EFFECTS OF NEST QUALITY ON INCUBATION AND REPRODUCTIVE SUCCESS IN CAROLINA CHICKADEES (*POECILE CAROLINENSIS*)

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Biology.

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ABSTRACT

EFFECTS OF NEST QUALITY ON INCUBATION AND REPRODUCTIVE SUCCESS IN CAROLINA CHICKADEES (*POECILE CAROLINENSIS*)

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The effects of parental care on reproductive success is well studied. Nest building is an important aspect of parental care in birds, but it is not well understood how variation in nest building behavior impacts their reproductive success. In this study, I address the effects of nest dimensions on incubation behavior and reproductive success in female Carolina chickadees (*Poecile carolinensis*). In Carolina chickadees, only females build nests, incubate eggs, and brood young nestlings. Larger, well-constructed nests can reduce the negative effects of cooling on eggs and nestlings as extensive cooling can result in delayed embryonic development, hatching asynchrony, or failure to hatch. However, larger nests are more energetically demanding for females to construct. Females therefore face tradeoffs between self-maintenance and incubation. In this study, I tested my hypothesis that nest quality would change incubation behavior and that investment in high quality nests would result in higher reproductive success in Carolina chickadees, a common breeding bird in western North Carolina. Throughout spring and summer 2016, I monitored nest boxes in Jackson and Macon counties, N.C. for reproductive activity. I quantified nest height, nest cup depth, and the amount of moss underneath the nest cup as nest dimensions. Incubation periods (on-bouts and off-bouts) were measured using iButtons (thermal data loggers) located both inside the nest cup and inside the nest box that collected nest temperature and ambient temperature every 5 minutes. Incubation behavior was quantified as total off-bout time and mean off-bout time. Reproductive success was quantified as the number of nestlings that fledged from individual nest boxes. I found statistically significant relationships between nest dimensions and reproductive success as well as non- statistically significant relationships between nest dimensions and incubation behavior. Together, my results suggest that females that invest in building high quality nests benefit by fledging more young and that females that build poor quality nests do not compensate by increasing incubation behavior.

INTRODUCTION

The complex nests of birds provide protection for eggs and young allowing bird species to occupy most terrestrial habitats (Collias 1997). Many studies have focused on how nest diversity influences success across species of birds (Major and Kendal 2000; Guillette and Healy 2015; Hilton et al. 2004). However, less is known about how variation within species in nest architecture contributes to reproductive success (Ardia et al. 2009; Windsor et al. 2013; Cooper and Voss 2013; Rodriguez and Barba 2016). Because eggs exchange heat with the nest environment (Collias and Collias 1984), features of nest architecture that contribute to a stable thermal environment may influence hatching and survival rates of nestlings (Møller 1984; Lombardo 1994; Alvarez and Barba 2008; Ardia et al. 2009). During incubation, parents actively exchange body heat with eggs and young to maintain temperature thresholds that ensures proper development of embryos and young (Webb 1993). A well-constructed nest can buffer eggs and young from cooling when parents are away from the nest (Ardia 2009). Thus, it is likely that nest architecture and incubation together contribute to nestling survival. In this study, I investigate how variation in nest quality impacts variation in incubation behavior and nestling survival.

Patterns of nest investment observed between species may have important implications for patterns of nest investment within species. Birds in environments with colder temperatures build larger nests while nests are smaller in warmer environments across species (Soler et al. 1998) because the nest environment provides a thermal buffer against low temperatures (Windsor et al. 2013). Across bird species, eggs and nestlings of altricial species are particularly vulnerable to temperature fluctuations in the nest, and high quality nests likely influence success by providing a stable thermal environment for eggs and nestlings (Collias and Collias 1984; Pereyra and Morton 2000). Investing in high quality nests may allow parents to reserve their own energy and resources by spending less time on the nest warming eggs or young and allowing more time for foraging. Well- constructed, thermally stable nests require significantly more time to build than do poorly- constructed, less thermally stable nests (Collias 1997; Hepp 2005; Pereyra and Morton 2000). A well-constructed nest can reduce exposure of eggs and young that may lead to arrested development or failure to thrive (Windsor et al. 2013). For example, nestlings are incapable of thermoregulation until they are 3 to 4 days old (Winkler 1993; Webb 1993; Rodriguez and Barba 2016; Pereyra and Morton 2000) and depend on incubation or heat and on insulatory properties of the nest to maintain heat when incubation is not possible (Webb 1993). To help compensate for periods of off-bouts by the female, Webb and King (1983) found that nestlings use a beneficial technique of huddling together to transfer heat between them. Females that invest time in building a thermally stable nest may be able to spend more time on self-maintenance and less time incubating (Møller 1987; Reid et al. 2000) or may benefit by increased reproductive success (Windsor 2013 et al.; Møller 1987; Reid 2000). Understanding how the environment of the nest influences incubation behavior of parents and proper development of young provides insight into how parents balance the costs of current and future reproduction (Reid et al. 2002). In this study, I investigate whether variation in nest quality within species provides benefits to females by allowing for less time invested in incubation, increased reproductive success, or both.

Incubation is energetically demanding but important because maintaining constant egg temperature above a minimal threshold is crucial to proper embryonic development (Hilton et al. 2004; Caragh 2013; Rodriguez and Barba 2016). Normal embryonic development requires that eggs remain at constant temperatures of around 100°F (Heenan et al. 2015; Voss 2002; Conway and Martin 2000 a; Conway and Martin 2000 b). For example, improper incubation can lead to hatching asynchrony which typically results in later hatched nestlings that are much smaller and less competitive than older nestlings (Magrath 1988). In most bird species, females are primarily responsible for incubation or brooding by transferring body heat to the eggs or young (McClintock et al. 2014; Webb 1993) via a highly vascularized and featherless area of the breast (brood patch) that allows for direct contact between females and eggs or young and for more efficient transfer of heat (Turner 1997; Webb 1993). Thus, incubation requires a large investment of time and energy from females to maximize reproductive success.

Energy investment by females in current reproduction can result in less energy available to invest in future reproduction (Hilton et al. 2004; Caragh 2013; Reid et al. 2000; Mainwaring and Hartley 2013). Nest building, egg production, and parental care are costly in terms of energy, especially for females. Trade-offs may arise because females must strike a balance between investing in current versus future broods (Windsor et al. 2013; Weathers et al. 2003). Investing in nest building and incubation may represent a compromise between self-maintenance and maintaining proper temperatures for developing eggs and nestlings (Carter et al. 2014; Hepp 2005; Alvarez and Barba 2008). Females can help mitigate some of the costs of incubation by investing in high quality nests; though, energy constraints may force some females to invest in constructing smaller, less thermally stable nests to allow more time for foraging (Collias 1997). Thus, females may face a trade-off between the cost of investing in nest building and the cost of investing in incubation and may not be able to invest in both.

In this study, I investigate how nest quality might predict incubation behavior and reproductive success in Carolina chickadees (*Poecile carolinensis*). Larger nests, with deeper nest cups and thicker walls, result in thermal stability (Alvarez and Barba 2008; Collias and Collias 1984; Hilton et al. 2004; Pinowski et al. 2006) but require a larger investment of time and energy. Females who are unable to invest in high quality nests could compensate by increasing incubation time. Furthermore, females who invest in high quality nests may be able to reduce investment in incubation and increase self-maintenance during the incubation period. If there is a trade-off between investment in nest quality and incubation, then I predict that females with low quality nests will exhibit increased investment in incubation and that females with high quality nests will exhibit decreased investment in incubation, with both scenarios allowing for the maintenance of a high reproductive output. Alternatively, high quality females could invest in both high quality nests and incubation to maximize reproductive success. If there is not a trade-off between nest building and incubation, and if variation in nest quality reflects variation in female quality, then I predict that high quality nests will influence reproductive success but not incubation behavior.

Carolina chickadees are an excellent species to test hypotheses about the relationship between nest quality, incubation, and reproductive success. They are cavity nesting birds that are common breeders in western North Carolina. They will take readily to artificial cavities (nest boxes) (Grubb and Bronson 1995; Christman and Dhondt 1997) that provide suitable habitat for Carolina chickadees and provide them easy access to their nests (Purcell et al. 1997). Females are solely responsible for nest building, for incubating eggs and nestlings, and for brooding young (Moreno et al. 2008; Moreno et al. 2010; Lambrechts et al. 2012). In this study, I tested whether investment in high quality nests by Carolina chickadees is evidence of a trade-off by measuring nest quality, incubation behavior, and nestling survival.

METHODS

Study Sites

In total, 150 nest boxes were dispersed across western North Carolina in the general areas of Glenville, (35.1684°N, 86.1278°W) Jackson County, N.C., Cashiers, (35.1119°N, 83.0996°W) Jackson County, N.C., Sapphire, (35.1084 °N, 83.0118°W) Jackson County, N.C., and Highlands, (35.0526°N, 83.1968°W) Macon County, N.C. (Figure 1). The majority of nest boxes were located in residential areas with varying amounts of trees, shrubs, grass, and moss present. Other nest boxes were located on commercial property such as golf courses and public schools though were still placed in close proximity to trees and shrubs. All nest boxes were built to the same dimensions (9" front height x 11 ¼" back height x 6" width x 6" depth x 1" depth) and installed on location by fastening each nest box to 5 foot sections of ½ inch aluminum conduit poles. Each nest box was assigned a unique nest identification number. Predator guards were not installed on nest boxes unless heightened risks of predation, such as snakes near a nest box, were observed. Beginning in March 2016, nest boxes were checked for signs of Carolina chickadee nesting materials and eggs. To ensure that all nest boxes were monitored effectively, I monitored nest boxes weekly. Early signs of Carolina chickadee nesting materials included small pieces of moss or fur in the bottom of the nest box cavity. Once nest building had begun, each nest box showing activity was checked every 4 days to look for the addition of nesting materials in the nest box. As nests were expected to be completed within 3 weeks of building activity (Hamilton Jr. 1943), active nests were checked daily nearing completion of building. I considered nests to be complete when they contained a bottom layer of moss with a nest cup typically consisting of woven grasses often topped with one or several materials such as fur, plant material(s), and pieces of animal hair (Andreas 2010).

Nest Quality (Dimensions)

Nest dimensions were quantified as nest height, nest cup depth, and the amount of moss underneath the nest cup. I recorded measurements of nest height and nest cup depth for each completed nest. As all nest boxes were built to the same dimensions, only the height of the nest and the nest cup depth varied among nests. Measurements were taken in centimeters using a small plastic ruler. The ruler was held up to the outside portion of the nest for outer measurements, and was placed inside the nest cup for depth measurements. As the height of many nests were uneven laterally at the nest's surface edge, 3 measurements were taken for nest height. The ruler was held to the front side of the moss nest, and a height measurement was taken on the left, center, and right edge of the front side, and then the average of the 3 measurements was calculated. The average height measurement was used in analysis. This technique for measuring nest height was used for all nests regardless if they were even or uneven laterally at the nest's surface edge. To quantify nest quality (referred to as nest dimensions), nest height, nest cup depth, and the amount of moss underneath the nest cup were used. The amount of moss underneath the nest cup was calculated by taking the height of the nest and subtracting from it the depth of the nest cup. After the nestlings fledged, each nest was collected in an individual storage bag, labeled, and stored at Highlands Biological Station until I could transfer all nests to Western Carolina University for further analysis.

Incubation Behavior

Upon completion of nest building, I checked nest boxes daily for eggs. Female Carolina chickadees typically lay one egg each morning until they have laid their full egg clutch. Carolina chickadee clutch sizes can range from 3 to 10 eggs (Tekiela 2004). The average clutch size of Carolina chickadees seen throughout this study was 5 eggs. Therefore, as soon as nests had at least 4 eggs in them, I installed Thermochron iButton DS1921 data loggers (iButtons) in the nest box. iButtons record temperatures with built-in memory of the device and store the temperature data until downloaded. Each nest box received two iButtons. The first iButton was inserted in the nesting material flush with the bottom of the nest cup and secured with a zip-tie around the perimeter of the iButton. The zip-tie allowed for easy removal and re-insertion of the iButton from the nest cup for the purpose of data downloading. To record ambient temperature inside the nest box, a second iButton, inserted into a fob, was installed inside the nest box using a zip-tie and was hung from a ventilation hole in the upper corner of the inside of the nest box. The zip-tie allowed for quick removal and re-installation of the iButton for the purpose of data downloading and did not interfere with the activities of the adults in the nest box. I simultaneously programed each iButton to record temperatures constantly in 5 minute intervals. To insure simultaneous activity, each iButton was programmed to begin recording temperatures at the same time. When installing iButtons, to minimize the influence of human body temperature on data recording, I placed each iButton on a 10 minute time delay before they began collecting temperature data. Using these parameters, each iButton could record data for 7 consecutive days, and data from the iButtons was downloaded every 6 days until the eggs hatched. The iButton program Thermodata Viewer was used to download each iButton's temperature data. The accessibility of downloading iButtons at the nest box site allowed for quick removal and re-insertion of the iButtons to the nest boxes to minimize any disturbance to the females or to the nestlings during incubation.

Female Carolina chickadees incubate their eggs for a period of 12-16 days (Tekiela 2004). Therefore, as the end of the incubation period approached, I checked each active nest box daily for signs of hatching. Hatching typically began in early morning hours and would continue throughout the day until each egg had hatched. Circumstances including inviability and asynchrony resulted in some eggs either not hatching or hatching a day later than all other nestlings. Upon hatching completion, I removed each iButton from both the nest and the nest box.

Temperature data from inside each nest and nest box allowed me to calculate periods of incubation by comparing the changes in nest temperature to ambient temperature (Hartman and Oring 2006). I quantified incubation behavior as mean off-bout time, and total off-bout time. Mean off-bout referred to the average amount of time in minutes that each female spent off of the nest per off-bout during the period of incubation. Total off-bout referred to the average amount of time as a proportion that each female spent off the nest per day during the period of incubation. I calculated mean off-bout and total off-bout individually by comparing the initial off-bout times per day of incubation per female with the final off-bout times per day of incubation per female. Identifying these two forms of incubation separately allowed for comparisons between the overall time that females spent incubating throughout the entire day verses the specific lengths of off-bout times throughout the day.

For analysis of incubation data, I used Raven Pro 1.4 and Rhythm (Cooper and Mills 2005) in conjunction with one another. Rhythm converts text files from iButtons into formatted files which can be opened using Raven (Cooper and Mills 2005). Raven allows analysis of data collected at consistent time intervals to depict off-bout and on-bout periods by incubating females (Cooper and Mills 2005). In relation to steady ambient temperatures, periods of decreasing nest temperatures indicated periods of off-bouts by the incubating female while periods of increasing nest temperatures indicated periods of on-bouts by the incubating female (Cooper and Mills 2005). Likewise, large spans of consistent nest temperatures during evening hours corresponded to the female incubating her eggs throughout the night (Cooper and Mills

2005). I used Raven to identify off-bouts in the incubation data and then visually inspected the data and made edits only when necessary. Necessary edits included predicted off-bout periods by Raven which were identified as non- off-bout periods by my written documentation of notes during nest box monitoring. When off-bouts were too long or when off-bouts were not identified at all in Raven, I also verified specific off-bout periods by my notes from nest box monitoring. iButton pairs remained in each nest box for the duration of incubation of the eggs.

To verify the accuracy of iButton data in showing incubation, a sample video was recorded during the period of incubation, and the video data was compared to the iButton incubation data of the nest box that had been recorded. I used a small PV 500 DVD player and button camera to collect the video imaging. The camera was secured to the top of the inside of the nest box using double sided Velcro to allow for viewing of nest activity. The recorder was secured to the nest box pole at the base of the nest box and connected to the camera via vents in the nest box. I collected sample video imaging on 2 different nest boxes. The cameras used would collect video imaging for a total of 6 hours. Once video data had been recorded, I would remove nest incubation iButtons from the nest and download them. The video data and the nest incubation iButton data were then compared to validate that off-bout periods detected by iButtons corresponded to periods when females were off the nest. I found that iButton data accurately estimated off-bout periods.

Reproductive Success

Carolina chickadee nestlings typically remain in the nest for 15-22 days (Gowaty and Plissner 1997). At day 12 after hatching, I re-installed both iButtons into each nest to record fledging data; such disturbances after day 12 can cause premature fledging of the nestlings. iButtons were used to accurately estimate fledging date. During the late nestling period, an iButton in the nest would record the presence of nestlings as being consistently warm (30- 35°C). Fledging was assumed to have occurred when the nest iButton temperature was correlated with the iButton measuring ambient temperature. The same method of video monitoring and analysis used for verifying incubation was also conducted during periods of fledging to test the accuracy of iButton fledging data collection. I compared the video data and the nest fledge iButton data to one another to check for accuracy. I found accuracy in the fledging iButton data collected as decreasing iButton temperatures correlated with video data fledging time. After re-installing both iButtons into each nest, I checked nest boxes bi-weekly for signs of nestling fledging. Visual evidence of fledging included fledglings on the ground near the box, feces on the inside and outside of nest box walls, a compacted nest cup, and adult Carolina chickadees feeding young in a nearby area. After nestling fledging had completed, I removed iButtons from the nest and download them. Thus, I felt confident that I was able to identify fledging versus predation if nestlings were in the box for at least 18 days and/or there was clear evidence of fledging. I used the number of fledglings as my measure for reproductive success in all of my analyses.

Statistical Analyses

I used the statistical software R for data analysis (R Core Team 2013). Using R, I constructed general linear models (GLM) representing the variables of nest dimensions, incubation behavior, and reproductive success. To analyze the relationship between reproductive success and nest dimensions, I constructed a GLM using the number of nestlings that fledged as the dependent variable, and using the height of the nest, the depth of the nest cup, and the amount of moss underneath the nest cup as co-variates. To understand the relationship between nest dimensions and incubation behavior, I constructed a GLM using the height of the nest as the independent variable and using the mean off-bout time and the total off-bout time as dependent variables.

<u>Figure 1</u> Study Sites

RESULTS

I monitored a total of 150 nest boxes. Of the 150 nest boxes monitored, 61 nest boxes were found to be occupied by Carolina chickadees and 89 were occupied by other species including eastern bluebirds (*Sialia sialis*), house wrens (*Troglodytes aedon*), and tree swallows (*Tachycineta bicolor*). The Carolina chickadee nest boxes were continually monitored whereas the remaining nest boxes containing other species were not continually monitored. Of the 61 Carolina chickadee nest boxes, 31 fledged with an average clutch size of 5.2, an average number of eggs hatched of 4.2, and an average number of fledglings per nest of 3.8. The nesting period was from the time of hatching to the time of fledging and averaged a total of 17.5 days. Thirty of the Carolina chickadee nests failed due to predation of eggs or nestlings by house wrens, bears, snakes, or failing to hatch, or nestling death, or abandonment for unknown reasons. As predated nests resulted in having no eggs to be considered for hatching, only nest boxes which contained successful nestling hatching were then considered for analysis of fledging success. Therefore, the sample size for reproductive success was 31. As incubation data, collected via iButtons, was gathered on 5 of the nest boxes that faced predation later in the brooding season, the sample size for incubation behavior was 36.

I checked the normality of my models by looking at the Normal Q-Q Plot and checked the assumption of homogeneity by looking at the Scale-Location Plot. I found that nest height was positively correlated with the number of nestlings that fledged the nest (Table 1, Figure 2a; GLM: $t = 3.883$, $P = 0.000574$, $N = 31$). Nest cup depth was negatively correlated with the number of nestlings that fledged the nest (Table 1, Figure 2b; GLM: $t = -2.375$, $P = 0.024634$, $N = 31$). The amount of moss underneath the nest cup was negatively correlated to nest cup depth

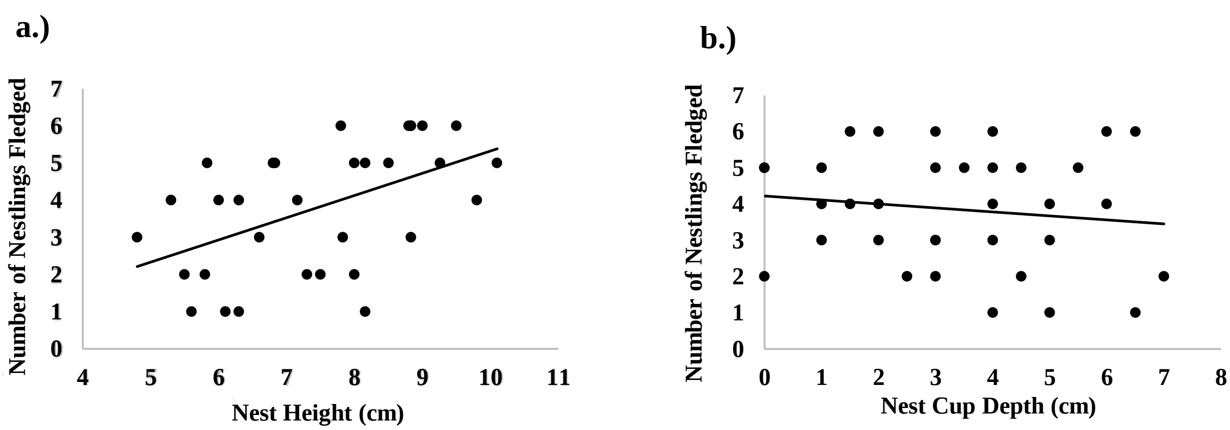
(Table 2, Figure 3; GLM: $t = -13.01$, $P = < 0.0001$, $N = 31$). The amount of moss underneath the nest cup was positively correlated to the number of nestlings that fledged the nest (Table 3, Figure 4; GLM: $t = 2.072$, $P = 0.04729$, $N = 31$). Nest height was not correlated to total off-bout time (Table 4, Figure 5; GLM: $t = 0.531$, $P = 0.60025$, $N = 36$). Nest height was not correlated to mean off-bout time (Table 5, Figure 6; GLM: $t = 1.196$, $P = 0.243$, $N = 36$).

Table 1

Individual results for overall GLM on nest dimensions and reproductive success. Nest dimensions are measured as nest height and nest cup depth. Reproductive success is measured as the number of nestlings that fledged the nest. There is a positive relationship between nest height and the number of nestlings that fledged the nest and a negative relationship between nest cup depth and the number of nestlings that fledged the nest.

	Estimate	Std. Error	t-value	p-value	df
Intercept	-0.2698	1.3065	-0.207	0.83788	29
Height	0.7061	0.1819	3.883	0.00057	29
Cup depth	-0.32	0.1347	-2.375	0.02463	29

Figure 2



a.) Statistically significant positive linear regression result comparing nest height to the number of nestlings that fledged the nest (GLM: t = 3.883, P = 0.000574, N = 31).

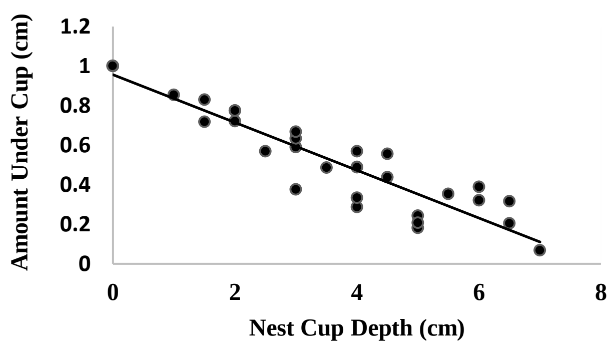
b.) Statistically significant negative linear regression result comparing nest cup depth to the number of nestlings that fledged the nest (GLM: t = -2.375, P = 0.024634, N = 31).

Table 2

Individual results for overall GLM on nest dimensions. Nest dimensions are measured here as the amount of moss underneath the nest cup and nest cup depth. There is a negative relationship between the amount of moss underneath the nest cup and nest cup depth.

	Estimate	Std. Error	t-value	p-value	df
Intercept	7.2736	0.3188	22.81	< 0.0001	29
Undercup	-7.0649	0.5431	-13.01	< 0.0001	29

Figure 3



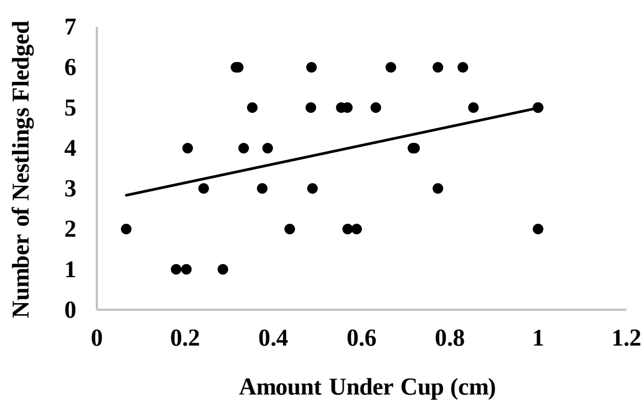
Statistically significant negative linear regression result comparing the amount of moss underneath the nest cup to the nest cup depth (GLM: t = -13.01, P = < 0.0001, N = 31).

Table 3

Individual results for overall GLM on nest dimension and reproductive success. Nest dimension is measured here as the amount of moss underneath the nest cup. Reproductive success is measured here as the number of nestlings that fledged the nest. There is a positive relationship between the amount of moss underneath the nest cup and the number of nestlings that fledged the nest.

	Estimate	Std. Error	t-value	p-value	df
Intercept	2.678	0.6557	4.084	0.00032	29
Undercup	2.3136	1.1167	2.072	0.04729	29

Figure 4



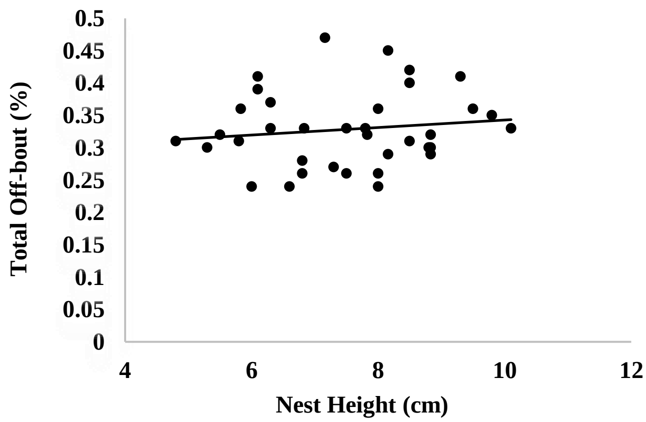
Statistically significant positive linear regression result comparing the amount of moss underneath the nest cup to the number of nestlings that fledged the nest (GLM: t = 2.072, P = 0.04729, N = 31).

Table 4

Individual results for overall GLM on nest dimension and incubation. Nest dimension is measured here as nest height. Incubation is measured here as total off-bout time. There is no relationship between nest height and the total off-bout time.

	Estimate	Std. Error	t- value	p- value	df
Intercept	0.26028	0.06216	4.187	0.00031	25
Height	0.00398	0.0075	0.531	0.60025	25

Figure 5



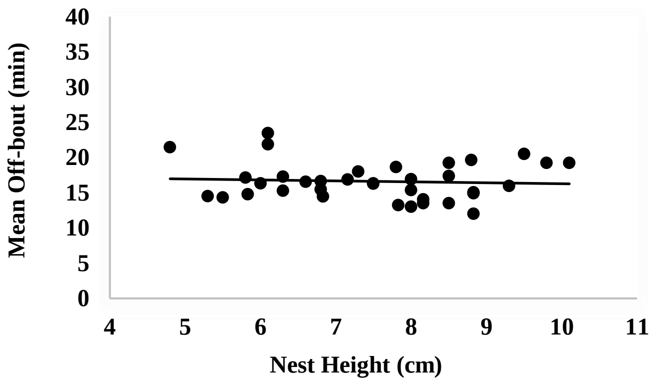
Non- statistically significant linear regression result comparing nest height to the total off-bout time (GLM: t = 0.531, P = 0.60025, N = 36).

Table 5

Individual results for overall GLM on nest dimension and incubation. Nest dimension is measured here as nest height. Incubation is measured here as mean off-bout time. There is no relationship between nest height and the mean off-bout time.

	Estimate	Std. Error	t- value	p- value	df
Intercept	9.5512	5.5387	1.724	0.097	25
Height	0.7991	0.6681	1.196	0.243	25

Figure 6



Non- statistically significant linear regression result comparing nest height to the mean off-bout time (GLM: t = 1.196, P = 0.243, N = 36).

DISCUSSION

I studied the effects of nest quality on incubation behavior and reproductive success in Carolina chickadees. I assayed nest quality by measuring nest dimensions; larger nests are considered higher quality nests (Alvarez and Barba 2008; Collias and Collias 1984; Hilton et al. 2004; Pinowski et al. 2006). I measured incubation behavior over most of the incubation period using thermal data loggers (iButtons). Incubation behavior was quantified in two ways: 1- average length of off-bouts per day; and 2- average amount of time spent off the nest per day. I predicted that quality of nest construction would change incubation behavior and that these changes would contribute to reproductive success. I found no correlation between nest quality and incubation behavior. I also predicted that high quality nest construction would influence reproductive success. I found a positive correlation between high quality nest construction and reproductive success. Nest height and the amount of moss underneath the nest cup predicted reproductive success, but there was no relationship between incubation behavior with reproductive success. Together, my results suggest that females that invest in building high quality nests benefit by fledging more young and that females that build poor quality nests do not compensate by increasing incubation behavior.

Nest Quality and Reproductive Success

In this study, the relationship between nest quality, measured here as the height of the nest, and reproductive success, measured here as the number of nestlings that fledged the nest, resulted in a significant positive relationship (Table 1, Figure 2a). Therefore, nest height predicts reproductive success in the Carolina chickadee. The second measure of nest quality I used was nest cup depth. In a study of great tits (*Parus major*), a close relative of Carolina chickadees, Alvarez and Barba (2008), found that nests with deeper cups were higher quality and more thermally stable than nests with shallow nest cups, as additional nest material would help provide more nest insulation. Nest insulation, which contributes to nest quality, is also an important factor in fledgling success. Better insulation in nest construction enables eggs and hatchlings to be kept warm in cold temperatures (Conway and Martin 2000 a; Conway and Martin 2000 b; Alvarez and Barba 2008). Consistency of proper temperature in the nest is necessary for successful hatching, development, and fledging (Alvarez and Barba 2008; Rodriguez and Barba 2016). Nest insulation is also important for the cushioning and protection of eggs and nestlings in the nest (Voss 2002; Collias and Collias 1984) and for thermal conductivity. If nests become wet, thermal conductivity increases (Hilton et al. 2004). Increased conductivity of nesting materials may have an adverse effect on eggs and nestlings because they will experience cooling at a faster rate. Many bird species will choose nesting materials that are less prone to absorbing water and will strategically place those materials in the nest box in a particular arrangement to reduce their exposure to water (Hilton et al. 2004). Similarly, material arrangement within the nest box can trap air layers to help aid in insulating the nest (Møller 1984). Trapped air layers can be significant for the nest (Deeming and Biddle 2015) similar to how animal down and animal fur function. The collected nests from my study differed mainly in the nest height and in the amount of moss underneath the nest cup. Animal fur and plant material(s), interlaced with moss, made-up the majority of the nests' outer composition, while the nest cups were lined with moss and animal fur to produce a soft and warm environment in the nest cup. This illustrates material selection by the female in nest construction. Biddle et al. (2015), found that common blackbirds (*Turdus merela*) used thicker, heavier materials for the outer nest wall compared to the interior of the nest cup which was composed of smaller, lighter materials. Heavier materials were also used at the base of the nest cup compared to the upper portions of the nest cup.

Likewise, in a study on bullfinches, material selection of thicker, heavier materials was used for the outer nest wall compared to the interior of the nest cup suggesting non-random material placement (Biddle et al. 2017). The chickadee nests in my study followed similar nest construction with heaviest, thickest materials to the outer-lower portion of the nest while the inside of the nest cups were built with finer, lighter materials. Bailey et al. (2014), found that material selection was influenced by the experience of the bird. This would suggest that nest quality and reproductive success would improve with the age and experience of the female chickadee.

As other researchers found that deeper nest cups were higher quality and helped provide more nest insulation contributing to fledgling success, I expected to find a positive correlation between nest cup depth and fledging success in my results. Instead, I found a statistically negative relationship between nest cup depth and fledging success (Table 1, Figure 2b). To explain my result, I suggest nest cup depth may not be the relevant factor for reproductive success, but rather, a similar form of nest quality may be. As nests lacking adequate insulating material at the bottom of the nest cup would make young birds more susceptible to heat loss (Voss 2002; Collias and Collias 1984), I chose to investigate the relevance of the amount of nesting material located below the nest cup. The statistical comparison of nest cup depth and the amount of moss underneath the nest cup resulted in a significant negative relationship (Table 2, Figure 3) indicating the amount of moss underneath the nest cup may be an important factor in nest quality. To further investigate nest quality in relation to reproductive success, I statistically compared the amount of moss underneath the nest cup to the number of nestlings that fledged the nest and found a significant positive relationship (Table 3, Figure 4). The positive relationship found emphasizes the amount of moss underneath the nest cup is an important factor in nest construction. In my study, shallower nest cups were found to provide better thermal insulation than deeper nest cups due to the larger amounts of moss present underneath the nest cup. More insulation between a shallow nest cup and the bottom of the nesting cavity results in increased thermal stability, in cushioning, and in protection of developing eggs and nestlings. Therefore, in relation to nest quality, my results indicate the amount of moss underneath the nest cup may be the relevant factor in reproductive success. As Carolina chickadees have only one brood per nesting season, females may choose to optimize their use of nesting materials and material placement to better cushion and insulate their clutch. In my study, placement of nesting materials was beneficial as 31 nest boxes had successful fledging with an average number of 3.8 fledglings per nest.

Nest Quality and Incubation Behavior

Incubation is demanding energetically for females in multiple ways (Rodriguez and Barba 2016). Energetic costs to females include the process of re-warming eggs and nestlings after returning from each off-bout (Collias and Collias 1984; Hilton et al. 2004). Energy demands on the female would also be related to female quality with higher energy expenditures associated with higher quality females. Age, and thus parental experience, would impact female quality and their energy demands for incubation and nest building (Conway and Martin 2000 a; Conway and Martin 2000 b; Collias and Collias 1984).

In my study, I found no correlation between nest dimensions and incubation behavior. I also found that nest quality had no effect on the amount of time Carolina chickadee females spent off their nest during the period of incubation (Table 4, Figure 5; Table 5, Figure 6).

Time Investment for Foraging and Incubation

Though adequate incubation is crucial to the development of eggs and nestlings, females must maintain their health and energy levels by procuring sufficient nutrients. They must spend time off the nest to satisfy their energy needs (Walters et al. 2016; Conway and Martin 2000 a; Conway and Martin 2000 b). To offset their time investment between incubation and foraging, different females have developed different techniques. In their study on Mediterranean great tits (*Parus major*), Rodriguez and Barba (2016) reported that, regardless of dropping temperatures, females did not increase the time that they spent incubating and instead continued to forage as needed. Similar findings to Rodriguez and Barba (2016) were reported by Møller (1987) and Reid et al. (2000). In their work, females spent more time off the nest foraging than they did on the nest incubating. Other females have chosen to build deeper nests which have greater insulatory properties when compared to shallower similar nests (Alvarez and Barba 2008). In some works, the females investing in the construction of deeper, better insulated nests spent less time on the nest incubating and more time off the nest foraging when compared to females that built shallow nests (Møller 1987; Reid et al. 2000). In my work with the Carolina chickadee, I found that the females invest more time on their nest incubating their eggs than they do off the nest participating in activities which may include foraging for self-maintenance.

Females have a variety of investment strategies that they can use to manage the trade-off between self-care and incubation/brooding. Females can invest solely in building a high quality nest, or in themselves by foraging more often and for longer periods of time, or they can maximize both self-care and care of their young. In my study, females that built more thermally stable, higher quality nests, created the possibility of leaving their nest more frequently and for longer periods of time and spending more time foraging than on incubation. However, my results show that female Carolina chickadees do not have more frequent off-bouts or for extended time periods based on the quality of their nests. If high quality females build high quality nests and continue to give high quality care until nestlings fledge, high quality females may be rewarded with higher reproductive success. It is possible that quality may vary between females or that females improve with age. I did not have the data to test that hypothesis, but this should be the subject of future studies.

Possible Investigations

Future work could involve investigation of nests by looking at characteristics of nest building materials such as, their composition, their quantity, and their location inside the nest box (Tomás et al. 2006; Hilton et al. 2004). Nests could be examined for their insulating properties and then compared to fledging success to determine if there is a correlation of nesting success to insulation characteristics of the nest. In their study on tree swallows (*Tachycineta bicolor*), Lombardo et al. (1995) found a relationship between reproductive success and nest quality where incubation behavior increased in nest boxes that had feather lining removed. Likewise, as well insulated nest cups positively influence the thermal properties of the nest for developing eggs and nestlings (White and Kinney 1974), it is understandable why Møller (1982) found decreased rates of hatching in barn swallows (*Hirundo rustica*) in smaller nests than in larger built nests. Through their work with rufous bush robins (*Cercotrichas galactotes*), Palomino et al. (1998) reported the thickness of the nest wall and nest bottom may be a key component in the thermoregulatory abilities of the nest environment. Interestingly, Palomino et al. (1998) and Alvarez and Barba (2008) did not find a positive relationship between reproductive success and the thickness of the nest bottom, though in my study of Carolina chickadees, I did find a positive result. My research resulted in a significant positive relationship between the amount of moss underneath the nest cup and the number of nestlings that fledged the nest. This result may show evidence that Carolina chickadees rely more heavily on the amount of moss underneath the nest cup for reproductive success than do other species of birds.

Furthermore, in contrast to my study, collection of nest measurements, incubation data, and reproductive success data, needs to be carried out more than once for the same female to establish repeatability. Repeatability serves as an approximate measure of heritability of behavior (Boake 1989), and females should exhibit repeatability in their nest measurements for each nesting attempt per brooding season (Stanback et al. 2013). Knowing a particular female's birth year would allow for tracking that female through repeated breeding seasons using identification banding and would permit investigation into whether female Carolina chickadee's age, quality, or both are related to nest quality. Two questions that could be answered are, 1- do female Carolina chickadees get better at nest building as they get older, and 2- do higher quality females build higher quality nests as indicated by variation in nest quality? In my study, the ages of the females were unknown, so no predictions could be made about the health and overall quality and breeding history of the females. Overall however, my study had beneficial implications in the study of Carolina chickadees as I found support that investment in high quality nest construction influences reproductive success while there was no support indicating a trade-off between incubation behavior and nest quality.

REFERENCES

Alvarez, E., Barba, E. 2008. Nest quality in relation to adult bird condition and its impact on reproduction in great tits (*Parus major*). Acta Ornithologica. 43(1): 3- 9.

Andreas, B. 2010. Use of bryophytes by Carolina chickadees (*Poecile carolinensis*) in nest construction. The American Bryological and Lichenological Society, Inc. Evansia 27(1): 23- 29.

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

Bailey, I., Morgan, K., Bertin, M., Meddle, S., Healy, S. 2014. Physical cognition: birds learn the structural efficacy of nest material. Proceedings of the Royal Society B- Biological Sciences. 281(1784): 20133225.

Biddle, L., Deeming, D., Goodman, A. 2015. Morphology and biomechanics of the nest of the common blackbird (*Turdus merula*). Bird Study. 62(1): 87-95.

Biddle, L., Goodman, A., Deeming, D. 2017. Construction patterns of birds' nests provide insight into nest-building behaviors. PeerJ. 5: e3010.

Boake, C. 1989. Repeatability: its role in evolutionary studies of mating behavior. Evolutionary Ecology. 3: 173-182.

Caragh, H. 2013. An overview of the factors influencing the morphology and thermal properties of avian nests. Avian Biology Research. 6: 104-118.

Carter, A., Hopkins, W., Moore, I., DuRant, S. 2014. Influence of incubation recess patterns on incubation period and hatchling traits in wood ducks (*Aix sponsa*). Journal of Avian Biology. 45: 273-279.

Christman, E., Dhondt, A. 1997. Nest predation in black-capped chickadees: how safe are cavity nests? The Auk. 114: 769-773.

Collias, N. 1997. On the origin and evolution of nest building by passerine birds. The Condor. 99: 253-270.

Collias, N., Collias E. 1984. Nest building behavior in birds. Princeton Univ. Press, Princeton, New Jersey.

Conway, C., Martin, T. a). 2000. Effects of ambient temperature on avian incubation behavior. Behavioral Ecology 11: 178–188.

Conway, C., Martin, T. b). 2000. Evolution of passerine incubation behavior: influence of food, temperature, and nest predation. Evolution. 54: 670-685.

Cooper, C., Mills, H. 2005. New software for quantifying incubation behavior from time-series recordings. Journal of Field Ornithology. 76(4): 352-356.

Cooper, C., Voss, M. 2013. Avian incubation patterns reflect temporal changes in developing clutches. PLoS One. 8(6).

Deeming, D., Biddle, L. 2015. Thermal properties of bird nests depend on air-gaps between the materials. Acta Ornithologica. 50(1): 121-125.

Gowaty, P., Plissner, J. 1997. Breeding dispersal of eastern bluebirds depends on nesting success but not on removal of old nests: an experimental study. Journal of Field Ornithology. 68: 323-502.

Grubb Jr., T., Bronson, C. 1995. Artificial snags as nesting sites for chickadees. The Condor. 97: 1067-1070.

Guillette, L., Healy, S. 2015. Nest building, the forgotten behavior. Current Opinion in Behavioral Sciences. 6: 90-96.

Hamilton Jr., W. 1943. Nesting of the eastern bluebird. The Auk. 60: 91- 94.

Hartman, C., Oring, L. 2006. An inexpensive method for remotely monitoring nest activity. Journal of Field Ornithology. 77: 418–424.

Heenan, C., Goodman, B., White, C. 2015. The influence of climate on avian nest construction across large geographical gradients. Global Ecology and Biogeography. 24: 1203-1211.

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

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

Lambrechts, M., Aime, C., Midamegbe, A., Galan, M., Perret, P., Gregoire, A., Doutrelant, C. 2012. Nest size and breeding success in first and replacement clutches: an experimental study in blue tits (*Cyanistes caeruleus*). Journal of Ornithology. 153: 173-179.

Lombardo, M. 1994. Nest architecture and reproductive performance in tree swallows (*Tachycineta bicolor*). The Auk: 814-824.

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

Magrath, R. 1988. Hatching asynchrony in altiricial birds: nest failure and adult survival. The American Naturalist. 131(6): 893- 900.

Mainwaring, M., Hartley, I. 2013. The energetic costs of nest building in birds. Avian Biology Research. 6(1): 12-17.

Major, R., Kendal, C. 2000. The contribution of artificial nests experiments to understanding avian reproductive success: a review of methods and conclusions. IBIS. 138: 298-307.

McClintock, M., Hepp, G., Kennamer, R. 2014. Plasticity of incubation behaviors helps wood ducks (*Aix sponsa*) maintain an optimal thermal environment for developing embryos. The Auk. 131: 672-680.

Møller, A. 1982. Clutch size in relation to nest size in the swallow (*Hirundo rustica*). Ibis. 124: 339–343.

Møller A. 1987. Nest lining in relation to the nesting cycle in the swallow (*Hirundo rustica*). Ornis Scandinavica. 18: 148–149.

Møller, A. 1984. On the use of feathers in birds' nests: predictions and tests. Ornis Scandinavica. 15: 38-42.

Moreno, J., Lobato, E., Gonzalez-Braojos, S., Ruiz-de Castaneda, R. 2010. Nest construction costs affecting nestling growth: a field experiment in a cavity-nesting passerine. Acta Ornithologica. 45: 139-145.

Moreno, J., Martinez, J., Corral, C., Lobato, E., Merino, S., Morales, J., Martinez-de la Fuente, J., Tomas, G. 2008. Nest construction rate and stress in female pied flycatchers (*Ficedula hypoleuca*). Acta Ornithologica. 43: 57-64.

Palomino J., Martí-Vivaldi M., Soler M., Soler J. 1998. Functional significance of nest size variation in the rufous bush robin (*Cercotrichas galactotes*). Ardea. 86: 177–185.

Pereyra, M., Morton, M. 2000. Nestling growth and thermoregulatory development in subalpine dusky flycatchers. The Auk. 118(1): 116- 136.

Pinowski, J., Haman, A., Jerzak L., Pinowska B., Barkowska M., Grodzki A., Haman K. 2006. The thermal properties of some nests of the Eurasian tree sparrow (*Passer montanus*). Journal of Thermal Biology. 31: 573-581.

Purcell, K., Verner, J., Oring, L. 1997. Comparison of the breeding ecology of birds nesting in boxes and tree cavities. The Auk. 114: 646-656.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.r-project.org/.

Reid, J., Monaghan, P., Nager, R. 2002. Incubation and the costs of reproduction. Avian Incubation: Behavior, Environment, and Evolution. Edited by Deeming, D. Oxford University Press: 314-325.

Reid J., Monaghan P., Ruxton G. 2000. Resource allocation between reproductive phases: the importance of thermal conditions in determining the cost of incubation. Proc. R. Soc. B 267: 37–41.

Rodriguez, S., Barba, E. 2016. Effects of cool nest microclimates on nestling development: an experimental study with Mediterranean great tits (*Parus major*). Ardeola. 63(2): 251-260

Soler, J., Møller, A., Soler, M. 1998. Nest building, sexual selection and parental investment. Evolutionary Ecology. 12: 427-441.

Stanback, M., Mercadante, A., Cline, E., Burke, T., Roth, J. 2013. Cavity depth, not experience, determines nest height in eastern bluebirds. Wilson Journal of Ornithology. 125: 301-306.

Tekiela, S. 2004. Birds of the Carolinas: field guide. Adventure Publications, Inc. pp. 219.

Tomás, G., Merino, S., Moreno, J., Sanz, J., Morales, J., García-Fraile, S. 2006. Nest weight and female health in the blue tit (*Cyanistes caeruleus*). Auk. 123: 1013–1021.

Turner, S. 1997. On the thermal capacity of a bird's egg warmed by a brood patch. Physiological Zoology. 70: 470-480.

Voss, M. 2002. Predictions and quantitative tests of optimal time and temperature allocation during intermittent incubation. Biology - Dissertations. 39. http://surface.syr.edu/bio_etd/39.

Walters, L., Webber, J., Jones, Brittany A., Volker, C. 2016. Taking a break: the relationship between ambient temperature and nest attendance patterns of incubating Carolina chickadees (*Poecile carolinensis*). The Wilson Journal of Ornithology. 128(4): 719-726.

Weathers, W., Davidson, C., Morton, M. 2003. Energetics of altricial nestlings in cold climates: insights from the mountain white-crowned sparrow. The Condor. 105(4): 707-718.

Webb, D. 1993. Maternal-nesting contact geometry and heat transfer in an altricial bird. Journal of Thermal Biology. 18(2): 117-124.

Webb, D., King, J. 1983. Heat-transfer relations of avian nestlings. Journal of Thermal Biology. 8(4): 301-310.

White, F., Kinney, J. 1974. Avian incubation. Science. 186: 107–115.

Windsor, R., Fegely, J., Ardia, D. 2013. The effects of nest size and insulation on thermal properties of tree swallow nests. Journal of Avian Biology. 44: 305-310.

Winkler D. 1993. Use and importance of feathers as nest lining in tree swallows (*Tachycineta bicolor*). The Auk. 110: 29–36.