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4 **Egg cooling associated with nest size in a passerine bird**

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18 **Abstract.** Breeders evolved adaptive responses to rapid changes in ambient temperature. In birds,
19 nests are expected to reduce egg cooling when the incubator is temporarily off the eggs. Here we
20 present the results of two complementary laboratory experiments aiming at testing the association
21 between egg cooling and the thickness of the nest under and surrounding the eggs in a non-
22 domesticated avian model species (great tit, *Parus major*). To simulate incubation behaviour, we
23 exposed nests with 4-egg clutches to a heat source until the eggs reached a normal incubation
24 temperature (ca. 39°C) and then recorded egg cooling eight minutes after removal of the heat
25 source, which corresponds to the time females generally leave eggs unattended during the
26 incubation period. Eggs cooled more quickly when the nest layer under the eggs was thinner and
27 when ambient temperature was cooler. We also show that the wall around the nest cup is important
28 to buffer egg cooling. It is hypothesised that in bird nests, both the thickness of the material under
29 the eggs, and the wall surrounding the nest cup interact to maintain a heat envelope around the eggs
30 for the time the incubating parent is foraging. This could explain why the thickness of the nest base
31 and wall are adjusted to the ambient temperature the birds experience during the nest building
32 phase, to anticipate the thermal conditions during incubation and preserve egg viability.

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36 **Key words** great tit, *Parus major*, nest size, egg cooling, temperature

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39 **1. Introduction**

40 Temperature is at the centre of studies that focus on biological consequences of climate change
41 (Parmesan and Yohe, 2003; White, 2008; IPCC Report, 2014; Deeming and Reynolds, 2015;
42 Gaughan et al. 2017). Temperature and its changes have a significant impact on how living beings
43 perform and breed (Visser et al., 2004; Cooper et al., 2005; Mainwaring et al., 2017; Andreasson et
44 al., 2018; Marrot et al., 2018; Page et al., 2022). However, animal breeders evolved numerous
45 proximate response mechanisms to changes in the thermal environment so that embryos and
46 offspring develop in optimal breeding environments. At macro- and micro-geographic scales, they
47 can indeed adjust their breeding site; modulate their timing of reproduction and embryonic
48 development; they can alter litter or clutch sizes; or they can even pause their breeding behaviours
49 in case of extreme temperatures (Baker, 1938; Drent, 1975; Wingfield et al., 1998; Visser et al.,
50 2004; Cooper et al., 2005; Caro et al., 2013; Deeming and Reynolds, 2015; Gładalski et al., 2018;
51 Kauffman et al., 2021; Sharpe et al., 2021).

52

53 In birds, larger nests with higher thermal insulation properties are often found in colder
54 breeding environments. One explanation is that these nests create a suitable environment for
55 incubated eggs, the development of which entirely depends on the surrounding temperature (Drent,
56 1975; Deeming and Reynolds, 2015). For instance, bigger nests are expected to protect developing
57 embryos against a decrease in egg temperature in cold environments (Gibb, 1950; Kluijver, 1950;
58 Drent, 1975; de Heij and Tinbergen, 2006; Deeming and Reynolds, 2015). In temperate zone
59 passerines, the temperature of incubated eggs is usually between 30 and 40°C (Gibb, 1950;
60 Kluijver, 1950; Drent, 1975; Haftorn, 1981b, 1988). During incubation, the surface temperature of
61 the nest cup (where the eggs sit) is >10°C higher than the temperature of the nest wall surrounding
62 the nest cup (Deeming and Pike, 2015; own observations). In addition, the temperature of the
63 incubated eggs is often more than 10 to 20°C higher than the temperature above or outside the nest
64 (Gibb, 1950; Drent, 1975; Haftorn, 1981a,b; Haftorn 1988; own observations). When an incubating
65 parent leaves the nest, incubated eggs are suddenly exposed to a colder environment that induces
66 egg cooling (Gibb, 1950; Haftorn, 1981a,b, Haftorn 1988). Egg cooling might hamper embryo
67 development when eggs are maintained below 34 to 38°C for prolonged periods of time (Drent,
68 1975; Mainwaring, 2015; Webb, 1987). In addition, low egg temperatures increase the energy
69 needed to re-heat the eggs when the incubator returns to the nest (reviewed in Drent, 1975;
70 Monaghan and Nager, 1997). We therefore hypothesised that, especially in colder breeding
71 conditions, bigger or thicker nests are designed to store more incubation-generated heat and that this
72 heat, when released progressively, will slow down egg cooling in the absence of the incubating
73 parent. However, few intraspecific investigations quantified associations between dynamics in egg
74 temperature and aspects of nest design across a wide range of thermal conditions.

75

76 Here we report results of a laboratory study of egg cooling in association with nest thickness
77 under the eggs of a non-domesticated model species, the great tit (*Parus major*). We heated 4-egg
78 clutches placed inside nests to reach a normal incubation temperature (ca. 39°C) (Gibb, 1950;
79 Kluijver, 1950; Drent, 1975; Haftorn, 1981b; Haftorn, 1988), and then removed the heat source to
80 monitor egg cooling for 8 minutes, which corresponds to a frequently observed off-bout duration in
81 natural conditions (Gibb, 1950; Kluijver, 1950; Haftorn, 1981a; Álvarez and Barba, 2014b;
82 Bambini et al., 2019). We performed those tests at various ambient temperatures, with nests of
83 different sizes, and by heating either entire nests, or nests in which the wall had been removed.
84 These different experimental conditions allowed us to test several factors that are expected to
85 influence egg cooling, including the role of the wall in the insulating properties of the nests. Based
86 on our findings on the basic thermal physics of nests, we provide a simple proximate explanation
87 for why colder environments might favour the construction of bigger and thicker nests.

88

89 **2. Methods**

90 The thermal properties of nests were investigated in laboratory conditions (cf. Lambrechts et al.,
91 2020). We tested 101 nests collected in 2018. Those nests had contained nestlings that successfully
92 fledged. Using nests that had been deserted, either before egg hatching (17 nests) or during the stage
93 of nestling rearing (3 nests), led to similar results (see supplementary material for more detail).
94 Nests had been constructed into four box types that have been described in Lambrechts et al.
95 (2017). Briefly, some nests were constructed inside wood-concrete Schwegler B1 boxes (internal
96 diameter 10.5 cm, bottom area 113 cm², volume of the nest chamber under the entrance hole: 1525
97 cm³) whereas the other collected nests were constructed inside one of three wooden box types that
98 differed in size. Wooden boxes (made of European larch, *Larix decidua*) were either small (bottom
99 area 36 cm², volume under the entrance hole 576 cm³), medium-sized (bottom area 121 cm²,
100 volume under the entrance hole 1936 cm³) or large (bottom area 210 cm², volume under the
101 entrance hole 3780 cm³). Nests from different box types differed in size (Lambrechts et al., 2017;
102 Holveck et al., 2019), and therefore the use of different box types only aimed at increasing the range
103 of nest sizes. We also used eggs from deserted clutches in 2018 (cf. Lambrechts et al., 2020). Eggs
104 had different stages of development, and included eggs with yolk and eggs with embryos. Nests and
105 eggs were from nest boxes erected in eight urbanised plots situated in the city of Montpellier
106 (southern France) and one forest plot situated at ca. 25 km from Montpellier (Demeyrier et al.,
107 2016; Lambrechts et al., 2016; 2017) (see table S1 for a description of the number of nests across
108 study plots and box types). Nests and eggs were transferred to the laboratory and kept at room
109 temperature before the start of the study.

110

111 We used the collected eggs to constitute three artificial 4-egg clutches, each clutch constituted of
112 eggs from the same nest of origin. We selected 4-egg clutches for the following reasons: 1) The size
113 of the incubation patch allows the female to simultaneously heat not more than four eggs via direct
114 contact with the skin (Löhr, 1986). Larger clutches lead to variation in surface temperature of
115 individual eggs, due to their position within the nest cup (Boulton and Cassey, 2012); 2) A clutch
116 size of four eggs per nest facilitated the measurement of temperature under the eggs; 3) Using one
117 single clutch size allowed to remove effects of clutch size on egg cooling rates (e.g. reviewed in
118 Cooper et al., 2005; Nord and Nilsson, 2012; Deeming and Reynolds, 2015). General experimental
119 procedures consisted in placing nests, each containing one 4-egg clutch, under an infrared lamp
120 (250 W, Philips, at 38 cm above the nest bottom) for the time needed to reach a temperature
121 observed during normal incubation (39 – 39.7°C, Haftorn, 1988; see also table 1 for a summary of
122 the heating duration needed across experimental conditions). Nests with eggs at incubation
123 temperature were then placed inside a closed insulated cabinet for eight minutes to monitor egg
124 cooling. By placing eggs in a closed cabinet, we could manipulate the ambient temperature and we
125 could also examine cooling in the absence of wind to simulate a nest-cavity environment
126 (Dickinson et al., 2019; Wachob, 1996). A fixed cooling period of eight minutes simulated the
127 duration that an incubating female is off the eggs in natural conditions (Gibb, 1950; Kluijver, 1950;
128 Haftorn, 1981a; Álvarez and Barba, 2014b; Bambini et al., 2019). Both the ambient temperature
129 and the temperature under the eggs were simultaneously measured using an Otio thermometer with
130 two temperature sensors (Model: EM713P, Auterive, France, www.otio.com). Nests and 4-egg
131 clutches were used multiple times, under different thermal conditions (see below). Because thermic
132 properties differ between eggs and are associated with egg development (Cooper and Vos, 2013),
133 the artificial 4-egg clutches were switched between nests so that different nests received the same
134 clutches.

135

136 Both the nest characteristics and the thermal environment were manipulated in two
137 experiments (Table 1). In a first experiment we heated both entire nests and nests from which the
138 wall had been removed. Cut nests were obtained by vertically sawing the nest wall with a hacksaw,
139 only leaving the nest material under the nest cup (figure S1). This way, we obtained nests all having
140 a surface of approximately 7 cm x 7 cm (Table 1). Nests with an even smaller nest floor surface (36
141 cm²) have been reported in our urban study site when great tits nested in a small box type
142 (Lambrechts et al., 2017). Intact and cut nests were heated, one at the time, with the eggs inside.
143 When the eggs reached a normal incubation temperature (39.0 – 39.7 °C) (Haftorn 1988), the nests
144 with eggs were placed for eight minutes inside an insulated cabinet that had an ambient temperature
145 that differed within and between days of testing. Ambient temperature inside the cabinet ranged
146 between 11 and 27°C. Each nest (entire and cut) was tested more than once with different clutches

147 of eggs that were chosen randomly among the three available clutches of four eggs, to avoid
148 possible egg characteristic effects on the temperatures measured. Measurements were taken
149 between 6:00 and 22:30, with sampling duration and sampling hours differing between sampling
150 days (1 to 12 measurements of egg cooling per nest). A thermometer was always placed next to the
151 nest inside the cabinet, and this value was used as a continuous variable in the statistical tests (see
152 below). A total of 462 egg cooling measurements were performed for this experiment.

153

154 In a second experiment, we also exposed nests to two different heating treatments. This time
155 however, nests were left intact, and the treatments consisted in placing, or not, a pierced insulation
156 layer in between the nest and the infrared lamp. The insulation layer consisted in a 5cm-thick foam
157 layer covered with cardboard. It was pierced in the middle in a way that, when placed over the nest,
158 the lamp only heated the nest cup containing the eggs, and not the nest rim. Such a procedure better
159 mimics natural conditions, in which an incubator only sits on the eggs, and not on the whole nest.
160 Like in experiment 1, nests were heated until eggs reach a normal incubation temperature (39.0 –
161 39.6 °C), and ambient temperature differed within and between days of testing. Each nest was tested
162 in both conditions (with and without the insulation layer) and more than once, with different
163 clutches of eggs that were chosen randomly among three of the available clutches of four eggs. A
164 total of 96 measurements were performed for this experiment.

165

166 *Statistics*

167 To analyse the data, we used mixed model procedures (lmer, package lmerTest, Kuznetsova et al.
168 2016, in R version 3.5.0, R-Core-Team, 2015) always using the level of egg cooling (i.e. differences
169 in egg temperature between the beginning and end of the eight-minute period of cooling) as the
170 dependent variable. We tested the effects of the treatment (entire vs. cut nests in exp. 1; with vs.
171 without insulation layer in exp. 2), the ambient temperature during the cooling phase (in °C), nest
172 thickness under the eggs (in mm), and bottom surface area of the nests (in cm²) to account for the
173 size of the different box types from which nests had been collected. Nest identity and clutch identity
174 were added as crossed random intercepts.

175

176 **3. Results**

177 In experiment 1, the nest surface area did not influence egg cooling rate ($p=0.122$, table 2). Egg
178 cooling was however strongly associated with the ambient temperature, and the thickness of nest
179 material under the eggs (all $p<0.001$, table 2, figure 1 left panel), with eggs cooling more rapidly as
180 temperature and nest thickness were low. Eggs also cooled down more in nests without a wall,
181 compared to entire nests (treatment effect, $p<0.001$, figure 1 right panel), which suggests that the
182 wall itself contributes to the thermal protection of the eggs. Birds however do not cover the entire

183 nest when they incubate, but mainly the nest cup. Therefore, to better understand whether the nest
184 wall buffers egg cooling naturally, or whether the treatment effect observed here partly results from
185 the fact that we heated the entire nests, we designed experiment 2. In this second experiment, nests
186 were left intact and we heated the entire nest, or only its cup, using insulating material above the
187 nest rim. While the effects of ambient temperature and nest thickness observed in experiment 1
188 were confirmed in this second experiment ($p < 0.001$), we found no effect of the nest surface area
189 ($p = 0.207$), and no effect of the treatment ($p = 0.273$, table 2). This confirms that the buffering effect
190 of the nest wall on egg cooling observed in experiment 1 was intrinsic and not because the nest wall
191 had also been exposed to heat.

192

193 **4. Discussion**

194 Deeming (2016) proposed the concept of the ‘bird-nest incubation unit’. In small passerines, like
195 *Paridae*, the incubator would require the nest surrounding the eggs to ensure that there is
196 maintenance of temperature. In this framework, thicker, larger or heavier nests would be more
197 efficient in maintaining egg temperature during the act of incubation to explain why larger nests are
198 found in colder environments (e.g. de Heij and Tinbergen, 2006; Crossman et al., 2011; Deeming et
199 al., 2012; Mainwaring et al., 2012, 2014; Heenan et al., 2015; but see Lambrechts and Caro, 2018).
200 Several techniques have been used to study the thermal insulation properties of passerine nests in
201 laboratory conditions, always in the absence of real eggs (e.g. Szentirmai et al., 2005; de Heij and
202 Tinbergen, 2006; Cruz et al., 2016; Akresh et al., 2017; Botero-Delgadillo et al., 2017; Gray and
203 Deeming, 2017; Biddle et al. 2018; 2019; Deeming and Humphreys, 2020). The laboratory studies
204 focused on several nest-size characteristics, including nest length, nest width, nest depth, inner cup
205 diameter, inner cup depth and nest dry weight (e.g. de Heij and Tinbergen, 2006; Botero-Delgadillo
206 et al., 2017). Associations between aspects of nest morphology and nest cooling have also been
207 reported in standardized laboratory conditions with relatively fixed ambient temperatures (e.g. Cruz
208 et al., 2016; Akresh et al., 2017; Botero-Delgadillo et al., 2017; but see de Heij and Tinbergen,
209 2006). Most of those studies however focused on the temperature of the nest itself, sometimes also
210 on dummy eggs, but not on the thermal dynamics of the eggs of the species that built the nest.

211

212 Here we hypothesized that larger nests might provide thermal benefits to the eggs once the
213 incubating female leaves the nest. To test this hypothesis, we heated nests that contained a 4-egg
214 clutch to monitor egg cooling after removal of the heat source. In our tests that simulated aspects of
215 natural incubation behaviour (natural incubation temperature, time that the incubator is off the eggs
216 during the egg cooling phase, dynamic environmental temperature within and across days), we
217 observed that eggs cooled more quickly when the nest layer under the eggs was thinner and when
218 the thermal conditions were cooler. A thicker nest layer under the eggs is thus able to store more

219 incubation-generated heat to be released for a longer period when the female is off the eggs. This is
220 a simple proximate explanation for why inside thicker nests, eggs cooled more slowly in our
221 different experimental set ups. That heated nests store heat to be released when suddenly exposed to
222 colder environmental conditions fits with our own observations that in thicker nests the temperature
223 under the eggs can continue to increase for up to several minutes after a heated nest is placed in
224 shade or cold conditions (M.M. Lambrechts, pers. obs.).

225

226 The important role played by the nest layers under the eggs does however not exclude the
227 possibility that the thickness of the nest wall might also contribute to the thermal insulation
228 properties of the nest, and this is what we showed with our experimental manipulation of the nest
229 wall. In experiment 1 we indeed showed that the presence of a nest wall is important to buffer egg
230 cooling, because eggs cooled down more quickly when the nest wall was removed. The presence of
231 a nest wall thus not only acts as a mechanical protection of the eggs against potentials shocks or to
232 hold eggs together in the centre of the nest, but it also maintains a heat envelope around the eggs,
233 preventing them to cool down rapidly when the nest is not attended. In experiment 2 we further
234 show that this heat envelope exists and operates efficiently even if only the centre of the nest is
235 heated, like it is the case in natural conditions when the incubating bird only heats its eggs and the
236 nest layers immediately below them. The brood patch of the incubator is indeed substantially
237 smaller than the size of the whole nest. The surface of four great tit eggs touching the incubation
238 patch is *circa* 10 cm² (Löhr, 1986), whereas the bottom surface of nests varied between *circa* 36
239 and 210 cm² in our study population. Altogether our experiments show that the nest is a complex
240 environment in which both the thickness of the material under the eggs, and the wall surrounding
241 the nest cup interact to maintain eggs at a viable range of temperatures for the time the incubating
242 parent leaves its nest for foraging and for other self-maintenance activities (see also Deeming et al,
243 2020).

244

245 Based on our laboratory results on egg cooling, we would expect that bigger or thicker nests
246 are built in colder environmental conditions, e.g. earlier in the season, at higher elevations or at
247 higher latitudes (Briggs et al., 2019; Deeming et al., 2012). Associations between aspects of nest
248 size and ambient temperature have been investigated in field studies (e.g. Deeming and Reynolds,
249 2015 for a review) and in laboratory conditions (Lambrechts and Caro, 2018). Consistent with these
250 expectations, we found in previous field studies that great tits invested more in nest building when
251 nests were constructed earlier in the season (Lambrechts et al., 2017). We also found that blue tits
252 built smaller replacement nests built later in the season than first nests built earlier in the same
253 breeding season (Lambrechts et al., 2012) (see also Deeming and Mainwaring, 2015). However, in
254 an 18-year field study that only focused on nests from first breeding attempts built in one box type

255 (Schwegler), we did not find a significant association between nest volume and the average ambient
256 temperature during the week preceding the onset of egg laying (Lambrechts et al., 2016). This
257 would imply that our long-term field measure of nest size (i.e. height of the nest rim at the entrance
258 door, ignoring the nest cup properties) does not necessarily reflect the functional thermic properties
259 of a nest (see also Deeming and Mainwaring, 2015; Cruz et al., 2016). Field studies interested in
260 thermic properties of nests influencing changes in egg temperature therefore should also take other
261 aspects of nest size into account, such as aspects associated with nest-cup characteristics (e.g.
262 thickness or composition of the material under the eggs) (e.g. de Heij and Tinbergen, 2006;
263 Deeming et al., 2012; 2020; Mainwaring et al., 2012; Deeming and Mainwaring, 2015). In addition,
264 in a previous laboratory study in great and blue tits (Lambrechts and Caro, 2018), we did not find an
265 association between ambient temperature and nest volume and nest weight. This was a bit
266 surprising given that nest weight and volumes probably better reflects nest thickness under the eggs,
267 which we measure here, than the sole measure of the external nest wall as done in the field study.
268 But we also observed that the captive birds only started to build when photoperiod was very long.
269 We therefore do not exclude that a very long photoperiod might change the sensitivity to ambient
270 temperature in breeders (e.g. Lambrechts and Perret, 2000) therefore perhaps also influencing
271 investment in nest building.

272

273 Future studies could focus on which egg characteristics proximately determine their thermal
274 properties and whether egg cooling might also be associated with aspects of nest morphology other
275 than mass or thickness, such as nest composition (Deeming et al., 2020). Egg warming following
276 egg cooling could also be investigated with similar approaches to investigate whether low egg
277 temperatures might increase a proximate cost of egg warming when the female returns to the cooled
278 eggs.

279

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282 monitoring.

283

284 **Conflicts of interest**

285 The authors declare that they have no conflicts of interest.

286

287

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449 **Table 1:** Laboratory tests used to study associations between nest-size characteristics and cooling of
 450 4-egg clutches (i.e. difference in egg temperature between the beginning and end of a fixed eight-
 451 minute period after heating). Experiment 1: Heating of entire nests *versus* heating of nests of which
 452 the nest wall was removed. Experiment 2: Heating of entire nests versus heating of only the nest
 453 cups of entire nests. Average values and their associated standard deviations, as well as the entire
 454 ranges of values for each of the measurements taken, are shown. Individual nests were tested
 455 multiple times varying temperature conditions across measurements.

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	Experiment 1		Experiment 2	
	Entire nest heated	Nests without wall heated	Entire nest heated	Nest cup heated
<i>Sample size</i>	336 measurements of egg cooling	126 measurements of egg cooling	48 measurements of egg cooling	48 measurements of egg cooling
<i>Nest mass (in g)</i>	23.4 ± 11.1 4.7 – 61.9	6.3 ± 2.8 1 – 13	23.9 ± 13.0 8.0 – 61.9	23.9 ± 13.0 8.0 – 61.9
<i>Nest thickness under eggs (in mm)</i>	21.5 ± 6.9 10.0 – 44.0	20.9 ± 6.7 10.0 – 44.0	22.3 ± 7.6 11.4 – 39.0	22.3 ± 7.6 11.4 – 39.0
<i>Duration of egg heating prior to cooling (in seconds)</i>	717 ± 266 324 – 3076	855 ± 303 418 – 2292	668 ± 184 324 – 1052	643 ± 148 377 – 995
<i>Temperature under heated eggs at the onset of egg cooling (°C)</i>	39.1 ± 0.1 39.0 – 39.7	39.1 ± 0.1 39.0 – 39.5	39.1 ± 0.1 39.0 – 39.6	39.3 ± 0.2 39.0 – 39.6
<i>Egg cooling (°C)</i>	7.0 ± 2.5 2.8 – 15.5	9.8 ± 3.4 4.9 – 18.7	6.5 ± 1.5 3.5 – 9.9	6.7 ± 1.6 3.4 – 9.9
<i>Air temperature during egg cooling (°C)</i>	19.9 ± 5.4 12.0 – 26.9	20.5 ± 5.4 11.0 – 26.5	24.2 ± 1.5 21.3 – 27.1	24.3 ± 1.4 21.2 – 26.3
<i>Measure day (Julian dates)</i>	170 – 246 43 days	175 – 279 26 days	237 – 246 20 days	237 – 246 20 days

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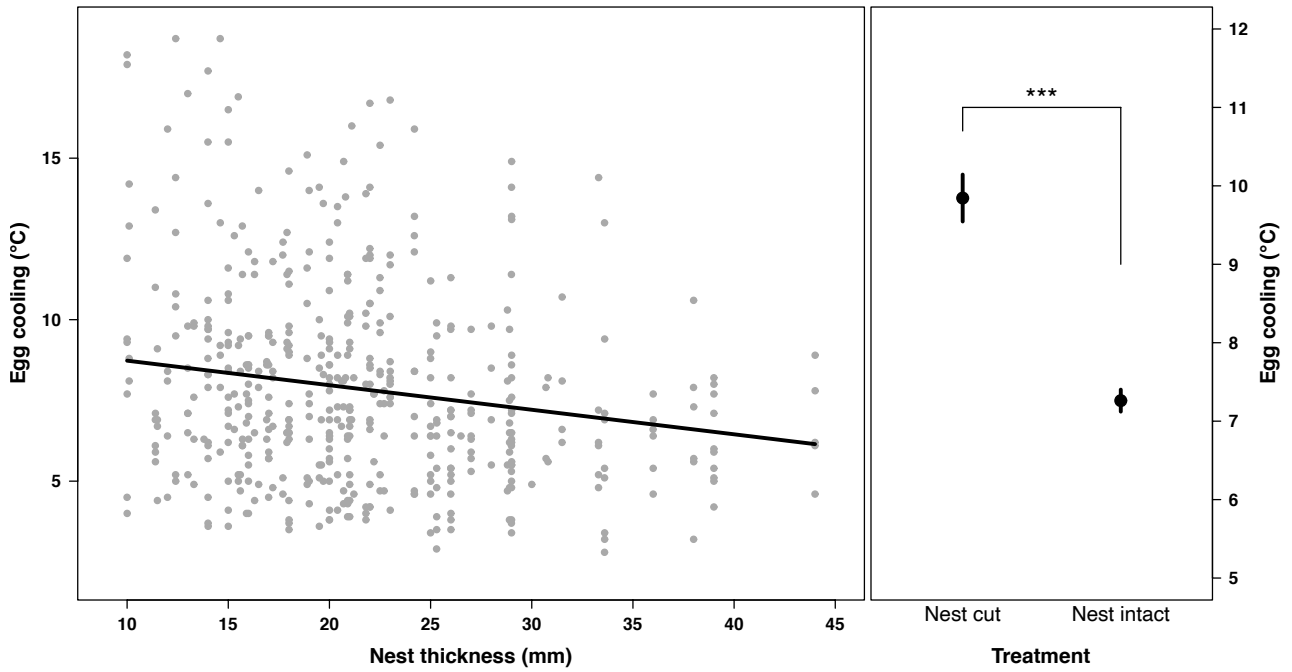
466 **Table 2:** Analyses of the variables that influence the level of egg cooling (i.e. difference in egg
467 temperature between the beginning and end of a fixed eight-minute period after heating). In experi-
468 ment 1 (n=462 measures), treatment consisted in heating entire nests or nests from which the wall
469 had been removed (intercept depicts cut nests, coded as 1 in the model). In experiment 2 (n=96
470 measures), treatment consisted in placing or removing a pierced insulation layer above the nest rim
471 in order to restrict heating to the nest cup (intercept is without the insulation layer, coded as 1 in the
472 model).

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Variable	Estimate	S.E.	F	p-value
<i>Experiment 1</i>				
(Intercept)	22.74	0.74		
Treatment	-2.99	0.15	410.42	<0.001
Ambient temperature	-0.39	0.01	1019.03	<0.001
Nest thickness under eggs	-0.07	0.01	21.09	<0.001
Nest surface area	-0.00	0.00	2.44	0.122
<i>Experiment 2</i>				
(Intercept)	21.82	1.54		
Treatment	0.10	0.09	1.21	0.276
Ambient temperature	-0.59	0.05	118.92	<0.001
Nest thickness under eggs	-0.08	0.02	13.62	<0.001
Nest surface area	0.00	0.00	1.64	0.208

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475 **Figure 1:** Influence of the thickness of nest material under the eggs (left panel, $p < 0.001$, the line
476 depicts the mixed-model output), and of the removal of the nest wall (right panel, $p < 0.001$) on egg
477 cooling in experiment 1. Eggs cool down more slowly when there is more material under the nest
478 cup, and when the nest wall is present.



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