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2 **Males feed their mates more and take**

3 **more risks for nestlings with larger**

4 **female-built nests: an experimental study**

5 **in the Nuthatch *Sitta europaea***

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28

29 **Abstract**

30 Nest size has been suggested to be a sexually selected signal, allowing individuals to  
31 obtain reliable information about partner quality and thereby optimize paternal  
32 investment in reproduction. Studies concerning the potential role of nests as signals are  
33 scarce for avian species in which the female is the only builder. We have aimed at  
34 understanding whether males' reproductive investment (e.g., provisioning rates and risk  
35 taking) change in relation to experimental manipulation of nest size in the Nuthatch  
36 *Sitta europaea*, as would be predicted if nest size is a sexually selected signal reflecting  
37 female's quality. To that end, we have experimentally manipulated nest size by  
38 approximately doubling its size and leaving other nests as controls. Experimental  
39 manipulation led males to increase their incubation feeding rates, and females from the  
40 experimental group showed higher levels of glutathione (tGSH), an important  
41 endogenous antioxidant whose synthesis may be enhanced indirectly through nutrition.  
42 Although male provisioning rates during the nestling stage did not differ between  
43 experimental groups, males responded to nest size manipulation by visiting nests sooner  
44 after human disturbance (an index of risk taking) as compared to control nests. Our  
45 study suggests that nest size constitutes a signal of female quality which elicits  
46 differential allocation of male resources both to females themselves and to their broods.  
47 Higher risk taking in favour of offspring by mates of strongly signaling females may  
48 denote an improvement in offspring survival chances in the field.

49

50 **Keywords:** extended phenotype, female traits, female signals, nest signalling, nest size,  
51 female quality

52

53 **Significance Statement**

54 Animals transfer information to other individuals through different traits. Given that nest  
55 building involve a large expenditure of time and energy for the builder, nest size may allow  
56 individuals to obtain reliable information about partner quality and thereby optimize  
57 paternal investment in reproduction. Despite the importance of females as nest-builders  
58 in a majority of avian species, studies concerning the potential role of nests as signals  
59 are scarce for avian species in which the female is the only builder. Our experimental  
60 study suggests that nest size constitutes a signal of female quality which elicits  
61 differential allocation of male resources both to females themselves and to their broods.  
62 Males from enlarged nests intensified their provisioning rates to females during the  
63 incubation stage and assumed higher risks for their broods after human disturbance.

64

65 **Introduction**

66 Animal communication is based on signals expressed by an individual that are  
67 perceived by another of the same or another species and induce a response that favours  
68 the first (Searcy and Nowicki 2005). Animals transfer information to other individuals  
69 through anatomical, physical and behavioral traits, such as colorful plumage,  
70 extravagant body ornaments or calls (Smith and Harper 1995; Maynard Smith and  
71 Harper 2003). These signals transmit information to the receptor that allows it to adjust  
72 its physiological and behavioral responses (Andersson 1994). Focusing on intra-specific  
73 communication, signaling is involved in influencing the behaviour of individuals from  
74 the same or different sex and may bring benefits in terms of survival and reproduction  
75 (Bradbury and Vehrencamp 1998). Signals may affect the disposition of mates to invest  
76 in common progeny according to the differential allocation hypothesis (Burley 1986;  
77 Sheldon 2000).

78 Nests are structures that animals build for reproduction (Collias and Collias  
79 1984; Hansell 2000), which may serve as signals (Sergio et al. 2011; Tomás et al. 2013;  
80 García-Navas et al. 2015; Møller and Nielsen 2015; see Moreno 2012 for a review).  
81 Some species are able to collect different materials to build complex structures or  
82 decorate their nests, aspects that are used as an extension of the phenotype of the builder  
83 (Dawkins 1982). In birds, nest building may involve a large expenditure of time and energy  
84 (Putnam 1949; Collias and Collias 1984; Nores and Nores 1994; Moreno et al. 2008),  
85 and it may even constrain the selection of nest site and nesting materials (Cantarero et  
86 al. 2015). The effort devoted to nest building and/or nest size may signal the quality or  
87 parental disposition of the builders, and thus affect the decisions of their mates on how  
88 much to invest in reproduction as proposed by the differential allocation hypothesis  
89 (Soler et al. 1998, 2001, 2007; Moreno 2012). Because reproduction is costly (Stearns

90 1992), individuals may find an optimal balance between the efforts they make and the  
91 negative impact those efforts have on their own long-term survival and future breeding  
92 efforts (Stearns 1992; Hanssen et al. 2005).

93         Several studies have previously demonstrated that nest size is used in the context  
94 of signaling after pair formation (Moreno et al. 1994; de Neve and Soler 2002; de Neve  
95 et al. 2004; Gill and Stutchbury 2005; Tomás et al. 2006, 2013; Mainwaring et al. 2008;  
96 Sergio et al. 2011; García-Navas et al. 2015). Thus, individuals could benefit from  
97 caring for the progeny shared with good nest-builders because only individuals in good  
98 condition would be able to build large nests (Zahavi 1977). If nests are reliable signals  
99 of builder quality, a direct relationship between this trait and the parental effort of its  
100 mate could be expected (Soler et al. 1998). Despite the importance of females as nest-  
101 builders in a majority of avian species, studies concerning the potential role of nests as  
102 signals are scarce for avian species in which the female is the only builder (but see  
103 Tomás et al. 2006, 2013; García-Navas et al. 2015). In these cases, males may respond  
104 to female nest building activity by optimizing paternal investment in reproduction  
105 (Borowiec et al. 2006).

106         Sexually monomorphic species, in which only females build nests, offer the best  
107 opportunity to test the differential allocation scenario with nests as signals. Moreover,  
108 species where males invest heavily in reproduction, such as socially monogamous  
109 species with prolonged pair bonds, constitute a suitable model for confirming the  
110 differential allocation hypothesis. The Eurasian Nuthatch *Sitta europaea* is a small  
111 sexually monomorphic hole-nesting passerine that defends territories throughout the  
112 year and presents long-term partnerships (Löhrl 1958; Matthysen 1998) and extremely  
113 low extra-pair paternity (Segelbacher et al. 2005). Nuthatch females build no structured  
114 nest, eggs and young being kept in a depression among loose pieces of rotten wood and

115 bark flakes of pine or other trees (Matthysen 1998; Wesolowski and Rowiński 2004;  
116 Cantarero et al. 2013, 2015). These nests occupy only a fraction of the bottom area of  
117 the cavity and have no clearly defined limits as to size or mass more than those defined  
118 by cavity dimensions, which is probably a response to two major challenges to  
119 successful nesting – the avoidance of predators while keeping the nest contents dry  
120 (Wesolowski and Rowiński 2004). Nuthatches females are known to travel far away to  
121 obtain these bark flakes which are carried singly (Löhrl 1958; Bohr 1962), and nest  
122 building activity typically takes some weeks (Enoksson 1990; Matthysen 1998). One  
123 nest in a large cavity contained no fewer than 11440 barks fragments and 850 fragments  
124 of wood (Olsson 1957). Mates may obtain information on nest size by relating cavity  
125 volume before and after nest construction given that loose nest materials adjust  
126 themselves to cavity walls. Our hypothesis about signaling through nest construction is  
127 based on the huge variation in nest size in our population and its doubtful relevance for  
128 the direct needs of holding and insulating eggs and nestlings. Thus, in other years we  
129 have weighed nuthatch nests in the range 11-112 g, a ten-fold difference (means and  
130 variances in Cantarero et al. 2014b). A good part of this variation could be related to  
131 females ‘showing off’ condition to mates rather than to strict reproductive requirements.  
132 No specific ornamentation of nests is required for them to function as signals (Moreno  
133 2012).

134         To test if nest size may constitute a signal of female quality and if males respond  
135 to it we experimentally manipulated nest size in a Spanish population of Nuthatches. In  
136 many avian species like the Nuthatch, females incubate alone and receive part of their  
137 food from their mates (Cantarero et al. 2013), a behavioral strategy to partly compensate  
138 the energetic limitations of females while incubating (Cantarero et al. 2014a). We  
139 estimated male investment in reproduction directly by recording male provisioning rates

140 to females during incubation and to nestlings 9 days after hatching. We also monitored  
141 risk taking by males as an index of disposition to invest in reproduction (e.g., Dale et al.  
142 1996; Lambrechts et al. 2000; Tilgar and Kikas 2009; Tomás et al. 2013). Risk taking  
143 was estimated in relation to trappability after disturbance by researchers at nest sites  
144 (e.g. Tomás et al. 2013).

145 We predicted following the differential allocation hypothesis that if nest size  
146 functions as a female signal to mates, our experimental manipulation of its size would  
147 lead males to invest more (increased provisioning rates and higher risk taking) as a  
148 response to female quality. Thus, female condition, nestling growth and reproductive  
149 success could be affected depending on the effects of the experiment on male  
150 investment decisions. For estimating female physiological condition, we have measured  
151 total glutathione (tGSH) levels in red blood cells. Glutathione is considered to be the  
152 most important endogenous antioxidant (López-Arrabé et al. 2014a, b, 2015, 2016), and  
153 birds may enhance its synthesis indirectly through nutrition (Eeva et al. 2010; López-  
154 Arrabé et al. 2015).

155

## 156 **Material and methods**

### 157 Study area and species

158 The study was carried out during the springs of 2014 and 2015 on a Nuthatch  
159 population breeding in wooden nest-boxes in a Pyrenean oak *Quercus pyrenaica* forest  
160 at 1200 m altitude in Valsain, central Spain (40° 54' N, 4° 01' W). Breeding activities  
161 are followed routinely every year and laying and hatching dates and brood sizes at  
162 hatching and fledging are determined. Nest-boxes are cleaned after the breeding season  
163 and again shortly before the next breeding season. Nuthatches are the first species to  
164 breed and to occupy nest-boxes in the spring, and are dominant over all other species

165 using nest-boxes in our study area. Egg laying in this population typically begins in late  
166 April, females laying a single clutch per year averaging 6.5 eggs (Cantarero et al. 2013).  
167 Nuthatch nests are totally unstructured (Cantarero et al. 2014b) and in our study area are  
168 mainly composed of loose pine *Pinus sylvestris* bark flakes or loose *Cistus laurifolius*  
169 bark strips (Cantarero et al. 2015). Besides bark, nuthatches use mud in nest building to  
170 narrow the entrance of cavities in order to exclude nest site competitors (Collias and  
171 Collias 1984; Matthysen 1998).

172 On day 18 (hatching day = day 1), we ringed nestlings and measured their tarsus  
173 lengths with a digital calliper (precision 0.01 mm) and their wing lengths with a stopped  
174 ruler to the nearest mm. Body mass was obtained with a digital balance to the nearest  
175 0.1 g.

176

#### 177 Nest size manipulation

178 The experiment was carried out on 30 nest-boxes (12 nests in 2014 and 18 nests in  
179 2015). All 300 nest-boxes in the area were inspected weekly from the first week of  
180 April and more frequently afterwards. In 2014, after laying of the first egg, nests were  
181 alternately assigned to one of the two experimental groups. In 2015, most pairs of  
182 nuthatches occupied the same or a neighbouring nest-box to those used in 2014. Given  
183 the intense territoriality of Nuthatches pairs and their inter-year fidelity to territories and  
184 mates (this was confirmed through rings), we assigned the opposite treatment to each  
185 territory in 2015 as in 2014. This avoids the problem of confounding treatment with pair  
186 or territory identity.

187 The experimental manipulation was conducted six days after clutch completion  
188 when incubation was advanced in order to avoid desertion. At all nest-boxes included in  
189 the study, eggs were removed, after which nest materials were extracted, weighed and

190 replaced, eggs being placed back at the end. The two treatment groups of nests did not  
191 differ with respect to laying date, hatching date, clutch size or brood size (all  $P > 0.60$ ).

192         The first group of nests was left unmanipulated (control group,  $N=14$ ). In the  
193 experimental group ( $N=16$ ), the size of nests was manipulated within its range of natural  
194 variation (Matthysen 1998 and references therein) by approximately doubling its size by  
195 inserting fresh nest materials at the bottom of the nest. These nest materials had been  
196 collected in previous seasons after some nests were abandoned prior to laying and had  
197 been kept frozen at  $-20\text{ }^{\circ}\text{C}$  until use. Through nest size manipulation, we aimed at  
198 creating perceptible differences in nest size between treatments which may provide  
199 useful information for males about the effort devoted by females to build the nest.  
200 Males may judge nest size by relating it to cavity dimensions.

201         Nest mass was recorded in both groups before and after the manipulation with a  
202 digital balance to the nearest 0.1 g. Before the manipulation, nest mass did not differ  
203 between nests subsequently assigned to control (mean  $47.22 \pm \text{SD } 15.84\text{ g}$ ) or  
204 experimental nests (mean  $43.00 \pm 15.33\text{ g}$ ;  $F_{2,28} = 1.069$ ,  $P = 0.466$ ). After the  
205 manipulation, nest size differed between groups as expected (control:  $47.22 \pm \text{SD } 15.84$   
206 g, and experimental:  $80.02 \pm \text{SD } 22.00\text{ g}$ ;  $F_{2,28} = 1.710$ ,  $P < 0.001$ ). Consequently, nest  
207 mass was increased in the experimental group to almost twice the original size.

208

#### 209 Risk taking index

210         On day 7 (hatching day = day 1), we captured parental individuals while they  
211 were provisioning nestlings in their nest-boxes. In the course of the breeding season,  
212 both male and female forage on their territory as the pair concentrates its activity around  
213 the nest site (Matthysen 1998); thus they witnessed a human observer manipulating  
214 their occupied nest-box as indicated by intense alarm-calling. We set up a conventional

215 nest-box trap inside the nest-box which was in operation for a maximum of 1 h to  
216 minimize disturbance to adult birds and nestlings. Traps were removed earlier if both  
217 adults were trapped before that time. The nest-box trap consisted of an aluminium door  
218 with a spring device which caught automatically the bird when it entered the nest-box  
219 (Stutchbury and Robertson 1986). Because focal birds could have seen parts of the trap  
220 from the outside at arrival, capture success could reflect the bird's reaction to both prior  
221 human proximity to the nest and the presence of a novel object inside the nest on arrival  
222 (Garamszegi et al. 2009; Tomás et al. 2013). Given the risks incurred by cavity nesters  
223 in being trapped inside the cavity by predators (e.g. Leader and Yom-Tov 1998), time  
224 until nest visits after predator disturbance at the nest could express the risk that birds are  
225 willing to incur to care for their offspring. The person setting up the trap did not wait  
226 around the nest-box until capture but observed nest visits with binoculars hidden and at  
227 a distance. On each trapping session we noted the time elapsed until capture of each  
228 individual and whether a male entered the nest-box before (1) or after his mate (0).

229 Parental individuals were ringed if necessary or identified, weighed and  
230 measured in the same way as nestlings. Each bird was also colour-ringed in order to  
231 identify the sex on films. Soon after capture a blood sample (about 50-150  $\mu$ l) was  
232 collected from the brachial vein in heparinized microcapillaries. Plasma was separated  
233 from blood by centrifugation (10 min at 12,000 rpm) and then both fractions were  
234 stored at  $-80$  °C until analysed for assaying total GSH in red blood cells (see below).  
235 All the procedure between capture and release of the bird took less than 10 minutes. It  
236 was not possible to record data blind because our study involved focal animals in the  
237 field.

238

239 Video recordings

240 During incubation, all nests were filmed 7 days after clutch completion (one day after  
241 the nest size manipulation). We filmed inside nest-boxes for periods of 90 min ( $92.06 \pm$   
242  $SD\ 12.01$  min,  $N=30$ ) with a video camera (Square SONY 1/3\* Super HAD CCD)  
243 connected to a 3G H.264 CCTV DVR 1 Tb digital recorder installed on the roof outside  
244 the nest-box. Both digital recorders and camcorders were powered by batteries (7.2 Ah  
245 12 V).

246 Nest-boxes were again filmed 9 days after hatching of the young for periods of  
247  $95.05 \pm SD\ 12.01$  min ( $N = 26$ ). In four nests all chicks died before day 9, but we have  
248 included observations taken during incubation. Nest desertions were associated with  
249 periods of cold and rainy weather in all cases. All films were randomly recorded  
250 between 08:00 and 14:00 h. Each sex could be distinguished on films due to the colour  
251 of their flanks (Matthysen 1990) and/or by its colour-ring as they usually climbed on the  
252 inside walls of the nest-box while provisioning nestlings. We excluded the mean time  
253 until the first nest visit by parents ( $6.58 \pm SD\ 4.43$  min,  $N = 56$ ). No evidence of stress  
254 or unnatural behaviour was observed after the first visit.

255

256 Behavioural data analysis

257 Recordings were displayed in the free VLC Media Player software. From films taken  
258 during incubation we estimated the proportion of time spent by the female inside the  
259 nest-box or “incubation attendance”, the mean duration of incubation sessions and  
260 recesses and the number of incubation feedings by males per hour. From films made  
261 during the nestling phase we obtained hourly provisioning rates by males and females.  
262 To minimize observer bias, blinded methods were use when all behavioral data were  
263 analyzed.

264

265 Determination of tGSH levels

266 tGSH levels in RBCs were determined as described in López-Arrabé et al. (2014a, b,  
267 2015). Briefly, red blood cell samples were diluted (1:20 w/v) and homogenized in a  
268 stock buffer (0.01 M PBS and 0.02 M EDTA) and mixed with 10% trichloroacetic acid.  
269 The mixture was mixed using a vortex, centrifuged (1,125 g for 15 min at 6° C), and the  
270 supernatant was separated. Three working solutions were created in a reaction buffer as  
271 follows: (1) 0.3 mmol·L<sup>-1</sup> NADPH, (2) 6 mmol·L<sup>-1</sup> DTNB, and (3) 50 U GSH  
272 reductase·mL<sup>-1</sup>. The next steps were performed on a Synergy HT Multi-Mode  
273 Microplate Reader (BioTek Instruments, Inc.). To 75 µL of sample (supernatant), we  
274 added 240 µL of the mixture of solutions 1 and 2. Afterward, 20 mL of solution 3 was  
275 added after 15 s and the absorbance at 405 nm was monitored after 15 and 45 s. The  
276 change in absorbance was used to determine the intracellular tGSH concentration by  
277 comparing the output with the results from a standard curve generated by serial dilution  
278 of GSH. A subset of samples assayed in duplicate showed high repeatability ( $R = 0.906$ ,  
279  $N = 18$ ,  $P < 0.001$ ). To minimize observer bias, blinded methods were use when all  
280 physiological data were analyzed.

281

282 Statistical analyses

283 One extreme outlier was removed from each group in the analyses of capture time and  
284 male provisioning rates during incubation. Statistical analyses were conducted using  
285 STATISTICA (version 7.0, StatSoft, Inc.). Hatching success only considers to eggs  
286 present at the time when treatments were assigned. Thus, hatching success and fledging  
287 success were calculated as the proportions of eggs that hatched and the proportion  
288 hatched chicks that fledged, respectively. Given the predictions presented and the  
289 experimental set-up we tested male, female and nestling variables with simple two-way

290 comparisons with treatment as factor. Non-normal variables were tested with non-  
291 parametric tests, while normally distributed variables were compared with parametric  
292 tests. Moreover, we used generalized linear models (GLZ) with binomial distributions  
293 to test whether the capture order of the pair (male before or after female) could be  
294 explained by nest treatment. Body condition was calculated as the residual mass  
295 corrected for tarsus length (Green 2001). We have included hatching date and brood  
296 size in the analyses of provisioning rates and nestling variables without finding any  
297 changes with respect to the effects of treatment which is the focus of the study. Thus,  
298 we will only present the tests without including these covariables.

299

## 300 **Results**

301 Males provisioned their mates during incubation at higher rates at the  
302 experimental nests (Fig. 1). The time needed for successful capture of females was  
303 similar in the two treatments (control:  $7.66 \pm \text{SD } 8.57$  min; experimental:  $9.72 \pm \text{SD}$   
304  $10.10$  min;  $U = 85.00$ ,  $P = 0.771$ ). However, males from the experimental group were  
305 captured significantly earlier than males from the control group (Fig. 2). Furthermore,  
306 males from the experimental group were captured more frequently before than after  
307 their mates (Fig. 3).

308 Treatment did not significantly affect incubation attendance (% incubation time)  
309 of females or incubation session duration (all  $P > 0.50$ ). However, the duration of  
310 incubation recesses was significantly shorter in control than in experimental nests  
311 (control:  $8.78 \pm \text{SD } 1.52$  min; experimental:  $11.87 \pm \text{SD } 5.17$  min;  $t_{26} = -2.120$ ,  $P =$   
312  $0.043$ ). Neither male nor female provisioning rates to nestlings were affected by  
313 treatment (all  $P > 0.40$ ), and they were strongly correlated (Spearman correlation:  $r_s =$   
314  $0.701$ ,  $P < 0.001$ ). The body condition of both adults were not affected by treatment

315 (female body condition,  $t_{26} = 0.651$ ,  $P = 0.520$ ; male body condition,  $t_{26} = -0.481$ ,  $P =$   
316  $0.635$ ). Compared with levels in control females, the concentration of tGSH in red  
317 blood cells was significantly higher in experimental females (Fig. 4). However, tGSH of  
318 males was not affected by treatment (control:  $2.68 \pm \text{SD } 1.22 \mu\text{mol g}^{-1}$ ; experimental:  
319  $3.05 \pm \text{SD } 0.96 \mu\text{mol g}^{-1}$ ;  $t_{26} = -0.848$ ,  $P = 0.405$ ). Nest mass showed a positive  
320 association with the tGSH levels of females in the control group (Pearson correlation:  $r$   
321  $= 0.74$ ,  $P < 0.05$ ).

322         There were no differences between treatments in nestling morphometric  
323 measurements on day 18 (all  $P > 0.20$ ). The nests from the two treatments did not differ  
324 in hatching success ( $U = 93.00$ ,  $P = 0.629$ ) or fledging success ( $U = 102.00$ ,  $P = 0.930$ ).  
325

## 326 **Discussion**

327 This study shows that nest size is a reliable signal of female quality which affects male  
328 reproductive investment in Nuthatches, thereby lending support to the ‘differential  
329 allocation’ hypothesis (Sheldon 2000). Males from enlarged nests intensified their  
330 provisioning rates to females during the incubation stage. Furthermore, these males  
331 responded to nest size manipulation by assuming higher risks after human disturbance  
332 as compared with control nests. Although parental provisioning rates to nestling were  
333 not affected by treatment, females from the experimental group showed higher levels of  
334 tGSH, an important endogenous antioxidant. Male and nestling condition were  
335 unaffected by the treatment.

336         The differential allocation scenario predicts that individuals are able to detect the  
337 quality of a mate on the basis of some traits that reliably signal its quality (Burley 1986;  
338 Sheldon 2000). Through these traits, individuals may adjust their reproductive  
339 investment and therefore optimize resources allocation. For example, Walters et al.

340 (2014) manipulated clutch brightness and found that male House Wrens *Troglodytes*  
341 *aedon* biased their parental care toward offspring whose egg brightness indicated that  
342 they would provide higher fitness returns. A different version of this scenario could be  
343 applied prior to egg laying, to signals related to nest or nest building (Andersson 1994;  
344 Soler et al. 1998). In this view, nests could be extended phenotypes used as signals  
345 (Moreno 2012). Most studies on nests as signals have dealt with males as potential  
346 signalers although females build nests singly or predominantly in a majority of avian  
347 species. A common finding in species where females build the nests is the presence of a  
348 positive relationship between female quality and nest size (Mainwaring et al. 2008;  
349 Lambrechts et al. 2012; Tomás et al. 2013; Jelínek et al. 2015). Thus, we could expect  
350 nests to signal female quality in species with female exclusive nest-building  
351 (Mainwaring et al. 2008; Møller and Nielsen 2015). This has been confirmed only in a  
352 few studies (Tomás et al. 2013; García-Navas et al. 2015). Our hypothesis about  
353 signaling through nest construction in nuthatches is based on the huge variation in nest  
354 size in our population and its doubtful relevance for the direct needs of holding and  
355 insulating eggs and nestlings. In the study year, the range of nest mass went from 19.4  
356 to 78.3 g, a fourfold difference. In other years we have weighed nuthatch nests in the  
357 range 11-112 g, a ten-fold difference (see Cantarero et al. 2014b for means and  
358 variances related to this range). Thus, a good part of this variation could be related to  
359 females ‘showing off’ condition rather than to strict reproductive requirements.  
360 However, we cannot exclude that nest volume is an ‘indicator’ according to Maynard  
361 Smith and Harper (2003). In both cases, the information could be used by males to  
362 adjust their level of parental effort.

363         Food provided by males during incubation is an important energy source for to  
364 compensate female activities (Cantarero et al. 2014a), and may improve female body

365 condition (Lifjeld and Slagsvold 1986) and thereby increase nest attentiveness (Moreno  
366 and Carlson 1989; Pearse et al. 2004; Matysioková et al. 2011). Competition for limited  
367 resources such as antioxidants (tGSH) implies that individuals should allocate resources  
368 to one component of fitness against others (de Ayala et al. 2006; Eeva et al. 2010).  
369 Females who received more male incubation feedings may use these increased plasma  
370 antioxidants to cope with costs related to reproduction and thereby enhance their own  
371 body condition or survival (López-Arrabé et al. 2014b). It is known that the depletion of  
372 cellular tGSH levels is produced as a consequence of metabolic activity (Finkel and  
373 Holbrook 2000). In fact, it has been recently shown in birds that flight effort may  
374 contribute to the depletion of the antioxidant defences in order to maintain redox  
375 homeostasis (Costantini et al. 2008). Thus, Nuthatch females would gain a fitness  
376 advantage through signaling. Indeed, female condition may be a primary target for male  
377 investment in species with long-term pair bonds where there may be long-term benefits  
378 from experience in breeding and defending a territory together (Matthysen 1998). Long-  
379 term pair bonds are considered in life-history theory to be associated with increased  
380 cooperation between mates.

381         An alternative interpretation posits that males may adjust mate provisioning to  
382 nest size in order to compensate for increased female effort and not as differential  
383 allocation of resources to high quality mates. As experimental females had not  
384 expended the effort indicated by nest size, this type of male compensation would lead to  
385 improved antioxidant condition as observed. This alternative scenario begs the question  
386 of why some females build nests ten times larger than others, a question which the  
387 signaling hypothesis tries to answer. To exclude this possibility, real construction effort  
388 would have to be manipulated, which is logistically impossible. We also know that  
389 larger nests in our population also imply a higher thermal buffering capacity for eggs

390 (Cantarero et al. 2014b). Thus, the increase in duration of incubation recesses observed  
391 in the experimental group could have contributed to improved female nutritional  
392 condition. However, we believe that increased mate provisioning during incubation is  
393 the main factor behind improved condition in experimental females as incubation  
394 attendance, which better represents the time available for foraging, did not differ  
395 between treatments.

396 Life-history theory posits that parental risk-taking responses may depend on how  
397 parents value their own survival versus the survival of their offspring (Dale et al. 1996;  
398 Tilgar and Kikas 2009; Colombelli-Negrel et al. 2010), and these decisions may vary  
399 both between and within individuals (Fernandez et al. 2015). Several studies have  
400 supported this concept in various species by finding positive relationships between  
401 parental risk taking and different measures of reproductive value (Ghalambor and  
402 Martin 2000; Lambrechts et al. 2000; Tilgar and Kikas 2009; Tomás et al. 2013),  
403 aggressiveness (Verbeek et al. 1996, 1999), or behaviour towards novel objects  
404 (Verbeek et al. 1994). An individual investing many resources in terms of risk and  
405 energy in offspring arising from low quality mates may lead to non-adaptive life  
406 histories (Moreno 2012). We have used rapidity in returning to the nest after  
407 disturbance as an index of risk taking instead of presenting predator models as in other  
408 studies (Garamszegi et al. 2009; Tomás et al. 2013). Human proximity to nests elicits  
409 intense alarm-calling in our population, indicating that humans may be considered as  
410 predators of nestlings and/or adults themselves. Risks of being trapped inside nest  
411 cavities may have selected for risk-awareness in this and other cavity nesters (e.g.  
412 Leader and Yom-Tov 1998). Moreover, predator models may suffer from rapid  
413 habituation due to immobility and unnaturalness. Thus, we assume that quickness in  
414 returning to the nest is a valuable index of male risk taking. Here, we demonstrate that

415 males respond to the experimental enlargement of nest size by taking higher risks after  
416 human disturbance at their nests as compared with control nests. This was shown by  
417 shorter capture time and a higher frequency of being first to visit the brood after  
418 disturbance, inverting the usual tendency to return to the nest after the mate. Apparently,  
419 males were able to calibrate risk according to the potential value of the brood as  
420 indicated by female quality (Fernandez et al. 2015).

421         Contrary to our prediction, but in agreement with the results obtained by Jelínek  
422 et al., (2015) in the Great Reed Warbler *Acrocephalus arundinaceus*, we found that  
423 parental provisioning rates to the chicks did not respond to nest size manipulation. The  
424 absence of any effect of treatment on nestling morphometric measurements is what we  
425 would expect if both partners do not changed their provisioning effort in response to our  
426 manipulation which was in fact the case. The absence of an association of improved  
427 female condition in the experimental group with higher provisioning rates to nestlings  
428 may be explained by females giving priority to their own immediate survival prospects  
429 rather than to improving offspring condition. However, nuthatch nestlings are brooded  
430 by females at least partly until the age of 16 days (Matthysen 1998), so experimental  
431 females may have used improved condition to increase brooding effort and thereby  
432 benefit nestling physiology instead of in increasing provisioning rates. These  
433 improvements may not have been detectable in nestling mass and size.

434         In summary, this study suggests that nest size constitutes a signal of female  
435 quality in Nuthatches which elicits differential allocation of male resources both to  
436 females themselves and to their broods. Male investment in incubation feeding allows  
437 females to maintain a high antioxidant condition during incubation. Higher risk taking  
438 by mates of strongly signaling females may denote an improvement in offspring

439 survival chances in the field. Females, as the main builders in most avian species, may  
440 express through their nests an extended phenotype which could act as a signal to mates.

441

#### 442 **Compliance with Ethical Standards**

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450 conflict of interest. Irene Saavedra-Garcés declares that she has no conflict of interest.  
451 Juan Moreno declares that he has no conflict of interest.

452 Ethical approval: All applicable international, national, and/or institutional guidelines  
453 for the care and use of animals were followed. Permissions for handling birds were  
454 provided by Consejería de Medio Ambiente de Castilla y León. J. Donés, director of  
455 “Centro Montes de Valsain”, allowed us to work in the study area. The study was  
456 ethically approved by the Ethical Committee of ‘Consejo Superior de Investigaciones  
457 Científicas’ (CSIC) and by the regional administration competent in matters related to  
458 animal protection in research according to Royal Decree 53/2013 (Dirección General de  
459 Producción Agropecuaria y Desarrollo Rural, Junta de Castilla y León).

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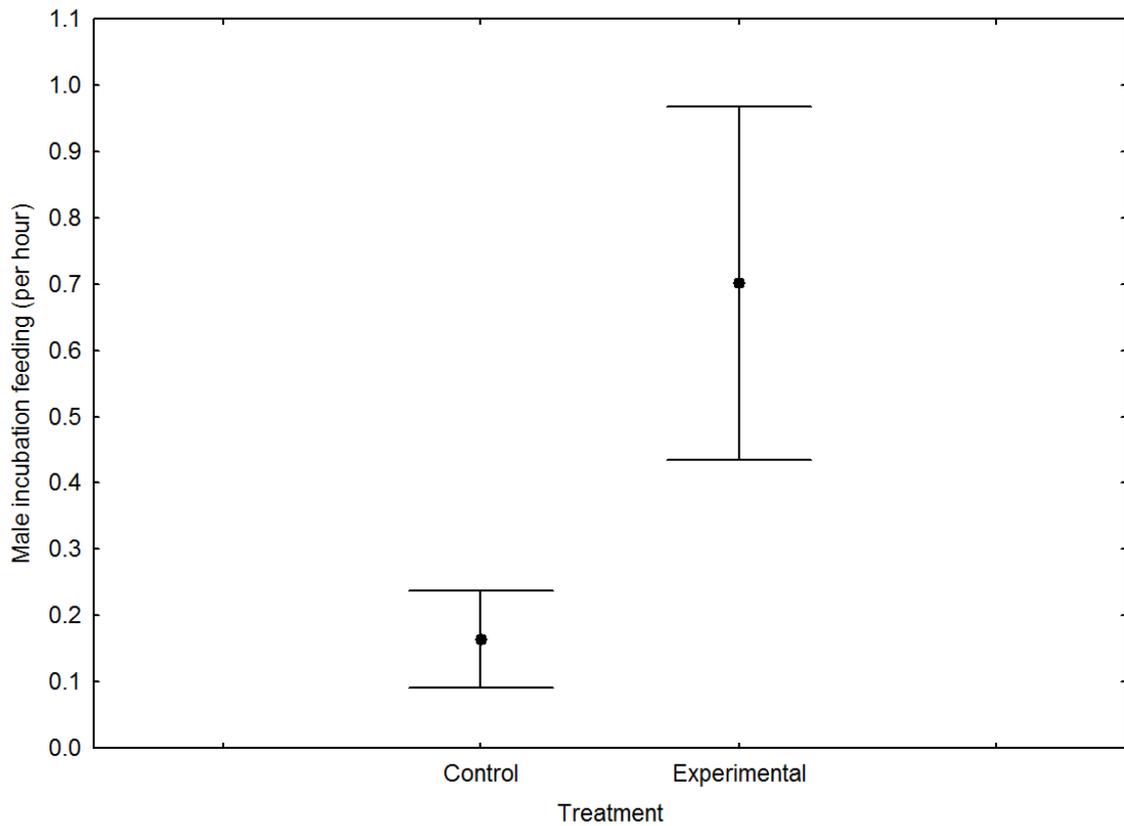
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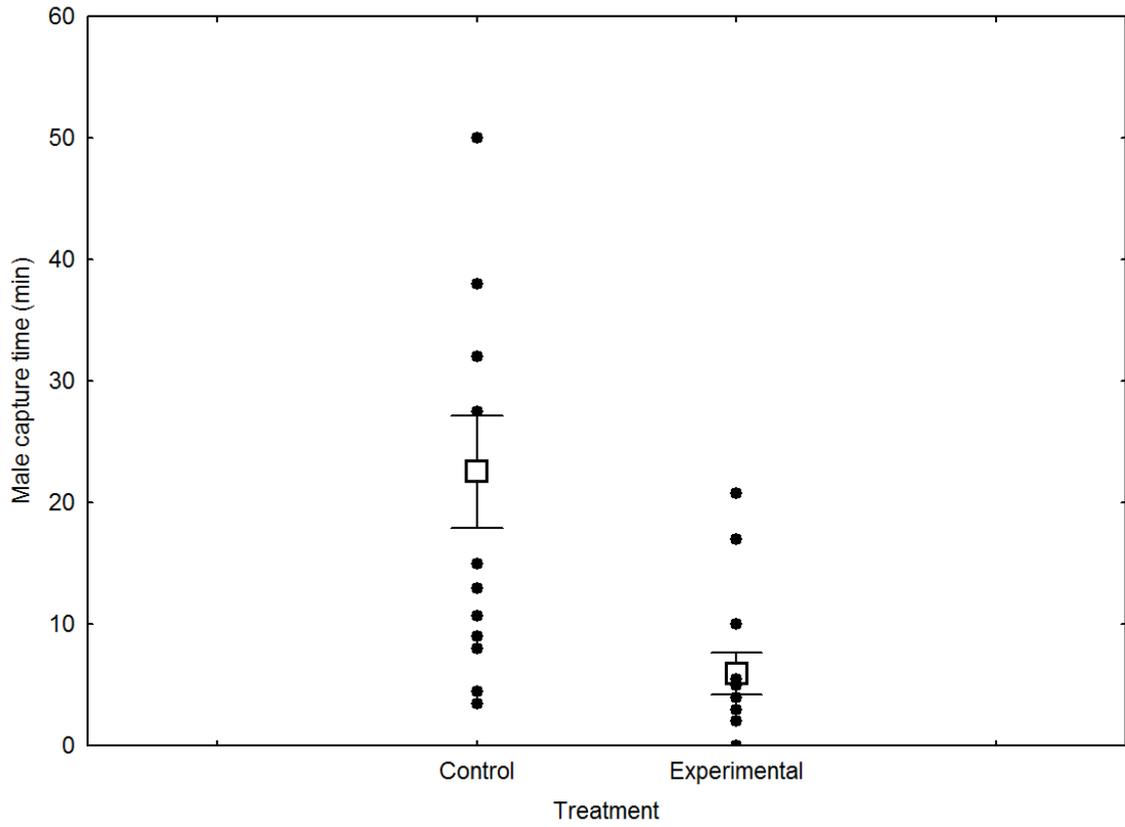
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629 Fig. 1 Differences in provisioning rates of nuthatch male during incubation, comparing  
630 control and experimental nests ( $U = 65.00$ ,  $P = 0.037$ ). Means ( $\pm$  SE,  $\square$ ) and individual  
631 data values ( $\bullet$ ) are shown for each treatment



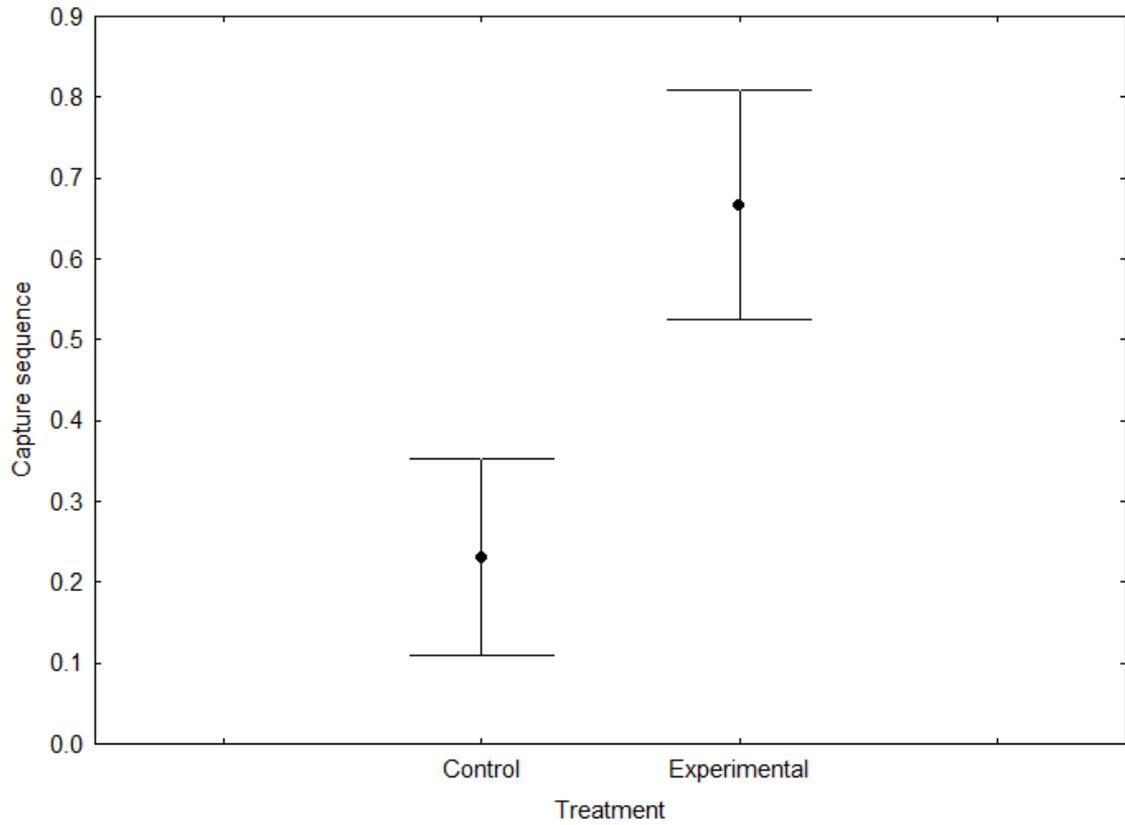
632  
633 Fig. 2 Differences in male capture time, comparing control and experimental nests ( $U =$   
634  $23.00$ ,  $P = 0.002$ ). Means ( $\pm$  SE,  $\square$ ) and individual data values ( $\bullet$ ) are shown for each  
635 treatment



636

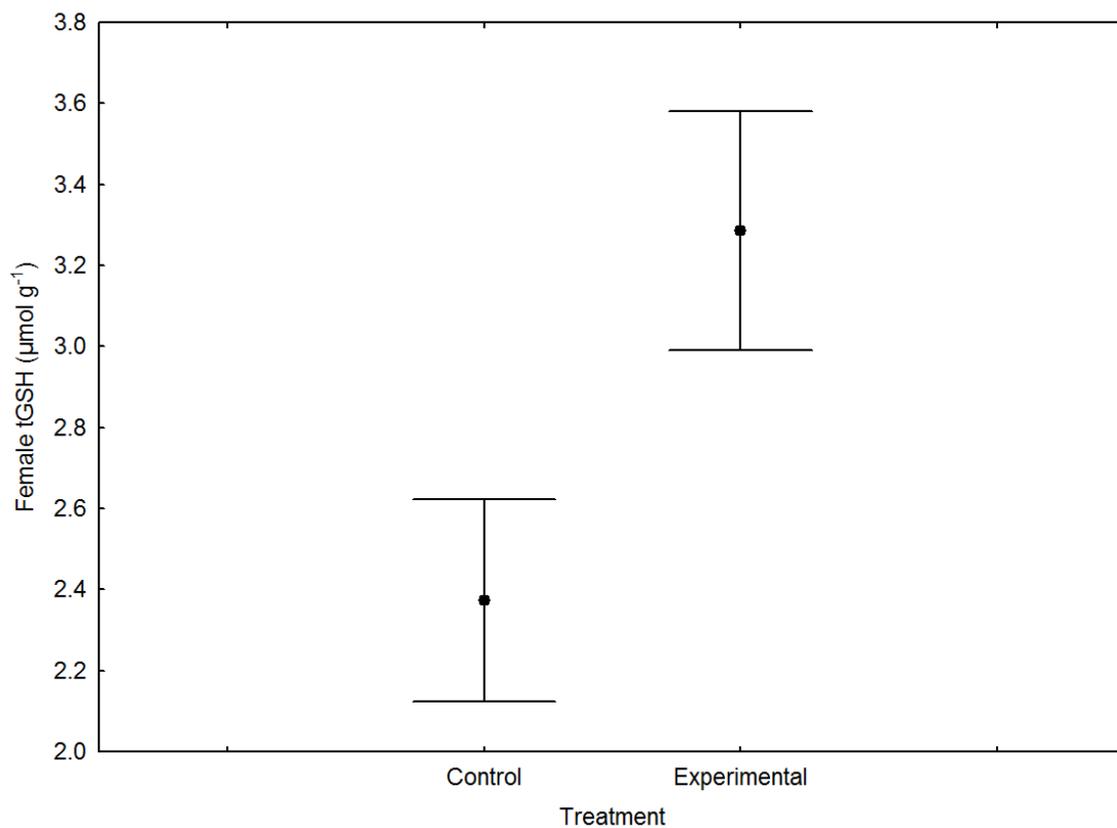
637 Fig. 3 Differences in capture sequence (male was captured at the nest-box before (1) or  
 638 after (0) his mate), comparing control and experimental nests ( $Wald = 4.452, P = 0.035$ ).

639 Means ( $\pm$  SE) are shown for each treatment



640

641 Fig. 4 Differences in total glutathione (tGSH) in red blood cells of females, comparing  
 642 control and experimental nests ( $t_{26} = -2.374$ ,  $P = 0.025$ ). Means ( $\pm$  SE,  $\square$ ) and individual  
 643 data values ( $\bullet$ ) are shown for each treatment



644