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Behavioural responses of great tits to experimental manipulation of nest temperature during incubation

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In several bird species only one member of the pair incubates the eggs. This individual should trade-off the time between caring for the eggs and leaving the nest to forage or perform other self-maintenance activities. Thus, daytime incubation is intermittent, and the number of "sessions" and "recesses", and their length, impact egg temperatures and therefore embryo development. We evaluated female investment decisions, and their reproductive consequences, by experimentally heating or cooling great tit *Parus major* nests during the incubation period in a Mediterranean population. Heated females were less often encountered within the nest incubating, and their recesses tended to be longer. Even though mean nest temperatures were higher in heated than in control nests, heating did not affect breeding success, size or condition of the nestlings, or parental condition or behaviour when feeding the nestlings. On the other hand, temperatures in cooled nests were lower than those in control nests during the daytime (but not during the night). However, females seemed able to compensate for the treatment, since breeding success was similar in cooled and control nests, and neither nestlings nor parents showed negative effects of the cooling treatment.

1. Introduction

Avian eggs need to be kept within a narrow range of temperatures (36–38 ºC) (Drent 1975, Webb 1987) for embryos to develop adequately. Though many species build well insulated nests, eggs are frequently exposed to ambient temperatures outside this range. In these cases, the parents should attend the eggs to prevent chilling or overheating, therefore assuming concurrent time and energy costs (Carey 2002, Deeming 2002, Tinbergen & Williams 2002, Turner 2002). In some species (mostly passerines; Deeming 2002), females should be constantly deciding whether to invest in themselves, foraging or performing other self maintenance activities, or in their eggs, taking care to keep them within the narrow range of temperatures appropriate for embryo development (Tinbergen & Williams 2002, Turner 2002). Thus, daytime incubation is intermittent, with incubation bouts ("sessions") followed by foraging trips out of the nest ("recesses"). This represents a good scenario to investigate life history trade offs (Ardia *et al.* 2009, 2010).

A way to evaluate female investment decisions, and their consequences for parents (especially females) and embryos or nestlings, is to modify the incubation costs. An effective way to do this is to manipulate the thermal environment (Reid *et al*. 2002, Nilsson *et al*. 2008). A handful of field studies have either provided supplemental heat (Davis *et al*. 1984, Haftorn & Reinertsen 1990, Reid *et al*. 1999, Bryan & Bryant 1999, Reid *et al*. 2000, Cresswell *et al*. 2004, Magrath *et al*. 2005, Londoño *et al*. 2008, Pérez *et al*. 2008, Ardia *et al*. 2009) or cooling (Davis *et al*. 1984, Haftorn & Reinertsen 1990, Nilsson *et al*. 2008, Ardia *et al*. 2010) during incubation, and examined the consequences for parents, embryos, and/or nestlings.

In spite of the experimental nature of these studies, relatively similar manipulations resulted in opposite results. Thus, providing extra heat could increase (Reid *et al*. 1999, Cresswell *et al*. 2004, Ardia *et al*. 2009), decrease (Davis *et al*. 1984, Haftorn & Reinertsen 1990, Londoño *et al*. 2008), or have no effect (Magrath *et al.* 2005) on female attentiveness (percentage of time spent incubating). Similar variation could be found in the duration of incubation sessions and recesses. The effects on female condition also seem diverse, since Magrath *et al*. (2005) reported that heated starling *Sturnus vulgaris* females were lighter than control females after the heating treatment, Pérez *et al*. (2008) found that heated tree swallow *Tachycineta bicolor* females gained mass, and Cresswell *et al*. (2004) found no differences in mass or metabolic rate between heated and control pectoral sandpiper *Calidris melanotos* females. Concerning embryo and nestling development, the results are also contradictory. Reid *et al*. (2000) examined a large number of parameters, and only found that breeding success (fledglings produced per egg laid) was higher in heated nests, and that females whose first clutch was heated had better hatching success in their second clutches. In contrast, Londoño *et al*. (2008) found that the size of 10-days old northern mockingbird *Mimus polyglottos* embryos was smaller in heated than in control nests. Pérez *et al*. (2008) used a cross fostering design to show that heating tree swallow nests mostly benefits females, so they could raise their nestlings more effectively; a direct effect on embryos was only apparent during the first week after hatching, disappearing later on.

Data from cooled nests are even scarcer, and their results also differ. For example, Davis *et al*. (1984) and Haftorn and Reinertsen (1990) reported an increase in attentiveness during the incubation period in cooled nests, while Ardia *et al*. (2010) found a reduction, with different responses in the duration of sessions and recesses. There is at least some consensus in the two studies with respect to the effects of cooling on nestlings and parents (Nilsson *et al*. 2008, Ardia *et al*. 2010), with both finding negative consequences (e.g., reduced body condition of females, reduced nestling growth rates) even though the details varied between them.

The basic prediction of the above studies was that "heating is good" (as it provides extra energy), and "cooling is bad" (as it drains energy). However, this was not supported, and it was even contradicted, by some of them. Therefore, more experiments with different approaches and/or under different environmental conditions would help to shed light on this topic. In particular, applying the two treatments (heating and cooling) simultaneously, in the same population and year, would help to understand how females respond to temperature manipulation under the same environmental conditions. Also, the effect of cooling or heating would partly depend on the prevailing ambient temperatures so, for example, heating could be detrimental under high ambient temperatures.

We present here the first experimental study where both treatments (heating and cooling) were applied simultaneously in a bird population. Moreover, the study was performed in the Mediterranean region, where temperatures in early spring are generally milder than those prevailing in the regions where previous experiments were conducted. For this, we heated and cooled great tit *Parus major* nests during the incubation period, and investigated the effect on different fitness related factors, including parental behaviour and condition, length of the incubation period, and breeding performance.

We predicted that experimental cooling would increase incubation costs, while heating would decrease incubation costs, and females would allocate these extra costs or savings among themselves and/or their offspring (Bryan & Bryant 1999, Ardia *et al*. 2010).

2. Material and methods

2.1. Study area and species

The study was conducted on a great tit population breeding in nestboxes within an extensive orange *Citrus aurantium* monoculture in Sagunto (Valencia), Eastern Spain (39°42’N, 0°15’W, 30 m a.s.l.), in 2009. Wooden nestboxes (see characteristics in Lambrechts *et al*. 2010) have been available in this area since 1986 (Encabo *et al*. 2001, Álvarez & Barba 2014). Great tits are uniparental incubators in which the female only incubates for about 66% of the daytime in the studied population (Álvarez & Barba, in press). The average clutch size is about 8 eggs (Atiénzar *et al*. 2012), the incubation period lasts around 13 days (Álvarez & Barba 2014), and males feed their mates during this period. After hatching, both sexes take part in feeding of the young.

2.2. Experimental design

We visited all nestboxes at least once per week, and daily in some periods, from late February to the end of the breeding season by mid July. This pattern of visits allowed us to estimate the date of laying of the first egg (assuming the laying of one egg per day), the date of the start of incubation (eggs uncovered and warm or female seen incubating; Álvarez & Barba 2014), and clutch size.

When incubation started (incubation day 1), nests where randomly assigned to one of the following treatments: heated (N = 12), control (N = 12), or cooled (N = 11). Only first clutches were used for the experimental treatments. Sample sizes varied as nests were lost along the nesting period.

On incubation day 1, nests were prepared for the treatments. Eggs and nests were carefully taken out of the nestbox, an inverted U-shaped wire mesh was placed on the nestbox floor, and the nest was placed again on the mesh. This left a “box” below the nest where cool, heat, or control pads could be placed. There was about 1 cm between the pad and the bottom of the nest; this was especially convenient for the correct functioning of the heat pads, which need oxygen for chemical reactions. We used commercial warming pads (Uniheat 72 h) that, when exposed to the air, produced elevated temperatures for 3 days due to the oxidation of iron powder. Cool pads (9-cell Flexible Ice Blankets of 12 ml each) were stored at –20°C and taken into the field in a portable freezer with ice pads to keep them frozen. Control pads were cool pads at ambient temperature.

Control, cool, or heat pads were placed below the nests around 07:00 on incubation day 2. Cool pads were replaced four times a day (around 07:00, 11:00, 15:00 and 19:00). Heat pads were changed every second day. Control pads were not changed. Nevertheless, to apply the same degree of perturbation to all the nests, they were visited, and the pads were removed and replaced or changed if necessary four times a day. Pads and wire meshes were removed by 07:00 on incubation day 13. Thus, heated nests experienced a continuously elevated temperature, while cooled nests experienced bouts of cold temperature when cool pads were replaced, which slowly disappeared as the ice melted (see below).

When the nests were taken out to place the wire mesh, we measured the thickness of the bottom (digital caliper, 0.01 mm accuracy) (see Álvarez & Barba 2008), as this might affect heat or cool transfer from the pads to the eggs. Nests were collected after fledging or failure, placed into sealed plastic bags, and stored at –20°C. After the breeding season the nests were dried in an oven (105°C, 12 h) and weighed (digital balance, 0.01 g accuracy) (see Álvarez *et al*. 2013 for details), in order to report the dry nest masses.

Two females (1 heated, 1 control) deserted after placing the wire mesh, while 8 females (2 heated, 3 control, 3 cooled) abandoned the nest during the experiment (days 2–11).

2.3. Breeding performance

The pattern of visits allowed us to estimate the date of hatching of the first egg, the length of the incubation period (from the date of the start of incubation to the date previous of the hatching of the first egg inclusive), the number of eggs hatched and the number of fledglings. From these, we estimated hatching success (proportion of eggs which hatched), fledging success (proportion of nestlings which fledged), and breeding success (proportion of eggs producing fledglings). These percentages were arcsin square root transformed for analyses (Zar 1996).

We individually marked the nestlings on day 5 by painting different parts of the body with a permanent non toxic felt pen, remarked them on day 7, and ringed them with individually numbered aluminum rings on day 10, so that they could be individually recognized during their nesting period. Nestlings were weighed (electronic balance, ± 0.1 g) and tarsus length measured (digital caliper, ± 0.01 mm), and an index of body condition for each individual was calculated as the mass to tarsus length ratio when they were 5, 10, and 15 days old. We calculated tarsus (mm/day) and mass (g/day) growth rates between days 5 and 10, and days 10 and 15, as a measure of nestling growth during the two phases of the nestling period.

Parents were captured at the nest when nestlings were 10–11 days old using nest door traps. We measured their wing (stopped rule, ± 0.5 mm) and tarsus length (digital caliper, ± 0.01 mm), weighed them (electronic balance, ± 0.1 g), and determined their sex and age (first year versus older birds) using plumage characteristics and the presence of brood patch (Svensson 1996). The index of body condition was also estimated. Males and females were ringed with individually numbered aluminum rings and fitted with a passive integrated transponder (see below).

2.4. Nest temperature and incubation behaviour

We used HOBO Pro v2 data loggers to document nest and ambient temperatures. One of the probes was usually attached to the back of the nestbox or to a close branch, so that it was permanently in the shade, while the other was placed directly above the wire mesh, but below the nesting material (Dawson *et al*. 2005). Temperature readings were recorded every 2 min during the sampling period.

We had four data-loggers, and these were moved between nests. We divided the incubation period into 2 stages (early: days 2–5, and late: days 9–12), and samples were taken from each active nest at least one day within each period. The times when females were on or off the nest generally showed as clear peaks and troughs on the resulting temperature traces, allowing the times of arrival and departure to be deduced (Holcomb 1974, Reid *et al*. 1999). This allowed us to calculate the number and duration of the incubation and foraging bouts, the percentage of the daytime spent incubating, and the duration of the female's overnight rest. As an additional estimation of attentiveness, the presence or absence of the female on the nest was noted each of the four times per day that each nest was checked, thus amounting to over 40 observations per nest during the incubation period.

To summarize the temperatures of the nests, we averaged the internal temperatures of each nest during each of the incubation stages, taking into account a "daytime" period (from 08:00 to 17:59, where females were entering and leaving the nest) and a "night" period (from 22:00 to 04:59, where females were within the nestbox). The times were selected to avoid the approximate periods when females start and finish the overnight period (Álvarez & Barba, in press).

Temperatures from one random day were taken if more than one day was available from a certain incubation stage. We also computed the difference between the external and internal temperatures at each 2 min interval, and averaged them for each nest and incubation stage.

We also checked in the lab if the treatments effectively affected the temperature within the nest cup. For this, we used the same nestboxes, wire meshes, and cool, heat, and control pads, along with great tit nests collected from the studied population. We used 5 nests for each treatment, and recorded the temperature at the nest cup during 2 hours.

2.5. Parental feeding frequencies

We used PIT (Passive Integrated Transponders) to study feeding frequencies (Freitag *et al*. 2001, Nilsson *et al*. 2008) when the nestlings were 11–12 days old, and feeding rates are around their maximum (Barba *et al.* 2009). When captured, each parent was fitted with an implantable PIT tag (Trovan ID 100, length: 11.5 mm, mass: 0.1 g) with unique codes. PIT tags were injected subcutaneously in the back of the birds in the featherless area above the scapula, following Nicolaus *et al*. (2008). Parent entrances to and leavings from the nests were recorded by means of a transponder reader system consisting of an antenna fitted to a nestbox door and connected to a data logger (Trovan LID–650) and a 12 V battery mounted in a plastic box covered with camouflage fabric (Álvarez & Barba, in press). When a tagged bird enters or leaves the nestbox, it interrupts the optical barrier, triggering the reading of the bird's corresponding PIT and the visit is stored in the memory, along with the exact time at which it occurred. The nestboxes which were used were opened at the front, so the original door was removed and the one fitted with the antenna placed when setting the reader.

The box with the reader was placed at the base of the tree (nestboxes were at most 1 m above the ground). The installation of this device took about 2 minutes. The system was typically mounted by 08:00 h in the morning of the sampling day (one day after the parents were captured), and it was left operating until the next morning (about 08:00 h). We assumed that each visit corresponded to a feeding trip. For each nest, we estimated the feeding rates per hour of males, females, and the total, as well as the feeding rate per hour per nestling (by dividing the total feeding rate by the number of nestlings present).

2.6. Statistical analyses

As there were no significant differences in the initial conditions (laying date, clutch size, date of the start of incubation, nest dimensions, parental characteristics; see Results) between treatments, there was no need to correct for these factors when comparing breeding performance between the experimental groups.

We generally used univariate general linear models to check for differences in parameters of interest between treatments (treatment as fixed factor), though there were some exceptions. To test for differences in nest temperatures and differences between the internal and external temperatures, both the treatment and incubation stages were included as fixed factors.

Also, we used linear mixed models, with nest identity as a random factor and treatment as a fixed factor, to check for differences in nestling tarsus length, mass, condition, and growth between treatments. Finally, we used chi-squared tests for examining differences between the frequencies of parental age classes.

Data were analysed by using the SPSS v. 19.0 statistical package. Means ± SD are shown where appropriate.

3. Results

3.1. Pre experimental data

The first egg of the experimental clutches was laid between 30 March and 18 April and there was no difference in the mean laying dates between the treatments ($F_{2,32} = 0.08$, $P = 0.92$). The clutch size varied between 6 and 12 eggs, with no difference in the mean clutch size between the treatments ($F_{2,32} = 0.32$, $P = 0.73$). Finally, incubation in these nests started between 8 and 24 April, and there were no differences in the mean starting dates between the treatments ($F_{2,32} = 0.07$, $P = 0.93$).

Nest mass ($F_{2,28} = 0.05$, $P = 0.95$) and thickness of the bottom ($F_{2,32} = 0.02$, $P = 0.98$) did not differ among nests allocated to the different treatments.

Tarsus length (males: $F_{2,18} = 0.49$, $P = 0.62$; females: $F_{2,18} = 0.18$, $P = 0.84$), wing length (males: $F_{2,18} = 0.09$, $P = 0.92$; females: $F_{2,18} = 0.25$, $P = 0.78$), and age class (males: $\chi^2 = 0.92$, df = 2, $P > 0.05$; females: $\chi^2 = 0.34$, df = 2, $P > 0.05$) of the parents did not differ between the treatments.

3.2. Nest temperature

In the lab, temperatures were higher in the heated nests (29.11 ± 2.07°C), intermediate in control nests (22.25 ± 0.23°C), and lower in cooled nests (19.08 ± 1.04°C; ANOVA, $F_{2,12} = 73.02$, $P < 0.001$, all *post hoc* Tukey tests $P < 0.05$). Therefore, the treatments effectively changed the temperature within the nest cup in the absence of incubating females.

Nest temperatures during the day differed between the incubation stages ($F_{1,32} = 6.43$, $P = 0.016$), and among treatments ($F_{2,32} = 64.13$, $P < 0.001$); the interaction was not significant ($F_{2,32} = 1.99$, $P = 0.15$). *Post hoc* Tukey tests revealed that temperatures were higher in heated nests, intermediate in control nests, and lower in cooled nests, while they were higher during the late incubation

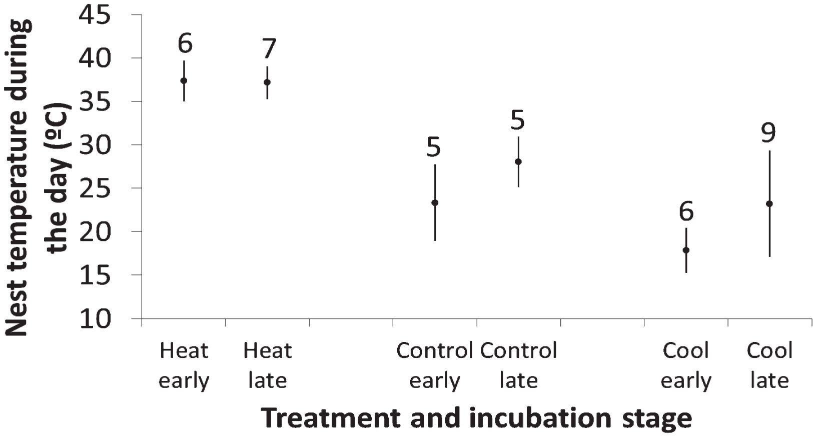

Fig.1. Mean nest temperatures (± SD) during the daytime (from 08:00 to 17:59) in heated, control and cooled nests during the early (days 2–5) and late (days 9–12) incubation stages. Sample sizes are shown above the bars.

stage (Fig. 1). The difference between the nest and ambient temperatures also differed between treatments ($F_{2,32}$ = 64.89, $P < 0.001$), being significantly higher (Tukey tests) in heated than in control and cooled nests, and significantly higher in control than in cooled nests. There were no differences between incubation stages ($F_{1,32}$ = 3.39, P = 0.08), and no interaction ($F_{2,32}$ = 2.42, P = 0.11).

During the night, nest temperatures differed between the treatments ($F_{2,31}$ = 19.64, $P < 0.001$) and incubation stages ($F_{1,31}$ = 11.17, P = 0.002); the interaction term was not significant ($F_{2,31}$ = 2.51, P = 0.10). Thus, nest temperatures in heated nests were higher than those in control and cooled nests, and higher during the late incubation stage. Also, the difference between the nest and ambient temperatures differed between the treatments ($F_{2,31}$ = 16.76, $P < 0.001$), and incubation stages ($F_{1,31}$ = 9.40, P = 0.004), with no interaction ($F_{2,31}$ = 2.58, P = 0.09). The difference in temperatures was higher in heated than in control or cooled nests (Tukey tests). Overall, it seemed that the treatment was effective in increasing nest temperatures in heated nests both during the day and during the night and in decreasing temperatures in cooled nests during the day. On the other hand, temperatures were higher late in the incubation period than in the early stage.

3.3. Breeding performance

We did not find significant differences between the treatments in the length of the incubation period, number of hatchlings or fledglings, and hatching, fledging or breeding success (Table 1). Tarsus length, mass, and growth rates of the nestlings were not affected by the treatments (Table 2).

3.4. Parental behaviour

Data from thermometers showed differences between the treatments in the mean duration of off-bouts ($F_{2,20}$ = 3.52, P = 0.049), with a mean duration of 20.9 ± 5.9 min (N = 9) in heated nests, 16.2

Table 1. Breeding parameters of heated, control and cooled nests during incubation. The values reported are Mean ± SD, with sample sizes in brackets. Differences between treatments were tested using one-way ANOVA (results reported are *F* and *P*).

	Heated	Control	Cooled	*F*	*P*
Incubation period (days)	12.44 ± 1.01 (9)	13.00 ± 1.16 (7)	12.29 ± 1.11 (7)	0.85	0.4
Number of hatchlings	5.75 ± 3.96 (12)	4.83 ± 4.32 (12)	4.82 ± 3.95 (11)	0.20	0.8
Number of fledglings	4.42 ± 3.90 (12)	3.64 ± 4.20 (11)	4.64 ± 3.91 (11)	0.19	0.8
Hatching success (%)	91 ± 19 (9)	95 ± 6 (7)	92 ± 14 (7)	0.01	1.0
Fledging success (%)	82 ± 26 (8)	100 ± 0 (5)	95 ± 9 (7)	2.82	0.09
Breeding success (%)	77 ± 30 (8)	95 ± 6 (5)	88 ± 17 (7)	0.93	0.4

Table 2. Mean ± SD (sample sizes in brackets) biometric parameters of nestlings on day 5, 10 and 15 experimentally assigned a heated, control or cooled environment during incubation on day 2 and 12. Differences between treatments were tested using one-way ANOVA (results reported are *F* and *P*).

Nestlings	Heated	Control	Cooled	*F*	*P*
Tarsus day 5 (mm)	10.95 ± 1.31 (60)	10.61 ± 1.37 (58)	11.30 ± 1.53 (51)	0.89	0.43
Mass at day 5 (g)	6.97 ± 1.43 (60)	6.93 ± 1.31 (58)	7.27 ± 1.43 (51)	0.36	0.70
Condition day 5 (g/mm)	0.67 ± 0.09 (60)	0.69 ± 0.06 (58)	0.68 ± 0.07 (51)	0.43	0.65
Tarsus day 10 (mm)	17.93 ± 1.20 (60)	18.29 ± 1.06 (54)	18.01 ± 0.89 (51)	0.37	0.70
Mass at day 10 (g)	14.05 ± 1.87 (60)	14.67 ± 1.37 (54)	14.88 ± 1.56 (51)	1.14	0.34
Condition at day 10 (g/mm)	0.81 ± 0.07 (60)	0.82 ± 0.05 (54)	0.85 ± 0.06 (51)	1.50	0.25
Tarsus at day 15 (mm)	19.49 ± 0.76 (59)	19.43 ± 0.63 (40)	19.38 ± 0.68 (48)	0.12	0.89
Mass at day 15 (g)	16.00 ± 1.83 (59)	16.98 ± 1.56 (40)	17.31 ± 1.29 (48)	1.94	0.17
Condition at day 15 (g/mm)	0.84 ± 0.08 (59)	0.89 ± 0.07 (40)	0.91 ± 0.05 (48)	2.83	0.09
Tarsus growth rate between days 5 and 10 (mm/day)	1.40 ± 0.18 (60)	1.52 ± 0.18 (54)	1.34 ± 0.23 (51)	2.34	0.12
Mass growth rate between days 5 and 10 (g/day)	1.43 ± 0.25 (60)	1.53 ± 0.19 (54)	1.52 ± 0.16 (51)	1.31	0.29
Tarsus growth rate between day 10 and 15 (mm/day)	0.31 ± 0.17 (59)	0.27 ± 0.16 (40)	0.27 ± 0.13 (48)	0.41	0.67
Mass growth rate between day 10 and 15 (g/day)	0.35 ± 0.37 (59)	0.49 ± 0.26 (40)	0.48 ± 0.18 (48)	1.03	0.38

± 3.3 min (N = 6) in control nests, and 15.3 ± 3.6 min (N = 8) in cooled nests. However, *post hoc* Tukey tests did not find statistically significant differences. Presence/absence data collected during systematic nest checking confirmed that heated females stayed in the nests less frequently (54%) than control (69%) or cooled females (66%; $F_{2,21}$ = 6.54, P = 0.006; Fig. 2). We found no differences between treatments in the number ($F_{2,20}$ = 0.98, P = 0.39) and duration ($F_{2,20}$ = 1.51, P = 0.24) of on-bouts periods along the day, nor in the duration of the overnight period ($F_{2,17}$ = 0.32, P = 0.73).

The mass and condition of the parents when feeding 10–11 days old nestlings did not differ between the treatments (Table 3). There were no significant differences in the feeding rates neither of males or females, nor in the combined rate or in the rate per nestling, between treatments (Table 3).

4. Discussion

4.1. Effects of heating

The heating treatment was effective in increasing the nest temperature, both during the day and dur-

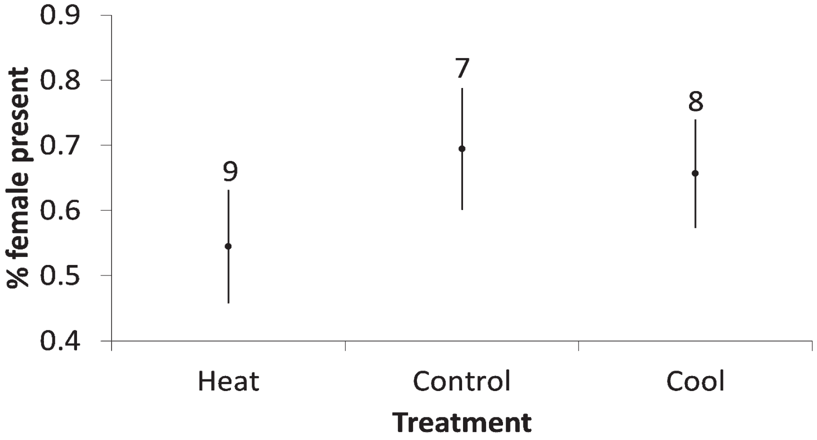

Fig.2. Percentage of presence at the nest (± SD) of females when nests were visited for the experimental treatments (4 times per day during the incubation period). Sample sizes are shown above the bars.

Table 3. Mass and condition of adults on day 10–11, and feeding frequency on day 11–12 at heated, control and cooled nests. The values reported are Mean ± SD, with sample sizes in brackets. Differences between treatments were tested using one-way ANOVA (results reported are *F* and *P*).

	Heated	Control	Cooled	*F*	*P*
Adults					
Female mass (g)	16.35 ± 0.39 (8)	16.40 ± 0.66 (7)	16.52 ± 1.07 (6)	0.10	0.9
Female condition	0.85 ± 0.03 (8)	0.87 ± 0.03 (7)	0.87 ± 0.05 (6)	0.36	0.7
Male mass (g)	17.43 ± 1.32 (8)	17.37 ± 0.50 (6)	17.34 ± 0.60 (7)	0.02	1.0
Male condition	0.88 ± 0.06 (8)	0.87 ± 0.03 (6)	0.87 ± 0.04 (7)	0.14	0.9
Feedings per hour					
Female	8.61 ± 3.41 (8)	6.71 ± 2.38 (5)	9.81 ± 4.26 (5)	1.04	0.4
Male	10.48 ± 4.10 (6)	8.54 ± 2.89 (5)	7.64 ± 2.92 (5)	1.01	0.4
Total	17.80 ± 5.69 (6)	15.26 ± 3.88 (5)	17.45 ± 5.68 (5)	0.37	0.7
Per nestling	3.36 ± 1.32 (6)	1.91 ± 0.46 (4)	2.82 ± 1.46 (5)	1.71	0.2

ing the night. Parents likely benefited and could allocate the saved energy into different activities. Options were increasing attentiveness, thus improving embryo development and/or shortening the incubation period, or allocating more time to foraging or self maintaining activities out of the nest. It seems that they chose the second option, as they seemed to perform longer off bouts, and reduced their presence at the nest, as shown by our regular nest visits.

Our results therefore suggest a decrease in nest attentiveness following the heating treatment. This agrees with the results of Davis *et al*. (1984), Haftorn and Reinertsen (1990), and Londoño *et al*. (2008) in heated nests of Belding's savannah sparrows *Passerculus sandwichensis beldingi*, pied flycatchers *Ficedula hypoleuca*, and northern mockingbirds respectively. Londoño *et al*. (2008) argued that females exposed to high temperatures would need to increase their foraging time, to cope with increasing metabolic rates, but they did not find this increase in foraging activity. Another factor which might be important in promoting longer off-bouts is avoiding predation at the nest. Nearly half of the predation events detected in nests in our study area (48%, *N* = 147 between 1997 and 2012) involved the predation of the female, either during incubation or brooding (own unpubl. data). Providing extra heat would allow females to leave the nest unattended for longer periods, thus reducing her predation risk.

Opposite results, i.e., increasing attentiveness in heated nests, have been found in other species, including great tits (Bryan & Bryant 1999, Reid *et al*. 1999, Cresswell *et al*. 2004, Ardia *et al*. 2009). The argument is that females could allocate this extra energy into improving embryonic development, increasing on-bout duration during the day and duration of the overnight incubation session, and therefore mean egg temperature (Bryan & Bryant 1999, Reid *et al*. 1999, Cresswell *et al*. 2004, Ardia *et al*. 2009). Moreover, Magrath *et al*. (2005) did not find differences in attentiveness between heated and control nests, though here both males and females incubated, and heated females decreased attendance while heated males increased it relative to controls, with the end result being a similar total attendance.

The lower attentiveness of females during the incubation period in heated nests was not clearly reflected in changes in breeding performance (hatching, fledging and breeding success) relative to the controls. This suggests that the decrease in attentiveness was more or less compensated by the extra heat provided. Among the studies which found a decrease in attentiveness when heating was applied, Londoño *et al*. (2008) only followed heated nests until the embryos were 10 days old, and they found that heated embryos were lighter than the control embryos. On the opposite extreme, studies reporting increased attentiveness in heated nests generally found improvements in the breeding performance (Reid *et al*. 2000, Ardia *et al*. 2009). Pérez *et al*. (2008) found that the positive effects on tree swallow nestlings was mediated by differences in attendance by the parents, since the final size of nestlings heated as embryos, but raised by control parents, did not differ from control nestlings, even though they had some advantages shortly after hatching (higher residual body mass on days 4 and 7).

The probable savings in energy experienced by females with heated nests was not reflected in their mass or body condition when feeding nestlings, so any possible improvement was very short term. Bryan and Bryant (1999) found that daily energy expenditure did not differ between heated and control great tit females on mild nights in Scotland (heating was applied only at night in this study). "Mild" conditions in Scotland mean minimum temperatures of 8–10°C. Minimum temperatures in Sagunto ranged between 3.7°C and 15°C (mean 10.6, SD = 2.8°C) during the incubation period of the clutches considered here (8th April to 7th May). Therefore, the lack of effect on females found in Sagunto could be explained by the (relatively) mild temperatures experienced there. The results from other studies also show striking differences, from heated females losing (Magrath *et al*. 2005) or gaining mass (Pérez *et al*. 2008), to no differences in mass between the control and heated females after the treatment (Bryan & Bryant 1999, Cresswell *et al*. 2004, Londoño *et al*. 2008).

The only study reporting feeding rates (Pérez *et al*. 2008) found that heated tree swallow females had higher feeding rates than control females, while no differences were found for males. We found that feeding rates in heated nests were only a bit, but not significantly, higher than those in control nests.

4.2. Effects of cooling

The cooling treatment was successful in decreasing the nest temperature during the day, but not during the night. On one hand, changes of the ice pads were more frequent during the day (every 4 h), while they were not changed from 19:00 to 07:00 h. Our data shows clear temperature drops after the ice pads were changed, but this effect disappeared with time and it was not detected when considering the "night period" (22:00–04:59; see Methods). On the other hand, female attentiveness is continuous during the night, while females make relatively frequent foraging trips during daytime. In any case, cooled females experienced lower temperatures than controls at least during the day.

Two studies which have experimentally cooled nests during incubation and examined female responses obtained opposite results: Davis *et al*. (1984) found an increase of attentiveness, and Ardia *et al*. (2010) a decrease. Davis *et al*. (1984) found an increase of on-bout duration and a reduction of off-bout duration in cooled nests, while Ardia *et al*. (2010) found a decrease in on-bout duration in cooled nests, and no change in off-bout duration. Though the experimental protocols were rather different, there is no clear explanation for these opposite results. Our study suggests that, in addition to changes in the duration of on or off-bouts, females could respond to the cooling treatment by modifying their behaviour once they are within the nestbox (e.g., the tightness of sit on the eggs), and/or through physiological mechanisms (e.g., by changing blood flow in the brood patch) (Ar & Sidis 2002, Deeming 2002).

The breeding performance of cooled and control nests were similar, suggesting that cooled females were able to effectively compensate for the treatment. Previous studies have found negative consequences of experimental cooling on nestlings. Thus, tree swallow nestlings cooled as embryos had lower body mass and poorer body condition at fledging than control chicks (Ardia *et al*. 2010). Similarly, Nilsson *et al*. (2008) found that blue tit *Cyanistes caeruleus* nestlings cooled as embryos had lower growth rates than control nestlings, though differences in fledging mass were not significant. There were discrepancies between these two studies in the effects on the duration of the incubation period: Ardia *et al*. (2010) found a longer period in cooled nests, while Nilsson *et al*. (2008) did not find any differences. Finally, hatching success, hatchling mass, and nestling survival did not differ between the control and cooled nests (Nilsson *et al*. 2008).

The probable extra effort of increasing the nest temperature after the replacement of the cool pads did not have consequences for the female condition in the short term (when feeding nestlings), as the mass and condition of the cooled females were almost the same when compared to control females. The experiment by Nilsson *et al*. (2008) exchanged hatchlings between nests which had been cooled during incubation with control nests, so that the "cooled" nestlings were attended by control parents, and control nestlings by "cooled" parents. They did not find conclusive evidence of the cooled parents being affected by the treatment (similar feeding rates compared to controls, and also similar, or even better, body condition), though they argued that females cooled during incubation dedicated more energy to themselves during the nestling period, and this had negative consequences for nestlings (lower body mass). Ardia *et al*. (2010), however, found clear negative effects on females (lower feeding rates and worse body condition than controls).

4.3. Conclusions

Female great tits responded to nest heating by decreasing their attentiveness. It seems therefore that they allocate the extra energy into foraging or self-maintaining activities out of the nest (among which reducing predation risk at the nest could be important), and not into improving nestling condition or survival. On the other hand, we were unable to detect behavioural responses of females in cooled nests, and they did not seem to experience negative consequences in the short time. Yet, the reproductive performance of cooled females did not differ from control females, suggesting that they were able to effectively compensate the effects of cooling.

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Talitiaisen käyttäytymisvasteet pesän lämpötilan kokeelliseen säätelyyn haudonnan aikana

Useilla lintulajeilla vain toinen emoista hautoo munia. Hautovan yksilön on tehtävä kompromissiratkaisuja ajankäytön suhteen, sillä munien hautomisen lisäksi on myös syötävä ja hoidettava itseään. Tästä johtuen päiväsaikainen haudonta on katkonaista. Haudontajaksojen lukumäärä ja pituus vaikuttavat munien lämpötilaan ja siten myös alkion kehitykseen. Seurasimme naaraiden investointipäätöksiä Espanjalaisessa talitiaispopulaatiossa, ja tarkastelimme miten ne vaikuttivat lisääntymiseen tilanteissa, jossa kokeellisesti lämmitettiin tai jäähdytettiin pesiä haudonnan aikana.

Lämmitettyjen pesien naaraat löytyivät harvemmin pesästä hautomasta ja niillä oli taipumus pitempiin poissaoloihin. Vaikka lämmittäminen selkeästi nosti pesien lämpötilaa (verrattuna kontrolleihin), se ei vaikuttanut pesimämenestykseen, poikasten kokoon tai kuntoon, emojen kuntoon tai ruokkimiskäyttäytymiseen. Pesien jäähdyttäminen laski pesien lämpötilaa päivisin kontrolleihin verrattuna, mutta pesien öisestä lämpötilasta ei löytynyt eroa. Naaraat ilmeisesti kykenivät kompensoimaan jäähdyttämisen vaikutuksia, sillä pesimämenestys oli samankaltainen jäähdytetyissä ja kontrollipesissä, eikä pesäpoikasilla tai emoilla havaittu negatiivisia vaikutuksia kyseisestä käsittelystä.

References

Álvarez, E. & Barba, E. 2008: Nest quality in relation to adult bird condition and its impact on reproduction in Great Tits. — Acta Ornithologica 43: 3–9.

Álvarez, E. & Barba, E. 2014: Incubation and hatching periods in a Mediterranean Great Tit *Parus major* population. — Bird Study 61: 152–161.

Álvarez, E. & Barba, E.: Within and between population variations of incubation rhythm of great tits *Parus major*. — Behaviour, in press.

Álvarez, E., Belda, E.J., Verdejo, J. & Barba, E. 2013: Variation in Great Tit nest mass and composition and its breeding consequences: a comparative study in four Mediterranean habitats. — Avian Biology Research 6: 39–46.

Ar, A. & Sidis, Y. 2002: Nest microclimate during incubation. — In Avian incubation: Behaviour, environment and evolution (ed. Deeming, D.C.): 143–160. Oxford University Press, New York.

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

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

Atiénzar, F., Álvarez, E. & Barba, E. 2012: Carbonero común – *Parus major*. — In Enciclopedia Virtual de los Vertebrados Españoles (ed. Salvador, A. & Morales, M.B.). Museo Nacional de Ciencias Naturales, Madrid. URL: www.vertebradosibericos.org/

Barba, E., Atiénzar, F., Marín, M., Monrós, J.S. & Gil-Delgado, J.A. 2009: Patterns of nestling provisioning by a single prey loader bird, Great Tit *Parus major*. — Bird Study 56: 187–197.

Bryan, S.M. & Bryant, D.M. 1999: Heating nest boxes reveals an energetic constraint on incubation behavior in great tits *Parus major*. — Proceedings of the Royal Society B 266: 157–162.

Carey, C. 2002: Incubation in extreme environments. — In Avian incubation: Behaviour, environment and evolution (ed. Deeming, D.C.): 238–253. Oxford University Press, New York.

Davis, S.D., Williams, J.B., Adams, W.J. & Brown, S.L. 1984: The effect of egg temperature on attentiveness in the Belding's Savannah Sparrow. — Auk 101: 556–566.

Dawson, R.D., Lawrie, C.C. & O'Brien E.L. 2005: The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine. — Oecologia 144: 499–507.

Deeming, D.C. 2002: Behaviour patterns during incubation. — In Avian incubation: Behaviour, environment and evolution (ed. Deeming, D.C.): 63–87. Oxford University Press, New York.

Drent, R. 1975: Incubation. — In Avian Biology (ed. Farner, D.S. & King, J.R.): 333–420. Academic Press, New York.

Encabo, S.I., Monrós, J.S. & Barba, E. 2001: Egg size variation in a Mediterranean Great Tit *Parus major* population. — Ardeola 48: 63–70.

Freitag, A., Martinoli, A. & Urelai, J. 2001: Monitoring the feeding activity of nesting birds with an autonomous system: case study of the endangered Wryneck *Jynx torquilla*. — Bird Study 48: 102–109.

Haftorn, S. & Reinertsen, R.E. 1990: Thermoregulatory and behavioral responses during incubation of free living female Pied Flycatchers *Ficedula hypoleuca*. — Ornis Scandinavica 21: 255–264.

Holcomb, L.C. 1974: Incubation constancy in the Red winged Blackbird. — Wilson Bulletin 86: 450–460.

Lambrechts, M.M., Adriaensen, F., Ardia, D.R., Artemyev, A.V., Atiénzar, F., Bañbura, J., Barba, E., Bouvier, J.-C., Camprodon, J., Cooper, C.B., Dawson, R.D., Eens, M., Eeva, T., Faivre, B., Garamszegi, L.Z., Goodenough, A.E., Gosler, A.G., Grégoire, A., Griffith, S.C., Gustafsson, L., Johnson, L.S., Kania, W., Keišs, O., Llambias, P.E., Mainwaring, M.C., Mänd, R., Massa, B., Mazgajski, T.D., Møller, A.P., Moreno, J., Naef-Daenzer, B., Nilsson, J.-Å., Norte, A.C., Orell, M., Otter, K.A., Park, C.R., Perrins, C.M., Pinowski, J., Porkert, J., Potti, J., Remeš, V., Richner, H., Rytkönen, S., Shiao, M.-T., Silverin, B., Slagsvold, T., Smith, H.G., Sorace, A., Stenning, M.J., Stewart, I., Thompson, C.F., Tryjanowski, P., Török, J., Van Noordwijk, A.J., Winkler, D.W. & Ziane, N. 2010: The design of artificial nestboxes for the study of secondary hole nesting birds: a review of methodological inconsistencies and potential biases. — Acta Ornithologica 45: 1–26.

Londoño, G.A., Levey, D.J. & Robinson, S.K. 2008: Effects of temperature and food on incubation behaviour of the northern mockingbird, *Mimus polyglottos*. — Animal Behaviour 76: 669–677.

Magrath, M.J.L., van Overveld, T. & Komdeur, J. 2005: Contrasting effects of reduced incubation cost on clutch attendance by male and female European starlings. — Behaviour 142: 1479–1493.

Nicolaus, M., Bouwman, K.M. & Dingemanse, N.J. 2008: Effect of PIT tags on the survival and recruitment of Great Tits *Parus major*. — Ardea 96: 286–292.

Nilsson, J.F., Stjernman, M. & Nilsson, J.-Å. 2008: Experimental reduction of incubation temperature affects both nestling and adult blue tits *Cyanistes caeruleus*. — Journal of Avian Biology 39: 553–559.

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

Reid, J.M., Monaghan, P. & Nager, R.G. 2002: Incubation and the costs of reproduction. — In Avian incubation: Behaviour, environment and evolution. (ed. Deeming, D.C.): 314–325. Oxford University Press, New York.

Reid, J.M., Monaghan, P. & Ruxton, G.D. 1999: The effect of clutch cooling rate on starling, *Sturnus vulgaris*, incubation strategy. — Animal Behaviour 58: 1161–1167.

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

Svensson, L. 1996: Guía para la Identificación de los Paseriformes Europeos. — SEO/BirdLife, Madrid.

Tinbergen, J.M. & Williams, J.B. 2002: Energetics of incubation. — In Avian incubation: Behaviour, environment and evolution. (ed. Deeming, D.C.): 299–313. Oxford University Press, New York.

Turner, J.S. 2002: Maintenance of egg temperature. — In Avian incubation: Behaviour, environment and evolution (ed. Deeming, D.C.): 119–142. Oxford University Press, New York.

Webb, D.R. 1987: Thermal tolerance of avian embryo: a review. — Condor 89: 874–898.

Zar, J.H. 1996: Biostatistical analysis. Third edition. — Pretince Hall, New Jersey.