Influence of Biting Insects on Growth and Condition of Nestling Eastern Bluebirds

Ella Bollinger
Arkansas Tech University
Master of Science in Fisheries and Wildlife Science
706 E N Street Apartment D, Russellville, AR 72801
Phone Number (225) 397-5040, Email: ebollinger@atu.edu

Preface

Highly pathogenic avian influenza (HPAI) is a highly contagious disease that can cause death in humans and poultry (Swayne et al. 2000). This disease causes mass die off in wild bird populations and has been detected in all 50 states as of 2022 (Centers for Disease Control and Prevention). In the last 30 days 12,510 cases of HPAI have been detected in wild birds in the lower 48 states (U.S. Department of Agriculture). HPAI is transmitted through bird to bird contact and bodily fluids (Kleyheeg et al. 2017). This means that handling infected birds, even if they are asymptomatic, can still spread the disease to other birds or to personnel. In order to collect the data for my master's thesis research we must handle birds. To minimize potential to contribute to the spread of HPAI, we request money to purchase sanitizing supplies. Below I provide background for the research and associated methods of the project. In the budget justification section, I provide additional details about the requested sanitizing supplies.

Introduction

Rate of growth and mass of avian nestlings at fledging are important predictors of post-fledging survival (e.g. Rodríguez et al. 2016 and many others). Consequently, factors that affect those parameters can indirectly influence avian population dynamics. Ectoparasites have frequently been shown to reduce nestling growth rates and size at fledging (e.g. Brown and Brown 1986; Johnson and Albrecht 1993; Richner et al. 1993) and therefore, potentially influence population dynamics in birds. Even in cases where ectoparasites do not influence fledgling mass, they can influence populations either by reducing post-fledging survival (e.g. Streby et al. 2009) or reducing lifetime reproductive success for adults that were parasitized as nestlings (e.g. Fitze et al. 2004). As a result, ectoparasites of nestlings sometimes influence population dynamics of birds and can have an even greater impact when coupled with the pathogens they often carry (Ladeau et al. 2007; George et al. 2015). The range, abundance, and season length of biting flies (Diptera) and the pathogens they carry are expected to increase in response to habitat alteration and climate change (Paull et al. 2017).

Because nestling birds are relatively naked and have limited capability for movement, they are particularly vulnerable to attack by hematophagous Diptera (Kale et al. 1972) such as mosquitoes (Culicidae) and blackflies (Simuliidae). In fact, nestlings may actually be preferred over adults (Blackmore and Dow 1958; Griffing et al. 2007). In response, many biologists and citizens have begun treating nests with chemical deterrents. This specifically includes such recommendations for Eastern Bluebirds from non-profit organizations (Sialis.org) and the commercial production of "blackfly resistant" bluebird boxes (Barn Owl Box Co).

Treating nests with insecticides has been shown to effectively reduce nest-dwelling parasites such as mites (Martinez-de la Puente et al. 2011) and larvae (Knutie et al. 2014). Pyrethroids are the most common insecticide used in studies that experimentally remove nest ectoparasites (Hund et al. 2015), although pyrethroid nest treatments have not been evaluated for blackflies (Hund et al. 2015). Two studies have tested whether other chemicals repel blackflies; one reported that insecticidal paint did not affect fly abundance (Rivero-de Aguilar et al. 2016), whereas the other found that citronella treatment reduced fly abundance and led to a resulting reduction in *Trypanosoma* blood parasites (Martinez-de la Puente et al. 2013). We do not know of any study that attempted to repel mosquitoes from nests with chemicals. Insecticidal chemicals are thought to have low toxicity to adult birds (Hund et al. 2015), though they do pose a risk of sublethal effects on the birds (Bulgarella et al. 2020). Nestlings typically have topical exposure to chemicals applied directly to them or to their nest, causing an underestimation of ectoparasite effects if chemical toxicity and ectoparasite burden elicit similar symptoms (Lopez-Arrabe et al. 2014).

Despite the ubiquity of biting flies and their potential to pose an energetic and immunological challenge to nestlings, studies investigating their association with and effects on breeding birds remain rare. This leaves several important knowledge gaps:

- Is the density of biting flies higher near nests, as would result if flies target their foraging toward stationary hosts at nests?
- Does the abundance of biting flies at the nest vary across breeding stages? As a result of changes in host number and vulnerability, we predict that nestlings should be more attractive than eggs, and that nestling attractiveness should reduce as feathers develop.
- Does chemical treatment deter biting flies? Recommendations for such treatments abound, but controlled studies of their effects on biting flies are limited.
- What effect do biting flies have on nestling growth, condition, disease prevalence, and survival? Experimental parasite reductions are necessary to separate these effects, though we only know of the one such study of blackflies and Blue Tits described above (Martinez-de la Puente, 2013).

Objectives and Methods

Consequently, questions aimed to elucidate the interplay between biting flies and nestling birds should receive more attention. We propose a series of observational and experimental field studies with Eastern Bluebirds (*Sialia sialis*). This research that will advance our basic understanding of host-parasite dynamics and inform applied strategies to reduce parasite pressures on wild birds. Our objectives are to:

1. Determine whether biting flies are attracted to active bird nests.
2. Establish whether the number of biting flies at nests varies according to nest stage.
3. Evaluate novel methods for deterring biting flies.
4. Quantify the effects of biting flies on nestling growth, condition, disease prevalence, and survival.

Nest boxes will be monitored from March to August of the 2025 and 2026 breeding seasons. Once eggs are present a nest box will be checked every other day to accurately determine hatching date and nest fate. Nests will be randomly assigned to either a control group or one of two insecticidal treatment groups: Permethrin impregnated cow tag or permethrin spray. Pyrethroids are the most common insecticide used in studies that experimentally remove nest ectoparasites because they have low toxicity in adult birds (Hund et al. 2015). The pyrethroid tags will be impregnated with 10% Permethrin and are advertised as effective for 5 months (GardStar® Plus, YTex Corporation). If effective, using insecticide impregnated cow tags would provide many benefits over insecticidal spray. First, it reduces chemical exposure to nestlings (Lopez-Arrabe et al. 2014). Second, it is less likely to impact parasites in the nest material (e.g., mites, lice) and will thus better focus our treatment effects on biting flies and mosquitos. Finally, by simply switching the tags between boxes we can reassign future nesting attempts to a different treatment. However, because this method has not been published, we are unsure whether it will be effective. We will evaluate the effectiveness of the tag by comparing the number of biting flies and mosquitoes trapped to the numbers of biting flies and mosquitoes trapped when spray used. This will ensure that we achieve the reduced parasite numbers necessary to still evaluate Objective 4. If treatments are found successful, we will compare the breeding and nestling success to the control nests to see if there is a correlation between reduced number of mosquitoes and nest success.

When nestlings are 6-11 days old, we will briefly remove them from the nest for banding, measurements, and blood collection. The birds will be banded with a single U.S. Fish and Wildlife Service metal band. Measurements will be used to gauge the birds' condition and will include mass, tarsus length, and fat reserves. We will also collect a small (up to 40 µl) sample of blood by pricking the tarsal or brachial vein of the nestlings. A small portion of the sample will be stored on a Whatman FTA Elute Micro Card to ensure stabilization of genetic material. The remainder of the sample will be centrifuged and the separated plasma stored at -20° C. These samples will be stored while we attempt to secure additional funding for the following laboratory analyses. First, we would use enzyme-immunoassays to quantify corticosterone concentrations in the blood; this "stress" hormone increases in response to nutritional or immunological challenges. Second, the samples will be tested for infection by mosquito borne illness such as West Nile virus and avian malaria pathogens carried by mosquitoes and blackflies by using qPCR.

Mosquitoes and biting flies will be trapped during both the incubating and nestling stages. By comparing these stages, we can isolate the influence of nestlings versus the incubating female on the number of biting flies visiting the nest. Each time we trap at a nest, we will also trap at the nearest empty nest to test the hypothesis that mosquitoes are attracted to active bird nests. Traps will be deployed for approximately 12 hours overnight to sample diurnal, crepuscular, and nocturnal species of mosquito. We will quantify the number of biting flies inside nest boxes by using established trapping methods (Tomás et al. 2008, Votýpka et al. 2009). In the nest boxes we will place a petri dish covered with sticky fly tape and surround by wire mesh (to prevent bird contact). Traps will be replaced at two days intervals. If the sticky trap method described by Votýpka (2009) is found to be ineffective we will then trap mosquitoes entering the nest boxes by using a CDC gravid mosquito trap. Captured insects will be killed by freezing them at -20° C, after which we will identify each insect to family or species and determine its sex. We will attempt to secure additional internal or external funding to process blood samples. D. Barron has extensive experience conducting hormone assays (e.g., Barron et al. 2013; Barron et al. 2015) and he would supervise me in conducting enzyme-immunoassays of corticosterone at ATU. Genetic analyses that test for avian malaria and identify sources of mosquito bloodmeals would be conducted in collaboration with Dr. Dylan Maddox at The Field Museum of Natural History.

To evaluate the efficacy of our treatments, we will use ANCOVA or mixed model repeated measure analysis to compare fly abundances and nestling measurements from treatment and control nest boxes. We will use the same approach to compare abundance of mosquitoes captured in control and treatment nest boxes during the incubation and nestling phase. To evaluate differences in mass of nestlings we will use a first or second-order polynomial model (whichever fits the data better) of mass against tarsal length.

Existing Support

Ella Bollinger has salary support through a graduate assistantship and associated teaching and tutoring duties each semester (~$13,200). This salary will offset my cost of living in Russellville including rent, utilities, groceries, and other expenses. Through this assistantship I am also provided with a tuition waiver. We do not have existing external support for this research project. Fortunately, however, we already own many of the supplies and equipment necessary to carry out this research including bird banding and measurement tools, blood sampling supplies (e.g. Whatman cards, centrifuge), microscopes for mosquito ID, binoculars and spotting scopes for bird ID, and laboratory supplies (e.g. freezer, microplate reader for corticosterone assays). The on-campus field site will also eliminate the need for transportation expenses. In October of 2024 we received a grant from the Arkansas Audubon Society totaling $949.20 to purchase and establish 60 nest boxes. We now have a total of 117 boxes spread across the Arkansas Tech University campus. Our field season began with the first nest checks on Monday March 10th. We have observed 4 completed nests and 2 more are being built, though none have eggs yet. We have received landowner/university permission, IACUC approval, and United States Fish and Wildlife banding permits.

Budget justification.

We are requesting a budget of $421.30 to purchase sterilization supplies to be used. Currently in the United States there is an outbreak of Highly Pathogenic Avian Influenza (HPAI). Wild Birds that are infected with HPAI are highly contagious but may be asymptomatic. To combat the transmission of this disease the EPA has provided a list of anti-microbial products that are effective against this disease, including alcohol-based hand sanitizer and sodium hypochlorite bleach (U.S. Environmental Protection Agency). We would use this money to purchase these cleaning products along with a small portable washing machine to clean and sanitize bird bags. We briefly hold birds in these bags during capture and processing, during which time they typically defecate. This washing machine would provide an efficient way to decontaminate bags between birds and would reduce risk to students and technicians of taking dirty bags home to wash in their personal machines. Our research aims to improve avian conservation, and we want to be certain that our actions do not harm our study birds. This grant would help us to protect our birds and our workers from HPAI.

Item	Cost (tax included)	Item Quantity	Total Cost
Portable Washing Machine	$357.04	1	$357.04
Bleach (1 gal.	$6.54	4	$26.16
Hand Sanitizer (2 Liters)	$38.10	1	$38.10
Total cost	N/A	7	$421.30

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DOUGLAS G. BARRON
Associate Professor of Wildlife Science
Arkansas Tech University

Phone: (479) 880-4182
Email: dbarron@atu.edu

ARKANSAS TECH UNIVERSITY

RE: Ella Bollinger Recommendation

March 14, 2025

To Whom It May Concern,

I am writing to wholeheartedly recommend Ella Bollinger for the Arkansas Audubon Society Trust Grant. As a new master's student in my lab at Arkansas Tech University, Ella has already made a remarkable impression with her intelligence, work ethic, and passion for avian research.

Ella's project focuses on establishing a long-term study population of Eastern Bluebirds by expanding the number of nest boxes on our campus. This initiative is not only crucial for her thesis but will also develop the infrastructure and foundational knowledge to be used by future students who will continue research with this population. Her thesis aims to explore the effects of biting flies on avian reproduction, an area that has profound implications for both bird populations and disease dynamics in wildlife. The outcomes of her work could lead to valuable insights that mitigate disease transmission, which is increasingly relevant in today's changing ecosystems.

Since joining my lab, Ella has exhibited a strong motivation to succeed. She has already taken the initiative to recruit and coordinate with undergraduate students to map existing nest boxes and digitize data from the 2024 breeding season. This proactive approach speaks volumes about her leadership qualities and her ability to foster collaboration within the research community. I have no doubt that her skills will translate effectively into fieldwork when she begins her studies during the 2025 breeding season.

Beyond her academic capabilities, Ella possesses a warm and engaging personality. She is highly personable, making her an excellent team member who encourages others and fosters a supportive environment. Her enthusiasm for her research and for avian conservation is infectious, inspiring those around her to share in her vision. It is this combination of technical skill and interpersonal abilities that will serve her well in her academic and professional endeavors.

Securing the Arkansas Audubon Society Trust Grant would enable Ella to purchase the necessary sanitizing materials for her project, allowing her to safely conduct her research. I firmly believe in the promise and feasibility of her novel research questions, and have confidence that the knowledge gained from her work will have far-reaching applications in conservation strategies and disease management.

In conclusion, I wholeheartedly support Ella Bollinger's application for the Arkansas Audubon Society Trust Grant. She is a dedicated and talented student whose research has the potential to make meaningful contributions to our understanding of avian reproductive ecology. I am confident that with the support of this grant, Ella will excel in her research and further the goals of avian conservation.

Thank you for considering this deserving candidate. If you have any further questions or require additional information, please feel free to contact me.

Sincerely,

Douglas G. Barron