Nest box-mounted PIT tag readers provide new insight on breeding behaviors of cavity-nesting waterfowl in Louisiana

by

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES	iii
ABSTRACT	1
INTRODUCTION	2
MATERIALS AND METHODS	4
RESULTS	14
DISCUSSION	19
CONCLUSION	22
ACKNOWLEDGMENTS	23
LITERATURE CITED	24

T	IST	\mathbf{OF}	FIG	HRES	AND	TABI	ES
1	/ 	\ / I '					/ L'/L 7

Sherburne Wildlife Area (North Farm and South Farm) outlined in yellow. Locations of nest box duplexes equipped with radio frequency identification (RFID) readers are represented by pink circles
Figure 2. Duplex-style nest box consisting of one new (left) and one old (right) nest box on a singular pole with a conical baffle. The waterproof container housing electrical components is ziptied below the nest boxes, while the solar panel is fastened to the top of a nest box with wood screws facing south. The loop-style antennas are zip-tied around the nest box entrance through four drilled holes, with antenna 1 affixed to the new nest box and antenna 2 affixed to the old nest box
Figure 3. Physical comparison of Black-bellied Whistling-Duck (<i>Dendrocygna autumnalis</i> [BBWD]; left) and Wood Duck (<i>Aix sponsa</i> [WODU]; right) ducklings. BBWD ducklings have a larger, broader bill and different facial markings than WODU, and their darker-colored markings are a distinct dark black compared to WODU, which are more of a dusty brown color
Figure 4. (A) Example of a web-tagged Wood Duck (<i>Aix sponsa</i> ; WODU) duckling. Half of all WODU ducklings received a web tag and half received a passive integrated transponder (PIT) tag as part of a tag retention study. (B) Example of the PIT-tagging process on a Black-bellied Whistling-Duck (<i>Dendrocygna autumnalis</i> ; BBWD) duckling. All BBWD ducklings were marked with PIT tags. Note that PIT tags cannot be visually observed after insertion because they are implanted under the skin
Figure 5. (A) Front of a radio frequency identification (RFID) circuit board with important components labeled. Note that the antenna leads are covered in electrical tape near the screw clamps to prevent the exposed wires from touching each other. (B) Back of RFID circuit board with important components labeled
Figure 6. Components of nest box-mounted radio frequency identification (RFID) readers. Note that the bulkier solar panel cable is run through a hole drilled into the container and sealed with epoxy putty, while the thinner antenna leads can be run through the lid or closure of the container without having to drill a hole. One of the antennas was color-coded with red tape to easily distinguish "Antenna 1" vs. "Antenna 2" when installing RFID readers in the field
Figure 7. Hand-wound loop antenna affixed to the nest box entrance with zip ties. I drilled four holes around the nest box entrance and passed a zip tie through each drilled hole and through the entrance itself to secure the antenna to the nest box

Figure 8. Density of nest initiation dates for BBWD and WODU from 2020-2022, calculated
using traditional box-monitoring methods, with the overlap between the two species highlighted
in gray. Note that although the two species overlap for a period of 95 days, BBWD did not begin
initiating nests until after WODU nest initiation reached its highest peak

Table 1. Total number of Wood Duck (*Aix sponsa*; WODU) and Black-bellied Whistling-Duck (*Dendrocygna* autumnalis; BBWD) adults detected by radio frequency identification (RFID) readers at Sherburne Wildlife Management Area (Iberville Parish, Louisiana) in 2022. Each species is categorized by whether individuals were captured by hand while physically monitoring nests in 2022. Note that a nontrivial percent of the individuals detected via RFID readers were not otherwise recaptured in 2022.

ABSTRACT

North American ducks are one of the most well-studied groups of organisms on the planet; however, we know astonishingly little about the nesting ecology of Black-bellied Whistling-Ducks (Dendrocygna autumnalis; hereafter, BBWD), which are rapidly expanding into the core of the eastern Wood Duck (Aix sponsa) range. Typical field methods used to study cavity-nesting waterfowl involve capturing and banding the incubating individual and collecting nest information (e.g., clutch size, nest age, presence of brood parasitism) at regular intervals. This precludes a full understanding of important breeding information including nest prospecting and parasitic egg laying and fails to detect nests that are terminated before discovery. Here, I quantified BBWD nest box visitation during the prospecting, laying, and incubation periods using subcutaneous passive integrated transponder (PIT) tags embedded in adults and ducklings and radio frequency identification (RFID) PIT tag readers mounted on nest boxes. I deployed 20 RFID readers on 40 duplex-style nest boxes from March–December 2022 at Sherburne Wildlife Management area with the potential to detect BBWD and WODU individuals that were previously marked with PIT tags from 2020–2022. I also conducted weekly nest visits where I recorded characteristics such as clutch size, nest age, incubation stage, and presence of intra- or interspecific nest parasitism. I detected 48 adults of both species via RFID readers, and 48% of individuals were never otherwise recaptured in 2022. I grouped BBWD into known pairs for analysis and found that pairs never visited nest boxes other than their own during the laying and incubation periods. I also determined that BBWD preferentially visited and nested in boxes that are >1 year old (t = 3.49, df = 20.30, p < 0.01), while WODU did not (t = 1.99, df = 18.37, p = 0.06). I found that traditional methods alone fail to document nearly half of the breeding population, meaning that recruitment estimates from previous nest box studies are woefully

underestimated. This, in addition to other breeding behaviors I observed, suggests that RFID technology can be used to reshape the way we study cavity-nesting waterfowl.

INTRODUCTION

North American ducks are one of the most well-studied groups of organisms on the planet; however, there is astonishingly little known about the ecology of Black-bellied Whistling-Ducks (*Dendrocygna autumnalis*; hereafter, BBWD), and most published studies are from south Texas populations in the 1970s. BBWD are a unique species, both taxonomically and behaviorally. The taxonomy of genus *Dendrocygna* (meaning "tree swan") has long been disputed but was more recently classified as separate from true ducks, geese, and swans (subfamilies Anatinae and Anserinae) and placed into the subfamily Dendrocygninae (Donne-Goussè et al. 2002). BBWD share many characteristics with geese and swans, including monomorphy, biparental brood care, long-term family grouping, and possibly perennial monogamy (Baldassarre 2014, Bolen 1971, Delnicki and Bolen 1976). BBWD have a more erect posture than most ducks, which is associated with a more terrestrial lifestyle similar to geese (Baldassarre 2014). However, BBWD are unique in that both sexes incubate the nest and they are facultative secondary cavity-nesters, with nests documented in natural cavities, manmade nest boxes, and on the ground (Bolen 1967, Delnicki and Bolen 1975, Markum and Baldassare 1989).

Intraspecific brood parasitism is a relatively common nesting strategy for waterfowl compared to other groups of birds (MacWhirter 1989, Yom-Tov 2001) and has been documented in BBWD on several occasions (James 2000, McCamant and Bolen 1979, Delnicki 1973).

Interspecific brood parasitism has been documented less frequently, with instances of BBWD parasitism of Wood Ducks (*Aix sponsa*; hereafter, WODU; Bolen and Cain 1968), Muscovy Ducks (*Cairina moschata*; Markum and Baldassare 1989a), and even Laughing Gulls (*Larus atricilla*; Ballard 2001). However, these instances were all documented in south Texas or

Mexico, within the historic range of BBWD. Recent research at Louisiana State University has indicated widespread and intensive rates of interspecific parasitism of WODU nests in Louisiana (Bakner et al. 2022), which has the potential to negatively affect WODU reproductive success as BBWD continue to expand into the United States.

Historically, BBWD occurred in Central and South America, with the northernmost breeding population occurring in south Texas (Baldassarre 2014). However, they have rapidly expanded their range into the United States in since the late 20th century (Cohen et al. 2019), with stable breeding populations throughout the southeast, extending as far north as Memphis, Tennessee (J. R. Henson, *unpublished data*) and wandering individuals observed as far north as central Quebec (eBird 2021). The expansion of BBWD, a generalist and highly competitive species, into the core breeding range of WODU is potentially a management concern for myriad reasons, including reduced egg hatchability and increased nest abandonment due to interspecific brood parasitism (Joyner 1976, Semel et al. 1988) and competition for nest sites (Charter et al. 2016) in areas where breeding phenology of both species overlaps (Croft et al. 2020). Very few peer-reviewed articles about BBWD nesting ecology outside of south Texas have been published (Bakner et al. 2022, Croft et al. 2020).

Traditional field methods used to study cavity-nesting waterfowl involve capturing and banding the incubating hen (both sexes in the case of BBWD) and collecting nest information such as clutch size, nest age, and apparent nest parasitism at regular (i.e., weekly) intervals. Because observations are only made at discrete intervals throughout the nesting period, these methods fail to capture potentially important breeding information, such as nest prospecting behavior, parasitic egg laying behavior, identification of nests that fail before they are discovered, and recruitment of non-incubating individuals. Failure to document non-incubating

individuals that may still contribute to population growth via parasitically-laid eggs may lead to inaccurate demographic estimates (i.e., recruitment) if a large portion of adults are missed.

Here, I used radio frequency identification (RFID) readers mounted on nest boxes to quantify breeding behaviors of BBWD and WODU marked with passive integrated transponder (PIT) tags, with an emphasis on BBWD. These RFID readers are continuously monitoring and record each time a PIT-tagged individual enters or leaves the nest box, potentially capturing information not possible with traditional methods alone. My objectives were to (1) quantify individuals not captured on a nest, (2) determine the duration of the BBWD nesting season using RFID data, (3) quantify BBWD behaviors during the prospecting, laying, and incubation periods, and (4) quantify nest box visitation for different box types.

MATERIALS AND METHODS

Study site

I monitored nest boxes installed by the Louisiana Department of Wildlife and Fisheries (LDWF) primarily for Wood Duck use, although Hooded Mergansers (*Lophodytes cucullatus*) and BBWD frequently nest in them. These nest boxes were located in Iberville Parish, Louisiana, on the Atchafalaya National Wildlife Refuge at two portions of Sherburne Wildlife Management Area known as "North Farm" and "South Farm", which are managed as moist soil impoundments for waterfowl (Figure 1). South Farm is open to the public, while North Farm is closed except for youth lottery hunts. Nest boxes were located on the side of levees and accessed by all-terrain vehicles or trucks. Sherburne WMA is one constituent site of a larger graduate research project on cavity-nesting ducks; since 2020, nests have been monitored weekly and ducks have been captured and marked in boxes.

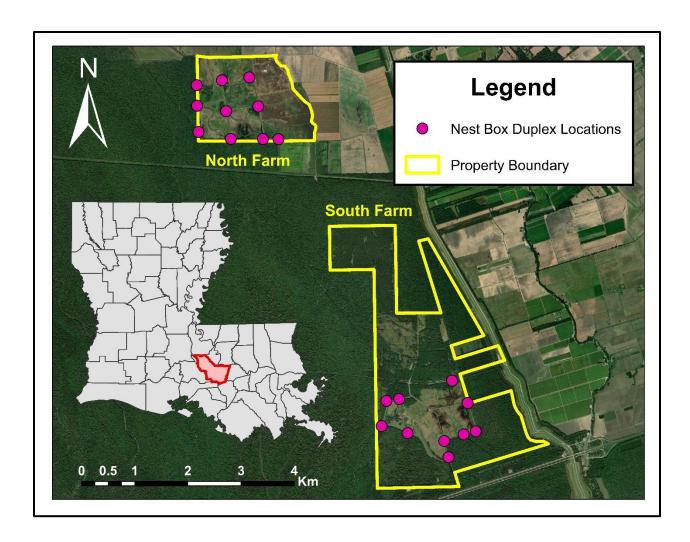


Figure 1. Map of Louisiana with Iberville Parish highlighted in red and study sites within Sherburne Wildlife Area (North Farm and South Farm) outlined in yellow. Locations of nest box duplexes equipped with radio frequency identification (RFID) readers are represented by pink circles.

In 2021 and 2022, I monitored 40 duplex-style nest boxes (two nest boxes mounted on either side of a pole). All units were singular nest boxes prior to February 2021; I added an additional nest box to each pole to accommodate the dual-antenna RFID readers at that time; new nest boxes were identical to the existing ones (Figure 2). I categorized nest boxes erected prior to 2021 as "old" and nest boxes erected in February 2021 as "new." 20 nest boxes were located at South Farm and 20 at North Farm; all duplexes were outfitted with a conical baffle predator guard (Figure 2).



Figure 2. Duplex-style nest box consisting of one new (left) and one old (right) nest box on a singular pole with a conical baffle. The waterproof container housing electrical components is ziptied below the nest boxes, while the solar panel is fastened to the top of a nest box with wood screws facing south. The loop-style antennas are zip-tied around the nest box entrance through four drilled holes, with antenna 1 affixed to the new nest box and antenna 2 affixed to the old nest box.

Nest monitoring

I collected data under U.S. Fish and Wildlife Service banding permit #06669 and Special Use Permit 43612-20-04; LDWF state collecting permits WDP-20-037 and WDP-21-060, and Wildlife Management Area Permit WL-Research-2020-03; protocols were approved by the Louisiana State University Institutional Animal Care and Use Committee Protocol A2019-27.

I visited nest boxes at approximately seven-day intervals in 2021 and 2022. I numbered each egg with a permanent marker in the order it appeared in the nest (Semel at al. 1988), determined the species of each egg (Bakner et al. 2022), and determined whether nests were active or inactive during each visit. I classified a nest as active if I observed a bird incubating,

new eggs were laid since the previous visit, or incubation progressed (Weller 1956). I classified inactive nests as abandoned, depredated, or successful. I considered a nest abandoned if egg laying or incubation were discontinued without sign of depredation. I classified a nest as depredated if egg laying or incubation ceased and eggs were destroyed or missing. Successful nests survived to hatch ≥ 1 egg; I counted the number of unhatched eggs and egg membranes to determine the number of eggs that hatched.

During nest visits, I attempted to capture the incubating individual by plugging the nest box entrance and removing the duck from the nest through the side door. If caught, I checked the individual for any markers, including passive integrated transponder (PIT) tags and leg bands. I detected PIT tags in the hand using a handheld reader (brand PROMAG) by running the device along the dorsal side of the bird several times. Individuals received the marker(s) they were lacking. I banded individuals using the appropriate-sized USGS band for their species and embedded 2×12 mm, 125kHz PIT tags (Cyntag part #601201) subcutaneously between the scapulae. I also took mass and tarsus measurements for each individual, determined age by plumage (Carney 1992), and determined sex by wing (WODU; Carney 1992) or cloacal examination (BBWD).

As the hatch date approached, I checked each nest every 1–3 days to ensure I was there to process ducklings because they typically leave the nest within 24 hours of hatching. For each duckling, I determined their species by plumage and bill shape (Figure 3), recorded mass and tarsus measurements, and marked them with the appropriate tag. I marked all BBWD ducklings with PIT tags in the same way as adults (Figure 4). As part of the ongoing graduate project, half of Wood Duck ducklings received a PIT tag, while the other half received a web tag applied using two small incisions on the webbing of the foot (Figure 4).



Figure 3. Physical comparison of Black-bellied Whistling-Duck (*Dendrocygna autumnalis* [BBWD]; left) and Wood Duck (*Aix sponsa* [WODU]; right) ducklings. BBWD ducklings have a larger, broader bill and different facial markings than WODU, and their darker-colored markings are a distinct dark black compared to WODU, which are more of a dusty brown color.

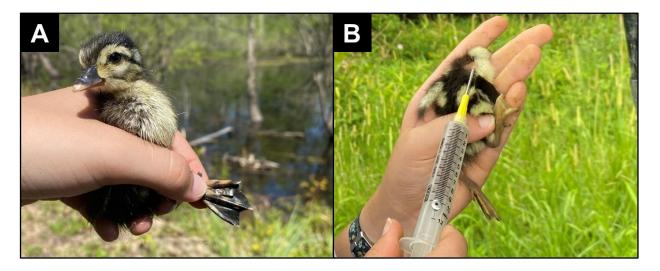


Figure 4. (**A**) Example of a web-tagged Wood Duck (*Aix sponsa*; WODU) duckling. Half of all WODU ducklings received a web tag and half received a passive integrated transponder (PIT) tag as part of a tag retention study. (**B**) Example of the PIT-tagging process on a Black-bellied Whistling-Duck (*Dendrocygna autumnalis*; BBWD) duckling. All BBWD ducklings were marked with PIT tags. Note that PIT tags cannot be visually observed after insertion because they are implanted under the skin.

RFID readers

I created stationary PIT tag readers using a custom radio frequency identification (RFID) circuit board (Bridge et al 2019). Each unit was equipped with two loop-style antennas, so that one circuit board could be used on a duplex-style nest box; antennas encircled the nest box entrance. I configured the units as a simple data logger that recorded the date, time, and alphanumeric ID of each individual's PIT tag as they entered or left the nest box.

I created the antennas using 26-gauge copper magnet wire and a 3D-printed jig to ensure the wire was wrapped in a uniform circle with an 11 cm diameter. PIT tags were particularly sensitive to antenna inductance, and deviation from optimal inductance resulted in failure of the antennas to detect PIT tags. Each antenna was tuned to 1.2 mH using a digital LCR meter and required 67–68 turns of the copper wire to reach that inductance. I found that different brands of wire varied in the number of turns needed to achieve 1.2 mH, so each brand was tested for the number of turns needed before creating a batch of antennas. Once the antenna wire was coiled, I wrapped it snugly with electrical tape (this step was important to keep consistent, as the tightness of the coil can change the antenna's inductance) and coated it with Plasti-Dip, leaving the free ends of the antenna exposed. I left the free ends to be ~1 meter long to ensure enough length between the antennas around the nest box entrance and the circuit board mounted below the box. Then, I used a fine-grit sandpaper to remove the insulation from the last ~1 cm of the antenna leads so that they could be connected to the circuit board. I attached the antenna leads to the circuit board's screw clamps with a small flathead screwdriver and covered any exposed areas of the wire that remained with electrical tape (Figure 5). I marked "antenna 1" with a small piece of red duct tape so they could be easily distinguished when installing the readers in the field (Figure 6).

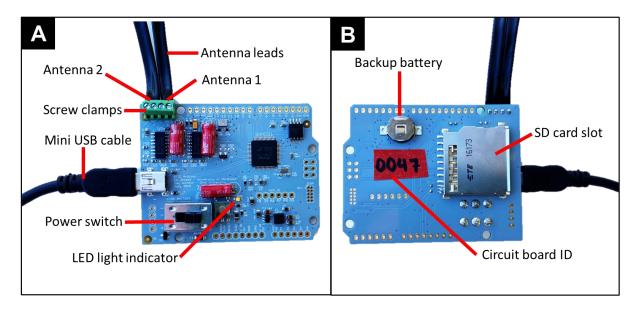


Figure 5. (A) Front of a radio frequency identification (RFID) circuit board with important components labeled. Note that the antenna leads are covered in electrical tape near the screw clamps to prevent the exposed wires from touching each other. (B) Back of RFID circuit board with important components labeled.

After attaching the antennas to the circuit board, I installed a 3V CR1025 battery to the back of the circuit board as a backup clock battery and loaded firmware to each board using the Arduino Integrated Development Environment. While installing the firmware, I ran a PIT tag through each antenna to ensure they both worked and assigned each circuit board a numeric ID that is displayed on the filename of data recorded to the SD card. I wrote the circuit board ID on a small piece of tape and attached it to the back of each circuit board.

To prepare each unit for the field, I first had to run a solar panel cable (Voltaic 3.5x1 mm extension cable – 4 ft) through a waterproof container so that the circuit board could be protected from water while the solar panel was mounted on top of the nest box. I used dry boxes from Outdoor Products initially but switched to Ziploc Twist 'n Loc (16oz) containers because they were much less expensive and easier to find. For both container types, I drilled a hole to run the solar panel cable through and sealed it using J-B Weld Waterweld epoxy putty. I then connected

the cable to the battery pack using an adapter (Voltaic V25 USB battery pack, 5V; Voltaic Female 3.5x1.1 mm MicroUSB adapter), connected the battery pack to the circuit board using a mini-USB cable, and inserted a FAT-formatted SD card into the circuit board. I left the antennas on the outside of the container; the antenna leads were able to run through the lids without having to drill a hole through the side of the container (Figure 6).

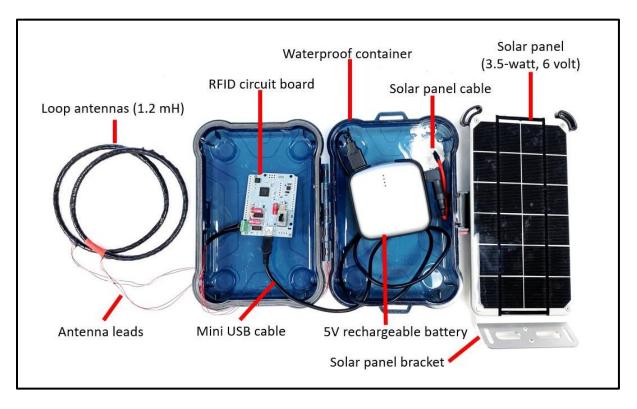


Figure 6. Components of nest box-mounted radio frequency identification (RFID) readers. Note that the bulkier solar panel cable is run through a hole drilled into the container and sealed with epoxy putty, while the thinner antenna leads can be run through the lid or closure of the container without having to drill a hole. One of the antennas was color-coded with red tape to easily distinguish "Antenna 1" vs. "Antenna 2" when installing RFID readers in the field.

I installed each unit on a duplex-style nest box in the field, using large zip ties to attach the waterproof container to the pole underneath the nest boxes. To attach the antennas, I drilled 4 holes around the next box entrance and zip tied the antenna around it (Figure 7). I then attached the cable extending from the waterproof container to the solar panel (Voltaic 3.5-watt, 6 volt; Voltaic solar panel bracket, small) and attached it to the top of the nest box using wood screws. I

installed each solar panel facing South (Figure 2). After installation, I turned on the circuit board and confirmed that it was working properly.



Figure 7. Hand-wound loop antenna affixed to the nest box entrance with zip ties. I drilled four holes around the nest box entrance and passed a zip tie through each drilled hole and through the entrance itself to secure the antenna to the nest box.

During weekly nest visits, I changed the SD cards in each unit and confirmed that the unit was still working properly. To do so, I turned off the unit, removed the SD card, turned it on, ran a designated PIT tag through each antenna, turned it back off, inserted a new SD card, and turned it back on. A small LED light on the circuit board indicated whether the tag was detected or not, and removing the SD card before testing ensured that the test PIT tag did not get recorded to the SD card memory, making it easier to input data later.

Post-processing and analysis

I conducted all analyses in R version 4.2.2 (R Core Team 2021). The original dataset generated by the RFID readers had numerous duplicate scans generated from an individual sitting at the nest box entrance for a long period of time. To remove these unnecessary detections, I removed consecutive scans within 15 seconds of one another, keeping the first one, using the dplyr package in R Wickham et al. 2022, R Core Team 2021). I assigned each BBWD individual as "paired" or "unpaired," with pairs defined as a male and female BBWD caught in the same nest box while the same nest was active. I could not conduct sex-specific analyses due to difficulty with accurate cloacal examination in previous field seasons, therefore, I assigned each pair an identification number for analysis. I also categorized each nest based on the presence or absence of conspecific brood parasitism. Parasitized nests received >1 BBWD egg per day during the laying stage (MacWhirter 1989) and/or additional eggs following day 4 of incubation, as BBWD initiate incubation about 3 days prior to laying the last egg (Delnicki 1973, Bakner et al. 2022).

I computed summary statistics of PIT tag detections for the prospecting, laying, and incubation periods. I defined each period based on empirical field data: the prospecting period is defined as the time period when a BBWD pair does not have an active nest of their own (this may include parasitic egg laying in addition to prospecting for their own nest site), the laying period as the interval between nest initiation and onset of incubation, and the incubation period as the time between start of incubation and the nest hatching or being abandoned/depredated. I calculated the duration of the BBWD nesting season in 2022 by subtracting the date of the earliest RFID BBWD observation from the latest and found the prospecting period for BBWD pairs by subtracting the earliest date from the latest date prospecting was observed by RFID readers for each pair. I calculated the duration of the 2022 WODU nesting season by subtracting

the date of the earliest WODU observation (determined from nest data, as RFID readers were not installed until after the first WODU nest was initiated) from the latest observation detected by RFID readers. Additionally, I conducted two-sample t-tests to determine whether BBWD and WODU preferred to visit old or new nest boxes during the prospecting period and used a p-value of 0.05 to indicate statistical significance. All data presented are mean \pm standard deviation.

RESULTS

I deployed 20 RFID reader units on 40 duplex-style nest boxes from March to December 2022, achieving 8,040 active scanner-nest box-days and 22,615 detections of BBWD and WODU. After excluding consecutive detections within 15 seconds of each other and detections of ducklings leaving the box post-hatch, there were 2,335 BBWD detections and 1,908 WODU detections remaining for analysis. I detected 48 total adults via RFID readers (Table 1). Given

Table 1. Total number of Wood Duck (*Aix sponsa*; WODU) and Black-bellied Whistling-Duck (*Dendrocygna* autumnalis; BBWD) adults detected by radio frequency identification (RFID) readers at Sherburne Wildlife Management Area (Iberville Parish, Louisiana) in 2022. Each species is categorized by whether individuals were captured by hand while physically monitoring nests in 2022. Note that a nontrivial percent of the individuals detected via RFID readers were not otherwise recaptured in 2022.

			Not captured in 2022		
Species	Total adults detected	Adults captured in 2022 ^a (%)	Adults ^b	Duckling recruits ^c	(%)
WODU	22	15 (68%)	6	1	(32%)
BBWD	26	10 (37%)	15	1	(62%)
Both	48	25 (52%)	21	2	(48%)

^a Adults captured in 2022 were captured by hand in a nest box but may have initially been tagged in 2020 or 2021.

^b Adults not captured in 2022 were captured in nest boxes and marked with passive integrated transponder (PIT) tags in 2020 or 2021 but were not captured by hand in 2022.

^c Duckling recruits were marked as day-old ducklings in 2020 or 2021 but were not captured by hand as an adult in 2022.

the scope of my study, my analyses included 9 confirmed pairs of BBWD unless otherwise noted; there are a few instances where I included WODU data to compare their behaviors to BBWD.

Duration of nesting season

RFID readers detected BBWD activity from 29 March to 16 September, indicating that the BBWD nesting season at these sites was 172 days long. RFID readers allowed me to detect an additional 49 days of nesting activity because field work ceased in July while BBWD were still initiating nests. I visualized the overlap in nest initiation for BBWD and WODU using data collected by traditional field methods from 2020–2022 (Figure 8).

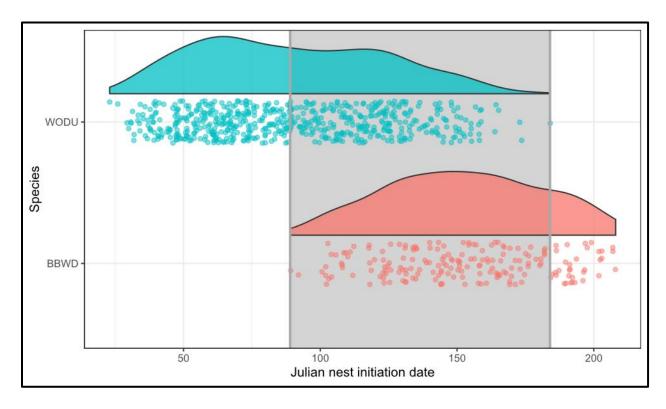


Figure 8. Density of nest initiation dates for BBWD and WODU from 2020–2022, calculated using traditional box-monitoring methods, with the overlap between the two species highlighted in gray. Note that although the two species overlap for a period of 95 days, BBWD did not begin initiating nests until after WODU nest initiation reached its highest peak.

I was unable to calculate the duration of the nesting season for WODU using RFID data because the first WODU nest was initiated 11 days before the first RFID readers were installed. However, the entirety of the WODU nesting season was captured by traditional nest monitoring, with nesting activity occurring from 11 February to 12 June, indicating that the WODU nesting season at my study sites was ~121 days long. This aligns with RFID data, which detected WODU activity from 11 February to 12 June, although 121 days is likely a low estimate because prospecting behaviors were missed by RFID readers preceding the earliest nest attempts.

RFID - prospecting period

RFID readers detected all 9 BBWD pairs during the prospecting period, garnering 228 observations of pair visits to nest boxes outside of their own nesting period. For BBWD pairs, the prospecting period was 74.2 ± 46.9 (range 8-131) days long, nearly double the span of a typical nesting attempt. Pairs visited an average of 5.9 ± 2.9 (range 2-11) unique nest boxes throughout the entire prospecting period and visited 0-4 boxes per day; on days when pairs were detected prospecting boxes, they visited an average of 1.3 ± 0.6 boxes that day.

RFID - laying period

RFID readers detected 5 BBWD pairs (3 additional pairs were not tagged until incubation) during the laying period at their own nests; pairs did not visit nest boxes other than their own during their laying period. Additionally, I documented 2 pairs and 2 unpaired BBWD individuals visiting laying-stage nests that were not their own (n = 6 nests); this occurred within their prospecting periods. These non-host pairs and individuals visited up to 2 laying-stage nests daily, and all (100%, n = 6) laying-stage nests visited by non-host BBWD were intraspecifically parasitized.

RFID – incubation period

RFID readers detected 8 pairs during the incubation period at their own nests; BBWD pairs did not visit nest boxes other than their own during their incubation period. Additionally, I observed 1 pair visiting incubation-stage nests that were not their own (n = 2 nests); this occurred within the pair's prospecting period. The non-host pair visited up to 2 incubation-stage nests daily, and all (100%, n = 2) incubation-stage nests visited by the non-host pair were intraspecifically parasitized. I also documented 3 unpaired BBWD individuals visiting incubation-stage nests that were not their own (n = 3 nests) during their prospecting periods; non-host individuals visited up to 1 incubation-stage nest per day. One (33%) incubation-stage nest visited by non-host BBWD individuals was intraspecifically parasitized.

Nest monitoring

In addition to collecting PIT tag reader data, I monitored nest boxes from 1 February until 28 July in 2022. I observed 17 BBWD nesting attempts in RFID reader-equipped nest boxes via weekly nest checks during the time they were installed; 70% (n = 12) nest attempts were detected by RFID readers. Nests that were not detected by RFID readers were either abandoned early into incubation (6%, n = 1) or initiated late enough into the field season (24%, n = 4) that the host pair were not captured and PIT-tagged. I determined fates for 11 (64%) nests. Of the nests with determined fates, 3 (27%) were abandoned and 8 (73%) were successful; no BBWD nests observed were depredated.

Nest box age

88% (n = 15) of all BBWD nests were initiated in old nest boxes. Excluding visits to their own nest box during the laying or incubation stage, BBWD individuals visited old nest boxes significantly more often than they visited new ones (Figure 9, t = 3.49, df = 20.30, p < 0.01).

50% (n = 10) of new nest boxes were visited by BBWD during the prospecting period, averaging 7.3 ± 13.4 visits per nest box. 90% (n = 18) of old nest boxes were visited by BBWD during the prospecting period, averaging 65.0 ± 72.7 visits per nest box.

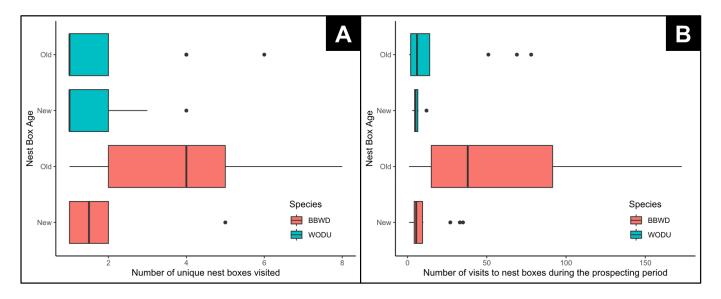


Figure 9. (A) Number of unique nest boxes visited by BBWD and WODU individuals across the entire nesting season in 2022, categorized by age of the nest box. Note that BBWD use a larger number of old nest boxes than new ones, while WODU visit a similar number of both. Across the entire breeding period, BBWD visited a greater number of nest boxes than WODU. (B) Number of unique visits to old and new nest boxes by BBWD and WODU individuals during the prospecting period only. BBWD visited nest boxes at a higher rate than WODU during the prospecting period.

This contrasts with WODU, where 52% (n = 15) of all nests were initiated in old nest boxes and they did not preferentially visit certain age nest boxes (t = 1.99, df = 18.37, p = 0.06). 45% (n = 9) of new nest boxes were visited by WODU during the prospecting period, averaging 1.6 ± 2.5 visits per nest box. 65% (n = 13) of old nest boxes were visited by WODU during the prospecting period, averaging 13.1 ± 25.0 visits per nest box. Overall, WODU individuals visited a significantly smaller number of unique nest boxes across the entire nesting season than BBWD (Figure 9, t = 3.01, df = 65.31, p < 0.01).

DISCUSSION

This is one of the first evaluations of BBWD nesting behaviors within the WODU core breeding range, and the first to use nest box-mounted RFID readers to observe BBWD behaviors not evaluated by traditional field methods. Nearly half of all adults detected by RFID readers were never otherwise recaptured in 2022, suggesting that traditional field methods for cavity nesting waterfowl are insufficient to fully understand their nesting strategies. These cryptic individuals indicate that recruitment estimates from previous nest box studies are biased low. RFID readers may be useful in creating correction factors for future studies to estimate recruitment more accurately in box-nesting populations.

I found that BBWD nesting activity occurred for a period of 172 days at my sites, beginning in March and concluding in September during the early teal hunting season. The first BBWD detection via RFID readers was on 29 March, nearly a month before the first nest was initiated on 27 April. Due to financial and logistical restraints, field work concluded in July while 4 BBWD nests were active; RFID reader data suggest that those nests were successful and an additional 2 nests were initiated and successfully hatched post-field season. Although nesting activity concluded at my sites following the onset of hunting pressure, class 1a broods (Southwick 1953) have been observed as late as November in urban areas where waterfowl hunting does not occur (K. E. Miranda and K. M. Ringelman, *personal observation*).

I found that unlike WODU, BBWD only visited their own nests during their laying and incubation periods. During the prospecting period, however, BBWD pairs visited up to 11 unique nest boxes, with ~1 nest box visited per day when pairs were actively prospecting. I also documented non-host BBWD pairs and individuals visiting 11 active BBWD nests during the laying or incubation period, and all but one of these nests were intraspecifically parasitized.

Because BBWD remain in family groups for long periods (Bolen et al. 1964), future research should aim to determine kinship of parasitic eggs and whether BBWD preferentially visit and/or lay in the nests of their kin (Nielsen et al. 2006). Blood samples were collected for every PIT-tagged adult and duckling in this study; thus, it would be possible to relate these results to kinship in the future.

The most surprising finding of this study was that BBWD strongly preferred older nest boxes, while WODU did not. This is interesting given that both old and new nest boxes were the same in every way except for weathering on the old boxes (Figure 2), although new boxes were weathered enough to look identical to old boxes by the end of the 2022 field season. Because each duplex consisted of one old and one new nest box mounted on the same pole, this phenomenon cannot be attributed to new nest box sites not being discovered by BBWD and suggests that fidelity is strongly associated with the specific nest box. Due to the generalist nature and rapid range expansion of BBWD, this preference was unexpected (Cohen et al. 2019). More research is needed to determine why this occurs and to explore the management implications of BBWD selection for older nest boxes.

Box-mounted RFID readers were particularly sensitive to construction parameters and environmental conditions. I attempted to conduct this study in 2021, but RFID reader performance was poor (they could not reliably read PIT tags in the field) and the data collected from them was unusable. After several months of trials and a new batch of circuit boards, I created new RFID readers that performed well just in time for the 2022 field season. I found that the antennas were particularly sensitive to inductance, and a deviation of ~0.1 mH from 1.2 mH rendered them unable to detect a PIT tag. I also tested several types of containers to house the circuit boards and found that the Outdoor Products dry boxes were the most durable overall but

let in more water than the less expensive Ziploc Twist n' Loc containers. When changing SD cards, I often found small amounts of condensation in the dry boxes, but moderate amounts of water did not seem to affect the performance of the RFID reader. The Ziploc containers, however, were more susceptible to UV damage and an RFID reader failed completely when a Ziploc lid disintegrated after several months of deployment and the container completely filled with rainwater. Overall, the dry boxes were more expensive but easier to use (they did not have a lid that could be lost) and more durable, but the Ziploc containers would work well for a short-term study or one in an area with less UV exposure.

An additional limitation of this study is the lack of sex-specific BBWD breeding behaviors. My analyses were focused at the pair level due to inconsistencies in determining an individual's sex in previous field seasons. Two confirmed pairs were sexed incorrectly, and not enough pairs were detected throughout all three periods of the nesting cycle to determine sex-specific patterns of prospecting and incubation. Clearly, cloacal examination is difficult for BBWD, so extra attention must be paid in the field when determining their sex.

Further research is needed to explore whether BBWD-WODU interactions, including competition for nest sites and interspecific nest parasitism, have significant effects on the WODU breeding population. Currently, BBWD do not begin initiating nests until after the peak of WODU nest initiation; this in conjunction with BBWD scarcely using newer nest boxes indicates that nest site competition is unlikely to negatively affect WODU, except at sites where boxes are particularly limiting. High rates of inter- and intra-specific nest parasitism, however, are more likely to negatively affect WODU reproductive output, but further investigation into this is beyond the scope of this study (Joyner 1976, Semel et al. 1988).

I observed several BBWD pairs that seemingly parasitized nests before initiating their own nests via RFID data; however, I have not confirmed maternity of the additional eggs laid to these nests. Nevertheless, this suggests a dual nesting strategy similar to Redheads (*Aythya americana*) parasitizing Canvasback (*Aythya valisineria*; Sorenson 1991) nests, but multiple years of data and genetically determined parentage are required to confirm this. In contrast to Sorenson (1991), I did not observe BBWD pairs that acted as parasites-only in 2022, but this may change between years as resource availability varies.

CONCLUSION

I observed WODU and BBWD nesting activity via traditional nest monitoring and nest box-mounted RFID readers from March–December 2022, with the potential to detect adults and ducklings that were marked with PIT tags since 2020. I found that nearly half of adults detected by RFID readers were cryptic; they were marked in previous years while nesting but were never otherwise recaptured in 2022. BBWD showed a strong preference for older nest boxes while WODU did not, which is surprising given that BBWD are generalists that are rapidly expanding their range. In addition, BBWD visit a larger number of nest boxes at a higher frequency than WODU; again, BBWD visitation is largely limited to older nest boxes. The observations made in this study would not have been possible using traditional methods alone, suggesting that RFID technology can reshape the way we study cavity-nesting waterfowl.

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LITERATURE CITED

- Bakner, D. L., K. E. Miranda, and K. M. Ringelman. 2022. Louisiana black-bellied whistling duck clutch characteristics in the presence of conspecific and interspecific brood parasitism. Journal of Field Ornithology 93(4):8.
- Baldassarre, G. 2014. Ducks, geese, and swans of North America. A Wildlife Management Institute Book, John Hopkins University Press, Baltimore, MD, USA.
- Ballard, B. M. 2001. Parasitism of a laughing gull nest by black-bellied whistling-ducks. Wilson Bulletin 113:339-340
- Bolen, E. G., B. McDaniel, and C. Cottam. 1964. Natural history of the black-bellied tree duck (*Dendrocygna autumnalis*) in southern Texas. Southwestern Naturalist 9(2):78-88.
- Bolen, E. G., and B. W. Cain. 1986. Mixed wood duck-tree duck clutch in Texas. Condor 70:389-390.
- Bolen. E. G. 1971. Pair-bond tenure in the black-bellied tree duck. Journal of wildlife Management 35:385-388.
- Bridge, E. S., J. Wilhelm, M. M. Pandit, A. Moreno, C. M. Curry, T. D. Pearson, D. S. Proppe,
 C. Holwerda, J. M. Eadie, T. F. Stair, A. C. Olson, B. E. Lyon, C. L. Branch, A. M.
 Pitera, D. Kozlovsky, B. R. Sonnenberg, V. V. Pravosudov, and J. E. Ruyle. 2019. An
 Arduino-based RFID platform for animal research. Frontiers in Ecology and Evolution
 7:257.
- Carney, S. M. 1992. Species, age, and sex identification of ducks using wing plumage. U. S. Department of the Interior, U. S. Fish and Wildlife Service, Washington, D. C., USA.

- Charter, M., I. Izhaki, Y. B. Mocha, and S. Kark. 2016. Nest-site competition between invasive and native cavity nesting birds and its implication for conservation. Journal of Environmental Management 181:129-134.
- Cohen, B. S., S. E. Askin, G. D. Balkcom, R. J. Benedict, J. A. Rader, J. D. James, B. A. Collier, and M. J. Chamberlain. 2019. Survival and distribution of black-bellied whistling-duck (*Dendrocygna autumnalis*) in the southeastern United States. Journal of the Southeastern Association of Fish and Wildlife Agencies 6:123-128.
- 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.
- Delnicki, D. 1973. Renesting, incubation behavior, and compound clutches of the black-bellied tree duck in southern Texas. M. S. thesis, Texas Tech University, Lubbock, USA.
- Delnicki, D., and E. G. Bolen. 1975. Natural nest site availability for black-bellied whistling-ducks in south Texas. Southwestern Naturalist 20(3):371-378.
- Delnicki, D., and E. G. Bolen. 1976. Renesting by the black-bellied whistling-duck. Auk 93(3):535-542.
- Donne-Goussè, C., V. Laudet, and C. Hanni. 2002. A molecular phylogeny of Anseriformes based on mitochondrial DNA analysis. Molecular Phylogenetics and Evolution 23:339-356.
- eBird. 2021. eBird: An online database of bird distribution and abundance [web application]. eBird, Cornell Lab of Ornithology, Ithaca, New York. http://www.ebird.org

- James, J. D. 2000. Effects of habitat and spatial characteristics on the incidence of conspecific brood parasitism and nest site selection in breeding black-bellied whistling-ducks. M. S. thesis, Texas A&M University, Kingsville, USA.
- Joyner, D. E. 1976. Effects of interspecific nest parasitism by redheads and ruddy ducks. Journal of Wildlife Management 40:33-38.
- MacWhirter, R. B. 1989. Minireview: on the rarity of intraspecific brood parasitism. Condor 91:485-492.
- Markum, D. E., and G. A. Baldassarre. 1989a. Breeding biology of Muscovy ducks using nest boxes in Mexico. Wilson Bulletin 101:621-626.
- Markum. D. E., and G. A. Baldassarre. 1989b. Ground nesting by black-bellied whistling-ducks on islands in Mexico. Journal of Wildlife Management 53:707-713.
- McCamant, R. E., and E. G. Bolen. 1979. A 12-year study of nest box utilization by black-bellied whistling ducks. Journal of Wildlife Management 43:936-943.
- Nielsen, C. R., R. J. Gates, and P. G. Parker. 2006. Intraspecific nest parasitism of wood ducks in natural cavities: comparisons with nest boxes. Journal of Wildlife Management 70:845-843.
- R Core Team. 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- Semel, B., P.W. Sherman, and S. M. Byers. 1988. Effects of brood parasitism and nest-box placement on wood duck breeding ecology. Condor 90:920-930.

- Sorenson, M. D. 1991. The functional significance of parasitic egg laying and typical nesting in redhead ducks: an analysis of individual behavior. Animal Behavior 42:771-796.
- Southwick, C. 1953. A system of age classification for field studies of waterfowl broods. Journal of Wildlife Management 17:1-8.
- Weller, M. W. 1956. A simple field candler for waterfowl eggs. Journal of Wildlife Management 20:111-113.
- Wickham, H., R. François, L. Henry, and K. Müller. 2022. dplyr: A grammar of data manipulation. R package version 1.0.10. https://CRAN.R-project.org/package=dplyr
- Yom-Tov, Y. 2001. An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. Ibis 143:133-143.