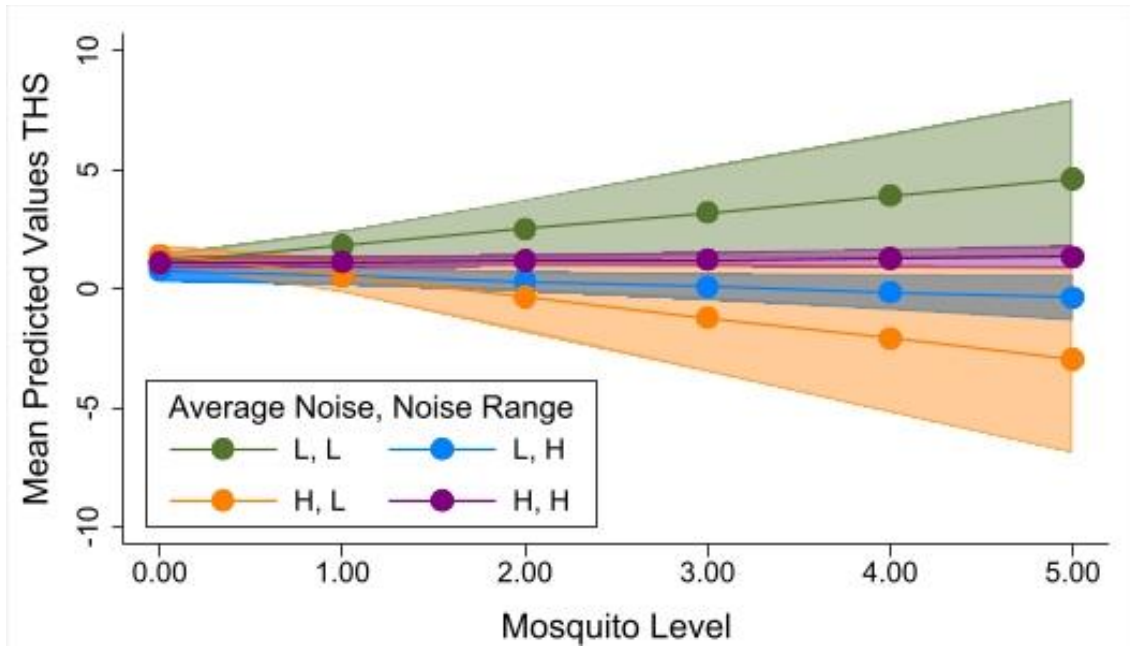


Figure 4-12. (H2) Marginal predicted mean values of transformed hatching success (THS) as a function of the interaction between average noise level, noise range, and mosquito level. When no mosquitos were present, all nests had similar hatching success. At high mosquito levels, quiet nests had the highest hatching success, and noisy nests had the lowest hatching success. Shaded areas represent 95% confidence intervals.

CHAPTER 5 DISCUSSION

Despite not originally expecting mosquitos to be a major source of disruption in the nest, my use of video data allowed me to identify mosquitos as a variable of interest, and as a result I have found strong evidence that mosquito incursions play an important role in modulating female incubation behavior—which in turn also impacts hatching success. The video data allowed me to define specific incubation behaviors beyond what could be gleaned from nest temperature data and deepened our understanding of the direct and indirect impacts of mosquitos and noise on hatching success. By including two different types of disturbance to the study, I was also able to reveal more complex interactive effects between noise range, noise average and mosquito harassment.

What is “Good” Incubation Behavior?

Arguably the most important task during incubation is maintaining the eggs at an optimal temperature for development, which is between 35 and 40°C (Lundy, 1969; Haftorn, 1988). With regard to the role of female behavior in maintaining the temperature of the eggs, most research is on diurnal incubation, and focuses on the frequency and duration of incubation bouts throughout the day (Conway & Martin, 1999; Wang & Weathers, 2009; Rosa et al., 2015). These studies generally define attentiveness, or “good” incubation behavior, as the amount of time spent incubating and/or the number of incubation bouts in a day—but since most of the night is spent sitting tightly on the eggs, length or frequency of incubation were not appropriate measures of attentiveness for my study. I instead stipulated that good nocturnal incubation behavior and attentiveness should relate to specific behavior that regulate small-scale temperature adjustments, such as turning the eggs over occasionally to ensure all eggs in the clutch are being properly warmed. PC_CHL, the principal component on which egg turning behavior loaded

strongly, was used as a proxy for attentiveness. Following my predictions, this indicator of “good” incubation behavior had a significant and positive effect on hatching success. However, I also found a significant and positive effect of PC_AG, the measure of agitation, which was contrary to what was expected. Rather than an indication of poor incubation behavior, I propose that agitations may in fact be the least disruptive way for females to react to a disturbance. Since agitations consist mainly of head movements, the female remains on the eggs and does not stand up nor interrupt her incubation. High levels of agitation in response to disturbances may also be an indication of a female’s high levels of attentiveness to her environment—which is key to good incubation.

Mosquitos as a Natural Nuisance to Incubating Females

Few studies have addressed the impacts of mosquitos as a behavioral disturbance, rather than just as a vector for disease, but many of which are focused on mammals, in particular ungulates (Joly et al., 2020; Koltz & Culler, 2021). Some research has been done on the anti-mosquito defense behaviors of birds (see Darbro & Harrington, 2007), but to my knowledge no research has been done linking mosquitos to specific incubation behaviors.

By collecting video data of incubation, rather than using nest temperature data to make assumptions about female behavior on the nest, I was not only able to fully characterize distinct behaviors but also define the role mosquitos play in modulating those behaviors. I found that mosquito levels are strongly correlated with incubation behavior, as it was the only variable that was significant in all three of the behavior models. In line with my predictions, high levels of mosquitos were associated with higher overall activity levels and agitated behaviors, while being strongly negatively correlated with the frequency of relaxed incubation behaviors. Noise also had an effect on behavior,

but it was to a much lesser extent, and mainly was expressed via an interaction with the level of mosquito harassment.

Mosquitos and hatching success. Elevated mosquito levels have been linked to decreases in reproductive success in various species. In wolves (*Canis Lupus*), mosquito abundance had a significant effect on denning behavior and decreased pup survival (Sidorovich et al., 2017). In the Waved Albatross (*Phoebastria irrorata*), high levels of mosquito harassment were linked to egg neglect and abandonment (Anderson & Fortner, 1988). I predicted a similar effect and hypothesized that higher mosquito levels would be associated with lower hatching success. In the hatching success model, mosquito level was significant both as a main effect and as an interaction with both average noise and noise range. In contrast to my prediction, as a main effect, mosquito level had a slight positive correlation with hatching success. However, I suspect this relationship may be coincidental rather than causal. Higher environmental temperatures have been demonstrated to increase hatching success in some bird species (e.g. House Sparrow (*Passer domesticus*), Pipoly et al., 2013), but higher environmental temperatures are also often highly correlated to greater abundances of mosquitos (see Roiz et al., 2014; Asgarian et al., 2021)—meaning there is a possibility that the effect observed in the model may in fact be a simple correlation, rather than possible evidence of causation. Within the interaction term, higher mosquito levels elucidated the differences in hatching success between the varying noise types. In the absence of mosquitos, there was no significant difference in hatching success regardless of the range or average noise levels. When mosquito levels were high, quiet nests with low noise range and low average noise levels had the highest hatching success, whereas chronically noisy nest (high average, low range) had significantly lower hatching success. Within the scope of hatching

success, the inclusion of mosquito level in the model allowed me to disentangle the effects of the two varieties of noise. Considering the role mosquito level was found to have in influencing all three types of behavior, it can also be concluded that mosquitoes had an indirect effect on hatching success through the modulation of behavior.

Interactive Effects of Disturbances

There is evidence in the literature that interactive effects between biotic and abiotic disturbances can have impacts on both behavior and reproductive success. Previous research on reindeer (*Rangifer tarandus*) found complex movement patterns modulated by combinations of disturbances from mosquito and different types of military exercises being performed in the area (Valente et al., 2020). During aerial military exercises, reindeer significantly reduced their movement rates, but conversely movement rates increased when military exercises coincided with an elevated mosquito presence. Gaston et al. (2002) found that the combination of elevated temperatures and high mosquito levels was associated with adult mortality and egg loss in Brünnich's Guillemot (*Uria lomvia*), while high temperature alone had no effect on either. In this study, I similarly explored the effects of two types of disturbances on behavior and hatching success—an anthropogenic disturbance, in the form of noise pollution, and a natural disturbance, through mosquito incursions.

By incorporating two types of disturbances in this study, I was able to find complex interactions that affect both incubation behaviors and hatching success. Two of the three models on behavior showed no significant effect of either type of noise, hinting that in contrast to my predictions, noise may not be a driving force in altering female behavior on the nest. Despite this, each behavior model did include a significant interaction between mosquito level and noise. While the models show that mosquito level

is the main driver of behavior, females in certain noise conditions appear to be more sensitive to mosquito incursions. As mosquito level increased, females in nest boxes with a high average noise level increased their activity at a higher rate than those in low average noise nest boxes. Paralleling these results, as mosquito level increases females in noisy nest boxes showed a steeper decrease in relaxed incubation behavior compared to their low average noise counterparts. This apparent higher sensitivity to mosquitos extends to hatching success, where those females inhabiting noisy nest boxes had significantly lower hatching success than females in quiet nest boxes. Although mosquito level had the strongest effect on incubation behaviors, this study suggests that higher noise levels may exacerbate the effects of increasing mosquito levels, pointing to the importance of the interactive effects in these sources of disturbance.

Limitations and Future Directions

Noise Measurements

Due to certain time, equipment, and personnel constraints, noise measurements were only taken during the daytime, and were taken sporadically across the duration of the breeding season. As a result, for the purposes of this study I made the assumption that noise levels at each nest box were relatively constant for the duration of the study period, and that the noise levels during the day were representative of the noise levels at night. Future studies should consider pairing nocturnal incubation recordings with audio recording equipment that can be left out overnight or for longer periods of time.

Personality

From personal observation, different females had different reactions to disturbances, pointing to the possible role of personality. Some females reacted very aggressively to a high mosquito presence—one female recorded 88 agitations in the span

of a ten-minute video. On the other hand, some females responded by sitting tightly on the eggs and tucking their head under their wing to avoid the mosquitos, and rarely exhibited agitated behaviors—one female inhabiting a comparable nest box, with similar average noise, noise range and mosquito levels, only had at most 17 agitations and exhibited low levels of activity overall despite the ongoing disturbances. The concept of personality has been explored in avian systems within multiple contexts; it has been found to play a role in nest selection (Zhao et al., 2016), in dominance and aggressive behavior (Verbeek et al., 1996), and even reproductive success (Collins et al., 2019; for review of research on avian personalities see van Oers and Naguib, 2013). Considering the wide-ranging role of personality in birds, it is likely that the influence of personality extends to incubation behavior, and future studies should consider the role of female personality type in modulating the behavioral response of females to disturbances.

Conclusion

My results demonstrate the central role mosquito abundance plays in altering the nocturnal incubation behaviors of Eastern Bluebirds, pointing to a need for further research on the role of mosquitos as a force of disturbance beyond vectors for disease. Elevated levels of mosquitos dramatically increased the frequency of agitated behavior, yet I found an unexpected positive relationship between agitation and hatching success—which may be due to the fact that agitations mainly consist of head movements, and do not halt or interrupt the female’s incubation. These agitations may also be a result of co-evolution with mosquitos, and evolved as an effective mitigation strategy for the disturbance. These agitations, however, are unlikely to work as well to mitigate abiotic disturbances such as noise. This can be seen in the hatching success data, where the combination of noise and high mosquito levels led to significant differences in hatching

success. When mosquito levels were elevated, any type of noise, whether intermittent (nest boxes exposed to high ranges of noise level) or chronic (nest boxes with high average noise, but low noise range) led to significantly lower hatching success as compared to quiet nest boxes. Despite neither noise nor mosquitos independently impacting the reproductive success of bluebirds, the significant effects of the interaction between these disturbances highlights the potentially additive nature of disturbances, and the need for multicausal studies.

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BIOGRAPHICAL SKETCH

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