8-2022

Reproductive Ecology and Microbial Communities From Wood Duck Nest Boxes in Georgia and Florida

Jake Shurba
jshurba@g.clemson.edu

REPRODUCTIVE ECOLOGY AND MICROBIAL COMMUNITIES FROM WOOD DUCK NEST BOXES IN GEORGIA AND FLORIDA

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

---

by
Jacob Anthony Shurba
August 2022

Accepted by:
Dr. Kyle Barrett, Committee Chair
Dr. Greg Yarrow
Dr. Kristi Whitehead

ABSTRACT

Wood ducks (*Aix sponsa*) are an important game species throughout the Atlantic Flyway that was nearly extirpated by the early 20th century due to overhunting and the loss of habitat. Wood ducks are secondary cavity-nesters that utilize artificial nest boxes and natural cavities. It is reported that the use of nest boxes is likely what led to re-establishment of the species in North America. Where boxes are numerous, overuse of boxes by multiple hens throughout a nesting season can occur and result in a buildup of bacteria, parasites and other potentially detrimental pathogens that can impact egg hatchability. No large-scale regional study of reproductive biology of box-nesting wood ducks has been conducted across multiple states. I performed a study examining the reproductive ecology of wood ducks and bacterial growth from nest boxes in Georgia and Florida between 2020 and 2021. My objectives were to 1) estimate the percent use of nest boxes by wood ducks, estimate percent nest success, and calculate an average number of ducklings that departed nest boxes within Georgia and Florida, 2) to calculate a cost per female recruit from nest boxes between Florida and Georgia, 3) determine if the use of different types of shavings have any effect on nest box use, nest success, and number of ducklings successfully exiting boxes, 4) determine if the use of different types of shavings has an impact on the growth of nest-box microbes, and 5) determine if there are preventative measures managers may use to keep microbes from negatively affecting eggs. I monitored 142 nest boxes in Florida and 123 nest boxes in Georgia in 2020 and 138 and 120 nest boxes in Florida and Georgia, respectively, during the 2021 field season. In Florida, 90.3% of nest boxes and in Georgia 60.5% of nest boxes were used by wood ducks across both years. Nest success across both years and states was 40.9% (n = 644 nests), and the primary cause of nest failure was abandonment for unknown reasons (37.6%). Nearly twice as many ducklings successfully exited nest boxes in Georgia than Florida with averages of 11.46 and 6.86 ducklings exiting boxes from Georgia and Florida, respectively ($P < 0.001$). I conducted a cost analysis of the use of nest boxes per female recruit in these states and calculated 0.08 and 0.10 yearling female recruits/box/year in Florida and Georgia. The calculated cost per yearling female wood duck recruit over 20 years was $108.35 in Florida and $86.68 in Georgia. The cost per recruit for Georgia was about half the cost for the box materials and annual maintenance for 20 years. More data is necessary to conclude if nest-box programs are cost effective in Georgia and Florida. I found that the type of shavings had no impact on box selection, nest success or ducklings successfully exiting nest boxes. Additionally, the use of different types of shavings had no impact on the growth of microbes collected from nest boxes. While there are no explicit recommendations for what type of shavings to use in nest boxes, it is recommended that managers regularly clean and provide maintenance to their nest boxes to avoid buildups of pathogens throughout the breeding season.

ACKNOWLEDGMENTS

The process of designing, executing, and writing a master's thesis is by far one of the most exciting, and yet, exhausting things that I have done as a professional. Without a doubt, it wouldn't have been finished without the help and support from many people. I want to first thank my colleague, mentor, and dear friend Dr. Shelli Dubay. From the first time we connected at UW-Stevens Point over "diseases and dead things," I knew this was a friendship that was going to last. You took a chance on me, a transfer student with bad grades, no research experience, and no clue what I was going to do after graduation because it all felt very out of reach. You showed me what research was like, helped me realize I loved it, and made getting a master's and a Ph.D. seem like something that was well within my grasp and ability to do. I will be forever grateful to you my friend, you gave me opportunities to grow as a scientist that have shaped my entire career, especially now as I move into a PhD studying disease ecology.

The Nemours Wildlife Foundation in Yemassee, SC is one of the primary funders of my research so in that regard, I want to thank Dr. Ernie Wiggers for his continued support of projects from Clemson and his effort to grow the partnership between the two entities. I also want to thank Mr. Beau Bauer from Nemours as well. We connected very quickly when I started working for you in 2019 and you rapidly became a mentor for me as a budding biologist, as well as a good friend. I will always cherish every bird walk, scientific discussion, and learning opportunity you gave me as I continue through my career.

My many field technicians put in endless hours of work to ensure that I had the best possible data to work with for my analysis, and so to Hannah Schley, Joseph Kizior, Dylan Sanborn, Chuck Farrell, Sam Sauber, Abby Weber, Hannah Caster, Hannah Imhoff, Rebeca Bravo, and Carter Freymiller, please know that I am forever grateful. This project has one of the most difficult field seasons I've ever worked, and you all did it with a smile and a fantastic attitude. Every year, you all set the bar for what to expect from technicians. I was so lucky to have a fantastic staff every year of my field season. Thank you as well to the South Carolina Dept. of Natural Resources for providing funding for my assistantship, the Georgia Dept. of Natural Resources and the Florida Fish and Wildlife Conservation Commission for providing equipment, boats, and biologists' time when we needed it.

Without my friends, none of this would have been possible. I am very lucky to have a group of friends who have either gone through graduate school or are currently going through it, and together we were able to support each other. Thank you to my best friends Dan Squires and Hannah Schley for being a listening ear, for proof-reading endless drafts, and for never getting tired of hearing me talk about ducks; I love you both. Katherine Rexroad and Janelle Taylor, I've had the chance to see you both battle your way through graduate school and the challenges that go with it, whether that be a difficult advisor or trouble with collaborators, but I have also had the pleasure of seeing both of you defend your respective theses and graduate. You're both such badasses, absolutely amazing scientists, and, most importantly, an example to other women in the field of natural resources and I'm so glad I get to call you my friends. Hannah Imhoff and Carter

Freymiller started off as field technicians for me and left as dear, dear friends. I will always be grateful for your friendship, our tiktokking adventures, and our fabulous little trailer in middle of nowhere Florida.

Finally, and most importantly, my family. Mom and Dad, I hope that you realize that you kicking me outside to play when I was a child was instrumental in getting me to where I am today. You helped garner that love and appreciation for nature. Thank you always for your love and support, even though I'm still not totally convinced you actually know what it is I do, you still are there to listen and help whenever I need it. I love you so much.

TABLE OF CONTENTS

LIST OF TABLES

List of Tables (Continued)

Table Page

LIST OF FIGURES

CHAPTER ONE

REPRODUCTION AND MANAGEMENT OF BOX-NESTING WOOD DUCKS IN GEORGIA AND FLORIDA

Wood ducks (*Aix sponsa*) are the only Nearctic *Aix* species of waterfowl (Anatidae) and an important game duck in North America (Bellrose and Holm 1994, Otis and Dukes 1995, Grado et al. 2011). In the early 20th century, it was noted by biologists that wood duck populations appeared to be declining (Grinnel 1901, Bellrose 1976). Market hunting and the loss of forested uplands and wetlands were detrimental as this is the habitat where wood ducks reside (Bellrose and Holm 1994). Forest loss was significantly detrimental as the wood duck is a secondary cavity-nesting species.

It is well documented that wood ducks will readily nest in artificial structures (hereafter nest boxes) across its range, especially in the absence of natural cavities (Lowney and Hill 1989, Bellrose 1990, Bellrose and Holm 1994, Croft et al. 2020). Artificial nest boxes have helped wood ducks recover in North America, especially in areas where natural cavities were limited (Lowney and Hill 1989, Fredrickson et al. 1990, Bellrose and Holm 1994, Stephens et al. 1998, Davis et al. 1999, Davis et al. 2007). Bellrose (1990) reported the number of nest boxes found in both the Atlantic and Mississippi Flyways in addition to their use rates and concluded, in addition to work by Hawkins and Bellrose (1940), that boxes were a valuable tool for the species' population growth (Table 1.1). Multiple states have reported numbers of boxes that have been deployed in addition to the success rate and the total number of ducklings that successfully exited nest boxes which showed increases in wood duck populations (Table 1.2). Wood ducks are philopatric, given that 79% of females returned annually to their natal locale for nesting, and 42% returned to the same box where they previously nested (Hepp et al. 1989, Hepp and Kennamer 1992). Given the efficacy of boxes for wood duck production, many state and federal agencies and private landowners have active nest-box programs for wood ducks in North America (Prevost et al. 1990, Heusmann 2000, Hepp et al. 2020, Croft et al. 2022).

Waterfowl population dynamics are influenced by recruitment (Cowardin and Blohm 1992, Davis et al. 2007). Recruitment refers to how young are added to the fall population through reproduction by adults in the spring population (Cowardin and Blohm 1992). Breeding season recruitment is the rate at which hatch- and after-hatch year females survive fall-winter and enter the spring breeding population (Hepp et al. 1989). The use of nest boxes can promote both fall and spring rates of recruitment (Bolen 1967*a*,*b*; Bellrose and Holm 1994). In states like South Carolina and New York where nest boxes were established, wood duck populations increased following the placement of these boxes as evidenced by breeding bird survey data (Nichols and Johnson 1990, Sauer and Droege 1990, Hepp et al. 2020). Multiple studies have documented this population increase, including 118 nest boxes in Missouri having an increase from 47 nests to 181 between 1966 and 1974 (Clawson et al. 1979), and 253 nest boxes increasing from 35 to 231 nests between 1966 and 1969 in Mississippi (Strange et al. 1971).

In 2018, about 30 waterfowl and wildlife professionals from state, federal, non-governmental organizations and universities assembled in a workshop at Nemours Wildlife Foundation in Yemassee, South Carolina (https://nemourswildlifefoundation.org/) to address priority research needs for waterfowl and wetland management (Schmidt et al. 2018, Wiggers et al. 2019). Despite voluminous research on wood ducks, participants concluded there remained a need to evaluate wood duck box-management programs across the southeastern United States, primarily to estimate the recruitment rate of females produced in nest boxes at a regional scale like seminal research conducted in South Carolina (Hepp et al. 1989, 2020). Like Hepp et al. (1989, 2020), I define recruitment as the proportion of female wood ducks, marked as day-old ducklings, that return as yearlings or older individuals to nest in boxes in their natal locale. No large-scale, regional study of reproductive biology of box-nesting wood ducks has been conducted across multiple states simultaneously in the United States or elsewhere in North America. This study was initiated to address wood duck breeding population ecology, especially recruitment by box-nesting females in eight states in the Atlantic and Mississippi flyways (Maryland, Delaware, North Carolina, South Carolina, Georgia, Florida, Mississippi, and Louisiana). The primary goal from the regional study was to determine if these populations were sustainable by native recruits returning to nest in boxes in their local natal area (Hepp et al. 1989, 2020; Wiggers et al. 2019; Bauer et al. 2021). With my collaborators, I designed and conducted a survey of 258 nest boxes distributed across four wildlife management areas (WMAs) in Florida and Georgia.

My objectives for this chapter were to 1) estimate percent use of nest boxes by wood ducks, estimate percent nest success, and calculate an average number of ducklings that departed nest boxes within and between Florida and Georgia, and 2) calculate a cost of maintaining a nest box per female recruit (Croft et al. 2022) in Florida and Georgia using collected wood duck reproductive data and a previously published estimate of recruitment for wood ducks in South Carolina (Hepp et al. 1989, 2020). I conclude by comparing the results from this research with similar studies in the partnering states and comparable published studies elsewhere.

STUDY AREA

My study area was in the Panhandle of western Florida (Fig. 1) and central Georgia (Fig. 2). In Florida, the study site was the Apalachee Wildlife Management Area (WMA; Sneads, Florida; 30.710599, -84.919843) and is managed jointly by the Florida Fish and Wildlife Conservation Commission (FWC) and the U. S. Army Corps of Engineers. The Georgia locations were the Redlands WMA (Greensboro, Georgia; 33.680115, -83.273044), B. F. Grant WMA (Eatonton, Georgia; 33.392210, -83.491500), and Cedar Creek WMA (Eatonton, Georgia; 33.229503, -83.523710), all of which were managed by the Georgia Department of Natural Resources (GA DNR) and the United States Forest Service. I did not randomly select study sites but chose sites with active nest box programs located in representative wood duck habitat.

Nest boxes were distributed among 15 emergent fresh marshes and ponds, and one lake in Florida. All boxes are located over water with 0.0–35.0% canopy cover and little to no vegetative cover surrounding the box. In Georgia, boxes were located on a number of managed wetland impoundments, two small ponds, one lake, and bottomland hardwood wetlands. Bottomland hardwood wetlands are influenced by the Oconoee River which dictate how much water is standing in them. Throughout periods of the summer, it is not uncommon for boxes in these locations to be over dry ground instead of water. Dominant flora in Florida and Georgia included duckweed (*Lemna minor*), cattail (*Typha* spp.), giant cutgrass (*Zizaniopsis milacea*), spikerush (*Eleocharis cellulosa*), alligator weed (*Alternathera philoxeroides*), horsetail (*Equisitum hyemale*), pickerelweed (*Pontederia cordata*), water lily (*Nymphea* spp.), bald cypress (*Taxodium distichum*), willows (*Salix* spp.), oaks (*Quercus* spp.), loblolly pine (*Pinus taeda)*, longleaf pine (*Pinus palustris*), and red maple (*Acer rubrum*).

I conducted my surveys during January through July 2020–2021. During the winter-summer period, long-term temperature and precipitation in Florida averaged 24°C (range = 15–3°C) and 78 mm (range = 47–127mm), respectively. During the winter-summer period in Georgia, long-term temperature and precipitation averaged 17°C (range = 10–24°C) and 72 mm (range = 63–81mm), respectively (U. S. Climate Data 2020*a*,*b*).

METHODS

Nest Boxes and Inspections

I began the 2020 field season with 142 nest boxes in Florida and 123 nest boxes in Georgia—all were erected at unknown times prior to this study. In May 2020, I installed four additional boxes at Redlands WMA to replace dilapidated boxes at this site. In January 2021 at all sites, I repaired structures, removed nest remains, and added wood shavings to all boxes to a depth of approximately 6–10 cm. In 2021 in Florida, I monitored 138 boxes and 120 boxes in Georgia. Boxes were monitored weekly. All boxes used in the study were wooden structures made of cypress or pine lumber described by Bellrose (1976) and were measured for internal volume (cm^3) prior to starting weekly box checks. In Florida, internal volume of boxes averaged 44,296 cm^3 (25.81 cm x 25.03 cm x 65.59 cm) with a maximum internal volume of 53,820 cm^3 and a minimum of 36,720 cm^3. All Florida boxes were erected on metal poles. Ninety-four boxes had a predator guard and 48 lacked one in 2020. In 2021, 94 boxes had predator guards and 44 lacked one after four boxes collapsed in a storm. Predator guards were metal cones with a diameter of approximately 91 cm. Georgia boxes averaged 37,923 cm^3 (24.59 cm x 27.26 cm x 57.13 cm) with a maximum internal volume of 54,270 cm^3 and a minimum of 22,344 cm^3. Georgia boxes were erected on wooden posts with 100 boxes protected by predator guards and 21 lacking one in 2020. In 2021, 100 were protected by predator guards, and 20 lacked one after a box had the floor collapse and was taken out of use.

During each inspection, I collected data on the following variables: 1) female duck species if present, 2) number of eggs by species, 3) number of live and dead ducklings, 4) number of egg-shell membranes to index ducklings that exited the box minus any dead ducklings remaining in a box (Davis et al. 1999), 5) number of unhatched eggs, and 6) number of depredated eggs and the predator if determinable (Bellrose and Holm 1994). Following termination of a nest and determination of its fate, I removed eggshell membranes, unhatched eggs, down, and wood shavings to promote subsequent nest box use and duckling production (Utsey and Hepp 1997, Davis et al. 2015). All boxes were randomly selected to receive either aspen or cedar shavings as part of an experimental design for a separate study (Chapter 2). Boxes were considered used when ≥1 egg was found during box checks and nest success was classified as ≥1 duckling successfully exiting the box. Dump nesting is a common practice seen in wood ducks and other secondary cavity-nesting species. Because of this, nests that were found with ≥13 eggs were classified as dump nests (Vritska 1995). Nest failure resulted from depredation, abandonment, or unknown causes of nest termination before hatching.

Statistical Analyses

Because mine was an inaugural study of box-nesting wood ducks in Florida and Georgia in association with six cooperating states in the Southeast region, I had no a priori predictions regarding reproductive performance by the wood ducks in my study. Thus, I tested the null hypothesis that nest box use, nest success and failures, and number of ducklings would not differ within or between years and between states (α = 0.05). To test these hypotheses, I first determined the frequencies of used (i.e., presence of ≥ 1 wood duck egg) and unused boxes and successful (≥ 1 egg hatched) and unsuccessful nests by wood ducks in both states and each year. I then tested if use of boxes and nest success were independent of states within years using chi-square tests (R Version 4.1.1, www.r-project.org, accessed 26 Nov 2021; R Core Team 2021*a*). I conducted tests within years, because I neither had endogenous data from breeding wood duck populations nor exogenous data to attempt explanation of any possible detected between-year differences in box use, nest success, and ducklings exiting nests during the study.

To account for Georgia's boxes being in three different WMAs, I performed a Kruskal-Wallis test (R Core Team 2021*b*; accessed 8 Jan 2022) to determine if the number of ducklings successfully exiting boxes between sites would preclude pooling data among Georgia WMAs for subsequent analyses of duckling data. I did not detect a difference among the three WMAs with the Kruskal-Wallis test (see Results); thus, I used a Mann-Whitney U-test to test if numbers of ducklings departing boxes differed between Florida and Georgia in 2020 and 2021 separately and combined (R Core Team 2021*c*; accessed 23 Nov 2021).

I performed a nest box cost-benefit analysis for wood ducks in each state by first determining the number of recruits per successful nest (% nest box use × % nest success × # of ducklings per successful nest × 50% females [1:1 assumed sex ratio, Hepp et al. 2020] × 6.8% yearling female wood duck recruitment rate per year [Hepp et al. 2020]), then I calculated the cost per yearling female wood duck recruit over the average nest box life of 20 years, accounting for costs of materials inflated to 2022 USA currency values (Barry 1992, Croft et al. 2022; $173.37 per box/[recruits per box per year × 20 years]). I used the published recruitment rate because no such data existed from Florida or Georgia. Additionally, previous related studies reported reproductive biology of box-nesting wood ducks and/or black-bellied whistling ducks (*Dendrocygna autumnalis*) (Bolen 1967*a*, *b*; Croft et al. 2020, 2022). However, there were no recruitment data from black-bellied whistling ducks, and I encountered only five nests of this species during my study in 2020 and 2021. Thus, my thesis addresses only wood ducks.

RESULTS

Nest-Box Use

I detected a difference in the frequencies of nest boxes used and not used by wood duck hens between Florida and Georgia in 2020 and 2021 ($P \leq 0.006$, Table 1.3). Use of boxes by hens in Florida was 91.5% and 89.1% in 2020 and 2021 (avg. 90.3%), respectively, compared to 61.8% and 59.1% in Georgia (avg. 60.5%) in the same years (Table 1.3).

Nest Success and Failures

I did not detect a difference in the frequencies of successful and unsuccessful nests between Florida and Georgia in 2020, 2021, or for both years combined ($P \geq 0.33$, Table 1.4). Nest success across years and states (which includes multiple nests per box) was 40.9% (n = 644 nests). Nest failures resulted from abandonment with no known cause (37.6%) and predation from woodpeckers, snakes, and other unknown predators (21.4% Table 1.5). Nests with ≥13 eggs were classified as dump nests as stated by Vritska (1995). In Georgia, 64.4% of successful nests were dump nests with a maximum clutch size being 49 eggs, 18 of which successfully hatched and exited the nest box. This is compared to Florida's rate of 32.4% of successful nests being dump nests with a maximum clutch size of 24 eggs, 15 of which successfully hatched and exited.

Ducklings

Number of ducklings exiting boxes among the three Georgia WMAs did not differ within years ($P \geq 0.13$). Nearly twice as many ducklings departed from successful nests in Georgia than Florida boxes in both years of the study ($P < 0.001$, Table 1.6). Overall, 11.46 and 6.86 ducklings/nest left boxes in Georgia and Florida, respectively.

Cost Analysis

The estimated cost of a nest box, plus its installation and maintenance over 20 years, was $173.37. I calculated 0.08 and 0.10 yearling female recruits/box/year in Florida and Georgia, respectively [i.e., Florida: 90.4% box use × 41.0% nest success × 6.86 ducklings per successful nest × 50% females (Hepp et al. 2020) × 6.8% yearling female wood duck recruitment rate per year (Hepp et al. 2020); Georgia: 60.5% box use × 41.2% nest success × 11.46 ducklings per successful nest × 50% females × 6.8% yearling female wood duck recruitment rate per year]. Thus, the calculated cost per yearling female wood duck recruit over 20 years was $108.35 in Florida and $86.68 in Georgia [$173.37 per box / (% recruit/box/year × 20 years = total recruits/box)]. Cost/recruit for Georgia was about half the cost for box and its annual maintenance for 20 years.

DISCUSSION

My estimate of the use of nest boxes by wood ducks in Georgia and Florida was about 60% and 90%, respectively. Bellrose and Holm (1994) reported a national box use rate of 42%, 40%, and 50% for the Atlantic, Mississippi, and Pacific flyways, respectively. They further explored box use rates in the Atlantic flyway, which averaged 36%, 42%, and 49% in the northern, central, and southern regions (Bellrose and Holm

1994). Georgia's estimated box use rate was lower than previously reported estimates by Utsey and Hepp (1997; 89%) and Croft et al. (2022; 61%) for wood ducks in coastal South Carolina. Compared to other states in our regional study, Florida had a greater box use rate, but South Carolina was greatest (97.8%, Table 1.7). South Carolina's greater box use rate can be attributed to the nest boxes being regularly maintained for three seasons (E. Miller, Clemson University, personal communication). This concept is also described by Utsey and Hepp (1997) where they report that regular maintenance schedules resulted in a greater box use rate. Delaware had the lowest box use rate of 54% (Table 1.5), which may have been related to many boxes being duplex style (i.e., two boxes per pole), aged over 10 years, proximity of boxes (e.g., 50 m) to each other, and perhaps too many boxes for the local population size (H. Schley, University of Delaware, personal communication). These are hypotheses that may be addressed through continuation of this study.

Florida's greater box use rate may be related to the significant loss of forested habitat, especially old-growth hardwoods, surrounding my study site. This loss of trees was due to the landfall of Hurricane Michael in October 2018. Following Hurricane Michael, FWC estimated a 90% loss of their mature longleaf pine (*Pinus palustris*) and other forest stands across Apalachee WMA. Many of these trees probably contained nesting cavities, and their loss may have driven wood ducks to artificial nest sites. Additional causes of this box use rate may include the earlier and extended nesting season for wood ducks in this region (Croft et al. 2020).

Georgia's use rate of 60% may be explained by habitat surrounding nest boxes in all three WMAs. Each WMA in Georgia is primarily comprised of bottomland hardwood swamps containing oak (*Quercus* spp.), loblolly pine (*Pinus taeda*), longleaf pine (*Pinus palustris*), and maple (*Acer* spp.). Wood ducks are secondary cavity nesters, using trees with a dbh of 40.6 cm, cavity entrances of ≥ 6 cm in diameter, vertical depths of >10 cm, and a nesting platform of ≥ 14 x 15 cm (Denton et al. 2012*a*, Zlonis et al. *in press*). Zlonis et al. (2020) described factors like dbh, tree health status, and tree species as predictors for whether a tree had developed one or more suitable cavities for wood ducks. Lowney and Hill (1989) described how the lack of suitable nesting cavities was a limiting factor for wood ducks in Mississippi. They concluded that nest boxes may augment wood duck production in locations where suitable natural cavities are lacking or in locations where egg depredation in natural cavities is high (Lowney and Hill 1989). Thus, a forest inventory in the Georgia WMAs may be advisable to determine the number of suitable natural cavities compared to the number of nest boxes.

Estimates of nest success varied between study states between 2020 and 2021. In Georgia, estimates of nest success (41%) were lower than previous reports for wood ducks nesting in both small (59%) and large nest boxes in Mississippi (66%; Stephens et al. 1998). Florida exceeded these estimates (69%) and was like recent estimates by Croft et al. (2022; 65%) for wood ducks nesting in boxes in South Carolina. Reasons for nest failure in both states included predation (21%) or abandonment for unknown reasons (38.6%); however, another explanation for Georgia would be the state of the boxes in 2020—the first year of the study. Nest boxes in Georgia were dilapidated and often missing important parts of the box like the roof or floor. Results from collaborating sites in North Carolina and South Carolina in 2021 included 129 successful nests in North Carolina (57%) and 190 successful nests in South Carolina (49%; Table 1.7).

I did not detect a difference in ducklings successfully exiting nest boxes among the three Georgia WMAs. My estimates of wood duck duckling production from nest boxes in Georgia ($\overline{x}$ = 11.46 ducklings/successful nest, SE = 0.48) were greater than previous studies in the Southeast. Specifically, in South Carolina, Croft et al. (2022) reported an average of 10.2 ducklings exiting successful boxes. Collaborating states reported an average of 10.10 ducklings in South Carolina and 11.94 ducklings in North Carolina (Bauer et al. 2021; Table 1.7). In 2021, Maryland reported the greatest successful duckling production at a rate of 12.25 ducklings/successful box, while Florida reported the lowest at 6.86 ducklings (Table 1.7).

One possible explanation for the greater duckling numbers in Georgia could be the clutch sizes of successful nests. Average clutch size in Georgia was 16 eggs, while in Florida there were 10.7 eggs/successful nest. Wood ducks commonly engage in nest parasitism (dump nesting) and can incubate large clutches of eggs successfully (Clawson et al. 1979, Davis et al. 2007). However, where dump nesting was prevalent, the productivity of the wood duck population decreased (Clawson et al. 1979), and dump nesting might regulate a population too high to sustain itself (Jones and Leopold 1969). Morse and Wight (1969) conversely reported how dump nesting acted in favor of a population of wood ducks in Oregon, by contributing 32% more ducklings to the population than that of average-sized clutches.

I have presented results that establish the cost of maintaining a nest box per female recruit over 20 years, using data from my study and a previously published estimate of a yearling female recruitment rate for box-nesting wood ducks from South Carolina (Hepp et al. 1989, 2020). In 2020, Hepp et al., concluded that recruitment rates of yearling females from boxes was insufficient (i.e., $\lambda < 1$) to sustain their nest-box populations without immigration of hens from natural cavities. I have calculated the cost of a female recruit in Florida ($108.35) and in Georgia ($86.68), however I do not believe there is enough data to conclude if nest box programs are cost-effective in these states. Georgia's cost was almost half the total cost of a box and its maintenance over 20 years ($173.37), but there is no evidence to conclude that either state's box program is cost-effective. Current studies are underway to provide recruitment estimates for these states, which can be used to better evaluate the cost-effectiveness of nest boxes per Croft et al. (2022). Additionally, more determination of what is considered "cost-effective" for the management of nest boxes should be explored.

Nest boxes are a management tool still widely used across the United States. Bellrose and Holm (1994) estimated that by the early 1980s, about 100,000 nest boxes had been erected and managed between the Atlantic and Mississippi flyways. In the Atlantic Flyway, every state has an active box-nesting program operated through state and federal agencies (Bellrose 1990). However, the true success of nest boxes is under debate, especially from a recruitment and population standpoint. Soulliere (1987) argues that with the availability of natural cavities, the hatchability and survival of ducklings would be the same in cavities as it is in nest boxes. Conversely, Hepp et al. (2020)

indicated that box-nesting populations of wood ducks from the Savannah River Site in South Carolina might not be self-sustaining without the immigration of hens from natural cavities. Expanding studies to natural cavities, in addition to nest-boxes, could provide new data on how sites with and without natural cavities influence competition for nest boxes compared to cavities as well as the influence on duckling success. At field sites in Louisiana, game cameras were attached to the inside of nest boxes to answer questions about egg loss during incubation (D. Bakner, Louisiana State University, personal communication). Placement of game cameras both inside and outside of boxes could help describe species causing unknown predation events, or perhaps why female wood ducks unexpectedly stop incubation and desert a nest. As previously mentioned, it would be beneficial to perform a forest inventory survey at field sites in Florida and Georgia to quantify the number of usable cavities compared to the number of nest boxes. Finally, continued research into the sustainability of nest boxes across the flyway is necessary to determine if nest boxes are acting as a source or a sink for wood duck populations. If it is the latter, more focus should be placed on the use of natural cavities and their current influence on wood duck populations.

LITERATURE CITED

Barry, S. C. 1992. Evaluation of a wood duck nest box program at Yazoo National Wildlife Refuge, Mississippi, 1987–1991. Thesis, Mississippi State University, Starkville, Mississippi, USA.

Bauer, B. A., D. Bakner, T. Gibson, E. Miller, H. L. Schley, J. A. Shurba, N. Simmons, and S. Sullivan. 2021. Regional examination of the contribution of nest boxes to wood duck recruitment in the Southeast and Mid-Atlantic United States. 2021 Annual Report. Available at Nemours Wildlife Foundation, Yemassee, South Carolina, USA, bbauer@nemourswildlife.org.

Bellrose, F. C. 1976. The comeback of the wood duck. Wildlife Society Bulletin 3: 107-110

Bellrose, F. C. 1990. The history of wood duck management. Pages 13-20 *in* Proceedings of the 1988 North American Wood Duck Symposium. L. H. Fredrickson, G. V. Burger, S. P. Havera, D. A. Graber, R. E. Kirby, and T. S. Taylor, editors. St. Louis, Missouri, USA.

Bellrose, F. C., and D. J. Holm. 1994. Ecology and management of the wood duck. Stackpole Books, Mechanicsburg, Pennsylvania, USA.

Bolen, E. G. 1967*a*. Nesting boxes for black-bellied tree ducks. Journal of Wildlife Management 31:794-797. Bolen, E. G. 1967*b*. The ecology of the black-bellied tree duck in southern Texas. Dissertation, Utah State University, Logan, Utah, USA.

Clawson, R. L., G. W. Hartman, and L. H. Fredrickson. 1979. Dump nesting in a Missouri wood duck population. Journal of Wildlife Management 43: 347-355.

Cowardin, L. M., and R. J. Blohm. 1992. Breeding population inventories and measures of recruitment. Pages 423-445 *in* B. D. J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu, editors. Ecology and management of breeding waterfowl. University of Minnesota Press, Minnesota, USA.

Croft, G. D., R. M. Kaminski, E. P. Wiggers, P. D. Gerard, and G. K. Yarrow. 2020. Nest-box use by wood ducks and black-bellied whistling ducks in coastal South Carolina. Wildlife Society Bulletin 44:662-669.

Croft, G. D., R M Kaminski, E. P. Wiggers, P. D. Gerard, and G. K. Yarrow. 2022. Box-nesting wood ducks and black-bellied whistling ducks in coastal South Carolina. Journal of the Southeastern Association of Fish and Wildlife Agencies 9:in press.

Davis, J. B., S. E. Stephens, B. D. Leopold, R. M. Kaminski, and P. D. Gerard. 1999. Wood duck reproduction in small and large nest boxes in Mississippi: A continued experiment. Proceedings Annual Conference of Southeastern Association of Fish and Wildlife Agencies 53:257-269.

Davis, J. B., R. R. Cox Jr., R. M. Kaminski, and B. D. Leopold. 2007. Survival of wood duck ducklings and broods in Mississippi and Alabama. Journal of Wildlife Management 71:507-517.

Davis, J. B., J. N. Straub, G. Wang, R. M. Kaminski, and B. D. Leopold. 2015. Simulations of wood duck recruitment from nest boxes in Mississippi and Alabama. Journal of Wildlife Management 79:907-916.

Denton, J. C., C. L. Roy, G. J. Soulliere, and B. A. Potter. 2012*a*. Change in density of duck nest cavities at forests in the north central United States. Journal of Fish and Wildlife Management 3: 76-88.

Florida Fish and Wildlife Conservation Commission [FWC]. 1999. Apalachee – Habitat and Management. Hurricane Michael. https://myfwc.com/recreation/lead/apalachee/habitat/. Accessed 7 Dec 2021.

Fredrickson, L. H., G. V. Burger, S. P. Havera, D. A. Graber, R. E, Kirby, and T. S. Taylor, editors. 1990. Proceedings of the North American wood duck symposium. St. Louis, Missouri, USA.

Grado, S. C., K. M. Hunt, C. P. Hutt, X. T. Santos, and R. M. Kaminski. 2011. Economic impacts of waterfowl hunting in Mississippi derived from a state-based mail survey. Human Dimensions of Wildlife 16:100-113.

Grinnell, G. B. 1901. American duck shooting. Forest and Stream Publishing Co., New York City, New York, USA.

Hawkins, A. S., and F. C. Bellrose. 1940. Wood duck habitat management in Illinois. Transactions of the North American Wildlife Conference 4: 392-395.

Hepp, G. R., R. A. Kennamer, and W. F. Harvey, IV. 1989. Recruitment and natal philopatry of wood ducks. Ecology 70:897-903.

Hepp, G. R., and R. A. Kennamer. 1992. Characteristics and consequences of nest-site fidelity in wood ducks. The Auk 4: 812-818.

Hepp, G. R., R. A. Gitzen, and R. A. Kennamer. 2020. Relative importance of vital rates to population dynamics of wood ducks. Journal of Wildlife Management 84:320-330.

Heusmann, H. W. 2000. Production from wood duck nest boxes as a proportion of the harvest in Massachusetts. Wildlife Society Bulletin 28: 1046-1049.

Jones, R. E., and A. S. Leopold. 1967. Nesting and interference in a dense population of wood ducks. Journal of Wildlife Management 31: 221-228.

Lowney, M. S., and E. P. Hill. 1989. Wood duck nest sites in bottomland hardwood forests of Mississippi. Journal of Wildlife Management 53: 378-382.

Morse, T. E., and H. M. Wight. 1969. Dump nesting and its effects on production in wood ducks. Journal of Wildlife Management 33: 284-293.

Nichols, J. D., and F. A. Johnson. 1990. Wood duck population dynamics: a review. Pages 83-105 *in* L. H. Fredrickson, G. V. Burger, S. P. Havera, D. A. Graber, R. E. Kirby, and T. S. Taylor, editors. Proceedings of the Annual Conference of Southeastern Association of Fish and Wildlife Agencies, St. Louis, Missouri, USA.

Nicolai, C. A. 2013. The Fallon wood duck project. U. S. Fish and Wildlife Service, Fallon, Nevada, USA.

Otis, D. L., and W. F. Dukes, Jr. 1995. Improving wood duck survival estimates in the Mid-Atlantic region by using nest box banding programs. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 49:618-628.

Prevost, M. B., T. H. Strange, and R. D. Perry. 1990. Management of a large-scale wood duck nesting project in South Carolina. Pages 297-302 *in* Fredrickson, L. H., G. V. Burger, S. P. Havera, D. A. Graber, R. E, Kirby, and T. S. Taylor, editors. 1990. Proceedings of the North American wood duck symposium. St. Louis, Missouri, USA.

R Core Team. 2021*a*. Kruskal-Wallis Test. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

R Core Team. 2021*b*. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

R Core Team. 2021*c*. Wilcoxon Rank Sum and Signed Rank Tests. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Sauer, J. R., and S. Droege. 1990. Wood duck population trends from the North American breeding bird survey. Pages 225-231 *in* L. H. Fredrickson, G. V. Burger, S. P. Havera, D. A. Graber, R. E. Kirby, and T. S. Taylor, editors. Proceedings of the 1988 North American Wood Duck Symposium. St. Louis, Missouri, USA.

Schmidt, P., R. M. Kaminski, and E. P. Wiggers. 2018. Call to action: identifying priority research questions for the southern Atlantic Flyway. Available at Nemours Wildlife Foundation, Yemassee, South Carolina 29945, USA, bbauer@nemourswildlife.org.

Soulliere, G. J. 1987. Distinguishing hooded merganser and wood duck nests by eggshell thickness. Journal of Wildlife Management 51:534-534.

Stephens, S. E., R. M. Kaminski, B. D. Leopold, and P. D. Gerard. 1998. Reproduction of wood ducks in small and large nest boxes. Wildlife Society Bulletin 26:159-167.

Strange, T. H., E. R. Cunningham, and J. W. Goertz. 1971. Use of nest boxes by wood duck sin Mississippi. Journal of Wildlife Management 35: 786-793.

U. S. Climate Data. 2020*a*. United States Climate Data for Eatonton, GA. https://www.usclimatedata.com/climate/eatonton/georgia/united-states/usga0877. Accessed 17 December 2021.

U. S. Climate Data. 2020*b*. United States Climate Data for Tallahassee, FL. https://www.usclimatedata.com/climate/tallahassee/florida/united-states/usfl0479. Accessed 17 December 2021.

Utsey, F. M., and G. R. Hepp. 1997. Frequency of nest box maintenance: effects on wood duck nesting in South Carolina. Journal of Wildlife Management 61:801-807.

Vrtiska, M. P. 1995. Aspects of reproductive and remige molt biology in wood ducks. Dissertation, Mississippi State University, Starkville, Mississippi, USA.

Wiggers, E. P., B. A. Bauer, P. Schmidt, R. M. Kaminski, and G. R. Hepp. 2019. Regional examination of the contribution of nest boxes to wood duck recruitment in the Southeast and Mid-Atlantic United States. Available at Nemours Wildlife Foundation, Yemassee, South Carolina, USA, bbauer@nemourswildlife.org.

Zampieri, N. E., S. Pau, and D. K. Okamoto. 2020. The impact of Hurricane Michael on longleaf pine habitats in Florida. Scientific Reports 10: 1-11.

Zlonis, E. J., J. B. Berdeen, and V. St-Louis. 2020. Forest inventory attributes predict the occurrence of cavities suitable for nesting wood ducks (*Aix sponsa*). Forest Ecology and Management 461: 117951.

Zlonis, E. J., R. Deo, and J. B. Berdeen. In press. LiDAR and multispectral imagery predict the occurrence of tree cavities suitable for a cavity-nesting duck. Remote Sensing in Ecology and Conservation.

Table 1.1. The number of nest boxes deployed for wood ducks reported by Bellrose (1990) in the Atlantic and Mississippi Flyways (*n*), and the percentage of used boxes (%) during that study.

Flyway	*n*	%
Atlantic	35,670	42%
Mississippi	58,192	40%

Table 1.2. The number of nest boxes deployed for wood ducks (*n*), percentage (%) of successful nests within each state, and the number of ducklings (*n* ducklings) that successfully exited next boxes.

State	Year	*n*	%	*n* ducklings
California	1994	2,295	73.0	9,103
Nevada	2013	344	66.0	2,177
South Carolina	2022	718	65.0	6,015

Table 1.3. Frequency (*n*) of nest boxes used and not used, percentage (%) of nest boxes used and not used, standard error (% SE) of the percentage of used and not used boxes, and chi-square (χ^2) test of the null hypothesis[a] that frequencies of nest boxes used and not used by wood ducks (*Aix sponsa*) would not differ between Georgia and Florida in 2020 and 2021.

		Georgia		Florida	
Boxes	Year	*n*	%	*n*	%
Used	2020	76	61.8	130	91.5
	2021	71	59.1	123	89.1
Not used	2020	47	38.2	12	8.4
	2021	49	40.8	15	10.8

[a]Reject the null hypothesis for 2020 ($\chi_1^2 = 32.03$, $P < 0.001$) and 2021 ($\chi_1^2 = 29.31$, $P < 0.006$), concluding that nest box use depended on state in each year.

Table 1.4. Frequency (*n*) of successful nests, percentage (%) of successful nests, standard error (% SE) of the percentage of successful nests, and chi-square (χ^2) test of the null hypothesis[a] that frequencies of successful and unsuccessful nests of wood ducks (*Aix sponsa*) would not differ between Georgia and Florida in 2020 and 2021.

		Georgia		Florida	
Nest	Year	*n*	%	*n*	%
Successful	2020	35	34.6	97	40.2
	2021	47	48.0	85	42.4
	2020-2021[b]	82	41.2	182	41.0

[a]Failed to reject the null hypothesis for 2020 ($\chi_1^2 = 0.843$, $P = 0.358$), 2021 ($\chi_1^2 = 0.955$, $P = 0.328$), and 2020-2021 combined, ($\chi_1^2 \leq 0.001$, $P = 0.998$), concluding that nest success was independent of states in both years.

Table 1.5. Percentages of successful nests by box-nesting wood ducks (*Aix sponsa*) and cause-specific nest failures in Florida and Georgia in 2020 and 2021.

				Nest failures			
State	Year[a]	*n*	Successful	Abandoned	Snake	Woodpecker	Mammal/Other
Florida	2020	242	40.1	26.4	19.8	2.9	10.7
	2021	202	42.1	45.0	1.0	3.0	8.9
Georgia	2020	102	34.3	45.1	6.9	2.9	10.8
	2021	98	47.9	41.8	0.0	0.0	10.2

[a]Nest fates differed between years for Florida ($\chi_4^2 = 46.12$, $P < 0.001$) and Georgia ($\chi_4^2 = 12.02$, $P = 0.017$)

[b]Nest failures assigned to snakes were by eastern rat snakes (*Pantherophis alleghaniensis*) observed in nest boxes consuming eggs and eggs pecked by woodpeckers (Picidae). Mammalian and other causes of predation are the result of unknown species.

Table 1.6. Mean ($\bar{x}$) number and standard error (SE) of wood duck (*Aix sponsa*) ducklings per successful nest (*n*) in Florida and Georgia in 2020 and 2021. Statistics are for Mann-Whitney U tests (*W*, *P*) that tested the null hypothesis of no difference in the number of ducklings departing nest boxes between states within and across years.

	Georgia		Florida			
Year	*n*	$\bar{x}$	*N*	$\bar{x}$	*W*	*P*
2020	35	10.65	97	6.49	2,802.5	< 0.001
2021	47	12.06	85	7.28	3,021.0	< 0.001
2020-2021	82	11.46	182	6.86	3,201.5	< 0.001

Table 1.7. Frequency (*n*) and percentage (%) of boxes used by wood duck females and containing ≥ 1 egg, *n* and % successful nests with ≥ 1 hatched egg and mean ($\bar{x}$) number of ducklings that exited boxes per successful nest from states involved in the wood duck recruitment project in 2021 (Note: Values in this table were computed from those in Bauer et al. 2021 or those reported in previous tables herein for Florida and Georgia).

	Boxes used		Successful nests		
State	*n*	%	*n*	%	$\bar{x}$ ducklings
Delaware	110	53.9	51	40.8	10.73
Florida	123	89.1	89	42.4	6.86
Georgia	71	59.2	47	48.0	11.46
Louisiana	320	96.7	173	40.5	9.58[b]
Maryland	86	88.7	48	51.1	12.25
Mississippi	149	85.6	51	25.0	8.31
North Carolina	162	84.8	129	56.8	11.94
South Carolina	178	97.8	190	48.7	10.10
$\bar{x} \pm$ SE[c]	81.98 ± 5.81		44.16 ± 3.38		9.69 ± 0.61

[a]Includes marked and unmarked ducklings exiting nest boxes minus any dead ducklings in nest boxes.
[b]Includes 505 web- and 643 PIT-tagged ducklings (Bauer et al. 2021).

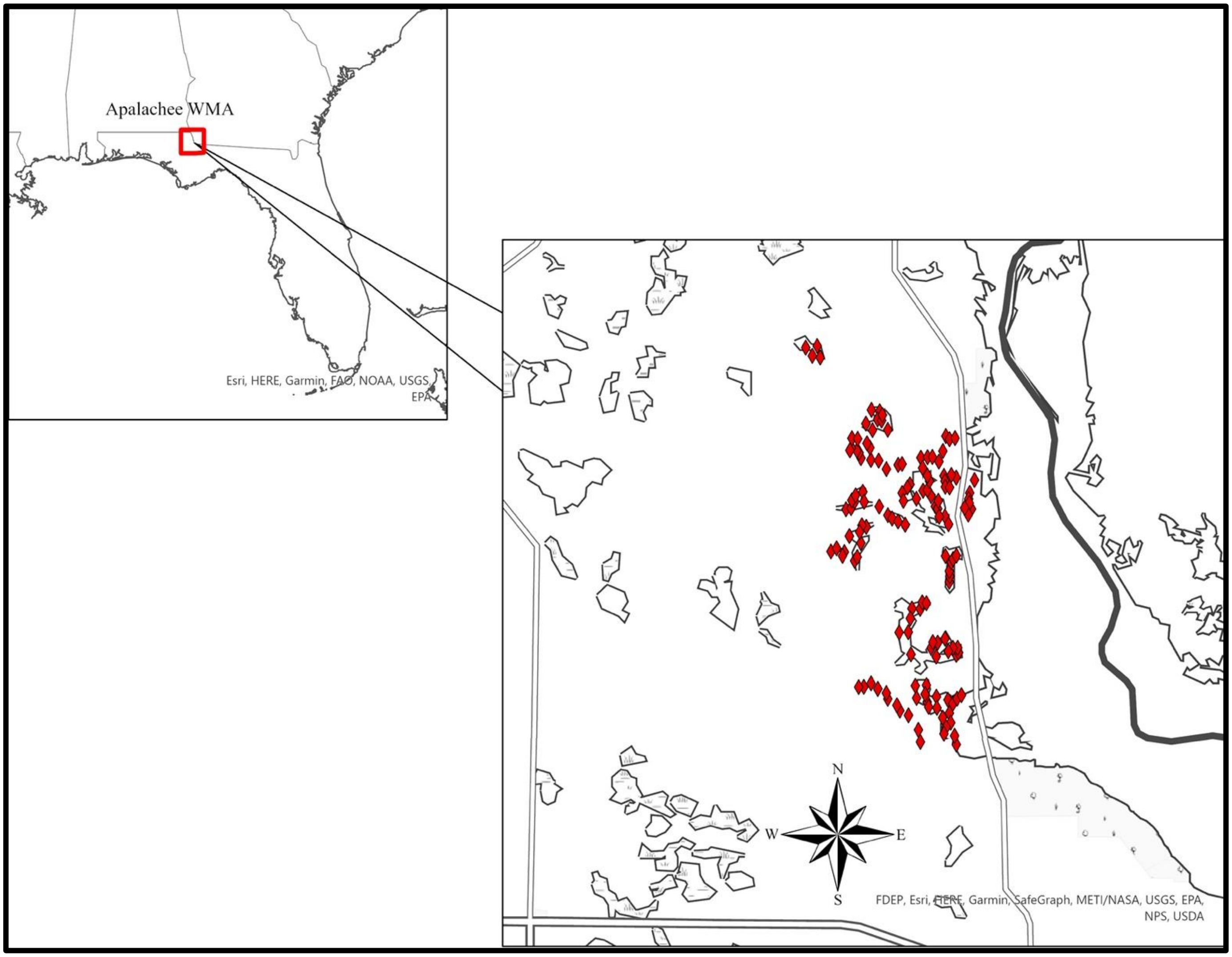

Figure 1.1. Locations of study areas and nest boxes (red diamonds) in the Apalachee Wildlife Management Area in Sneads, Florida in 2020-2021.

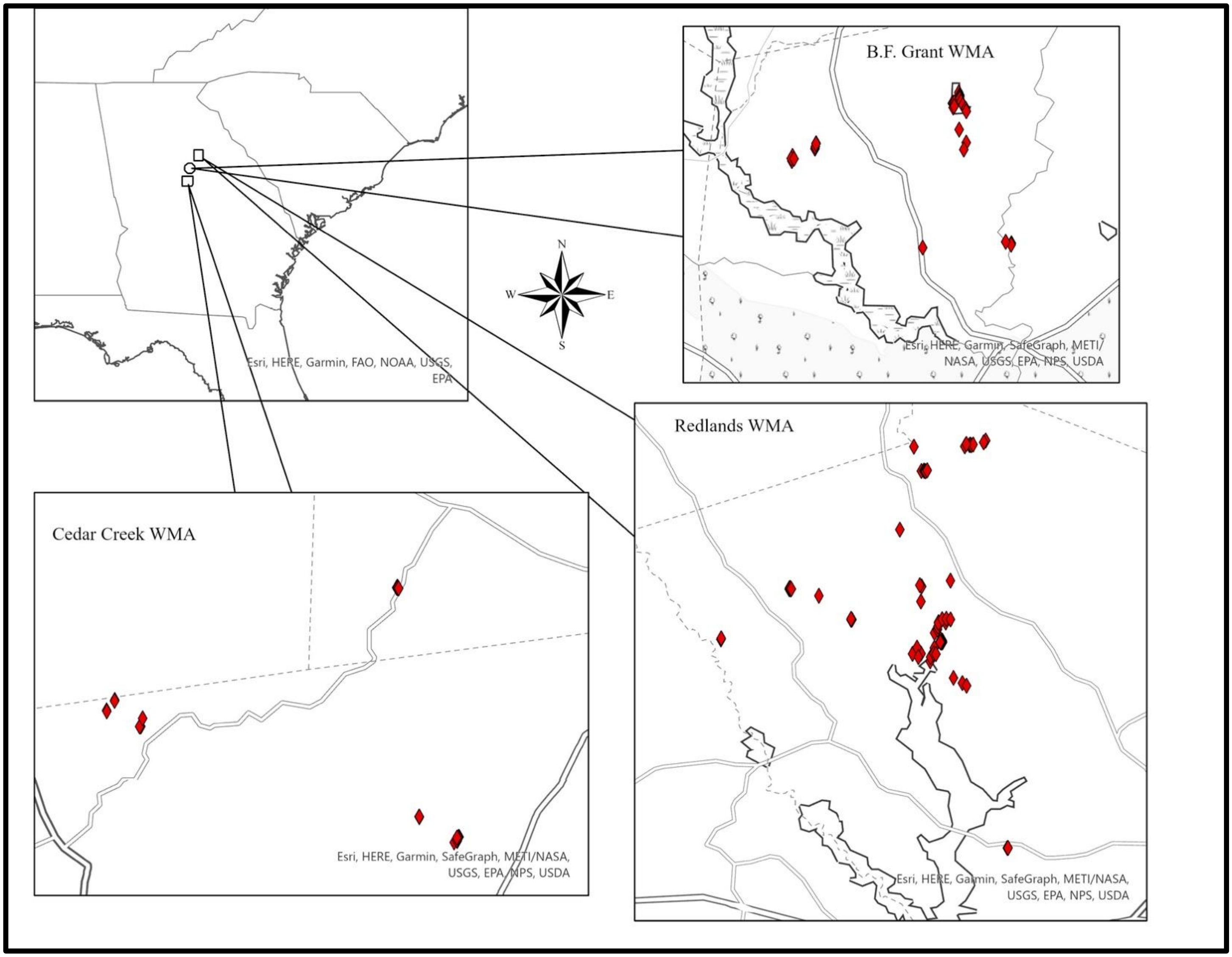

Figure 1.2. Locations of study areas and nest boxes (red diamonds) in the B. F. Grant, Cedar Creek, and Redlands Wildlife Management Areas in Eatonton, GA in 2020-2021.

CHAPTER TWO

EFFECT OF SHAVINGS TYPE ON BOX SELECTION, NEST SUCCESS, AND MICROBIAL GROWTH FROM NEST BOXES IN GEORGIA AND FLORIDA

Wood ducks (*Aix sponsa*) are a secondary cavity-nesting species that use both natural cavities and artificial structures (hereafter nest boxes) across their range (Bellrose 1990, Bellrose and Holm 1994, Croft et al. 2020). After nearly being extirpated in the early 20th century from market hunting and loss of habitat, research suggests that nest boxes helped wood ducks to recover and increase across North America, especially in areas where natural cavities are limited (Jones 1964, Lowney and Hill 1989, Fredrickson et al. 1990, Bellrose and Holm 1994, Stephens et al. 1998, Davis et al. 1999, Davis et al. 2007, Nicolai 2013).

Wood duck females are the sole caretakers of the young and are responsible for the incubation and brood-rearing (Hepp and Bellrose 1995). They are also philopatric with reports of 79% returning annually to the same wetland for nesting and 42% to the same box, especially if they had nested successfully in that box the previous year (Hepp et al. 1989, Hepp and Kennamer 1992). Average clutch size in wood ducks is debated with estimates ranging from 10-15 eggs by Bent (1925, while Clawson et al. (1979) reported a mean clutch size of 7-14 eggs. Vrtiska (1995) reported a mean clutch size of 10 eggs for captive females in the absence of nest parasitism and concluded that 9-12 eggs are an ideal clutch size range for wood ducks, which was also like other duck species (Rohwer and Anderson 1988, Bellrose and Holm 1994, Croft et al. 2022).

In nest boxes that produce successful nests, multiple hens will utilize them. The continued use of nest boxes by multiple hens can often lead to a buildup of bacteria, parasites, and other potentially detrimental pathogens that could decrease egg hatchability and nesting success (Clark and Mason 1985, Walls et al. 2012). This is of concern for species like wood ducks and other cavity-nesting waterfowl species whose populations are driven by recruitment, which I define as the proportion of female wood ducks marked as day-old ducklings, that return as yearlings or older individuals to nest in boxes in their natal locale (Hepp et al. 1989, Cowardin and Blohm 1992, Davis et al. 2007, Hepp et al. 2020). One well-documented method used by many cavity-nesting avian species to minimize bacterial infection of eggs is incubation. By incubating, adult birds can potentially minimize the buildup of detrimental pathogens by reducing moisture on the eggshell (Jacob 1978, Menon and Menon 2000, Shawkey et al. 2003, Sweeney et al. 2004, Cook et al. 2005*a, b*). Some bird species utilize nest materials to minimize pathogens in their nests. Clark and Mason (1985) describe how European starlings (*Sturnus vulgaris*) utilize green vegetation in their nests which act as natural fumigants for insect pests and pathogens alike. Wood ducks, however, do not bring in their own nesting material and rely on what is left behind in natural cavities, or what is placed in boxes by managers. Little is also published on the potential effects that nesting material may have on microbial communities from wood duck nest boxes.

To my knowledge, no large-scale, multi-state study of microbial communities from wood duck nest boxes has been conducted in the United States or elsewhere in North America. My objectives for this chapter were to 1) determine if use of different types of shavings have any effect on nest box use, nest success, and number of ducklings successfully exiting boxes; 2) determine if the use of different types of shavings has an impact on the growth of nest-box microbes; and 3) determine if there are preventative measures managers may use to keep microbes from negatively affecting eggs.

STUDY AREA

My study area was in the Panhandle of western Florida and central Georgia. In Florida, the study site was the Apalachee Wildlife Management Area (WMA; Sneads, Florida: 30.710599, -84.919843) and is managed jointly by the Florida Fish and Wildlife Conservation Commission (FWC) and the U. S. Army Corps of Engineers. The Georgia locations were the Redlands WMA (Greensboro, Georgia 33.680115, -83.273044), B. F. Grant WMA (Eatonton, Georgia: 33.392210, -83.491500), and Cedar Creed WMA (Eatonton, Georgia: 33.229503, -83.523710), all of which were managed by the Georgia Department of Natural Resources (GA DNR). I did not randomly select study sites but chose sites with active nest box programs deemed appropriately located in representative habitats of both states by FWC and GA DNR.

Nest boxes were distributed among emergent fresh marshes and ponds in Florida. In Georgia, boxes were located on managed wetland impoundments, ponds, lakes, and forested wetlands. Dominant flora in Florida and Georgia included duckweed (*Lemna minor*), cattail (*Typha* spp.), giant cutgrass (*Zizaniopsis milacea*), spikerush (*Eleocharis cellulose*), alligator weed (*Alternathera philoxeroides*), horsetail (*Equisitum hyemale*), pickerelweed (*Pontederia cordata*), water lily (*Nymphea* spp.), bald cypress (*Taxodium distichum*), willows (*Salix* spp.), oaks (*Quercus* spp.), loblolly pine (*Pinus taeda*), longleaf pine (*Pinus palustris*), and red maple (*Acer rubrum*). All boxes used in the study were wooden structures made of cypress or pine lumber described by Bellrose (1976). All Florida boxes were erected on metal poles. Predator guards were metal cones with a diameter of approximately 91 cm. Ninety-four boxes had a predator guard and 48 lacked one in 2020. In 2021, 94 boxes had a predator guard and 44 lacked one after four boxes collapsed in a storm. Georgia boxes were erected on wooden posts with 100 boxes being protected by predator guards and 21 lacking one in 2020. In 2021, 100 boxes were protected by predator guards, and 20 lacked one after one box had the floor collapse and was taken out of use.

I conducted my surveys from January through July 2020–2021. Long term temperature and precipitation in Florida during the winter-summer period averaged 24°C (range = 15–33°C) and 78 mm (range = 47–127 mm), respectively. During the winter-summer period in Georgia, long-term temperature and precipitation averaged 17°C (range = 10–24°C) and 72 mm (range = 63–81 mm), respectively (U. S. Climate Data 2020*a*, *b*).

METHODS

Nest Box and Egg Field Protocols

I began the 2020 field season with 142 nest boxes in Florida and 123 nest boxes in Georgia—all of which were erected before my study. In May 2020, I installed four additional boxes at Redlands WMA to replace dilapidated boxes at this site. In January 2021 at all sites, I repaired structures, removed nest remains, and added wood shavings to all boxes to a depth of 6-10 cm. In 2021 in Florida, I monitored 138 boxes and 120 boxes in Georgia. Boxes were randomly selected to receive either aspen or cedar shavings. In Florida, 75 boxes contained cedar and 67 contained aspen in 2020, while in 2021 74 boxes contained cedar and 64 contained aspen (Table 2.1). In Georgia, 69 boxes contained cedar and 52 with aspen in 2020, while in 2021 69 contained cedar and 51 had aspen (Table 2.1). These shavings were selected as they are common shavings used by managers in nest boxes. Aspen was selected for its non-odorous makeup, while cedar was selected because it contains natural oils that can help promote an anti-microbial environment (Johnston et al. 2001).

I collected sterile swabs when incubation was initiated in a nest box. Initiation of incubation was determined as when the hen pulled down from her breast and placed it on top of the eggs, usually occurring after the laying of the penultimate egg (Kennamer et al. 1990, Wilson and Verbeek 1995, Manlove and Hepp 2000, Hepp et al. 2006, Walls et al. 2012). I collected swabs during weekly box checks. I first captured the hen if she was in the box, applied a leg band and took morphometric data (age, tarsus length and weight). The hen was then released, after which three eggs were individually selected at random from the nest. In boxes where swabs were being taken, this occurred before any other activities to avoid environmental or human contamination. In Florida I collected samples from 16 boxes in 2020 and 13 boxes in 2021. In Georgia, I collected samples from 8 nest boxes in 2020 and 13 nest boxes in 2021. I swabbed each egg twice for future laboratory methods. Once I swabbed the egg, I placed the swab in a tube containing 1–2-ml of sterile phosphate buffered saline (PBS) and placed in a cooler until it could be frozen. I labeled tubes with the state, nest box identification number, the type of shavings used in the box, and the egg number. Tubes were kept in a -20°C freezer until they were brought to Clemson University to be stored in a -80°C freezer.

Bacterial Culturing

I collected 24 samples from nest boxes in 2020 and 25 samples from nest boxes in 2021. I initially cultured microbes by plating two 0.1-ml samples of the PBS supernatant on each of the two-growth media. I used MacConkey agar (MAC) and Tryptic Soy Agar (TSA) as these are often media that are used to detect the groups of bacteria most associated with bird eggs (Cook et al. 2003, Walls et al. 2012). Forty grams per liter of MAC base powder (Ward Science, Ontario, Canada) and 50 grams per liter of TSA base powder (Hardy Diagnostics, Santa Maria, California, USA) was dissolved in the appropriate volume of distilled water, and the mixture was autoclaved at 121°C for 15-30 minutes and poured into Petri dishes (100 mm in diameter).Cultures were incubated (Symphony Gravity Convection Incubator, E191047, VWR Inc., Radnor, Pennsylvania, USA) aerobically: MAC was incubated at 35°C and TSA at 23°C, for 48 hours. Cultures were checked at 24 hours to ensure fungal contamination did not occur. After 48 hours, I counted microbial colony-forming units (CFUs; CFUs/0.1 ml) on each plate and marked individual colonies of each type of microbe grown.

I removed individual colonies with a sterile loop and streaked onto new plates with MAC and TSA media. Cultures of the pure colonies were incubated again at 35°C for MAC, and 23°C for TSA for another 48 hours, again being checked at 24 hours for fungal contamination. Following incubation, I removed the plates and checked for additional growth besides the initial microbial CFU. If the cultures were pure based on visual inspection, then they were cultured for a final time. The last culture was performed using Tryptic Soy Broth (TSB). Thirty grams per liter of TSB base powder (VWR Inc., Radnor, Pennsylvania, USA) was dissolved in the appropriate volume of distilled water, and the mixture was autoclaved at 121°C for 15-30 minutes. Pure CFUs were removed from plates using a sterile loop and added to 15 mL tubes containing 10 ml of TSB. I then placed the tubes in the incubator at 23°C for 48 hours.

Following incubation, I removed the TSB cultures and checked them for growth which is indicated by the presence of turbidity, specks, or flocculation in the medium while an uninoculated culture remains clear and without turbidity. If growth was determined, I Gram-stained the samples. Gram staining is often the first test performed in the process of identifying organisms, with those retaining the primary color and appearing purple-brown being Gram-positive while those that appear red are Gram-negative (Bartholomew and Mittwer 1952, Beveridge and Davies 1983, O'Toole 2016). I recorded the Gram stain results for each bacterial species grown as well as the number of CFUs counted per egg from each box where samples were taken.

Statistical Analyses

Because mine was an inaugural study of microbes in wood duck nest boxes in Florida and Georgia, I had no a priori predictions regarding the effects of shavings on bacterial growth from the nest boxes in my study. Additionally, it was not examined in Chapter One how the use of these different types of shavings might impact nest box use, nest success and failures, and the number of ducklings successfully exiting nest boxes.

Thus, I tested the null hypothesis that nest box use, nest success and failures, and number of ducklings would not be affected by the type of shavings used within or between years and states ($\alpha = 0.05$). To test these hypotheses, I first determined the frequencies of boxes with either aspen or cedar shavings, and then of those boxes, I determined the frequencies of used (i.e., presence of ≥ 1 wood duck egg) and unused boxes and successful (≥ 1 egg hatched) and unsuccessful nests by wood ducks in Georgia and Florida between 2020 and 2021. I then tested if use of boxes, and success of nests were independent of type of shavings using the chi-square test (R Version 4.1.1, www.r-project.org, accessed 15 Feb 2022; R Core Team 2021*a*). I conducted tests within years, because I neither had endogenous data from breeding wood duck populations nor exogenous data to attempt explanation of any possible detected between-year differences in box use, nest success, and ducklings exiting nests during the study.

To account for Georgia's boxes being in three different WMAs, I performed a Kruskal-Wallis test (R Core Team 2021*b*) to test if the number of ducklings successfully exiting boxes between sites would preclude pooling data among Georgia WMAs for analyses. I did not detect a difference among the three WMAs with the Kruskal-Wallis test (see Chapter One Results); thus, I used a Mann-Whiney U-test to test if numbers of ducklings departing boxes was affected by the type of shavings used between Florida and Georgia in 2020 and 2021 (R Core Team 2021*c*). To test the effects of shavings type on the number of microbial species and CFUs within boxes, I performed a non-parametric Mann-Whitney U test.

RESULTS

Nest-Box Use

Nest box use was significantly greater in boxes with cedar shaving relative to boxes with aspen shavings in Georgia during 2020 ($P = 0.004$). I found no evidence of differences as a function of shaving type in Georgia 2021 ($P = 0.116$), Florida 2020 ($P = 0.108$), and Florida 2021 ($P = 0.09$; Table 2.1). Use of boxes between 2020 and 2021 in Florida averaged 44.6% and 45.7% in aspen and cedar-filled boxes, respectively. From 2020 through 2021 in Georgia, box use averaged 19.8% and 40.7% in aspen and cedar filled boxes respectively (Table 2.1).

Nest Success and Failures

I did not detect a difference in the success of nest boxes and the use of different types of nest shavings in Florida and Georgia during both 2020 and 2021 ($P = 0.109$; $P = 0.958$; $P = 0.586$; $P = 0.756$; Table 2.2) and concluded that nest success was independent of the type of shavings used in nest boxes. Nest success between 2020 and 2021 in Florida averaged 22.7% and 18.4% in aspen and cedar filled boxes respectively. From 2020 through 2021 in Georgia, nest success averaged 16.0% and 25.1% in aspen and cedar filled boxes, respectively (Table 2.2).

Ducklings

As stated in Chapter One, the number of ducklings exiting boxes among the three Georgia WMAs did not differ within years ($P \geq 0.13$). Nearly twice as many ducklings departed from successful nests filled with cedar compared to aspen in both Florida and Georgia in both years, but duckling success was independent of the type of shavings used in nest boxes (Table 2.3). An average of 6.6 and 10.7 ducklings exited nest boxes filled with aspen and cedar respectively, in Florida, while in Georgia, an average of 7.3 and 12.1 ducklings exited boxes with aspen and cedar, respectively.

Microbial Densities

The use of different types of shavings in nest boxes had no impact on the number of microbial species grown in 2020 in Florida (P = 0.55) and Georgia (P = 0.29) nor in 2021 in Florida (P = 0.16) and Georgia (P = 0.31). Additionally, there was no impact from different shavings on the number of CFUs grown in 2020 in Florida (P = 0.31) and Georgia (P = 0.29) nor in 2021 in Florida (P = 0.35) and Georgia (P = 0.33; Table 2.4). In total, 29 boxes were sampled in Florida and 20 boxes in Georgia between 2020 and 2021. Numbers of species grown ranged from 1 to 8 morphologically different species in Florida and 3 to 8 morphologically different species in Georgia. The numbers of CFUs/egg grown in Florida ranged from 3 to 382 individual CFUs/egg, while in Georgia the amounts ranged from 1 CFU to 123 CFUs/egg.

DISCUSSION

In the Atlantic Flyway, every state currently has an active box-management program that is operated through both state and federal agencies (Bellrose 1990). Because wood ducks do not carry nesting material into boxes or natural cavities, Bellrose and Holm (1994) recommend covering the floor of each nest box with 8-10 cm of sawdust, wood shavings or wood chips. Further investigation concluded that sawdust should be avoided in nest boxes as it can lead to the suffocation of ducklings and addling of the eggs depending on the external temperature and humidity (Jones and Leopold 1967).

No other studies have compared box use between multiple nest mediums. Both estimates of box use for aspen and cedar filled boxes in Florida, and the cedar filled boxes in Georgia are like previously reported box use rates in the Atlantic Flyway of 36%, 42%, and 49% in the northern, central, and southern regions (Bellrose and Holm 1994). During both years of the study, I found that only boxes selected and used in Georgia during the 2020 season were dependent upon the type of shavings used in the boxes.

The dependence of box selection and use on shavings type in Georgia during 2020 is likely due to 2020 being the first year where nest boxes were actively being cleaned and managed at the three WMAs. In both Florida and Georgia boxes were cleaned at the start of the nesting season and the only data collected was the number of eggshell fragments (G. Balkom, Georgia Department of Natural Resources, personal communication; N. Bunting, Florida Fish and Wildlife Conservation Commission, personal communication. Boxes filled with aspen and cedar were selected at almost the same rate in Florida. All boxes are actively managed and cleaned by staff through the year which makes the boxes readily accessible for use.

I did not detect a relationship between the type of shavings used in nest boxes and the success of a nest. Estimates of nest success decreased in boxes with aspen and increased in boxes with cedar from 2020 to 2021 in Florida, but increases were seen in both aspen and cedar filled boxes in Georgia between 2020 and 2021. Boxes with aspen contained more successful nests only in Florida compared to Georgia where more successful nests came from boxes with cedar shavings. Despite this, there was no statistical difference discovered. While no significant correlation between the shavings type and nest success was determined, there are trends that can be explored in future studies as these results were likely impacted by unknown or untested endogenous and exogenous variables. At the start of the project, nest boxes were randomly selected to receive either treatment of aspen or cedar shavings. In Georgia, I believe that the selection of boxes was likely biased during the randomization in a way that caused one of the WMAs in Georgia to receive more cedar treatments than aspen, making it difficult to really compare between the two.

I did not detect a relationship between the type of shavings used in nest boxes and the successful exiting of ducklings from nest boxes. Across states and years, the mean number of ducklings exiting cedar-filled boxes were higher, but not statistically different form ducklings leaving aspen-filled boxes. The types of shavings used had no impact on the number of species, nor the CFUs/egg grown in either Florida or Georgia. While there was significant bacterial growth from nest boxes sampled in both 2020 and 2021, there is little evidence to conclude that the bacteria sampled are responsible for nest failures through the years.

The reuse of nest boxes and natural cavities is a commonly observed practice in many secondary-cavity nesting species like wood ducks (Walls et al. 2012). There are documented benefits to utilizing the nesting strategy, including the allowance for early knowledge about a location and subsequent nest initiation, the knowledge of the success or failure of a particular location, and the increase of reproductive success because of the prior mentioned benefits (Greenwood and Harvey 1982, Hepp and Kennamer 1992, Wiebe et al. 2007, Walls et al. 2012). Despite the knowledge that wood ducks reuse cavities and nest boxes, Utsey and Hepp (1997) reported that nest boxes that did not have previous nesting material cleaned out were used less and had fewer successful nests than those that were cleaned at least once during a nesting season.

Nest boxes managed during my study were maintained and cleaned throughout the breeding season. Whenever a nest was terminated, the box would be cleaned out and new shavings added to it; this likely led to significantly less bacterial loads in the boxes (Walls et al. 2012). Another factor to consider is the eggs themselves. Wood duck eggs, along with many other cavity-nesting species, contain levels of defense against microbial infection. These include high levels of antimicrobial proteins in both the albumen inside the egg and the cuticle of the eggshell itself (Wellman-Labadie et al. 2008*a, b*). It is likely that these adaptations to the eggs of cavity-nesting species were developed to account for the selection of nesting locations where high humidity and microbial contamination may influence egg survival. Wood ducks appear to have antimicrobial proteins in the albumin to ensure the development of the embryo in humid conditions (Wellman-Labadie et al. 2008*b*).

Box management programs are active across the United States, but especially in the Mississippi and Atlantic Flyways (Bellrose and Holm 1994). For box programs to be successful, it is estimated that regular maintenance and cleaning is required to increase hatching success (Utsey and Hepp 1997). While the nature of my field season allowed for nest boxes to be cleaned and refilled with new nesting material at the termination of every nest, this may not be feasible for managers operating box programs for state and federal agencies. At minimum, it is recommended by Utsey and Hepp (1997), that nest boxes be cleaned once at the start of the breeding season, again after the peak of nesting, and one final time immediately after the breeding season concludes.

The results of my study conclude that the types of shavings used in the nest boxes have no impact on box selection, nest success, ducklings exiting, or microbial communities collected from nest boxes. While Walls et al. (2012) found no relationship between egg viability and infection of eggshells by bacteria, their study was focused solely at the Savannah River Ecology Site in South Carolina. An expansion of this study design to include nest boxes across the Atlantic Flyway to examine microbial growth and its impact on eggs at different latitudinal levels. Additionally, it would be worth examining differences in microbial communities and the impacts they have on incubating eggs between nest boxes, which are cleaned out regularly, and natural cavities, which are not capable of being cleaned out. This comparison could then be used to determine nest success and subsequent recruitment of young females from natural cavities vs. nest boxes and if there is any impact from microbial communities.

The temperature that cultures were incubated at (23°C and 35°C) were both lower than the average nest temperature during incubation (36.3°C–37.4°C; Hepp et al. 2005). Future studies should increase the culture incubation to match this as there is a chance that my methods caused me to not grow microbes of concern. Another expansion should include the examination of oxygen levels within a nest and from there, attempting microbial growth in anaerobic conditions. Finally, no study has been done on the impacts of beneficial bacteria from wood duck nest boxes and the effects they have on development of embryos within eggs.

It would be worth examining differences in the antimicrobial capabilities of different species of cavity-nesting waterfowl at different latitudinal levels. It is reported that both wood ducks and hooded mergansers (*Lophodytes cucullatus*) have particularly strong antimicrobial proteins in their egg whites and eggshell cuticles (Wellman-Labadie et al. 2008*a*, *b*), but no research has been done on black-bellied whistling ducks (*Dendrocygna autumnalis*). Comparing the antimicrobial properties of the eggs of these three species across the Atlantic Flyway could provide more information on how these cavity-dwelling birds combat bacterial infection.

LITERATURE CITED

Bartholomew, J. W., and T. Mittwer. 1952. The Gram stain. Bacteriology Reviews 16: 1-29.

Bellrose, F. C. 1976. The comeback of the wood duck. Wildlife Society Bulletin 3: 107-110.

Bellrose, F. C. 1990. The history of wood duck management. Pages 13-20 *in* Proceedings of the 1988 North American Wood Duck Symposium. L. H. Fredrickson, G. V. Burger, S. P. Havera, D. A. Graber, R. E. Kirby, and T. S. Taylor, editors. St. Louis, Missouri, USA.

Bellrose, F. C., and D. J. Holm. 1994. Ecology and management of the wood duck. Stackpole Books, Mechanicsburg, Pennsylvania, USA.

Bent, A. C. 1925. Life histories of North American wild fowl. Order: Anseres (Part II). U. S. National Museum Bulletin 126. Washington, D. C., USA.

Beveridge, T. J., and J. A. Davies. 1983. Cellular responses of Bacillus subtilis and Escherichia coli to the Gram stain. Journal of Bacteriology 156: 846-858.

Clark, M., and J. R. Mason. 1985. Use of nest materials as insecticidal and anti-pathogenic agents by the European starling. Oecologia 67: 169-176.

Clawson, R. L., G. W. Hartman, and L. H. Fredrickson. 1979. Dump nesting in a Missouri wood duck population. Journal of Wildlife Management 43: 347-355.

Cook, M. I., S. R. Beissinger, G. A. Toranzos, R. A. Rodriguez, and W. J. Arendt. 2003. Trans-shell infection by pathogenic microorganisms reduces the shelf life of nonincubated bird's eggs: a constraint on the onset of incubation? Proceedings of the Royal Society of London. Series B: Biological Sciences 270: 223-224.

Cook, M. I., S. R. Beissinger, G. A. Toranzos, R. A. Rodriguez, and W. J. Arendt. 2005*a*. Microbial infection affects egg viability and incubation behavior in a tropical passerine. Behavioral Ecology 16: 30-36.

Cook, M. I., S. R. Beissinger, G. A. Toranzos, and W. J. Arendt. 2005*b*. Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. Ecology Letters 8: 532-537.

Cowardin, L. M., V. Carter, F. C. Golet, and E. T. LaRoe. 1979. Classification of wetlands and deepwater habitats of the United States. U. S. Fish and Wildlife Service. Washington, D.C., USA.

Cowardin, L. M., and R. J. Blohm. 1992. Breeding population inventories and measures of recruitment. Pages 423-445 *in* B. D. J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu, editors. Ecology and management of breeding waterfowl. University of Minnesota Press, Minnesota, USA.

Croft, G. D., R. M. Kaminski, E. P. Wiggers, P. D. Gerard, and G. K. Yarrow. 2020. Nest-box use by wood ducks and black-bellied whistling ducks in coastal South Carolina. Wildlife Society Bulletin 44:662-669.

Croft, G. D., R. M Kaminski, E. P. Wiggers, P. D. Gerard, and G. K. Yarrow. 2022. Box-nesting wood ducks and black-bellied whistling ducks in coastal South

Carolina. Journal of the Southeastern Association of Fish and Wildlife Agencies 9:in press.

Davis, J. B., S. E. Stephens, B. D. Leopold, R. M. Kaminski, and P. D. Gerard. 1999. Wood duck reproduction in small and large nest boxes in Mississippi: A continued experiment. Proceedings Annual Conference of Southeastern Association of Fish and Wildlife Agencies 53:257-269.

Davis, J. B., R. R. Cox Jr., R. M. Kaminski, and B. D. Leopold. 2007. Survival of wood duck ducklings and broods in Mississippi and Alabama. Journal of Wildlife Management 71:507-517.

Fredrickson, L. H., G. V. Burger, S. P. Havera, D. A. Graber, R. E, Kirby, and T. S. Taylor, editors. 1990. Proceedings of the North American wood duck symposium. St. Louis, Missouri, USA.

Greenwood, P. J., and P. H. Harvey. 1982. The natal and breeding dispersal of birds. Annual Review of Ecology and Systematics 13: 1-21.

Hepp, G. R., R. A. Kennamer, and W. F. Harvey, IV. 1989. Recruitment and natal philopatry of wood ducks. Ecology 70:897-903.

Hepp, G. R., and R. A. Kennamer. 1992. Characteristics and consequences of nest-site fidelity in wood ducks. The Auk 4: 812-818.

Hepp, G. R., and F. C. Bellrose. 1995. Wood duck (*Aix sponsa*). The birds of North America, number 169. The American Ornithologists' Union, Washington D.C., USA, and The Academy of Natural Sciences, Philadelphia, Pennsylvania, USA.

Hepp, G. R., T. Hayes-Folk, and C. A. Manlove. 2005. Nest temperature, incubation period, and investment decisions of incubating wood ducks *Aix sponsa*. Journal of Avian Biology 36: 523-530.

Hepp, G. R., R. A. Kennamer, and M. H. Johnson. 2006. Maternal effects in wood ducks: incubation temperature influences incubation period and neonate phenotype. Functional Ecology 20: 307-314.

Hepp, G. R., R. A. Gitzen, and R. A. Kennamer. 2020. Relative importance of vital rates to population dynamics of wood ducks. Journal of Wildlife Management 84:320-330.

Jacob, J. 1978. Uropygial gland secretions and feather waxes. Pages 165-211 *in* Chemical Zoology, Aves. Florkin, M., B. T. Sheer, and A. H. Brush, editors. Academic Press, New York City, New York, USA.

Johnston, W. H., J. J. Karchesy, G. H. Constantine, and A. M. Craig. 2001. Antimicrobial activity of some Pacific Northwest wood against anaerobic bacteria and yeast. Phytotherapy Research 15: 586-588.

Jones, R. E. 1964. Reproduction of the wood duck, *Aix sponsa*, in the Sacramento Valley, California. Thesis, University of California – Berkeley, Berkeley, California, USA.

Jones, R. E., and A. S. Leopold. 1967. Nesting interference in a dense population of wood ducks. Journal of Wildlife Management 31: 221-228.

Kennamer, R. A., W. F. Harvey IV, and G. R. Hepp. 1990. Embryonic development and nest attentiveness of wood ducks during egg laying. Condor 92: 587-592.

Lowney, M. S., and E. P. Hill. 1989. Wood duck nest sites in bottomland hardwood forests of Mississippi. Journal of Wildlife Management 53: 378-382.

Manlove, C. A., and G. R. Hepp. 2000. Patterns of nest attendance in female wood ducks. Condor 100: 688-693.

Menon, G. K., and J. Menon. 2000. Avian epidermal lipids: functional considerations and relationship to feathering. American Journal of Zoology 40: 540-552.

Nicolai, C. A. 2013. The Fallon wood duck project. U. S. Fish and Wildlife Service, Fallon, Nevada, USA.

O'Toole, G. 2016. Classic Spotlight: How the Gram stain works. Journal of Bacteriology 198: 3128.

R Core Team. 2021*a*. Kruskal-Wallis Test. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

R Core Team. 2021*b*. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

R Core Team. 2021*c*. Wilcoxon Rank Sum and Signed Rank Tests. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rohwer, F. C., and M. G. Anderson. 1988. Female-biased philopatry, monogamy, and the timing of pair formation in migratory waterfowl. Pages 187-221 *in* Current ornithology, Vol. 5. R. F. Johnston, editor. Plenum, New York, USA.

Shawkey, M. D., S. R. Pillai, and G. E. Hill. 2003. Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. Journal of Avian Biology 34: 345-349.

Stephens, S. E., R. M. Kaminski, B. D. Leopold, and P. D. Gerard. 1998. Reproduction of wood ducks in small and large nest boxes. Wildlife Society Bulletin 26:159-167.

Sweeney, R. J., I. J. Lovette, and E. L. Harvey. 2004. Evolutionary variation in feather waxes of passerine birds. Auk 121: 435-445.

U. S. Climate Data. 2020*a*. United States Climate Data for Eatonton, GA. https://www.usclimatedata.com/climate/eatonton/georgia/united-states/usga0877. Accessed 17 December 2021.

U. S. Climate Data. 2020*b*. United States Climate Data for Tallahassee, FL. https://www.usclimatedata.com/climate/tallahassee/florida/united-states/usfl0479. Accessed 17 December 2021.

Utsey, F. M., and G. R. Hepp. 1997. Frequency of nest box maintenance: effects on wood duck nesting in South Carolina. Journal of Wildlife Management 61: 801-807.

Vrtiska, M. P. 1995. Aspects of reproductive and remige molt biology in wood ducks. Dissertation, Mississippi State University, Starkville, Mississippi, USA.

Walls, J. G., G. R. Hepp, and L. G. Eckhardt. 2012. Effects of nest reuse and onset of incubation on microbial growth and viability of wood duck eggs. The Condor 114: 720-725.

Wellman-Labadie, O., J. Picman, and M. T. Hincke. 2008*a*. Antimicrobial activity of the anseriform outer eggshell and cuticle. Comparative Biochemistry and Physiology 149: 640-649.

Wellman-Labadie, O., J. Picman, and M. T. Hincke. 2008*b*. Enhanced c-type lysozyme content of wood duck (*Aix sponsa*) egg white: an adaptation to cavity nesting? Physiological and Biochemical Zoology 81: 235-245.

Wiebe, K. L., W. D. Koenig, and K. Martin. 2007. Costs and benefits of nest reuse versus excavation in cavity-nesting birds. Annales Zoologici Fennici 44: 209-217.

Wilson, S. F., and N. A. M. Verbeek. 1995. Patterns of wood duck nest temperatures during egg-laying and incubation. The Condor 97: 963-969.

Table 2.1. Frequency (*n*) of boxes selected by wood ducks that contained aspen and cedar, percentage (%) of boxes selected by wood ducks that contained aspen or cedar, standard error (% SE) of the percentage of selected boxes, and chi-square (χ^2) test of the null hypothesis[a] that frequencies of nest boxes used and not used by wood ducks (*Aix sponsa*) are independent of the type of wood shavings (nonaromatic aspen or aromatic cedar) used in the nest boxes in Georgia and Florida in 2020 and 2021.

			Aspen		Cedar	
Boxes	State	Year	*n*	%	*n*	%
Used	Florida	2020	64	45.1	66	46.5
		2021	61	44.2	62	44.9
	Georgia	2020	22	17.9	54	43.9
		2021	26	21.7	45	37.5
Not used	Florida	2020	3	2.1	9	6.3
		2021	4	2.9	11	8.0
	Georgia	2020	26	21.1	21	17.1
		2021	25	20.8	24	20.0

[a]Reject the null hypothesis in Georgia 2020 (χ_1^2 = 2.82, *P* = 0.004) and conclude that nest box use was dependent upon the type of shavings used, however I fail to reject the null hypothesis in Florida 2020 (χ_1^2 = 2.59, *P* = 0.108), Florida 2021 (χ_1^2 = 2.82, *P* = 0.09) and Georgia 2021 (χ_1^2 = 2.46, *P* = 0.116) and conclude that nest box use was independent of the type of shavings used in the nest boxes.

Table 2.2. Frequency (n) of successful nests containing aspen and cedar shavings, percentage (%) of successful nests containing aspen or cedar, standard error (% SE) of successful nests containing aspen or cedar, and chi-square (χ^2) test of the null hypothesis[a] that frequencies of successful and unsuccessful nests of wood ducks (*Aix sponsa*) would be independent of the type of shavings used within nest boxes between Georgia and Florida in 2020 and 2021.

			Aspen		Cedar	
Nest	State	Year	n	%	n	%
Successful	Florida	2020	57	23.6	40	16.5
		2021	44	21.8	41	20.3
	Georgia	2020	15	14.7	20	19.6
		2021	17	17.3	30	30.6

[a]Fail to reject the null hypothesis in Florida 2020 ($\chi_1^2 = 2.56$, P = 0.109), Florida 2021 ($\chi_1^2 = 0.003$, P = 0.958) Georgia 2020 ($\chi_1^2 = 0.296$, P = 0.586), and Georgia 2021 ($\chi_1^2 = 0.097$, P = 0.756) and conclude that nest success was independent of the type of shavings used in the nest boxes.

Table 2.3. Mean ($\bar{x}$) number and standard error (SE) of wood duck (*Aix sponsa*) ducklings per successful nest (n) in Florida and Georgia in 2020 and 2021. Statistics are for Mann-Whitney U tests (W, P) that tested the null hypothesis that the number of ducklings departing nest boxes would be independent of the type of shavings used in the nest boxes.

		Aspen		Cedar			
State	Year	n	$\bar{x}$	n	$\bar{x}$	W	P
Florida	2020	57	6.8	40	10.8	1,242.5	0.452
	2021	44	6.3	41	10.5	819.0	0.467
Georgia	2020	15	6.9	20	12.0	152.0	0.960
	2021	17	7.7	30	12.2	253.5	0.982

Table 2.4. Average amounts of colony forming units (CFUs) and morphologically different bacterial species (Spp.) from individual boxes (*n*) in Georgia and Florida between 2020 and 2021. Statistics are for Mann-Whitney U tests (*W*, *P*) that tested the null hypothesis that microbial growth would be independent of the type of shavings used in the nest boxes.

State	Treatment	Year	*n*	CFU	Spp	*W*	*P*
Florida	Aspen	2020	10	66.3	3.5	36	0.55
		2021	5	99.4	2.2	20	0.31
	Cedar	2020	6	149.7	2.6	30	0.16
		2021	8	24.0	1.7	27	0.35
Georgia	Aspen	2020	2	100.7	5.7	10	0.29
		2021	2	72.3	3.3	9.5	0.31
	Cedar	2020	6	45.7	3.0	10	0.29
		2021	11	31.6	0.7	10	0.33