DISEASE ECOLOGY OF A POPULATION OF EASTERN BLUEBIRDS (*SIALIA SIALIS*) IN GEORGIA: A STARTING POINT

by

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(Under the Direction of Sara H. Schweitzer)

ABSTRACT

A population of Eastern Bluebirds (*Sialia sialis*) nesting within three grass-dominated agricultural sites in northwestern Georgia was monitored to estimate survival and reproduction, establish baseline physical and hematological parameters, and survey infectious agents as a preliminary investigation of the effects of infectious agents on bluebird population dynamics. Adult and nestlings were captured, marked with aluminum leg bands, and released during the breeding seasons (April through August) of 2004 through 2006 in order to estimate survival. Adult survival was not associated with nesting site, whereas nestling survival varied by site. Adult males and females differed in body condition and packed cell volume only. Adult packed cell volume and total plasma protein was higher than that of nestlings. Ten species of macroparasites, 5 species of protozoa, 43 species of bacteria, and 1 virus were detected by live examination, examination of blood smears and feces, necropsy, DNA extraction, and examination of nesting materials. Exploratory analyses suggested differences in parasite prevalences may have been associated with site characteristics. Reproduction varied among years but not among sites. Hematophagous nest mite populations were

manipulated within nest boxes using a pyrethrin treatment to evaluate the effect of blood loss on nestlings. Nestlings in both treatment and control groups survived to fledging and did not differ in body mass or tarsus length, but nestlings from treated boxes had higher levels of hemoglobin and lower numbers of immature erythrocytes than nestlings from untreated boxes.

INDEX WORDS: Agricultural, Eastern Bluebird, Condition, Conservation, Hematological, Parasite, Reproduction, Survival.

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DEDICATION

This work is dedicated to my husband, Jon Fredric Carleton, whose endless love and support made this possible.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

EASTERN BLUEBIRD POPULATION DYNAMICS

Eastern Bluebirds (*Sialia sialis*) are recognized as a species of great public appeal (Zeleny 1977; Gowaty and Plissner 1998). This member of the thrush family (Turdidae) is distributed throughout the eastern United States, eastern Mexico, and southern Canada. Eastern Bluebirds are commonly found in open habitats including pastures and tree-lined fields, orchards, recent clear-cuts or burned tracts, pine savannahs, newly planted pine plantations, parklands, and large lawns. These habitats provide foraging areas, perching sites for foraging attempts, insect prey, and cavity-bearing structures for nesting (Pinkowski 1977; Gowaty and Plissner 1998; Jasmer 1999; Zeleny 1977).

Predation, competition, nesting site availability, and severe weather events are major population regulators of Eastern Bluebirds (Robbins et al. 1986; Sauer and Droege 1990; Gowaty and Plissner 1998). Since the early 1900s, populations have been subject to moderate increases and sometimes drastic decreases (Zeleny 1977; Robbins et al. 1986; Sauer and Droege 1990). Loss of habitat and nesting sites due to conversion of old pastures, fields, and orchards to development, agricultural intensification, and some forms of silviculture, have been identified as a significant cause of population reductions. Widespread use of pesticides and competition with other cavity-nesting species including European Starlings (*Sturnus vulgarus*) and English House Sparrows (*Passer domesticus*) were also implicated (Zeleny 1977; Robbins et al. 1986; Sauer and Droege 1990; Jasmer 1999). Decline of many local populations have been associated with high mortality rates during severe-climatic events. For example, reductions in abundance were observed following hurricanes in

1968 and 1972 (Sauer and Droege 1990). Massive die-offs have also been documented following extremely cold weather (Williams 1904; Pinkowski 1979; Pitts 1981).

Recent trends, based on Breeding Bird Survey (BBS) information, indicate most populations are rebounding or increasing. During the ten-year period, 1978-1987, BBS data demonstrated overall recovery with no U.S. state experiencing significant losses (Sauer and Droege 1990). Current information indicated continued increases in Eastern Bluebird numbers through 2001, including a 3.19% increase in Georgia (Sauer et al. 2005). Efforts by conservation groups, such as the establishment of bluebird trails and placement of nest boxes by the North American Bluebird Society, contributed to this recovery in many areas (North American Bluebird Society 2005).

Local populations may remain subject to decline in spite of these encouraging trends. Harsh weather conditions and habitat loss and degradation will continue to pose risks to Eastern Bluebirds (Sauer and Droege 1990). Although the effect of unprecedented hurricane activity in 2005 has yet to be established, the impact on Eastern Bluebird populations in parts of Louisiana, Mississippi, and Alabama may be severe.

In addition to extreme weather events, other environmental changes are occurring. In the southeastern United States, reforestation of farm land and urbanization of rural areas has escalated dramatically. Georgia, for example, is currently the sixth fastest growing state since 1990 (Censusscope.org 2005). Likewise, the metropolitan-Atlanta area recently increased in population by 40% (Yang and Lo 2003). With such explosive growth resulting in an estimated daily conversion of 20.2 ha

of rural and open space to development (Bouvier and Stein 2005) and reforestation, habitat loss appears inevitable.

Within the past 20 years, ecologists began to recognize that the ability of infectious agents to produce disease was influenced not only by ecological and evolutionary changes in host populations, but also by environmental changes induced by climate and human activity (Anderson and May 1986; Real 1996; Tompkins and Wilkins 1998; Altizer et al. 2003; Holt et al. 2003; Lafferty and Holt 2003). Although the literature is replete with studies examining Eastern Bluebird reproductive and behavioral ecology, the role of infectious agents and diseases in bluebird population regulation has not been systematically explored. It is possible that infectious agents and parasites exert a greater pressure than previously realized. Because transmission increases with increased host density and often increases with stress (Anderson and May 1986; Lafferty and Gerber 2002; Lafferty and Holt 2003), local bluebird populations should experience greater risk of parasite transmission and possibly disease if birds concentrate into reduced or fragmented habitat resulting from urban expansion and agricultural intensification.

HOST-PARASITE INTERACTIONS

An estimated 58,000 or more parasite species use the approximately 10,000 known species of birds as hosts (Crompton 1997). These parasite species include micro parasites (viruses, bacteria, fungi, and protozoa) and macro parasites (nematodes, cestodes, trematodes, acanthocephans, ticks, lice, mites, and some flies). Parasites may regulate host populations by density-dependent mechanisms, including direct host morbidity and mortality, indirect host mortality caused by increased

predation, decreased host fecundity and reproductive success, parasite avoidance behavior, and natural selection (Anderson and May 1978; Hamilton and Zuk 1982; Møller et al. 1990; Møller 1991; Toft 1991; Brown et al. 1995; Korpimaki et al. 1995; Goater and Holmes 1997; Hudson 1986; Hudson and Dobson 1997; Stanback and Dervan 2001; Bize et al. 2003; Holt et al. 2003). A classic example of parasite-mediated regulation in birds is the Red Grouse-*Trichostrongylus tenuis* system. Cyclical decline of Red Grouse (*Lagopus lagopus scoticus*) has been directly attributed to infection with the caecal nematode, *T. tenuis*. Experiments employing parasite removal demonstrated conclusive evidence for this interaction (Hudson 1986; Hudson et al. 1992). Studies of certain swallow species and their ectoparasites demonstrate the negative effect of parasitism on reproduction and survival (Møller 1991; Burtt et al. 1991; Brown et al. 1995). Individuals suffering from any form of disease or large numbers of parasites were likely to produce fewer offspring, if at all, and experience reduced survival, especially during adverse weather.

Environmental stressors can significantly affect parasite prevalence in vertebrates (Lafferty 1997; Lafferty and Holt 2003). Common Lizards (*Lacerta vivpara*) kept in poorer quality enclosures were more frequently infected with a blood parasite than those kept in high quality enclosures (Opplinger et al. 1998). Several studies found that increased prevalence of parasitic infections in fish were associated with sewage effluent (Lafferty 1997; Landsberg et al. 1998; Coyner et al. 2003). Anthropogenic habitat disturbance is implicated as a contributing factor in the decline of Ugandan Redtail Guenons (*Cercopithecus ascanius*). Studies found gastrointestinal helminth and protozoal infections occurring at higher prevalences in logged versus un-

logged forests. It is thought these increases in parasitic infections were associated with greater contact with humans and domesticated animals (Gillespie et al. 2005).

It is difficult to predict the effect of emerging diseases on Eastern Bluebirds or other native songbird species. Much evidence suggests that introduced pathogens have dramatically negative effects on naive hosts (Davis 2003). Following the release of non-native birds and subsequent introduction of a malaria-causing blood parasite, several native Hawaiian birds are now endangered (van Riper et al. 1986). Another example is West Nile Virus which since its introduction in 1999, killed thousands of birds, including Eastern Bluebirds (Centers for Disease Control 2006).

DISEASES, PARASITES, AND EASTERN BLUEBIRDS

Eastern Bluebirds are undoubtedly subject to all classes of infectious agents. Most individuals within any bluebird population are expected to harbor one or more parasite species without exhibiting overt symptoms of disease. As with other species, occurrence of parasites within a bluebird population is expected to follow a negative binomial distribution with a relatively small number of individuals harboring the majority of parasites (McCallum and Scott 1994). While the term “infection” implies presence of an infectious agent or agents on a host, not all infections result in disease. Many agents, especially certain viruses, exist as commensals. Disease occurs when some aspect of a host’s normal functions are impaired, modified, or ceases to function resulting in morbidity of some degree or mortality (Forrester and Spalding 2003). Environmental factors, malnutrition, certain contaminants, stress, trauma, and developmental anomalies also produce disease but are not infectious. Disease may

also result from an interaction between infectious agents or an infectious agent and some noninfectious factor (Forrester and Spalding 2003).

Field recovery of diseased or deceased individual birds is extremely unlikely, making detection of active disease processes difficult. Few mass die-offs of Eastern Bluebirds have been documented and these have been attributed to exceptionally cold weather rather than an epizootic (Williams 1904; Pinkowski 1979; Pitts 1981). Our current knowledge of diseases and parasites affecting Eastern Bluebirds is based on examination of a relatively small number of individuals and therefore is incomplete. The most comprehensive source of information on parasites and other factors resulting in mortality of Eastern Bluebirds is contained within a volume published by Forrester and Spalding (2003). This volume, however is limited to data collected within the state of Florida. A 26 year-old report describing most of the known parasites infecting Eastern Bluebirds (Roberts 1981) reviewed the literature from 1936 through 1977. Based on results of a 2007 database search, including the National Parasite Collection database, 36 species of parasites or infectious agents were reported from Eastern Bluebirds (Tables 1.1 and 1.2). Any of the generalist parasites of passerines or other closely related family of birds is likely to infect Eastern Bluebirds. As insectivores, bluebirds are exposed to certain parasites, such as nematodes and acanthocephalans, through ingestion of arthropods acting as intermediate hosts or mechanical transports for infective stages of the parasite. Infection, therefore, is likely to be associated with insect prey availability. Vegetation characteristics influence insect diversity and availability. Arthropods and birds were more diverse and abundant in cotton (*Gossypium hirsutum*) fields managed by conservation tillage and stripcover cropping than those managed by

conventional methods featuring high intensity tillage (Cederbaum et al. 2004). Such greater abundance of insect prey and avian predator should reduce the risk of consuming an insect harboring infective parasite stages by the dilution effect (Schmidt and Ostfeld 2001). Other environmental factors also determine exposure risk. Seasonal environmental factors, such as precipitation and temperature, influence transmission rates of vector-borne viruses and helminth larval development (Altizer et al. 2006). For example, Red Grouse living in wet moors experienced a higher prevalence of *T. tenuis* infection than those living in dry moors (Hudson et al. 1985). Many more ecological and evolutionary aspects of bluebirds and their parasites are likely involved in the dynamics of infection and disease, including genetics-based host immune response, population density of bluebirds and conspecifics, and host and prey behavior.

SUMMARY OF OBJECTIVES

To better comprehend the dynamics of any population, an understanding of diseases and environmental factors influencing disease prevalence in populations is necessary, therefore an investigation of such factors affecting Eastern Bluebirds is timely and has implications for conservation and habitat management. Quantification of parasite prevalence and evaluation of survival, reproduction, and health parameters in association with intensity of parasitism may demonstrate if infectious agents act as population regulators in bluebirds. Awareness of infectious agents currently associated with eastern bluebirds would facilitate diagnosis and identification of emergent agents should disease-related die-offs occur. The association between habitat heterogeneity or intensity of habitat disturbance and parasite prevalence is also an important

consideration. Habitat quality is likely affected by differing agricultural practices and this in turn, may affect parasite and disease prevalence within bluebird populations inhabiting these environments. If this is the case, such knowledge would be useful in decisions regarding optimal habitats for bluebird conservation.

The objectives of this study were to investigate the effects of infectious agents on bluebird reproductive success and survival and the influence of habitat disturbance associated with agricultural enterprises on disease and parasite transmission. I hypothesized that differences in infectious agent prevalence and species abundance within the population, parasite intensity within individual bluebirds, and overall health and reproductive success of bluebirds would be affected by intensity of habitat disturbance. Objectives were accomplished by 1) identifying infectious agents and disease processes occurring within the population; 2) estimating reproductive success and survival relative to habitat disturbance and parasite prevalence, intensity, and species abundance; 3) comparing clinical health indicators, including hematological parameters, relative to parasite species abundance, prevalence, and intensity, and reproductive success of bluebirds residing within grass-dominated agricultural habitats subject to various levels of disturbance; and 4) manipulating nest mite populations to determine hematological and growth effects on nestlings. Information gained from this study was used to propose management recommendations incorporating an emphasis on disease prevention. Such management decisions should benefit current and future bluebird conservation by maximizing survival and reproduction of individuals and decreasing disease risks within populations.

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Table 1.1. Micro parasites and helminths detected in Eastern Bluebirds from North America from the literature (1941-2004) based on a 2007 database search.

Parasite	Reference
Viruses	
Eastern Equine Encephalitis	Favorite 1960
West Nile Virus	CDC 2006; Forrester and Spalding 2003
Protozoa	
Leucocytozoan sp.	Wetmore 1941
Leucocytozoan dulreuili	Greiner et al. 1975
Leucocytozoan majoris	Greiner et al. 1975
Haemoproteus oryzivorae	Greiner et al. 1975
Haemoproteus fallisi	Greiner et al. 1975; Mataxas and Pung 1999; Carleton et al. 2004
Haemoproteus sp.	Ricklefs and Fallon 2002.
Trypanosoma avium	Wetmore 1941; Greiner et al. 1975; Mataxas and Pung 1999; Carleton et al. 2004
Plasmodium sp.	Wetmore 1941; Greiner et al. 1975
Nematodes	
Dispharynx nasuta	Wehr 1971
Oxysoirura pusillae	Pence 1972
unidentified microfilaria	Love et al. 1953; Robinson 1954; Greiner et al. 1975
Cestodes	
Hymenolepis sp.	Wehr 1934
Trematodes	
Lutztrema monenteron	Price and McIntosh 1935
Collyriclum faba	Kibler 1968; Pinkowski 1975

Table 1.2. Parasitic arthropods detected on Eastern Bluebirds from North America from the literature (1940-2007) based on a 2007 database search.

Parasite	Reference
Nest mites	
Analgopsis sp.	Peters 1936
Dermanyssus prognephilus	Peters 1936; Mataxas and Pung 1999
Dermanyssus hirundinis	Burtt et al. 1991; Chow et al. 1983
Nasal mites	
Sternostoma sialiphilus	Pence 1973; Hyland and Ford 1961
Boydaia spatulata	Pence 1973
Fleas	
Ceratophyllus idius	Fox 1940
Ceratophyllus niger	Fox 1940
Ceratophyllus diffinis	Fox 1940; Benton and Shatrau 1965
Ceratophyllus gallunae	Boyd 1951
Lice	
Philopterus sialii	Peters 1936; Malcomson 1960; Mertins and Dusek 1999; Price, et al. 2003; Reeves, et al. 2007
Ricinus sp.	Peters 1936
Hippoboscid flies	
Ornithomyia anchineuria	Peters 1936
Blowfly fly larvae	
Apaulina sp.	Mason 1944; Kenaga 1961; Pinkowski 1977
Protocalliphora sialia	Rogers et al., 1991; Hannam 2006
Phaenicia coeruleiviridis	Mertins and Spalding 2001
Philornis porteri	Spalding et al. 2002
Synthesiomyia nudiseta	Spalding et al. 2002
unidentified Calliphoridae	Chow et al. 1983

CHAPTER 2

CONDITION, REPRODUCTION, AND SURVIVAL OF AN EASTERN BLUEBIRD POPULATION NESTING ON GRASSY AGRICULTURAL HABITATS IN GEORGIA[1]

[1] Carleton, R. E. To be submitted to *The Oriole*

ABSTRACT

An Eastern Bluebird (*Sialia sialis*) population from northwestern Georgia was monitored during breeding season months (April through August) of 2003 through 2006. The population was divided among three sites; fields managed for hay production, cattle pasture, and an old field. Body mass, amount of subcutaneous fat, amount of pectoral muscle mass, and erythrocyte packed cell volume, total plasma protein, buffy coat layer height, and percentage of polychromatic erythrocytes in adults and nestlings were measured as indices of condition. Survival estimates based on numbers of banded adults recaptured in nest boxes were calculated using Program Mark. Reproduction was measured as the number of eggs, number of eggs hatched, number of nestlings fledged, and number of nests lost to predation per nest box per year. Condition indices appeared normal and the population showed no signs of low survival or reproduction. There were no differences in morphological and hematological parameters of adults and nestlings among sites, although erythrocyte packed cell volume and total plasma protein values were higher in adults than nestlings. Adult survival did not differ among sites, but varied between years, whereas nestling survival was lowest on the old field site. Reproduction was lowest in 2005 and may have been associated with abnormal weather patterns early in the breeding season.

Key words: agricultural, Eastern Bluebird, Georgia, hematological, reproduction, survival

INTRODUCTION

Eastern Bluebirds (*Sialia sialis*) are distributed throughout most of eastern North America and parts of Mexico and Central America. These easily recognizable thrushes inhabit open grassy areas such as pastures and fields, orchards, recent clear-cuts or

burned tracts, pine savannahs, parklands, and large lawns providing foraging areas, perching sites for foraging attempts, insect prey, and nesting sites (Gowaty and Plissner 1998). Eastern Bluebirds form monogamous pair bonds during the breeding season although extra-pair paternity occurs with some regularity (Gowaty and Bridges 1991). As secondary-cavity nesters, bluebirds require snags, partially rotted fence posts, or other structures supporting cavities for reproduction (Gowaty and Plissner 1998). Pairs may produce up to five broods per season although two successful broods are typical (Gowaty and Plissner 1998).

Eastern Bluebird populations have been subject to moderate increases and sometimes drastic decreases since the early 1900s (Robbins et al. 1986; Sauer and Droege 1990). Loss of habitat and nesting sites due to urbanization, agricultural intensification, including conversion to monocultures, pesticide use, competition with other cavity-nesting species, and severe climatic events have contributed to reductions in Eastern Bluebird populations (Robbins et al. 1986; Sauer and Droege 1990). Beginning in 1978, Breeding Bird Survey (BBS) data indicated recovery over a 10 year period with no U.S. state experiencing significant losses. Efforts by conservation groups, such as the North American Bluebird Society, contributed to this recovery in many areas (North American Bluebird Society 2005). Recent BBS information demonstrated continued increases in Eastern Bluebird numbers through 2001, including a 3.19% increase in Georgia (Sauer et al. 2005).

Although trends indicate recovery, Eastern Bluebird populations remain subject to severe climatic events, habitat degradation, and competition with other species (Sauer and Droege 1990). For example, reductions in Eastern Bluebird abundance

were observed following hurricanes in 1968 and 1972 (Sauer and Droege 1990). Although the effect of unprecedented hurricane activity in 2005 has yet to be established, the impact on Eastern Bluebird populations in Louisiana, Mississippi, and Alabama may be severe. In addition to extreme weather events, other environmental changes are occurring.

Increased loss and fragmentation of wildlife habitat occurs with increased urbanization (Soulé 1991). Human population growth escalated dramatically throughout the southeastern United States during the 1990s (Censusscope.org 2005). For example, metropolitan Atlanta experienced a population increase of 40% during approximately the same period (Yang and Lo 2003). Bluebird habitat may become decreasing available with urban expansion and changes in the agricultural landscape of Georgia.

The purpose of this exploratory study was to assess the general condition, short-term survival, and reproductive success of a population of Eastern Bluebirds nesting on three grass-dominated agricultural sites in Georgia. Establishment of baseline parameters and health screening is an important component for monitoring population changes and disease surveillance (Hartup et al. 2004). Data from this study provided previously undocumented baseline condition parameters for the species. Further information on bluebird ecology within agricultural habitats would be useful for decision-making processes regarding habitat selection for bluebird conservation.

METHODS

The study area was located within the 11,331 ha land tract of Berry College, Mount Berry, Georgia, USA. Much of the campus property has been associated with

agricultural enterprises since its establishment in 1905. Approximately 1,200 ha of suitable bluebird habitat was located among three non-contiguous sites of grass-based agricultural usage; (HA) approximately 40 ha of Bermuda grass (*Cynodon* spp.) fields used for hay production, (LM) approximately 60 ha of old fields with features of early succession, and (PA) approximately 40 ha of mixed-grass pasture supporting the college's beef cattle operation. Each site had resources required by bluebirds, including grassy vegetation, perching sites used to locate prey and structures supporting nesting sites (nest boxes) making these sites ideal for the study.

Data collection took place during breeding season months (April through August) of 2003 through 2006. Adults and 10 day old nestlings were banded with individually numbered United States Geological Survey aluminum bands from 2004 through 2006. Capture of breeding pairs was attempted within five days of hatching of each clutch using a trip trap door system.

Condition Assessment. Adults were weighed to the nearest 0.5 g and examined for anomalies or injuries. Age of adults was estimated as second year (SY) or after second year (ASY) using coloration and shape of the 10th primary covert feather (Plissner et al. 1994). A subjective body condition score (BCS) based on pectoral muscle mass (0 = emaciated, 1 = thin, 2 = moderately muscled, 3 = well muscled, 4 = robust) (Graham 1993) and amount of subcutaneous fat (SFS) observed in the furcular region (0 = no fat or trace amounts, 1 = fat concave to furcula, 2 = fat convex to furcula) (Helms and Drury 1960) were recorded during examinations. A 10 µl blood sample was drawn via right jugular venipuncture during the first capture occasion. The sample was placed into a microhematocrit tube for packed cell volume (%) (PCV), total plasma protein (g/dl) (TP),

and buffy coat height (mm) (BC) determination. Buffy coat referred to the band of white blood cells located above packed erthrocytes. Total plasma protein was measured with a Reichert temperature-compensated clinical refractometer (Reichert, Inc., Depew, NY) and buffy coat height was measured with a digital caliper. A blood smear was made and stained with a Giemsa-type stain (Hemacolor, EM Science, Gibbstown, NJ) to determine the percentage of polychromatic erythrocytes (PPC). Smears were examined at 1000x magnification and oil immersion for quantification of polychromatic or immature cells per 100 erythrocytes. Presence of hematozoa was analyzed elsewhere (Ch. 3).

The left first rectrix was collected from adults for digital image analysis. Studies demonstrating a positive correlation between color brightness and parasite load suggest pigment measurements may be used to assess feather quality and health in birds (Burley et al. 1991; Korpimaki et al. 1995). Image editing software has been used to quantify pigmentation from biological sources (Davis et al. 2004; Mays et al. 2004). A digital image of each feather was created using a Canon CanoScan 4400F digital scanner (Canon, USA, Inc., Lake Success, NY) at 1200 dpi. Individual feathers were placed on grid-lined paper for consistency and the scanned images were cropped by identical grid locations approximately 5mm from the tip. Average color value, a percentage measure of brightness, and RGB model, a measure of red, green and blue in an image, were obtained using the Gnu Image Manipulation Program 2.2 photo editing software (Free Software Foundation, Inc., 2006). Measurements of males were compared to females to evaluate usefulness of the program and then comparisons were made separately among males and females.

During the final six weeks of the study, 14 adult males were sacrificed for complete necropsy and collection of a sufficient amount of blood for viral screening. The amount of serum required for screening precluded safe sampling of living birds. Necropsy results were reported elsewhere (Ch. 3, Ch. 5). Serum was separated from 2.0 ml of blood from each bird and frozen at 4° C. Samples were submitted to the Southeastern Cooperative Wildlife Disease Study (University of Georgia, College of Veterinary Medicine, Athens, Georgia, USA) for West Nile Virus (WNV) and St. Louis Encephalitis Virus (SLEV) screening.

A 10 µl blood sample was obtained from 10 day old nestlings for measurement of packed cell volume, total plasma protein, and buffy coat height using the same methods as previously described. Percentage polychromasia was not examined for nestlings.

Reproduction. Nest boxes constructed of untreated white pine (*Pinus strobus*) wood were mounted on power line poles or wooden fence posts located within each site (HA n = 17, PA n = 16, LM n = 12). Nest boxes were put in place in 2002 with a minimum separation of 150 m. During the breeding season months of 2003 through 2006, nest boxes were monitored weekly for signs of nesting activity. Monitoring continued twice weekly following initiation of a nest and then daily after the first egg was produced. Boxes were not checked during the estimated incubation period of 12 days after the last egg was laid. From estimated hatch date until three days after the last egg hatched, boxes were monitored daily and the number of nestlings was recorded. Daily monitoring resumed 19 days after hatching and until all nestlings fledged. Empty nests with signs of disturbance observed with 19 days of hatching were considered lost to predation. Nesting material was removed after nestlings fledged or in the case of

predation or other nest failure. The number of eggs produced, number hatched, and number of nestlings fledged were recorded for each nest box. Hatch or fledge dates were estimated by last observance when observations were incomplete. Nests missing all eggs prior to estimated hatch date or missing all nestlings prior to estimated fledge date were recorded as lost to predation.

Survival. Capture-recapture histories were recorded for all recaptured banded bluebirds over three seasons. ASY birds captured in 2004 were considered present but unobserved in 2003. Because few dead birds were reliably recovered from the field, only live recapture data was used to estimate apparent survival. Survival was estimated separately for birds banded as adults and nestlings. Data were combined for adults within each site (HA, LM, PA) and nestling data were combined only by site because nestling sex was unknown in some cases. A candidate model set based on *a-priori* hypotheses of site- and time-related differences in survival was defined for each age category although many more models were possible. Data were encoded into a standard encounter history format; 1 = capture and 0 = no capture (Lebreton et al. 1992).

Statistical analyses. Replication at the site level was not possible on the study area. Site was included as a model effect in analyses only as a preliminary investigation of relationships between the parameters investigated and habitat.

Health assessment data were combined for all seasons and compared among sites and between sex and age categories using Factorial ANOVAs or Pearson χ^2. Bivariate fit of color measurements and packed cell volume, total plasma protein, and percentage of polychromatic cells were performed to explore relationships. Data were

combined to produce mean hematological and morphological values for the study population. Reproductive data were analyzed by site for each season using one way ANOVA or Pearson χ^2 and comparisons among seasons were made using repeated measures MANOVA. Differences were further explored using Tukey's honestly significant difference tests or contrasts. JMP-IN Statistical Software (SAS Institute, Inc., Cary, NC) was used for analyses with *a-priori* significance set at $\alpha = 0.05$.

Program Mark v.4.2 (White and Burnham 1999) was used to estimate apparent survival based on maximum-likelihood estimates for each model. Bootstrap goodness of fit simulations (500) were used to assess the fit of a general model and adjust the variance inflation factor for model comparisons. The most parsimonious model was chosen using corrected Akaike's Information Criterion ($QAIC_c$) and $QAIC_c$ weights (Anderson and Burnham 2002).

RESULTS

Sample Size. One hundred forty-four adults (84 females, 60 males) were captured and examined during the study; HA *n* = 66 (36 females, 30 males), LM *n* = 28 (17 females, 11 males), PA *n* = 50 (31 females, 19 males). Most of the birds (92) captured were aged as second year adults; 39 from the HA site, 21 from the LM site, and 32 from the PA site. Body mass was recorded for all birds whereas the remainder of morphological and hematological measurements were obtained from smaller sample sizes (Table 2.1).

Condition Assessment. Adult body weight (Table 2.1) was not influenced by site ($F_{2,144} = 2.72$, $P = 0.07$), age ($F_{1,144} = 0.67$, $P = 0.79$), or sex ($F_{1,144} = 0.0001$, $P = 0.99$), and there were no interactions. Body condition scores of females were different than that of males (Pearson $\chi^2 = 16.82$, 2 df, $P = 0.0002$), but body condition did not differ by age

(Pearson χ^2 = 3.68, 2 df, P = 0.1586) or site (Pearson χ^2 = 6.91, 4 df, P = 0.14) (Table 2.1). Subcutaneous fat scores did not differ among sexes (Pearson χ^2 = 4.13, 2 df, P = 0.13), sites (Pearson χ^2 = 7.63, 4 df, P = 0.106), or ages (Pearson χ^2 = 4.14, 2df, P = 0.13) (Table 2.1). Most birds were scored as 1 (59.4%), 32.8% were scored as 0, and 7.7% were scored as 2. A χ^2 test of independence indicated a relationship between subcutaneous fat score and body condition score (Pearson χ^2 = 16.04, 1 df, P = 0.003).

There was no difference in adult packed cell volume (Table 2.2) among sites ($F_{2,124}$ = 0.1902 P = 0.83) or age categories ($F_{1,124}$ = 0.027, P = 0.87), but there was a difference between sexes ($F_{1,124}$ = 8.47, P = 0.004). There were no interactions between model effects. Nestling packed cell volume (Table 2.2) did not differ among sites ($F_{2,71}$ = 2.79, P = 0.07) but strongly differed from adult packed cell volume ($F_{1,194}$ = 151.91, P < 0.0001) (Fig. 2.1).

Adult total plasma protein (Table 2.2) did not differ among sites ($F_{2,82}$ = 2.79, P = 0.17), between sexes ($F_{1,82}$ = 0.22, P = 0.64) or by age ($F_{1,82}$ = 2.14, P = 0.15). Nestling total plasma protein did not vary among sites ($F_{2,38}$ = 1.75, P = 0.19) (Table 2.2). There was a difference in total plasma protein between adults and nestlings ($F_{1,162}$ = 19.56, P < 0.0001) (Fig. 2.2). Adult percentage of polychromatic cells (Table 2.2) differed among sites ($F_{2,47}$ = 3.84, P = 0.03), but not between sexes ($F_{1,47}$ = 0.002, P = 0.98) or age categories ($F_{1,47}$ = 3.11, P = 0.08). Percentage of polychromatic cells was greater among adults nesting in the LM site than those nesting in the PA site. Adult buffy coat height (Table 2.2) did not differ among sites ($F_{2,87}$ = 0.81, P = 0.45) , or between sexes ($F_{1,87}$ = 0.24, P = 0.62) or ages ($F_{1,87}$ = 0.86, P = 0.62). Nestling buffy coat height did

not differ among sites ($F_{2,42}$ = 0.47, P = 0.63) or from adult buffy coat height ($F_{1,131}$ = 3.26, P = 0.07) (Table 2.2).

Mean feather red-green-blue color model (Table 2.1) differed appropriately between males and females ($F_{1,72}$ = 16.86, P = 0.0001). There was no difference in red-green-blue color model of males among sites ($F_{1,72}$ = 1.72, P = .24) or females among sites ($F_{1,72}$ = 3.21, P = 0.05). Color value also differed by sex ($F_{1,72}$ = 37.11, P < 0.0001) but not for males among sites ($F_{1,72}$ = 1.21, P = 0.31) or females among sites ($F_{1,72}$ = 1.54, P =0.43). There were no relationships between hematological parameters and color measurement values.

One (7.1%) of 14 serum samples tested strongly positive for West Nile Virus and weakly positive for Saint Louis Encephalitis virus. The positive bird was collected from the PA site.

Reproduction. The number of eggs produced differed among years ($F_{3,33}$ = 6.83, P = 0.001), but did not differ among sites ($F_{2,35}$ = 0.740, P = 0.48) and there were no interactions between time and site ($F_{6,66}$ = 0.80, P = 0.57). The number of eggs produced in 2003 was less than in 2004 ($F_{1,35}$ = 6.01, P = 0.019) and less than 2006 ($F_{1,35}$ = 11.06, P = 0.002) but not different than 2005 ($F_{1,35}$ = 0.104, P = 0.74). More eggs were produced in 2004 than in 2005 ($F_{1,35}$ = 4.35, P = 0.04) but there was no difference compared to 2006 ($F_{1,35}$ = 0.281, P = 0.59). Fewer eggs were produced in 2005 than in 2006 ($F_{1,35}$ = 9.37, P = 0.004) (Fig. 2.3).

There was a difference in the number of eggs hatching among years ($F_{3,33}$ = 5.29, P = 0.004) but not among sites ($F_{2,35}$ = 0.818, P = 0.45) and no interaction of time and site ($F_{6,66}$ = 0.949, P = 0.47). There were differences in the number hatching

between 2003 and 2006 ($F_{1,35}$ = 4.38, P = 0.04), 2004 and 2005 ($F_{1,35}$ = 4.35, P = 0.04), and 2005 and 2006 ($F_{1,35}$ = 10.62, P = 0.002), but not between 2003 and 2004 ($F_{1,35}$ = 2.90, P = 0.09), 2003 and 2005 ($F_{1,35}$ = 0.175, P = 0.678), or 2004 and 2006 ($F_{1,35}$ = 0.281, P = 0.59).

The number of nestlings fledging successfully also differed among years ($F_{3,33}$ = 3.52, P = 0.02) but not among sites ($F_{2,35}$ = 2.41, P = 0.104) and there was no interaction between time and site ($F_{6,66}$ = 0.522, P = 0.79). More nestlings fledged in 2004 compared to 2005 ($F_{1,35}$ = 9.10, P = 0.005) and 2006 than 2005 ($F_{1,35}$ = 5.33, P = 0.027). There were no differences between 2003 and 2004 ($F_{1,35}$ = 3.18, P = 0.08), 2003 and 2005 ($F_{1,35}$ = 1.02, P = 0.32), 2003 and 2006 ($F_{1,35}$ = 0.836, P = 0.37), or 2004 and 2006 ($F_{1,35}$ = 0.630, P = 0.43).

Nest predation did not vary among years ($F_{3,33}$ = 2.47, P = 0.07) or among sites ($F_{2,35}$ = 0.818, P = 0.45) and there was no interaction between either effect ($F_{6,66}$ = 0.608, P = 0.72). For all years combined, there were no differences among sites in the number of eggs produced ($F_{2,37}$ = 0.51, P = 0.60), number of eggs hatched ($F_{2,37}$ = 1.01, P = 0.32), number of nestlings fledged ($F_{2,37}$ = 2.43, P = 0.10), or number of nests lost to predation ($F_{2,37}$ = 1.39, P = 0.27).

Survival. The nestling survival model that suggested survival differed among sites and recapture differed by time received the greatest $QAIC_c$ weight (48.8%) among all models tested. Based on this model, nestlings raised in the LM site had an estimated 17.6% probability of surviving to the next year compared to a 22.8% probability for nestlings raised in the HA site, and a 41.2% probability for nestlings raised in the PA site. Recapture probability increased with time to 49.9%. The model receiving the

greatest $QAIC_c$ (61.5%) among adult models tested suggested survival differed with time and recapture probability remained constant. This model supported the hypothesis of no site effect.

DISCUSSION

Condition parameters and adult survival were constant among this population. Lack of replication at the habitat level precludes a meaningful statistical evaluation of differences among the three sites, however further investigation is warranted based on these preliminary findings. There was no evidence suggesting the population was in poor condition or had low levels of reproduction or survival. Although parasites were detected in the population (Chapters 3, 4, 5, and 6), status of parasitism was not included in the analysis because in most cases, intensity of parasitism within individuals could not be determined. The sensitivity of methods used to detect infections in living birds is often low (Waldenström et al. 2004) and results may not reliably indicate direct effects of disease, namely increased mortality. Indirect effects, such as decreased fecundity and survival, are best evaluated using controlled studies involving removal of parasites from a segment of the population (McCallum and Dobson 1995).

Survival after fledging appeared to differ among sites, with the lowest estimated survival probability associated with the LM site, but the model supportive of the differences received a relatively low $QAIC_c$ weight (48.8%) and should be considered suspect. Lack of site replication, small sample size and low number of sampling seasons are plausible reasons.

The population experienced a dramatic decrease in reproduction during 2005 although reproduction rebounded the following year. Heavier than normal precipitation

(+ 0.69 in) occurred during April when the first clutches were produced. It was also the wettest April during the study. Lower than normal precipitation (-1.83 in) followed in May of 2005 (National Oceanic and Atmospheric Administration, 2007) when first broods would have normally fledged. Departure from the normal weather pattern during the early breeding season may have been associated with lower reproduction in 2005.

Differences in body condition scores between males and females are possibly the result of physiological stress experienced by males defending territories and mate guarding (Gowaty et al. 1989). Based on evidence of a relationship between amount of subcutaneous fat and pectoral muscle mass, it is unexpected that the subcutaneous fat scores of males were not different than subcutaneous fat scores of females.

Mean adult packed cell volume was similar to packed cell volumes reported for similar species (48%) (Fair et al. 2007). Packed cell volume was the only hematological parameter differing in males and females. Most studies show no differences between males and females or report higher packed cell volumes in males. A study reviewing variation in packed cell volumes among species had no mention of Eastern Bluebirds (Fair et al. 2007) and no other studies were available for comparison. Decreased packed cell volume occurs during egg-laying in American Kestrels (*Falco sparverius*) (Rehder and Bird 1983) and Red-billed Queleas (*Queleas queleas*) (Jones 1983). Females in this study were captured between 12 and 17 days after the start of incubation. Measurements during non-breeding months may provide more insight into reasons for sex-related differences.

The buffy coat height, an indirect measure of white blood cell volume, and total plasma protein were measured to provide estimates of immune function. Increases in

white blood cell volume and total plasma proteins are associated with inflammatory or infectious diseases (Gustafsson et al. 1994). Although no adults exhibited symptoms of ill-health, undetected infections may have been present. Data presented here suggests no differences in estimated immune function existed within the population.

Increased percentage of polychromatic cells or immature erythrocytes, signify a regenerative bone marrow response. Because packed cell volumes in birds show little change with blood loss, increased polychromasia is a more reliable indicator (Campbell 1995). The percentage of polychromatic cells of birds from the LM site was 20.0% greater than the population mean. It is unknown whether the difference is due to increased regeneration in one site, suppressed regeneration in another site, or sampling error due to lack of site replication. No baseline values were available for comparison.

There was no apparent relationship between feather color measurements and hematological parameters. Successful procurement and defense of nest boxes by male Eastern Bluebirds is associated with degree of plumage coloration (Siefferman and Hill 2005). Variations in color reflectiveness associated with structural characteristics of feather barbs suggest color is indicative of condition in Eastern Bluebirds (Shawkey et al. 2003). Although the red-green-blue color model and color value correctly identified differences between males and females, these measurements may not have been sufficiently sensitive to detect condition-associated variation.

Few differences in condition, survival, or reproduction were found within the study population. These preliminary results suggest grass-dominated agricultural habitats are acceptable sites for bluebird conservation; however further investigation

with replication at the habitat level is needed to investigate whether or not differences truly exist between sites. Work involving more diverse habitats and other species is needed to evaluate the effects of other forms of agricultural disturbance on avian populations.

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Table 2.1 Mean morphological measurements of body mass, color value, and red-blue-green color model (RGB) of adult Eastern Bluebirds nesting on grass-dominated agricultural habitats in Georgia (2004-2006).

	Body Mass (g)	Color value	RGB Model Value
Adult Female			
Mean ± SD	30.2 ± 1.52	78.6 ± 12.7	62.2 ± 11.2
(n)[a]	(84)	(47)	(47)
Adult Male			
Mean ± SD	30.0 ± 1.39	100.5 ± 10.99	75.7± 12.68
(n)	(60)	(26)	(26)
All Adults			
Mean ± SD	30.15 ± 1.46	86.4 ± 16.03	67.0 ± 13.36
(n)	(144)	(73)	(73)

[a] = number sampled

Table 2.2 Mean hematological parameters, including packed cell volume (PCV),total plasma protein (TPP), percentage of polychromatic cells (PC), and buffy coat layer height (BC) of adult and 10 day old nestling Eastern Bluebirds nesting within grass-dominated agricultural sites in Georgia (2004-2006).

	PCV (%)	TP (g/dl)	PC (%)	BC (mm)
Nestling				
Mean ± SD	42.3 ± 4.5	4.8 ± 0.43	nm[b]	0.68 ± 0.33
(n)[a]	(72)	(38)	(0)	(45)
Adult Female				
Mean ± SD	49.5 ± 2.63	4.8 ± 0.44	8.2 ± 3.2	0.60 ± 0.22
(n)	(73)	(52)	(25)	(49)
Adult Male				
Mean ± SD	47.8 ± 3.22	4.7 ± 0.47	8.3 ± 3.7	0.58 ± 0.19
(n)	(53)	(31)	(23)	(39)
Adults				
Mean ± SD	48.8 ± 2.97	4.8 ± 0.45	8.2 ± 3.4	0.59 ± 0.29
(n)	(126)	(83)	(48)	(88)

[a] = number sampled, [b] = not measured

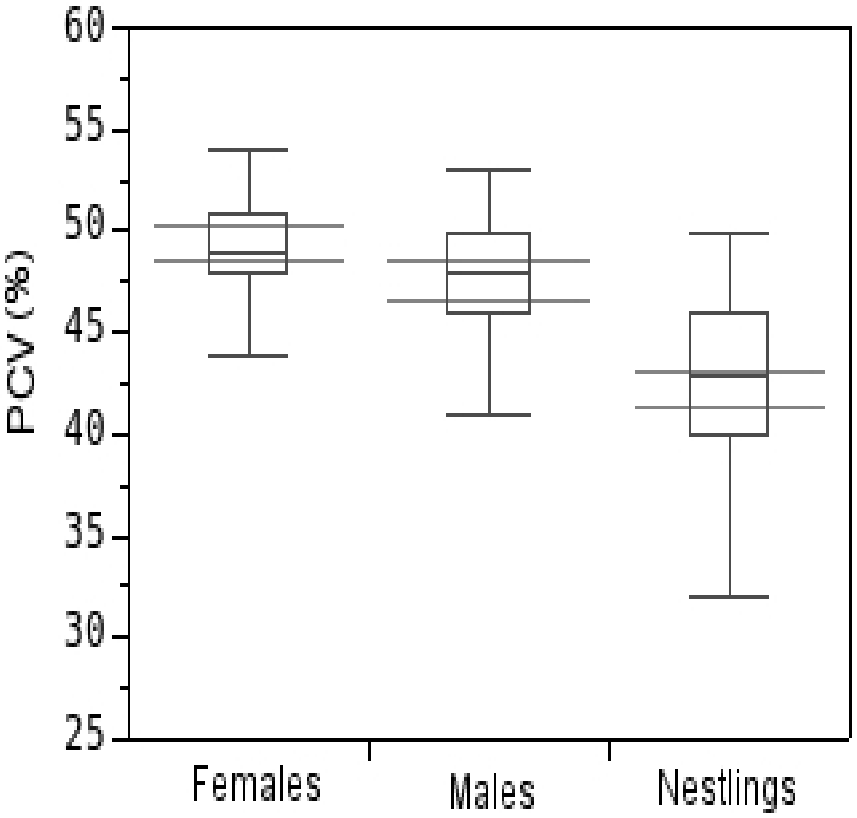

Figure 2.1. Mean packed cell volumes (PCV) of female (*n* = 73) and male (*n* = 53) adult and 10 day old nestling (*n* = 72) Eastern Bluebirds nesting on grass-dominated agricultural sites in Georgia (2004-2006) shown as box plots with 95% confidence interval bars.

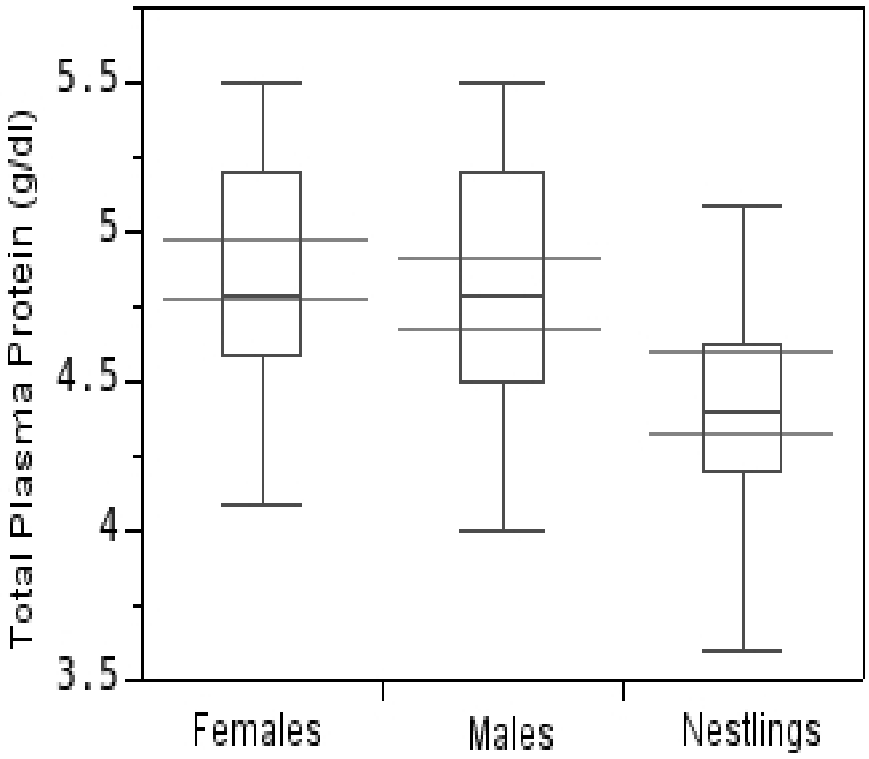

Figure 2.2. Mean total plasma protein of female (*n* = 52) and male (*n* = 31) adult and 10 day old nestling (*n* = 38) Eastern Bluebirds nesting on grass-dominated agricultural sites in Georgia (2004-2006) shown as box plots with 95% confidence interval bars.

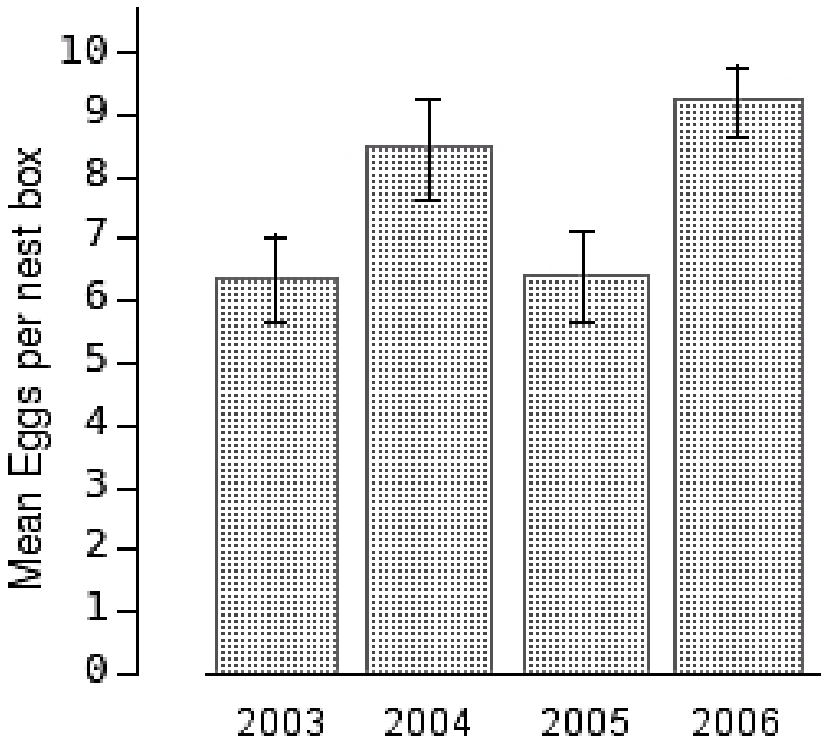

Figure 2.3. Mean number of eggs produced per nest box by year (*n* = 50) by a population of Eastern Bluebirds nesting on grass-dominated agricultural sites in Georgia (2003 - 2006).

CHAPTER 3

PROTOZOAN PARASITES OF EASTERN BLUEBIRDS INCLUDING TWO NEW HOST RECORDS FOR *SARCOCYSTIS* SP. AND *ATOXOPLASMA* SP.[2]

[2] Carleton, R. E. and M. J. Yabsley To be submitted to *Comparative Parasitology*

ABSTRACT

A population of Eastern Bluebirds nesting within three grass-dominated agricultural sites in Georgia were surveyed for parasitic protozoa (2004-2006). We detected four species by blood smear examination, necropsy, and PCR methods; *Atoxoplasma* sp., *Haemoproteus fallisi, Plasmodium relictum,* and *Sarcocystis* sp. These findings represent new host records for *Atoxoplasma* sp. and *Sarcocystis* sp. We examined effects of *Haemoproteus* infection on selected hematological parameters and found no difference between infected and non-infected birds or between sexes.

INTRODUCTION

Protozoan parasites (Apicomplexa) are commonly associated with both wild and domesticated birds (Atkinson and van Riper III 1991). This diverse array of organisms has been isolated from virtually every organ and tissue type, including the blood and nervous system (Doster and Goater 1997). Pathogenesis varies from negligible to severe depending on host, species of parasite, and site of infection (Atkinson and van Riper 1991; Forrester and Spalding 2003). For example, some species of hematozoa apparently cause no adverse effects on host body mass (Bennett et al. 1993). Studies have also found no evidence that *Haemoproteus* infection reduces reproduction in Pied Flycatchers (*Ficedula hypoleuca*) (Siikamäki et al. 1997) or American Kestrels (*Falco sparverius*) (Dawson and Bortolotti 2001). Other hematozoa, such as *Plasmodium relictum*, may cause high rates of mortality and in some cases, extinction (Atkinson et al. 2000).

Galliforms, including chickens, pheasants, turkeys, and quail infected with certain *Eimeria* spp. experience increased morbidity and mortality (Todd and Hammond 1971).

Disseminated Visceral Coccidiosis, also caused by *Eimeria* spp., is associated with granulomatous tissue lesions and mortality in Sandhill Cranes (*Grus canadensis*) and endangered Whooping Cranes (*Grus americana*) (Forrester and Spalding 2003).

Other protozoa, such as *Sarcocystis* sp. and *Toxoplasma gondii*, are capable of inducing serious infections. Encephalitis associated with *Sarcocystis* sp. has been implicated in the death of a Golden Eagle (*Aquila chrysaetos*) (Dubey et al. 1991), a Wild Turkey (*Meleagris gallopavo*) (Teglas et al. 1998), a Northern Gannet (*Morus bassanus*) (Spalding et al. 2002), and a Northern Goshawk (*Accipiter gentilis atricapillus*) (Aguilar et al. 1991). *Sarcocystis* spp. have also been isolated from pulmonary tissue of psittacines that died acutely (Smith et al. 1989). Several orders of birds, including Ciconiiformes (Spalding et al. 1994), Anseriformes (Drouin and Mahrt 1979), Columbiformes (Conti and Forrester 1981; Box and Smith 1982), and Passeriformes (Duszynski and Box 1978; Box and Smith 1982; Dame et al. 1995; Luznar et al. 2001) serve as intermediate hosts for numerous species of *Sarcocystis* with typically no pathological effect (Thornton 1972). *Toxoplasma gondii* is frequently isolated from tissues of many species of wild birds (Dubey 2002). Infection with *T. gondii* may have serious consequences, as in the case of a Bald Eagle (*Haliaeetus leucocephalus*) that died of necrotizing myocarditis (Szabo et al. 2004). There are other reports of disease or mortality associated with *T. gondii* or *Toxoplasma*-like organisms, including death in pigeons and blindness in canaries (reviewed in Dubey 2002).

Protozoal infections of poultry and game species are widely studied while less is known about protozoal infections of passerine birds. Most research has been directed toward protozoal diseases affecting endangered species (Atkinson et al. 2000) or easily

detectable hematozoan infections (Janovy 1997). Knowledge of pathogen prevalence is valuable because it provides information on the associations between disease and population dynamics (McCallum and Dobson 1995).

Information on protozoal infections of Eastern Bluebirds (*Sialia sialis*) is limited to reports of hematozoa (Table 3.1). Expansion of the current knowledge base may increase our understanding regarding the role of infectious agents in bluebird population dynamics. The objectives of this study were to survey protozoan parasites infecting a population of Eastern Bluebirds, estimate infection prevalence and intensity, and examine the effects of hematozoan infection on hematological parameters.

METHODS

This study was conducted on the land tract of Berry College, Mount Berry, Georgia (USA: 34.282799 ^{o}N, 85.191803 ^{o}W) during the breeding seasons (April through August) of 2004 through 2006. Bluebird habitat within the study area was located within three independent sites of grass-dominated agricultural use; Bermuda grass (*Cynodon dactylon*) field under hay production (HA), fescue (*Festuca* sp.) and mixed grass cattle pasture (PA), and a low management field (LM) with characteristics of early succession.

Breeding Eastern Bluebirds were captured inside nest boxes using a trap door system and banded with individually numbered with United States Geological Survey (USGS) aluminum bands. A blood sample was obtained by right jugular venipuncture and a smear was made immediately and air-dried. We placed the remaining blood sample into a microhematocrit tube for measurement of packed cell volume (PCV) and total protein (TP). Blood smears were stained with Hemacolor Staining Solution (EM

Science, Gibbstown, NJ) after fixation and examined for presence of hematozoa at 1,000X magnification and oil immersion. We estimated intensity of infection by counting the number of infected cells per 100 high power fields (approximately 10,000 erythrocytes) and percentage of polychromatic cells by counting the number of immature erythrocytes per 100 cells.

Fecal material was collected during capture occasions was held at room temperature for 24 hours to allow for sporulation of oocysts. We added two drops of 10% buffered formalin as a preservative prior to storage at 4° C. Fecal samples were prepared by centrifugation in Sheather's sugar solution and examined for the presence of oocysts at 400x magnification.

During the final 6 weeks of the study, we harvested 14 adult male bluebirds for complete necropsy. A complete necropsy was performed following the method described by van Riper, III and van Riper (1980). Partial necropsy findings from two additional bluebirds found dead in the field were included in the study. Fecal material was collected and stored as previously described. To conduct a PCR-based survey for protozoa, the spleen was harvested and frozen at -20° C until analysis.

DNA was extracted from ~5mg of homogenized spleen tissue using the GFX Genomic Purification kit (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. A nested polymerase chain reaction (PCR) was used to amplify a portion of the18S rRNA gene of all species of *Eimeria, Isospora, and Atoxoplasma*. Primary PCR amplification consisted of 10 µl of DNA in a 25 µl reaction containing 10mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate (dNTP), 2.5 units Taq DNA Polymerase (Promega Corp.,

Madison Wisconsin), and 25 pmol of primers EIMF (5'— ACCATGGTAATTCTATG) and 990 (5'— TTGCCTYAAACTTCCTT). Secondary PCR amplification was identical except for the primers, EIMR (5'— CTCAAAGTAAAAGTTCC) and 989 (5'— AGTTTCTGACCTATCAG) (Yabsley and Gibbs 2006). Reaction conditions consisted of 30 cycles of 94° C for 30 sec, 40° C for 1 min, and 72° C for 1 min plus 1 sec/cycle, followed by 72 min for 12 min. A nested PCR was used to amplify a portion of the *Haemoproteus* and *Plasmodium* cyt b genes using primers HAENF and HAEMNR2 in a primary reaction and HAEMF and HAEMR2 in a secondary reaction (Waldenstrom et al. 2004). Amplified products were separated in 2% agarose gels, stained with ethidium bromide, and visualized with UV light. Products were purified with a Microcon spin filter (Amicon Inc., Beverley, Massachusetts), sequenced at the University of Geogia Integrated Biotech Labs (Athens, Georgia).

Skeletal muscle of three necropsied birds (18.7%, n = 16) harbored rice-shaped structures resembling sarcocysts (Fig. 3.1). Representative sections of muscle with cysts were collected from each bird and placed in 10% buffered formalin for histopathology and frozen at – 20° C for PCR analysis. Histologic sections were stained with H & E and examined at 400x magnification. A portion of the 18S rRNA gene was amplied as described using primers 18S9L and 18S1H (Li et al. 2002). Amplicons were purified and sequenced as described above.

JMP-IN Statistical Software (SAS Institute, Cary, NC) was used for all statistical analyses. Differences in prevalence and intensity of infection between sexes were compared by Pearson Chi square analysis. As a preliminary investigation, differences among the three sites was compared using one and two way ANOVA platforms;

replication at the habitat level was not possible on the study area. ANOVA was also used to test for differences among hematological parameters of infected and non-infected bluebirds. *A-priori* signifcance was set at $\alpha = 0.05$. Significant ANOVAs were further examined by Tukey's honestly significant difference tests.

RESULTS

No oocysts were detected in any fecal samples ($N = 98$). *Haemoproteus fallisi* gametocytes were detected in 81 of 135 (59.0%) blood smears examined. Prevalence of infection was independent of sex (females (58.2%, $n = 79$) and males (60.7%, $n = 56$) (Pearson $\chi^2 = 0.025$, $P = 0.87$), prevalence was similar among the sites; HA 54.1%, $n = 61$, PA 63.8%, $n = 47$, LM 62.9%, $n = 56$ (Table 3.2). Intensity of infection was low with a mean of 3.4 (± 6.2 SD) infected cells per 10,000 cells and did not differ between males and females ($F_{1,135} = 2.78$, $P = 0.097$). Percentage of polychromatic cells was not significantly different between infected and non-infected birds ($F_{1,47} = 0.387$, $P = 0.538$) and there was no effect on packed cell volume or total protein (PCV: $F_{1,118} = 0.19$, $P = 0.65$; TP: $F_{1,76} = 0.55$, $P = 0.46$).

Four (3.2%) of the smears examined had detectable *Trypanosoma avium* infections. All four were from male birds; three were captured in the HA site and one was captured in the LM site (Table 3.2).

Three species of parasites were identified by PCR analysis of splenic tissue. One (7.1%) necropsied bird was infected with *Haemoproteus* sp., ten (71.4%) were infected with *Plasmodium relictum*, and seven (50.0%) were infected with *Atoxoplasma* sp. (Table 3.2). Sequences from nine *Plasmodium* infected birds were 99% identical to a *P. relictum* (GenBank AY733088) sample obtained from a captive Jackass Penquin

(*Spheniscus demersus*). The sequence from the remaining bluebird had several polymorphic bases but was 98% identical to *P. relictum.* Interestingly, no *Plasmodium* infections were detected in blood smears including smears from birds examined by necropsy. The single *Haemoproteus* infection was 98% identical to a *Haemoproteus* sp. haplotype 2 (GenBank AF465563) obtained from a Yellow-throated Warbler (*Dendroica dominica*) and a Northern Parula (*Parula americana*). This sequence was also 98% identical to a *Haemoproteus* sp. haplotype 1 (GenBank AF465562) obtained from an Eastern Bluebird and a Western Bluebird (*Sialia mexicana*). Sequence analysis of products produced by the *Eimeria/Isopora/Atoxoplasma* reaction was 98% identical to the only *Atoxoplasma* sp. sequence published in GenBank (GenBank AY331571). This sequence was obtained from a Southern Cape Sparrow (*Passer melanurus melanurus*). All *Atoxoplasma* sequences from positive bluebirds were identical to each other.

Birds with grossly visible sarcocysts were collected only from the PA site. Cysts were found within all major muscle groups of two birds and only in wing, leg, and back muscles of the third. Representative muscle cysts measured 2.16mm x 0.74mm, 2.52mm x 0.77mm, and 1.84mm x 0.77mm. We also found cysts within connective tissue associated with the caudal surface of one eye of the bird harboring the largest number of cysts (Fig. 3.2). No inflammation was associated with the muscle cysts. Sequences of the partial 18S rRNA gene (792bp) of the *Sarcocystis* sp. from the three bluebirds were identical. The sequences were 100% identical to *S. falcatula* (of the 359 available overlapping bases) (AY628220) and 100% identical to a *Sarcocystis* sp. AGP-1 (of 752bp overlapping bases) reported from a captive African Grey Parrot (*Psittacus*

erithacus) (DQ768305; Dubey, et al. 2006). The bluebird *Sarcocystis* sp. was only 99.7% identical to *S. neurona* (SNU07812) (790 of 792 bases).

DISCUSSION

We detected five species of protozoal parasites within the bluebird population under study. Two of the protozoa, *Atoxoplasma* sp. and *Sarcocystis* sp., represent new host records for these species.

Atoxoplasma sp. was commonly detected in necropsied bluebirds by PCR. *Atoxoplasma* spp. have been reported to cause disease in Canaries (*Serinus canaria*) (Sánchez-Cordón et al. 2007), Greenfinches (*Carduelis chloris*) (Cooper et al. 1989), Bullfinches (*Pyrrhula pyrrhula*) (McNamee et al. 1995), and Bali Mynahs (*Leucopsar rothschildi*) (Partington 1989). None of the infected bluebirds exhibited signs or symptoms of disease, however the consequences of either a chronic infection or infection compounded by environmental stresses are unknown.

Sarcocystis spp. have not been previously reported in Eastern Bluebirds. Although our sequences matched the sequence available for *S. falcatula*, there is a high degree of genetic conservation in the 18S rRNA (Dame et al. 1995) which hinders our ability to obtain a specific identification of the *Sarcocystis* sp. Other genetic targets possibly could provide more specific identification information (Tanhauser et al. 1999). Brown-headed Cowbirds (*Molothrus ater*), which were occasionally seen within the study area, have been found harboring muscle cysts of *S. falcatula* (Luznar et al. 2001). Virginia Opossums (*Didelphis virginiana*), a definitive host (Box et al. 1984) of several *Sarcocystis* spp., were also observed foraging in areas of the PA site where supplemental cattle feed was frequently scattered. Presumably, bluebirds from this site

acquired the infection after consuming oocysts directly or indirectly from insects in the vicinity of opossum feces. Insects may have acted as mechanical carriers of oocysts as cockroaches have been shown experimentally to transmit *Sarcocystis muris* oocysts to mice (Smith and Frenkel 1978). Similar evidence of mechanical transport showed that filth flies carried *Cryptosporidium parvum* oocysts on their exoskeletons (Graczyk et al. 2000). The only birds harboring sarcocysts were collected from the PA site, however our sample sizes for the both HA and LM sites were very small. Opossums were only observed in the PA site and it is likely that these animals avoid the lack of cover characterized by the HA site. A larger sample of birds harvested for necropsy would be necessary to determine whether or not prevalence within the PA site is actually higher.

Haemoproteus fallisi was previously detected in this population during an initial survey in 2003 (Carleton et al. 2004). This identification was based on morphological characteristics of gametocytes in blood smears and association of the species with the subfamily Turdinae (Greiner et al. 1975; Bennett and Pierce 1988). In the current study, we found no negative effect of *Haemoproteus* infection on hematological parameters however the intensity of infection in all sampled birds was very low indicating that infections were likely chronic.

The prevalence of *Atoxoplasma*, *Sarcocystis*, and *Plasmodium* within the population could not be determined as these organisms were only detected by necropsy of a small number of birds. Despite the large proportion of *Plasmodium* infections detected by PCR, none were detected in blood smears. This is not unexpected as chronic *Plasmodium* infections are very difficult to detect by this method (Atkinson and

van Riper III 1991). Examination of blood smears was found to be much less sensitive compared to two PCR methods (Waldenström et al. 2004).

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Table 3.1. Parasitic protozoa in Eastern Bluebirds from North America as reported in literature (1941-2004) and based on a 2007 database search.

Species	Reference
Leucocytozoan sp.	Wetmore 1941
Leucocytozoan dulreuili	Greiner et al. 1975
Leucocytozoan majoris	Greiner et al. 1975
Haemoproteus oryzivorae	Greiner et al. 1974
Haemoproteus fallisi	Greiner et al. 1975; Mataxas and Pung 1999; Carleton et al. 2004
Trypanosoma avium	Wetmore1941; Greiner et al. 1975, Mataxas and Pung 1999; Carleton et al. 2004
Plasmodium sp.	Wetmore 1941; Greiner et al. 1975

Table 3.2. Method of detection and prevalence of parasitic protozoa associated with Eastern Bluebirds nesting within grass-dominated agricultural sites in Georgia, USA (2004-2006).

Species	Method	HA %	LM %	PA %	Total %
Atoxoplasma sp.	pcr	66.7 (3)	75.0 (4)	28.6 (7)	50.0 (14)
Haemoproteus fallisi	bs	54.1 (61)	62.9 (56)	63.8 (47)	59.0 (135)
	pcr	33.3 (3)	0 (4)	0 (7)	7.1 (14)
Plasmodium relictum	pcr	66.7 (3)	100 (4)	57.1 (7)	71.4 (14)
Sarcocystis sp.	n	0 (5)	0 (4)	42.8 (7)	18.7 (16)
Trypanosoma avium	bs	4.9 (61)	1.8 (56)	0 (47)	2.9 (135)

Methods: bs = blood smear, n = necropsy, pcr = polymerase chain reaction
HA = hay field, LM = low management, PA = pasture
( ) = number of birds examined

Figure 3.1 Sarcocysts (arrows) in skeletal musculature of an adult Eastern Bluebird from a cattle pasture in Georgia (2006).

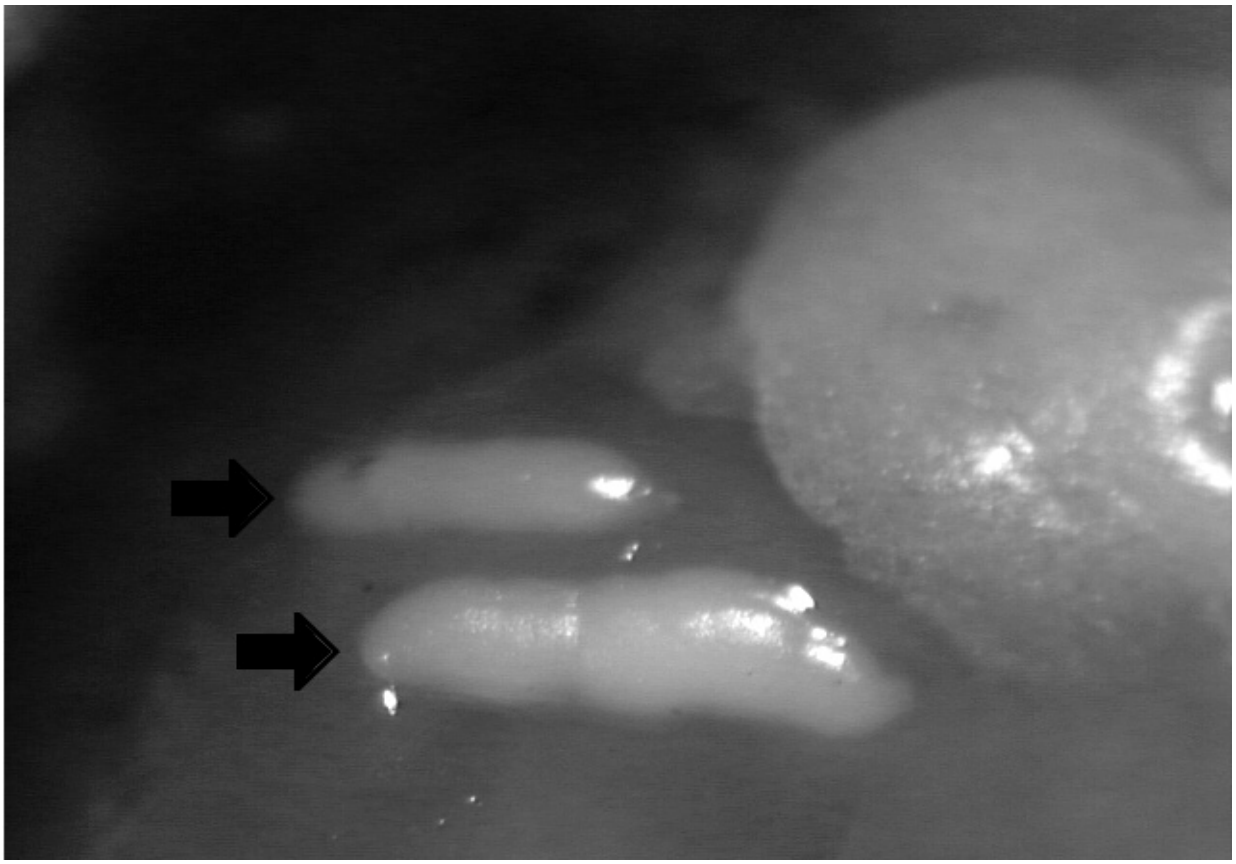

Figure 3.2 Sarcocysts (arrows) in connective tissue associated with the caudal surface of one eye of an adult Eastern Bluebird from a cattle pasture in Georgia(2006).

CHAPTER 4

BACTERIA ISOLATED FROM A POPULATION OF EASTERN BLUEBIRDS (*SIALIA SIALIS*) NESTING WITHIN THREE GRASS-DOMINATED AGRICULTURAL SITES IN GEORGIA[3]

[3] Carleton, R. E. To be submitted to *The Journal of Field Ornithology.*

ABSTRACT

Eastern Bluebirds (*Sialia sialis*) breeding in northwestern Georgia, USA, in hayfield, pasture, and low-intensity managed old-field sites, were surveyed to assess their associated bacterial communities. From March through April 2005 and May through June 2007, oral and cloacal swabs were taken from 33 adult bluebirds and submitted for culture and identification. No *Salmonella* spp. or other significant avian pathogens were among the 43 species isolated. Species richness of bacteria, number of unique species, and number of pathogens varied among by site. Nineteen potential human pathogens were isolated. Because bluebirds are popular songbirds that readily accept man-made nest boxes, handling nesting material may present an exposure risk to humans from these or other pathogens. Several species of bacteria isolated may cause opportunistic infections in bluebirds subjected to environmental stress.

INTRODUCTION

Infectious agents of free-ranging birds are studied frequently; however, much is still unknown about bacterial species associated with passerine birds and the accompanying prevalence of infection (Reed et al. 2003). Certain bacteria are well documented as significant avian pathogens, including *Salmonella* spp. (Refsum et al. 2003), *Yersinia pseudotuberculosis* (Kapperud and Rosef 1983), *Pasteurella multiocida* (Friend 1999), *Escherichia coli* (Nuttall 1997), and *Mycobacteria avium* (Nuttall 1997). Wild and domestic birds are also subject to diseases caused by the bacteria-like organisms, *Mycoplasma gallisepticum* (Hartup et al. 2001) and *Chlamydiophilia psittaci* (Kaleta and Taday 2003).

Studies of bacterial ecology and epidemiology are important not only because of their possible role in host population regulation (Anderson and May 1979) but also because of the zoonotic potential of certain bacterial species (Steele et al. 2005). *Salmonella* spp. (Harris 1991) and *Chlamydiophilia psittaci* (Kaleta and Taday 2003) are notable examples of zoonotic bacteria isolated from birds. Anthropogenic activities, such as maintenance of backyard bird feeders and nest boxes, likely put humans in contact with these infectious agents (Friend et al. 2001). Feeders may also increase the likelihood of disease transmission among birds that congregate at a readily available food source (Dobson and Foufopoulos 2001).

To better understand the dynamics of host-pathogen relationships, documentation of agents infecting a population is necessary. Such knowledge may also be a useful tool in the management of free-ranging populations (Dobson and McCallum 1997). The objectives of this study were to survey the bacterial species associated with a population of Eastern Bluebirds (*Sialia sialis*) nesting on grass-dominated agricultural sites and estimate prevalence of each species isolated and species richness of bacteria among the sites.

METHODS

Study Area. This survey took place on the Berry College campus, Mount Berry, Georgia (USA: 34.282799 °N, 85.191803 °W). The campus encompassed approximately 1,200 ha of bluebird habitat composed of three non-contiguous 40- to 60-ha sites. Site management and level of disturbance differed according to agricultural use: (HA) production of Bermuda grass (*Cynodon dactylon*) for hay, (PA) moderately-grazed cattle pasture, and (LM) low-intensity management. The HA site was intensively

managed; fields were harvested two to three times during each spring and summer, depending on amount of rainfall. Inorganic fertilizer was applied immediately after removal of hay bales. Except for spot treatment of fire ant (*Solenopsis invicta*) mounds, no pesticides were applied. The PA site was divided into three sections by electrical wire fencing. Cattle were rotated into a different section every two weeks. Vegetation consisted primarily of perennial fescue grass (*Festuca arundinacea*) with scattered low-growing plants, such as Carolina horsenettle (*Solanum carolinense*) and wild rose (*Rosa* sp.), small (<1 ha) stands of oaks (*Quercus* spp.), and solitary loblolly pine (*Pinus taeda*) trees. The LM site was separated from the HA site by approximately 10 km comprised of mixed hardwood and pine forest. This site consisted of about 60 ha of old field, early succession habitat. Although 25% of the LM site was mowed once during each summer, it was subject to no other management or livestock grazing. The site received runoff from the adjacent Lavender Mountain and retained water in lower areas following heavy rainfall. Mixed grasses (e.g., perennial fescue, Johnson grass (*Sorghum halepense*), and broomsedge (*Andropogon* spp.), rushes (*Juncus effusus*), maple (*Acer* spp.), sweet gum (*Liquidambar styraciflua*) saplings, and occasional shrubs, including wild rose and privet (*Ligustrum sinese*), grew within the site.

Nest boxes used to attract bluebirds were constructed of untreated white pine (*Pinus strobus*) following a North American Bluebird Society design (NABS 2002). Nest boxes were mounted on power line poles or fence posts, and separated by approximately 150 m. Box density varied slightly by site: 12 boxes were placed within the LM site, 16 boxes were placed within the HA site, and 18 boxes were placed within

the PA site. Some nest boxes were not used by bluebirds. Nesting material was removed and discarded after each brood fledged.

Bacterial Survey. Adult Eastern Bluebirds were captured inside nest boxes using a trap door apparatus. The survey took place during two non-consecutive breeding seasons: March through August 2005, and May and June 2007. Birds were identified by individually numbered United States Geological Survey aluminum leg bands or, if unbanded, by nest box identification number only. Each bird was examined for anomalies or signs of illness. A sample of bacteria from the oral cavity was obtained by rubbing a sterile culture swab (Fisherfinest Microorganism Collection and Transport System, Fisher HealthCare, Houston, TX) across the oral mucosa and choanae. A cloacal sample was obtained with a separate sterile swab by gently inserting the tip into the cloaca. Swabs were placed in individual sleeves containing modified Amies clear transport medium and kept inside a cooler supplied with frozen refrigerant gel packs (Dry Pack Industries, Encino, CA) for transport from the field. All samples were maintained under refrigeration and submitted within three days of collection to the Athens Diagnostic Laboratory (University of Georgia, College of Veterinary Medicine, Athens, GA) for culture and identification of bacteria.

Prevalence was estimated for the population and each site. The number of bacteria species detected per bird per study site was used to estimate species richness (Clayton and Moore 1997). For each study site, species evenness, the proportion of individuals of each species isolated, and species richness were used to calculate a Shannon index to estimate bacteria diversity (Odum and Barrett 2005).

Statisical analyses. Data from the two breeding seasons were pooled then analyzed using JMP IN Statistical Software (SAS Institute, Inc., Cary, NC) in order to examine the make-up of cloaca and oral cavity bacterial communites. Lack of additional sites of each category precluded analysis (Dowdy et al. 2004). Diversity indices per site and prevalence per site were not compared.

RESULTS

Forty-three bacterial species were cultured from the sample population (*n* = 33, Table 4.1). Most (86%) of these species were Gram-negative bacteria; Gram-positive bacteria accounted for only six of the species isolated. The most common species (*Enterococcus* sp., Gram-positive *Bacillus* sp., *Enterobacter* sp., *Escherichia coli*, and *Pantoea agglomerans*) were isolated from all sites. Prevalence was estimated for each species; however, because of the small sample size, prevalences of individual species among sites were not compared (Table 4.1). Twenty-three species were isolated from the HA site, 29 species were isolated from the PA site, and 30 species were isolated from the LM site. Sixteen species were common among all three sites and seven species were common to two of three sites. Twenty species were unique to at least one site; nine species were unique to the LM site, eight species were unique to the PA site, and three species were unique to the HA site. The number of species isolated per bird ranged from three to 13, with a mean of 5.57(SD ±1.92). The number of species per bird . Diversity indices ranged from 2.82 for the HA to 3.25 for the LM site = 3.25; diversity index for the PA site was 2.96.

Twelve species were cultured from the oral cavity only, 12 species were cultured from the cloaca only, and 14 species were cultured from their hosts' oral cavity and

cloaca (Table 4.1). Five species were cultured either from the oral cavity or cloaca of their hosts, but not simultaneously from the same host.

Nineteen of the species cultured from bluebirds, excluding those isolates identified only by genus, are potential human pathogens. Most were isolated from the PA (14) and LM (13) sites, while the fewest were isolated from the HA (eight). The number of potential pathogens isolated per bird ranged from zero to four with a mean of 1.91 (1.18 ± SD) and did not differ between sexes ($F_{1,32}$ = 0.899, P = 0.352). No *Salmonella* spp. was isolated from any samples and no anomalies or signs of illness were identified in any of the birds.

DISCUSSION

Forty-three bacterial species were isolated from the bluebirds under study. *Entercoccus* sp., Gram-positive *Bacillus* sp., and *Enterobacter* sp. were cultured from nearly all birds sampled suggesting that these bacteria are probably part of the normal oral and intestinal flora of bluebirds. In previous surveys for bacteria conducted in Ohio and California, *E. coli* was commonly isolated from insectivorous, passerine birds (Morishita et al. 1999; Rogers 2006). In this study, it was also commonly isolated in bluebirds. The significance of isolates of *E. coli* in this local population is unknown as none of the sampled birds showed overt signs of disease and all successfully reared young. Many of the isolates likely represent environmental contamination or temporary infections obtained while capturing or consuming insect prey. Stressed or otherwise compromised birds, however, may develop serious infections from otherwise non-pathogenic commensals (Smith et al. 2002). Mortality in bluebirds has been associated with extreme weather conditions (Sauer and Droege 1990) and interspecific competition

for nesting sites (Gowaty 1984). Bacterial infections associated with reduced immunocompetence may be a consequence of such events (Smith et al. 2002). Three bacterial species isolated in this study, *Citrobacter freundii, Pseudomonas* spp., and *Klebsiella* spp., cause significant opportunistic infections in canaries and psittacines (Dorrestein 1997). Pathogenicity may be promoted by other factors as well. *E. coli*, a common isolate in this study, was cultured from the liver of a dead Western Bluebird (*Sialia mexicana*) harboring acanthocephalan worms. The cause of death in this and similar cases was attributed to intestinal ulceration and penetration resulting from endoparasitism (Thompson-Cowley and Helfer 1979). Necrotic hemorrhagic enteritis has also been implicated as the cause of Western Bluebird mortality. *Clostridium perfringens* was isolated from both liver and intestinal tissue in several of these cases (Bildfell et al. 2001).

Cloacal sampling exclusively, as in previous surveys (Brittingham et al. 1988; Lombardo et al. 1996), may not yield a thorough representation of bacterial communities within a host species. In this study, 12 bacterial species were cultured only from oral cavity swabs and six of those were potential human pathogens. Future bacterial surveys of any vertebrate host should take samples from both the cloaca and oral cavity.

Despite some commonality, the bacterial communities isolated from bluebirds appeared to vary among the three sites; because replication at the site level was not possible on the study area a true statistical analysis is precluded (Table 4.1). The HA site, the most disturbed and having the lowest plant diversity, had lower bacteria species richness and fewer numbers of unique species than either the PA or LM sites.

Diversity indices were similar among sites. Species richness and number of unique bacterial species were similar between the PA and LM sites. Both sites had greater plant diversity and received less disturbance than the HA site. Because biodiversity within agriculture ecosystems is influenced by agricultural practices (Altieri 1999), the differences in bacterial species richness, numbers of unique species, and unique species isolated among the sites in this study, may be a reflection of management. Plant diversity has a significant effect on the composition of soil bacterial communities. Litter decomposition rates also increase in association with greater plant diversity (Carney and Matson 2005). It should be noted, however that relationships between plant diversity and animal pathogens has not been examined. Hay harvest within the HA site regularly removed all but approximately 25 mm of top growth. The resulting loss of organic matter may have depressed soil bacterial community diversity by reducing soil nutrient content. Cattle manure within the PA site and greater plant diversity with less removal of organic matter within the PA and LM sites may have likewise promoted bacterial diversity. Further investigation of site-bacterial community relationships is required before any association between site characteristics and bluebird bacterial communities can be made.

Bacteria species associated with birds that cause zoonotic diseases, such as *S. typhimurium* (Harris 1991), were not isolated from the study population; however, nearly one half of the species isolated are opportunistic human pathogens. These species include, *Acinetobacter* spp. (Bergogne-Bérézin and Towner 1996), *Burkholderia cepacia* (Holmes et al. 1998), *Chryseobacterium meningosepticum* (Bloch et al. 1997), *Chryseomonas luteola* (Chihab et al. 2004), *Citrobacter* spp. (Badger et al. 1999),

Enterobacter spp. (Sanders and Sanders 1997), *E. coli* (Nataro and Kaper 1998), *Hafnia alvei* (Janda and Abbott 2006), *Klebsiella* spp. (Podschun and Ullmann 1998), *Leclercia adecarboxylata* (Temesgen et al. 1997), *Pantoea agglomerans* (Cruz et at. 2007), *Proteus* sp. (O'Hara et al. 2000), *Pseudomonas aeruginosa* (Bodey et al. 1983), *Serratia liquefaciens* (Mossad 2000), *S. marcescens* (Hejazi and Falkiner 1997), *Shewanella putrefaciens* (Khashe and Janda 1998), and *Stenotrophomonas maltophilia* (Denton and Kerr 1998). Because bluebirds readily nest in artificial cavities, backyard or bluebird trail maintainance of nest boxes is common (Zeleny 1977; Gowaty and Plissner 1998). Routine cleaning of nest boxes, as recommended by the United States Geological Survey's Northern Prairie Wildlife Research Center (2006), may present a risk of exposure to these or other potential pathogens. Individuals, especially those who may be immunocompromised, should take reasonable precautions when handling used nesting materials.

Further investigation into relationships between bacterial communities and habitat is needed before specific management recommendations can be formulated; the presence of potentially pathogenic bacteria within a free-ranging population is a matter of concern. Measures designed to mediate physiological stress, such as reducing inter- and intraspecific competition for nesting sites and other resources or providing roosting shelters for use during extremely cold weather, may reduce the occurrence of opportunistic infections. Conservation efforts directed at other species may also be improved by including awareness of associated bacterial communities.

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Table 4.1. Bacteria isolated from an Eastern Bluebird (*Sialia sialis*) population nesting on three grass-dominated agricultural sites in northern Georgia (2005 and 2007) sorted by location *in situ* and by site (HA = hayfield, PA = pasture, LM = low management). Prevalence (%) is given for each species isolated from the population.

Species isolated	Location *in situ*			Study Site			Population	
	Oral	Cloaca	Both	HA (10)[a]	PA (12)	LM (11)	Total (33)	Prevalence
Acinetobacter sp. [b p]	1	0	0	0	1	0	1	3.0
Acinetobacter baumannii [b p]	4	1	0	1	3	1	5	15.1
Acinetobacter lwoffii [b]	1	0	0	0	1	0	1	3.0
Bacillus sp. [c]	3	2	9	6	4	4	14	42.4
Non-enteric Bacilli [b]	5	2	0	3	1	3	7	21.2
Burkholderia cepacia [b]	0	1	0	1	0	0	1	3.0
CDC Enteric Group 74 & 75 [b]	0	1	1	1	0	1	2	6.1
C. meningosepticum [b p]	1	0	1	0	0	2	2	6.1
Chryseomonas luteola [b]	1	0	0	0	1	0	1	3.0
Citrobacter sp. [b]	0	5	0	1	1	3	5	15.1
Citrobacter amalonaticus [b]	0	1	0	0	0	1	1	3.0
Citrobacter freundii [b p]	0	3	3	0	1	2	3	9.1
Enterobacter sp. [b p]	4	7	1	4	6	2	12	36.4
Enterobacter aerogenes [b p]	0	3	0	1	1	1	3	9.1
Enterobacter cloacae [b p]	3	7	1	4	4	3	11	33.3
Enterobacter sakazakii [b p]	1	0	0	0	1	0	1	3.0
Enterococcus sp. [c]	6	13	7	9	10	7	26	78.8
Escherichia coli [b p]	1	5	3	2	4	3	9	33.0
Hafnia alvei [b p]	0	2	0	0	0	2	2	6.1
Klebsiella sp. [b]	0	3	0	1	1	1	3	9.1
Klebsiella oxytoca [b]	2	0	5	2	2	3	7	21.2

Table 4.1. Continued.

Species isolated	Location *in situ*			Study Site			Population	
	Oral	Cloaca	Both	HA (10)[a]	PA (12)	LM (11)	Total (33)	Prevalence
Klebsiella pneumoniae [b p]	0	8	0	2	4	2	8	24.2
Leclercia adecarboxylata [b p]	1	0	0	0	1	0	1	3.0
Micrococcus sp. [c]	2	0	0	0	0	2	2	6.1
Pantoea sp. [b]	2	2	0	1	1	2	4	12.1
Pantoea agglomerans [b p]	5	3	1	2	3	4	9	33.0
Pasturella-like organism [b]	0	1	0	1	0	0	1	3.0
Proteus sp. [b p]	0	0	1	0	1	0	1	3.0
Providencia rettgeri [b]	0	0	1	0	1	0	1	3.0
Pseudomonas sp. [b]	2	1	0	1	1	1	3	9.1
Pseudomonas aeruginosa [b p]	0	1	1	1	1	0	2	6.1
Pseudomonas mendocina [b]	0	2	0	0	0	2	2	6.1
Pseudomonas putida [b]	3	0	0	1	2	0	3	9.1
Serratia sp. [b]	0	4	0	0	2	2	4	12.1
Serratia liquefaciens [b p]	0	1	0	0	1	0	1	3.0
Serratia marcescens [b p]	1	2	0	0	1	2	3	9.1
Serratia odorifera [b]	1	0	0	1	0	0	1	3.0
Shewanella putrefaciens [b p]	1	0	0	0	0	1	1	3.0
Sphingomonas paucimobilis [b]	0	1	0	0	0	1	1	3.0
Staphylococcus sp. [c]	6	0	1	1	3	3	7	21.2
Stenotrophomonas maltophilia [b]	1	0	0	0	0	1	1	3.0
Streptococcus sp. (alpha) [c]	2	0	0	1	0	1	2	6.1
Streptomyces sp. [c]	2	0	0	0	0	2	2	6.1
Total = 43								

[a] = number sampled, [b] = Gram negative, [c] = Gram positive, [p] = potential pathogen

CHAPTER 5

MACROPARASITES OF EASTERN BLUEBIRDS (*SIALIA SIALIS*): A REVIEW AND SURVEY OF A POPULATION NESTING WITHIN GRASS-DOMINATED AGRICULTURAL HABITATS IN GEORGIA[4]

[4] Carleton, R. E., M. J. Yabsley, and J. W. Mertins. To be submitted to *Comparative Parasitology.*

ABSTRACT

A review of parasites associated with Eastern Bluebirds (*Sialia sialis*) is dated and excludes many species reported since 1977. We compiled an updated list of macroparasites recovered from Eastern Bluebirds using reports from the literature and database searches. Because bluebirds are subject to infection with many generalist parasites, additional species were likely to be reported. We surveyed the macroparasite community associated with a population of Eastern Bluebirds (*Sialia sialis*) in northern Georgia, during the 2004, 2005, and 2006 breeding seasons by live examination, fecal examination, necropsy, and examination of nesting material. Ten species of macroparasites were detected including five species not previously reported in Eastern Bluebirds: *Plagiorhyncus cylindraceus*, *Capillaria* sp., a species of Stronglyloid nematode, a species of Spiruroid nematode, and *Carnus floridensis*. Prevalence based on pooled data over 3 years was low (19.7%) and did not vary among our three study sites, each characterized by grass-dominated agriculture. We found preliminary evidence of associations between some parasite species and the type of agricultural habitat.

INTRODUCTION

Eastern Bluebirds (*Sialia sialis*) are one of the most readily recognized songbird species of North America due to their wide distribution, bright coloration, relative tameness, and ready acceptance of nest boxes (Gowaty and Plissner 1998). The popularity of this species is evidenced by its adoption as the official state bird of New York and Missouri. During most of the twentieth century, bluebird populations declined due to interspecific competition, habitat loss, and severe weather events (Sauer and

Droege 1990). Public awareness and concern over dwindling populations resulted in the formation of The North American Bluebird Society, establishment of bluebird trails, and increased use of nest boxes as a replacement for natural cavities (Janetatos 1996). Bluebird numbers have rebounded in most areas; however, local populations remain at risk to environmental changes (Sauer and Droege 1990). To better understand pressures affecting this species and to anticipate, monitor, and manage changes in Eastern Bluebird populations, updated and novel information is needed to provide greater insight into their population dynamics.

Host-parasite interactions are recognized as important regulators of vertebrate populations either through direct or indirect effects (Toft 1991). Decreased survival and fecundity associated with macroparasite infestation have been documented in Red Grouse (*Lagopus lagopus scoticus*) (Hudson 1986) and Cliff Swallows (*Hirundo pyrrhonota*) (Chapman and George 1991). The limited information on parasites affecting Eastern Bluebirds has presented no evidence that infectious agents play a role in population regulation (Gowaty and Plissner 1998). Continuing loss of habitat may force Eastern Bluebirds into smaller, less optimal areas which may increase the risk of intra- and interspecific parasite transmission and disease (Holmes 1996). An investigation of bluebird parasites and environmental conditions promoting parasite transmission is timely and has implications for conservation and habitat management.

Current knowledge is based on the occasional opportunistic recovery of dead, injured, or sick bluebirds, observational studies of nesting adults and nestlings, and recoveries of parasites during non-species-specific surveys. A 26 year-old report describing most of the known parasites infecting Eastern Bluebirds (Roberts 1981)

reviewed the literature from 1936 through 1977. Many papers have been published since that report, including new host records of burrowing fly larvae (Mertins and Spalding 2001; Spalding et al. 2002) and infection with West Nile Virus (Forrester and Spalding 2003). Based on results of a 2007 database search, including the National Parasite Collection database, macroparasites reported from Eastern Bluebirds include 5 species of gastrointestinal or subcutaneous helminths and 21 species of parasitic arthropods (Tables 5.1 and 5.2).

The objectives of this study were to identify macroparasites associated with a population of Eastern Bluebirds nesting on grass-dominated agricultural habitats in Georgia and estimate parasite prevalence and species richness within the population. Because the study population was potentially susceptible to a multitude of generalist parasites and relatively few of those have been reported in Eastern Bluebirds, we expected to recover macroparasites not previously documented.

METHODS

Study Area. The study was conducted during three breeding seasons (March - August), 2004 through 2006, within the 11,331-ha land tract of Berry College, Mount Berry, GA; approximately 104 km northwest of Atlanta, Georgia (USA: 34.282799 ^{o}N, 85.191803 ^{o}W). Berry College has been associated with agricultural enterprises since its establishment in 1905. Approximately 1,200 ha were suitable bluebird habitat divided among three non-contiguous study sites of grass-dominated agricultural usage: approximately 40-ha Bermuda grass (*Cynodon dactylon*) hay field (HA), approximately 40-ha moderately-grazed cattle pasture (PA), and approximately 60-ha low management fields (LM) with characteristics of early succession. Each site had habitat

characteristics required by bluebirds, namely open areas of primarily grassy vegetation, perching sites used to locate prey, and structures supporting nesting sites (Gowaty and Plissner 1998). Nest boxes constructed from untreated white pine (*Pinus strobus*) and mounted on power-line poles or fence posts were located within each site and had been in place since 2002. All boxes were monitored for nesting activity throughout the breeding seasons and old nesting material was removed after a brood fledged or was lost to predation.

Breeding adults (*N* = 130; HA *n* = 59; LM *n* = 23; PA *n* = 48) were captured inside nest boxes using a trap door system. Captures of both members of a nesting pair were attempted following successful hatching of each clutch. Adults were banded on the first capture occasion with individually numbered United States Geological Survey (USGS) aluminum leg bands for future identification. Each bird was briefly examined for ectoparasites by gently ruffling through its feathers and exposing featherless tracts. To minimize stress and reduce the risk of nest abandonment, birds were released within eight min of capture. Ectoparasites, when detected, were placed into a vial containing 70% ethanol (ETOH) pending identification.

A sample of feces was collected in a vial containing two drops of 10% buffered formalin as a preservative. Fecal samples were prepared by centrifugation and flotation with Sheather's sugar solution (Georgi and Georgi 1990), and then examined at 100x magnification with a light microscope. Samples were denoted as positive if one or more ova were detected. Ova were identified to the level of family or genera as possible.

Because only limited sampling could be performed safely on living birds, 14 males were sacrificed for necropsy during the final weeks of the 2006 breeding season:

PA *n* = 7, HA *n* = 3, LM *n* = 4. Bluebird offspring survival is not significantly reduced following loss of the male from a breeding pair (Gowaty 1983), therefore we anticipated no or minimal negative effects to the population. Birds were euthanized by exsanquination or carbon dioxide intoxication following capture. Each carcass was placed in a sealable plastic bag and refrigerated until necropsy to prevent ectoparasites from escaping. Necropsies were completed within 24 hours of collection following a standard necropsy protocol (see van Riper III and van Riper 1980). Nasal passages were flushed by forcing a water and alcohol solution through the choanal opening. Wash solution was captured in a petri dish and examined under a dissecting microscope at 40x magnification. After sealing the pharyngeal area and nares with cotton, each carcass was washed with a water-isopropyl alcohol-soap solution then rinsed thoroughly (Clayton and Walther 1997). Ectoparasites were separated from the wash solution by vacuum filtration and placed into vials containing 70% ETOH. Techniques described by Doster and Goater (1997) were followed for the detection, recovery, and quantification of endoparasites. Fecal material present in the large intestine was collected and examined as previously described.

Over the course of the study, five dead adults and one fledgling were recovered in the field; advanced autolysis prevented necropsy of the fledgling and two of the adults. A minimal state of autolysis allowed partial necropsy of the remaining two adults; two females (HA and PA sites) and one male (HA sites). Body washes were not performed on these birds as any ectoparasites present would have moved from the carcasses.

Nestlings were examined for ectoparasites and lesions suggestive of parasite-induced injury at ten days of age. Representative samples of ectoparasites, if present, were collected from one or more nestlings within each clutch. Nests were collected after nestlings fledged, placed into sealed plastic bags, and stored under refrigeration before examination. Because of excessive amounts of feather dust and other fine debris within the nesting material, an alcohol solution was added to each bag to wet the contents. Nesting material was then placed into a shallow pan and examined for arthropods that could be seen with the naked eye. The alcohol solution was decanted following disposal of nesting material and filtered for recovery of microscopic arthropods. All ectoparasites collected from nests or nestlings were placed in vials containing 70% ETOH.

Data were pooled over the three years of the study. Prevalence and species richness was estimated for the population. As a preliminary investigation, prevalence and richness of parasites was compared among sites by χ^2 and ANOVA analyses with *a-priori* significance set at $\alpha = 0.05$. Because no replication of habitat types was possible, analyses of site comparisons were exploratory in nature. JMP-IN Statistical Software (SAS Institute, Inc., Cary, NC) was used for all analyses. A likelihood ratio test was used when insufficient data (< 5 observations per contingency table cell) precluded χ^2 validity (Sall et al. 2005). Macroparasite species prevalence by recovery method was estimated as the percentage of infected birds divided by the number of birds sampled using a particular method. Prevalence estimations for each site and the study area were based on parasite detection by all applicable recovery methods excluding nest and nestling examination. Species prevalence by site was determined

by the number of birds per site infected with a particular species divided by number of birds sampled per site. Species prevalence within the study area equaled the number of birds infected with a particular parasite species divided by total number of birds sampled. Macroparasite prevalence within the study area equaled the number of birds within the study area infected with any macroparasite divided by total number of birds sampled. Prevalence of nest mites was determined by dividing the number of infested nests by the number of nests examined. Species richness for both individual sites and the study area was defined as the number of macroparasite species detected by all methods per study site or over all study sites (study area), respectively.

RESULTS

Few macroparasites were detected by live examination of adult bluebirds (*n* = 130; Table 5.3). One partially engorged tick (*Ixodes brunneus*) was found attached near the aural canal of a female nesting within the LM site. One chewing louse (*Philopterus sialii*) was collected from the axillary area of a male nesting within the PA site. Feather mites, although commonly found, were not included in the survey because they are considered non-parasitic. Small subcutaneous nodules, similar in description to nodules associated with *Collyriclum faba* (Blankespoor et al. 1985), were found on five (3.8%) females captured within the LM site (60.0%), the HA site (20.0%), and PA site (20.0%). The nodules were not excised for identification.

One tick, *I. brunneus*, was found attached to the head of a dead female. This bird was discovered inside the nest box in which she had raised young the previous year. The body was emaciated and showed no signs of trauma. Based on the bird's body condition and presence of the tick, starvation as a consequence of tick paralysis

was the presumptive cause of death (Luttrell et al. 1996). Two additional adults, both males, were also found dead within nest boxes and had no signs of trauma. No ticks were found on either bird; however, both carcasses were in advanced states of decomposition and cause of death could not be determined. Chewing lice (*P. sialii*) were recovered from four (28.5%) males sacrificed for complete necropsy (n = 14). Birds harboring lice were taken from PA (75.0%) and LM (25.0%) sites only and the number of lice per bird ranged from one to five. Prevalence among sites (Table 5.3) was not compared because the number of lice recovered was very small. No other parasitic arthropods were recovered during necropsies.

Acanthocephalan worms, identified as *Plagiorhyncus cylindraceus*, were recovered from the small intestines of six (37.5%) of 16 birds necropsied. Most (66.7%) of the birds harboring *P. cylindraceus* were nesting within the HA site. No birds from the LM site harbored acanthocephalans. Prevalence of acanthocephalans differed among sites (Likelihood ratio G^2 = 7.79, P = 0.023, df = 2), and the number of worms per bird differed among sites ($F_{16,2}$ = 4.81, P = 0.02). Birds harboring the largest number of worms (three, four and six) were from the HA site.

A strongyloid nematode recovered from the ventriculus of a bird taken from the PA site was the only nematode recovered by necropsy. This bird also harbored two acanthocephalans. A partially intact cestode was the only gastrointestinal helminth recovered from any of the birds nesting in the LM site. All helminths were found only within the gastrointestinal tract (Table 5.3). Fecal samples were collected from 86 birds by live examination or necropsy. Multiple samples from the same bird were not counted as additional samples. Parasite ova were detected in 11 (12.8%) samples (Table 5.3).

Ova of *Capillaria* sp. were detected in five samples (5.8%), *Hymenolepis* sp. ova were detected in four samples (4.6%), ova of a Spiruroid nematode was detected in one sample (1.2%), and three samples (3.5%) contained Strongyloid nematode ova. One sample contained ova of two species, *Hymenolepis* sp. and a *Capillaria* sp. Prevalence of ova did not differ among sites (Likelihood ratio G^2 = 1.54, P = 0.56, df = 2). Samples from necropsied birds harboring acanthocephalans or nematodes contained no ova.

Many (43.7%) nests (n = 103) contained numerous hematophagous nest mites identified as *Dermanyssus prognephilus* (Table 5.3). There was no difference in the number of infested nests among sites (Pearson χ^2 = 3.43, 2 df, P = 0.18). No other parasitic arthropods were recovered from nests.

Two arthropod species were detected during examinations of nestlings (Table 5.3). Nest mites (*D. prognephilus)* were found on nestlings from all sites and all came from nests in which mites were recovered during nest examination. Three Florida nestling bird flies, *Carnus floridensis*, were collected from a single clutch of heavily infested nestlings reared within the HA site. No blow fly larvae or lesions suggestive of blow fly larvae activity were found on any nestlings.

Twenty-nine (19.7%) of the adult bluebirds sampled (n = 147), including all necropsies, live examinations, and fecal examinations, harbored one or more macroparasite. Prevalence was 34.5% in the LM site (n = 29), 20.7% in the PA site (n = 53), and 15.4% in the HA site (n = 65), but there was no difference in prevalence among sites (Pearson χ^2 = 4.40, 2 df, P = 0.11). There was no difference in prevalence between females (16.7%, n = *84*) and males (17.6%, n = 51) based on a χ^2 test of

independence (Pearson $\chi^2 = 0.022$, 1 df, $P = 0.88$); males harvested for necropsy were excluded to remove sampling bias.

Ten species of macroparasites were recovered from the study population, including a cestode, an acanthocephalan, three species of nematodes, possibly one trematode, one tick and chewing lice species, one species of nest mite, and one bird fly. Five of these species have not been reported previously in Eastern Bluebirds (*C. floridensis*, *P. cylindraceus*, *Capillaria* sp., a Strongyloid nematode and a Spiruroid nematode). Excluding nest associated parasites, species richness did not vary among the three sites; eight species were recovered from both the LM and PA sites, and seven were recovered from the HA site. Some species were detected in all sites and others were detected in only one or two of the sites (Figs. 5.1 and 5.2).

DISCUSSION

Overall prevalence of macroparasites within the adult study population (19.7%) was low; however, this probably represents an underestimate due to the modest number of necropsies and low sensitivity of fecal and live examinations. Because no similar investigations are available for comparison, we can only speculate that overall prevalence would have increased with additional necropsies. Several parasite species previously reported in Eastern Bluebirds were not detected during this study. This may be due to the above mentioned limitations or differences in geographical distribution of certain parasite species and their hosts or a dilution effect (Schmidt and Ostfeld 2001).

Highly prevalent parasitic species occurring at high intensity within one host species should successfully colonize many other host species (Brown 1984; Poulin 1990). American Robins (*Turdus migratorius*), belong to the same taxonomic subfamily

as bluebirds, were commonly seen on all of our study sites. A necropsy-based investigation of robins in Ohio recovered 43 species of helminth and 96.0% prevalence among adults and juveniles (Cooper and Crites 1974). If prevalence in robins is generally as high as those in Ohio, Eastern Bluebirds comprising the study population should have many helminth species in common with resident robins. This conjecture in conjunction with the small number of helminth species recovered in our study further suggests an underestimation of overall prevalence and species richness.

Nests and nestlings were frequently infested with *D. prognephilus*, a hematophagous nest mite commonly found in bluebird nests (Moss et al. 1970; Pinkowski 1977). As with the adult population, macroparasite prevalence among nestlings was also lower than that found in other studies (Burtt et al. 1991). For example, Mataxas and Pung (1999) estimated prevalence of *D. prognephilus* among Eastern Bluebird nestlings at 98.0%. Blowfly larvae, another common parasite of bluebird nestlings, were notably absent from nests on our study site. As a comparison, 85.0% of bluebird nests in Michigan contained larvae (Pinkowski 1977).

The Florida nestling bird fly (*C. floridensis*), one of the five species previously unreported macroparasites in Eastern Bluebirds, were recovered from one nest. This blood-sucking fly is one of three described species in the genus occurring in North America. Adults of the genus parasitize nestlings of wild birds; larvae live in the nest materials, probably as scavengers. Florida nestling bird flies have only been collected twice; both times in Florida. The original discovery was collected from a woodpecker nestling in 1992 (Grimaldi 1997). *C. floridensis* was also collected from nestling Great-crested Flycatchers (*Myiarchus crinitus*) (Mertins and Miller 1997).

Recovery of arthropods by the live examination method was extremely low compared to recovery by body washing before necropsy, although only one species of parasitic arthropod (*P. sialii*) was recovered by both methods. In one study, fewer lice lice were detected on Rock Doves (*Columba livia*) by visual examination than by fumigation (Walther and Clayton1997). Although the dust-ruffling method that incorporates a pyrethrin-based insecticide, enhances the collection of ectoparasites from live birds, we elected not to use this method because of increased handling time (Walther and Clayton 1997). In one study, body washing recovered greater than 95% of lice from sparrows (McGroarty and Dobson 1974). Assuming recovery rates would be similar for bluebirds, our estimated prevalence of lice (28.5%) is likely representative of this population.

The tick species (*I. brunneus)* recovered during the study was plausibly responsible for the death of at least one bird and possibly the other dead birds discovered within nest boxes. Each of these deaths occurred early in the breeding season which coincides with the seasonally highest recoveries of this tick species (Luttrell et al. 1996). Birds serve as the only host for all stages of *I. brunneus.* Passerines have been found with the tick more frequently than any of the other 64 known host species. Because relatively few dead birds are recovered from the field or are in a state of autolysis preventing determination of death, the incidence of tick paralysis in this population is unknown. The discovery of additional carcasses within nest boxes raises considerable suspicion that the presence of *I. brunneus* is significant. The dead female successfully fledged three broods during the previous breeding season (2005) and died before nesting in 2006.

Subcutaneous nodules suggestive of *C. faba* were only detected during live examination. Definitive identification would only have been possible by excision of one or more nodules. Eastern Bluebirds are known hosts of *C. faba* as the trematode was found within nodules on several birds captured in western New York (Kibler 1968). A higher prevalence of *C. faba* has been reported in female birds (Blankespoor et al. 1985). Prevalence also appeared higher in females within our study population. Assuming nodules discovered during this study contained *C. faba,* our findings represent the second report of this trematode in Eastern Bluebirds and a new host distribution record. Little is known about the life history of *C. faba*, but it may be transmitted by semi-aquatic insects as there is some evidence of a higher prevalence in birds nesting near fresh water (Blankespoor et al. 1985).

Fecal examinations were valuable in identifying species not recovered during necropsy. But it is likely that many birds harboring gastrointestinal helminths were not identified as hosts due to single worm infections, worm infections consisting of a single sex, or infection with species having low fecundity or irregular ovulation. For example, six of the birds we examined by necropsy harbored acanthocephalans yet no ova were detected in any fecal examinations.

Plagiorhyncus cylindraceus represents one of two new host records resulting from our study. This acanthocephalan was previously reported in Western Bluebirds (*Sialia mexicana*) and implicated as a contributory cause of death in a small number of cases (Thompson-Cowley and Helfer 1979; Bildfell et al. 2001). One of the dead bluebirds recovered in our study harbored six *P. cylindraceus.* All of the worms were located in close proximity and appeared to occlude the small intestinal lumen. This bird,

a female, died following a routine capture, examination, and collection of a blood sample. It is possible that this heavy worm burden was a confounding factor as this was the only capture-associated death during the study.

We found evidence suggestive of relationships between the presence of certain parasite species and habitat characteristics based our limited sample size. Acanthocephalans and *Capillaria* sp. were detected more frequently in the HA site. Although it is unclear whether disturbance in the form of routine hay harvest was a factor, there is evidence suggesting that parasite transmission patterns change with many forms of disturbance (Lafferty and Holt 2003). For example, gastrointestinal helminth and protozoal infections of Ugandan Redtail Guenons (*Cercopithecus ascanius*) occur at a higher prevalence in logged forests than unlogged forests (Gillespie et al. 2005). In a similar study, frogs collected from pastures had greater parasite abundance and species richness than those collected from forests (McKenzie 2007).

The strong association of *P. cylindraceus* with the HA site may be due to a relationship between changes associated with hay production and those of its isopod intermediate host (*Armadillidium vulgare*) (Schmidt and Olsen 1964). Isopods infected with *P. cylindraceus* are more active, tolerate drier conditions, are less likely to seek shelter, and prefer lighter colored backgrounds than non-infected conspecifics (Moore 1983). These behavioral changes along with a greater likelihood of exposure or enhanced visibility following hay harvest may increase the rate of predation by bluebirds. Further study would be necessary to evaluate this hypothesis. Reasons for

the strong association between *Capillaria* nematodes and the HA site are less clear as no intermediate host is involved.

The prevalence of macroparasites in the study population was relatively low, although it is likely many infected birds were not detected. With the exception of *I. brunneus* and *P. cylindraceus*, the impact of parasites on individual birds appeared to be minimal. Indirect effects on reproduction and long-term survival are unknown. Concurrent infections or those in combination with physiological stress may reduce survival during severe weather conditions or reduce fecundity as reported in other species (Hudson 1986; Chapman and George 1991). Controlled studies comparing anthelminthic-treated birds with untreated birds would be required to determine these effects (Hudson and Dobson 1997).

Our study extends the checklist of reported parasites associated with Eastern Bluebirds and suggests there may be an association between certain agricultural habitats and specific parasites. Although further study is warranted, it appears advisable to locate nest boxes in more diverse. With this information in hand, individuals and organizations may make better decisions regarding site selection for the promotion of bluebird residency.

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Table 5.1. Helminths detected in Eastern Bluebirds as reported in literature from 1934-1999 based on a 2006 database search.

Parasite	Location in situ	Host location	Reference
Nematodes			
Dispahrynx nasuta	ventriculus	ns[a]	Wehr (1971)
Oxysoirura pusillae	eye	LA	Pence (1972)
microfilaria[a]	blood	GA	Love et al. (1953)
	lung	ns	Robinson (1954)
	blood	ns	Greiner et al. (1975)
	blood	GA	Mataxas and Pung (1999)
Cestodes			
Hymenolepis sp.	intestine	VA	Wehr (1934)
Trematodes			
Lutztrema monenteron	gall bladder	VA	Price and McIntosh (1935)
Collyriclum faba	subcutis	NY	Kibler (1968)
		MI	Pinkowski (1975)

[a] ns = not specified

Table 5.2. Parasitic arthropods detected in Eastern Bluebirds (*Sialia sialis*) as reported in literature from 1936-2007 based on a 2007 database search.

Arthropod	Host location	Reference
Ticks		
Ixodes brunneus	GA, TN	Luttrell et al. (1996)
Amblyomma americanum[a]	GA	Mataxas and Pung (1999)
	GA	Durden et al. (2001)
A. maculatum	GA	Durden et al. (2001)
Nest mites		
Analgopsis sp.	NC	Peters (1936)
Dermanyssus prognephilus	SC	Peters (1936)
	GA	Moss et al. (1970)
	GA	Mataxas and Pung (1999)
Dermanyssus hirundinis	OH	Burtt et al. (1991)
	MI	Chow et al. (1983)
Nasal mites		
Sternostoma sialiphilus	LA	Pence (1973)
	MI	Hyland and Ford (1961)
Boydaia spatulata	LA	Pence (1973)
Fleas		
Ceratophyllus idius	ns[b]	Fox (1940)
C. niger	ns	Fox (1940)
C. diffinis	ns	Fox (1940)
	ns	Benton and Shatrau (1965)
C. gallunae	ns	Boyd (1951)

Table 5.2. Continued.

Arthropod	Host location	Reference
Lice		
Philopterus sialii	FL, NC, NH, NY, OH, SC, VA	Peters (1936)
	ns	Malcomson (1960)
	FL	Mertins and Dusek (1999)
	ns	Price et al. (2003)
	TN	Reeves et al. (2007)
Ricinus sp.	FL	Peters (1936)
Hippoboscid flies		
Ornithomyia anchineuria	NH	Peters (1936)
Myiasis flies		
Apaulina sp.	MI	Mason (1944)
		Kenaga (1961)
		Pinkowski (1977)
unidentified Calliphoridae	OH	Chow et al. (1983)
Protocalliphora sialia	ns	Rogers et al. (1991)
	NY	Roby et al. (1992)
	NY	Wittmann and Beason (1992)
	PA	Hannam (2006)
Phaenicia coeruleiviridis	FL	Mertins and Spalding (2001)
Philornis porteri	FL	Spalding et al. (2002)
Synthesiomyia nudiseta	FL	Spalding et al. (2002)

[a] = nymph, [b] ns = not specified

Table 5.3. Macroparasites recovered from an Eastern Bluebird population nesting on three grass-dominated agricultural sites in Georgia (2004 - 2006). Macroparasites were recovered by necropsy (N), fecal examination (F), live examination of adults (LEA), live examination of clutch (LEC), or examination of nest materials (NE). Prevalence (%) of macroparasites was determined for birds or nests examined, then sorted by study site (HA = hayfield, PA = pasture, LM = low management).

Macroparasite	Method	Prevalence (n[a])	Prevalence (n) by study site HA	PA	LM
Acanthocephalans					
Plagiorhyncus cylindraceus[b]	N	37.5 (16)	80.0 (5)	14.3 (7)	0 (4)
Cestodes					
Hymenolepis sp. [c]	N	6.2 (16)	0 (5)	0 (7)	25 (4)
	F	3.5 (86)	2.4 (41)	7.4 (27)	0 (18)
Nematodes					
Spiruroid nematode[b]	F	1.2 (86)	0 (41)	0 (27)	5.5 (18)
Stronglyloid nematode[b]	N	6.2 (16)	0 (5)	6.2 (7)	0 (4)
	F	8.1 (86)	2.4 (41)	0 (27)	11.1 (18)
Capillaria sp.	F	3.6 (86)	9.7 (41)	3.7 (27)	0 (18)
Trematodes					
Collyriclum faba[c]	LEA	3.8 (130)	1.7 (59)	2.1 (48)	13.0 (23)
Arthropods					
Ixodes brunneus	N	5.9 (17)	0 (5)	12.5 (8)	0 (4)
	LEA	0.69 (130)	0 (59)	0 (48)	4.3 (23)
Philopterus sialii	N	28.5 (14)	0 (3)	42.8 (7)	25.0 (4)
	LEA	0.7 (130)	0 (59)	2.1 (48)	0 (23)
Dermanyssus prognephilus	NE	43.7 (103)	53.2 (47)	38.7 (31)	32.0 (25)
Carnus floridensis[b]	LEC	0.75 (133)	2.1 (47)	0 (31)	0 (25)

[a]n = number of birds or nests examined
[b] denotes new host record
[c]presumptive identification

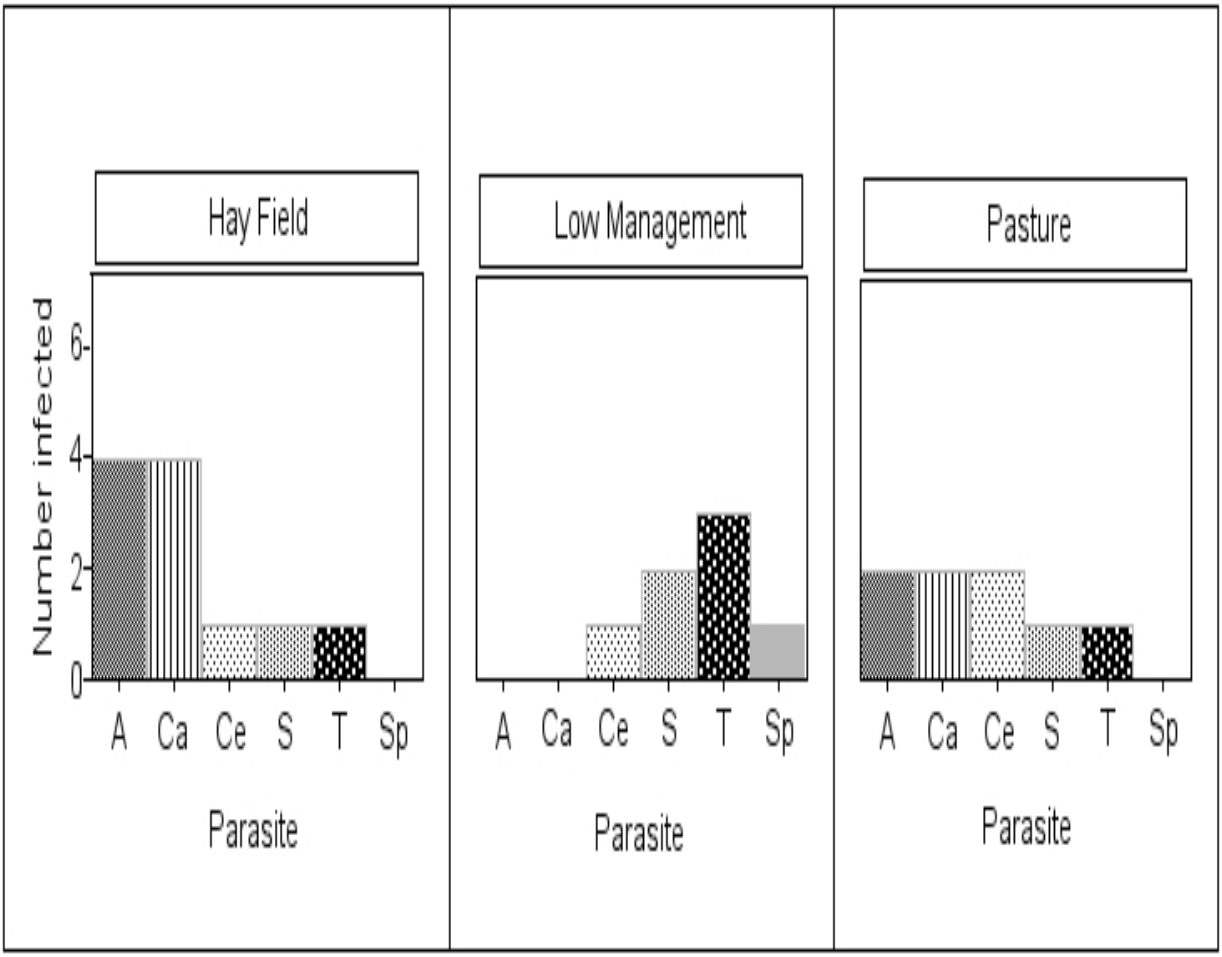

Figure 5.1. Number of adult Eastern Bluebirds (*n* = 144) nesting on three grass-dominated agricultural sites in Georgia that were infected with gastrointestinal or subcutaneous helminths. Parasites were detected by live examination (144), fecal examination (86), or necropsy (16) (2004-2006). A = acanthocephalan, Ca = capillaria sp., Ce = cestode, S = strongyloid nematode, T = trematode, Sp = spiruroid nematode.

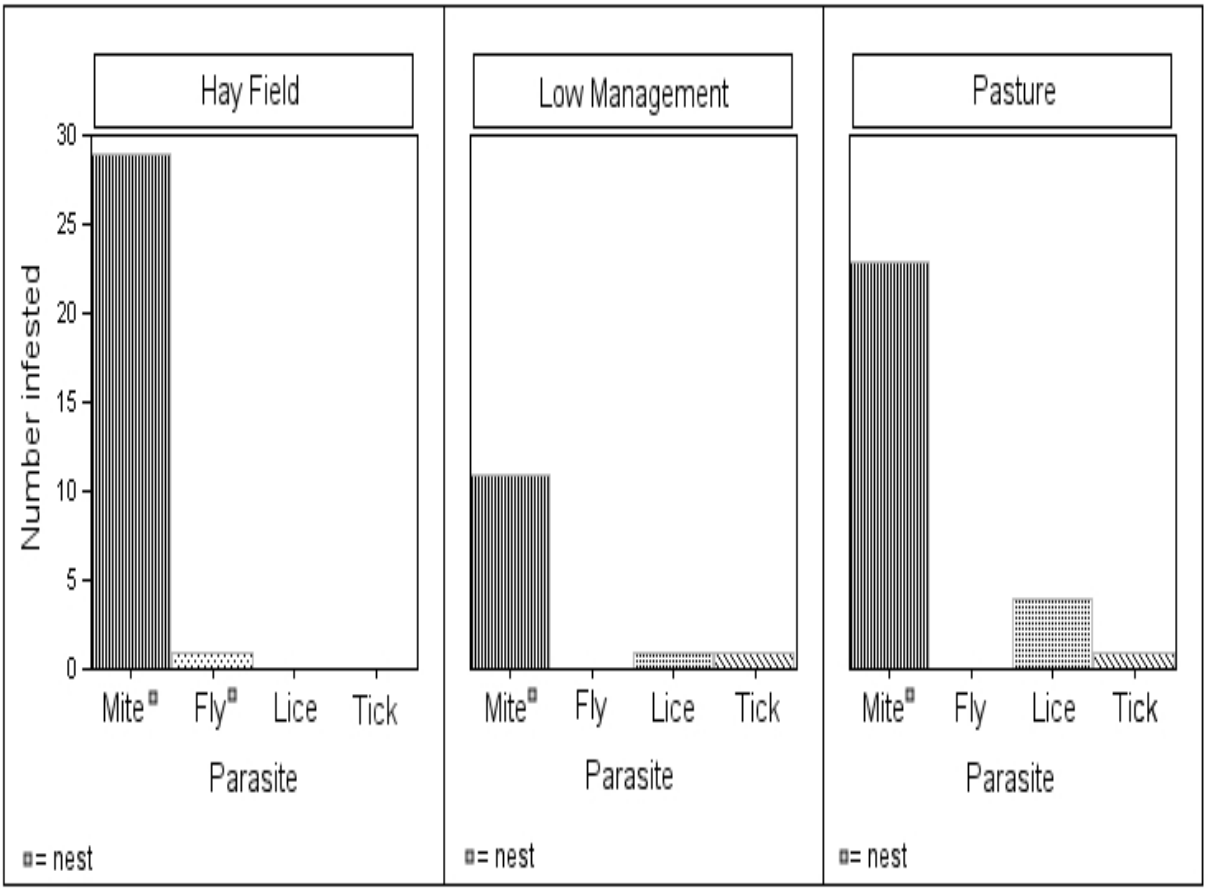

Figure 5.2. Number of adult Eastern Bluebirds (*n* = 144) or nests (*n* =103) found to be infested with parasitic arthropods; sorted by grass-dominated agricultural nesting sites. Parasites were collected by live examination (144) or necropsy (15) of adults and visual examination of nests that fledged young (2004-2006).

CHAPTER 6

EFFECT OF HEMATOPHAGOUS NEST MITES ON GROWTH AND HEMATOLOGICAL PARAMETERS OF EASTERN BLUEBIRD (*SIALIA SIALIS*) NESTLINGS[5]

[5] Carleton, R. E. Submitted to *The Wilson Journal of Ornithology* 10/15/2007

ABSTRACT

Many studies suggest there is an association between nest-dwelling ectoparasites and reduced reproductive success in many avian species. In contrast, other studies do not support this conclusion and further find no negative impact on certain growth and hematological parameters of parasitized nestlings. I manipulated populations of the hematophagous mite, *Dermanyssus prognephilus*, within Eastern Bluebird nests using a pyrethrin-based insecticide to examine effects on nestling growth, selected hematological parameters, and fledging success. Results demonstrated no difference in growth, fledging success, or estimated white blood cell counts and packed cell volumes of infested and non-infested nestlings, however infested nestlings had lower hemoglobin and increased percentages of immature erythrocytes. These findings are suggestive of a physiological response to blood loss which may effect post-fledging survival.

INTRODUCTION

Nestling birds are subject to a large number of ectoparasites, including hematophagous mites and ticks (Acari) (Møller 1990, 1991; Burtt et al. 1991; Richner et al. 1993), fleas (Siphonaptera) (Richner et al. 1993; Brown et al. 1995), blowfly larvae (Diptera: Calliphoridae) (Pinkowski 1977), and bugs (Hemiptera) (Loye and Carroll 1991; Brown et al. 1995). Nest infestations are associated with reduced reproductive success in Barn Swallows (*Hirundo rustica*) (Møller 1990, 1991), Cliff Swallows (*Hirundo pyrrhonata*) (Chapman and George 1991, Loye and Carroll 1991), Great Tits (*Parus major*) (Richner et al. 1993), Sand Martins (*Riparia riparia*) (Santos Alves 1997), and House Martins (*Delichon urbica*) (Lope et al. 1993). These findings, along with the

work of Anderson and May (1978) and Hudson and Dobson (1991), provide strong evidence of parasite-mediated population regulation through reduced host fecundity.

Direct effects of ectoparasites on nestlings have been studied frequently but often with contradictory conclusions (Møller 1990, 1991; Wittman and Beason 1992; Johnson and Albrecht 1993; Richner et al. 1993; Tompkins et al. 1996; Mataxas and Pung 1999; Weddle 2000; O'Brien et al. 2001). Some studies concluded that nest infestations were associated with greater nestling mortality or smaller size and weight at fledging (Møller 1990; Richner et al. 1993; Weddle 2000). In contrast, others attributed no negative effects to nest ectoparasites (Wittman and Beason 1992; Tompkins et al. 1996; Mataxas and Pung 1999).

Many studies of effects of ectoparasites on nestling and fledgling survival focused on growth parameters (i.e., tarsus length and weight), but some examined hematological effects such as hematocrit (packed cell volume) and hemoglobin levels (Powleslard 1977; Chapman and George 1991; Whitworth and Bennett 1992; Johnson and Albrecht 1993; O'Brien et al. 2001; Hannam 2006). For example, in a study comparing blood hemoglobin levels of House Wren (*Troglodytes aedon*) nestlings, parasitized nestlings had significantly lower hemoglobin levels. The investigators hypothesized such parasite-mediated effects may have significant impacts on post-fledge survival by reducing flight ability due to reduced oxygen availability. Increased predation risk and decreased foraging efficiency are theoretical consequences of decreased hemoglobin resulting from parasitism (O'Brien et al. 2001). Eastern Bluebird (*Sialia sialis*) nestlings parasitized by blow files were found to have lower

hematocrit and hemoglobin levels compared to non-parasitized nestlings (Hannam 2006).

Eastern Bluebird nests are frequently infested with one or more species of ectoparasites. Blood-feeding mites belonging to the genus *Dermanyssus* (*D. hirundinis, D. prognephilus)* are common inhabitants of bluebird nests (Burtt et al. 1991; Mataxas and Pung 1999). Likewise, larvae of *Protocalliphora* blowflies are occasionally found in nesting materials or attached to nestlings (Pinkowski 1977; Roby et al. 1992; Wittman and Beason 1992; Mataxas and Pung 1999). While parasitism by *Protocalliphora* larvae has not been associated with nestling mortality (Pinkowski 1977; Mataxas and Pung 1999), larvae of *Philornis porteri* were the suspected cause of death of 59 Eastern Bluebird nestlings in Florida (Spalding et al. 2002).

In many bluebird breeding areas, wooden nest boxes are promoted as replacements for naturally-occurring cavities (Zeleny 1977). A study in Michigan found no difference in blow fly parasitism of eastern bluebird nestlings raised in wooden nest boxes or naturally occurring cavities (Pinkowski 1977). Improper management of nest boxes, such as leaving old nesting material in place, often results in tremendously large parasitic mite populations (Burtt et al. 1991). Such practices could significantly reduce nestling survival in populations dependent on artificial nesting sites if parasitism causes a negative impact to growth and condition. This study investigates effects of a commonly occurring hematophagous nest mite (*Dermanyssus prognephilus*) on hematological and physical parameters of Eastern Bluebird nestlings by manipulation of mite populations within artificial nesting sites (wooden nest boxes). I expected

hematological and physical parameter values would differ in nestlings from miticide-treated nest boxes compared to nestlings from non-treated nest boxes.

METHODS

Study Area. The study took place on a land tract located in the Ridge and Valley physiographic province of northwestern Georgia, USA (34^{o} 28' N, 85^{o} 19' W) during the 2006 breeding season (March through August). Study sites consisted of two 40-ha sites providing suitable bluebird habitat; open areas of grassy vegetation with perching sites used to locate prey and structures providing nesting opportunities (nest boxes).

Nestling Manipulation. Nest boxes used in the study were constructed from unpainted white pine (*Pinus strobus*) lumber. All boxes had been in place since 2002 and contained bluebird nests in previous breeding seasons. Because nest mites were regularly seen on nestlings and within recently fledged nests, I removed old nestling material each time a nest box was vacated to prevent large accumulations of mites. Fifteen nest boxes within each site were monitored for nesting activity beginning in early March 2006. To ensure consistent conditions of a minimal mite infestation, only boxes successfully fledging one brood were designated as candidate boxes for the study. Old nesting material was removed following fledging of the first brood. Candidate boxes were then monitored for second clutches. Boxes with clutches consisting of at least three eggs were randomly assigned to either a control (no miticide application) or treatment (miticide application) group.

Two days before the estimated hatch date, approximately 28 gm of Johnson's Rid Mite Powder For Birds (Johnson's Veterinary Supply, UK), a pyrethrin-based miticide, was applied to treatment group nests after temporarily removing the eggs.

Dust was reapplied to nests, and nestlings were lightly dusted with approximately 5 gm of powder on day 3, 6, and 10 post-hatching. Nests and nestlings assigned to the control group were similarly manipulated except for application of powder. The miticide powder likely prevented infestation of treated boxes with fly larvae, lice, or winged parasitic arthropods in addition to mites.

On day 12 post-hatching, three nestlings from candidate boxes were chosen at random for measurement. Because the number of nestlings per nest box varied, brood size for each was recorded. Individually numbered United States Geological Survey aluminum leg bands were placed on nestlings for identification. Body mass and right tarsus length were measured, and a 20-µl blood sample was obtained by jugular venipuncture. A small quantity of the sample was used to make a blood smear and fill one hematocrit tube. The remainder was placed into an anticoagulant-containing vial for hemoglobin measurement and an estimated white blood cell count. Nestlings were examined for the presence or absence of *D. prognephilus* or other species of hematophagous mites and other ectoparasites, such as lice or blow fly larvae, and returned to the nest box following measurement and sample collection. Boxes were monitored for an additional 10 days to document successful fledging. All nesting material was removed from vacated boxes and visually examined for presence or absence of mites.

Hematological Parameters. I measured hemoglobin indirectly by colorimetric determination using a QuantiChrom Hemoglobin Assay Kit (BioAssay Systems, Inc. Hayward, CA). The assay was performed on 10 µl of whole blood diluted 100-fold with distilled water (F. Huang, BioAssay Systems, Inc., pers. comm.). Absorbance at 400

nm of the hemoglobin standard supplied with the kit and the diluted sample was measured with a Spectronic 20D+ spectrophotometer (Thermo Electron Corp. Waltham, MA). Hemoglobin absorbance of samples from individual nest boxes was standardized by dividing the absorbance of the sample by the absorbance of the standard.

Packed cell volume (% packed red blood cells per blood volume) (PCV) was measured using centrifuged whole blood in standard 50-µl capillary tubes and a Critocaps micro-hematocrit capillary tube reader (Oxford Labware, St. Louis, MO).

Estimated white blood cells count (10^6 WBC/µl) and differential were performed using the Unopette 5877 system (Becton-Dickinson, Rutherford, NJ), Neubauer hemacytometer, and the stained blood smear. The estimated white blood cell count was calculated following a standard procedure (Redig, 1997).

Percentage of polychromatic cells or number of immature erythrocytes per 100 erythrocytes counted was determined using the stained blood smear examined at 1000x.

Statistical analyses. Hematological and physical parameter values of the three nestlings measured per nest box were used to calculate a mean value for each nest box. Mean nest box value was used as the unit of analysis to avoid pseudoreplication and negate individual differences among nestlings due to competition. Condition was calculated by dividing mean body mass by mean tarsus length. A Shapiro-Wilk W Test was performed to assess normality on all units before analyses. All data were normally distributed except for percentage polychromasia which was square-root transformed. Group (control and treatment), site, and brood size (three, four, or five nestlings) were included as main effects in the original model; however, site and brood size were highly

correlated. Because site contributed relatively less to the r^2 value than brood size, it was deleted from the final model. Results were analyzed using an A x B factorial ANOVA (*a-priori* α = 0.05). All analyses were performed using JMP IN 5.1 Statistical Software (SAS Institute Inc., Cary, NC).

RESULTS

Sample Size. Of 30 nest boxes monitored, 18 contained broods of three to five nestlings which survived predation for the duration of the study. Eight boxes were assigned to the treatment group and 10 were assigned to the control group.

Physical Parameters. Neither group differed in body mass or tarsus length (body mass: $F_{1,18}$ = 0.22, P = 0.64; tarsus length: $F_{1,18}$ = 0.78, P = 0.39) (Table 6.1). Condition of treated nestlings was not different from that of non-treated nestlings ($F_{1,18}$ = 0.01, P = 0.89). Neither brood size or an interaction between group and brood size produced significant effects for any of the physical parameters.

Hematological Parameters. There was no difference in main effects or interactions for estimated white blood cell count or packed cell volume between nest boxes treated for mites and non-treated nest boxes (WBC: $F_{1,18}$ = 0.05, P = 0.82; PCV: $F_{1,18}$ = 0.89, P = 0.36) (Table 6.2). The analysis revealed a significant main effect for group (treatment vs. control) for both hemoglobin absorbance (Fig. 6.1) and percentage of polychromasia (Fig. 6.2) (Hemoglobin: $F_{1,18}$ = 10.64, P = 0.006; Percentage of polychromasia: $F_{1,18}$ = 6.23, P = 0.028) (Table 6.2). There were no effects of brood size and no additive effects.

Fledging Success. All nestlings (n = 69) monitored in this study fledged successfully. Mites (*D. prognephilus*) were recovered from all control group nests. Nests treated with

the miticide powder were free of mites and no other species of ectoparasites were recovered from control or treatment group nests.

DISCUSSION

The results of this study demonstrated a direct effect of pyrethrin application and blood loss on Eastern Bluebird nestlings. The increased numbers of immature erthrocytes in non-treated nestlings, as measured by percentage of polychromatic cells, was representative of a physiologic response to blood loss (Campbell 1995). Because birds naturally possess a great capacity for erythropoesis, parasitized nestlings may be expected to maintain a normal erythrocyte volume (Campbell 1995). This likely explains the lack of difference in control and treatment group packed cell volumes, but a significant difference in percentage of polychromasia. Also indicative of blood loss in the non-treated nestlings is the lower hemoglobin level. A study examining the effect of blowfly larvae on nestling House Wrens found similar reductions in hemoglobin levels (O'Brien et al. 2001).

Body mass, tarsus length, and condition were not affected by parasitism in this study, suggesting that either blood loss does not negatively impact growth or parasitized nestlings are able to sufficiently compensate physiologically. Estimated white blood cell counts were likewise not affected by parasitism which suggested no increase in immune response was elicted.

The lack of difference in fledging success of parasitized nestlings compared to non-parasitized nestling, suggests that hematophagous mites have no direct effect on nestling mortality. Survival following fledging was not examined, however. As suggested in a similar study, post-fledging survival of parasitized nestlings may be

negatively affected by decreased flight capability resulting from reduced hemoglobin levels (O'Brien et al. 2001). Fledgling bluebirds fly to the tops of trees upon leaving the nest and remain near or within cover for approximately seven days (Gowaty and Plissner 1998). Fledglings with decreased flight capacity may be unable to reach cover or avoid predation until hemoglobin level normalizes with erythrocyte maturation.

Additional studies focusing on post-fledging survival of parasitized and non-parasitized nestlings would add greatly to our understanding of nest parasite-mediated effects on this species. Because pyrethrin-based pesticides are acceptably safe for use on birds (Theodorides 1990), treatment of vacated nest boxes following removal of old nesting material is recommended for the eradication of residual mite populations and reduce negative effects on future broods.

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Table 6.1. Mean (± standard deviation) body mass, right tarsus length, and condition of 12-day-old Eastern bluebird nestlings raised in non-treated (control) vs. pyrethrin-treated (treatment) nest boxes in Georgia (2006).

Parameter	Control (n = 10)	Treatment (n = 8)
Body mass (g)	24.84 ± 2.03	25.66 ± 2.68
Tarsus Length (mm)	23.08 ± 0.93	23.47 ± 0.69
Condition (g/mm)	1.07 ± 0.08	1.09 ± 0.09

Table 6.2. Mean (± standard deviation) packed cell volume, estimated white blood cell count, hemoglobin absorbance, and percentage of polychromatic or immature erythrocytes of 12-day-old Eastern Bluebird nestlings raised in non-treated (control) vs. pyrethrin-treated (treatment) nest boxes in Georgia (2006).

Parameter	Control (n = 10)	Treatment (n = 8)
Packed cell volume (%)	43.9 ± 3.17	42.3 ± 3.39
Estimated WBC (10^6 WBC/µl)	86.9 ± 29.37	78.1 ± 26.38
Hemoglobin absorbance (nm)[a]	1.2 ± 0.28	1.5 ± 0.24
Percentage polychromasia (%)[a]	39.0 ± 0.06	31.0 ± 0.53

[a] = significantly different

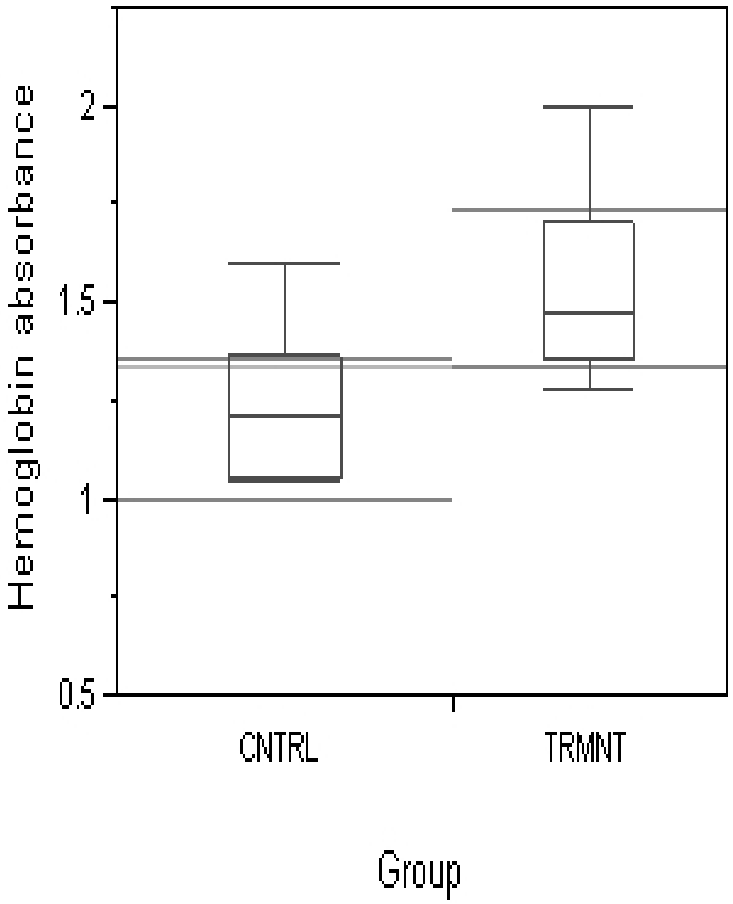

Figure 6.1. Box plots and 95% confidence interval bars showing differences in mean hemoglobin absorbance (nm) of 12-day-old Eastern Bluebird nestlings raised in pyrethrin-treated (TRMNT) and non-treated (CNTRL) nest boxes in Georgia (2006). Grand mean bars shown for both groups.

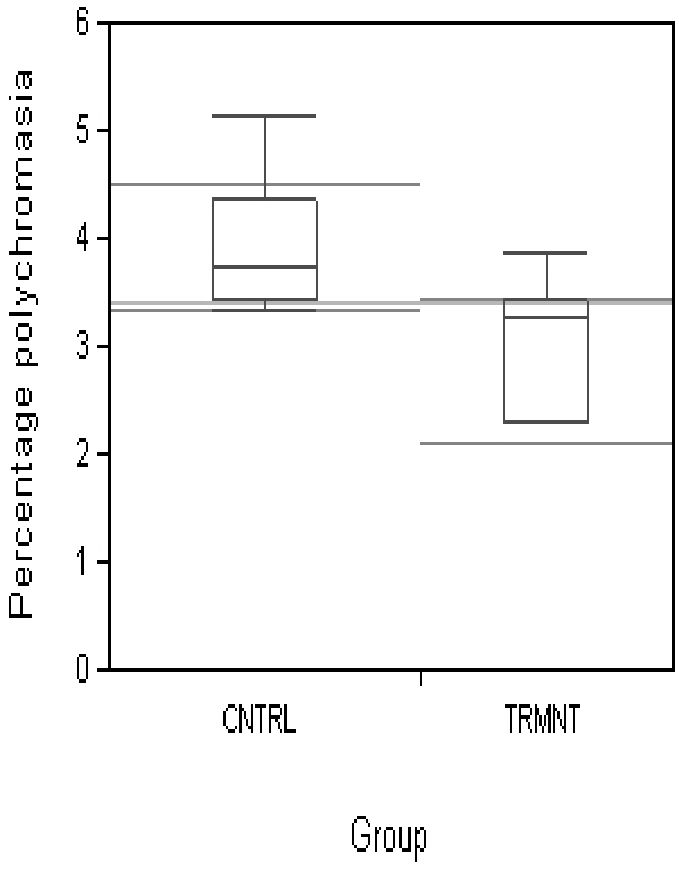

Figure 6.2. Box plots and 95% confidence interval bars showing mean percentage of polychromatic (immature) erythroctyes (after square root transformation) of 12-day-old Eastern Bluebird nestlings raised in pyrethrin-treated (TRMNT) and non-treated (CNTRL) nest boxes in Georgia (2006). Grand mean bar shown for both groups.

CHAPTER 7

SUMMARY

CHAPTER SUMMARIES

Chapter 1. Introduction and Literature Review. Eastern Bluebird (*Sialia sialis*) populations fluctuated dramatically during most of the 20th century due to habitat loss, severe weather events, competition with introduced species, and pesticide use (Zeleny 1977; Sauer and Droege 1990). Predation, competition, nesting site availability, and severe weather events appear to be the major population regulators of Eastern Bluebirds (Sauer and Droege 1990; Gowaty and Plissner 1998). Information about infectious agents and diseases affecting bluebird population is limited and less is known relationships between habitat and bluebird parasites. The combined objectives of this study were to survey parasites associated with a population of Eastern Bluebirds inhabiting sites of differing agricultural use and plant diversity and explore relationships between bluebird health, reproduction, and survival among the three sites.

Chapter 2. Condition, Reproduction, and Survival of an Eastern Bluebird Population Nesting on Grassy Agricultural Habitats in Georgia. There was no indication that the study population was in poor health or experienced low levels of reproduction or survival. I found no difference in physical or hematological parameters of adults or nestlings among sites, although packed cell volume and total protein values were significantly higher for adults compared to nestlings. One of 14 birds tested serologically positive for West Nile Virus, however the bird did not appear to be negatively affected and sired young.

Survival estimates for adults were based on a model supportive of differences in survival over time, but not among sites. Nestling survival differed by site; nestling survival was greatest in the site used for cattle grazing.

Reproduction did not vary among sites although there were differences in the numbers of eggs produced, numbers of eggs hatching, and number of successful broods among years. All measures of reproduction were significantly lower in 2005. Reproductive success, as measured by the number of successful broods per number of eggs hatched, did not vary among years but differed among sites in 2005. A higher than normal amount of rainfall early in the breeding season may have contributed to the reductions. Based on these results, no site was deemed inappropriate for bluebird conservation.

Chapter 3. Protozoan Parasites of Eastern Bluebirds, Including Two New Host Records for Sarcocystis sp. and Atoxoplasma sp. Five species of protozoal parasites were detected among an Eastern Bluebird population by examination of blood smears, necropsy, and polymerase chain reaction (PCR) amplication using splenic tissue harvested during necropsy; *Atoxoplasma* sp., *Plasmodium relictum*, and *Haemoproteus fallisi* were detected by PCR, *Atoxoplasma Sarcocystis* sp. was detected by necropsy, and *H.* fallisi and *Trypanosoma avium* detected in blood smears. Detection of *Atoxoplasma* sp. and *Sarcocystis* sp. represented new host records for the parasites. Sample size of precluded statistical comparisons of PCR results among study sites. Based on combined data from both methods, two species were absent from at least one site; *Sarcocystis* sp. was only detected in the cattle pasture site and *T. avium* was only detected in the low management and hay field sites. Concurrent infections were detected in 10 (71.2%) birds by a combination of blood smear examination, necropsy, and PCR. More species were detected by necropsy and PCR than by blood smear examination.

Haemoproteus fallisi was commonly detected in blood smears. No difference in prevalence was detected among study sites. Intensity of infection was low and had no apparent effect on packed cell volume, total plasma protein, or percentage of polychromatic cells.

Chapter 4. Bacteria Isolated from a Population of Eastern Bluebirds (Sialia sialis) Nesting within Three Grass-dominated Agricultural Sites In Georgia. Forty-three species of bacteria were isolated from an Eastern bluebird population nesting within three grass-dominated agricultural sites in Georgia. Fewer bacterial species and fewer bacterial species unique to one site were detected in the site with the highest level of management and disturbance and lowest plant diversity; the Bermuda grass (*Cynodon dactylon*) hay field. Organic matter removed during hay harvest may have reduced soil nutrient content and suppressed bacterial community diversity (Carney and Matson 2005). Nearly equal numbers of bacterial species and numbers of bacterial species unique to one site were isolated from the low management and pasture sites. Shannon-Weiner indexes ranged from 2.82 for the HA site to 2.96 for the PA site and 3.25 for the LM site. In this study, 12 bacterial species were cultured only from oral cavities of bluebirds and six of those were potential human pathogens. This finding suggests studies that restrict sampling to the cloaca underestimate a host's bacterial community.

Nineteen of the bacteria detected in bluebirds were potential human pathogens, but no *Salmonella* spp. isolated. Based on these findings, immunocomprised persons should avoid contact or take precautions when cleaning used bluebird nest boxes to avoid exposure to potential pathogens (Friend et al. 2001).

None of the birds sampled showed signs or symptoms of disease, however an opportunistic infection could result from endoparasitism (Thompson-Cowley and Helfer 1979), injury, or physiological stress associated with severe weather events or intra- or interspecific competition (Smith et al. 2002). Management practices should include measures to reduce stress, including sufficient nest box density to reduce competition and roosting shelters for use during extremely cold weather.

Chapter 5. Macroparasites of Eastern Bluebirds: A Review and Survey of a Population Nesting Within Grass-dominated Agricultural Habitats in Georgia. A population of Eastern Bluebirds (*Sialia sialis*) nesting within three sites experiencing different agricultural use and levels of plant diversity was surveyed for macroparasite presence. Methods of detection included live examination, examination of fecal material, and necropsy. Ten species of macroparasites were recovered from the study population; detection of five species represents new host records for *Carnus floridensis*, *Plagiorhyncus cylindraceus*, *Capillaria* sp., *Strongyloid* sp. and *Spiruroid* sp. A cestode (*Hymenolepsis* sp.), possibly one species of trematode (*Collyriclum faba*), one species of tick (*Ixodes brunneus)*, one species of chewing lice (*Philopterus sialii*), and one species of nest mite (*Dermanyssus prognephilus*) were also detected but were previously reported in the literature (1934-2007).

Macroparasite prevalence did not vary among sites, but some species were detected in only one or two of the sites. There appeared to be a strong association between *P. cylindraeceus, Capillaria* sp. and the site with the lowest plant diversity and most disturbance; other species and site associations were less evident. Although indirect effects of macroparasites on bluebird survival and reproduction could not be

established, it appeared advisable to locate nest boxes in more diverse habitats in order to avoid infection with acanthocephalans.

Chapter 6. Effect of hematophagous nest mites on growth and hematological parameters of Eastern Bluebird (Sialia sialis) nestlings. Nest mite populations within nest boxes used by Eastern Bluebirds were manipulated by application of a pyrethrin-based insecticide to nests and nestlings within designated treatment boxes. I made comparisons of mean physical measurements and hematological parameters of nestlings by box and sorted by main effect of group (treated vs. control). There was no difference in physical measurements of the two groups. Packed cell volume and total plasma protein did not differ among groups, but hemoglobin measured by absorbance and percentage of polychromatic cells differed significantly. Lower hemoglobin and higher percentage of polychromatic cells in the control group was highly suggestive of physiological compensation to blood loss (Campbell 1995). All nestlings fledged successfully, however survival after fledging may have been reduced in non-treated birds because of lower hemoglobin levels as suggested by other researchers (O'Brien et al. 2001).

FUTURE DIRECTIONS

This study resulted in an increase in information regarding the range of infectious agents affecting Eastern Bluebirds. Because of the small scale of the study area, replication at the habitat level was not possible and comparisons of parasite prevalence and bluebird condition, reproduction, and survival among sites were limited in value. These comparisons however indicated that differences among sites may exist and further investigation with replication at the habitat level is warranted. A related future

study may investigate the possible relationship between a higher prevalence of *Plagiorhycus cylindraeus*, its intermediate host, and hay field habitats.

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