

Is the evolution of clutch size limited by incubation ability in shorebirds?



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Front page photo: ©Jessica Beatty, enlarged, five-egg clutch including two temperature logging eggs

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Abstract

Incubation is a crucial aspect of avian life history where differences in incubation techniques and investments can have long lasting effects on offspring and parental well-being and reproductive success. The factors limiting why some birds, such as shorebirds, have fixed clutch sizes has intrigued life history theorist to propose different hypotheses about the evolution of clutch size. Lack's "incubation limitation hypothesis," suggesting that clutch size is limited by the amount of eggs a parent can successfully cover during incubation, laid the foundation for many studies regarding clutch size evolution.

The aim of this study was to investigate possible ways in which clutch size affects incubation temperature in shorebirds. To do so, I enlarged nests of Northern Lapwing *Vanellus vanellus* in Rogaland, Norway to five-egg clutches with control four-egg clutches of the same individual nest occurring for three-day periods per clutch size. Incubation temperatures were found to be significantly higher in enlarged clutches compared to natural-sized clutches indicating that incubation may be more efficient in enlarged clutches. However, this study was conducted over a small portion of the entire incubation period and I suspect that the high incubation temperatures in enlarged clutches found here, would not be maintained throughout the incubation period. I propose that costs to both parents and offspring associated with enlarged clutch size may outweigh potential reproductive benefits.

Introduction

Life history theory seeks to explain the evolution of different traits which influence fitness, such as lifespan, reproduction, clutch and brood size, developmental rate, and quality of offspring (Bennett & Owens 2002). A central notion of life history theory is that reproduction is costly, therefore there is a trade-off between current and future reproduction (Winkler & Walters 1983; Roff 1992; Bennett & Owens 2002). When reproductive factors such as the number of eggs laid and hatched, and young raised to independence has the potential to vary, natural selection acts through selective pressures to create optimal investment strategies (Bennett & Owens 2002). Although there is a long history of research focusing on avian life history strategies, many hypotheses are still debated among life history theorists (Bennett & Owens 2002).

One life history strategy that traditionally received much attention by evolutionary ecologists is the variation in avian clutch sizes. While there are both proximate (e.g. physiological mechanisms halting egg laying) and ultimate (e.g. selective pressures) level causes which could explain why birds lay a given number of eggs in a single nesting event, hypotheses of ultimate level causes continue to be of much research and debate in terms of clutch size evolution (Winkler & Walters 1983). Lack (1947) hypothesized that clutch size is limited by the number of chicks a parent can provide with food and raise to independence. However, it is commonly observed that the optimal clutch size is lower than the size that gives the highest number of recruits (Roff 1992; Vander Werf 1992; Sandercock 1997) referred to as Lack's clutch size (Sandercock 1997). The reason for this is likely that the optimal clutch size results from a trade-off between the number of offspring in the current reproductive event and the future survival and fecundity of parents (Roff 1992).

A characteristic of avian reproduction is the need to keep the egg's temperature within the suitable range required for embryonic development. This is typically achieved through incubation where the parent bird transfers heat to the embryo (White & Kinney 1974, Deeming 2002). With the exception of a few bird species, most incubating parents develop a special area of skin devoid of feathers and well-vascularized, referred to as the brood patch (Deeming 2002). This patch is used specifically to increase the heat of eggs during incubation and is believed to be used by birds to monitor egg temperature (Deeming 2002). This type of incubation, referred to as contact incubation, results in temperature gradients throughout the egg, therefore parents typically move and turn eggs in order to more equally distribute heat (Deeming 2002). In species where both sexes incubate, both the males and females will develop this patch of skin, although size and quantity of patches may vary and tends to be larger in the dominant incubator when there is a difference in nest attentiveness per sex (Wiebe & Bortolotti 1993).

In intermittent incubation egg temperatures are not constant throughout the incubation period, rather the eggs cool and rewarm after the incubating bird leaves and returns to the nest after foraging, guarding the nest, or performing other necessary behaviors (Deeming 2002). Compared to constant incubation, intermittent incubation is believed to compromise the embryonic development in exchange for the parents' welfare (Deeming 2002; Reid et al. 2002; Olson et al. 2006).

Birds are commonly grouped into altricial and precocial species. Altricial birds require more parental care than precocial ones and are born naked and unable to forage on their own after hatching. Precocial species are born with their eyes open, have a well-developed downy plumage, and are generally further developed than altricial birds. Hence, Lack's idea that clutch

size is determined by limits to post-hatching demands of parental care seems unlikely in precocial species where chicks are not fed by their parents (Sandercock 1997).

Historically, little focus has been placed on the role of incubation in clutch size theory, but more recent work has demonstrated that this important avian reproductive phase should not be neglected. Incubation is energetically costly and the costs increase with clutch size (Thomson et al. 1998). Consequently, incubating a large clutch may have a negative impact on the incubator's success in the subsequent chick rearing phase (Heaney & Monaghan 1995; Lengyel et al. 2009) or on future fitness (de Heij et al. 2006). Another possibility that could explain clutch size evolution is the "incubation limitation hypothesis" which proposes that clutch size is limited by the amount of eggs that can be efficiently covered by the incubating parent (Lack 1947, Arnold 1999).

Shorebirds are of special interest in this respect. Although the species in this bird group are behaviorally and ecologically diverse (Lengyel et al. 2009), shorebirds commonly lay a fixed four-egg clutch with little variation within populations (MacLean 1972; Winkler & Walters 1983). They also lay unusually large eggs in relation to female body size (Arnold 1999).

Andersson (1978) found that due to shorebirds' pyriform (pear-shaped) eggs, the most energetically advantageous and heat conservative clutch size is four eggs. Accordingly, adding another large egg would disrupt the ideal heat conservation of the four-egg configuration. Lack (1947) predicted that the ability to successfully incubate a clutch of eggs depends on the brood patch size relative to the area of eggs needing to be covered. Since shorebirds typically have two brood patches, each with the believed capacity to contact incubate two eggs, it has been proposed

that enlarged clutches in shorebirds fail due to the ability of the parent's brood patch to successfully cover more eggs during incubation (Sandercock 1997).

The role of incubation ability as a limiting factor in clutch size evolution has typically been tested by comparing the success of natural and experimentally enlarged clutches. In shorebirds, experimentally enlarged clutches have been shown to have longer incubation periods (Székely et al. 1994; Sandercock 1997), increased hatching asynchrony (Arnold 1999; Lengyel et al. 2009), decreased hatchability (Larsen et al. 2003), and reduced hatchling condition (Larsen et al. 2003). Importantly, all these findings indirectly indicate that incubation is less efficient and eggs are exposed to generally lower temperatures in enlarged clutches. However, to my knowledge only the study by Yogeve et al. (1996) has directly tested the key prediction that egg temperatures are reduced in larger shorebird clutches. Moreover, contrary to what has been traditionally hypothesized and previously found in shorebirds, Reid et al. (2000) found that enlarged clutches showed increased incubation temperatures in starlings *Sturnus vulgaris* which led to higher water loss rates and reduced hatchability in eggs.

In this study, I experimentally manipulated clutch sizes in a Palearctic shorebird, the Northern Lapwing *Vanellus vanellus*, and explored the effects that clutch size may have on egg temperatures. I envision two possible ways that temperature may vary with clutch size. First, if more eggs lead to increased heat retention within the clutch (Reid et al. 2000), temperatures would be higher, temperature differences between eggs in the same clutch would be lower, and cooling rates would be slower in enlarged clutches. Alternatively, if adding an additional egg disrupts the normal heat storing configuration (Andersson 1978), then egg temperatures should be lower and both between egg differences in temperature and cooling rates should be higher in

enlarged clutches. Reid et al. (2000) suspected that the increased variation and greater within-nest temperature differences found in enlarged clutches was due to the inability of the incubating parent to cover all eggs equally at once. As this also follows the belief that enlarged clutches will disrupt the ideal clutch configuration, I expect higher variation in egg temperature in the enlarged clutches in both hypotheses. I control for ambient temperature in my analyses, as previous studies have shown that it affects egg temperatures (Biebach 1986; Nord et al. 2010). Finally, I looked at the effects of clutch size on parental behavior, assuming that enlarging a clutch leads to increased parental stress (Thomson et al. 1998), while minimizing individual behavior differences by using the same nests as both natural and enlarged clutch sizes.

Methods

Study Species

The Northern Lapwing *Vanellus vanellus* is a medium-sized shorebird widely distributed in the Palearctic region (Cramp & Simmons 1983). In Europe, lapwings typically nest in open farmlands and breed in the early spring. In Norway, the agricultural areas of Jæren in Rogaland are particularly important breeding grounds for lapwings. However, both in Norway and many other European countries, the species has declined considerably in the last 15 years potentially due to agriculture intensification during lapwing nesting and fledging periods (Petersen 2009; Byrkjedal et al. 2012).

Males can be differentiated from females as having longer crests and more pronounced plumage contrast on the face and chest (Cramps & Simmons 1983), and individuals may be identified by variable plumage drawings (Byrkjedal et al. 1997). During the breeding season males defend territories where one to four females may nest simultaneously and 30-50% of males are polygynous (Berg 1993; Byrkjedal et al. 1997; Parish et al. 1997). Northern Lapwings typically lay four eggs, sometimes three eggs and more rarely two or five eggs (Cramp & Simmons 1983; Grønstøl et al. 2006). The species has been experimentally shown to be able to successfully incubate five eggs (Larsen et al. 2003), therefore making an ideal study species to look at enlarged clutch sizes in shorebirds. The incubation period lasts on average 28 days (Cramp & Simmons 1983; Shrubbs 2007; Petersen 2009). The main role of males is copulation and guarding of territory although both sexes incubate and tend the precocial young (Cramp & Simmons 1983). Steady incubation begins after the clutch is complete (Cramp & Simmons 1983). During

daylight hours male incubation effort varies considerably among individuals and is lower than that of females (27% vs. about 50-64%, respectively; Liker & Székely 1999; Lislevand & Byrkjedal 2004; Jongbloed et al. 2006). At night males only incubate sporadically (Lislevand & Thomas 2006; Jongbloed et al. 2006).

Study Site

The study was conducted at Støldmyra in Orre, Rogaland (58°42'27.5"N 5°33'46.3"E) in the spring of 2014, lasting from 11 April until 7 May. The study area now has one of the highest densities of breeding lapwings in the Jæren area (Mjølvsnes 2014; Figure 1).

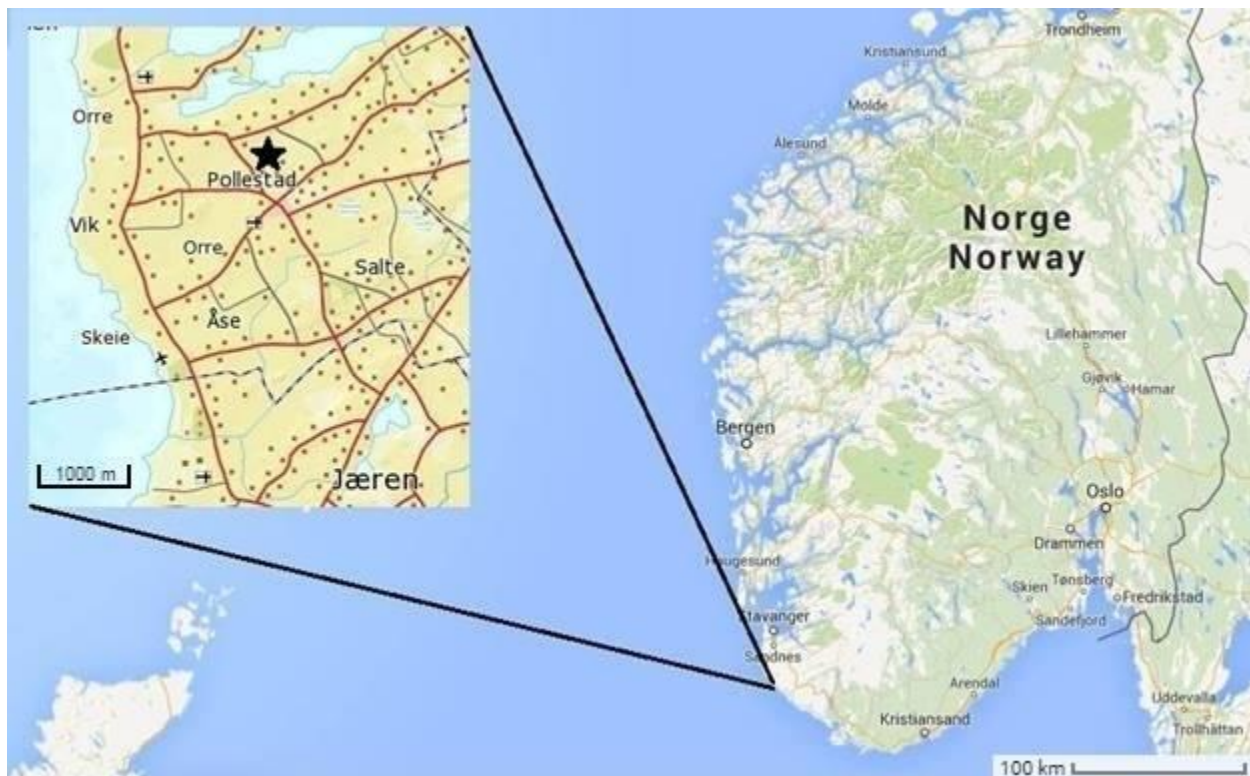


Figure 1. Map of field site, Støldmyra, marked by a star (created with maps from <http://www.norgeskart.no/>)

The study field was cultivated grassland with some scattered wet areas. Each summer grasses are typically harvested for the first time in June, no later than the 20th each year to be used as fodder for cattle (Elizabeth Erga pers. comm. 9 May 2014). It is estimated that the eggs were first laid in the week prior to the start of the study. The first eggs hatched on the field on 20 April 2014; this hatching was the first to be identified on the Norwegian bird database for the year (www.artsobservasjoner.no).

Field Procedures

Nests were found by scanning the field for incubating and nest scraping lapwings using binoculars and a spotting scope. The twenty six nests were then marked at a distance of approximately one meter in a standard direction, with a thin wooden post. New nests were marked and added to the map throughout the study as needed to fulfill experimental requirements (see below). Nest marking co-occurred on days with experimental nest manipulation in order to reduce disturbance. I estimated incubation stages by using the egg floatation method as described by Van Päässen et al. (1984), in which angle and height above the water surface at which the egg floats is used to estimate the stage of embryonic development. I used the information about developmental stages to determine an order in which the nests should be used in the experiment to reduce the risk that eggs would hatch before the temperature recording was completed. All the nests used for experimental and host nest purposes were completed, four-egg clutches prior to nest manipulations.

Each of the experimental nests (n=10) was observed for six consecutive days during the 26 days that the study lasted. Each nest acted as its own control clutch (four eggs) for three of the six days of observations and as an experimentally enlarged clutch (five eggs) for the other three

days. In order to reduce possible effects of experimental design, a coin was flipped to determine the starting clutch size. With this randomization, the first experimental nest started as a four-egg clutch and the order alternated thereafter to secure a balanced sample of each clutch size.

Egg temperatures ($^{\circ}\text{C}$) were recorded every minute for the six day experimental period using fake eggs, hereafter referred to as logger eggs, which contained a temperature sensor connected to a data logger (Tinytag Talk2, Gemini Data Loggers Ltd., UK) via a 50 cm long flexible cable. The fake eggs were made from Fimo modeling clay (Staedtler Mars GmbH and Co., Germany) which has a similar heat capacity to real eggs (Reid et al. 2000). Fake eggs were painted with acrylic paints to resemble real lapwing eggs. A hole was drilled into the center of the thickest part of the egg along the longitudinal axis, and the temperature sensor was inserted and glued into place. The lead cable ran from the sensor inside the egg to the recording device that was sealed in two plastic bags in order to exclude moisture. Two logger eggs were placed into each nest, separated by a real egg (see Appendix II for examples of nest configurations). I let the cable run through the floor of the nest cup, and both the recording device and the cable were buried next to the nest. The cable was slackened to allow some egg movement within the nest, but not enough to allow it to be repositioned within or outside of the clutch. Eggs removed from experimental nests to give place for the artificial logger eggs were placed in neighboring nests to continue incubation, hereafter referred to as host nests. For each of the ten experimental nests, the logger eggs and cameras (described below) were removed after the six day period, and the eggs that were incubated in host nests were returned to their natal nests except for an occasion when two eggs were cracked and one case when the host nest was abandoned and the egg was left cold and no longer viable. Of the ten experimental nests, all birds accepted clutch manipulations without abandoning their nests.

I recorded ambient temperature (°C) once every minute using a separate temperature logger (MSR Mini Data Logger MSR145WTH, MSR Electronics GmbH, CH) placed on the northern side of the field. This measurement was recorded approximately 30 cm above the ground, shaded by a hedgerow at the end of the field. This logger remained on the same site for the entire 26 day period that the study lasted.

To monitor incubation behavior of the parent birds, the experimental nests had a wildlife camera (Dovrefjell Basic IR 5MP) setup next to the nest marking post throughout each six day period (see Appendix II for examples of wildlife camera photos taken during the study). The cameras were set to take three photos every five minutes for the extent of each experimental six day period. Unfortunately, due to technical difficulties, most of the expected photos were not captured. Full photo data sets were only available for two nests although there were some gaps where there was an error with lighting and photos were blank; the remaining nests had partial photo sets. Therefore the analyses that were supposed to be performed such as investigating differences in incubation temperatures and nest attentiveness between sexes were not possible.

Field Observations

The nests were observed daily from a car parked along the perimeter of the field to ensure that an individual bird was present at the nests each day. This observation typically occurred between 9:00 and 14:00 as weather permitted, unless the nest was planned to be manipulated in the evening, in that case, no observation was made earlier in the day. Unnatural disturbances, such as human interactions including farming activities and my nest manipulations, were noted to potentially account for extended off-nest periods when the lapwings would leave the nests unattended, a period hereafter referred to as a recess.

Data Analysis

Data was analyzed using the software R, version 3.0.2 (<http://www.rproject.org/>). As nest visits may have temporarily affected the birds' incubating behavior, a preset amount of data was excluded from data analysis; typically at the beginning of recorded data on the first day and an hour surrounding the time of manipulation on the third day. This amount was adjusted if necessary to include more time when I was at the field for longer periods of nest manipulation or nest marking.

In order to test for effects of clutch size on egg temperatures during incubation, I focused on nocturnal incubation bouts when incubation is most constant (i.e. without recesses) and similar between nests. Periods of constant nocturnal incubation were determined by looking at stable plateaus on the temperature logger graphs during overnight hours (between sunrise and sunset) for each of the six days when egg temperature was monitored in each nest (Figure 2)

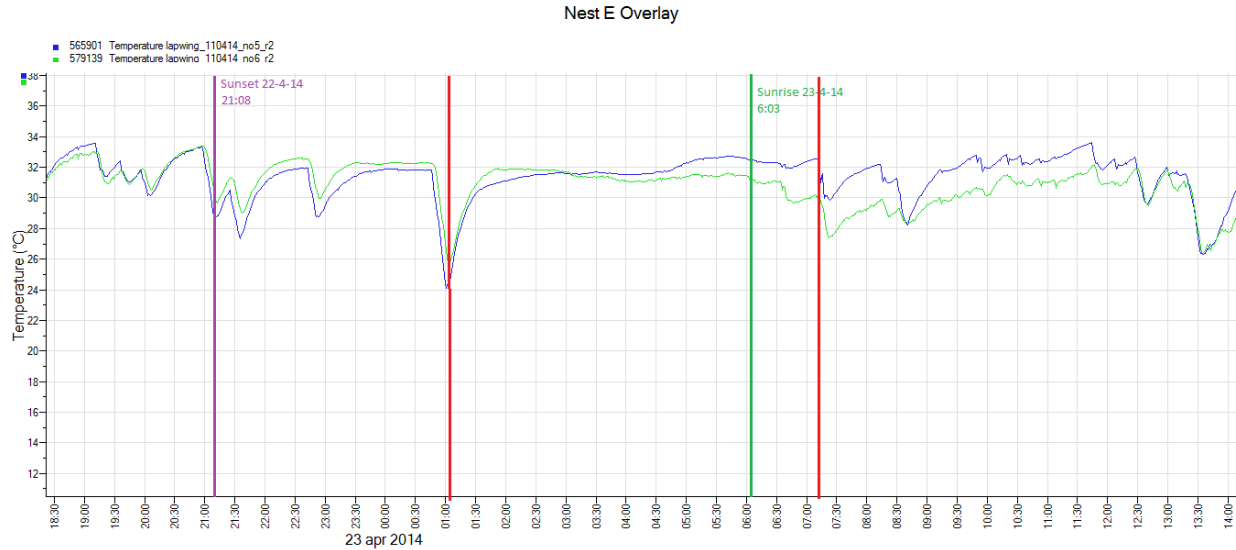


Figure 2. Example of constant nocturnal incubation period taken from the Tinytag Explorer plots. The red lines denote the period taken into consideration as constant incubation. This period represents where the raw data would be taken before subtracting an hour at the beginning and a half hour at the end. Sunset and sunrise data were taken into consideration when looking for the overnight period.

These plateaus were assumed to be periods of time where there was one individual constantly incubating the clutch without recesses or periods where two individuals changed so quickly that the temperature did not drop. The constant incubation period started when the temperature started increasing after the last recess before the long period of constant incubation began and ended when there was a significant drop in the temperature that was easily visible on the temperature logging graph (Tinytag Explorer). If there were recesses in the period of typical constant incubation (between sunset and sunrise) then the longest incubation bout was chosen for analysis.

To ensure that the data that was used for analysis of constant nocturnal incubation did not include the warming period where the eggs were increasing in temperature before reaching constant incubation, an hour of data was removed from the beginning of the identified incubation bout. This amount was determined by looking at the amount of time it appeared to take to reach

constant incubation on the logger graphs. To exclude possible recesses where temperature decreased after the incubating individual left the nest, a half hour of data was excluded from the end of the nocturnal incubation bout. Three nests did not fit these requirements possibly due to nocturnal disturbance, resulting in seven nests with constant incubation data, each with data from six nocturnal incubation periods.

To test for differences in egg cooling rates of logger eggs during recesses, I determined periods when the birds had left the nest by calculating the change in temperature between the current minute and the previous minute (just as was done with the temperature difference data) and by looking for a preset amount of decrease. These recesses were found by closely looking at the temperature logger graphs (Figure 3) for easily seen temperature drops and then confirmed as a recess if the temperature decreased at a rate of at least 0.1°C for four consecutive minutes. The end of the recess was noted as when the temperature increased by at least 0.05°C .

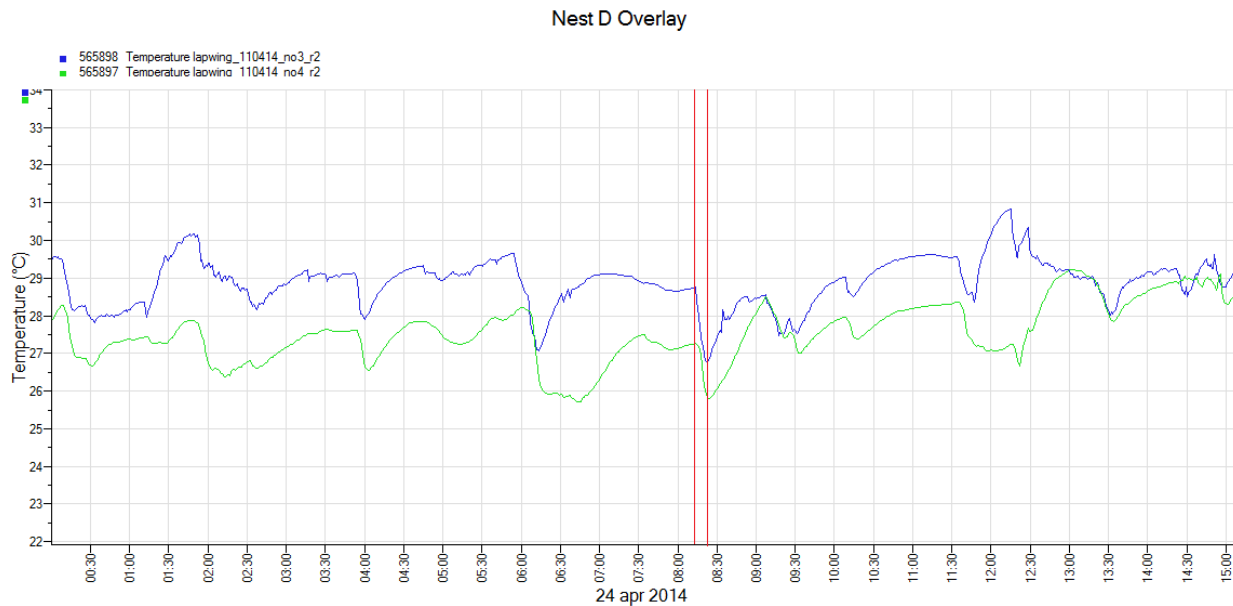


Figure 3. Example of recess period used to calculate cooling rate taken from the Tinytag Explorer plots. The red lines denote the period taken into consideration as a recess. The rate was calculated from the temperatures between the two red lines.

I extracted data for as many recesses as possible per clutch size per nest, with most nests having about 20 recess periods. There were a few nests that had less recesses identified because some recesses that followed the present decrease/increase requirement only occurred in one logger. If the cameras had worked as planned, it may have been possible to determine what was going on at the nest during the time where one egg experienced a recess.

Egg cooling rates follow Newton's Law of Cooling (Reid et al. 2002) which is expressed as follows:

$$k = \frac{\ln\left(\frac{(T_0 - T_a)}{(T_t - T_a)}\right)}{t}$$

Where k = cooling rate, T_0 = initial temperature (start) of recess period, T_t = final temperature at the end of recess, T_a = ambient temperature (average over the specific cooling period), t = total time of recess period, i.e. the time when eggs were exposed to the ambient temperature.

The cooling periods used in calculating the cooling rate did not significantly differ in length of time between clutch sizes (mean duration: 4 egg=14.28 minutes, 5 eggs=14.08 minutes; Welch two sample t-test: $t=0.2108$, $df=171.857$, $p=0.8333$)

The absolute values were taken of the temperature differences between eggs, as difference was independent of individual loggers. The temperature was registered to five decimal places. Of the 87,324 temperature recordings, 13 calculated to zero difference. This is however, not possible as there must be some amount of difference smaller than able to be recognized by the loggers, therefore those temperature differences were adjusted to be 0.00005. The log was taken of the

absolute temperature differences in order to create a model that would only predict positive temperature differences.

Full photo data sets were only available for two nests although there were some gaps where there was an error with lighting and photos were blank; the remaining nests had only partial photo sets. The number of photos taken were counted and evaluated in terms of whether a lapwing was present and the sex was identified when possible; depending on lighting and angle of bird, it was sometimes impossible to determine the sex. Nest attentiveness for each sex per nest and clutch size was calculated as a proportion of photos where the bird was present out of total photos for that clutch size and nest. If an individual was incubating during one of the three photos taken at the five minute mark, it was tallied as one count. The count data was then compiled into a table but due to the lack of full data sets, statistical tests were not possible. When possible, comparisons were made between clutch sizes within each sex and between the sexes at each clutch size.

I used linear mixed effects models (lme, nlme package in R) to test for effects of clutch size on temperature differences between the two fake eggs, egg temperatures and variance during periods of stable incubation, and egg cooling rates during recesses. I chose to use linear mixed effect models because I had clustered observations (by nests) with several replicates. When the effect of different nests is included in a model, it is always added as random effect. To account for possible effects of times when the nest was visited for clutch size manipulation, a categorical factor “disturbance” which represented disturbed (zero) and undisturbed (one) periods was included in the model. This factor did not include other disturbances such as farming activities, due to lack of complete records of these occurrences.

The average, minimum, and maximum egg temperatures were calculated for each period of constant nocturnal incubation for each nest. To determine the spread of data during constant incubation, I calculated the variance in egg temperatures for both loggers each night. The average of the variance for both loggers within each nest was calculated and used as the dependent variable in the analyses. To account for possible effects of different experimental days on these four dependent variables, I included the night number as a factor in these models. Moreover, I included the average ambient temperature as an independent variable, calculated from the recorded ambient temperatures during each constant incubation bout.

In order to account for the time series in which the data occurred and the relationship of each temperature to the previous temperature, the previous minute's temperature difference was included as an independent variable in the model on temperature differences between the two fake eggs. To account for the effects that ambient temperature might have on incubation temperatures, I added ambient temperature both as a linear function and as a quadratic function to the model. The quadratic function makes it possible to account for a non-linear relationship that the ambient temperature might exhibit.

Models to test within-nest temperature difference between the two logger eggs, were built using the dependent variable temperature difference and the independent variable last temperature difference and then by adding in factors and interaction terms to test for significance. To find the best fitting model for all analyses, the AIC (Aikaike Information Criterion) values were compared. Since the AIC values were very close in size when testing within-nest temperature difference, the AIC weights (Wagenmakers & Farrell 2004) were calculated for each model and used to determine the best fitting, most representative model. The significance level was set to

alpha=0.05 for all analyses and tests are two-tailed. R scripts for all analyses in this study are given in the Appendix III.

Results

Temperature Differences within Nests

There was significantly less temperature difference between the two logger eggs of the five-egg clutch compared to the four-egg clutch ($\beta = -0.01729$, $SE = 0.0009$, $p = 0.0141$; Table 1). Models were built by adding ambient temperature as a linear and a quadratic factor, disturbance, clutch size, and interaction terms between each factor (see Appendix III for R script). The simplest model, referred to as lme3, without interaction terms was found to have the lowest AIC value and a significantly higher AIC weight when compared to the other four models (Appendix Table A3.1). Therefore, model lme3 was used for analysis of differences in egg temperatures within the nests.

Table 1. Effects of clutch size on within-nest temperature difference from linear mixed effects model lme3. The dependent variable (temperature difference) is included in the intercept, with factors of last temperature difference (last.temp.diff) to account for the previous temperature influencing the current temperature, ambient temperature as a linear and quadratic function (Amb.temp; I(Amb.temp²)), and absence of disturbance (Disturbance1). Nest identity is included as a random effect in the model.

Factor	Value	S E	D F	t	p
Intercept	-0.01506	0.003956	87309	-3.8067	0.0001
last.temp.diff	0.961734	0.000926	87309	1038.42	<0.0001
Amb.temp	0.001373	0.000248	87309	5.5268	<0.0001
Clutch.size5	-0.00223	0.00091	87309	-2.4541	0.0141
Disturbance1	0.014518	0.003079	87309	4.716	<0.0001
I(Amb.temp ²)	-6.2E-05	1.16E-05	87309	-5.3276	<0.0001

A response curve of predicted temperature differences from model lme3 was plotted against ambient temperature (Figure 4). This curve shows predictions based on the model (lme3) for the temperature difference between loggers when the nest is not disturbed for each clutch size. The difference between the two clutch sizes in terms of temperature difference appears to decrease at

extreme ambient temperatures, but there was no significant interaction with temperature difference and ambient temperature in the model. The relationship is curved showing that there is more within-nest temperature difference during the ambient temperature range observed in the field. As the ambient temperature reaches the further temperature extremes, the difference in temperature between the two logger eggs within the same nest decreases; represented here by the curved relationship. The magnitude of difference in the four-egg clutch is consistently greater than the five-egg clutch.

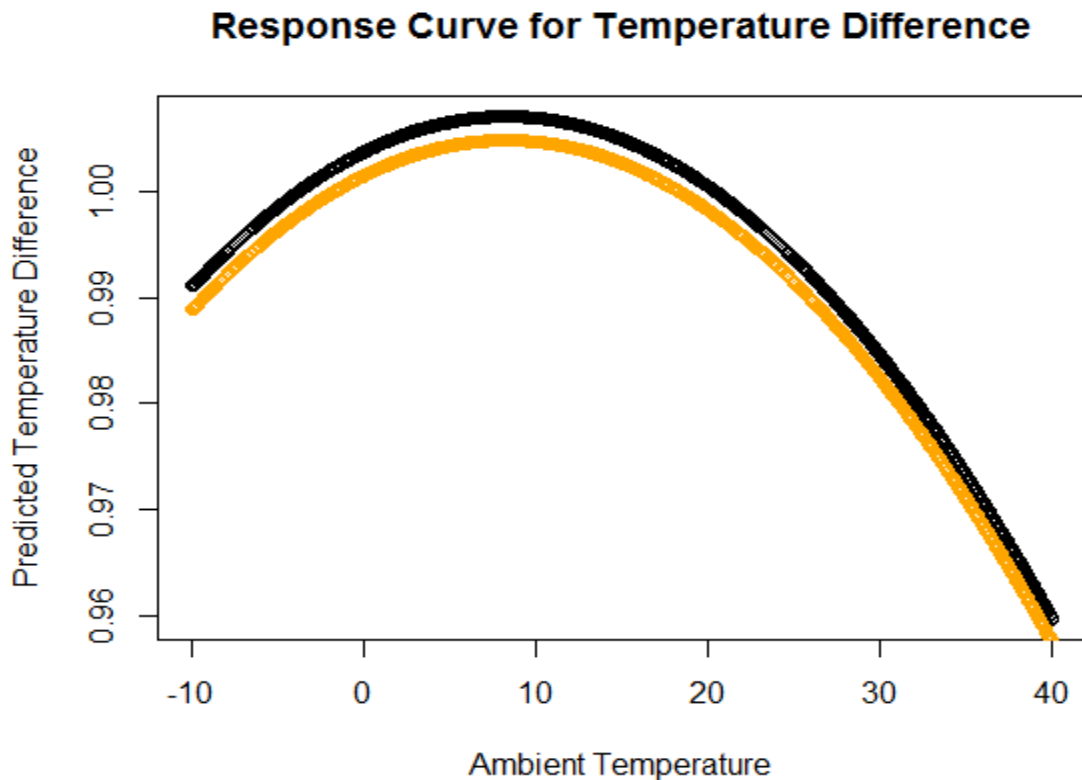


Figure 4. Predicted temperature difference between loggers during undisturbed conditions compared to ambient temperature for four-egg (black) and five-egg (orange) clutches. The ambient temperature range observed in the field was between -5.9°C and 25.4°C . The plot shows interpolation for a larger temperature range of -10°C to 40°C in order to predict for within-nest temperature differences at more extreme temperatures than observed in the field.

Cooling Rates

I identified a total of 386 recess periods, including both loggers within each nest and both clutch sizes. There was no significant difference in cooling rates between four- and five-egg clutches ($\beta=0.0120$, $SE=0.0009$, $p=0.1658$). There was also no significant difference in the cooling rates of the two logger eggs within the same nest ($\beta=0.0125$, $SE=0.0009$, $p=0.0602$). Therefore, the cooling rate observed when the incubating bird leaves the nest, is independent of clutch size and does not vary between eggs within the same nest.

Egg Temperature in Relation to Clutch Size

During constant nocturnal incubation ($n=42$ constant incubation periods, 21 for each clutch size) minimum temperatures per nest was significantly higher in five-egg clutches than in four-egg clutches ($\beta=25.3459$, $SE=0.5462$, $p=0.0002$; Figure 5). The second and third nights had significantly higher minimum temperatures than the first night for both clutch sizes ($\beta=25.0175$, $SE=0.6666$, $p=0.0072$; $\beta=25.4149$, $SE=0.6685$, $p=0.0013$, respectively). There was no significant difference in the minimum temperature between the second and third nights.

Minimum Temperature at Constant Incubation

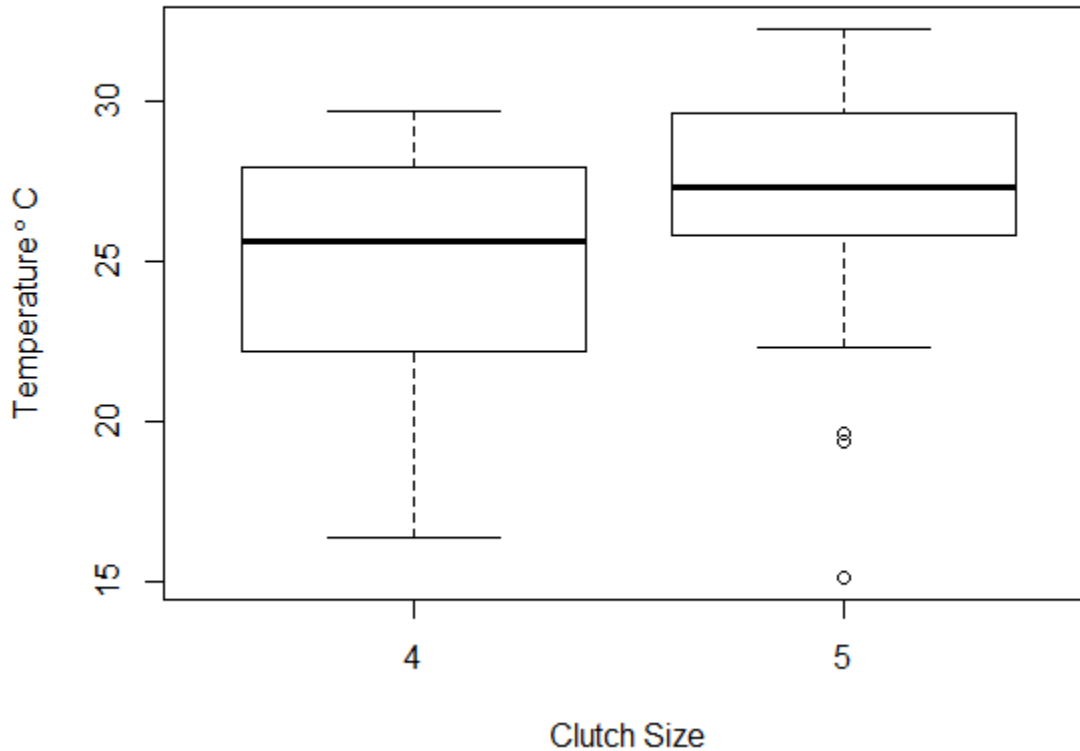


Figure 5. Box plot of minimum egg temperatures during constant nocturnal incubation for lapwing clutches with four- and five-eggs. Data for all nests is grouped together for this plot as opposed to the in the model where nest identity was treated as a random effect.

Also the maximum temperature during constant nocturnal incubation was found to be significantly higher in five-egg clutches than in four-egg clutches ($\beta=27.4860$, $SE=0.442$, $p=0.0021$; Figure 6). Following the same pattern as minimum temperature, the second and third nights had higher maximum temperatures than the first night ($\beta=27.184$, $SE=0.5403$, $p=0.0442$; $\beta=27.877$, $SE=0.54178$, $p=0.0014$, respectively) and there was no significant difference between the second and third night's maximum temperature.

Maximum Temperature at Constant Incubation

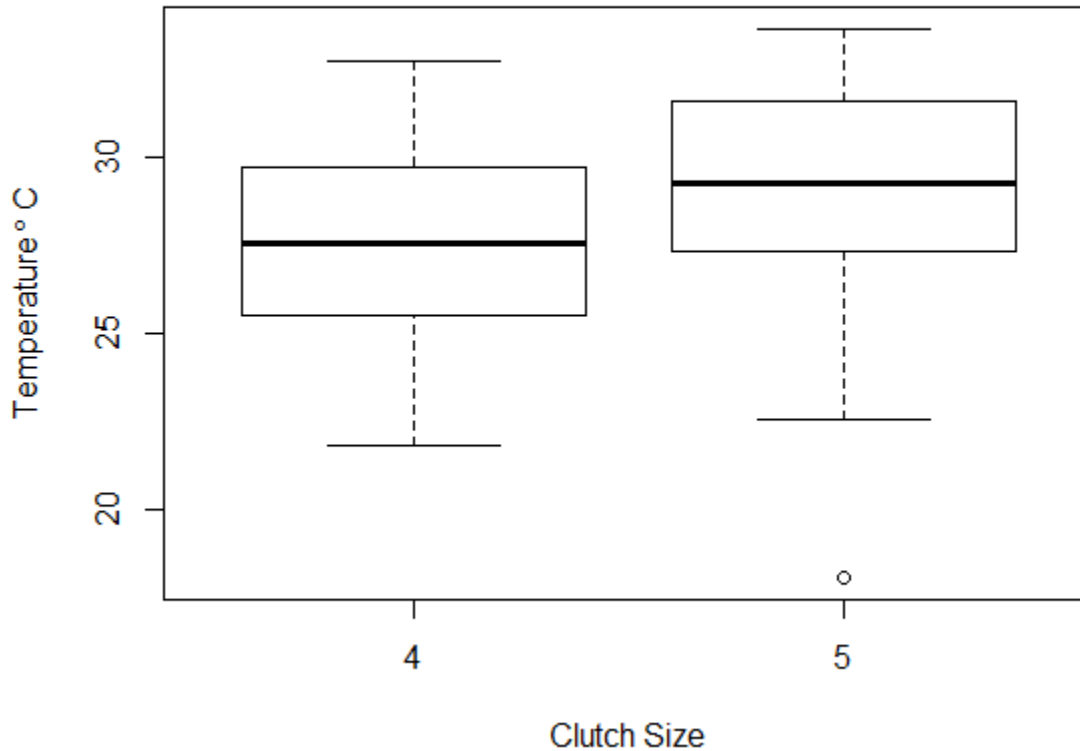


Figure 6. Box plot of maximum temperatures in loggers during constant nocturnal incubation for both clutch sizes. Data for all nests is grouped together for this plot as opposed to the in the model which has nest identity as a random effect.

The overall temperature range during constant nocturnal incubation was about 24.5°C to 27.5°C in four-egg clutches, and about 27°C to 29°C in five-egg clutches. The mean egg temperature recorded in four-egg clutches was about 26°C, which was significantly lower than in in a five-egg clutch (28°C; $\beta=28.028791$, $SE=0.496207$, $p=0.0003$; Figure 7).

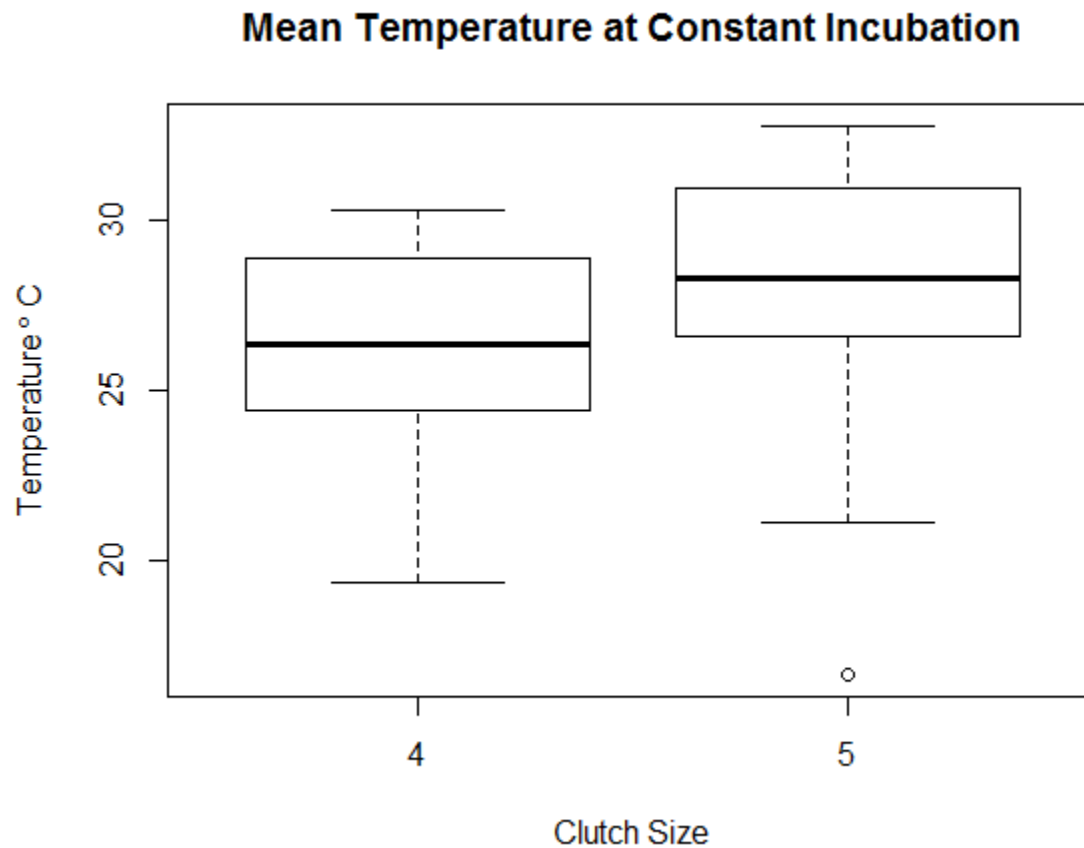


Figure 7. Box plot of mean temperatures during constant nocturnal incubation for each clutch size. Data for all nests is grouped together for this plot as opposed to the in the model which has nest identity as a random effect.

Variance in Egg Temperatures in Relation to Clutch Size

There was a significantly higher variance in egg temperatures in five-egg clutches compared with four-egg clutches ($\beta=0.20642247$, $SE=0.011490974$, $p=0.0008$, Table 2). In the third night there was significantly higher variance compared to the first and second nights, but no significant difference was found in variance between the first and second nights.

Table 2. Statistical outputs from a linear mixed effects model testing temperature variance for both clutch sizes at constant overnight incubation including re-leveling for different nights.

The dependent variable (logged average variance) is included in the intercept, with factors for each night (Night1, Night2, Night3) and average ambient temperature (Ave.amb.temp) throughout the overnight incubation bout for each night. Nest identity is included as a random effect in the model.

Factor	Value	SE	DF	t	p
Intercept	0.1638423	0.013928567	31	11.76304	<0.0001
CS5	0.04258017	0.011490974	31	3.705532	0.0008
Night2	0.00972333	0.00972333	31	0.693111	0.4934
Night3	0.04592243	0.01406287	31	3.265509	0.0027
Ave.amb.temp	0.03225163	0.001667483	31	19.3415	<0.0001
Intercept	0.17356564	0.014132045	31	12.28171	<0.0001
CS5	0.04258017	0.011490974	31	3.705532	0.0008
Night1	-0.0097233	0.014028533	31	-0.69311	0.4934
Night3	0.03619909	0.014095022	31	2.568218	0.0153
Ave.amb.temp	0.03225163	0.001667483	31	19.3415	<0.0001

Nest Attentiveness

Due to a small data set (five nests with varying amounts of photos and not all a complete sets of photos for both clutch sizes) it was not possible to test statistically for differences in nest attentiveness related to clutch size. It was also not possible to use the photos to match specific times that a male was incubating with the temperature data to compare the efficiency of male versus female incubation with the different clutch sizes. However, with the photos that were captured, it can be seen that females spend more time incubating than males in all nests for both clutch sizes (Table 3). There is also a trend of increased incubation in females on the five-egg clutches in all four of the nests that had photos for both clutch sizes. Although there is increased male attentiveness in nest B from four- to five-egg clutches, male attentiveness was lower in five-egg clutches in nest N. With only two nests having a representative photos set for males, it is not possible to determine if this the higher proportion of male attentiveness in nest B

represents an overall trend. Nest C only had 28 photos captured because the camera only captured photos at random times during the study. At nest C, only the female was identified, with some photos showing an individual present, but it was not possible to tell which sex due to poor lighting.

Table 3. Nest attentiveness calculated for each sex as the percentage of all photos captured for each nest and each clutch size. NA shows that no photos were available due to technical difficulties. Proportion of counts for each sex are taken out of the total counts for that clutch size per nest; F4= proportion of counts with a female present during a 4 egg clutch, F5= proportion of counts with a female present during a 5 egg clutch, M4= proportion of counts with a male present during a 4 egg clutch, M5= proportion of counts with a male present during a 4 egg clutch, TNA4= total proportion of counts with an individual present including both sexes and unidentified sexes during a 4 egg clutch, TNA5= total proportion of counts with an individual present including both sexes and unidentified sexes during a 5 egg clutch, TC4= total amount of photos captured during a 4 egg clutch, TC5= total amount of photos captured during a 5 egg clutch. Actual counts of photos for each proportion are written in parentheses.

Nest	F4	F5	M4	M5	TNA4	TNA5	TC4	TC5
A	0.74 (648)	NA	0.02 (16)	NA	0.88 (779)	NA	881	NA
B	0.59 (632)	0.73 (423)	0.04 (28)	0.25 (145)	0.87 (696)	0.99 (575)	800	580
C	0.07 (1)	0.14 (2)	NA	NA	0.14 (2)	0.21 (3)	14	14
K	0.45 (47)	0.56 (451)	NA	0.03 (24)	0.50 (53)	0.84 (677)	105	804
N	0.14 (79)	0.52 (404)	0.08 (44)	0.05 (40)	0.58 (329)	0.81 (625)	569	772

Discussion

By experimentally enlarging normal four-egg clutches of Northern Lapwings by one egg, I found that clutch size influences egg temperatures during incubation in several ways. As far as I know, only the study by Yogeve et al. (1996) has directly tested this key prediction of Lack's (1947) "incubation limitation hypothesis" in a shorebird species. The findings in the current study may therefore have further implications for our understanding of the factors that lead to the evolution of a fixed clutch size of four eggs in shorebirds.

Egg Temperatures during Incubation

The current study showed both higher temperatures and less within-nest differences in egg temperatures of enlarged clutches compared to natural-sized clutches. Both findings agree with the hypothesis that larger clutches retain heat better (Reid et al. 2000). However, the result does not support the "incubation limitation hypothesis" (Lack 1947; Arnold 1999), or the hypothesis that a four-egg clutch is optimal in terms of heat conservation due to the pyriform shape of shorebird eggs (Andersson 1978). Many shorebird studies have indirectly suggested that enlarged clutches have longer incubation periods than natural-sized clutches (Székely et al. 1994; Yogeve et al. 1996; Sandercock 1997; Arnold 1999; Wallander & Andersson 2002; Larsen et al. 2003) which concur with the only previous study where egg temperatures have actually been measured in enlarged clutches (Yogeve et al. 1996).

I would predict that the increased egg temperatures in enlarged clutches as demonstrated in this study should result in shorter incubation periods than in normal four-egg clutches. However, this is opposite to the findings by Larsen et al. (2003) who reported prolonged incubation periods in

five-egg clutches of the same species. A possible reason for this discrepancy could be differences in experimental design between the two studies. Since Larsen et al. (2003) studied nests where clutch sizes were manipulated for the extent of the whole incubation period and I only looked at egg temperatures during a three-day period, it is possible that the overall abilities of parents to keep the eggs warm differed between studies. Adding an extra egg to the lapwing nests may make the parents work harder to keep the eggs warm, by for instance sitting more tightly on the clutch or increasing blood flow to the brood patch. This could result in an initial rise in egg temperature as observed in my study. Yet, as the costs of incubation are higher in large clutches (Thomson et al. 1998; Deeming 2002; Boulton & Cassey 2012) the parents may not be able to keep up this high effort for more than a few days after which incubation conditions deteriorate. If this is the case, the final result might be prolonged incubation periods even if egg temperatures are kept relatively high in the beginning of the incubation period.

One result of the current study which may indicate that incubation ability was reduced by an extra egg in the clutch, was the increased variance in egg temperatures in five-egg clutches. The variance was highest in the third night of the experimental treatment. In many birds, embryonic development benefits most from constant stable temperatures (White & Kinney 1974; Olson et al. 2006; Deeming 2002) therefore the more fluctuations, as seen in five-egg clutches in this study, may affect the development of the chick during incubation and possibly lead to the longer incubation periods observed in many shorebird studies (Székely et al. 1994; Yogeve et al. 1996; Sandercock 1997; Arnold 1999; Wallander & Andersson 2002; Larsen et al. 2003). If energetic demands in parents increase from maintaining higher incubation temperature it may be hard for the parents to keep up continuously high incubation temperatures. The result could be greater temperature variance within the clutch. This suggests that egg temperature may be high in the

enlarged clutch for a short period, but it may not be possible for the parents to maintain over the entire incubation period.

There is a narrow range of temperature in which incubation should occur for ideal embryonic development (Deeming 2002; Durant et al. 2010). Slight differences of less than 1°C in average temperature during incubation has been shown to significantly impact growth, stress (Durant et al. 2010), and locomotor performance in ducklings (Hopkins et al. 2011). In the current study, I found an average temperature difference of 2°C between clutch sizes, a magnitude large enough to produce differences in chick development between clutches of different size if this temperature difference remains throughout the incubation period. High incubation temperatures may have additional effects accompanying change in the incubation period and parental well-being.

Reid et al. (2000) suggested that the higher egg temperatures found in enlarged clutches resulted in a higher water loss rate in eggs thereby reducing egg mass. Since egg mass is positively correlated to chick mass (Ricklefs 1984), reduced egg mass in enlarged clutches was suggested to result in reduced hatchability (Reid et al. 2000). Therefore it is possible that the lower chick body mass found in the enlarged clutches of Larsen et al. (2003) resulted from increased water loss causing dehydration due to higher incubation temperatures. However, as described above, it is possible that the high egg temperatures in enlarged clutches is only a temporary effect and that temperatures are actually lower than natural-sized clutches when viewed over the full incubation period due to incubation stress in parents. Reduced incubation temperatures may have a wide array of effects on the developing bird embryos (Olson et al. 2006; Durant et al. 2010; Hopkins

et al. 2011), probably also accounting for reduced body condition in lapwing hatchlings from five-egg clutches (Larsen et al. 2003).

Cooling Rates

Contrary to what I predicted, cooling rates of eggs during recesses were not related to clutch size in the current study. Based on results in previous studies where clutch sizes were altered I envisioned two possibilities, either 1) that eggs cooled more slowly due to heat retention in larger clutches (Reid et al. 2002) or 2) that eggs cooled more quickly due to altered clutch configuration and larger parts of the eggs being directly exposed to the colder ambient air temperatures (Andersson 1978). While no significant effects were found, I suspect that there may be some aspect of the physical egg configuration within the nest that affected the enlarged clutch making the cooling rate similar to the four-egg clutch, but specific nest configurations were not investigated in the current study. Since I found no effects on egg cooling rates between clutch sizes, the high incubation ability resulting in higher egg temperatures in enlarged clutches is likely not a result of physical properties in the clutch of eggs but rather behavioral or physiological responses to the increased incubation demands in parent birds (Carter et al. 2014).

Nest Attentiveness

Though there was a small sample size due to technical problems with my cameras, the data indicated a trend towards increased nest attentiveness in females in the enlarged clutches. It is possible that increased attentiveness could create the higher temperatures observed in the enlarged clutches, but this cannot be directly confirmed as the data used in calculating the temperature ranges was taken from overnight incubation while the photos were taken throughout

the day and night. In addition, the nocturnal incubation bouts used in the analyses were not found to differ significantly in length between clutch sizes, which would be expected if there was a difference in nocturnal attentiveness.

Larsen et al. (2003) found that clutch size did not affect male nest attentiveness in lapwings, but such a relationship could have been masked by the high between-individual variation in male incubation behavior (Lislevand & Byrkjedal 2004). In contrast, I used the same nests for both clutch sizes to account for the problems of individual variation. Moreover, if there are sex differences in incubation efficiency in lapwings (e.g. due to sex differences in brood patch size (Deeming 2008; Wiebe & Bortolotti 1993)), and male incubation effort is altered in relation to clutch size, this may have influenced my results on incubation temperatures. More studies are needed to make this comparison in incubation abilities between sexes. Also, it is believed that male lapwings may incubate more towards the end of the incubation period (Cramp & Simmons 1983). Therefore it would be interesting to see if this held true for both clutch sizes. To do so, it would be advisable to observe the incubating birds throughout the entire incubation period to see if overall incubation changed in males.

Future Research

While overall the results of this study indicate that the incubation temperature is higher and more consistent within the nests of enlarged clutches, there are many possible costs that can lead to selection for a four-egg clutch. These costs may affect 1) the incubating parent in their current and future reproductive success, 2) the developing embryos or 3) both (see Table A.1 in Appendix for more alternative explanations and hypotheses about clutch size evolution).

Whereas Lack's "incubation limitation hypothesis" may account for some possible limitations of

clutch size during incubation, the revision of this hypothesis by Monaghan & Nager (1997) to include the amount of eggs the parent can successfully lay, incubate, and raise to independence may better encompass the total costs and benefits of different clutch sizes throughout the entire breeding period.

Since parental behavior plays a major role in temperature regulation during incubation (Boulton & Cassey 2012), there are many more aspects that can be researched further in future studies.

One interesting aspect of egg temperature to investigate would be to measure egg surface temperatures (Boulton & Cassey 2012). It is commonly believed that incubating parents use cues from the egg to sense temperature and know when they are able to leave the nest during intermittent incubation (Deeming 2002; Boulton & Cassey 2012). I suggest future research studying recess periods, cooling rates, and egg temperatures consider using egg surface temperatures as well as using logger eggs to get a better understanding of the cues parents might use during incubation in regards to temperature regulation. Future studies should also investigate rewarming rates after a recess period as enlarged clutches have previously been shown to rewarm slower than natural-sized clutches (Reid et al. 2000).

Previous studies have shown that larger clutch sizes increase the energetic demands of the incubating parents in birds (Haftorn & Reinertsen 1985) and although there might be positive results initially there may be unforeseen later negative consequences (carry-over effects) in terms of parental and chick well-being and future fitness. For instance, Lengyel et al. (2009) found that incubating enlarged clutches caused increased strain on the parents who were then less able to defend a good quality territory. Future studies should consider monitoring temperature over the

entire incubation period, still incorporating each nest into being its own control for half the period, as well as study the post-incubation effects.

Conclusion

The results of this study did not support Lack's traditional "incubation limitation hypothesis" because incubation temperatures were higher and more consistent within experimentally enlarged clutches. However, previous studies found prolonged incubation periods in five-egg clutches of Northern Lapwings (Larsen et al. 2003), suggesting that eggs were exposed to reduced incubation temperatures. My "snapshot" of data from a restricted part of the incubation period might not have been long enough to observe possible energetic stress in parents incubating on enlarged clutches. I therefore suggest that future studies address how egg temperatures vary in different clutch sizes over longer parts of the incubation period.

Furthermore, regardless of the "incubation limitation hypothesis" it is interesting that Northern Lapwings were able to maintain higher egg temperatures in larger clutches. The ways in which lapwings are able to up-regulate their incubation efficiency like this is unclear though, and needs further studies.

My results may appear to lean towards enlarged clutches being optimal by having higher incubation temperature which should lead to faster embryonic development (Deeming 2002).

Interestingly, lapwings incubating five eggs are also previously found to have increased productivity (on average one egg more hatched in enlarged nests in a study by Larsen et al. 2003). However, there may be other significant costs of incubating five eggs which have not yet been addressed. First, enlarged clutches may lead to increased energetic demands for incubating parents which lead to reduced parental abilities or increased mortality in the future (Deeming 2002; Boulton & Cassey 2012). Second, since chicks in five-egg clutches hatch in reduced body

condition (Larsen et al. 2003) it is possible that they survive less well than chicks from normal four-egg clutches.

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Appendix I

Table A1.1. Other proposed hypotheses to explain clutch size limitations

Note: “Supported” means results are more consistent with this hypothesis, does not directly disprove the hypothesis, or provides support for hypothesis as a potentially stronger limitation than other hypothesis. This does not mean the hypothesis has been proven true.

“Rejected” means results show little to no support for hypothesis or shows very strong support for other hypotheses. This does not mean this hypothesis has been proven false.

“Inconclusive” means specific results do not necessary support or reject the hypothesis and/or result may be tied to other factors

Hypothesis – clutch size limitation due to:	Prediction/Outcome	Species	Support or reject hypothesis	Reference
Egg Formation/Production	Extra eggs or successive clutches of lower quality	Sandpipers	Supported	(Sandercock 1997)
	Constrained by food	Kentish Plover	Rejected	(Székely et al. 1994)
	Able to re-lay clutches	Kentish Plover; Spur-winged Plover	Rejected	(Székely et al. 1994; Yogeve et al. 1996)
Nest Predation	Increased laying time	Review of shorebird studies	Supported	(Walters 1984)
	Predation rates independent of clutch size	Kentish Plover; Redshank; Northern Lapwing	Rejected	(Székely et al. 1994; Wallander & Andersson 2002; Larsen et al. 2003)
Parental Care	Less protection in enlarged clutches/broods	Review of shorebird studies	Supported	(Safriel 1975; Walters 1984)
	Parents have lower quality territories during chick rearing after incubating enlarged clutches	Pied Avocet	Supported	(Lengyel et al. 2009)
	Higher chick mortality in enlarged clutches	Pied Avocet	Supported	(Lengyel et al. 2009)
	Parents able to raise enlarged broods	Review of shorebird studies	Rejected	(Winkler & Walters 1983)
Reduced parent/chick condition (often linked to incubation parental care)	Lower body mass of hatchlings in enlarged clutches	Northern Lapwing	Supported	(Larsen et al. 2003)

	Lower hatchability in enlarged clutches	Northern Lapwing	Supported	(Larsen et al. 2003)
	Lower hatching success in enlarged clutches	Spur-winged Plover; Sandpipers	Supported	(Yogev et al. 1996; Sandercock 1997)
	Reduced survival in enlarged broods	Review of shorebird studies	Supported	(Safrieli 1975)
	Hatchling body size independent of clutch size	Pied Avocet	Rejected	(Lengyel et al. 2009)
	Number of eggs hatched per nest and per egg independent of clutch size	Kentish Plover	Rejected	(Székely et al. 1994)
	Hatchability independent of clutch size	Redshank	Rejected	(Wallander & Andersson 2002)
	Hatching success higher in enlarged clutches	Redshank; Northern Lapwing; Pied Avocet	Rejected	(Wallander & Andersson 2002; Larsen et al. 2003; Lengyel et al. 2009)
	Hatching success independent of clutch size	Sandpipers	Rejected	(Sandercock 1997)
Incubation Ability - Lack's "incubation limitation hypothesis"	Brood patch size	Sandpipers	Supported	(Sandercock 1997)
	Longer incubation period in enlarged clutches	Kentish Plover, Spur-winged Plover; Sandpipers; Northern Lapwing	Supported	(Székely et al. 1994; Yogev et al. 1996; Sandercock 1997; Larsen et al. 2003)
	Increased hatching asynchrony in enlarged clutches	Review of shorebird studies; Pied Avocet	Supported	(Arnold 1999; Lengyel et al. 2009)
	Increased hatching success but poorer quality chicks	Northern Lapwing	Inconclusive	(Larsen et al. 2003)
	No difference in	Pied Avocet	Inconclusive	(Lengyel et al. 2009)

	incubation period length			
	Slower rate of weight loss in eggs of enlarged clutches	Kentish Plover	Inconclusive	(Székely et al. 1994)
	No difference in nest attentiveness	Kentish Plover; Sandpipers; Northern Lapwings	Inconclusive	(Székely et al. 1994; Sandercock 1997; Larsen et al. 2003)
	Weight change in parents not affected during incubation	Kentish Plover	Rejected	(Székely et al. 1994)
	Increased reproductive success	Redshank	Rejected	(Wallander & Andersson 2002)

Appendix II



Figure A2.1. A natural four-egg clutch, pre-experimental manipulation (left) a nest with two logger eggs along with two real lapwing eggs, making control four-egg clutch (top right), and a nest with two logger eggs and three real lapwing eggs, making an enlarged five-egg clutch (bottom right).



Figure A2.2. Photos from the wildlife cameras; described clockwise from the top left: Female incubating (Nest B), Male incubating (Nest B), Female returning after recess (Nest A), Nocturnal incubation by female (Nest B), Female returns after recess during nocturnal incubation (Nest K), Many other animals were seen on the study field including Curlews (seen here), Golden Plovers, Godwits, other birds, and hares (personal observation).



Figure A2.3. Adult female Northern Lapwing (left) and a lapwing chick from first hatched nest of the year (right) as seen through a spotting scope from the parked car in which observations for lapwing presence at nest sites were performed. The first chicks hatched April 22, 2013.

Appendix III

Table A3.1. R-Script for the five models testing temperature differences between logger eggs within the same nest (The model used for analysis, lme3, is in bold).

The linear mixed effects models were built from dependent variable of temperature difference (Temp.diff) and independent variable last temperature difference (last.temp.diff) and then adding possible factors and interaction terms: ambient temperature as a linear and quadratic term (Amb.temp; I(Amb.temp²), respectively), Clutch size (Clutch.size), Disturbance, and nest was added as a random factor in all models.

Model	Script	AIC Weight
1	<code>lme1<-lme(Temp.diff~last.temp.diff+Amb.temp+Amb.temp*Clutch.size+I(Amb.temp^2)*Clutch.size+Clutch.size+Disturbance+I(Amb.temp^2),random=~+1 Nest,data=myData)</code>	8.07E-07
2	<code>lme2<-lme(Temp.diff~last.temp.diff+Amb.temp+Amb.temp*Clutch.size+I(Amb.temp^2)*Clutch.size+Clutch.size+Disturbance+I(Amb.temp^2)+Disturbance*Clutch.size,random=~+1 Nest,data=myData)</code>	5.44E-09
3	<code>lme3<-lme(Temp.diff~last.temp.diff+Amb.temp+Clutch.size+Disturbance+I(Amb.temp^2),random=~+1 Nest,data=myData)</code>	0.9999992
4	<code>lme4<-lme(Temp.diff~last.temp.diff+Amb.temp+Amb.temp*Clutch.size+I(Amb.temp^2)*Clutch.size+Clutch.size+I(Amb.temp^2),random=~+1 Nest,data=myData)</code>	3.07E-09
5	<code>lme5<-lme(Temp.diff~last.temp.diff+Amb.temp+Amb.temp*Clutch.size+I(Amb.temp^2)*Clutch.size+Clutch.size+Disturbance+I(Amb.temp^2)+Disturbance*Amb.temp+Disturbance*I(Amb.temp^2),random=~+1 Nest,data=myData)</code>	1.10E-11

Table A3.2. Statistical outputs for the five models from Table A.2 (above). Estimates(value) are presented so that a positive sign indicates a faster rate of egg surface cooling (steeper negative slope) and a negative sign indicated a slower rate of egg surface cooling (shallower negative slope).

Model	Factor	Value	SE	DF	t	p
1	Intercept	-0.01869	0.004113487	87307	-4.5436	<0.0001
	last.temp.diff	0.961472	0.000929537	87307	1034.356	<0.0001
	Amb.temp	0.002121	0.000339619	87307	6.2447	<0.0001
	Clutch.size5	0.005512	0.002533725	87307	2.1756	0.0296
	Disturbance1	0.01473	0.003079282	87307	4.7835	<0.0001
	I(Amb.temp ²)	-9.1E-05	1.547E-05	87307	-5.8856	<0.0001
	Amb.temp:Clutch.size5	-0.00164	0.000514046	87307	-3.1829	0.0015
	Clutch.size5:I(Amb.temp ²)	6.44E-05	0.000023755	87307	2.7095	0.0067
2	Intercept	-0.02055	0.00517258	87306	-3.9731	0.0001
	last.temp.diff	0.961464	0.000929635	87306	1034.239	<0.0001
	Amb.temp	0.002124	0.000339655	87306	6.2526	<0.0001
	Clutch.size5	0.009108	0.006567067	87306	1.3869	0.1655

	Disturbance1	0.016603	0.004409221	87306	3.7654	0.0002
	I(Amb.temp^2)	-9.1E-05	0.000015474	87306	-5.8845	<0.0001
	Amb.temp:Clutch.size5	-0.00164	0.000514047	87306	-3.1828	0.0015
	Clutch.size5:I(Amb.temp^2)	0.000064	0.000023762	87306	2.6944	0.0071
	Clutch.size5:Disturbance1	-0.00364	0.006130742	87306	-0.5935	0.5529
3	Intercept	-0.01506	0.0039561	87309	-3.8067	0.0001
	last.temp.diff	0.961734	0.000926151	87309	1038.42	<0.0001
	Amb.temp	0.001373	0.000248396	87309	5.5268	<0.0001
	Clutch.size5	-0.00223	0.000909746	87309	-2.4541	0.0141
	Disturbance1	0.014518	0.003078566	87309	4.716	<0.0001
	I(Amb.temp^2)	-6.2E-05	0.000011612	87309	-5.3276	<0.0001
4	Intercept	-0.004	0.002698414	87308	-1.466	0.1427
	last.temp.diff	0.96187	0.000925922	87308	1038.827	<0.0001
	Amb.temp	0.00208	0.000339539	87308	6.1225	<0.0001
	Clutch.size5	0.00528	0.002533473	87308	2.0851	0.0371
	I(Amb.temp^2)	-9E-05	1.54752E-05	87308	-5.8706	<0.0001
	Amb.temp:Clutch.size5	-0.0016	0.000513942	87308	-3.0723	0.0021
	Clutch.size5:I(Amb.temp^2)	6.2E-05	2.37498E-05	87308	2.5956	0.0094
5	Intercept	-0.0547	0.018714672	87305	-2.9227	0.0035
	last.temp.diff	0.96125	0.000931977	87305	1031.404	<0.0001
	Amb.temp	0.00685	0.003334472	87305	2.0539	0.04
	Clutch.size5	0.005638	0.002534388	87305	2.2246	0.0261
	Disturbance1	0.050805	0.018557468	87305	2.7377	0.0062
	I(Amb.temp^2)	-0.00021	0.000126288	87305	-1.6531	0.0983
	Amb.temp:Clutch.size5	-0.00164	0.00051413	87305	-3.1969	0.0014
	Clutch.size5:I(Amb.temp^2)	0.000064	0.00002376	87305	2.6916	0.0071
	Amb.temp:Disturbance1	-0.0047	0.003333628	87305	-1.409	0.1588
	I(Amb.temp^2):Disturbance1	0.000115	0.000126402	87305	0.9085	0.3636

Table A3. 3. Linear mixed effects model testing average variance in temperatures during constant nocturnal incubation and statistical outputs including re-leveling for nights where CS= clutch size, Night= night number, log.ave.vari= logged average variance, Ave.amb.temp= average ambient temperature during each selected nocturnal incubation bout.

Model: avevari.lme<-lme(log.ave.vari~CS+Night+Ave.amb.temp,random=~+1|Nest,data=varilog2.df)

Factor	Value.	SE	DF	t	p
Intercept	0.163842	0.013928567	31	11.76304	<0.0001
CS5	0.04258	0.011490974	31	3.705532	0.0008
Night2	0.009723	0.00972333	31	0.693111	0.4934
Night3	0.045922	0.01406287	31	3.265509	0.0027

Ave.amb.temp	0.032252	0.001667483	31	19.3415	<0.0001
Intercept	0.173566	0.014132045	31	12.28171	<0.0001
CS5	0.04258	0.011490974	31	3.705532	0.0008
Night1	-0.00972	0.014028533	31	-0.69311	0.4934
Night3	0.036199	0.014095022	31	2.568218	0.0153
Ave.amb.temp	0.032252	0.001667483	31	19.3415	<0.0001

Table A3.4. R-Script for cooling rates linear mixed effect model; where K= cooling rate, Clutch.size.cool= clutch size, Logger= logger egg (as both loggers were used in analysis), nest was added as a random effect.

Script

```
coolio1.lme<-lme(K~Clutch.size.cool,random=~+1|Nest,data=Cool3.df)
coolio2.lme<-lme(K~Clutch.size.cool+Logger,random=~+1|Nest,data=Cool3.df)
```

Table A3.5. Statistical outputs for cooling rate linear mixed effect models, Clutch.size.cool5 represents the cooling rate for five-egg clutches and the intercept includes the cooling rate for the natural-sized four-egg clutch.

Factor	Value	SE	DF	t	p	AIC
Intercept	0.013257	0.001152	375	11.5075	<0.0001	-2487
Clutch.size.cool5	-0.00129	0.000927	375	-1.38857	0.1658	
Intercept	0.014125	0.00124	374	11.3885	<0.0001	-2476.5
Clutch.size.cool5	-0.00129	0.000924	374	-1.39287	0.1645	
Logger2	-0.00174	0.000922	374	-1.88484	0.0602	

Temperature Difference Residuals per Nest

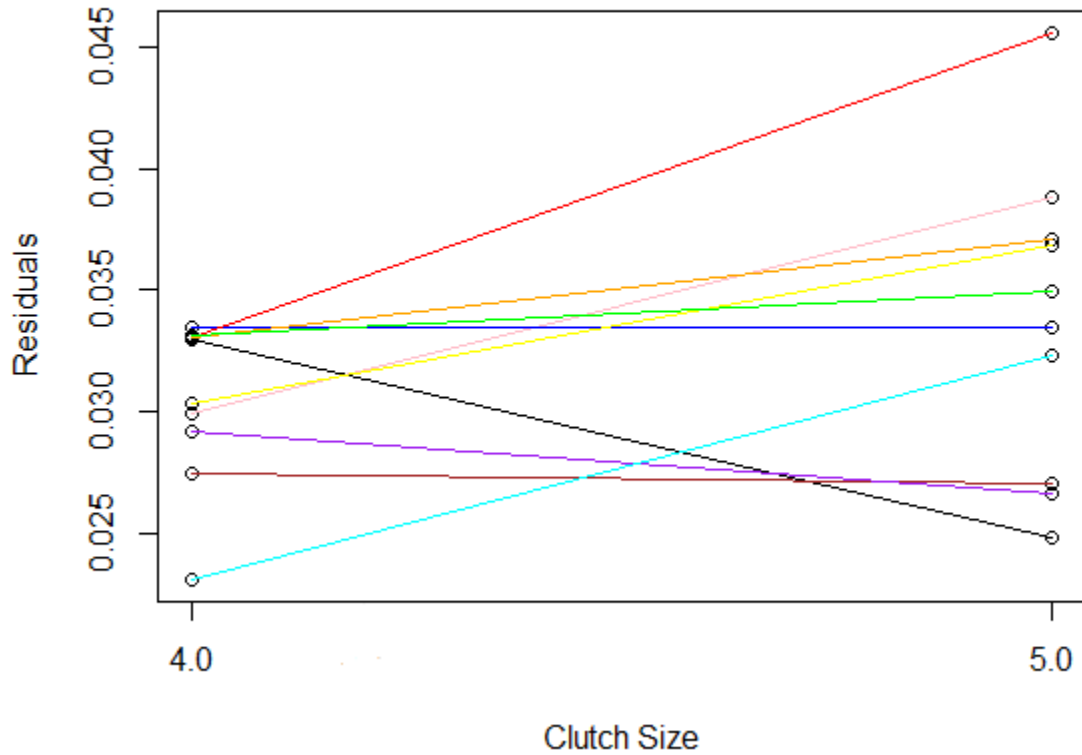


Figure A3.1. Temperature difference residuals (difference between the predicted values from the model and the observed data points) for all nests. By plotting the residuals from model lme3 against clutch size, I found that the model can be used to predict temperature difference equally as well for both clutch sizes. Lines are drawn between the residual for each clutch size for each nest. Since there is not a strong directional trend to higher or lower residuals for either clutch size, the model predicts equally as well for both four- and five-egg clutches.