The Cost of Incubation: Manipulating Nest Microclimate and Examining Nest Site Selection to Understand Energetic Tradeoffs during Incubation in Wood Ducks (*Aix sponsa*)

by

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Abstract

Incubating Wood Ducks (*Aix sponsa*), face tradeoffs between time spent incubating with time spent foraging. To explore the costs and investment strategies of incubating female wood ducks I (a) manipulated the microclimate of nests by reducing down insulation from 4.0 g to 0.5 g, and (b) examined the relationship between nest-site microclimate with nest site selection. Females with reduced down faced increased cooling rats, and therefore shortened morning recesses and increased daily incubation constancy. Behavioral changes occurred progressively through the incubation period and were not influenced by female body mass. Overall clutch temperatures did not differ between treatments and correspondingly, there were no differences in length of the incubation period, hatching success, or ducking phenotypes. I detected a novel level of plasticity in daily incubation behaviors of female wood ducks in response to variations in clutch cooling rates and ambient temperatures. Nest site selection was positively associated with higher early season nest-box temperature variability. Further, warmer nest-boxes were initiated earlier in the early-breeding season and were less likely to be abandoned. Females who had previously experience in nest-boxes selected nest-boxes that were cooler than available nearby boxes, where new females or females without previous experience in selected nest-boxes selected boxes randomly without relation to nest-box microclimate. In this study, I found evidence of female wood ducks making investment decisions, both in changing incubation behaviors and selecting for warm nesting boxes, in order to balance energy between conflicting needs between them and their offspring.

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Plasticity of incubation behaviour: using nest microclimate to understand energetic tradeoffs of female wood ducks

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Abstract

Optimal development of avian embryos occurs within a narrow range of incubation temperatures. Most incubating parents are challenged to balance their time on the nest while taking foraging recesses to satisfy their energetic requirements. To explore the costs and investment strategies of incubating female wood ducks (*Aix sponsa*), we manipulated the microclimate of nests by reducing down insulation from 4.0 g to 0.5 g. Cooling rates of clutches during morning recesses increased when down insulation was reduced, especially at low ambient temperatures. Females with reduced down responded to increased cooling rates by shortening morning recesses and increasing daily incubation constancy. Behavioural changes occurred progressively through the incubation period; however, the ability to make these changes was not influenced by female body mass. Females in both groups also responded to changes in ambient temperature by spending less time incubating as ambient temperatures increased. These behavioural modifications influenced incubation temperature. Clutch temperatures at the end of morning recesses were similar for females with reduced and normal insulation. Further, average clutch temperatures for the full incubation period did not differ between treatments and correspondingly, there were no differences in length of the incubation period, hatching success, or ducking phenotypes. Our study was able to detect a novel level of plasticity in daily incubation behaviours of female wood ducks in response to variations in clutch cooling rates and ambient temperatures. Behavioural adjustments made by females kept the incubation environment in the optimal range for embryo development without negatively impacting female body condition.

Keywords: Aix sponsa; cost of reproduction; incubation behaviours; incubation temperature; trade-offs; wood duck.

Introduction

Incubating birds must balance the competing demands of satisfying their own energetic requirements with maintaining proper thermal environments for developing embryos. Avian eggs begin to develop at 24-27°C, but optimal growth and development of embryo occurs between 36-38°C (Webb 1987). Reduced incubation temperature affects offspring phenotype and slows embryonic development leading to longer incubation periods and increased risk of nest predation (Reid et al. 2002; Hepp et al. 2006; Olson et al. 2006). Incubation is metabolically expensive, and incubation recesses allow time for females to forage and meet energetic costs (Reid et al. 2002; Tinbergen and Williams 2002). Evolution of passerine incubation rhythms between species have been shown to be shaped by various constraints, including food availability, temperature, and nest predation risk (Conway and Martin 2000a). Within species, avian incubation rhythms also appear to be plastic, and have been seen to respond to variables such as female condition (Crisculo et al. 2002), predation risk (Ghalambor et al. 2013), ambient temperature (Conway and Martin 2000b), and egg temperature (Reid et al. 2002). Number, length, and timing of recesses can influence incubation temperatures and metabolic cost of incubation (Deeming and Ferguson 1991; Martin et al. 2007).

Reproductive costs often are best estimated using manipulative experiments (Reznick 1985). Studies of incubation costs, for example, have manipulated clutch size, length of the incubation period, or thermal property of nests. Enlarging clutch size resulted in longer incubation periods for pied flycatchers (*Ficedula hypoleuca*; Nord and Nilsson 2012) and northern lapwings (*Vanellus vanellus;* Larsen et al. 2003), reduced immune function, body mass, and future fecundity of incubating common eiders (*Somateria mollissima;* Hanssen et al. 2005), and decreased future survival and reproduction in great tits (*Parus major;* de Heij et al. 2006). Manipulative lengthening of incubation periods caused an increased loss of body mass and reduced hatching success in barnacle geese (*Branta leucopsis;* Tombre and Erikstad, 1996). Artificial removal of contour feathers have been shown to increase nest attendance and shorten length of daily recesses in barn swallows (*Hirundo rustica;* Moller 1991), as well as slow hatching growth (Lombardo et al 1995, Dawson et al. 2011). Perez et al. (2008) and Ardia et al. (2009) heated nest boxes to change the thermal property of nests and therefore reduced energetic constraints of incubating barn swallows (*Hirundo rustica*). Females of heated nest boxes increased incubation constancy and egg temperature, ended incubation in better body condition, and raised nestlings that were larger and in better condition than females of unheated nest boxes (Perez et al. 2008, Ardia et al. 2009). Recent studies make it clear that incubation is an important reproductive cost in birds that can limit both current and future reproductive success and potentially influence the evolution of avian life histories (Heaney and Monaghan, 1996, Reid et al. 2000, 2002, Hepp and Kennamer 2011, 2012, DuRant et al. 2013).

The wood duck (*Aix sponsa)* is a relatively small-bodied, cavity-nesting species. Females alone incubate, take two recesses each day, and spend > 80% of the day on the nest (Manlove and Hepp 2000, Folk and Hepp 2003). During egg-laying, females remove feather down from their belly and breast to cover eggs during incubation recesses. Incubation temperature ranges between 34.8-37.8^{o}C, and incubation period averages 32 days (Hepp et al. 2006). Low incubation temperature has been shown to negatively impact offspring quality in wood ducks. Embryos of eggs that were artificially incubated at temperatures < 35.9°C developed slowly, expended more energy, and hatched with reduced nutrient reserves compared to those incubated at higher temperatures (Hepp et al. 2006; Durant et al. 2011a). Further, these ducklings had higher baseline and stress induced corticosterone levels (Durant et al. 2009), decreased growth and acquired immunity responses (Durant et al. 2011b), reduced locomotor performance (Hopkins et al. 2011), and expended more energy to thermoregulate (Durant et al. 2011c) than ducklings from eggs incubated at higher temperatures. Effects of low incubation temperature (< 35.9°C) carried-over to subsequent life-history stages and negatively impacted survival, recruitment to the breeding population, and reproductive success of female wood ducks (Hepp and Kennamer 2012). Current evidence suggests that incubation is an important reproductive cost in wood ducks, but nesting date and female quality may help to mediate these costs (Hepp et al. 1990, 2005, 2011).

In this study, we further investigated incubation costs of wood ducks by altering the thermal properties of naturally-incubated nests. We manipulated the amount of down insulation in nests and examined trade-offs between female incubation behavior, incubation temperature, and length of the incubation period. We compared females with reduced levels of insulative down (0.5 g) to females with normal levels of down (4.0 g). We predicted that nests with reduced insulation would cool faster during incubation recesses. Because we know that low incubation temperature in wood ducks lengthens the incubation period and influences offspring quality (DuRant et al. 2013), we predicted that females with reduced insulation would seek to maintain optimal incubation temperature by taking shorter incubation recesses and spending more time on the nest. However, investment decisions of incubating females should be condition-dependent. Therefore, we predicted that females starting incubation at relatively low body mass would be more likely to invest in self-maintenance than in maintaining proper egg temperatures.

Methods

We conducted the study at the Department of Energy's Savannah River Site in west-central South Carolina (33^{o}1'N, 81^{o}3'W) in January-July 2011 and 2012. Nest boxes at Par Pond (N = 87) and Carolina Bays (N = 24), were checked for nesting activity every four days. When nests were located, eggs were measured (length and breadth, mm), and chronologically labeled with a permanent marker to record laying order within the clutch. Wood ducks lay one egg per day (Drobney 1980), and date of nest initiation was estimated by subtracting the number of eggs in the nest when it was first discovered from the date the nest was checked (Hepp et al. 1990). Brood parasitism is common in wood ducks, so when number of eggs in initiated nests outnumbered the days between nest checks, the day after previous check was assumed to be the day of nest initiation.

Incubating females were captured during the first and last week of incubation. At first capture, unmarked females were banded with U.S. Fish and Wildlife Service leg bands and aged as adults (ASY) or yearlings (SY; Harvey et al. 1989). All females were weighed to the nearest 5 g with a 1,000 g Pesola scale, and tarsus length (nearest 0.1 mm), wing length from wrist to edge of longest primary (nearest 1 mm), and culmen length (nearest 0.1 mm) were measured.

Incubation behaviour, clutch temperature and cooling rate, and ambient temperature.-- We installed temperature data loggers (HOBO U23, Pro v2, Onset Computer Corp, Bourne, MA, USA) in nests during egg-laying to monitor incubation behaviours (Manlove and Hepp 1998, 2000). Briefly, all nesting material was removed, and a platform containing a wooden egg with an embedded thermistor was placed in the nest box. The wooden egg was securely fastened to the platform so that the female could not reposition the egg, and the egg with the thermistor was placed in the center of the nest. Nesting material and eggs were returned to the nest, and the wooden egg was positioned so that the thermistor would come in direct contact with the incubating female's brood patch. These loggers recorded temperatures every 6.4 minutes (N = 225 day^{-1}). Temperature data were downloaded and plotted for each 24-hr period. Data from these plots provided an accurate method of determining presence and absence of incubating females (Manlove and Hepp 2000). We used these data to compute morning recess length (min), incubation constancy (% of day spent on the nest incubating), and average daily clutch temperature. Any days that we disturbed the hen's natural incubation rhythm, including days when captures were attempted, were not used in our analysis. In 2012, an iButton (i-Button, Maxim Integrated Products, Sunnyvalle CA) was attached with epoxy to the blunt end of the wooden egg to calculate clutch cooling rates (°C/minute) during morning incubation recesses. This data logger was positioned in the nest at the same level as the rest of the eggs and recorded temperature every 6 minutes (N = 240 day^{-1}).

We installed iButtons inside nest boxes in a standardized location at the back of the box 10 cm below the entrance hole to measure ambient nest box temperature (± 0.5 °C) every 30 minutes (N= 48 day^{-1}). From these, daily ambient temperature and morning ambient temperature were calculated for each box. We used a time period from 5am to 10am for morning temperature calculations to mimic morning activity period (Bellrose and Holm 1994).

Manipulation of down insulation.— We considered full incubation to have started when females began spending portions of both the day and night on the nest. On the morning of the fifth day of full incubation, we manipulated the amount of down insulation in nests to either normal (4.0g down) or reduced amounts (0.5g down). All insulation in the nest was removed and replaced with cleaned, pre-measured down. The normal amount of down was similar to amounts measured previously in natural nests on Par Pond (3.8 g ± 1.2; N = 12). Manipulations of down were completed when females were away from the nest during early morning recesses to minimize disturbance to incubating females. A pilot study in 2011 showed that females (*N* = 10) with reduced down compensated very little for the loss of down, adding just 0.03 ±0.17g. At this point, we also standardized the clutch to the first 12 viable eggs. In subsequent visits to the nest, any new eggs were removed, because nests are sometimes parasitized after the start of incubation.

We chose to reduce down to 0.5 g because it successfully increased cooling rates across different ambient temperatures while still leaving enough down to allow for a thin layer to cover the eggs. Egg cooling rates for 4.0 g and 0.5 g of down were tested in environmental chambers (Environmental Growth Chambers, Chagrin Falls, OH) with temperature set at 7, 15, and 22°C (*N* = 6, *N* = 10, and *N* = 10, respectively). Clutches of 12 undeveloped wood duck eggs were warmed in incubators to an average temperature of 37.6°C. To accurately measure egg temperatures, we drilled a small hole in one of the eggs, placed a thermistor in the albumen and sealed the hole with silicone gel. Warmed clutches were placed immediately into nest boxes in the environmental chamber. Egg temperature was recorded every 1.2 min (Stowaway, Onset Computer Corp, Bourne, MA, USA), and eggs were allowed to cool for two hours. Cooling rates from these clutches were linearly estimated and averaged for each treatment and ambient temperature group.

Body composition of ducklings- Eggs were randomly selected from both early (days 2-5) and late (days 7-9) laying sequence and removed from each experimental nest when females were captured during the last week of incubation in 2012. Eggs were brought to the lab and placed in an incubator at 37.6 °C. When eggs pipped, they were transferred to a brooder to hatch. Hatched ducklings were weighed, sexed, and tarsus and culmen were measured within 12 h of hatching. Ducklings were euthanized using CO_2 inhalation and cervical dislocation in accordance with approved IACUC (PRN 2010-1691) protocols. Ducklings were placed in individually-labeled plastic bags and frozen for later processing.

Duckling body composition was determined using methods of Hepp et al. (2006). Ducklings were thawed and dried (65°C) to constant mass. Dry ducklings were ground and neutral lipids were extracted from whole ducklings in a Soxhlet apparatus using petroleum ether (Dobush et al. 1985). Lean samples were dried to constant mass, and lipid content was the difference in mass of the dry sample before and after lipid extraction. Total lipid mass was calculated by multiplying the proportion of lipid in the dry, ground sample by the dry mass of the whole duckling. Lean dry mass was calculated by multiplying the proportion of non-lipid material in the dry, ground sample after lipid extraction and dry duckling mass. Lean dry samples were burned in a muffle furnace at 550°C for 10 h to determine ash content. Ash-free lean dry mass (AFLDM) was calculated as the difference between ash content of burned sample and lean dry mass. The proportion of AFLDM in the sample multiplied by the dry mass of the duckling provided an estimate of duckling’s protein mass.

Statistical analysis- All statistical tests were completed using R 2.15.2 for Windows (The R Foundation for Statistical Computing, 2012). Cooling rates of clutches in nest boxes during morning recesses in 2012 were estimated using mixed linear models (program NLME in R

2.15.2; Pinheiro and Bates 2000) and restricted maximum-likelihood (REML) methods. Insulation treatment and ambient temperature were fixed effects, nests were random effects, and repeated measures were used within nests. Interaction of insulation treatment and ambient temperature during the morning activity period tested whether slopes were homogeneous.

Mixed linear models with repeated measured were fitted with REML and used to test effects of the insulation treatment on daily morning recess length, incubation constancy, and average clutch temperature. Models included fixed effects of insulation treatment, average daily ambient nest box temperature, day of incubation, early incubation body mass, as well as the interaction between incubation day and treatment. Individual females and repeated daily measures within nests through the incubation period were accounted for using random effects. Data were considered to be autoregressive in consecutive incubation days, so an appropriate correlation structure was used (corAR1, Pinherio and Bates 2000). Least-squares means were calculated and Tukey-Kramer methods were used to test for differences between means (program lsmeans in R12.15.2; Lenth 2013).

Linear models (program LM, R2.15.2) were built to characterize responses of incubation period, change in female body mass during incubation, and hatching success to insulation treatment while accounting for variation due to date of nest initiation. Body composition of ducklings included measures of wet mass, dry mass, lipid, protein, and ash content. Effects of insulation treatment on duckling body components were analyzed using mixed linear models. These models accounted for the fixed effect of insulation treatment and fresh egg mass, the random effect of nest, and repeated measures of eggs within nests.

Results

Nests were randomly assigned to treatments in 2011 and 2012, and we manipulated the amount of insulation in wood duck nests (normal insulation, 4.0 g, $N = 16$; low insulation, 0.5 g, $N = 18$) on day 5.12 ± 0.16 of incubation. Nest initiation date did not differ between treatments ($F_{1,32} = 0.32$, $P = 0.58$) and averaged 12 March ± 12 days (range 19 February- 12 April). Early incubation body mass of females was 567.4 ± 5.47 g and did not differ between treatments ($F_{1,32} = 0.06$, $P = 0.81$).

Cooling Rate

We measured cooling rates of 19 clutches (N = 8 normal and N = 11 reduced insulation) during 446 morning recesses. Insulation treatment ($F_{1,17} = 10.61$, $P = 0.001$), ambient box temperature ($F_{1,425} = 118.81$, $P <0.0001$), and the interaction of treatment and ambient box temperature ($F_{1,425} = 10.62$, $P = 0.001$) influenced clutch cooling rates. Clutch temperature declined more slowly with increasing ambient box temperature for nests with normal insulation (-0.00334 ± 0.00031 °C/min) than for nests with reduced insulation (-0.00460 ± 0.00039 °C/min. Least-squares mean cooling rate was greater for clutches with reduced insulation (-0.082 ± 0.004°C/min) compared to clutches with normal insulation (-0.068 ± 0.005°C/min; $Z = 2.33$, $P = 0.02$).Our experiments with egg cooling rates in environmental chambers showed similar effects of insulation treatment and ambient temperature on cooling rate (Fig. 1). Cooling rates were greater for eggs with reduced insulation, and differences increased as ambient temperature declined.

Morning Recess Length and Incubation Constancy

Daily incubation behaviours were measured for 34 nests ($N = 16$ normal and $N = 18$ reduced insulation) for a total of 686 days. Insulation treatment ($F_{1,31} = 0.51$, $P = 0.48$) and incubation day ($F_{1,649} = 1.49$, $P = 0.22$) did not affect morning recess length, but there was a significant interaction of treatment and incubation day ($F_{1,649}$ = 7.66, P = 0.006). Morning recess length did not vary with incubation day for females with normal levels of insulation (β = -0.35 ± 0.29); however, morning recess length of females incubating nests with reduced insulation declined as incubation day progressed (*β* = -1.08 ± 0.39 min; Figure 2.). Incubating females were sensitive to ambient temperature, and length of morning recesses increased with increasing ambient temperatures (β = 0.69 ± 0.33 min, $F_{1,649}$ = 4.27, P = 0.039). Female body mass did not affect morning recess length ($F_{1,31}$ = 0.12, P = 0.73). Least-squares mean morning recess length was shorter for females with reduced insulation (93.50 ± 5.73 min) than for females with normal insulation (106.72 ± 5.80 min) but differences were not significant (Z = 1.62, P = 0.11). Temperature of clutches at the end of morning recesses did not differ between nests with normal (30.19 ± 0.18 ºC) and reduced insulation (30.23 ± 0.16 ºC; $F_{1,17}$ = 0.017, P = 0.90).

In these same nests, daily incubation constancy averaged 83.0 ± 6% (range 59.5% to 97.3%). Insulation treatment ($F_{1,31}$ = 0.27, P = 0.61) and incubation day ($F_{1,649}$ = 0.04, P = 0.84) did not affect daily incubation constancy, but there was a significant interaction between insulation treatment and day of incubation ($F_{1,649}$ = 9.71, P = 0.002). Incubation constancy did not vary with day of incubation for females with normal amounts of insulation (β = -0.0093 ± 0.045); however, incubation constancy increased with day of incubation for females with reduced insulation (β = 0.190 ± 0.061; Figure 3). Decreasing ambient temperatures lead to an increase in incubation constancy (β = 0.304 ± 0.047, $F_{1,649}$ = 44.22, $P \leq 0.0001$), but female body mass had no effect on incubation constancy ($F_{1,31}$ = 0.61, P = 0.44). Least-squares mean incubation constancy was greater for females with reduced insulation (84.6 ± 0.1%) than for females with normal insulation (81.6 ± 0.1%; Z = -2.13, P = 0.03).

Incubation Temperature, Incubation Period, and Duckling Phenotype

Daily clutch temperature for both treatment groups averaged 36.79 ± 0.93°C ($N = 34$; range = 29.04°C - 38.87°C). Insulation treatment ($F_{1,31} = 1.38$, $P = 0.25$), incubation day ($F_{1,649} = 2.49$, $P = 0.93$), and the interaction of treatment and incubation day ($F_{1,649} = 1.25$, $P = 0.26$) did not affect daily clutch temperature. Ambient temperature had strong positive effects on daily clutch temperature ($\beta = 0.057 \pm 0.007$°C, $F_{1,649} = 63.55$, $P < 0.0001$), but there was no effect of female body mass on daily clutch temperature ($F_{1,31} = 0.007$, $P = 0.94$).

Nest-averaged incubation temperature for all nests (both normal and reduced insulation) ranged from 35.33 - 38.32°C, averaging 36.81 ± 0.70°C. Average incubation temperature was not affected by insulation treatment ($F_{1,31} = 0.29$, $P = 0.59$), even after accounting for nest initiation date. Length of the incubation period ($F_{1,31} = 0.17$, $P = 0.68$), hatching success ($F_{1,31} = 0.24$, $P = 0.63$), and percentage change in female body mass during incubation ($F_{1,31} = 1.20$, $P = 0.28$) were not affected by insulation treatment (Table 1). Using fresh egg mass as the covariate, duckling body mass ($F_{1,23} = 0.05$, $P = 0.82$) and body components (lipid: $F_{1,21} = 0.40$, $P = 0.53$; protein: $F_{1,21} = 1.50$, $P = 0.23$; and ash: $F_{1,21} = 0.58$, $P = 0.45$) also did not vary with insulation treatment (Table 2).

Discussion

Our results show that reducing down insulation increased clutch cooling rates in wood duck nests. Females responded to increased cooling rates by shortening morning recesses and increasing incubation constancy, and this relationship grew stronger as incubation progressed. Changes in incubation behavior maintained a proper thermal environment for developing embryos. Plasticity of behaviors allows for adaption and evolution, particularly in rapidly changing environments (Snell-Rood 2013). Plasticity in various aspects of parental care and reproductive investment has been exhibited in many different species, such as timing of nesting in great tits (*Parus major;* Nussey et al. 2005), feeding frequency in bell miners (*Manorina melanophrys*; Wright et al. 2010), and changing feeding rates in various avian species in relation to predation risk (Ghalambor et al. 2014).

Cooling rates of eggs in the nests changed with differences in both ambient temperature and nest insulation. Previous studies also have reported the importance of nest materials or ambient temperatures in influencing clutch temperatures (Conway and Martin 2000b, Hilton et al. 2004, Heenan 2013); however, our findings show novel interactions between the two factors within manipulated nests. This interaction of the ambient environment and the nest microclimate illuminate the thermal challenges experienced by females incubating in cold environments. Further studies of natural variation in nesting materials, nest site microclimate, and ambient temperatures effects on nesting timing and incubation regimes will continue to help explain the costs of early season incubation.

We recorded high levels of plasticity in length of morning recess and daily incubation constancy in respect to the ambient temperatures and insulation treatment. Nest with reduced insulation experienced shortened morning recess lengths and increased incubation constancy as the incubation period progressed. This led to different averages of incubation constancy between the two treatment groups, although these changes did not led to significant differences in mean morning recess length for the two treatment groups. This is contrasted by studies of tree swallows (*Tachycineta bicolor)*, where experimental box cooling led to decreases in daily attendance (Ardia et al. 2010), and experimental heating of boxes led to increased time spent on nest (Ardia et al. 2009). Similarly, daily incubation constancy increased in heated nest boxes with common starlings (*Sturnus vulgaris*; Reid et al. 2000). Previous evidence of incubation plasticity has focused on manipulating physical temperature of the nest site, or looking at natural correlations between temperatures and incubation behavior. Our methods directly manipulated temperatures experienced by the eggs while not changing the environmental temperatures incubating females would experience. If plasticity in incubation behaviours were only dependent on ambient temperatures, or only dependent on clutch temperatures upon return from recesses, we would expect either no change or immediate changes in behaviors to our experiment, respectively. However, the gradual change we observed suggests that wood duck incubation regimes are dictated by both stimuli as returning females learn to adopt their incubation patterns to new egg cooling rates.

Changes in incubation behaviors ensured no effect of insulation treatment on average nest clutch temperatures. Average nest clutch temperatures were within the range previously recorded at our site (Hepp et al. 2006) and above that of which had been found to have negative effects on duckling phenotype. In a study of duckling quality in artificially incubated eggs from this population, duckling quality declined when incubation temperatures were kept at or below 35.0°C compared to eggs incubated at 35.9°C or 37.0°C which were not detrimentally effected (Hepp et al. 2006). Although there was significant daily clutch temperatures variation, manipulated clutch temperatures were not below this critical temperature point. This could explain why we found no significant effect of manipulative clutch temperatures on incubation length, duckling hatching rates, or duckling size and composition.

Despite increased daily incubation effort by females incubating with reduced insulation, we did not detect significant mass loss in females because of these increased costs. While we were able to produce changes in daily incubation behaviours in manipulated nests, measurements of daily constancy are within those previously recorded in natural nests (Hepp et al. 2006). This could explain why these females did not incur greater body mass loss. Experimental manipulation of our experiment was designed to exasperate already high early season cost of incubation due to cold ambient temperatures, yet it is possible that temperatures were not naturally cold enough to challenge the energy balance for nesting females due to abnormal weather patterns. If experiments were repeated in colder temperatures, we would expect that some individuals would continue to increase incubation effort and incur more energetic costs, while lower-quality individuals would not be able to continue to increase effort, and would sacrifice clutch temperatures and quality of their clutch.

Early incubation female body mass was used as an estimate of female condition; however, it was not seen to affect investment as we predicted. Previous studies of avian incubation investment have shown that individual quality can affect investment decisions. Studies using body mass in wood ducks have shown mixed effects on reproductive investment. Body mass has been seen to effect nesting initiation date (Hepp and Kennamer 1993; 2011), but did not influence incubation length or hatching success (Hepp et al. 1990). Changes in annual and inter-annual body mass in wood ducks vary greatly, and have been linked to annual precipitation patterns more strongly than incubation efforts (Harvey et al. 1989). Additionally, as our study was limited to only early season breeders, who have been shown to be of higher quality (Hepp and Kennamer 2011), and variation within these early season breeders should be lower than the population Further exploration of condition-specific female investment during a larger portion on the breeding season would further explain reproductive trade-offs and effort.

Our findings continue to suggest an energetic cost of incubation for female wood ducks nesting in the southeast US, but a cost that is generally managed successfully. The plasticity of incubation behaviour in wood ducks nesting in the southeastern United States allows for behavioural adaption to the large variation of ambient temperatures during the breeding season.

Despite not detecting evidence for overall consequences of this energetic trade-off, either in hen or offspring health, we were able to produce female incubation behaviour changes. Previous studies have shown the sensitivity of incubating females to naturally variable nest microclimates, however these studies have primarily focused on passerine incubation behaviours (Reid et al. 2002). Continued research on the extent of behavioural plasticity in response to ambient and clutch temperatures, as well as possible influences of female condition, are needed to understand to effects of investment on current and future reproductive efforts, particularly in larger bodied species.

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References

Ardia, D.R., Perez, J.H., Chad, E.K., Voss, M.A., & Clotfelter, E.D.. 2009. Temperature and life history: experimental heating leads female tree swallows to modulate egg temperature and incubation behavior. *Journal of Animal Ecology*, **78**, 4-13.

Ardia, D.R., Perez, J.H., & Clotfelter, E.D. 2010. Experimental cooling during incubation leads to reduced innate immunity and body condition in nestling tree swallows. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1881-1888.

Arnold, T.W. 1993. Factors effecting egg variability and incubation time in dabbling ducks. *Canadian Journal of Zoology*, **71**, 1146-1152.

Bellrose, F.C., & D.J. Holm. 1994. Ecology and management of the Wood Duck. Stackpole Books, Mechanicsburg, PA.

Cartar, R.V., & Montgomerie, R.D.. 1987. Day-to-day variation in nest attentiveness of white-rumped sandpipers. *Condor,* **89**, 252-260.

Conway, C.J. & Martin, T.E. 2000a. Evolution of passerine incubation behavior: Influence of food, temperature, and nest predation. *Evolution*, **54**, 670-685.

Conway, C.J. & Martin, T.E. 2000b. Effects of ambient temperature on avian incubation behaviour. *Behavioural Ecology*, **11**, 178-188.

Criscuolo, F., Gabrielsen, G.W., Gender, J., & Le Maho, Y. 2002. Body mass regulation during incubation in female common eiders (*Somateria mollissimas*). *Journal of Avian Biology*, **33**, 83-88.

Dawson, R. D., O'Brien, E. L., & Mlynowski, T. J. 2011. The price of insulation: costs and benefits of feather delivery to nests for male tree swallows (*Tachycineta bicolor*). *Journal of Avian Biology*, **42**, 93-102.

De Heij, M. E., Van den Hout, P. J., & Tinbergen, J. M. 2006. Fitness cost of incubation in great tits (*Parus major*) is related to clutch size. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2353-2361.

Deeming, D. C., & Ferguson, M. W. 1991. Egg incubation: its effects on embryonic development in birds and reptiles. Cambridge: Cambridge University Press.

Dobush, G.R., Ankney, C.D., & Krementz, D.G.. 1985. The effect of apparatus, extraction time, and solvent type on lipid extractions of snow geese. *Canadian Journal of Zoology,* **63**, 1917-1920.

Drobney, R.D. 1980. Reproductive energetics of wood ducks. *Auk,* **97**, 480-490.

Durant, S.E., Hepp, G.R., Moore, I.T., Hopkins, B.C., & Hopkins, W.A. 2009. Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (*Aix sponsa*) ducklings. *Journal of Experimental Biology,* **213**, 45-51.

Durant, S.E., Hopkins, W.A. & Hepp, G.R.. 2011a. Embryonic developmental patterns and energy expenditure are affected by incubation temperature in wood ducks (*Aix sponsa*). *Physiological and Biochemical Zoology*, **84**, 451-457.

Durant, S.E., Hopkins, W.A., Hawley, D.M., & Hepp, G.R.. 2011b. Incubation temperature affects multiple measures of immunocompetence in young wood ducks. *Biology Letters*, **8**, 108-111.

Durant, S.E., Hopkins, W. A., Wilson, W.A., & Hepp, G.R.. 2011c. Incubation temperature affects the metabolic cost of thermoregulation in a young precocial bird. *Functional Ecology*, **26**, 416-422.

DuRant, S. E., Hopkins, W. A., Hepp, G. R., & Walters, J. R. 2013. Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biological Reviews,* **88**, 499-509.

Feldhiem, C. 1997. The length of incubation in relation to nest initiation date and clutch size in dabbling ducks. *Condor,* **99**, 997–1001**.**

Folk, T.H. & Hepp, G.R.. 2003. Effects of habitat use and movement patterns on movement patterns on incubation behavior of female wood ducks (*Aix sponsa*) in southeast Alabama. *Auk*, **120**, 1159-1167.

Ghalambor, C.K., Peluc, S.I., and Martin, T.E. 2013. Plasticity of parental care under the risk of predation: how much should parents reduce care?. *Biology Letters*, **9**, e20130154

Harvey, W.F., Hepp, G.R., & Kennamer, R.A.. 1989. Age determination of female wood ducks during the breeding season. *Wildlife Society Bulletin*, **17**, 254-258.

Hanssen, S.A., Hasselquist, D., Folstad, I., & Erikstad, K.E. 2005. Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. *Proceedings of the Royal Society of London. Series B: Biological Sciences***, 272**, 1039-1046.

Heaney, V., & Monaghan, P. 1996. Optimal allocation of effort between reproductive phases: the trade-off between incubation costs and subsequent brood rearing capacity. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **263**, 1719-1724.

Heenan, C . B. 2013. An overview of the factors influencing the morphology and thermal properties of avian nests. *Avian Biology Research*, **6**, 104-118.

Hepp, G. R., & Kennamer, R. A. 1993. Effects of age and experience on reproductive performance of wood ducks. *Ecology*, **74**, 2027-2036.

Hepp, G.R. & Kennamer, R.A. 2011. Date of nest initiation mediates incubation costs of wood ducks (*Aix sponsa*). *Auk*, **128,** 258-264.

Hepp, G.R. & Kennamer, R.A. 2012. Warm is better: incubation temperature influences apparent survival and recruitment of wood ducks (*Aix sponsa*). *PLoS One*, **7**, e47777.

Hepp, G. R., Kennamer, R. A., & Harvey, W. F. 1990. Incubation as a reproductive cost in female wood ducks. *Auk*, **107**, 756-764.

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

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

Hilton, G. M., Hansell, M. H., Ruxton, G. D., Reid, J. M., & Monaghan, P. 2004. Using artificial nests to test importance of nesting material and nest shelter for incubation energetics. *Auk*, **121**, 777-787.

Hopkins, B. C., DuRant, S. E., Hepp, G. R., & Hopkins, W. A. 2011. Incubation temperature influences locomotor performance in young wood ducks (*Aix sponsa*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **315**, 274-279.

Larsen, V. A., Lislevand, T., & Byrkjedal, I. 2003. Is clutch size limited by incubation ability in northern lapwings?. *Journal of Animal Ecology*, **72**, 784-792.

Lenth, R. V. 2013. lsmeans: Least-squares means. R package version 1.06-05.

Lombardo, M.P., Bosman, R.M., Faro, C.A., Houtteman, S.G., & Kluisza, T.S. 1995. Effect of feathers as nest insulation on incubation behavior and reproductive performance of tree swallows (*Tachycineta bicolor*). *Auk*, **112**, 973-981.

Manlove, C. A., & Hepp, G. R. 1998. Effects of mate removal on incubation behavior and reproductive success of female wood ducks. *Condor*, **100**, 688-693.

Manlove, C.A. & Hepp, G.R.. 2000. Patterns of nest attendance in female wood duck. *Condor*, **102**, 286-291.

Martin, T. E., Auer, S. K., Bassar, R. D., Niklison, A. M., & Lloyd, P. 2007. Geographic variation in avian incubation periods and parental influences on embryonic temperature. *Evolution*, **61**, 2558-2569.

Moller, A.P. 1991. The effect of feather nest lining on reproduction in the swallow *Hirundo rustica*. *Ornis Scandinavica*, **22**, 396-400.

Nord, A., Sandell, M.L., & Nilsson, J.. 2010. Female zebra finches compromise clutch temperature in energetically demanding incubation conditions. *Functional Ecology*, **24**, 1031-1036.

Nord, A., & Nilsson, J. Å. 2012. Context-dependent costs of incubation in the pied flycatcher. *Animal Behaviour*, **84**, 427-436.

Nussey, D.H., Postma, E., Gienapp, P., & Visser, M.E. 2005. Selection on heritable phenotypic plasticity in a wild bird population. *Science*, **310**, 304-306.

Olsen, C.R., Vleck, C.M., & Vleck, D.. 2006. Periodic cooling of bird eggs reduces embryonic growth efficiency. *Physiological and Biochemical Zoology*, **79,** 927-936.

Pérez, J. H., Ardia, D. R., Chad, E. K., & Clotfelter, E. D. 2008. Experimental heating reveals nest temperature affects nestling condition in tree swallows (*Tachycineta bicolor*). *Biology Letters*, **4**, 468-471.

Pinheiro, J. C., & Bates, D. M. 2000. *Mixed-effects models in S and S-PLUS*. New York: Springer Verlag.

Reid, J. M., Monaghan, P., & Ruxton, G. D.2000. Resource allocation between reproductive phases: the importance of thermal conditions in determining the cost of incubation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **267**, 37-41.

Reid, J. M., Ruxton, G. D., Monaghan, P., & Hilton, G. M. 2002. Energetic consequences of clutch temperature and clutch size for a uniparental intermittent incubator: the starling. *Auk*, **119**, 54-61.

Reznick, D. 1985. Costs of reproduction: an evaluation of the empirical evidence. *Oikos*, **44**, 257-267.

Snell-Rood, E.C. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. *Animal Behaviour*, **85**, 1004-1011.

Tinbergen, J. M., & Williams, J. B. 2002. Energetics of incubation. In: *Avian Incubation Behaviour, Environment, and Evolution*. (Ed. by DC Deeming.) pp. 299–313. Oxford: Oxford University Press.

Tombre, I.M, & Erikstad, K.E.. 1996. An experimental study of incubation effort in high-Arctic Barnacle Geese. *Journal of Animal Ecology*, **65**, 325-331.

Webb, D.R. 1987. Thermal tolerance of avian embryos: a review. *Condor*, **89,** 874-898.

Wright, J., **McDonald, P.G., te Marvelde , L., Kazem, A.J.N., & Bishop, C.M.** 2010. Helping effort increases with relatedness in bell miners, but ‘unrelated’ helpers of both sexes still provide substantial care. *Proceedings of the Royal Society B.,* **277**, 437-445.

Yerkes, T. 1998. The influence of female age, body mass, and ambient conditions on redhead incubation constancy. *Condor*, **100**, 62-68.

Fig. 1: Mean (±SE) cooling rates of wood duck eggs housed in environmental chambers at 7°C, 15°C, and 22°C with normal (4.0 g) and reduced (0.5 g) feather down insulation.

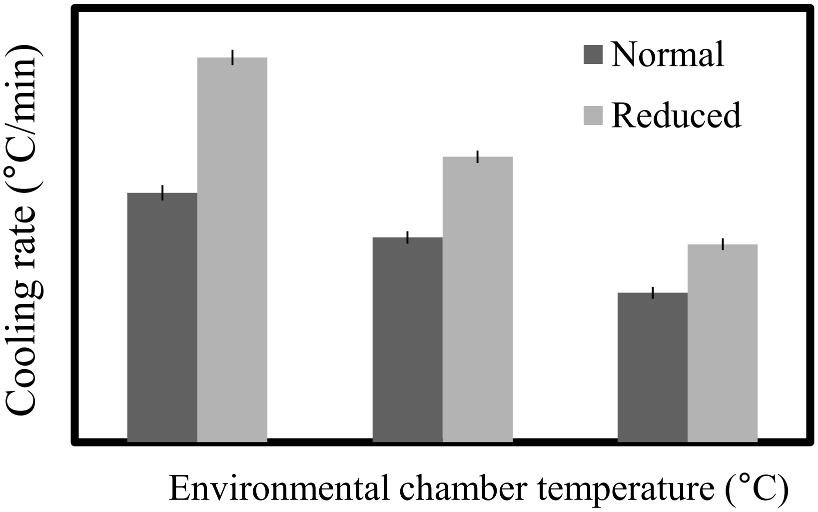

Figure 2. The relationship between morning recess length and incubation day (± 95% confidence interval) for female wood ducks with normal (4.0g) and reduced (0.5g) feather down insulation.

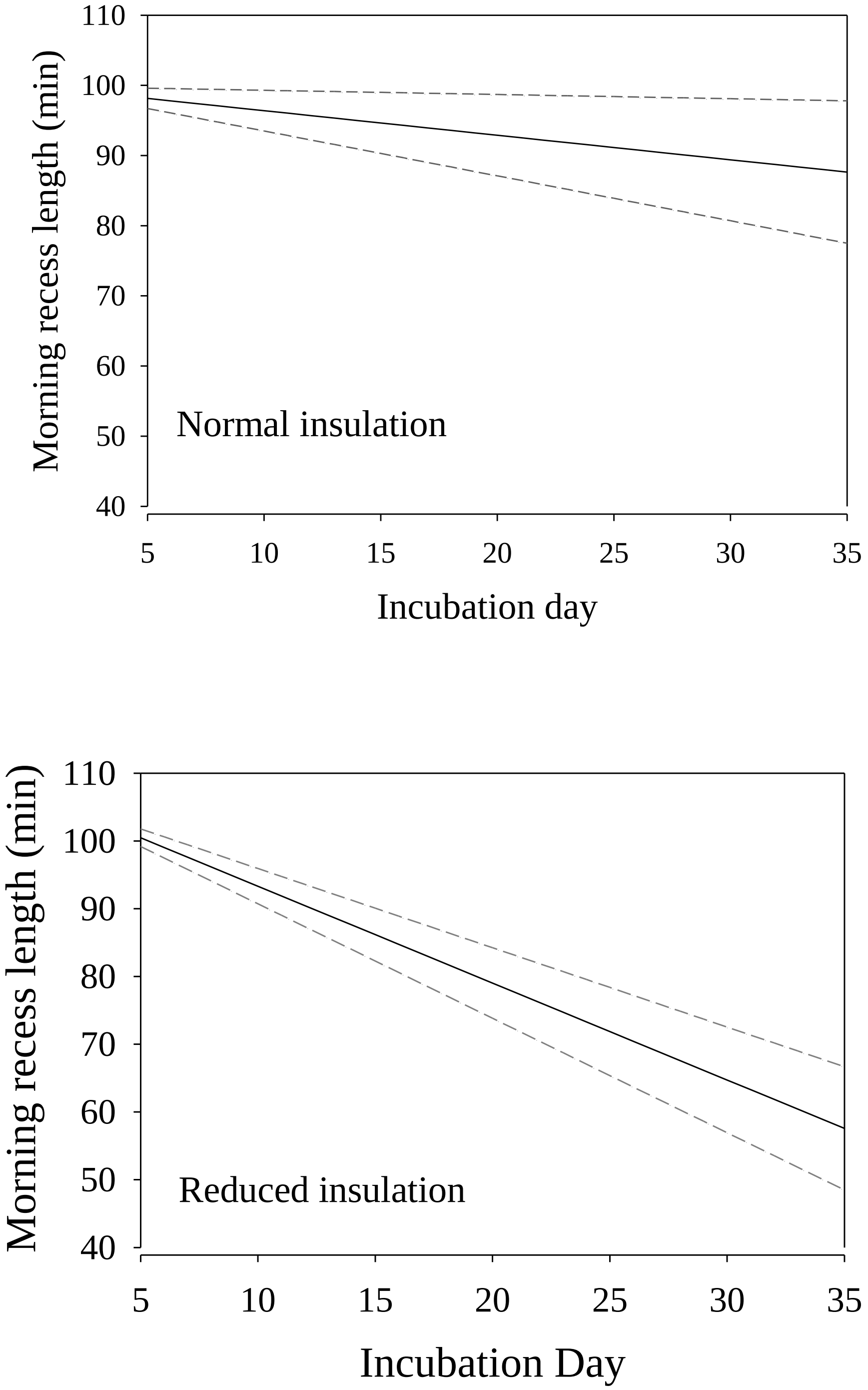

Figure 3. The relationship between incubation constancy and incubation day (± 95% confidence interval) for female wood ducks with normal (4.0g) and reduced (0.5g) feather down insulation treatments.

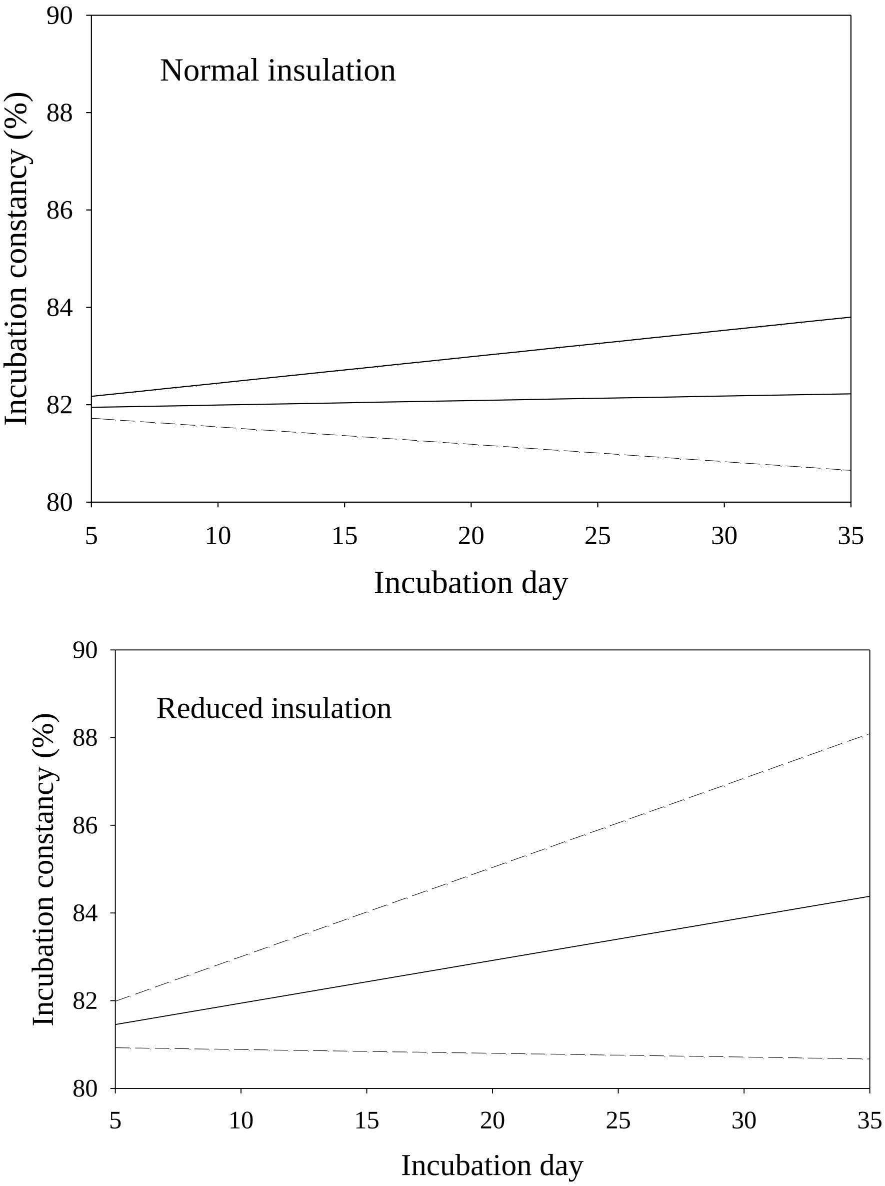

Table 1. Characteristics (means ± SE) of wood ducks nests for which level of down insulation was manipulated (Reduced = 0.5 g; Normal = 4.0 g).

	Reduced insulation (N = 18)	**Normal insulation (N = 16)**
Incubation period (days)	32.12 ± 0.40	31.80 ± 0.42
Female body mass loss (g)	8.93 ± 6.82	20.57 ± 7.24
Female body mass loss (%)	1.55 ± 1.17	3.47 ± 1.24
Hatching success (%)	91.86 ± 0.02	89.97 ± 0.04

Table 2. Body mass and body composition (least-squares mean ± SE) of day-old wood duck ducklings hatched from nests with reduced (0.5 g) and normal (4.0 g) levels of down insulation. Fresh egg mass (g) was used as covariate.

	Reduced insulation (N = 25)	**Normal insulation (N = 24)**
Fresh egg (g)	40.78 ± 0.77	42.73 ± 0.78
Wet duckling (g)	26.29 ± 0.41	26.15 ± 0.42
Dry duckling (g)	7.93 ± 0.15	7.89 ± 0.15
Lipid (g)	2.36 ± 0.10	2.45 ± 0.10
Protein (g)	4.97 ± 0.07	4.84 ± 0.07
Ash (g)	0.57 ± 0.01	0.56 ± 0.01

Influences of nest microclimate and nest fidelity on nest site selection in early-season breeding Wood Ducks (*Aix sponsa*)

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Abstract

Wood Ducks (*Aix sponsa)* nesting during the cool, early portion of the breeding season should select nest sites that minimize energetic costs of incubation. In this study, we calculated the mean and standard deviation of nest box temperature (15 January – 30 April), and examined the effects of nest site microclimate on nest site selection, timing of nest initiation, and the probability that nests were successfully incubated. We found nest site selection was positively associated with greater variation in nest box temperature, but not with average nest box temperature. However, females initiated nests earlier in warm nest boxes than in cool nest boxes, and these nests were less likely to be abandoned. Next, every 4 days, we compared microclimate of nest boxes that were selected by females during that period of time with nearby nest boxes that were available but were not selected. We included in this analysis the previous nesting history of incubating females, because previous nesting experience can influence nest site selection in Wood Ducks. Females selecting nest boxes that they previously had used, chose nest boxes that were cooler than available nearby boxes. For new females and females without previous nesting experience in the selected nest boxes, microclimate did not differ between nest boxes that were selected and those that were not selected. Our results suggest that nest site selection during the early season favors warmer boxes with more variable temperatures; however, this system is complicated by nest site fidelity and brood parasitism.

***Key words**: Aix sponsa*, nest-microclimate, nest selection, nest fidelity, nest parasitism, Wood Duck.

Introduction

Nest sites should provide protection and a stable thermal environment for incubation (McComb and Noble 1981, Paclík and Weidinger 2007, Coombs et al. 2010). Eggs are in continuous contact with the thermal environment of the nest; therefore, the nest's microclimate plays an important role in egg heat loss and overall clutch temperature (Collias and Collias 1984, Hilton et al. 2004). According to the thermoregulatory hypothesis of nest site selection, nest site and nesting material should be selected so that the nest microclimate helps to buffer against temperature fluctuations and maintain egg temperatures (White and Kinney 1974, Kern 1984). Nest structure and thermal performance influences clutch temperature both when incubating parents are on and off the nest (Hilton et al. 2004). High quality nests should reduce energetic demands of incubation (Weathers 1985); therefore, selecting nest sites with favorable microclimate should mitigate incubation costs (Ar and Sidis 2002).

Selection of thermally beneficial nest sites evolved in many species to mitigate costs of incubation (Kern 1984, Burton 2006). Evidence of both preferences and benefits of favorable nest sites have been seen across many taxa. Rhodes et al. (2009), examining nest microclimate of South Island Saddleback (*Philesturnus carunculatus),* a secondary cavity-nesting bird, found that nesting females selected warmer chambers with more stable temperature profiles. Further, factors influencing nest microclimate, such as cavity tree quality in Northern Flickers (*Colatpes auratus*; Wiebe 2001), shade and nest orientation in Horned Lark (*Eremophila alpestris*; Hartman and Oring 2003), and nest box orientation in woodland passerines (Goodenough et al.

2008) were seen to influence nest selection. Additionally, Ardia et al. (2006) found seasonally dependent nest box selection; boxes that faced thermally optimal directions were selected strongly by tree swallows (*Tachycineta bicolor*) in the early, cold breeding season.. Female mate, and therefore nest choice, can be influenced by nest quality in species that build their own nest structures, such as Penduline Tits (*Remiz pendulinus*; Hoi et al. 1994) and Baya Weavers (*Ploceus philippinus*; Quader 2006).

If nest site selection is adaptive, selection should be positively associated with nest success. Microclimate can have independent influences on hatching success and nest site selection. Álvares and Barba (2008) failed to find evidence of female nest site selection or males' nest building investment in Great Tits (*Parus major*); however they did find a significant relation between nest size and thermal quality and breeding performance. Nest box orientation had no effect on nest selection in American Kestrels (*Falco spaverius*); however, nest box orientation affected temperature and humidity of nest sites and hatching success (Butler et al. 2009).

Widespread evidence of nest site philopatry in waterfowl suggests that this behavior must be advantageous, as it has been shown to influence nest success (Greenwood and Harvey 1982). In philopatric species, such as the Wood Duck, individuals utilize previous nesting experiences to make informed decisions. This should lead to greater fitness compared to those that made these choices without prior experiences (Doligez et al. 2003). Nest site fidelity can interact with nest site quality, and influence subsequent nest site selection (Weatherhead and Boak 1986, Bollinger and Gavin 1989). Previous studies with Wood Ducks showed that 41.9% of females returned to the same nest site, and these females nested earlier than those that changed nest location (Hepp and Kennamer 1992.) Similarly, Common Goldeneyes (*Bucephala clangula*) that changed nest boxes between breeding attempts nested later, had smaller clutches, and had overall lower nesting success than females that exhibited nest site fidelity (Dow and Fredga 1983).

In this study, we examined nest-site selection during the early portion of the breeding season in a population of Wood Ducks in the southeastern United States. We predicted average daily temperature would vary among nest boxes, and that females would initiate in boxes that were warmer overall than boxes that were not selected. We predict that timing of nest initiation should be dependent on 24-hour daily and morning average temperatures. Morning time period was included because it encompassed the morning activity period when female Wood Ducks prospect for nest site (Bellrose and Holm 1994). Further, we predicted that nest-box selection would be influenced by the interaction of nest-box microclimate and the previous nesting experience of females, because nest site fidelity is high in this population (Hepp and Kennamer 1992).

Methods

This study was conducted at the Department of Energy's Savannah River Site in west-central South Carolina (33°1'N, 81°3'W) in January-July 2011 and 2012. The study site, Par Pond, is split into three distinct arms which provide distinct regions to test for nest box selection in (Figure 1). Nest boxes at Par Pond (*n* = 82) were checked for nesting activity every four days during the breeding season. When nests were located, eggs were chronologically labeled with a permanent marker to record laying order within the clutch. Wood Ducks lay one egg per day (Drobney 1980), and date of nest initiation was estimated by subtracting the number of eggs in the nest when it was first discovered from the Julian date the nest was checked (Hepp et al. 1990). Brood parasitism is common in Wood Ducks, so when number of eggs in initiated nests outnumbered the days between nest checks, the day after previous nest check was assumed to be the day of nest initiation.

Incubating females were captured during the first and last week of incubation. At first capture, unmarked females were banded with U.S. Fish and Wildlife Service leg bands and aged as adults (ASY) or yearlings (SY; Harvey et al. 1989), and band numbers of females were recorded. Virtually all incubating females have been captured on Par Pond since 2006, allowing us to identify females that had previously nested on the lake and the nest boxes they used.

We installed iButtons (i-Button, Maxim Integrated Products, Sunnyvalle CA) inside nest boxes in a standardized location at the back of the box 10 cm below the entrance hole to measure ambient nest box temperature (± 0.5 °C) every 30 minutes. This position was selected to avoid direct contact with the incubating female and minimize direct influence of the sun. Temperature loggers were changed about every 30 days during the breeding season to maintain continuous measurements for all boxes. All iButtons were placed in an environmental chamber (Environmental Growth Chambers, Chagrin Falls, OH) at constant temperature of 37°C for 4 hours. Differences in accuracy for each iButton (± 0.05°C) were measured and controlled for in all subsequent analyses.

To examine whether incubating females significantly warmed nest boxes, mean temperatures were calculated for 10 nest boxes for a five-hour overnight period (2000-0300) within the same cove of the lake from 10-30 March. Comparing nest boxes in the same cove with and without incubating females at night allowed us to minimize temperature differences caused by differences in location and exposure, and only examine temperature effects of incubating females. For each night in each box, we characterized occupancy, using only nights that we knew females had initiated full incubation (occupied; *n* = 47), or nights where we knew the nest box was empty (empty; *n* = 134).

We defined the early breeding season as beginning with the first nest initiation each year (25 February 2011 and 3 March 2012), and ending on 30 April after the first pulse of nest initiations (Fig. 1). To test whether for how box microclimate affects the probability of nest-box selection, the timing of selection, and the probability of a nest being incubated, we first summarized nest box microclimate for the early breeding season. For each day we calculated average daily (0000-2359) and morning (0500-1000) temperatures for each nest box. We used these values to calculate single early season average daily and morning temperatures for each box. Standard deviations of daily and morning temperatures of each box also were calculated to help capture variability due to conditions such as sun exposure that may affect nest box selection. Repeatability was measured using methods described in Lessells and Boag (1987) to look for differences in seasonal box temperatures. These measures of box microclimate were compared with the box utilization during the early breeding season (a binomial variable of if eggs were found in the nest box before 30 April), relative nest initiation of the first nesting attempt (relative date of nest initiation of first nest in each nest box), and the probability of initiation of incubation of first nesting attempts at selected boxes (a binomial variable of if signs of full incubation were seen, and nest was not abandoned during egg laying).

To test effects of nest-site fidelity and nest microclimate on nest box selection, we examined nest microclimate surrounding each nest initiation in which we were able to identify the incubating female. Temperatures for selected nest-boxes during the four-day period that surround nest detection were compared to boxes within the same arm of the lake which were not selected (*n* = 22 4-day periods). Relative box temperatures, the difference between average temperatures during the 4-day periods for the selected boxes with the unoccupied boxes were calculated for each nest initiation. Effect of previous nesting experiences of females were utilized to account for nest site fidelity. Three categories of female experience were utilized; (1) unbanded females with no previous nesting experience, (2) banded females who had previously nested on Par Pond, but never in selected box, or (3) banded females who have previously nested on Par Pond in the selected box.

All statistical tests were completed using R 2.15.2 for Windows (The R Foundation for Statistical Computing, 2012). Mixed linear models with repeated measures tested for differences between occupied and empty boxes, while including temporal fixed effects to control for nightly variation. Effects of nest temperature (mean and standard deviation) on the probability of boxes of being used, and the probability of nests being abandoned were tested using generalized linear models using binomial variance (program GLM, R 2.15.2). Relationships between nest box microclimate and timing of nest initiation was examined using simple linear models (program LM, R2.15.2). To examine whether nest site fidelity and nest microclimate influenced nest selection, mixed effects linear models were built (program LME, R2.15.2) that allowed for the random effects of year and portion of the lake and tested relative box temperature's relationship with timing of nest initiation, female experience, and the interaction of the two.

Results

Daily temperatures were recorded for all boxes (*n* = 87) from 15 January to 30 April in 2011 and 2012. Daily box temperatures for this time period were 15.56 ± 0.78°C, range: 12.52-17.89°C, and morning box temperatures were 12.21 ± 1.16°C, range: 8.49-17.50°C. In 2011, females initiated nests in 72 nest boxes and 35 of these nests were abandoned before being incubated during the early breeding season. In 2012, females initiated nests in 72 nest boxes and

37 of these nests were abandoned before being incubated during the early breeding season. Nest box temperatures varied through the early breeding season, but there was a general warming trend for both 24-hour and morning temperatures (Fig. 2). Average daily temperatures did not show repeatability between years (r = -0.102) but average morning temperatures were highly repeatable between years (r = 0.667). Presence of incubating females did not increase nest box temperatures (*T* = 1.33, df = 237, *P* = 0.19).

Comparison of temperatures of used and unused nest boxes during the early part of the breeding season showed that selection of nest boxes was related to variation in daily nest box temperatures (*Z* = 1.96, df = 1 and 163, *P* = 0.050). Nest boxes were 4.72 times (95% CL = 1.03-23.54) as likely to be selected for each 1°C increase in standard deviation. The probability of females selecting nest boxes was not related to average daily nest box temperature (*Z* = 0.66, df = 1 and 163, *P* = 0.51), average morning nest box temperature (*Z* = 1.11, df = 1 and 163, *P* = 0.27), or variation in morning temperature (*Z* = 0.81, df = 1 and 163, *P* = 0.42).

Timing of nest initiation during the early breeding season was influenced by average nest box temperature. First nests were initiated 4.19 ± 1.62 days earlier for every 1°C increase in average nest box temperature (*T* = -2.60, df = 137, *P* = 0.011). Variation in daily box temperature also influenced timing of first nests (β = -8.11 ± 3.75, *T* = -2.16, df = 137, *P* = 0.032), with greater variation in temperature leading to earlier nest initiation. There were no relationships between average morning box temperatures (*T* = -0.83, df = 137, *P* = 0.41) and variation in morning temperatures (*T* = -1.35, df = 137, *P* = 0.18) and date of first nests.

The probability of nests being abandoned marginally decreased with increasing daily average box temperatures. For each 1°C increase in daily nest box temperature, nests were 0.605 (95% CL = 0.354 – 0.998) times as likely to be abandoned (*Z* = -1.91, df = 1 and 129, *P* =

0.056). Increased variation in daily nest box temperatures decreased the probability of a nest being abandoned (*Z* = -2.13, df = 1 and 129, *P* = 0.03). For every 1°C increase in standard deviation, nests were 0.257 (95% CL = 0.069 - .866) times as likely to be abandoned. Variation in morning box temperatures also decreased the probability of nests being abandoned (*Z* = -2.84, df = 1 and 129, *P* = 0.005), as every 1°C increase in standard deviation made it 0.248 (95% CL = 0.089 – 0.617) times as likely that a nest was abandoned. There was no relationship between average morning box temperatures and the probability that nests were incubated (*Z* = -0.87, df = 1 and 129, *P* = 0.38).

For females with previous histories of using the selected nest box, nest boxes were cooler than neighboring boxes that were not selected. These experienced females selected boxes that were 5.75 ± 2.29°C cooler then neighboring empty boxes (*T* = -2.51, df = 15, *P* = 0.02. For females without previous nesting experience (i.e., either new to the population or new to nesting in the selected box), there was no difference in temperatures between nest boxes that were selected and not selected (*T* = -0.86, df = 15, *P* = 0.40). Date of nest initiation had no effect on the relationship (*T* = -1.31, df = 15, *P* = 0.21).

Discussion

Nest box use was high during the early seasons of the 2011 and 2012 breeding season, with nests being initiated during the early season in 82.8% of nest boxes and 54.0-62.1% of these nests were incubated. Probability of nest boxes being selected, date of nest initiation, and probability of nests being incubated during the early breeding season were all significantly affected by variation in daily nest box temperatures. Increased variation in these temperatures led to higher probability of boxes being selected earlier within the breeding season, and these nests were more likely to be incubated. Further, timing of nest box selection increased with increasing

24-hour box temperatures. We show that female Wood Ducks select more variable, potentially warmer boxes for nesting during the early season.

Nest box temperatures varied between years with 2011 being cooler than 2012. We did not find evidence of daily temperature repeatability between the two study years, as boxes' temperature rankings were inconsistent due to high variability of seasonal temperatures; however morning temperatures were highly repeatable. If box temperatures are reliably favorable in different years, even in widely variable ambient conditions, nest site fidelity would be encouraged. As nest microclimate appears to not be predictable between breeding seasons, nest site fidelity must be linked to other aspects of nest-site quality, such as previous successful experiences (Hepp and Kennamer 1992). Boxes that provided favorable conditions for successful incubation should be recognized from previous experiences, and would be utilized for further nesting attempts. As the two study years both had irregular temperatures for early breeding season at our study site, further studies examining the relationships between nest box temperature and success, specifically looking at early season average temperature or variation in temperature, would help further disentangle the effects of nest microclimate and nest site fidelity on nest site selection (Clark and Shutler, 1999).

During the early breeding season, boxes with higher variation in day-to-day 24-hour temperature were more likely to be utilized, although average 24-hour temperatures were not significant. As boxes have different exposures to ambient conditions, such as afternoon sunlight, boxes have high variability in both overall temperature averages and temperature variability. The only nest microclimate property that increased probability that nest boxes were selected was 24-hour nest box temperature variability; higher standard deviations in box temperatures increased probability of nest boxes being selected. We suggest that this could be because of warm afternoon sun exposure on sunny days effected the variability between days more strongly than overall average 24-hour temperatures. Additionally, boxes with higher temperature variability could be located on geographic parts of the lake with high exposure to ambient conditions as well as high visibility, leading to the apparent correlation between temperature variability and nest box selection.

Date of relative nest initiation was positively correlated with increased average 24-hour nest box temperatures, as nests were initiated earlier in warmer boxes. Additionally, nests were initiated earlier in boxes with higher temperature variation. Opposite as predicted, morning temperatures did not affect timing of nest box selection. Date of nest initiation in our population of wood ducks has been shown to effect costs of incubation. Larger females nest earlier in the breeding season, spend more time incubating on the nest, and lose more body mass during the incubation period (Hepp and Kennamer 2011). Our results support these findings, as warmer nest box temperature lead to decreased incubation effort and therefore reproductive costs (McClintock et al., *in press*). As earlier nesting females are selecting warmer boxes, this is evidence of females selecting boxes which should help mitigate some of the costs associated with early season incubation, particularly if they don’t have previous nesting experiences.

Probability of nests being abandoned decreased with increased variability of both 24-hour temperatures and morning temperatures. We found that boxes with higher 24-hour average temperatures were less likely to be abandoned. Previous studies at our study site show that nests abandoned during egg laying are most likely due to intra-species nest parasitism, either as too many eggs were laid in the nest to make incubation profitable, or eggs were laid by females who did not intend to incubate. As previous experiences between these groups are different, we expect them to have different nest selection criteria. Warmer nests are less likely to be abandoned, and they provide a more favorable microclimate for incubation. This further supports nest box selection as a method to mitigate early breeding season's costs.

For incubated nests with identified females, nest site fidelity had a strong effect on nest initiation, potentially swamping the effects of box microclimate. Females nesting in boxes they previously used selected for boxes that were cooler than empty boxes nearby. Females that were either new to the breeding population or did not have previous experience in selected nest box seemed to choose nest boxes randomly. Most females that breed during the early season are high quality, and often experienced as to meet the high costs of early season breeding (Hepp and Kennamer 2011). Yet these female did not seem to be preferentially choosing boxes that were warmer then available neighbors. Nest site fidelity has been shown to have a strong effect on nest site selection in Wood Ducks (Roy et al. 2009), and this system is further complicated by the high levels of nest parasitism in our population. As we are seeing differential methods of selection between experienced and inexperienced females, further investigation of the importance of nesting experience, particularly on abandoned nests, needs to be investigated.

Given our results, we suspect that there may be an effect of nest microclimate effect on nest site selection; however effects of previous experience and nest parasitism complicate the system. We chose to examine early breeding season, as colder and more variable temperatures should increase the benefit of nesting in a box with favorable temperatures. However, females who nest at this time are either experienced and select boxes based off previous experience, or are nesting parasitically possibly because their physical condition precludes incubation during the cold early season. Further examination of the system, either by having the ability to identify females associated with abandoned or parasitic nests, or examining nest initiations later in the breeding season where more inexperienced females may attempt to incubation, could shed further light on nest microclimate's effect on nest-site selection.

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Works Cited

Álvares, E. and E. Barba. 2008. Nest quality in relation to adult bird condition and its impact on reproduction in Great Tits (*Parus major*). Acta Ornithologica 43: 3-9.

Ar, A. and Y. Sidis. 2002. Nest microclimate during incubation. Oxford Ornithology Series 13; 143-160.

Ardia, D.R., J.H. Pérez, and E.D. Clotfelter. 2006. Nest box orientation affects internal temperature and nest site selection by Tree Swallows. Journal of Field Ornithology 77: 339-344.

Bellrose, F.C., and D.J. Holm. 1994. Ecology and management of the Wood Duck. Stackpole Books, Mechanicsburg, PA.

Bollinger, E.K. and T.A. Gavin. 1989.The effects of site quality on breeding-site fidelity in Bobolinks. Auk 106: 584-594.

Burton, N.H.K. 2006. Nest orientation and hatching success in the tree pipit (*Anthus trivialis*). Journal of Avian Biology 37: 312-317.

Butler, M.W., B.A. Whitman, and A. M Duffy. 2009. Nest box temperatures and hatching success of American Kestrels caries with nest box orientation. The Wilson Journal of Ornithology 121: 778-782.

Clark, R. G., & Shutler, D. 1999. Avian habitat selection: pattern from process in nest-site use by ducks?. Ecology, 80: 272-287.

Collias N.E and E.C. Collias. 1984. Nest building and bird behavior. Princeton University Press, Princeton, N.J.

Coombs, A.B., J. Bowman, and C.J. Garroway. 2010. Thermal properties of tree cavities during winter in a northern hardwood forest. Journal of Wildlife Management 74: 1875-1881.

Doligez, B., C. Cadet, E. Danchin, and T. Boulinier. 2003. When to use public information for breeding habitat selection? The role of environmental predictability and density dependence. Animal Behaviour 66: 973-988.

Dow, H. and S. Fredga. 1983. Breeding and natal dispersal of the Goldeneye, *Bucephala clangula*. Journal of Animal Ecology 52: 681-695.

Drobney, R.D. 1980. Reproductive energetics of wood ducks. Auk, 97: 480-490.

Goodenough, A.E., D.P. Maitland, A.G. Hart, and S.L. Elliot. 2008. Nestbox orientation: a species-specific influence on occupation ad breeding success in woodland passerines. Bird Study 55: 222-232.

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

Hartman, C.A. and L.W. Oring. 2003. Orientation and microclimate of Horned Lark nests: the importance of shade. Condor 105: 158-163.

Harvey, W.F., G.R. Hepp, and R.A. Kennamer. 1989. Age determination of female wood ducks during the breeding season. Wildlife Society Bulletin 17: 254-258.

Hepp, G.R., and R.A. Kennamer. 1992. Characteristics and consequences of nest-site fidelity in Wood Ducks. Auk 109: 812-818.

Hepp, G.R., and R.A. Kennamer. 2011. Date of nest initiation mediates incubation costs of wood ducks (*Aix sponsa*). Auk 128: 258-264.

Hepp, G.R., R.A. Kennamer, and W.F. Harvey. 1990. Incubation as a reproductive vost in female wood ducks. Auk 107: 756-764,

Hilton, G.M., M.H. Hansell, G.D. Ruxton, J.M. Reid, and P. Monaghan. 2004. Using artificial nests to test importance of nesting material and nest shelter for incubation energetics. Auk 121: 777-787.

Hoi, H., B. Schleicher, and F. Valera. 1994. Female mate choice and nest desertion in Penduline Tits (*Remiz pendulinus*); the importance of nest quality. Animal Behaviour 48: 743-746.

Kern, M.D. 1984. Racial differences in nest of white-crowned sparrows. Condor 86: 455-466.

Lessells, C.M. and Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. Auk 104: 116-121.

McComb, W.C., and R.E. Noble. 1981. Nest-box and natural-cavity use in three mid-south forest habitats. Journal of Wildlife Management 45:93-101.

Paclík, M. and K. Weidinger. 2007. Microclimate of tree cavities during winter nights-implications for roost site selection in birds. International Journal of Biometeorology 51: 287-293.

Quader, S. 2006. What makes a good nest? Benefits of nest choice to female Baya Weavers (*Ploceus philippinus*). Auk 123: 475-486.

Rhodes, B., C. O’Donnell, and I. Jamieson. 2009. Microclimate of natural cavity nests and its implications for a threatened secondary-cavity-nesting passerine of New Zealand, the South Island Saddleback. Condor 111: 462-469.

Roy Nielsen, C, P.G. Parker, and R.J. Gates. 2006. Intraspecific nest parasitism of cavity-nesting wood ducks: costs and benefits to hosts and parasites. Animal Behaviour 72: 917-926.

Roy, C., J.M Eadie, E.M. Schauber, N.S. Odell, E.C. Berg, and T. Moore. 2009. Public information and conspecific nest parasitism in wood ducks: does nest density influence quality of information. Animal Behaviour 77: 1367-1373.

Weatherhead, P.J. and K.A. Boak. 1986. Site infidelity in Song Sparrows. Animal Behaviour 34: 1299-1310.

Weathers, W.W. 1985. Energy cost of incubation in the canary. Comparative Biochemistry and Physiology Part A: Physiology 81: 411-413.

Weibe, K.L. 2001. Microclimate of tree cavity nests: is it important for reproductive success in Northern Flickers?. Auk 118: 412-421.

White, F.N.and J.L Kinney. 1974. Avian Incubation. Science 186 107-115.

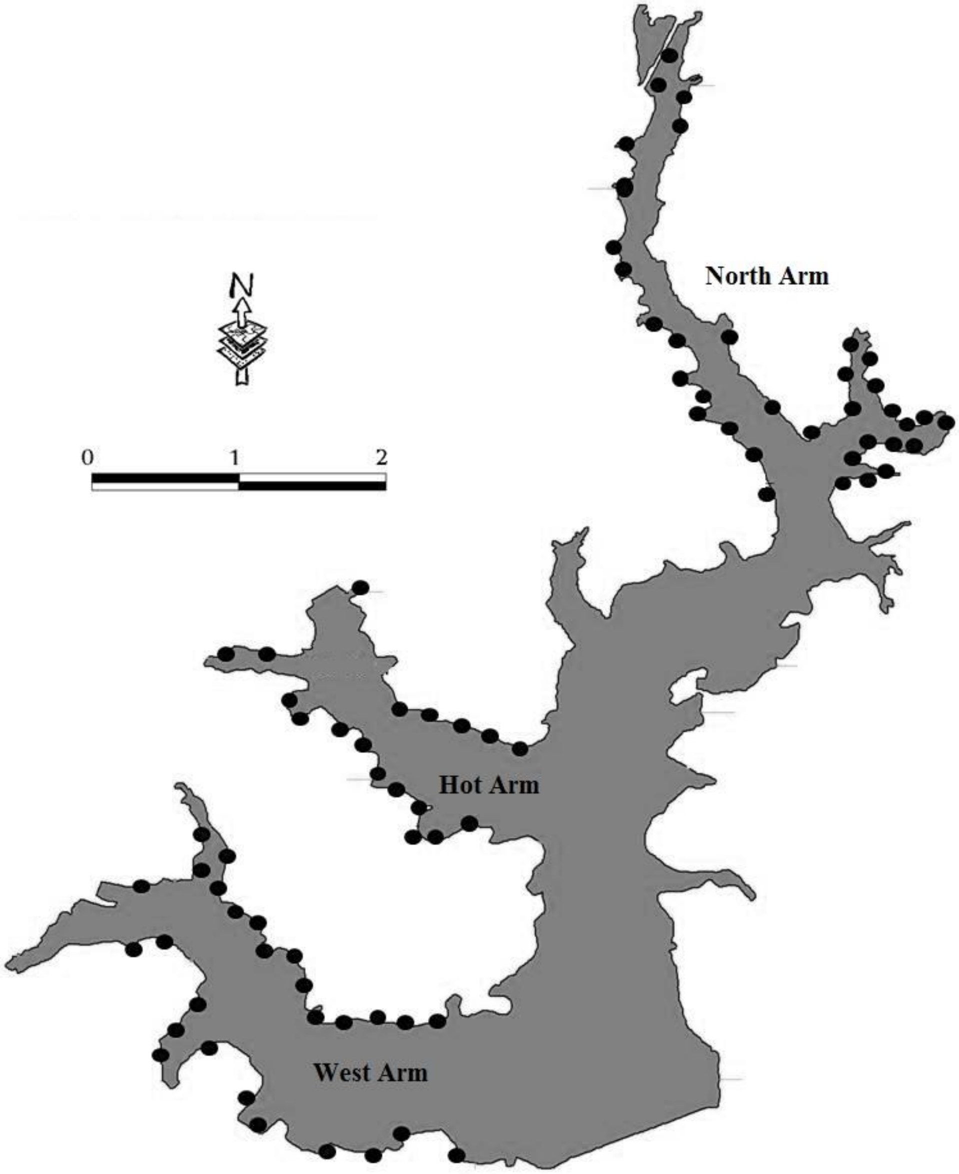


Figure 1. Map of Par Pond, with distinct regions of the pond..

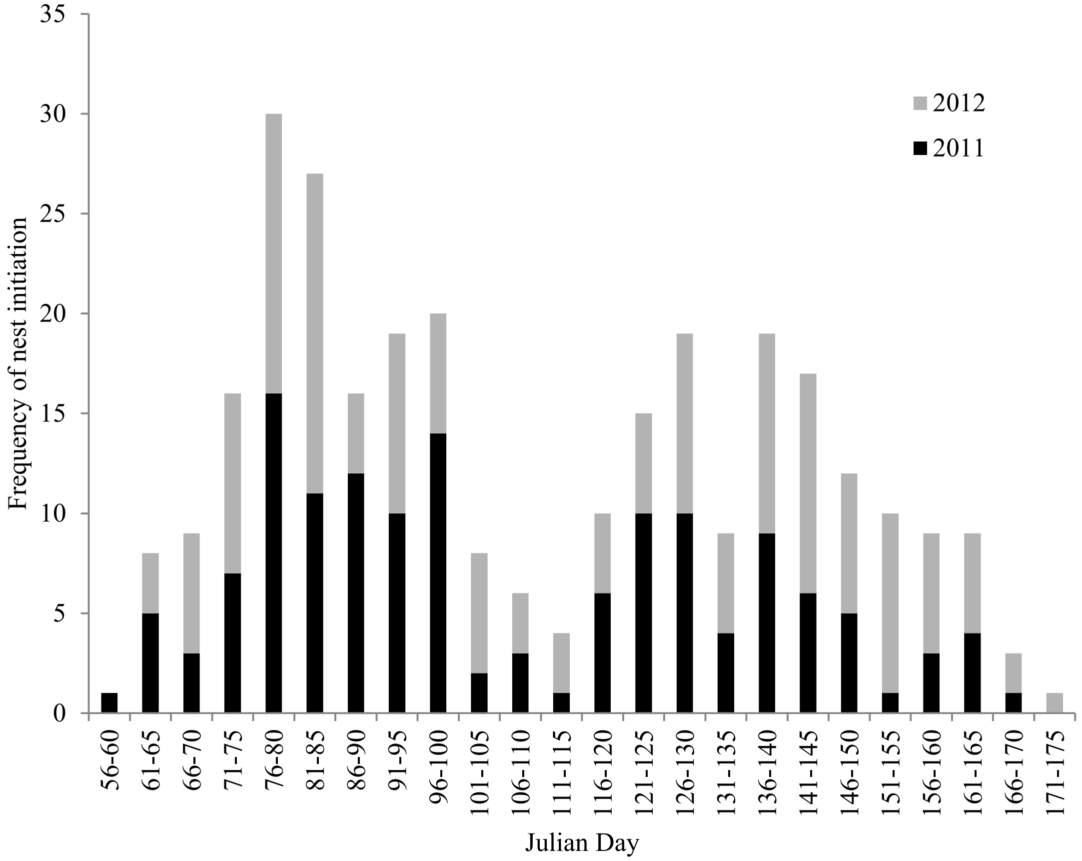
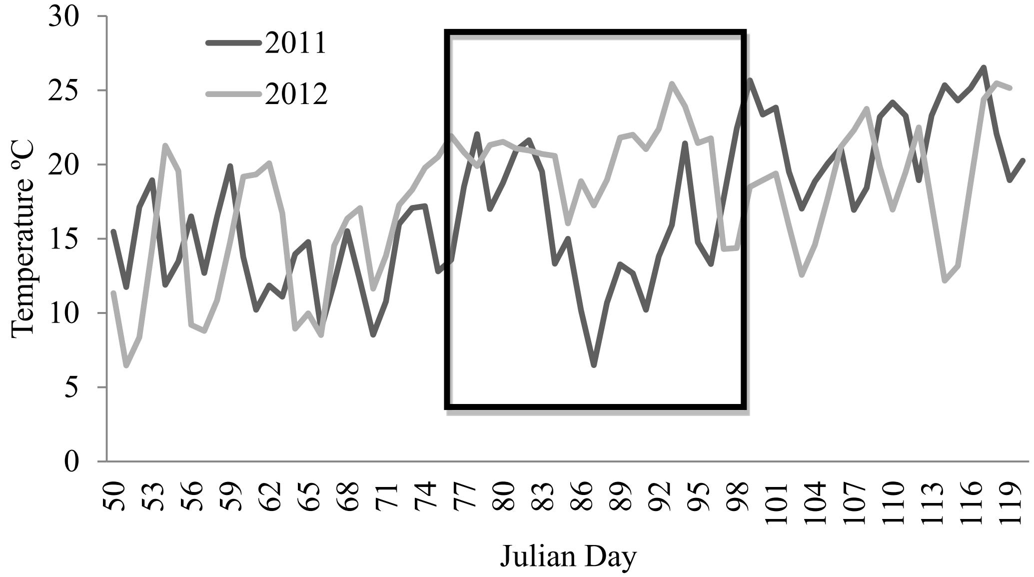


Figure 2. Frequency of Wood Duck nest starts by 5-day periods during the 2011 and 2012 breeding seasons.



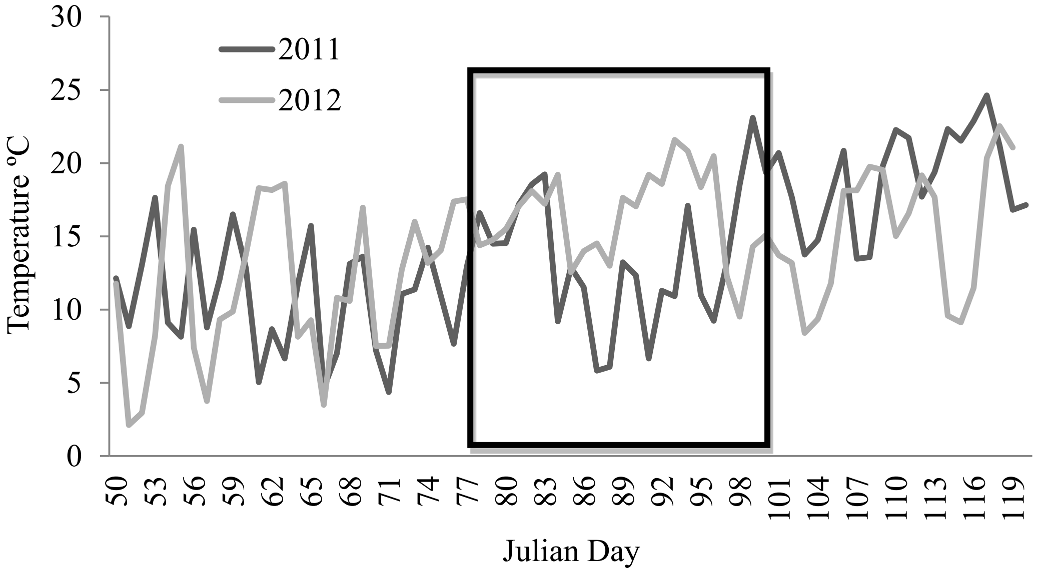


Figure 3. Temperature profiles for average daily (top) and average morning (bottom) nest box temperatures in 2011 and 2012. Area in box highlights the peak of nest initiation.