

RESPONSES OF COASTAL NESTING KING RAILS (*RALLUS ELEGANS*) TO
ENVIRONMENTAL THREATS OF RISING WATER AND TEMPERATURE

By

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King rail (*Rallus elegans*) populations are experiencing long-term declines attributable to habitat loss. Sparse breeding populations in coastal areas may represent the last strongholds for this species, but climate change models predict these are at risk from extreme weather events, including rising temperatures and higher frequencies and intensities of storms, with associated risks of storm surge and flooding. A breeding population at Mackay Island National Wildlife Refuge (NWR) in coastal North Carolina likely represents an important source population for the region. I investigated the effects of elevated temperatures and rising water on the nesting success of king rails at Mackay Island NWR, and whether they have evolved behavioral plasticity in nesting behavior to mitigate these threats. I further tested the alternative hypothesis that variation among parents in nesting effort may be related to individual differences in body condition.

Clutch temperatures that exceed acceptable limits for embryo development may result in embryo mortality. I monitored clutch and ambient temperatures simultaneously using Thermochron iButtons, and I documented parental incubation behavior at king rail nests. Video recordings reveal parents adjust their incubation behavior in response to ambient temperatures, and shading their nest may allow king rails to maintain their clutches below lethal temperatures

in hot conditions. However, king rails tend to take more frequent recesses of nest attendance at higher temperatures, exposing their eggs to adverse conditions for embryo development. Clutches that reached higher temperatures for longer periods of time tended to hatch fewer chicks. Additionally, seasonal variation in temperature exposed late season nests to hotter conditions. Parents begin incubating earlier and hatching became more asynchronous later in the breeding season. Although parents show plasticity in incubation behavior, hatching failure related to high clutch temperatures suggests king rails may be constrained in their ability to cope with high temperatures experienced on the nest.

Coastal marsh breeders must also contend with water level variation, and other rails (Family: Rallidae) add material to their nests throughout incubation to prevent nest flooding. Water levels at Mackay Island NWR are influenced by wind-driven tides and, consequently, are exceptionally variable. King rails build taller nests in areas with greater ranges in water levels. Parents spent proportionately more time building when the water level approached the top of the nest. However, individual nest height remained relatively constant and did not increase considerably during the laying period, suggesting that the majority of nest building occurred before laying began. Nest building during the laying and incubation periods may therefore function mainly to counteract settling and improve the structural integrity of the nest. Although they continue to build throughout the nesting cycle, parents may not be able to prevent nest flooding when faced with rapidly rising water.

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Chapter 1

Plasticity in incubation behavior by nesting king rails in response to temperature variation

ABSTRACT

King rails (*Rallus elegans*) nesting in coastal freshwater marshes experience a wide range of temperatures during the course of the breeding season. Birds are adapted to keep their clutch of eggs within an acceptable temperature range for embryo development, but king rail clutches are at risk of exceeding lethal temperature limits in the latter half of the nesting season. King rail parents were found to modify their behavior at the nest in relation to ambient temperature by shading their eggs. I tested whether onset of shading was consistent with a direct response to ambient temperature (adaptive plasticity), or whether their investment in this and other nest behaviors was limited by body condition. I monitored clutch and ambient temperatures simultaneously, and documented nesting behavior at Mackay Island National Wildlife Refuge in 2014 and 2015. Parents spent proportionately more time shading and less time incubating their eggs at higher ambient temperatures, and distribution of effort among tasks was not related to body condition. However, parents also took more frequent recesses in hotter conditions, often exposing their eggs to direct sunlight. Clutches that both reached and remained at high temperatures tended to hatch fewer chicks. Because mean ambient temperature increases throughout the breeding season, I investigated seasonal patterns in onset of incubation and its effect on hatching rate. I found that in this population, hatching asynchrony increases significantly later in the season. Together these results suggest that breeding king rails may be

constrained in their ability to cope with sustained high temperatures should seasonal maxima continue to rise as predicted.

INTRODUCTION

The king rail (*Rallus elegans*) is a secretive marshbird found in freshwater and brackish wetlands in the eastern United States (Cooper, 2008; Meanley, 1969; Taylor & van Perlo, 1988). It lives and nests in marshes with dense emergent vegetation (Pickens & King, 2013). Rails are wetland obligates and are sensitive to habitat disturbance (Cooper, 2008). Therefore, they are excellent indicators for habitat health (Péron et al., 2013; Woodrey et al., 2012).

King rail populations have been exhibiting long-term declines attributable to habitat loss, particularly in migratory populations that breed in the interior parts of the species' range (Bolenbaugh et al., 2012; Cooper, 2008). Marshbirds are considered indicator species for wetland ecosystem quality (Conway, 2011). King rails are listed as a Species of Greatest Conservation Need in 30 State Wildlife Action Plans and as threatened or endangered in 12 states (Cooper, 2008). Remaining strongholds of king rails are restricted to coastal areas, where the populations are typically resident (Bolenbaugh et al., 2012). Coastal populations of king rails may thus constitute source populations (*sensu* Pulliam, 1988), crucial to the survival of the species.

Coastal ecosystems are among the most threatened in the world (Millennium Ecosystem Assessment, 2005) due to increasing risk of changes to the physical habitat associated with climate change, including sea level rise and marsh erosion (Michener et al., 1997; Ravens et al., 2009). Coastal wetlands will likely experience more frequent, severe and unpredictable storms (Erwin, 2009; IPCC, 2014). Moreover, mean global temperatures are predicted to rise up to 4°C

by the end of the 21st century (IPCC, 2014), and global warming is expected to exacerbate the loss of wetland species (Millennium Ecosystem Assessment, 2005).

During the incubation period, avian parents are selected to keep their clutches within a temperature range ideal for embryo development, about 35-37°C (DuRant et al., 2013; Stoleson & Beissinger, 1999). Below this range, embryo development is slowed, and below physiological zero, about 26°C (Conway & Martin, 2000), development is suspended (DuRant et al., 2013; Webb, 1987). Avian embryos of various species have been found to tolerate cool temperatures below physiological zero, only to perish after remaining below physiological zero for long periods of time or reaching exceptionally low temperatures. On the other hand, eggs are more adversely affected by exposure to hot temperatures (Webb, 1987). If the eggs reach temperatures that exceed the upper limit of the critical window for embryo development, about 40.5°C (Conway & Martin, 2000), the embryos are prone either to develop deformities that will impede their future fitness or to perish (DuRant et al., 2013; Webb, 1987). The temperatures reported above are conservative estimates and likely vary across species and with the duration of exposure (Reyna & Burggren, 2012; Webb, 1987). For example, embryo mortality in Adelie penguins can occur between 38 and 39 °C (Webb, 1987), whereas Northern bobwhite embryos can survive temperatures up to 50 °C for up to one hour (Reyna & Burggren, 2012). We do not currently know the lethal temperature for king rail embryos.

To understand the effects of changing climate variables on vulnerable species, it is necessary to study the effects on those species directly. Egg temperature may respond differently to variation in environmental conditions, based on egg and nest characteristics as well as parental care strategies. I made use of natural fluxes of ambient temperature and exposure to direct solar radiation to understand whether egg temperature impacts hatching success in king rails. There's

reason to assume that late in the king rail's breeding season, during mid-summer periods of hot weather, eggs would be at a higher risk of reaching temperatures exceeding the lethal temperature, resulting in hatching failure.

Development and expression of behavior can be sensitive to environmental change, and behavioral plasticity can be adaptive in variable environments (Moran, 1992; Snell-Rood, 2013). Coastal environments are inherently variable, so king rails nesting in coastal areas should exhibit flexibility in breeding behavior. During the incubation period, parent birds must balance the thermal requirements of the eggs and their own energetic needs (Conway & Martin, 2000; Hepp & Kennamer, 2012). Some species have evolved behavioral strategies that prevent overheating of their eggs in hot conditions, such as belly-soaking in Charadriiformes (Grant, 1982), and hovering over the nest and shading their eggs in ground-nesters (Downs & Ward, 1997; DuRant et al., 2013; Ward, 1990; White & Kinney, 1974). To keep clutch temperatures consistent, incubating parents of a wide variety of species modify their behavior as ambient temperatures fluctuate (e.g. plovers (*Vanellus* spp., Ward, 1990), wood ducks (*Aix sponsa*, Hepp & Kennamer, 2012), passerines (Passeriformes, DuRant et al., 2013). If king rails are selected for plasticity in incubation behavior, individuals should behave in similar ways under similar conditions.

Alternatively, level of effort and behavioral variation in incubation may be related to the individual parent's body condition (Chastel et al., 1995; Dearborn, 2001; DuRant et al., 2013). Incubation is crucial for reproductive success, but the energetic cost to parents can limit both current and future reproductive success (Deeming, 2002; DuRant et al., 2013; Heaney & Monaghan, 1996; Reid et al., 2000). Individuals in relatively poor physical condition may be required to spend more time foraging and less time attending their nests to support their own survival and thus, future reproductive attempts. Poor body condition has been demonstrated to be

correlated with smaller clutch sizes (Bolton et al., 1993), nest desertion (Chastel et al., 1995; Drent & Daan, 1980), low hatching and fledging probabilities (Chastel et al., 1995), and incubation inconstancy (Yerkes, 1998).

The viability of unincubated eggs declines over time. Microbial growth and abnormal development may occur during exposure to temperatures unsuitable for embryo development (Ardia et al., 2006; Cook et al., 2003; Webb, 1987). To preserve viability and prevent deformities in early-laid eggs under hot conditions, parents may begin to incubate their eggs before the clutch is complete (Arnold et al., 1987; Lord et al., 2011; Stoleson & Beissinger, 1999; Veiga, 1992). Warmer ambient temperatures may accelerate embryo development, minimizing the duration of incubation, and hence, the exposure of the nest to predators. However, early onset of incubation is the proximate cause of within-clutch hatching asynchrony (Ardia et al., 2006; Martin et al., 2007; Stoleson & Beissinger, 1999). King rail chicks are nidifugous (Meanley, 1969). Parents in this population are known to move their broods substantial distances (over 300 m, on average) from the nest within a few days of hatching (Kolts, 2014). If hatching occurs asynchronously, parents may desert the unhatched eggs in favor of moving the hatched chicks to better habitat. Thus, there may exist a tradeoff between maintaining the viability of early-laid eggs via early onset of incubation and the benefits of synchronous hatching.

A breeding population of king rails at Mackay Island National Wildlife Refuge (NWR), located in the northeast corner of North Carolina, has been the focus of a breeding ecology and population genetics study for the past five years. I made use of the wide range of seasonal temperatures over the course of the king rail's April – July nesting season to examine the effects of temperature on hatching success. Over the course of two breeding seasons, I simultaneously

monitored clutch and ambient temperatures at a selection of nests to measure the temperature ranges to which nesting king rails and their eggs are exposed, and to understand the degree to which parental behavior impacts egg temperature. I made focal observations of parental behavior to determine whether time budgets vary among king rails attending nests. I observed that king rails shade their eggs as part of a repertoire of behaviors at the nest. I asked whether king rails vary in their investment in specific behaviors such as shading, and if the variance is correlated with environmental variables (adaptive plasticity) or attributable to individual differences.

Downs & Ward (1997) demonstrated in crowned plovers (*Vanellus coronatus*) that eggs shaded by a model of a parent were about 10°C cooler than ambient temperatures. Under the hypothesis that king rails show adaptive plasticity, parents should perform shading more at high ambient temperatures that exceed lethal temperatures, and should incubate traditionally when ambient temperatures are below lethal temperatures, assuming shading in king rails effectively cools their eggs. Alternatively, under the hypothesis that parental behavior is related to individual condition, parents in poorer condition may not be able to withstand attending the nest during extreme heat, and may not engage in shading behavior as much as parents in superior condition. Parents may be able to maintain their clutches below lethal temperatures by attending their nest, but nests with clutches that exceed lethal temperatures for long periods of time will experience hatching failure.

To understand the effects of seasonal variation on hatching success, I used data collected at Mackay Island NWR between 2011-2015. I predicted that late season nests would exhibit a decline in hatching success. To maintain egg viability, parents of late-season nests should begin to incubate and attend the nest continuously before clutch completion, and these nests should

exhibit greater hatching asynchrony than nests that are initiated in the cooler, earlier part of the season.

METHODOLOGY

STUDY SITE

Mackay Island National Wildlife Refuge (NWR), located along the Atlantic flyway at the border between North Carolina and Virginia, comprises 4,774 acres of freshwater marshes interspersed with impoundments. Water level and salinity (<5 ppm) are both influenced by wind-driven tides (USFWS, 2008). The marsh vegetation is dominated by black needlerush (*Juncus roemerianus*), cordgrass (*Spartina sp.*), cattail (*Typha sp.*), and the invasive common reed (*Phragmites australis*) (Kolts 2014). To maintain the marshes at an early successional stage, the refuge is managed through prescribed burns in three- to five- year cycles. Most burns occur in the winter, but occasionally they have been conducted as late as April (Rogers, 2011; USFWS, 2008).

NEST FINDING AND MONITORING

King rail nests were found throughout the breeding seasons (April-July) in 2014 and 2015 using transects and systematic search methods that have been employed successfully in finding king rail nests at this site in previous years (Brackett, 2013; Kolts, 2014). Search efforts were focused in areas in which king rails had been visually or audibly detected. King rails at this site are known to nest in black needlerush and were often found in association with muskrat (*Ondatra zibethicus*) activity (Brackett, 2013; Cooper, 2008; Kolts, 2014). Therefore, these areas were searched regardless of whether king rails were detected.

All nests that contained at least one king rail egg or egg shells were documented and monitored. Eggs were individually marked with indelible ink, and physical appearances were described. Length and width (± 0.1 mm) were measured with calipers, and weight (± 1 g) with a 100g-Pesola, if laid within 24 hours. In an effort to document eggs laid by different females within and between years, as well as within nests, each clutch was photographed in a standardized manner upon completion of laying. Nests found during laying were visited daily until clutch completion, then every 2-3 days until pipping of the first egg. Every effort was made to minimize both the rate and duration of nest visits. Without monitoring clutch temperatures directly, incubation was determined by tactile sensing of the eggs above ambient temperature, or by observing an incubating adult. Nests were monitored until completion and nest fate was recorded (hatched, deserted or depredated). Predator type was noted where possible based on whether eggshells were left in or around the nest, the presence of predator footprints near the nest site, and whether the nest itself was damaged. Nest monitoring yielded clutch size, initiation date, the start of incubation, and duration of the incubation period (for hatched nests found during laying). Initiation dates of nests found during incubation could be estimated by floating eggs to assess the developmental stage (Rush et al., 2010).

Once hatching began, I returned to the nest daily to capture chicks until all eggs had either hatched or were deserted. Because king rail chicks are nidifugous (Meanley, 1969), I approached nests stealthily and rapidly to capture chicks before they exited the nest. Blood samples of up to 30 μ L were drawn from the femoral artery, and the weight (± 0.1 g) and tarsus length (± 0.1 mm) were recorded. To avoid resampling in subsequent captures, I applied a small dab of non-toxic acrylic paint on the nape feathers of each sampled chick. Monitoring nests

closely during hatching yielded the number of eggs hatched and the degree of hatching asynchrony.

MONITORING CLUTCH AND AMBIENT TEMPERATURE

To monitor the incubation temperatures of each clutch, I placed in each active nest a model egg containing a Thermochron iButton (Embedded Data Systems; model: DS1921G-2048). Previously, experimental model eggs placed in nests had all been accepted (Brackett, 2013). The eggs were made of plaster-of-Paris and shaped using silicon molds matching the dimensions of king rail eggs. When set, the plaster egg was painted realistically to resemble a king rail egg using a nontoxic acrylic paint base and brown indelible ink spots. A hole was drilled into the center of the egg to fit an iButton. To keep the iButton dry, it was wrapped in a thin layer of plastic food wrap before being placed in the model egg, and the hole sealed with hot glue. Once the hot glue cooled, the surface of the glue was smoothed and painted to prevent the glue from sticking to the real eggs. To measure simultaneously the local ambient temperature, a control iButton was placed within two meters of the nest in a lidded, plastic box (6 cm x 5 cm x 3.5cm) with holes drilled in the sides and bottom for ventilation. To keep the iButton dry, it was secured to the lid using Velcro. All iButtons were pre-programmed to measure temperature at five-minute intervals.

The accuracy of each iButton was tested in relation to a mercury thermometer over a temperature range of 2°C to 60°C using a refrigerator and a drying oven. All iButtons were found to be accurate to $\pm 1^\circ\text{C}$, as per the manufacturer's specifications. I then determined if iButtons inside of model eggs were accurately capturing the temperature eggs experienced in the nest. To adjust the temperatures recorded inside model eggs, I fitted egg models with an iButton

and exposed them to a range of temperatures (2-60°C) in a drying oven. The temperatures recorded inside the model eggs were correlated to the temperatures recorded by a mercury thermometer (JMP Pro 11.2.0, SAS Institute Inc.) next to the egg and were fit (Fig. 1.1; $R^2 = 0.99$) to the following linear equation:

$$\text{Model Egg } ^\circ\text{C} = 0.395 + 0.967(\text{Thermometer } ^\circ\text{C})$$

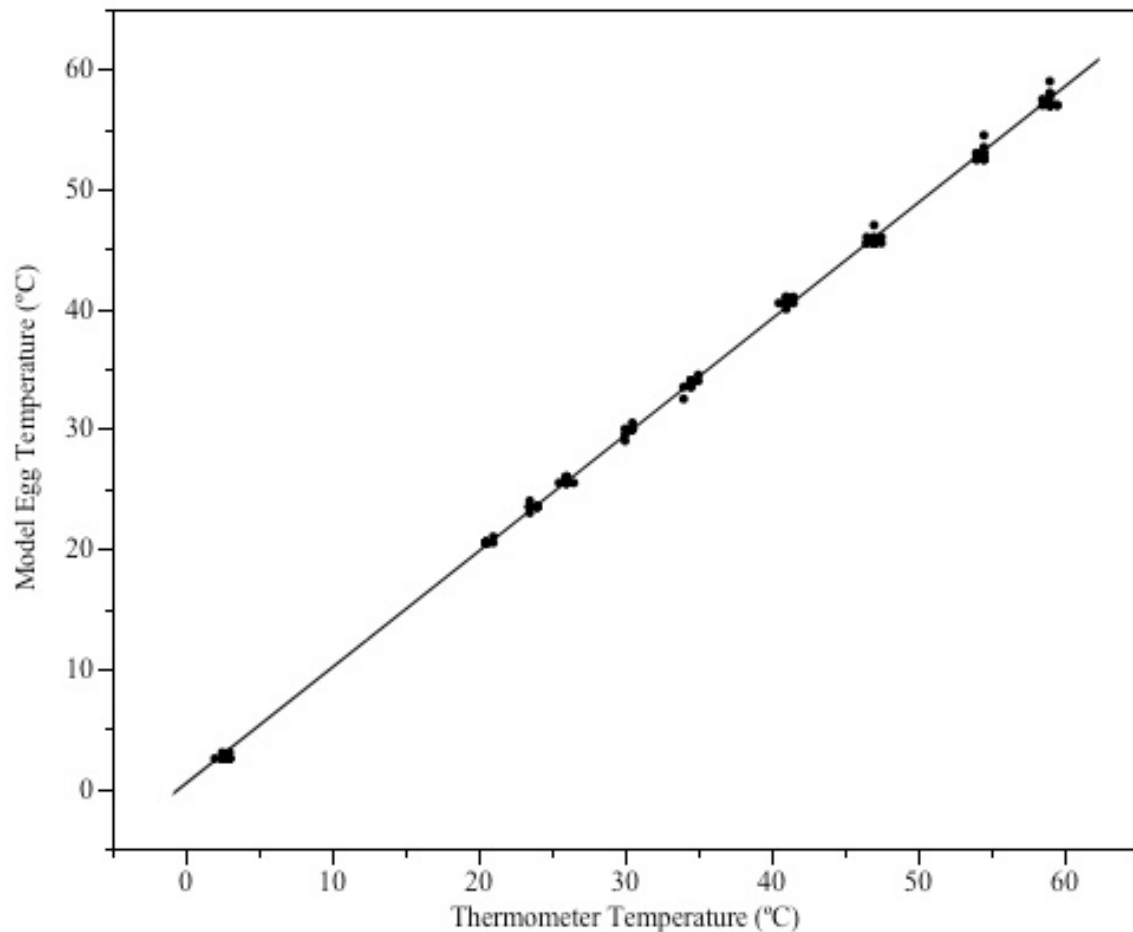


Figure 1.1. Temperatures recorded inside model eggs compared to the ambient temperature, as measured by a mercury thermometer (°C; regression line).

As the temperature was raised, model egg temperatures were slightly cooler than the temperatures measured by the mercury thermometer, particularly at hotter temperatures. This equation was therefore applied as a correction factor to all temperatures measured in model eggs

in the field to account for insulation by the plaster-of Paris during ramping.

CAPTURING AND BANDING TECHNIQUES

Using a method established during the course of previous work (Brackett, 2013; Kolts and McRae, in prep), I captured breeding adult king rails at their nests late in the incubation stage, when adults were ‘sitting tight’ on their nest (see Appendix A for the IACUC approval letter). I discretely surrounded the nest with overlapping mist nets and secured the bottom edge of the nets with tent stakes. I then flushed the incubating adult into the nets. If an adult was captured, they were extracted from the mist nets and processed immediately. If the capture attempt was unsuccessful, I raised the bottom edge of one mist net. I then left the nest area for up to 40 minutes to allow the adult to return to the nest before attempting the capture again. If an adult was not captured within three attempts in a day, the mist nets were removed and I retreated from the area. To prevent nest desertion, I only used this method within the last week of incubation.

Once an adult was captured, I banded them with a uniquely numbered U.S. Geological Survey aluminum band and a unique combination of three colored leg bands, distributed as two bands on each leg. This allowed for visual re-identification of individuals without recapture, including during video analysis (see below). The color bands were spiral flat bands made of darvic (A.C. Hughes, United Kingdom), sealed as a closed ring using a portable welding iron. Body mass was measured with a 500g Pesola (± 5 g). Flattened wing chord (± 1 mm) was measured with a 300-mm wing rule, and bill length (± 0.1 mm) and tarsus length (± 0.1 mm) with calipers. A blood sample of up to 50 μ L was drawn from the brachial vein and stored in about 1.5 mL 100% ethanol. Most adult king rails can be assigned sex based on morphometrics, but size

distributions for males and females overlap (Perkins et al., 2010). A PCR-based diagnostic test was therefore used to confirm the sex of all sampled individuals (Brackett, 2013).

DOCUMENTING NESTING BEHAVIOR

To observe parental behavior, I placed tripod-mounted digital video cameras at active nests (Sony Handycam, Sony Handycam HD, Go-Pro Hero 3, or Go-Pro Hero 3+). Filming occurred between 0600 and 1900 when weather permitted (i.e. not raining). Each recording lasted between 2 and 10 hours (the extent of the battery or data storage). Filming for up to 10 hours at a time reduced the number of visits and disturbance to the nest while capturing the full repertoire of attendance behaviors.

SEASONAL TRENDS

Mean ambient temperature generally increases over the course of the nesting season (early April - mid July). I used five years of available nesting data at Mackay Island (2011-2015) to analyze seasonal trends in incubation patterns, hatching synchrony, and nesting success. The estimated first egg date (Julian date) of each nest was used as a measure of relative timing of nesting within a season.

RESULTS

REPRODUCTIVE ECOLOGY AND CAPTURE EFFORT

King rail nests were found between 14 April and 16 July in 2014 and between 17 April and 14 July in 2015. A detailed account of the nests found in 2014 and 2015 can be found in

Table 1.1. Thirteen eggs from 5 nests found deserted were opened, and none contained signs of embryo development.

Table 1.1. Summary of nest parameters in 2014 and 2015. Unless otherwise indicated, percentages and numbers of nests are reported.

	2014	2015
Earliest estimated first egg date	9 April	9 April
Total nests found	41	28
Nests found active	66% (27)	46% (13)
Nests found depredated	24% (10)	40% (11)
Nests found deserted	10% (4)	11% (3)
Mean (range) number of eggs in nests found deserted	2.3 (1-5)	2.3 (1-4)
Nests found with unknown fates	0% (0)	3% (1)
Nests found in area burned within 1 year	12% (5)	21% (6)
Active nests that hatched at least 1 chick	22% (6)	46% (6)
Mean (range) percent eggs hatched in successful nests	81.8% (71.4-90.9%)	70.2% (14.3-100%)
Active nests that were subsequently deserted	19% (5)	0% (0)
Active nests that were subsequently depredated	59% (16)	54% (7)
Mean (range) clutch size	9.1 (5-12)	8.9 (7-12)

King rails built nests and chose nesting sites that varied in exposure (Fig. 1.2). Most nests were made of and placed in primarily black needle rush (81%; Fig. 1.2A), but some also incorporated or were found in dense patches of cattail (*Typha* spp.; Fig. 1.2B) or common three square (*Scirpus pungens*). Some pairs placed their nest under a canopy of vegetation (Fig. 1.2C), or created a canopy themselves by bending grasses over the nest (Fig. 1.2D).



Figure 1.2. King rail nests and nesting sites in 2015. Arrows point toward nest cup. **A.** Very exposed nest (post-hatching) with little to no natural protection from the sun. **B.** Well-concealed nest with natural canopy. **C.** Nest built under and shaded by rose mallow (*Hibiscus moscheutos*). **D.** Nest over which the parents bent grasses, creating a canopy.

NESTING BEHAVIOR AND SUCCESS

Late summer ambient temperatures can far exceed optimal incubation temperatures. For example, a peak ambient temperature of 51°C was recorded at a nest on 17 June 2014. To monitor the temperatures that king rail clutches are reaching, a model egg was introduced into each of 20 active nests in 2014 (90% of which were accepted by the parents) and 8 active nests in 2015 (100% of which were accepted). In two instances in 2014 parents rejected the model egg, one by deserting the nest (model introduced on Day 2 of laying sequence), and the other by removing the model egg (model introduced during incubation).

Twenty-seven nests were filmed in the 2014 and 2015 breeding seasons, for a total of 375 hours of video recordings. Filming for up to 10 hours at a time reduced the number of visits and disturbance to the nest while capturing a wide variety of attendance behavior. To more accurately describe the variation in diurnal scheduling of attendance and behavior, daily video recordings were broken down into roughly 2-3 hour segments. Detailed time budgets of attending adults allowed me to assess variation in the duration of nest attendance and behavior of each parent during the incubation period. I calculated both the mean ambient and mean clutch temperatures that were recorded during each video segment.

To determine the degree to which ambient temperatures affected clutch temperatures, and whether parents were able to behaviorally mitigate the harmful effects of hot temperatures, I related the mean clutch temperatures to the mean ambient temperatures at each nest using a linear mixed effects model. Mean ambient temperature was included as a fixed effect, and nest was included as a random effect to account for repeated measures on the same nest. Only data from the incubation period, and when the adults were present (determined by video evidence), were included. I excluded periods of time in which both parents were in recess at least 50% of

the time recorded (3 instances), because the temperatures recorded during these time periods did not accurately reflect the impact of parental behavior on clutch temperature. Mean clutch temperature increased significantly with mean ambient temperature (Linear mixed effects model: $F_{1,52} = 27.31, p < 0.0001$) but was dampened at higher ambient temperatures (Fig. 1.3). Even when ambient temperatures exceeded purportedly lethal levels (about 40.5°C; Conway & Martin, 2000; DuRant et al., 2013), mean clutch temperature on average remained within reasonable limits for embryo development. An example of a temperature profile for a nest can be found in Appendix B (Fig. B.1). To determine if the measurement of clutch temperature was affected by the location of the model egg within the clutch (surrounded on all sides by real eggs versus on the perimeter of the clutch), I performed a mixed effects ANOVA including model egg location as a fixed effect and nest as a random effect to account for multiple measures on the same nest. The location of the model egg within the clutch did not significantly influence clutch temperature readings (Mixed effects ANOVA: $F_{1,47} = 0.03, p = 0.86$).

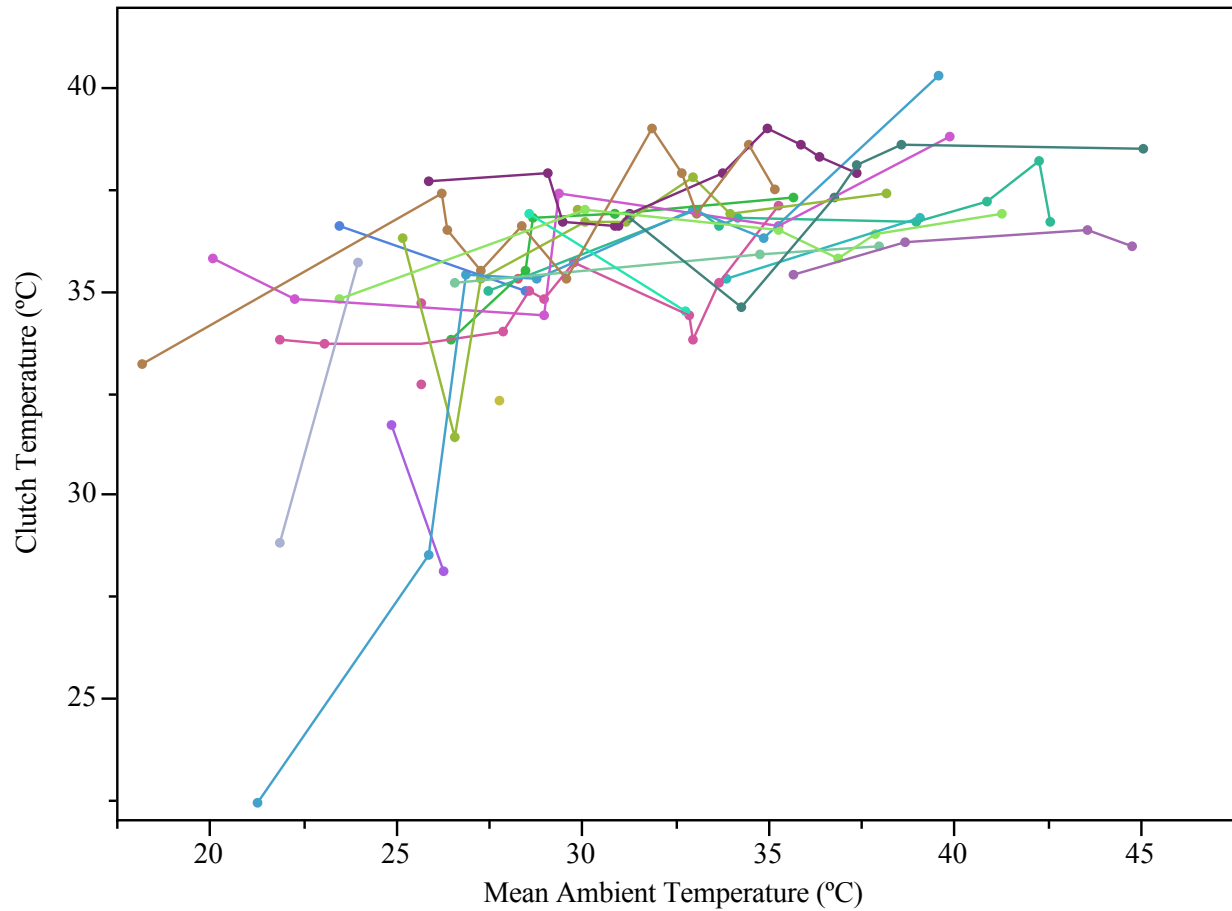


Figure 1.3. Mean clutch temperatures increased significantly with mean ambient temperatures (Linear mixed effects model: $F_{1,101} = 48.55$, $p < 0.0001$). Multiple measures are indicated by nest-specific colors ($n = 24$ nests).

Video sequences from nine nests, representing 180 hours, featured at least one color-banded adult, which allowed me to distinguish individuals attending the nest (Fig. 1.4). Males have significantly longer bills than females (Two-sample t-test: $T_{58} = 7.26$, $p < 0.0001$; $n = 30$ males and 30 females; Fig. 1.5). Thus, when neither parent was banded, I distinguished between members of mated pairs by comparing relative bill lengths in profile, using the ratio of the diameter of the eye to the distance from the corner of the eye to the tip of the bill. Mates could also be distinguished if they differed greatly in body size, particularly when both were in the same frame. No instances of more than two individuals at the nest were recorded.



Figure 1.4. A banded male, captured at his nest in 2014, filmed attending his nest.

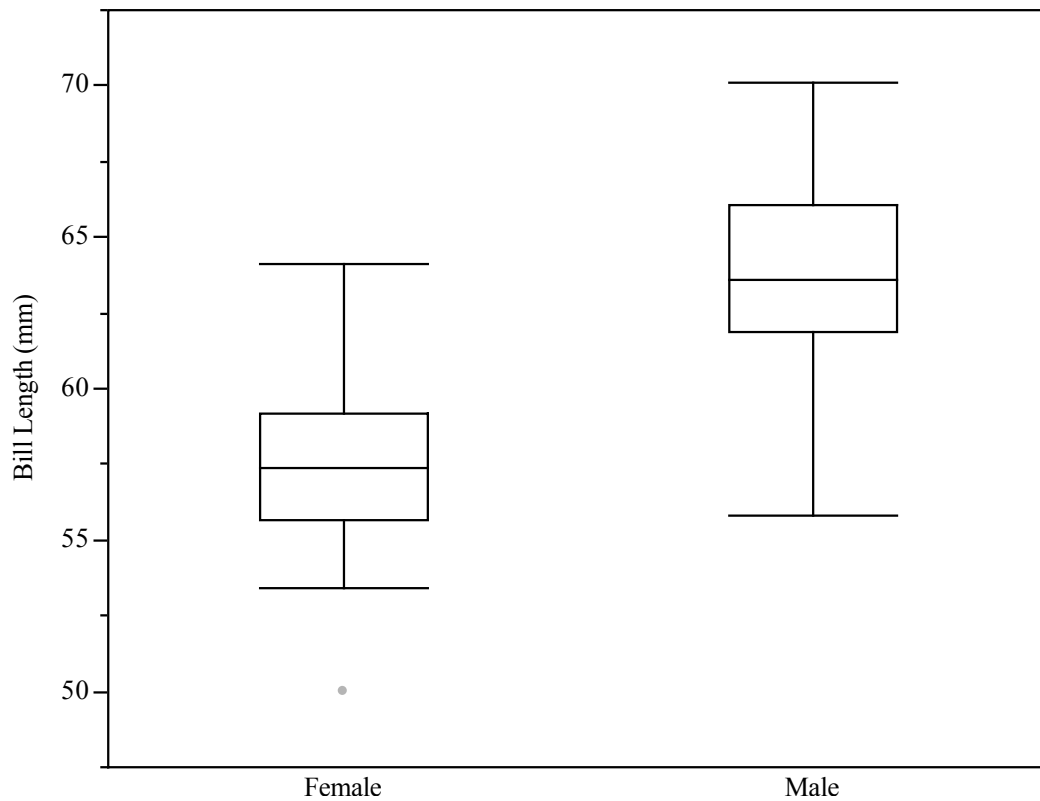


Figure 1.5. Males on average have significantly longer bill lengths than females (Two-sample t-test: $T_{58} = 7.26$, $p < 0.0001$). The point represents an outlier.

Both male and female parents displayed a variety of nesting behaviors including incubating, shading, rotating their eggs, preening, sleeping, vocalizing, and nest-building. Parents also left their nest unattended, referred to as ‘recess’ time. Individuals varied in their wariness of the cameras, and their return to the nest ranged from 1 to 75 minutes after a camera was deployed. Therefore, I excluded this period of time from all video analyses. Adults took nest-attendance recesses for periods up to 91 minutes, so the length of their absence from the nest after camera deployment was within the observed variation of their typical recess duration. An example of a daily behavior profile for a nest can be found in Appendix B (Fig. B.2). Most bouts of parental attendance exceeded the full duration of video recordings, and full bout durations could not be determined. For each 2-3 hour video segment, I calculated the proportion

of time each parent attended the nest and the proportion of time it was unattended (recess). For each parent, I calculated the proportion of time spent performing specific attendance behaviors (incubating, shading, nest-building).

I used morphometric data of 38 individuals captured at their nests from 2011 to 2015 to calculate the body condition of banded individuals. I developed a principal component score of “body size” using measures of tarsus length, wing length, and bill length. I used PC1 because it explained the majority of the variation in the data (80.9% of the variance). I then regressed body mass against PC1-body size, and recorded the body mass residuals as ‘body condition’ (Amat & Masero, 2004). Individuals with relatively high body mass residuals were considered to be in better condition, as they had a high score when controlling for their body size.

To determine whether parents incubated less during hotter ambient conditions, or alternatively if incubation effort is related to body condition, I used a linear mixed effects model. The proportion of time incubating was the response variable, and mean ambient temperature, body condition (body mass residuals), and an interaction term of mean ambient temperature*body condition were included as fixed effects. Individual was included as a random effect to account for repeated measures on the same individuals. The proportion of time individuals spend incubating was not related to body condition (Linear mixed effects model: $F_{1,48} = 2.22, p = 0.14$), nor the interaction between body condition and ambient temperature ($F_{1,53} = 2.06, p = 0.15$), but individuals spent significantly less time incubating as ambient temperature increased ($F_{1,50} = 26.93, p < 0.0001$; Fig. 1.6).

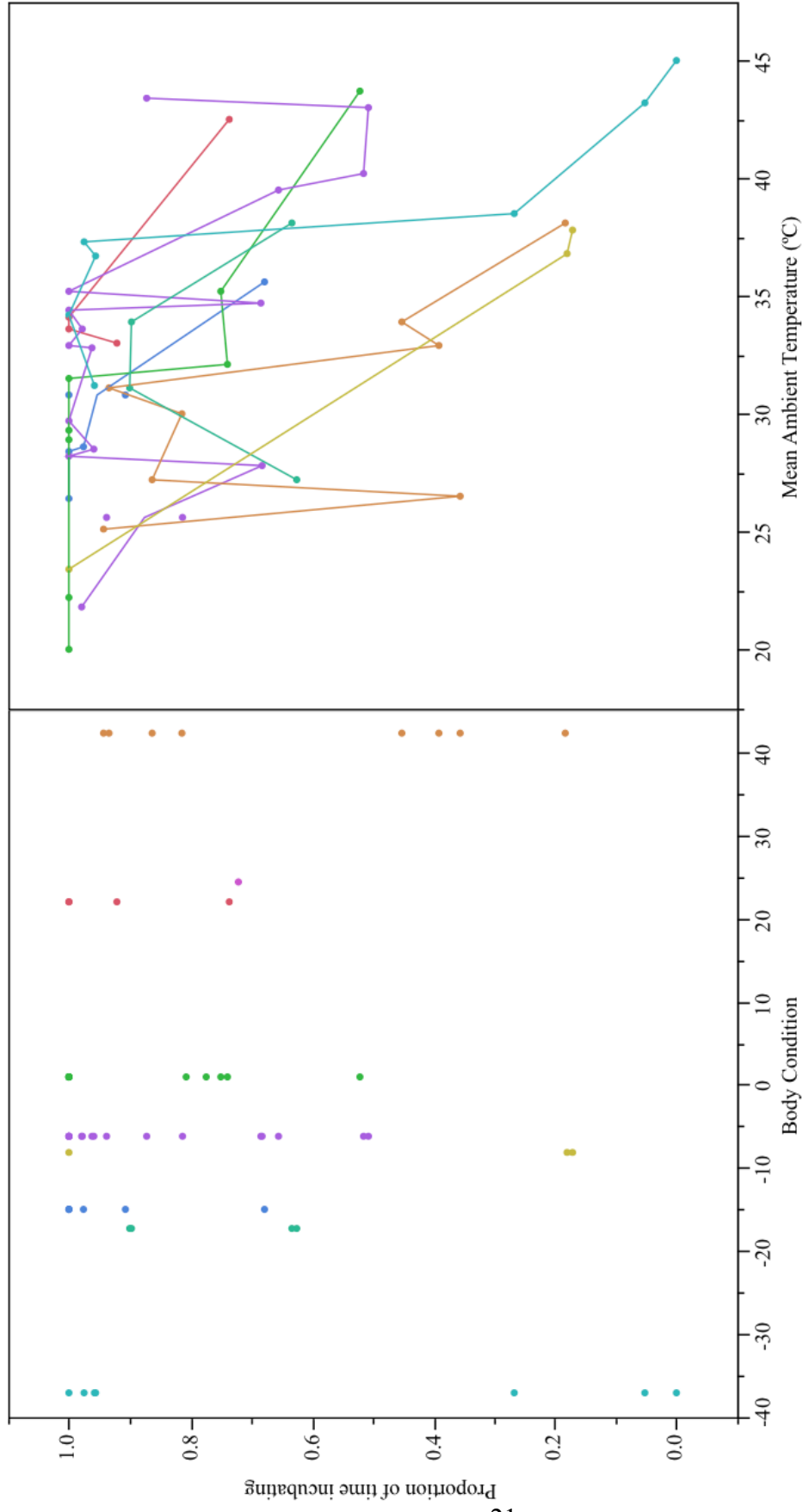


Figure 1.6. The proportion of time individual parents spent incubating decreases significantly with mean ambient temperature (Linear mixed effects model: $F_{1,50} = 26.93$, $p < 0.0001$), but is not related to body condition ($F_{1,48} = 2.22$, $p = 0.14$) or the interaction between condition and ambient temperature ($F_{1,53} = 2.06$, $p = 0.15$). Multiple measures are indicated by individual-specific colors ($n = 9$ parent king rails).

Because the proportion of time parents spent incubating was not related to their body condition or the interaction term, I removed these as fixed effects, allowing more individuals (i.e. unbanded parents) to be included in the analysis ($n = 34$). To account for instances of repeated measures on both parents at each nest, I also included pair as a random effect. Individuals spent significantly less time incubating as mean ambient temperature increased (Linear mixed effects model: $F_{1,138} = 51.08, p < 0.0001$; Fig. 1.7).

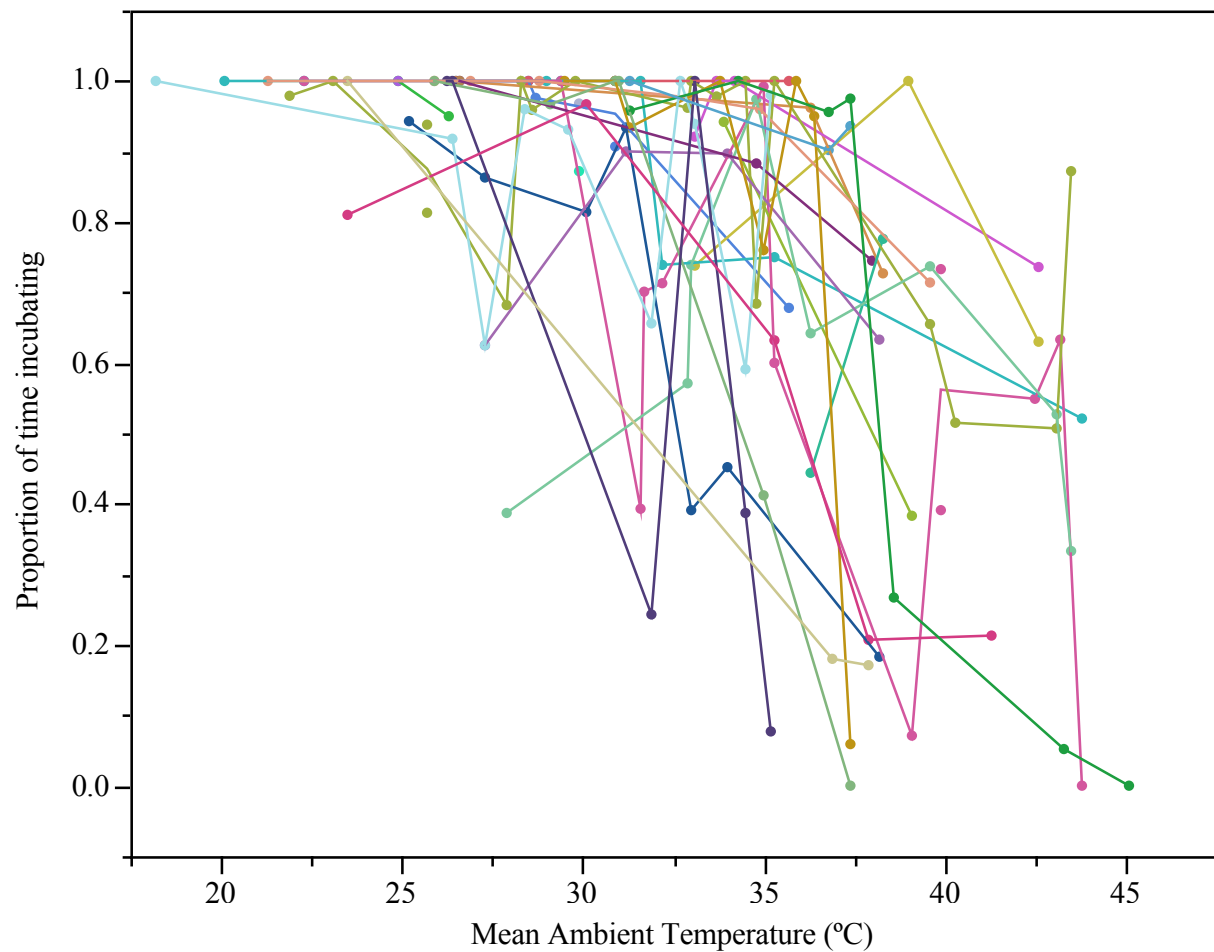


Figure 1.7. The proportion of time individual parents spent incubating decreased significantly with mean ambient temperature (Linear mixed effects model: $F_{1,138} = 51.08, p < 0.0001$). Multiple measures are indicated by individual-specific colors ($n = 34$ parent king rails).

Video recordings revealed that king rail parents also shade their eggs by elevating their body above their clutch and holding wings askew (Fig. 1.8). Shading parents showed a range of signs of heat stress, including opening their bills, panting heavily, and ruffling their feathers.



Figure 1.8. Female shading her clutch by elevating her body above her eggs and holding her wings askew. Ruffled feathers and panting indicate heat stress. Out of 24 nesting pairs filmed, 83% were observed shading their clutches.

To determine if variation in shading is also explainable by variation in ambient temperature, I related the proportion of time individual parents spent shading to both the mean ambient temperature at their nest and their body condition (body mass residuals) using a linear mixed effects model. The proportion of time shading was the response variable, and mean ambient temperature, body condition (body mass residuals), and an interaction term of mean ambient temperature*body condition were included as fixed effects. Individual was included as a random effect to account for repeated measures on the same individuals. Proportion of time spent shading was not related to body condition (Linear mixed effects model: $F_{1,51} = 0.05$, $p = 0.83$) or the interaction between body condition and mean ambient temperature ($F_{1,53} = 0.19$, $p = 0.66$),

but individuals spent significantly more time shading when ambient temperatures were higher ($F_{1,49} = 51.31, p < 0.0001$; Fig. 1.9).

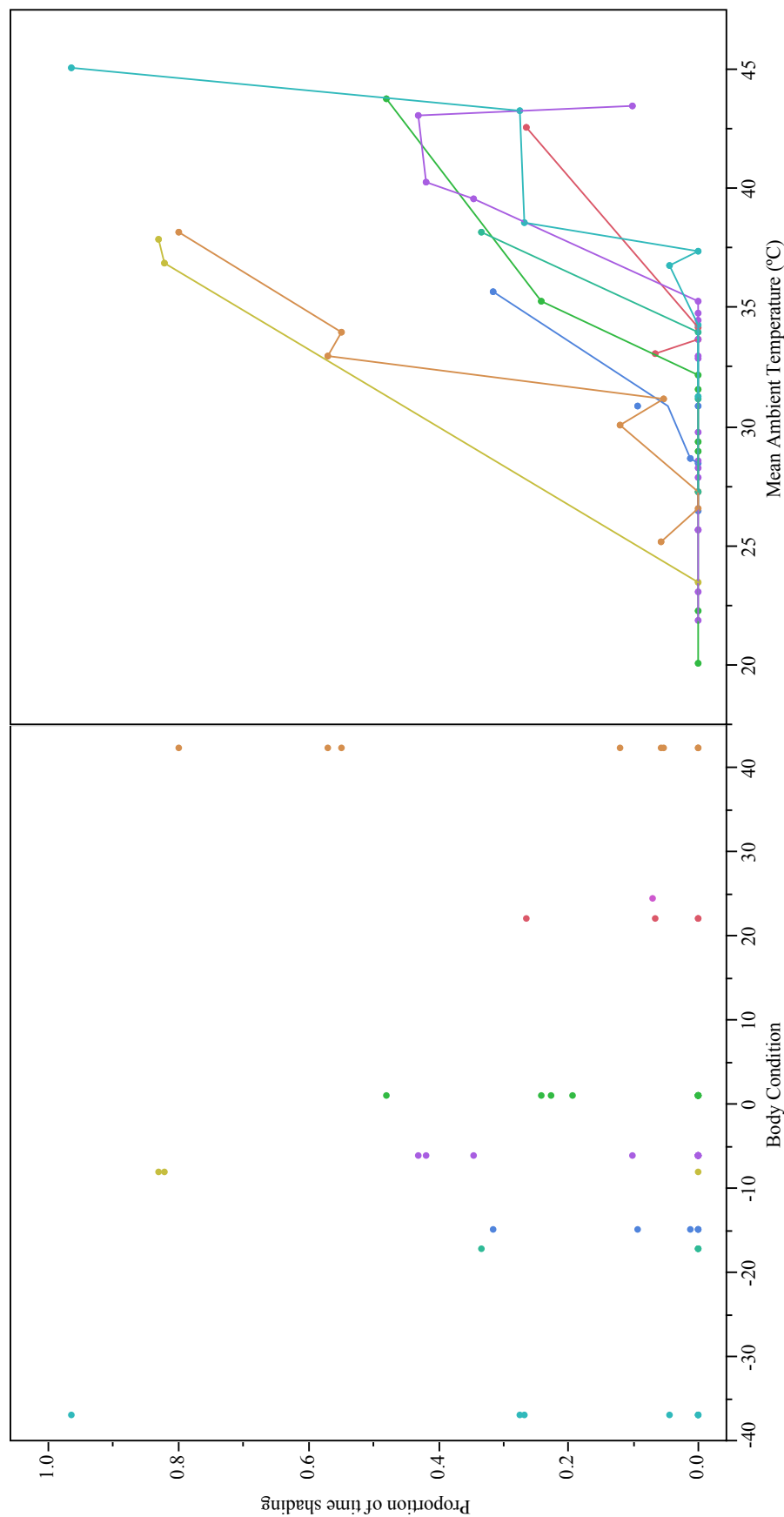


Figure 1.9. The proportion of time individual parents spent shading increased significantly with mean ambient temperature (Linear mixed effects model: $F_{1,49} = 51.31, p < 0.0001$), but was not related to body condition ($F_{1,51} = 0.05, p = 0.83$) or the interaction between body condition and mean ambient temperature ($F_{1,53} = 0.19, p = 0.66$). Multiple measures are indicated by colors specific to each individual ($n = 8$ parent king rails).

Because the proportion of time parents spent shading was not related to their body condition or the interaction term, I removed these as fixed effects, allowing more individuals to be included in the analysis ($n = 34$). To account for instances of repeated measures on both parents at each nest, I also included pair as a random effect. Incubating adults spent proportionately more time shading at higher ambient temperatures (Linear mixed effects model: $F_{1,141} = 70.5, p < 0.0001$; Fig. 1.10).

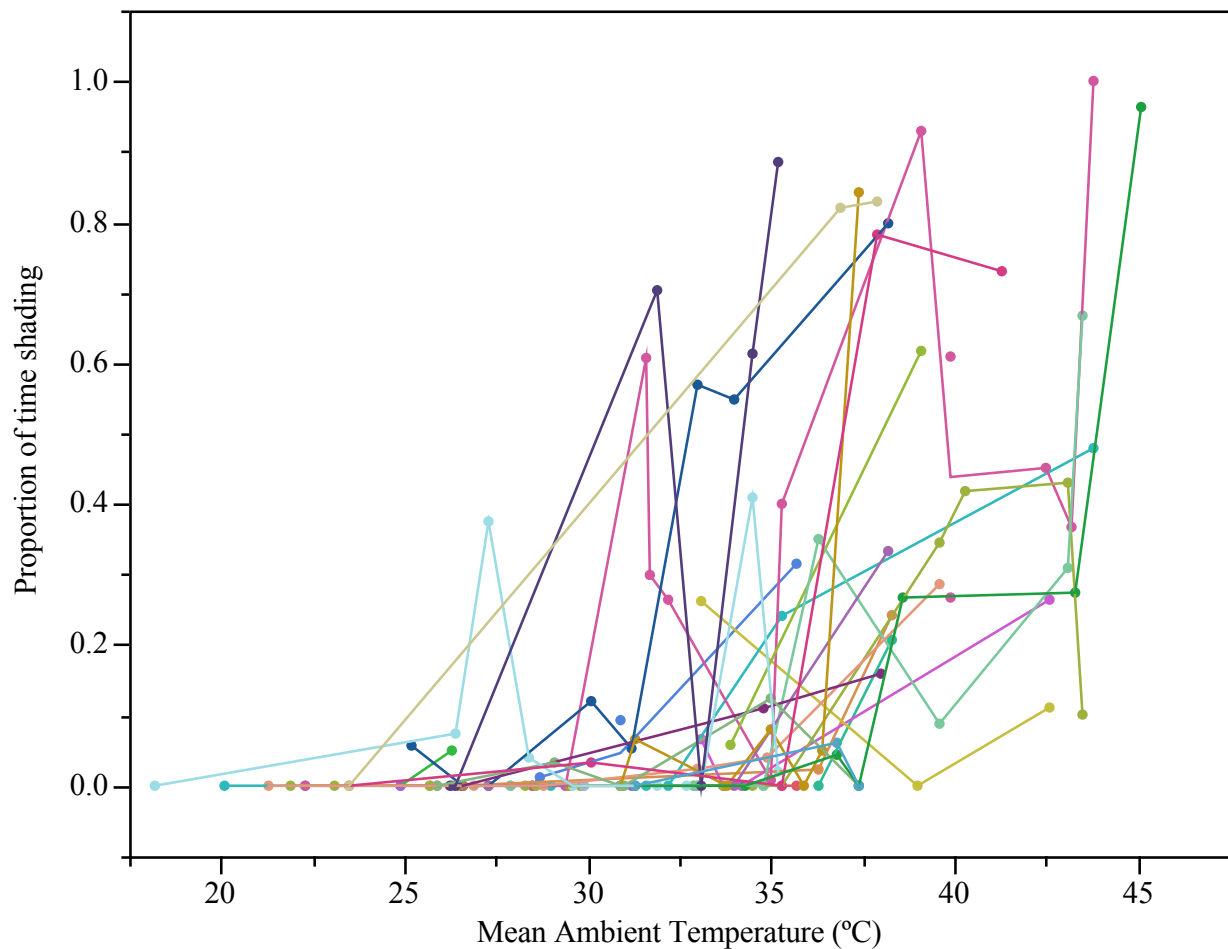


Figure 1.10. The proportion of time individual parents spent shading increased significantly with mean ambient temperature (Linear mixed effects model: $F_{1,141} = 70.5, p < 0.0001$). Multiple measures are indicated by colors specific to each individual ($n = 34$ parent king rails).

Parents also took recesses from the nest, leaving their clutches unattended and exposed to ambient conditions. Adults may temporarily desert their eggs when energetic constraints are high. I calculated recess duration and frequency only for recesses bracketed by parental attendance (i.e. a parent was observed both leaving and returning to the nest). In biparentally incubating birds, it is unclear which parent, the attending parent or the off-nest parent, determines the duration of bouts and recesses (Bulla et al. 2015; Bulla et al., 2014). Therefore recesses could not be attributed to either parent for the purpose of relating duration and frequency to body condition. I observed 112 total recesses among 23 nesting pairs, and both recess duration (mean: 7.1 +/- 15.2 minutes) and frequency (mean: 0.40 +/- 0.88 recesses per hour) varied among pairs. Eighty-six percent of recesses were 10 minutes or shorter in duration.

To determine whether parents took longer or more frequent recesses during hot ambient temperatures, I related the duration of time nests were left unattended and the number of recesses taken per hour to mean ambient temperature using linear mixed effects models. Mean ambient temperature was included as a fixed effect, and pair was included as a random effect to account for repeated measures on the same pairs. Parents took shorter (Linear mixed effects model: $F_{1,27} = 4.4, p = 0.05$; Fig. 1.11), but more frequent (Linear mixed effects model: $F_{1,111} = 17.7, p < 0.0001$; Fig. 1.12) recesses when ambient temperatures were warmer.

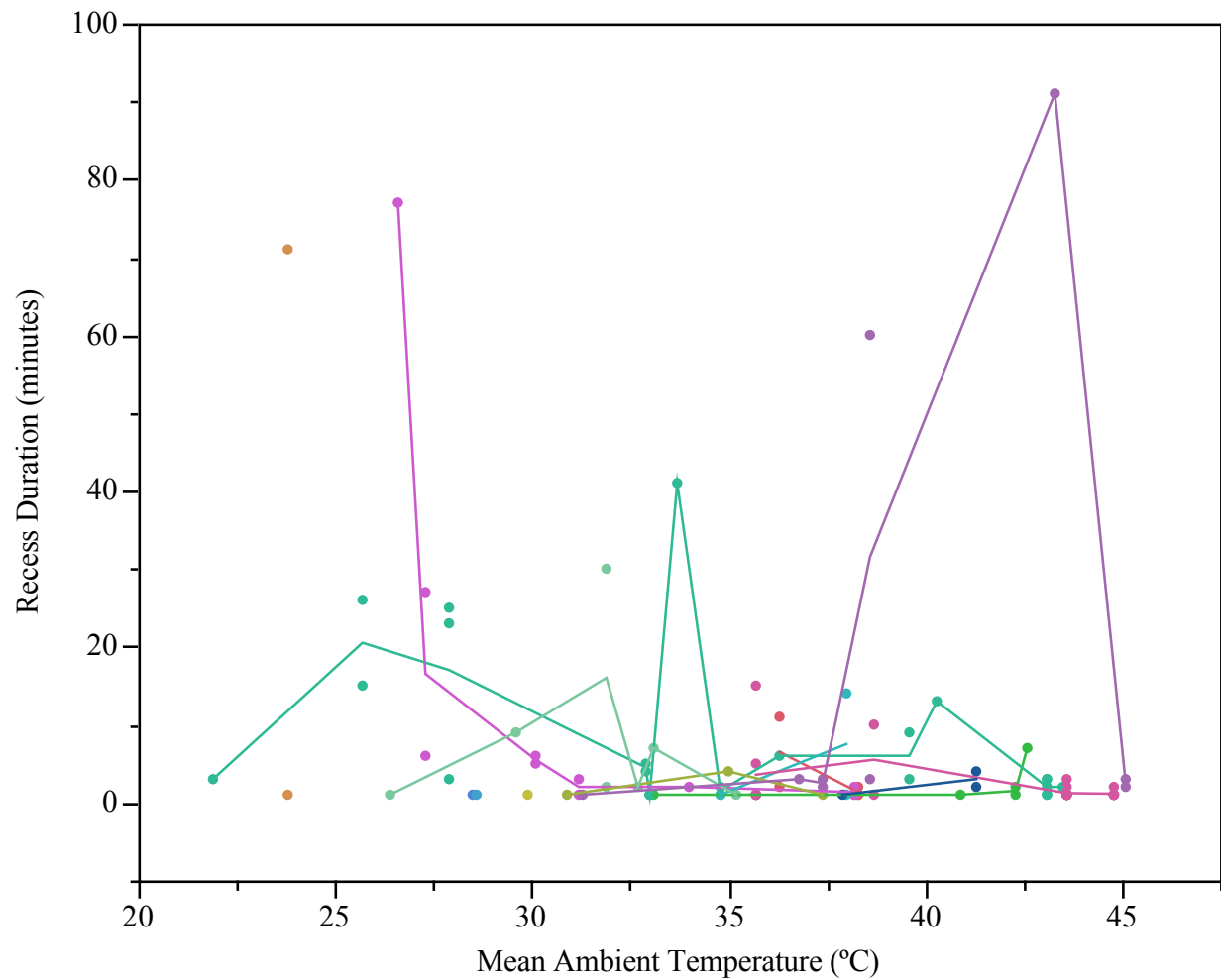


Figure 1.11. Nesting pairs took significantly shorter recesses as ambient temperature increased (Linear mixed effects model: $F_{1,27} = 4.4$, $p = 0.05$). Multiple measures are indicated by colors specific to each pair ($n = 23$ pairs).

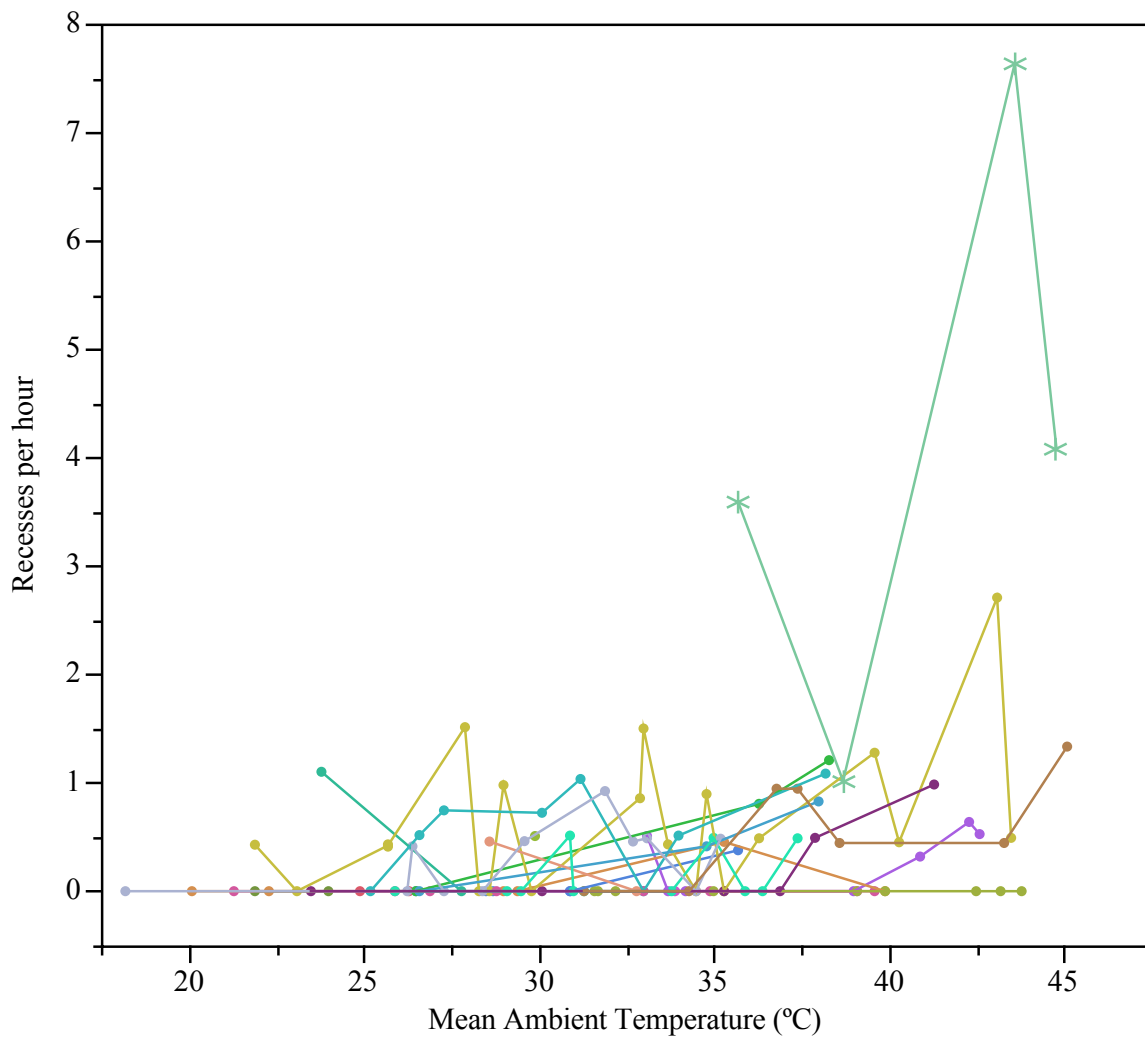


Figure 1.12. Nesting pairs take recesses significantly more frequently as ambient temperature increases (Linear mixed effects model: $F_{1,111} = 17.7, p < 0.0001$). Multiple measures are indicated by colors specific to each pair ($n = 23$ pairs). Even when I remove from the analysis data from one pair with unusually high recess rates represented by green asterisks, the result does not change ($F_{1,110} = 14.4, p = 0.0002$).

As parents take more frequent recesses at higher ambient temperatures, the eggs are more exposed to ambient temperatures that exceed the upper limit for embryo survival. To determine if hatching failure is related to high clutch temperatures, I related the proportion of eggs that hatched within each clutch to the maximum temperature each clutch reached during incubation. Because the sample size is relatively small, I used the Wilcoxon rank-sum test, a non-parametric

test. Nests that were depredated while parents were attending the nest were excluded from this analysis, as it was not known whether the eggs were alive or not at the time of predation. Nests with maximum clutch temperatures that exceeded 43.5°C hatched significantly fewer eggs within each clutch (mean = 0.21) than nests that did not exceed 43.5°C (mean = 0.76) (Wilcoxon rank-sum: $p = 0.04$; Fig. 1.12). In three instances, the maximum clutch temperatures occurred during time periods captured on video. In each case, the nest was unattended, and the eggs were exposed to direct sunlight.

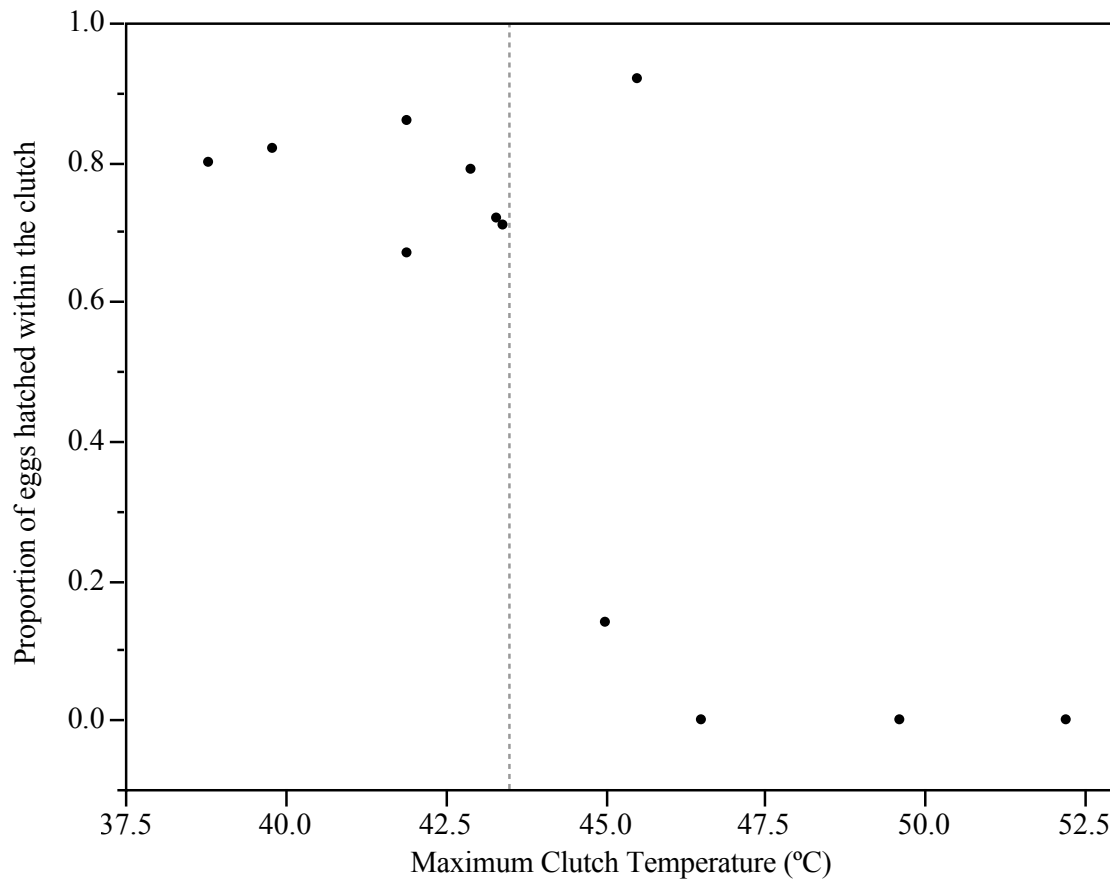


Figure 1.13. Clutches with maximum clutch temperatures that exceeded 43.5°C, demarcated by the dashed line, hatched significantly fewer chicks within each clutch than nests that did not exceed 43.5°C (Wilcoxon rank-sum: $p = 0.04$, $n = 12$). Only nests that either hatched or were deserted by the parents were included in this analysis.

In one of five clutches that exceeded 43.5°C, the majority of the eggs still hatched (Fig. 1.13). It is possible that hatching failure is also affected by the duration of time clutches spend at high temperatures. To determine if hatching success declined the longer the clutch temperature remained above 43.5°C, I related the proportion of eggs that hatched within each clutch to the duration of time clutch temperatures exceeded 43.5°C during the incubation period. Clutches that exceeded 43.5°C for over 20 minutes hatched on average significantly fewer chicks per clutch (mean = 0.04) than clutches that exceeded 43.5°C for less than 20 minutes (mean = 0.79; Wilcoxon rank-sum: $p = 0.002$; Fig. 1.14).

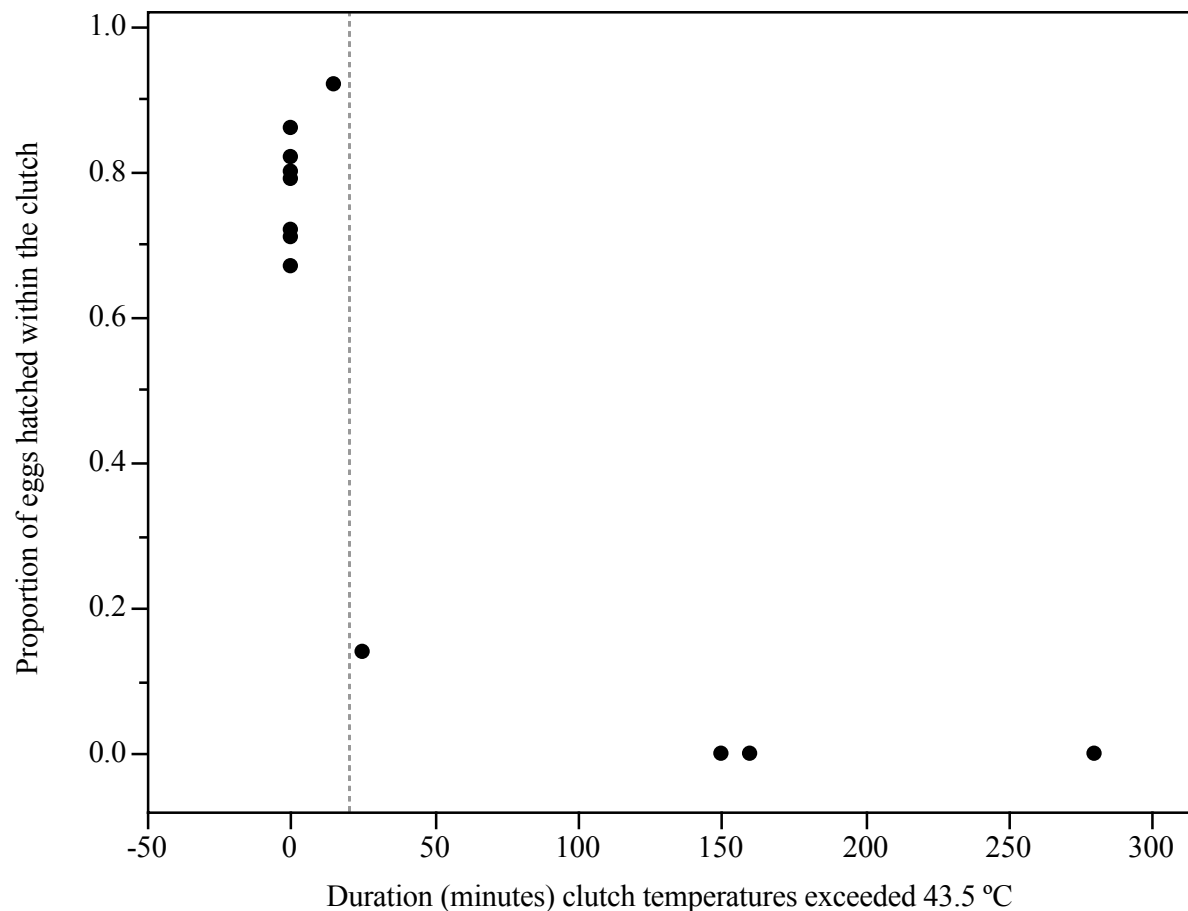


Figure 1.14. Clutches that exceeded 43.5°C for over 20 minutes hatched significantly fewer chicks per clutch than clutches that exceeded 43.5°C for less than 20 minutes (Wilcoxon rank-sum: $p = 0.002$, $n = 12$). Depredated nests were excluded from this analysis.

SEASONAL TRENDS

Mean ambient temperature increased over the course of the nesting season (early April to mid-July; Fig. 1.15) in 2011-2015. I used nesting data from 2011-2015 at Mackay Island NWR to detect seasonal trends in nest success and incubation patterns. To determine if early-season nests were more successful than late-season nests, I related the proportion of eggs that hatched within each clutch to the first egg date (Julian date) using a linear mixed effects model, including year as a random effect. I excluded nests that were found already depredated or deserted because the first egg date was unknown and their fates are unclear (fully depredated, partially hatched and then depredated, or deserted and then depredated). Although we do not know whether the eggs were alive or dead at the time of predation, only six out of 107 nests were deserted (without a predation event) during five years of study, so depredated nests were not excluded from this analysis. The proportion of eggs that hatched within each clutch was not related to first egg date (Linear mixed effects model: $F_{1,105} = 2.54$, $p = 0.11$; Fig. 1.16).

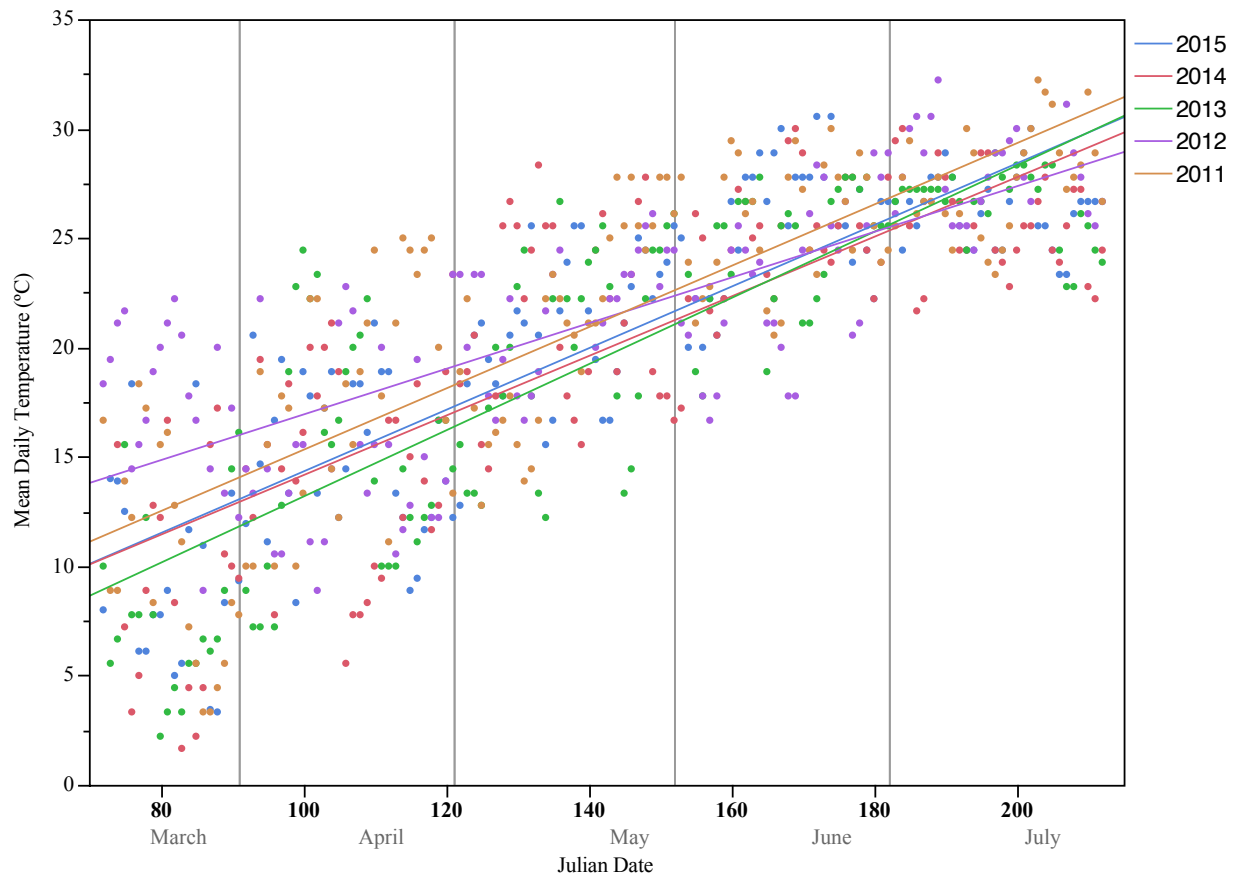


Figure 1.15. Mean daily temperature at Mackay Island NWR increases over the course of the king rail's nesting season (Linear mixed model: $F_{1,688} = 1326.84$, $p < 0.0001$; www.wunderground.com, KNCKNOTT 1).

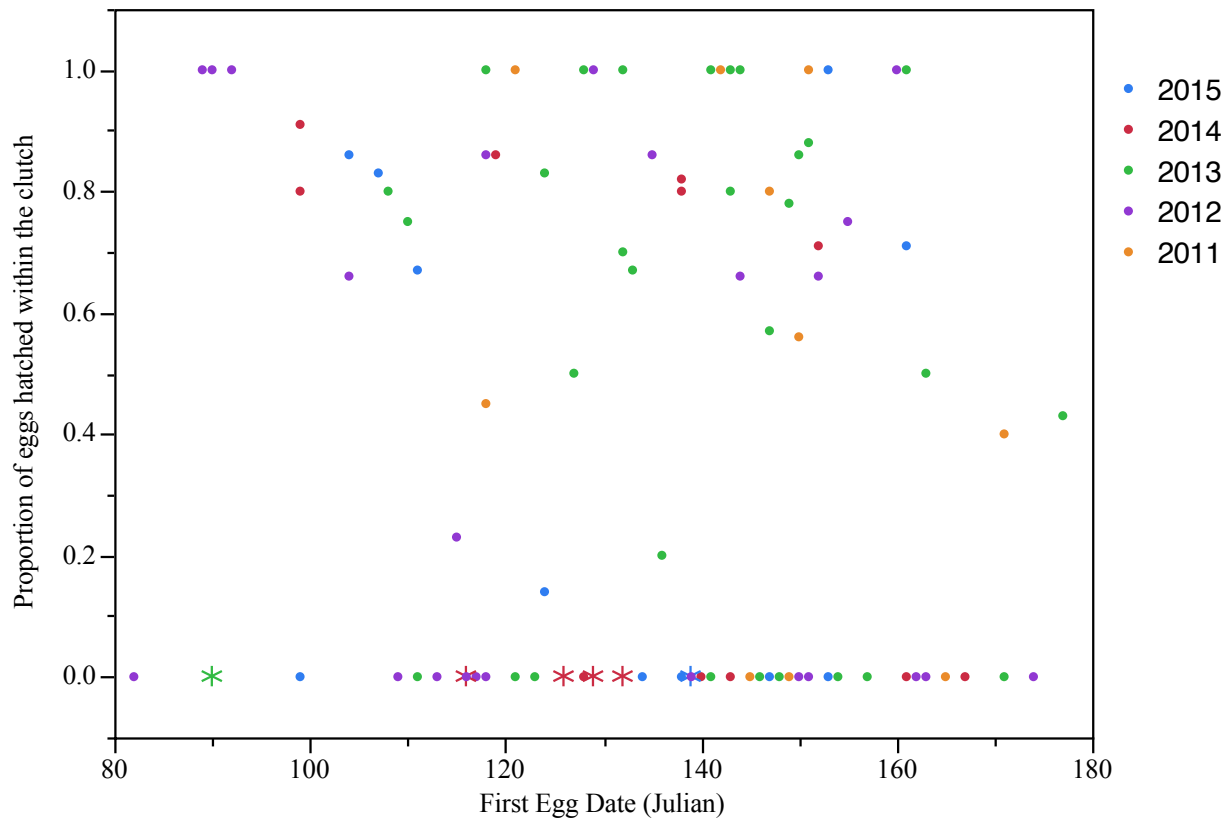


Figure 1.16. The proportion of eggs that hatched within each clutch was not related to first egg date (Julian date) (Linear mixed effects model: $F_{1,105} = 2.54$, $p = 0.11$, $n = 107$). Six nests were deserted, indicated by asterisks. The year of each nest is indicated by color.

Warmer temperatures later in the season could accelerate embryo development and shorten the incubation period (Martin et al., 2007). I calculated the duration of the incubation period as the number of days between the start of incubation and the first day of hatching. Thus, only 7 nests met the criteria of being found during the laying period, when the start of incubation could be determined, and that survived to hatch. Though the sample size is small, the duration of the incubation period seems consistent over the course of the season (mean = 20.9 days, range = 20-22 days).

King rails can only lay one egg per day. To preserve egg viability in hotter conditions, parents may begin incubating early-laid eggs before clutch completion (Ardia et al., 2006;

Stoleson & Beissinger, 1999). I related the number of days parents began full-time (24-hour) incubation after nest initiation to the first egg date (Julian date). I used a linear mixed effects model with the first egg date (Julian date) as a fixed effect and year as a random effect. Adults tended to begin incubating earlier relative to the first egg date the later it got in the breeding season, thus reducing the amount of time early-laid eggs were exposed to ambient conditions, although this result was marginally not significant (Linear mixed effects model: $F_{1,16} = 3.5$, $p = 0.07$; Fig. 1.17A).

Early onset of incubation is expected to increase hatching asynchrony, and result in earlier hatch dates for the first laid eggs. I calculated the degree of hatching synchrony as ‘hatch rate’: the number of eggs that hatched divided by the number of days over which they hatched. To determine if hatching became more asynchronous later in the breeding season, I used linear mixed effects models with the first egg date (Julian date) as a fixed effect and year as a random effect. Hatching became significantly more asynchronous later in the breeding season (Linear mixed effects model: $F_{1,37} = 6.7$, $p = 0.01$; Fig. 1.17B).

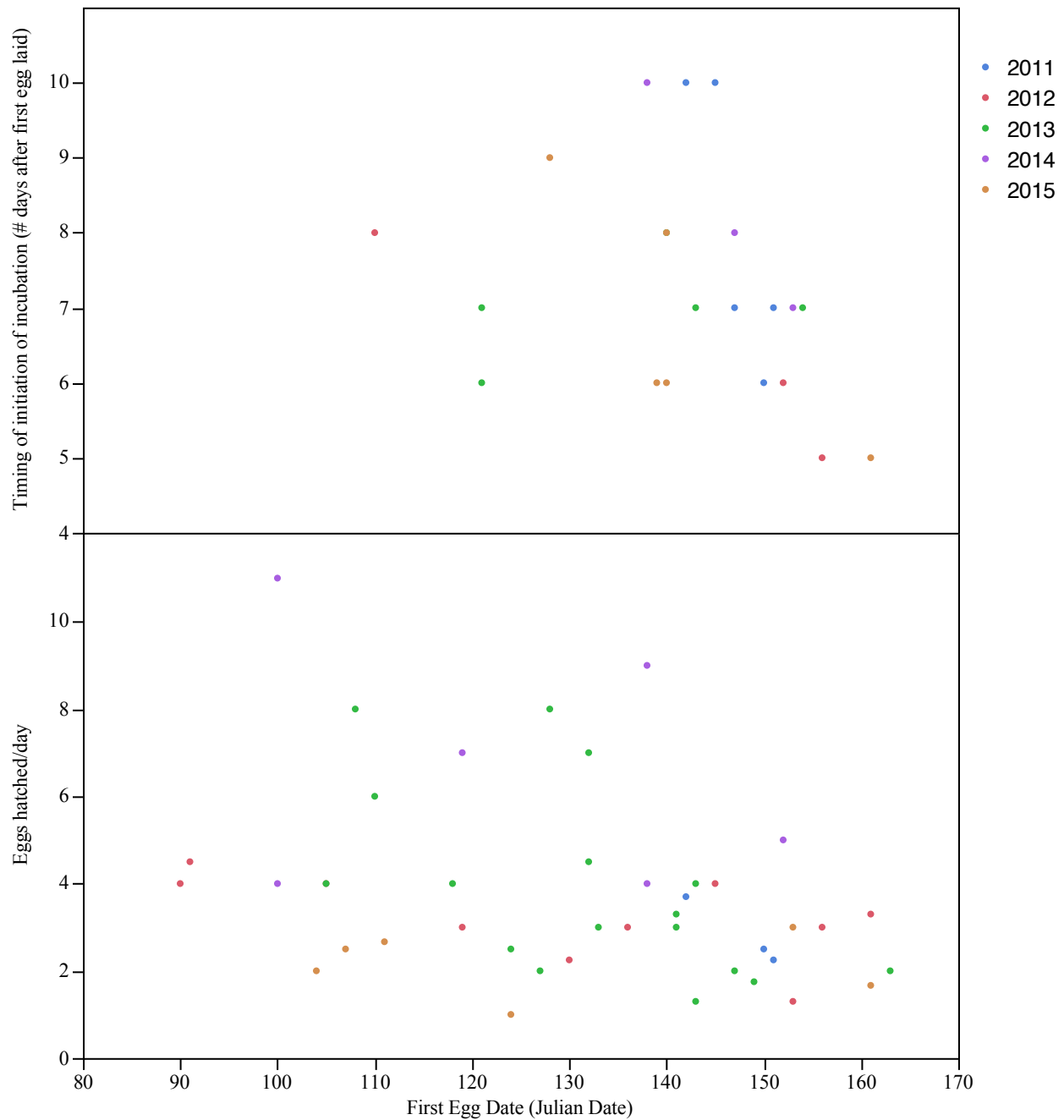


Figure 1.17. Within-season trends by first egg date (Julian date) in incubation and hatching synchrony for nests occurring in 2011-2015. **A.** Parents began full time nest attendance earlier (days after the first egg) later in the breeding season (Linear mixed effects model: $F_{1,16} = 3.5$, $p = 0.07$; $n = 20$ nests). **B.** Hatching was more asynchronous in late-season nests (Linear mixed effects model: $F_{1,37} = 6.7$, $p = 0.01$; $n = 42$ nests).

SEX DIFFERENCES IN INCUBATION SCHEDULING AND NOCTURNAL INCUBATION

To determine sex differences in attendance patterns throughout the day, I compared the proportion of time the male parent spent at the nest in the morning (approximately 06:00-10:00), mid-day (10:00-14:00), afternoon (14:00-17:00), and evening (17:00-21:00). Within these periods of time, the time when nests were left unattended was excluded from the analysis, so the sum of the male and the female proportions of time attending the nest was equal to one. To stabilize the variances between these temporal periods, I used an arcsine square root transformation, commonly used for proportional data (Gotelli & Ellison, 2013). I performed a mixed-effects ANOVA with the time of day as a fixed effect and individual as a random effect to account for repeated measures on the same individuals. Males attended their nests significantly more in the evening than earlier in the day (Mixed effects ANOVA: $F_{3,117} = 31.3$, $p < 0.0001$; Fig. 1.18). Additionally, 75% of the parents captured at the nest in 2011-2015 were females, a process that usually took place between 07:00 and 14:00.

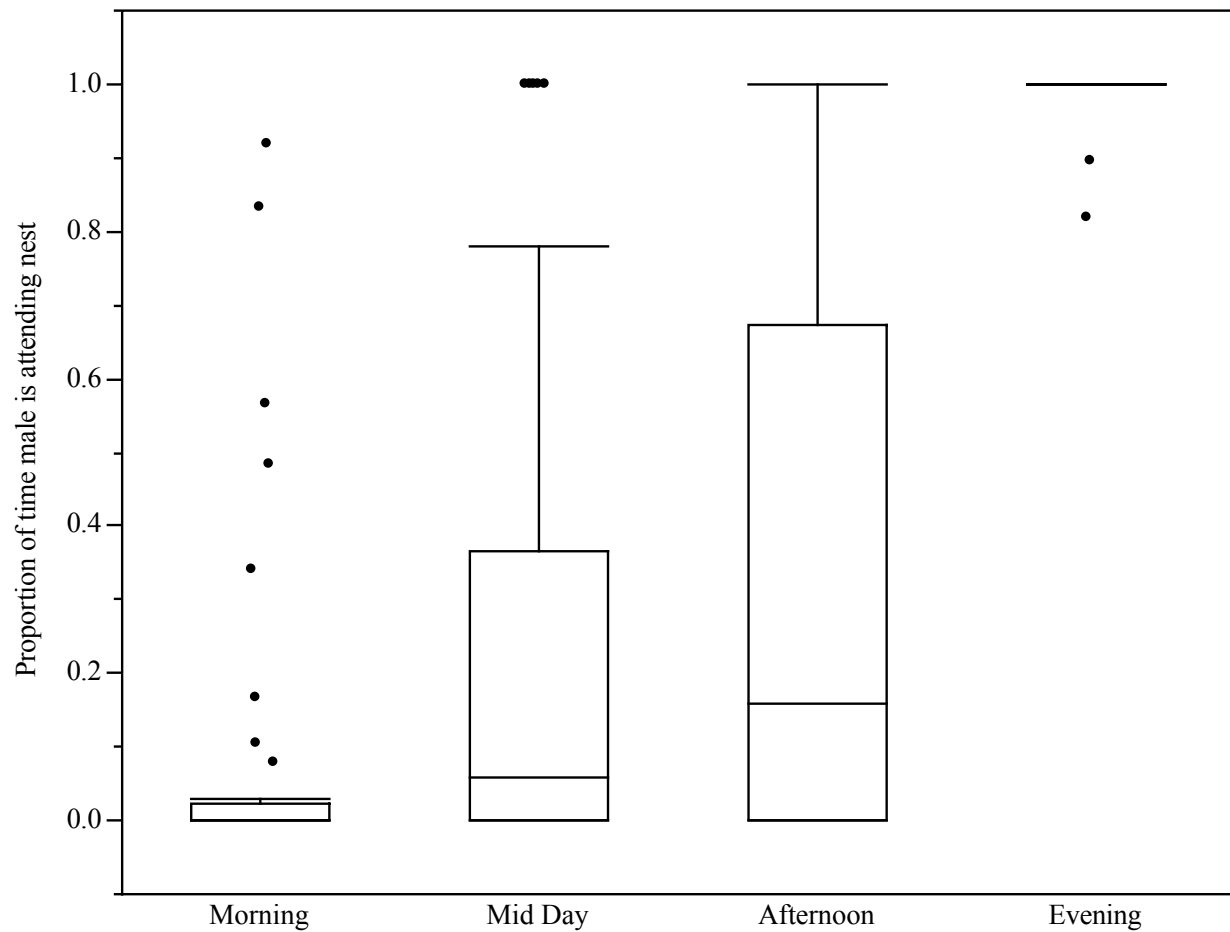


Figure 1.18. Males were significantly more likely than females to be attending the nest in the evening (17:00-21:00) and significantly less likely in the morning (06:00-10:00), midday (10:00-14:00), and afternoon (14:00-17:00) (Mixed effects ANOVA: $F_{3,117} = 31.3$, $p < 0.0001$; $n = 18$). Points represent outliers.

At 9 nests in 2014 and 2015, the start of incubation could be determined by comparing synchronized 5-minute clutch and ambient temperature readings. At 4 of these nests, temperature readings revealed that parents began to incubate their clutches at night 1 to 5 days before beginning full-time, 24-hour incubation. Daily patterns of nest attendance between males and females may explain overnight incubation patterns.

DISCUSSION

SHADING AND INCUBATING AS FLEXIBLE BEHAVIORS

Nesting king rails have evolved a behavioral strategy of shading their clutches at high ambient temperatures. It appears that this behavior is flexible because all individuals tended to shade for proportionately more time at higher ambient temperatures and traditionally incubated for proportionately more time at lower ambient temperatures. Neither shading nor incubation time budgets were related to parental condition. Instead, my results support the hypothesis that variation in these behaviors is a result of adaptive plasticity and is not attributable to individual differences. The flexibility of incubating and shading behaviors effectively dampened the effect of high and low ambient temperatures on clutch temperatures to keep the clutch temperatures relatively consistent. Thus, with few exceptions, parents in this population were able to maintain their eggs at a relatively constant temperature under hot conditions while attending their nests.

In species that have evolved the behavior of shading, it is thought that the primary purpose of shading is to control egg temperatures by preventing the eggs from overheating in hot conditions (DuRant et al., 2013). If this is the case, parents should simply shade constantly in hot conditions, allowing air to pass over their eggs and to cool via convective cooling (Downs & Ward, 1997). This relies on the assumption that parents are sensing and responding to the temperatures of their eggs. In a study by (White & Kinney, 1974), after injecting the brood patches of herring gulls with a local anesthetic, they found that the parents would incubate their eggs more intensely than birds without anesthetized brood patches, and their eggs reached higher temperatures. This suggests that adults are able to use their brood patches not just to transfer heat to their clutches, but also to sense the temperature of the clutches, allowing them to adjust their posture accordingly. If king rails are also capable of sensing and responding to clutch

temperatures, shading may have evolved for the purpose of keeping clutches below lethal temperatures.

On the other hand, shading may actually be an adaptation for cooling the parent with the beneficial side effect of preventing overheating of the eggs (Grant, 1982). This does not rely on the assumption that king rails are capable of sensing their clutches and perceiving whether to adjust their behavior. By monitoring body temperatures of shading crowned plover (*Vanellus coronatus*) parents, studies have demonstrated that adult internal temperatures were always cooler when they were shading versus when they were incubating (Brown & Downs, 2003; Downs & Ward, 1997). Additionally, there was no evidence to suggest the eggs were kept cooler when the adult was shading, and in some instances the eggs actually reached higher temperatures when being shaded than when they were being tightly incubated. In support of this scenario in king rails, I observed parents shading intermittently, in that they would alternate incubating and shading when ambient temperatures were high. There were cases in which the adults would shade for longer bouts in exceptionally hot conditions, panting heavily and ruffling their feathers, signs of extreme heat stress. However, these parents would occasionally temporarily abandon their nest-attendance duties (in contrast to shading continuously, as would be expected if shading were primarily to cool the eggs), and would leave their eggs exposed to the direct sunlight. The attending parents may be forced to find a cooler location, eventually returning to the nest once it has cooled itself sufficiently.

Attending an open nest when ambient temperature greatly exceeds body temperature is likely costly and stressful to parent king rails. Energy expenditure during the incubation period in birds has largely been studied in the context of the need to keep eggs warm in cool environments, but not vice versa. Traditional incubation (sitting) has been shown to be costly in terms of the

trade-off between the need to keep eggs warm with the energetic needs of the parent (i.e. foraging) (Bulla et al., 2015; Cresswell et al., 2004). The degree of energy expenditure during incubation is affected by the rate at which heat is lost from the clutch, the insulation properties of the nest itself, and environmental temperature. By experimentally reducing the costs of incubation in first clutches of starlings (*Sturnus vulgaris*), parents were able to reallocate those resources to future reproductive attempts, experiencing higher hatching success and a smaller decrease in clutch sizes in second broods when compared to control nests (Reid et al., 2000). Moreover, it has been argued that incubating birds are more stressed during the hotter part of the season, when cooling of the eggs is necessary (Downs & Ward, 1997). Parent king rails can maintain constancy by shading up to a point, but prolonged high temperatures may push their tolerance limits. Future studies should attempt to better understand the energetic demands of shading, and whether the costs of shading affect parental fitness.

RECESSES AND NEST SUCCESS

King rails tended to take shorter, but more frequent recesses at high ambient temperatures, likely in response to heat stress from remaining at the nest. Unfortunately, recess duration cannot be attributed to individual birds during changeovers, as it is unknown whether the attending parent or the off-nest parent initiates them (Bulla et al., 2015). To determine whether body condition impacts recess duration and frequency, future studies should attempt to better understand the changeover and bout dynamics within king rail pairs, and which parent initiates recesses.

The amount of recess time avian parents take has been shown to have an effect on the incubation temperature (Martin et al., 2007). This was also the case for king rails. In three

instances where nests were filmed at the time when the maximum incubation temperature occurred, neither of the parents was attending the nest, and the eggs were exposed to direct sunlight. Some clutches reached excessively high temperatures, and the majority of clutches that exceeded temperatures of 43.5°C experienced complete hatching failure. A single nest that reached a high of 45.5°C hatched the majority of its eggs, but it exceeded 43.5°C for less than 20 minutes. Clutches that remained at high temperatures for extended periods were at greater risk of hatching failure. This is evidence that Atlantic coast king rails are experiencing ambient temperatures during the breeding season that are pushing the limits of their tolerance levels and have a direct impact on reproductive success.

It remains puzzling that some king rail clutches exceeded the estimated lethal temperature of most avian embryos (40.5 °C) and still hatched. In some species, embryos may be able to partially regulate their own temperatures or may be more tolerant of extreme temperatures, accounting for interspecific differences in incubation temperature (Du & Shine, 2015). It is possible that king rail embryos are capable of tolerating or avoiding hotter conditions for limited periods of time. Embryos may be able to control their exposure to heat behaviorally to an extent by moving within the egg to avoid hotspots. The embryo may then be limited in the amount of room inside of the egg, both by the size of their egg and the embryo itself (Du & Shine, 2015). More developed embryos may make sounds to prompt more attentive behavior from the parents (Brua et al., 1996). It has also been suggested that heat shock proteins, a subset of molecular chaperones that help organisms cope with thermal stress (Sørensen et al., 2003), may play an important role in the tolerance of embryos to heat, but this is largely unstudied in birds (Du & Shine, 2015). Embryos may also improve their thermal tolerance by increasing their oxygen supply or changing the density or enzyme activity of respiring mitochondria, as it has been

suggested that temperature-dependent limitations on oxygen supply and demand influence thermal tolerance (the hypothesis of oxygen- and capacity-limited thermal tolerance; Du & Shine, 2015; Portner, 2010). Embryos may also adjust blood parameters (such as facultative cardiac function and hematocrit) to enhance oxygen supply, though this has only been demonstrated in reptile embryos and avian nestlings (Ardia, 2013; Crossley & Altimiras, 2005; Du et al., 2010). Further research should address whether king rails have behavioral or physiological adaptations to withstand high temperatures.

VARIATION IN NESTS AND NEST SITE SELECTION

Parents can influence the microclimate of the nest and the temperatures the clutches reach by adjusting the overall nest construction and selection of nesting sites (Webb, 1987). In species that nest in colder climates, nests that provide better insulation have been shown to reduce the energy required to maintain suitable egg temperatures and to reduce parental attendance at the nest, allowing more time for foraging (Reid et al., 2000; White & Kinney, 1974). Hence there should be selection for an optimal allocation of resources to nest site selection and construction, and the outcome of a breeding attempt may be influenced by the availability of good nest sites and materials (Reid et al., 2000). King rails build nests and choose nesting sites that vary considerably in exposure. To tolerate hotter conditions, king rails may adaptively select more protected sites and build less exposed nests to reduce heat stress (Walsberg & King, 1978).

In shorebirds, it has been suggested that nesting in exposed sites facilitates early detection of predators. However, there may be a tradeoff in choosing between a thermally superior nesting site and a site that allows for better detection of predators (Amat & Masero, 2004). Because of the physiological cost of attending a nest in a hot, exposed environment, body condition may

influence where parents choose to put their nests. Individuals in better condition may choose more exposed sites because they are able to cope with the heat stress and are able to detect predators earlier. On the other hand, individuals in poorer condition may have to nest in concealed areas for the thermoregulatory benefits, while accepting that they may be more vulnerable to predators (Amat & Masero, 2004). Future studies should attempt to distinguish how nest construction and nest site selection affect both nest microclimates and predation risk in king rails and other species that breed in hot climates.

SEASONAL TRENDS

Ambient temperatures increase over the course of the king rail's nesting season, and it stands to reason that clutches initiated later in the season would be more likely to reach lethal temperatures and experience higher levels of hatching failure as a result. Seasonal decreases in hatchability have been reported for other species (Koenig, 1982). Though no significant seasonal trend in hatching failure was observed in this study, I was unable to determine initiation date of a large proportion of nests found inactive with unclear fates, and only six nests were confirmed as deserted by the adults.

I predicted that the incubation period of nesting attempts later in the season would be shorter, as eggs may experience accelerated embryo development in warmer conditions (Martin et al., 2007). This was also not the case: the duration of the incubation periods did not change through the course of the season. However, I could only determine the complete duration of incubation of nests that were found early enough in the laying period and hatched, yielding a sample size of only 7 nests, which may have been insufficient to detect a seasonal trend. Alternatively, parental attendance patterns may stabilize egg temperature regimes, preventing

acceleration in embryonic development in nests that occur later in the season. I suggest future studies address this issue by determining whether embryo development is accelerated or whether parental buffering of warmer ambient temperatures prevents acceleration.

Later in the season, king rails began incubating earlier relative to the first egg date, although this trend was marginally non-significant. This reduced the time early-laid eggs were exposed to ambient conditions. Egg viability decreases with time after laying, and incubation before clutch completion may be necessary to ensure hatching of the earlier laid eggs (Arnold, 1993; Stoleson & Beissinger, 1999). Early onset of incubation is the proximate cause of hatching asynchrony. Based on five years of nesting data for king rails, hatching became significantly more asynchronous later in the season. It was previously reported that hatching occurs over 1-2 days in king rails (Meanley, 1969), but late broods hatched over a period of up to 4 days at Mackay Island NWR. Because king rails move their chicks substantial distances within the first few days after hatching (Kolts, 2014), hatching asynchrony may cause parents to desert some unhatched eggs in favor of moving the hatched chicks to better habitat. Thus, there may be a tradeoff between the benefits of early onset of incubation and the benefits of synchronous hatching. Asynchronous hatching can result in brood reduction, but may serve to give older chicks a better chance of survival. Survival of broods after they leave the nest is not well-understood in king rails, but is thought to be a limiting factor in population growth (Cooper, 2008). Smaller broods due to hatching asynchrony may decrease the chances of at least some chicks surviving to adulthood.

NOCTURNAL INCUBATION

In 2014 and 2015, continuous monitoring of clutch and ambient temperatures at 9 nests revealed that in 4, parents began nocturnal incubation 1 to 5 days before beginning diurnal incubation. Studies that record details of nest attentiveness have found that parents will gradually increase incubation constancy instead of converting directly into full time incubation (Anderson, 1997; Arnold, 2011; Haftorn, 1981; Loos & Rohwer, 2004). Nocturnal incubation prior to clutch completion may allow sufficient embryo development to decrease hatching synchrony (Lord et al., 2011). Video observations revealed that male parents were significantly more likely to be in attendance in the evening than females. Therefore, it is reasonably likely that males attended nests throughout the night and were responsible for initiating nighttime incubation. Incubation patterns between the sexes in rails are poorly known, but male nocturnal incubation has been documented in several species (Gullion, 1954; McRae, 1996; Vehrencamp, 2000). It has been suggested that nocturnal incubation prior to clutch completion may reduce the risk of nest predation by parents actively defending the nest (Basso & Richner, 2015; Clotfelter et al., 1999; Montgomerie & Weatherhead, 1988), or it may serve to prevent brood parasitism (Stoleson & Beissinger, 1999). The reasons behind male nocturnal incubation prior to clutch completion are not clear in king rails and should be explored in future studies.

CONCLUSIONS

Hatching failure was related to both high clutch temperatures and the duration of time clutches spent at high temperatures. King rail parents showed plasticity in both incubation and shading effort in response to temperature variation, but parents took more frequent recesses at higher ambient temperatures. All king rails may have an upper limit in their ability to tolerate

high temperatures experienced while attending an open nest, and we may be starting to see these limits. However, parents may be able to adapt to higher temperatures predicted for the future by selecting nesting sites that are more protected from the direct sunlight, though this may tradeoff with the ability to detect predators earlier. King rail embryos may also adapt to avoid or to tolerate hotter conditions behaviorally (e.g. avoiding hot spots within the egg) or physiologically (e.g. enhancing oxygen supplies).

Nests initiated later in the breeding season experienced significantly warmer temperatures than nests that were initiated earlier. Parents of late-season nests began incubating sooner after their first egg was laid, possibly to preserve the viability of early-laid eggs. Nocturnal incubation began prior to diurnal incubation in four out of nine nests, and males likely attended the nests overnight. Hatching also became more asynchronous later in the breeding season, a consequence of the early onset of incubation, which may result in the desertion of some unhatched eggs. I suggest future studies explore the consequences of brood reduction on survival rates of chicks during the brood-rearing period, thought to be a limiting factor in the population growth of this species.

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Chapter 2

King rails vary nest height in relation to the range of water level at the nest site, but building effort is not related to female condition

ABSTRACT

Most rails nest above or adjacent to standing water in wetland breeding habitat. Coastal marshes are prone to variation in water level influenced by both estuarine effluent and storm surge. King rails have become more reliant on coastal marshes, since inland wetland habitat is greatly decreased. Nest-building behavior in king rails was studied in a natural coastal marsh subject to a wind-driven tide system. Rapid water level rise from storm surge at this site has led to nest inundation. I examined whether all individuals exhibit flexibility in nest building related to water level variation (adaptive plasticity) or, alternatively, whether individuals in superior body condition tend to build more. I monitored nest height and water level and quantified nest building behavior at king rail nests in 2014-2015. King rails constructed taller nests on average in areas with a wider range of water level. Parents also spent proportionately more time building when water levels were closer to their nests, but individual nest height remained relatively constant in relation to the substrate. Nest height also did not increase considerably during the laying period, suggesting that the majority of nest accumulation occurred before laying began. Nest building during the laying and incubation periods may function mainly to counteract settling and improve the structural integrity of the nest. King rails exhibit plasticity in average nest height, indicating variance in overall effort. Though king rails continue to build through the nesting cycle, they may not be able to increase nest height rapidly enough to avoid flooding when faced with rapidly rising water. Wetland managers might improve king rail nest success by drawing down water levels in managed impoundments earlier, creating additional breeding habitat less vulnerable to flooding.

INTRODUCTION

Most rails (Family Rallidae) nest in emergent vegetation, above or adjacent to standing water (Taylor & van Perlo, 1988). Nesting above water may deter some nest predators, but this is a trade-off with flooding risk. In Missouri, nest success was positively correlated with water depth underneath or directly adjacent to the nests (Darrah & Krementz, 2011; Reid, 1989). Some species of rails are known to continually build up their nests in response to rising water levels (Robertson & Olsen, 2015; Taylor & van Perlo, 1988). In a study of Kentish plovers (*Charadrius alexandrinus*), when the insulation properties of nests were manipulated by decreasing or increasing the amount of nest material, parents would actively adjust their nest and restored the original amount of nesting material within 24 hours (Szentirmai & Szekely, 2002). This suggests that parents maintain the amount of material in their nest and are flexible in their building behavior.

We observed a wide range of king rail nest heights at Mackay Island National Wildlife Refuge (NWR) (Fig. 2.1A and B). Meanley (1969) noted that king rail nests in relatively dry areas were not elevated, whereas nests placed above water could be elevated as much as 30 cm above the water surface. Marsh water levels at Mackay Island NWR are influenced by a wind-driven tide system and, consequently, are highly variable (Rogers, 2011). Recent climate change models are predicting more intense and more frequent extreme precipitation events and storm surges in the future, particularly for the southeast U.S. (IPCC, 2014; Melillo et al., 2014). Sudden flooding associated with storm surge may cause nest loss if adults are not capable of building their nests up quickly enough. Rapid water level rise due to storm surge at Mackay Island NWR resulted in the inundation of two nests in the 2013 breeding season, leading to the desertion of

the nest by the parents (Fig. 2.1B). Studies measuring nest height and nest building in wind-driven tide systems seem to be missing from the literature.



Figure 2.1. Variation in height of king rail nests. **A.** A nest of relatively average height. **B.** A relatively built up nest. **C.** A low nest that became inundated and was subsequently deserted.

Continuous nest building and bigger nests may alternatively be associated with the parents being in better condition (Lens et al., 1994; Soler et al., 1998), as nest building can be costly in terms of time and energy (Szentirmai & Szekely, 2002). Studies have found that nest size is positively related to other fitness related parameters such as clutch size (Soler et al., 2001), hatching success (Moller, 1982), and breeding success (Álvarez & Barba, 2008). This would predict that nest building in king rails may not be related to water level variability, but may be a function of body condition (Soler et al., 1998).

King rails may prefer nest sites with a lower risk of nest flooding (i.e. lower water level variability). When considering a nest site, birds consider a variety of factors including the proximity to feeding areas, thermoregulatory benefits (i.e. protection from sun), camouflage

against predators, and the ability to detect predators early (Barati et al., 2012). A study of clapper rails (*Rallus longirostris*) found that parents tend to construct nests further away from tidal influences in an effort to avoid nest inundation (Gaines et al., 2003). However, if nesting in areas with deep water reduces nest predation, this may tradeoff with reducing flood risk.

To determine whether rails adaptively adjust nest height, I monitored both nest height and water level at king rail nests in coastal marshes, and quantified nest building behavior of king rail parents. I determined whether king rails built taller nests in response to wider ranges of water level (adaptive plasticity), or alternatively, whether taller nests were built by king rails of superior body condition. If king rail parents built up their nests in direct response to rising water, then I expected parents to devote more time to nest building and to build up their nests when water levels were closer to the nest rim. I determined whether parents in better condition selected nest sites with lower ranges in water level since these areas may be less prone to flooding, or if they selected sites with deeper water, which might deter most ground predators.

METHODOLOGY

STUDY SITE

Mackay Island NWR, on a coastal island within the Currituck Sound, comprises 4,774 acres of freshwater marshes interspersed with impoundments. Water levels in the marshes are strongly influenced by wind-driven tides (USFWS, 2008). Because there are no inlets along the northern portion of the Currituck Sound (the barrier island completely encloses Back Bay), the Currituck Sound acts similarly to a bathtub. When the wind blows predominately from a southwesterly direction, the water is pushed into Back Bay, and water levels in the marshes increase. When the wind blows from the northeast, the water is pushed back into Currituck

sound, and water levels in the marshes decrease. The microtopography and irregular channeling inside the marshes also influence the degree of water level variation. Thus, the water levels in the marshes are highly variable through time and space. Mackay Island NWR has four impoundments, in which the water level is managed. Water level is raised in the impoundments for overwintering waterfowl, and then lowered in spring to promote vegetation growth for grazing waterfowl. For a full study site description, refer to Chapter 1.

NEST AND WATER LEVEL MONITORING

King rail nests were found throughout the breeding seasons (April-July) in 2014-2015, and they were monitored until hatching or failure. Nests found during laying were visited daily until clutch completion, then every 2-3 days until pipping of the first egg. For a full description of nest monitoring, refer to Chapter 1.

I installed a notched, discrete garden pole mounted in the reeds next to the nest to measure nest height and water level at each nest. The standard measuring stick increased consistency in measurements between nest visits. During each nest visit, 'Water Level' was measured from the substrate level to the water surface (± 2 cm; Fig. 2.2). 'Nest Height' was measured from the substrate level to the top rim of the nest that was closest to the garden pole (± 2 cm). From water level and nest height, I calculated the daily 'Proximity of Water' to the nest by subtracting Nest Height from Water Level. For each nest, I also calculated the 'Water Level Range' by subtracting the minimum Water Level that was measured at the nest site, throughout the period of time it was monitored, from the maximum Water Level measured at the nest site.

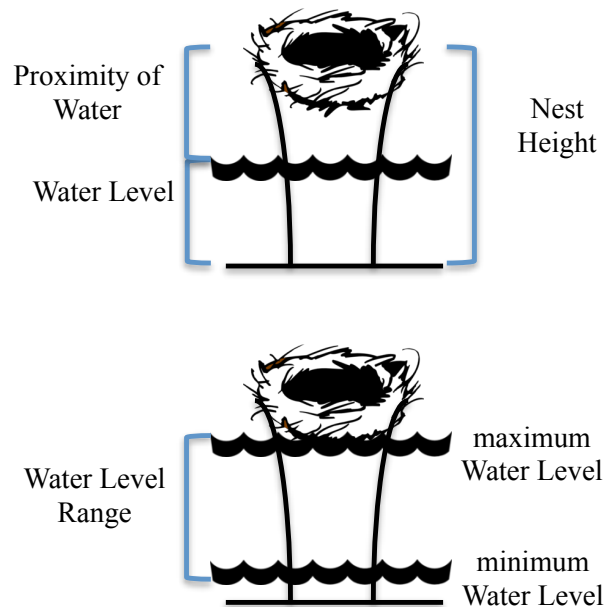


Figure 2.2. Water and nest measurements at each nest. ‘Nest Height’ was the distance from the substrate to the top rim of the nest, and ‘Water Level’ was the distance from the substrate to the water surface. From these measures, the ‘Proximity of Water’ (the distance from the water surface to the top rim of the nest) and the ‘Water Level Range’ (the total change in water level over the course of the nest cycle) were determined.

CAPTURING AND BANDING TECHNIQUES

I captured breeding adult king rails late in the incubation stage, when adults were ‘sitting tight’ on their nest. I surrounded the nest with overlapping mist nets, and then flushed the incubating adult into the nets. Once an adult was captured, I banded it with a uniquely numbered U.S. Geological Survey aluminum band and a unique combination of three colored leg bands, distributed as two bands on each leg. Body mass (± 5 g), flattened wing chord (± 1 mm), bill length (± 0.1 mm), and tarsus length (± 0.1 mm) were measured, and a blood sample was drawn to confirm the sex of each individual. For a full description of capturing and banding, refer to Chapter 1.

DOCUMENTING NESTING BEHAVIOR

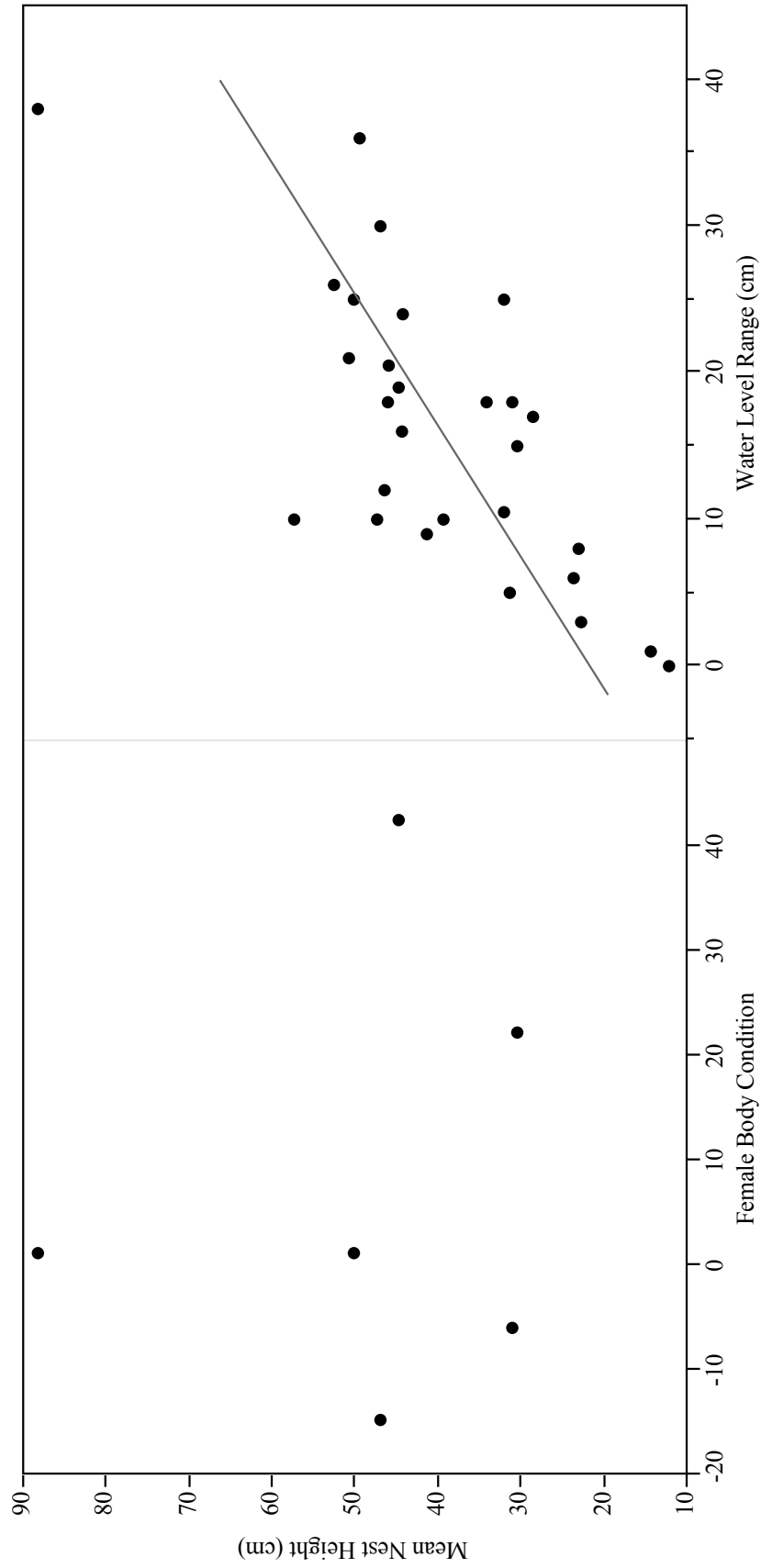
To observe parental behavior, I placed a tripod-mounted digital video camera at active nests. Detailed daily time budgets of attending adults allowed me to assess variation in the nest building behavior of each parent during the laying and incubation periods. I distinguished adults at 9 of 24 nests by identifying banded individuals, or comparing relative bill lengths in profile when neither adult was banded. For a full description of documenting nesting behavior and distinguishing adults, refer to Chapter 1.

RESULTS

ARE NEST HEIGHT AND BUILDING RELATED TO GREATER VARIATION IN WATER LEVELS OR INDIVIDUAL DIFFERENCES?

Nest Height measured from the rim down to the solid substrate varied considerably among pairs; mean ($\pm$ SD) = 39.5 ± 15.1 cm (range: 12 - 93 cm). To determine whether individual differences in body condition or variation in water levels affect nest height, I used a Spearman rank-order correlation and linear regression, respectively. Both males and females were observed nest building. However, morphometrics were only obtained from three males in 2014-2015, so there were insufficient data to incorporate male body condition into my analyses. Most nests were in natural marsh that is subject to an irregular pattern of wind-driven tides. Water Level change was highly variable, and due to the nature of these fluctuations I measured difference in level among nest visits rather than the rate of change as an independent variable. Therefore, predictor variables of female body condition and Water Level Range were used to test for effects of condition and water level variability on nest height. Nest Height was not related to female body condition (Spearman rank-order correlation: $r_s = -0.23$, $p = 0.66$), although the sample size is small and there may not be enough statistical power to detect a trend. However,

parents built significantly higher nests on average at sites with a greater Water Level Range
(Linear regression: $F_{1,27} = 28.8$, $p < 0.0001$, $R^2 = 0.53$; Fig. 2.3).



Clutch size was related to female body condition (Linear regression: $F_{1,24} = 7.18$, $p = 0.01$, $R^2 = 0.24$; Fig. 2.4). To include more individuals in the analyses, I used clutch size as a proxy for female condition for females with known clutch sizes. Mean nest height was also not related to clutch size (Linear regression: $F_{1,24} = 0.08$, $p = 0.78$, $R^2 = 0.003$, $n = 24$).

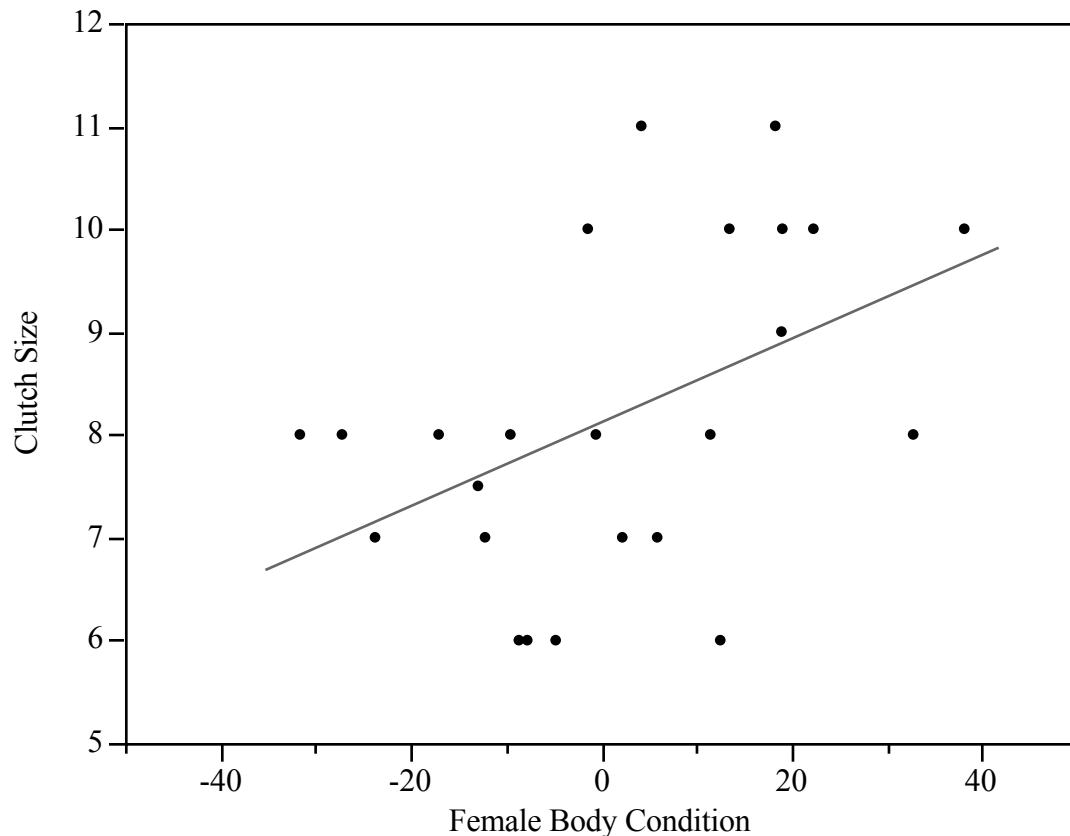


Figure 2.4. Clutch size was significantly positively related to female body condition (Linear regression: $F_{1,24} = 7.18$, $p = 0.01$, $r^2 = 0.24$, $n = 25$ females). Only females with known clutch sizes caught during the incubation period from 2011-2015 were included in this analysis.

To determine whether king rails build more when the water level approaches the top of their nest, I related the proportion of time each pair spent building to the Proximity of Water to the top of the nest using a linear mixed model. The proportion of time spent nest building was based on 2.3 to 47.6 hours (mean $\pm$ standard deviation = 28.7 ± 12.0 hours) of videotaped observations per nest, and was calculated by dividing the duration of time either parents spent

nest building divided by the amount of time the nest was filmed per day. The Proximity of Water was included as a fixed effect, and pair was included as a random effect to account for multiple measures on the same pair. I used an arcsine square root transformation on the response variable to ensure a linear relationship. Pairs spent proportionately more time nest building when water levels were closer to their nest (Linear mixed model: $F_{1,39} = 8.71$, $p = 0.005$; Fig. 2.5).

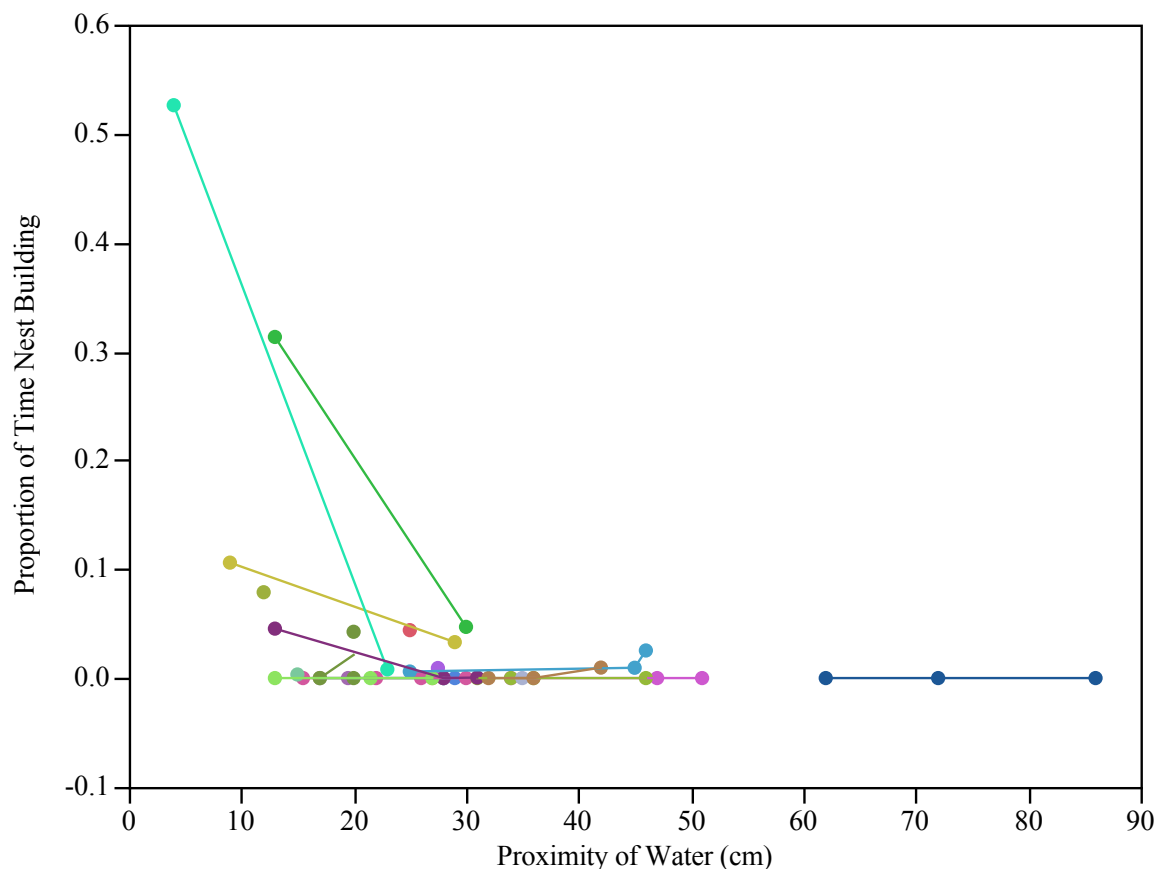


Figure 2.5. The proportion of time pairs spent nest building was significantly negatively related to the proximity of water to the top of the nest (Linear mixed model: $F_{1,39} = 8.71$, $p = 0.005$). Multiple measures are indicated by colors specific to each pair ($n = 24$ pairs).

To determine if changes in Nest Height were related to the Proximity of Water to the nest, I performed a linear mixed effects model. The change in nest height was calculated by subtracting the Nest Height measured during the previous nest visit from the Nest Height measured during the following visit. The Proximity of Water to the top of the nest was included

as a fixed effect, and nest was included as a random effect to account for multiple measures on the same nest. When water levels were closer to the nest rim, parents did not increase the height of their nests (Linear mixed effects model: $F_{1,182} = 0.88, p = 0.35$; Fig. 2.6).

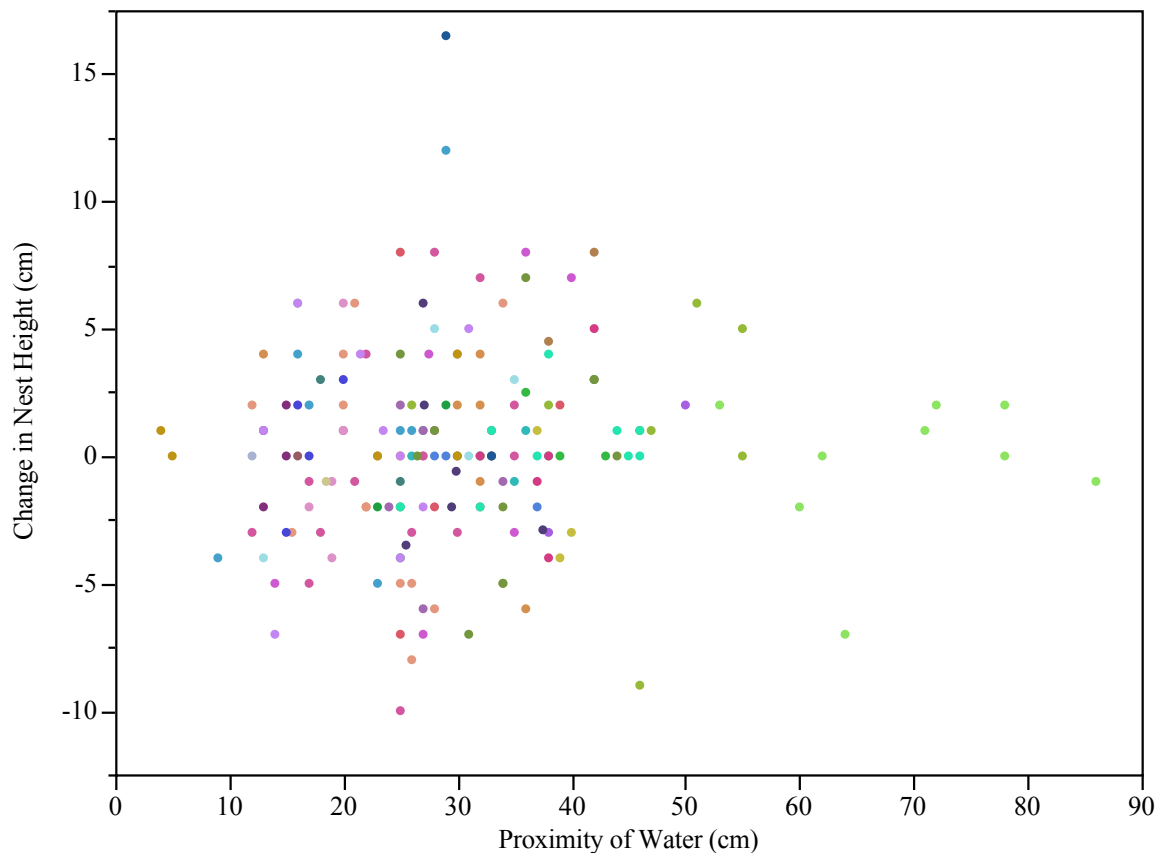


Figure 2.6. Mean change in nest height from the previous nest check did not increase significantly in relation to the proximity of water to the top of the nest. (Linear mixed effects model: $F_{1,182} = 0.88, p = 0.35$). Multiple measures are indicated by colors specific to each pair ($n = 33$ pairs).

Since king rails did not build up their nests in response to water levels during incubation, I focused on observations taken daily during the laying period. I compared daily Nest Heights for 11 nests found during the laying period. I used a linear mixed effects model with day of laying (number of eggs) as a fixed effect and pair as a random effect to account for multiple measures on the same pair. Nest Heights did not change significantly during the laying period (Linear

mixed model: $F_{1,34} = 1.16, p = 0.29$; Fig. 2.7). Instead, it seemed as though king rail nest construction was most often complete before laying began, and that additional building was only to maintain the existing nest height.

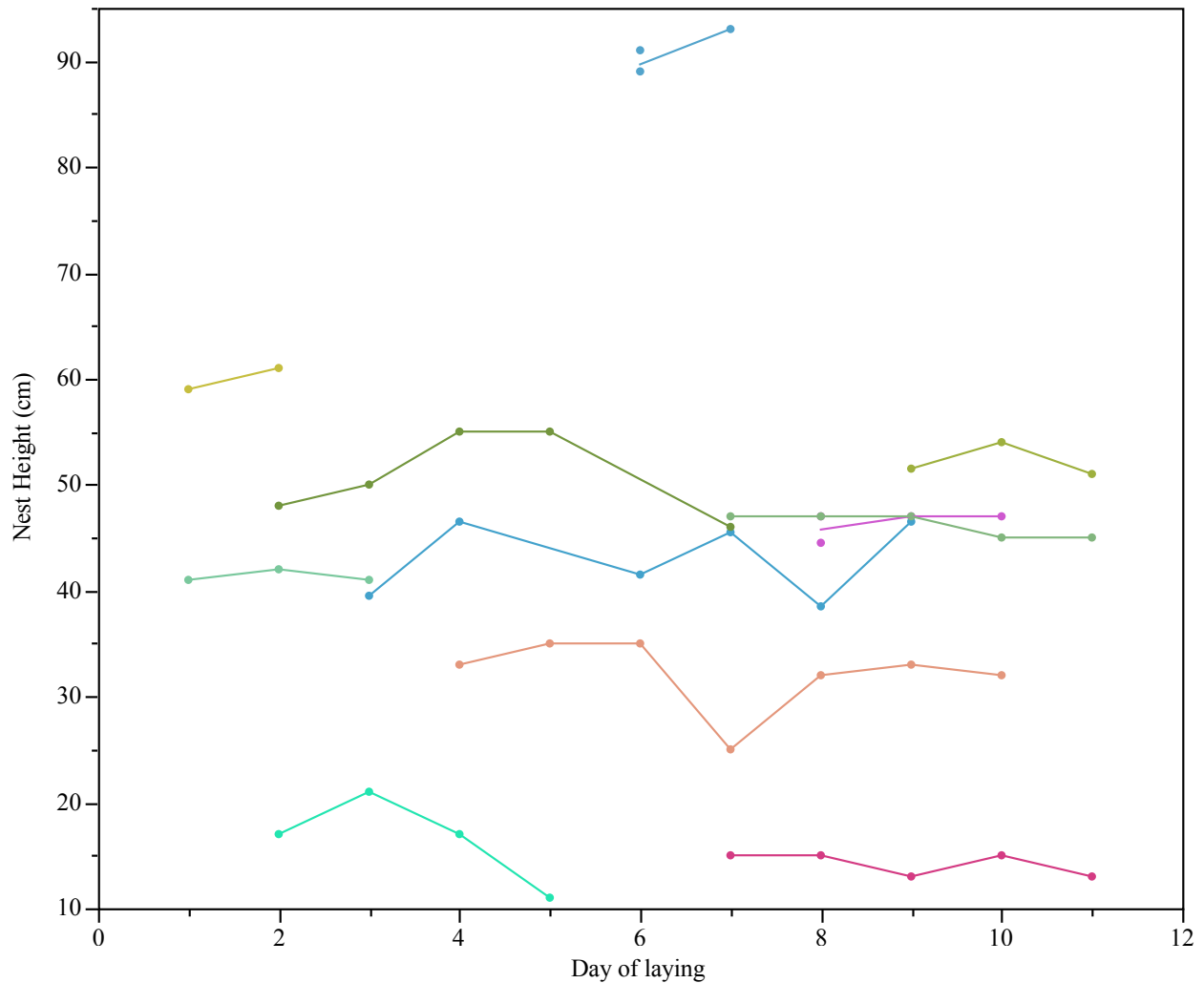


Figure 2.7. Relative nest heights of 11 nests during laying. Individual Nest Heights did not change significantly over the course of the laying period (Linear mixed model: $F_{1,34} = 1.16, p = 0.29$). Multiple measures are indicated by colors specific to each nest.

NEST SITE SELECTION IN RELATION TO WATER LEVEL RANGE

The majority of nests in 2014 and 2015 were found in the natural marsh, but two (7%) and 3 (7%) nests, respectively, were found in impoundments after drawdown had occurred, but where the influence of the wind-driven tides on the water levels was buffered. Water Level Range varied among nesting sites and over time (mean $\pm$ SD = 16.4 ± 9.6 cm, range: 0 – 38 cm). To determine whether parents in better condition selected nest sites in areas less prone to flood, I correlated the Water Level Range at their nests to female body condition using Spearman's rank-order correlation. Females in better condition did not nest in areas with a smaller range of water levels ($r_s = -0.36$, $p = 0.46$; Fig. 2.8), although the sample size is small and there may not be enough statistical power to detect a trend. I also correlated the Water Level Range at each nest to clutch size, an indicator of female condition, but the result remained non-significant (Linear regression: $F_{1,24} = 0.02$, $p = 0.89$, $r^2 = 0.0009$, $n = 25$).

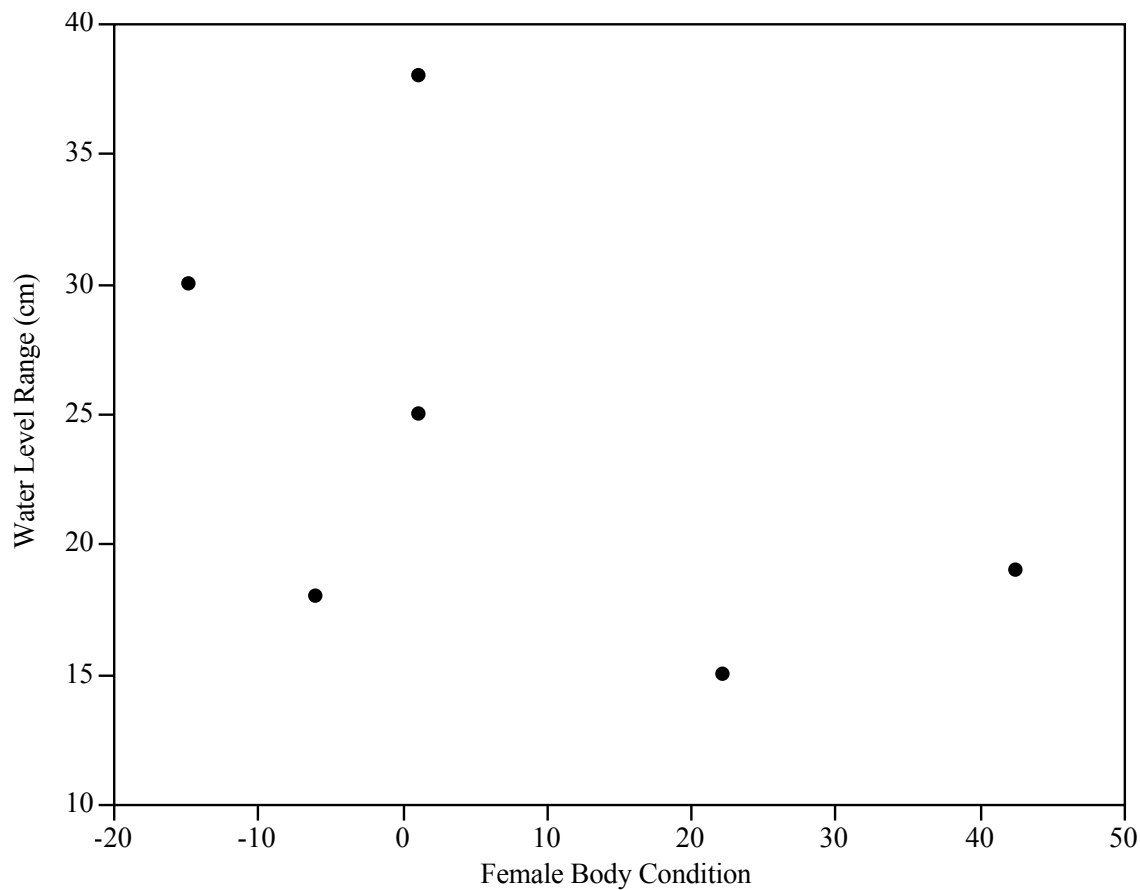


Figure 2.8. The range of water levels at nesting sites was not related to female body condition (Spearman's rank-order correlation: $r_s = -0.36$, $p = 0.46$, $n = 6$).

Rather than selecting nest sites with a lower Water Level Range, king rails may choose sites with deeper water to deter nest predators. I correlated the mean Water Level measured at each nest to female body condition using Spearman's rank-order correlation. Females in better condition did not select nest sites with deeper water ($r_s = -0.23$, $p = 0.66$; Fig. 2.9), although the sample size is small and there may not be enough statistical power to detect a trend. I also correlated mean Water Level to clutch size, but the result remained non-significant ($F_{1,24} = 0.71$, $p = 0.41$, $r^2 = 0.03$, $n = 25$).

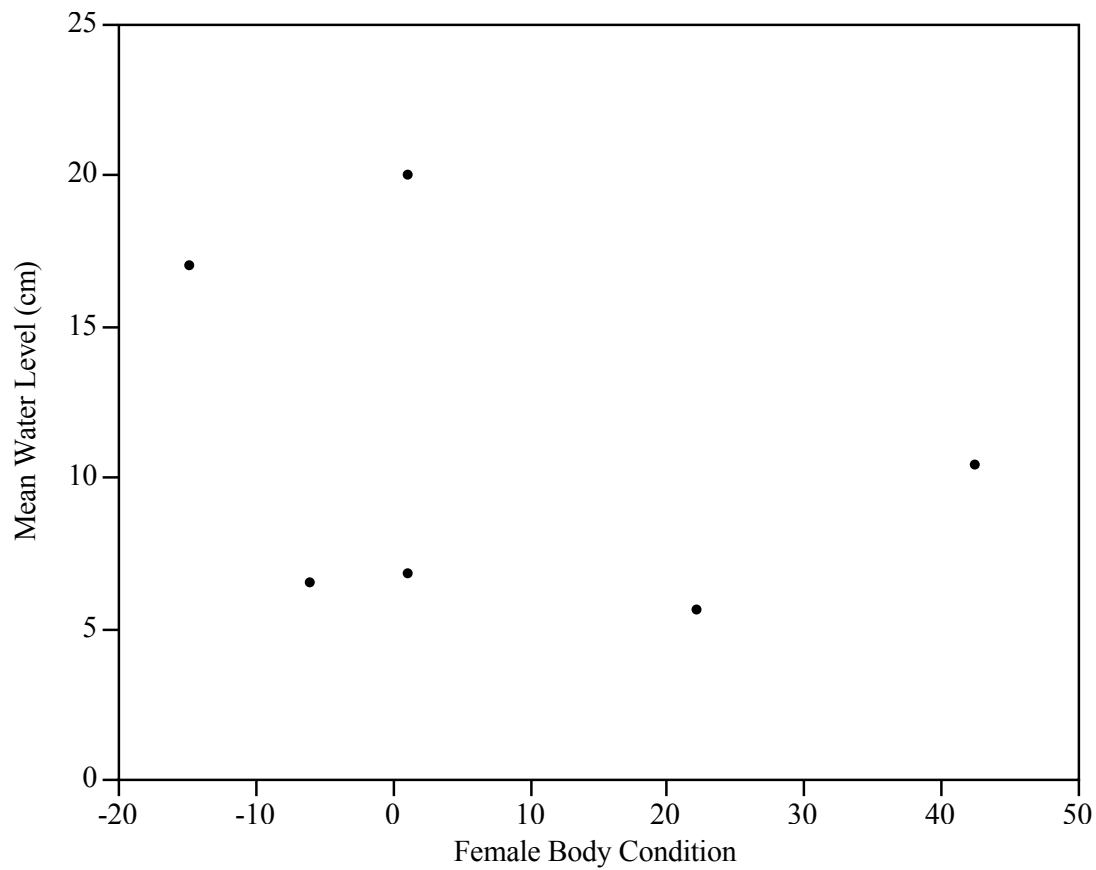


Figure 2.9. Mean water level at nest sites was not related to female body condition (Spearman's rank-order correlation: $r_s = -0.23$, $p = 0.66$, $n = 6$).

To determine if nests in shallower water were more likely to be depredated, I performed a logistic regression. I related mean Water Level at each site to nest fate, which was broken into two categories: depredated and all other fates. Nests in areas of shallower Water Levels were not more likely to be depredated ($\chi^2 = 0.34$, $p = 0.56$, $df = 1$; Fig. 2.10).

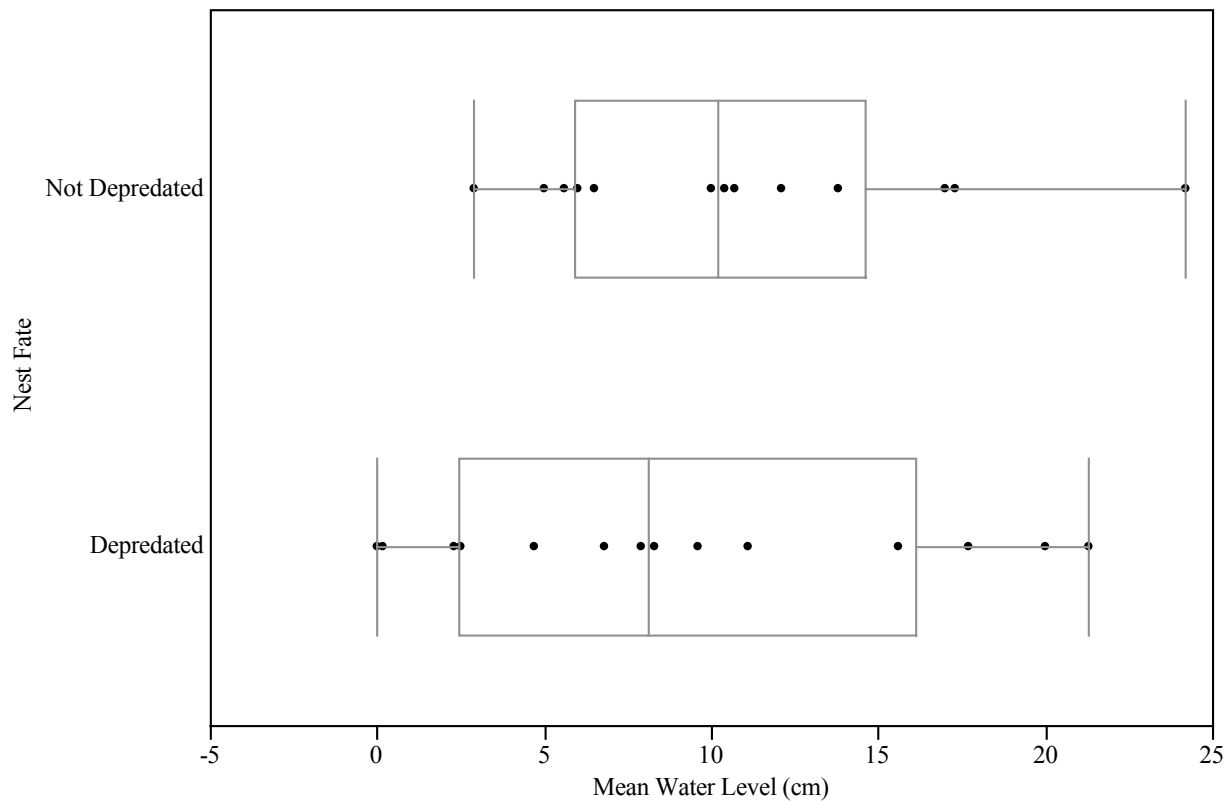


Figure 2.10. Nest fate (depredated vs. all other nest fates) was not related to the mean Water Level at each nest site (Logistic regression: $\chi^2 = 0.34$, $p = 0.56$, $df = 1$, $n = 28$ nests). Points represent different nests.

DISCUSSION

PLASTICITY IN AVERAGE NEST HEIGHT, BUT NOT CONTINUAL HEIGHT

ADJUSTMENTS

King rails exhibited plasticity in nest building by constructing taller nests in areas with greater water level ranges. Females in better condition did not tend to build taller nests, though my ability to test this was limited due to sample size and lack of data from male parents. Parents invested proportionately more effort building when the water level was closer to the top of their nest, but mean nest height did not increase significantly when water levels approached the top of their nests. Nests both increased and decreased in height between visits. Decreases were likely a

result of the nest settling under the weight of the eggs and the incubating adults. Nest height did not increase significantly during the laying period, suggesting that the majority of nest building occurred before laying began. Nest building during the laying and incubation periods may therefore be used to counteract settling and to improve the structural integrity of the nest.

In the two instances of inundation of king rail nests that we have observed at Mackay Island NWR, the nests were subsequently deserted by both parents. However, clapper rail (*Rallus crepitans*; Chesser et al., 2010) eggs that were partially submerged in water still hatched, suggesting submerged eggs may remain viable for short periods of time (Kozicky & Schmidt, 1949; Rush et al., 2010). Clapper rails have also been observed retrieving their eggs after they had been displaced via flooding (Rush et al., 2010), but this behavior has not been observed in the king rail. Further studies on the viability of submerged king rail eggs and parental behavior after nest inundation are needed to determine if complete nest failure can be avoided.

Parental experience may play a role in nest building expediency. It is possible that king rail parents learn the structural properties of appropriate nesting materials, and how to best use those materials to create a sturdier nest (Bailey et al., 2014; Collias & Collias, 1964). In captive village weaverbirds (*Ploceus cucullatus*), younger and less-experienced males tend to build messier nests with a looser weave than do older and more-experienced males (Collias & Collias, 1964). Bailey et al. (2014) demonstrated that nest building in captive zebra finches (*Taeniopygia guttata*) also improves with experience: individuals learned to choose stiffer, more preferable nesting materials and to avoid building with more flexible materials. It has also been demonstrated that experienced blackbird parents (*Turdus merula*) tend to build their nests larger and more quickly than less experienced parents (Wysocki et al., 2015). If more experienced king rails are able to build more quickly, they may be able to respond to rapidly rising water and build

their nests up more quickly than inexperienced parents, rather than changes in nest height being related only to the proximity of water to their nest. If this is the case, experience rather than body condition may play a more important role in nest building. Future studies should attempt to determine what role experience plays in nest quality and building expediency, and how this may affect reproductive success.

Having a taller nest may prevent nest flooding, but it is possible that taller nests are more conspicuous and may have a greater likelihood of being depredated by avian predators (Mainwaring et al., 2014). In a controlled study by Piper & Catterall (2004) using artificial nests of varying heights, elevated nests were more often depredated than ground nests, even though mammalian predation was more likely for ground nests. Avian nest predators, such as fish crows (*Corvus ossifragus*), were common throughout the refuge, and depredated king rail eggs were occasionally found with small holes, indicative of avian predation. I suggest studies investigate the possible tradeoff between the benefits of having a taller nest to prevent nest flooding and having a shorter nest to prevent avian predation.

It is possible that males may take the more active part in building the nest prior to the start of laying. Meanley (1969) suggested that the male is the primary nest builder in king rails, as he observed males gathering grasses and bringing them back to their nest site. On one occasion, a female was briefly observed building a nest, but the male quickly came to relieve her (Meanley, 1969). I observed both males and females building during the laying and incubation period, but I did not observe building prior to the beginning of laying. This is apparently when the majority of the nest is constructed. If males are doing the majority of nest building prior to laying, nest size and structure may be related to male condition rather than female condition. In blue tits (*Cyanistes caeruleus*), a species in which the female alone builds the nest, nest weight

was positively related to female body mass, but not male body mass (Mainwaring et al., 2008). This is thought to help the non-building partner assess their partner's willingness to invest in reproduction and parental care. Future studies should explore the division of labor, and whether there are sex-related differences in nest building prior to the start of laying.

NEST SITE SELECTION

Female body condition did not seem to be related to nest site selection based on water level variation, although the sample sizes for these analyses were relatively small. It is conceivable that males ultimately select the nest site, based on Meanley's (1969) observations that males contribute substantially to the early stages of building. Moreover, based on his study in which king rails were tracked at Mackay Island NWR, Kolts (2014) observed that overwintering resident males occupy the same home range they defend in the breeding period, suggesting they may have greater familiarity with the territory. Thus, higher quality nest sites, based on water depth and variability, may be related to male condition rather than female condition. In general, male king rails play a large role in parental care, and the extent of their contributions warrants further study.

Other factors, rather than water level variation, may play a greater role in nest site selection. When selecting a nesting site, birds must consider many factors, including the proximity of feeding areas, thermoregulatory benefits (e.g. protection from sun), camouflage against predators, and the ability to detect predators early (Barati et al., 2012). King rails may need to compromise the risk of flooding when considering other factors that may more strongly determine hatching success. Rather than water depth, proximity to human-related activities may prevent nest predation at Mackay Island NWR. King rail nests were often found within 10 meters

of refuge roads that were open to the public and were used frequently by refuge visitors.

Goławski & Mitrus (2014) found that red-backed shrikes nesting closer to buildings achieved higher nesting success than those that nested further from buildings, suggesting predation pressure was lower when nesting closer to buildings. Whether king rails in superior condition select nest sites closer to human-related activities should be investigated in future studies.

If water level variation becomes more stochastic and extreme as predicted (IPCC, 2014), coastal king rails may have better nesting success in areas with a lower water level variation, such as non-tidal marshes or managed impoundments. Studies comparing nesting success in impoundments versus natural marshes have considered wading birds, waterfowl, and shorebirds (Breininger & Smith, 1990; Erwin, 1996), but either did not consider rails or were not able to survey them sufficiently. At Mackay Island NWR, water levels in the impoundments are elevated in the winter for wintering waterfowl, and are drawn down in the spring to promote the growth of vegetation as food for waterfowl (USFWS, 2008). King rails nest in the impoundments later in the breeding season after the growth of vegetation has become sufficient for nesting cover. By drawing down water levels in impoundments earlier in the spring, managers can provide additional habitat for earlier nesting king rails to enhance population growth.

CONCLUSIONS

King rails exhibit plasticity in nest building by constructing taller nests in areas with greater water level fluctuations. Nests in coastal marshes are vulnerable to flooding, and parents may be constrained in their ability to build quickly enough to keep pace with rapidly rising water. King rail parents continue building throughout the nesting period, but this seems to contribute to maintenance of the structural integrity and to compensate for settling rather than

resulting in an increase in nest height. This suggests nest height may be fixed. It is possible that the level of experience of the parents may play a role in nest building, as parents may learn to construct nests more efficiently using better materials. There may also be a tradeoff between the benefits of having a taller nest to avoid inundation and the benefits of having a shorter nest to avoid detection by avian nest predators. I suggest future studies examine the influence of parental experience and nest predation on nest height and building effort.

Females in better condition did not nest in sites with a lower variation in water levels (lower risk of flooding) or with deeper water (possible lower risk of predation). Males may play a greater role in nest site selection, and the quality of nest sites may be related to male body condition. Nest predation is not influenced by water depth, but nest predators may be deterred by human activity, a question for future studies to answer. King rail nests could be less vulnerable to flooding in managed impoundments, and drawing down water levels earlier in the spring may enable managers to provide additional nesting habitat for king rails.

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APPENDIX A: IACUC APPROVAL LETTER



East Carolina University.

**Animal Care and
Use Committee**

212 Ed Warren Life
Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

December 17, 2013

Susan McRae, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. McRae:

Your Animal Use Protocol entitled, "Ecology and Genetics of the King Rail" (AUP #D253a) was reviewed by this institution's Animal Care and Use Committee on 12/17/13. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

A handwritten signature in dark ink, reading 'Dorcas O'Rourke'.

Dorcas O'Rourke, DVM
University Veterinarian

DO/jd

Enclosure

APPENDIX B: Examples of temperature and behavior profiles

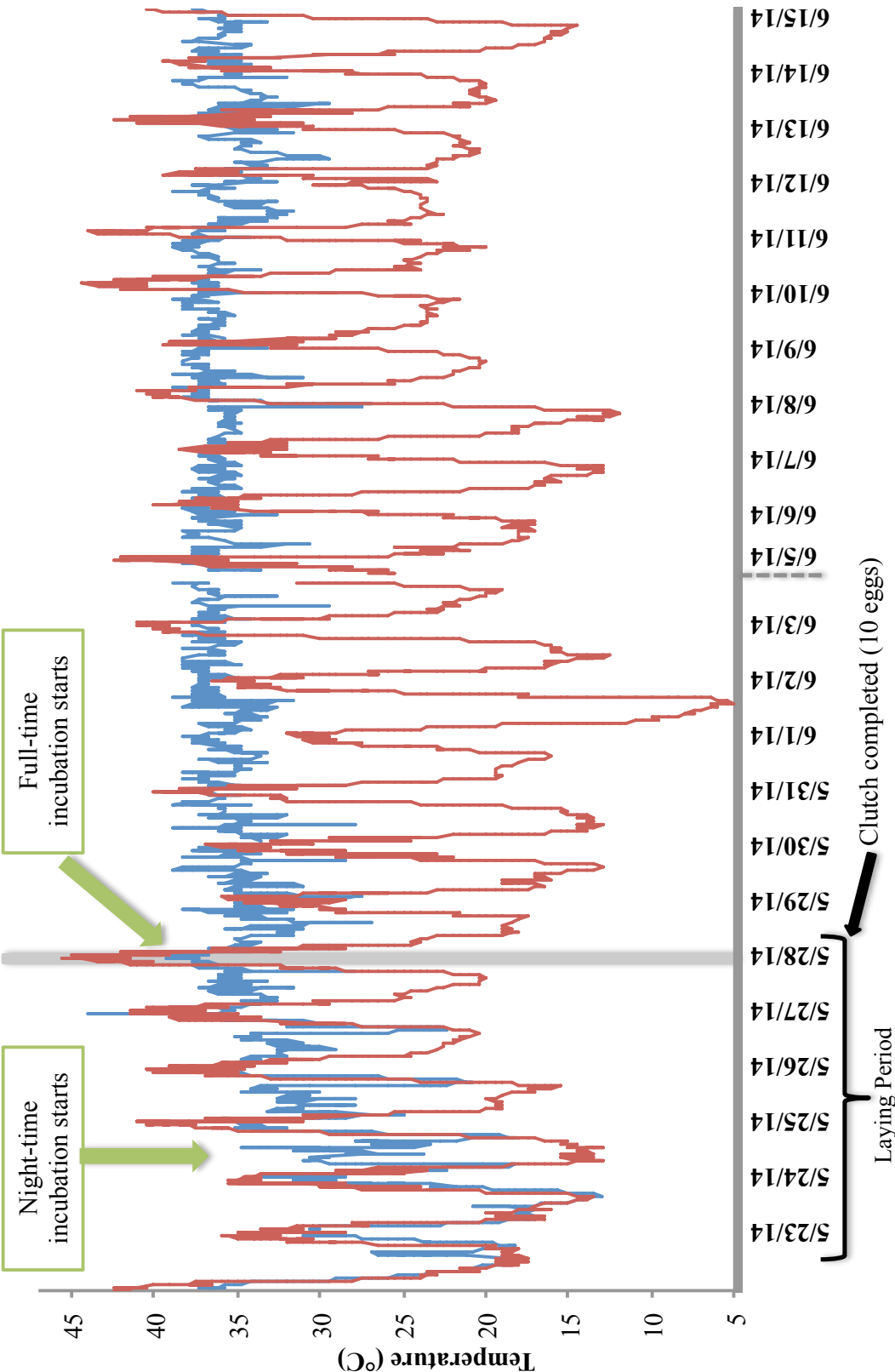


Figure B.1. Temperature profile of nest 14-17 from the laying period into the 2nd week of the incubation period. Clutch temperature (blue) and ambient temperature (red) can be compared to determine when the parents began incubating. The gray box denotes the time period of the behavioral profile in Fig. A.2.

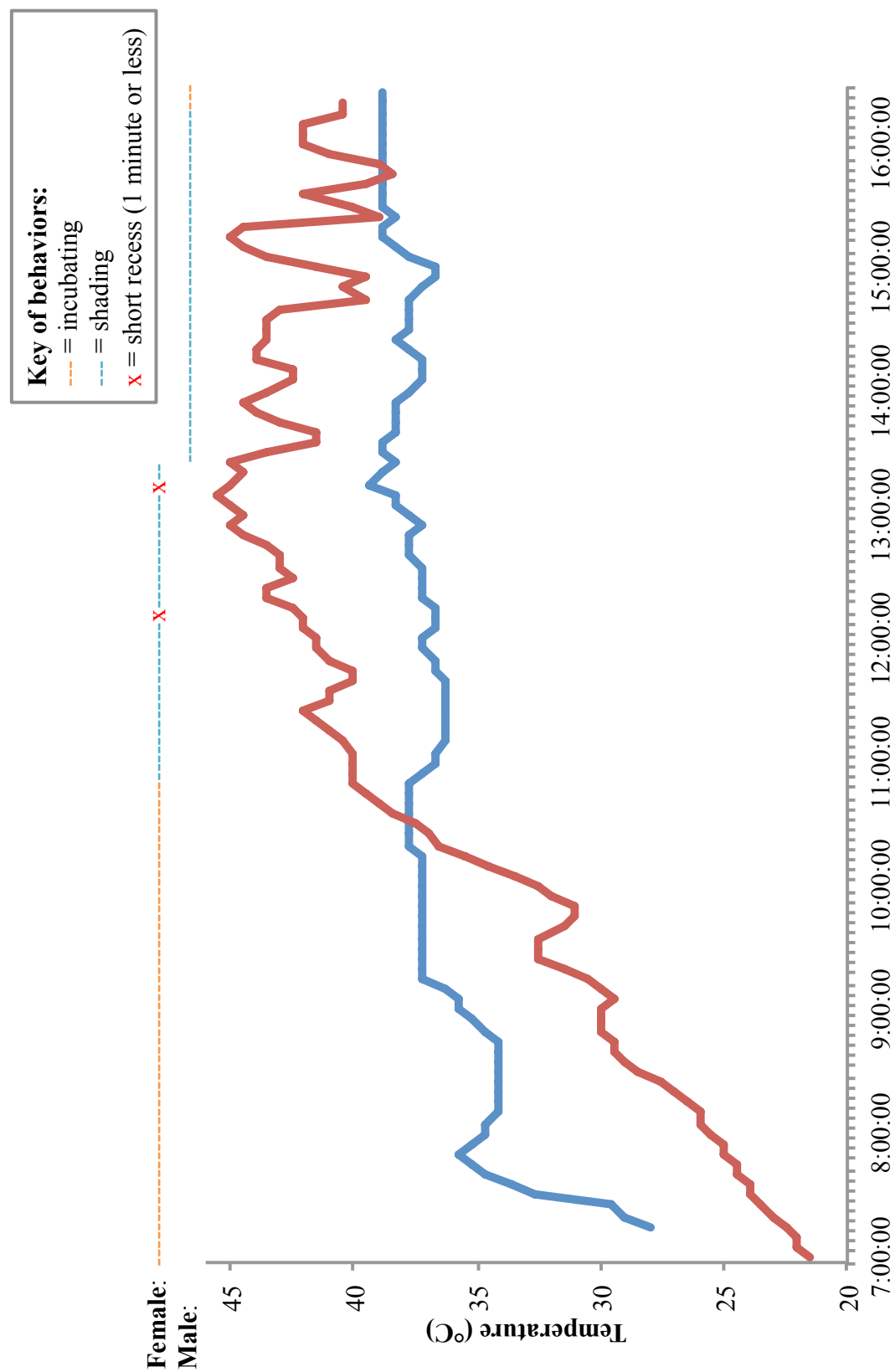


Figure B.2. Temperature and behavior profile for nest 14-17 on 28 May 2014, the first day of full-time incubation. The nest was filmed from 7:00-16:20. The top denotes the behavior of the attending adults, male or female, and the bottom shows the clutch temperatures (blue) and ambient temperatures (red) during this time period.