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7 Mating success increases alarm calling effort in male fowl, *Gallus gallus*

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17 **WILSON & EVANS: MATING INCREASES ALARM CALLING IN MALE FOWL**

18

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25 Investment in mates and offspring typically reflects a tradeoff between survival and
26 reproductive potential. Individuals should therefore invest according to potential fitness
27 benefit. Males typically cannot ascertain their probability of paternity directly, but this can
28 often be approximated from mating success. In fowl, mating frequency and fitness are both
29 predicted by the rate at which males produce alarm signals. These novel phenomena suggest
30 insights into the evolution of apparently altruistic signals. However, like virtually all studies
31 examining the adaptive value of alarm calling, the relations between behaviour and its
32 possible benefits are correlative. Demonstration of a causal relationship requires
33 experimental evidence. There are two likely models of the relation between alarm calling
34 and reproductive success. Calling, like other costly traits, might be attractive to females.
35 Alternatively, calling might result from recent mating success and hence be a form of mate
36 investment. Here, we test the latter possibility by manipulating the mating success of male
37 fowl. Results show that males increase their alarm calling effort as a function of their recent
38 mating success. This provides strong support for the mate investment hypothesis and is the
39 first demonstration of a causal link between mating frequency and alarm calling.

40

41 *Keywords:* alarm signal; altruistic behaviour; animal communication; antipredator behaviour;
42 mate investment

43 Investing in mates and offspring can be one of the most energetically demanding and
44 time-consuming activities that animals engage in (Clutton-Brock & Godfray 1991). In
45 providing critical resources, such as food, territory, and protection from predators, investors
46 forego additional mating opportunities and risk both injury and death (Clutton-Brock 1991).
47 These costs can be offset by increases in the probability of survival and future reproduction
48 by recipients, but such fitness compensation is contingent upon young or prospective young
49 being genetically related to the investor (Trivers 1972). Optimal investment is especially
50 problematic for males, because paternity is often compromised by mate infidelity, sperm
51 competition, and cryptic female choice (Møller & Birkhead 1993). Mate guarding can be
52 surprisingly ineffective (e.g. Gowaty & Bridges 1991; Kempenaers et al. 1992), and males of
53 only a few species have been shown to be capable of recognizing and investing selectively in
54 their own offspring (Davies & Brook 1989; Davies et al. 1992).

55
56 Given that direct measures of paternity are typically unavailable, decisions about
57 when and how much to invest can only depend upon a proxy (Trivers 1972). A male's first
58 mating affords him the possibility of siring offspring, but the probability of success is less
59 certain. A comparative study involving 52 bird species found that paternal investment was
60 positively associated with certainty of paternity (Møller & Birkhead 1993), as estimated from
61 the relative frequency of extra-pair offspring. Investment strategies can also vary within a
62 species. Female dunnock's (*Prunella modularis*), for example, reside and mate with either
63 one or two males, and males, provided they have mated with the female, provision her
64 chicks with food (Burke et al. 1989). Unlike monogamous males, however, polyandrous
65 males adjust their chick feeding effort according to the share of matings obtained by their
66 competitors during the period in which the eggs are fertilized (Davies et al. 1992).

67

68 Identifying the adaptive utility of alarm calling is a classic problem in behavioural and
69 evolutionary biology. Signallers endanger themselves by warning conspecifics of impending
70 danger, yet they receive no obvious fitness payoff in return (Marler 1955; Sherman 1977;
71 Alatalo & Helle 1990; Wood et al. 2000). Individual selection (Woodland et al. 1980), kin
72 selection (Maynard Smith 1965) and reciprocal altruism (Trivers 1971) have all been invoked
73 as explanations. However, empirical tests of these theories have been complicated by the
74 challenge of quantifying signalling costs and controlling confounding factors in the field, such
75 as the presence and proximity of kin, individual experience, and recent mating history.
76 Furthermore, calling may serve multiple functions, and functions may vary across species
77 and contexts. Most inferences about call function have necessarily been based upon
78 observations of the classes of callers and call recipients (e.g. sex; mating status; parental
79 status; kinship; residency) present during predator encounters (Dunford 1977; Sherman
80 1977; Smith 1978; Schwagmeyer 1980; Woodland et al. 1980; Cheney & Seyfarth 1981;
81 Hoogland 1983, 1996; Blumstein et al. 1997; Haftorn 2000). This approach identifies putative
82 functions of calling based on the conditions conducive to each, but experimental
83 manipulations of the relevant factors are lacking.

84

85 Here we focus on a subset of potential functions, that of warning mates and
86 prospective offspring (Witkin & Ficken 1979). This makes the clear prediction that propensity
87 to alarm call should depend on the signaller's reproductive potential (Trivers 1972).
88 Supporting evidence comes from studies of socially monogamous willow tits (*Parus*
89 *montanus*). During the winter, males are more likely to alarm call if they can see their mate
90 (Hogstad 1995). Willow tits form pair bonds that persist throughout the year, so a male that

91 protects his mate during the non-breeding season increases the probability that she will
92 survive until spring and raise his offspring (Alatalo & Helle 1990; Hogstad 1995). Hogstad
93 (1995) interprets this as evidence for mate investment. While the data are certainly
94 consistent with such a function, they do not exclude an alternative explanation, which is that
95 costly alarm calling (Alatalo & Helle 1990) functions instead to attract females (Zahavi 1975).
96 The precise adaptive value of alarm calling hence remains unclear.

97

98 Fowl (*Gallus gallus*) are an ideal system for examining the function(s) of antipredator
99 signalling. They live in stable social groups for most of the year and both sexes establish
100 pronounced dominance hierarchies. During the breeding season, males defend territories
101 and escort sexually receptive females (McBride et al. 1969). Furthermore, males emit
102 functionally referential alarm calls that indicate the presence of terrestrial and aerial
103 predators (Evans 1997; Evans et al. 1993). Remarkably, the rate at which males emit aerial
104 alarm calls is the single best predictor of their mating success (Wilson et al. In press). This
105 correlation has not been observed in any other species. It thus offers a potential novel
106 insight into the evolution of aerial alarm calling.

107

108 In the present study, we manipulated the mating success of male fowl over a period
109 of several weeks to test for a causal effect on the production of aerial alarm calls. To verify
110 that any change was specific to antipredator signals, we also monitored crowing, an
111 energetically cheap territorial signal. To evaluate whether changes in behaviour were
112 categorical or continuous, we tracked every mating achieved and also measured the number
113 of zygotes fertilized by each male. We predicted that because male fowl are highly
114 polygamous and cannot recognize their own offspring (Ligon & Zwartjes 1995), they should

115 use mating frequency as a proxy for reproductive success and adjust alarm calling effort
116 accordingly.

117

118 **METHODS**

119

120 Subjects were sexually mature fowl (*Gallus gallus*) derived from a population of golden
121 Sebrights that had been interbreeding freely for several generations. This strain has not
122 been artificially selected for rapid growth or egg production and, although morphologically
123 distinct from junglefowl, their behavioural and vocal repertoires are very similar (Kruijt 1964;
124 Collias 1987; Stevens 1991). Fowl are easily manipulated and tolerant of experimental
125 conditions; they have proven to be an ideal system for studies of sexual selection and
126 acoustic signalling (Evans & Marler 1991; Cornwallis & Birkhead 2008; Wilson et al. In press).

127

128 A total of 30 males and 30 females were assigned randomly to mixed-sex pairs. All
129 birds were fitted with numbered leg bands to allow individual identification, and each was
130 tested only once. For four weeks before entering the experiment, individuals were denied
131 physical contact with the opposite sex to standardize their recent mating history and to
132 ensure that female sperm storage tubules were evacuated (Lodge et al. 1971). During this
133 time, hens were placed in a female-only outdoor aviary, and males were housed singly
134 indoors to minimize the effects of social dominance (Parker & Ligon 2002). Birds not being
135 tested were housed in an indoor colony (see Evans & Evans (1999) for details). Immediately
136 before testing, we obtained morphometric data from each male, including body weight,
137 tarsus length, and ornament size (see Wilson et al. (In press) for details).

138

139 Pairs of birds were housed in one of six adjacent outdoor pens (3.5 x 1.5 x 1.5-m l x w
140 x h; Fig. 1*a-c*). Each pen had a dirt substrate to facilitate food searching, scratching, and
141 dustbathing behaviour, a coop at one end that contained straw bedding, opaque walls to
142 prevent visual contact with adjacent pens, and a transparent wire roof that afforded a view
143 of the sky. Partitions between pens were sufficient to attenuate the calls of neighbouring
144 males by approximately 8 dB. All alarm calls recorded were evoked by naturally occurring
145 events, such as birds flying over the birds' pens (see Gyger et al. 1987). A removable
146 partition divided the pen longitudinally (Fig. 1*a, b*) and prevented physical interaction and
147 mating between the male and female occupants. This was constructed of galvanized mesh,
148 which allowed the two birds to view each other and to interact vocally. Food, water, and a
149 perch for roosting were available on both sides of the partition throughout the experiment.

150

151 We adopted a randomized complete block design, in which five cohorts (blocks) were
152 tested sequentially in the six pens between 13 September 2006 and 6 March 2007. This
153 period corresponds to the breeding season of fowl in the southern hemisphere. In each
154 cohort, a pair of birds was assigned at random to each pen (males and females were
155 assigned randomly to opposite sides of the partition) at approximately 1600 h. Data
156 collection began the following morning and continued for 28 days (see Fig. 2*a*). During the
157 first week, the male was prevented from mating so that we could measure his pre-mating
158 calling effort (baseline period; Fig. 2*a, b*). We then manipulated male mating success during
159 weeks two and three by either removing the wire partition to allow mating (experimental
160 group, 3 per cohort, $N_{total} = 15$ males) or leaving it in place to prevent mating (control group,
161 3 per cohort, $N_{total} = 15$ males). Within each cohort, 3 pairs were assigned at random to each
162 treatment. Whenever the partition was removed or installed in the experimental group

163 pens, we did a sham manipulation in the control group pens to control for disturbance.
164 Finally, in week four, we examined the effect of recent mating experience on male calling
165 effort by re-installing the partition and returning all pairs to baseline conditions (Fig. 2*a, b*).
166

167 We recorded each male's vocal behaviour every day for 2 h beginning at local sunrise
168 (determined using Geoscience Australia for latitude: -33° 50' 00" longitude: 151° 15' 00").
169 During this time, both subjects and predators are active, windspeed is low, and
170 anthropogenic disturbance is minimal. Vocalizations from all six males were acquired
171 simultaneously using Behringer C-2 studio condenser microphones (frequency response: 20
172 Hz - 20 kHz; pickup pattern: cardioid) suspended from the centre of each pen roof (Fig. 1*a, b*).
173 Signals were digitized using an 8-channel, 24 bit/96 kHz interface (PreSonus FirePod) and
174 recorded as separate channels within WAVE files (16 bit, 44.1 kHz sampling rate) using Boom
175 Recorder software (version 7.5, VOSGAMES) on a Macintosh computer. A seventh channel
176 comprising a mix of the other six was also created to facilitate subsequent scoring of
177 vocal activity.

178
179 We recorded 56 h of audio from each of the 30 males, totalling 1680 h in all. Raw
180 audio files were processed using automated sound detection software (ISHMAEL, © David K.
181 Mellinger) which used an energy summation algorithm to extract all clips containing sound
182 in at least one channel that exceeded a user-specified detection threshold for > 0.25 s and <
183 2.0 s. To improve selectivity, signal amplitude was calculated from the 700 - 1800 Hz band
184 only. These detection criteria were based on previous measurements of aerial alarm call
185 structure and ensured that all alarm calls were extracted (Bayly & Evans 2003). The resulting
186 files contained all seven channels, plus 0.25 s preceding and following the vocalization(s).

187 Clips were collated according to recording day, and scored using Raven Interactive Sound
188 Analysis Software (version 1.3 Pro, © Cornell Lab of Ornithology Bioacoustics Research
189 Program). Although we specified an extremely sensitive detection threshold (0.02), we
190 confirmed that ISHMAEL had extracted all of the relevant signals by periodically scoring
191 vocalizations from the raw files. In all cases, results were identical.

192

193 Our response variables were the total number of aerial alarm calls and crows
194 produced daily by each male. Crowing is an energetically inexpensive dominance-related
195 signal directed towards other males (Chappell et al. 1995; Horn et al. 1995). We would
196 consequently not expect crowing rate to vary as a function of recent mating experience.
197 Comparisons between these two signals were designed to test the specificity of any
198 detected change in alarm calling and, in particular, to distinguish this from a more global
199 change in vocal activity.

200

201 We scored signals and signallers by simultaneously viewing the six pens' audio
202 channels as scrolling real-time spectrograms (512 sample, 50% overlap, Hamming window),
203 while at the same time listening to the time-locked mix channel at approximately natural
204 amplitude. Females do not produce crows or aerial alarm calls and so were excluded as
205 possible signallers. When only one microphone detected a vocalization, the male in the pen
206 corresponding to that microphone was deemed the caller. When multiple microphones
207 detected the same vocalization, the pronounced amplitude differences between channels
208 corresponding to adjacent pens allowed us to reliably identify the calling male.

209

210 We also obtained an estimate of reproductive potential for each male in the
211 experimental group. A Panasonic colour CCTV security camera (model WV-CF212E) mounted
212 on the back wall of each pen provided a complete view of the interior, which we recorded
213 from 0.5 hours before sunrise until 0.5 hours after sunset using a D-Teg 8-channel digital
214 video recorder (model SRXM5008-DVD, mpeg-4 compression, 12 frames per second, 720 x
215 288 lines of resolution). This corresponded to the entire period in which birds were active.
216 For each male, we scored all copulations achieved during the two weeks in which he had
217 access to the female. This amounted to an average of 201 h of footage for each of the 15
218 males in the experimental group, totalling 3015 h in all. Scoring this volume of footage was
219 made possible by viewing it at 16x normal speed. We confirmed that we could accurately
220 and reliably identify all copulations by periodically scoring footage at the normal speed. In all
221 cases, the results were identical.

222

223 Birds were disturbed as little as possible during the experiment. We entered the pens
224 at the end of each week to collect eggs and replenish food and water. Eggs were
225 subsequently incubated at 38.3°C and 85% relative humidity. Incubation was stopped by
226 chilling at 72 hours and eggs were tested for the presence of an embryo. The total numbers
227 of copulations, number of eggs laid by the female, and number of eggs fertilized by the male
228 provided us with three non-independent estimates of reproductive success.

229

230 We calculated the total number of alarm calls and crows produced weekly by males.
231 For each vocalization, we considered each male's calling effort in week one to be 100%, and
232 expressed calling in subsequent weeks relative to this baseline performance. Calling effort
233 during weeks two and three was then compared between treatments using a repeated

234 measures ANOVA, with cohort as a blocking factor. To check whether differences between
235 treatments in weeks two and three reflected differences in housing conditions, as distinct
236 from the mating success of males, we performed a separate comparison of calling behaviour
237 in week four, when both treatment groups had been returned to baseline conditions and
238 males differed only in their recent mating experience. In all analyses, the data conformed to
239 the parametric assumptions of normality and homoscedasticity.

240

241 For all comparisons, we describe the magnitude of differences between treatments
242 using Cohen's *d*. Effect sizes larger than 0.8 are considered 'large' (Cohen 1988). Finally, we
243 used linear regression to test whether the number of copulations, eggs produced, or number
244 of embryos affected the magnitude of the change in male alarm calling effort. Effect sizes
245 were calculated manually according to Cohen (1988); all other analyses were conducted
246 using SPSS for Mac (software, version 16). Tests were 2-tailed, with an alpha level of 0.05.

247

248

RESULTS

249

250 Treatments effectively manipulated male mating and reproductive success. In the
251 experimental group, males mated an average (\pm SE) of 35.6 (\pm 7.9) times and fertilized an
252 average of 6.0 (\pm 1.5) eggs out of a total of 12.1 (\pm 2.0) eggs laid over the four weeks. Mating
253 frequency and the number of eggs laid were correlated (Pearson correlation: $r_{15} = 0.584$,
254 $p = 0.022$) and together were an excellent predictor of the number of eggs fertilized
255 (multiple regression: $F_{2,12} = 7.682$, $p = 0.007$, $R^2_{\text{adjusted}} = 0.488$). No eggs were fertilized in the
256 control group, although the total number of eggs laid in the control group was very similar

257 (12.5 ± 2.1, mean ± SE) and did not differ significantly from that in the experimental group
258 (unpaired *t*-test: $t_{28} = -0.114$, $p = 0.910$).

259

260 Males permitted to mate increased their alarm calling effort an average of 31.5%
261 relative to control males during weeks two and three (Fig. 3a). This was reflected in a
262 significant treatment effect ($F_{1,20} = 14.211$, $p = 0.001$) of large size (week 2: $d = 1.1$, region of
263 nonoverlap between distributions = 58.9%; week 3: $d = 0.9$, region of nonoverlap between
264 distributions = 51.6%). Importantly, the effect of being allowed to mate was also clearly
265 apparent in week 4, after all males had been returned to identical housing conditions (Fig.
266 3a). Alarm calling in the experimental group was still 20.9% greater than in controls
267 ($F_{1,20} = 9.413$, $p = 0.006$) and effect size was similar to that during the differential treatment
268 phase ($d = 0.9$, region of nonoverlap between distributions = 51.6%).

269

270 In contrast, crowing was unaffected by mating performance (ANOVA: all $p \geq 0.35$;
271 effect sizes: all $d < 0.4$, all regions of nonoverlap between distributions < 27%; Fig. 3b),
272 demonstrating that the effect of mating was specific to alarm calls. Miscellaneous grouping
273 variables, including pen number and the side of the partition that the male was placed in,
274 had no effect on the production of either call type (all $p > 0.05$).

275

276 None of our measures of male reproductive success (number of copulations, eggs
277 laid by the female, and eggs fertilized) predicted the magnitude of increase in alarm calling
278 effort (simple linear regressions: all $p > 0.15$, $R^2 < 0.15$). The experience of mating thus
279 appears to have a categorical effect. Finally, we could find no strong relationship between

280 alarm calling effort and any aspect of male morphology (body weight, tarsus length, and
281 ornament size; simple linear regressions: all $p > 0.25$, $R^2 < 0.1$).

282

283 **DISCUSSION**

284

285 We manipulated the mating and reproductive success of male fowl to examine their effects
286 on alarm call production. In each week, males in the experimental group alarm called almost
287 one-third more than controls, which were prevented from mating (Fig. 3a). Both current
288 access to a receptive hen and recent experience of one were sufficient to induce this effect.
289 This latter comparison is important because the only difference between the groups in week
290 four was previous mating success. Furthermore, increases in alarm calling were specific.
291 Mating had no effect on the rate of crowing (Fig. 3b), allowing us to reject the possibility of a
292 more global increase in vocal activity. Similarly, differences between the treatments do not
293 reflect an audience effect (Karakashian et al. 1988; Evans & Marler 1991, 1992). This is an
294 immediate potentiation of aerial alarm calling in the presence of any adjacent conspecific
295 and hence quite distinct from the much slower divergence between experimental and
296 control groups in the present study (Fig. 3a), both of which effectively had a female audience
297 throughout. We conclude that males selectively increased production of a costly signal in
298 response to the possibility of reproductive success. This provides the first experimental
299 evidence that mating has a causal effect on alarm calling effort.

300

301 Mating frequency and the number of eggs laid by a female were both excellent
302 predictors of male reproductive success, yet alarm calling did not vary in relation to the
303 magnitude of these potential cues. Instead, mating caused a categorical increase in alarm

304 calling effort. This is perhaps surprising, and may reflect the high certainty of paternity
305 afforded by the absence of competitors in this experiment. Nevertheless, our experimental
306 conditions were not unrealistic. Fowl live in social groups consisting of several males during
307 the non-breeding season, but frequently form subgroups containing a single male and one or
308 more females (Collias et al. 1966; Collias & Collias 1967). During the breeding season, males
309 become highly territorial. During this time, flocks disperse and breeding pairs form for the
310 nest building and egg laying periods (McBride et al. 1969). The female access treatment
311 hence reproduced the social conditions under which reproductive success is
312 naturally achieved.

313

314 Discrete investment in alarm calling effort could also reflect well-documented
315 post-copulatory mechanisms that disrupt the link between mating and reproductive success.
316 Females in large social groups, for example, selectively eject the sperm of non-preferred
317 males (Pizzari & Birkhead 2000). Sperm competition (Froman et al. 2002) and differential
318 sperm allocation (Pizzari et al. 2003) further diminish a male's certainty of paternity. Females
319 can store the sperm of preferred males (Brillard 1993; Pizzari & Birkhead 2000), so a single
320 mating may fertilize many eggs or none at all. The probability of extrapair fertilizations could
321 be so high under these conditions that a male would have no way of estimating his
322 probability of paternity and would, instead, invest on the basis of a threshold function
323 reflecting the mere possibility of paternity (i.e. whether or not he had mated at all).

324

325 We suggest two possible mechanisms underlying the observed increase in alarm
326 calling effort. First, calling may reflect endocrine state. Testosterone affects the production
327 of alarm calls in male fowl; that is, calling is abolished by castration and is reinstated by

328 androgen therapy (Gyger et al. 1988). Furthermore, it is well documented that territory
329 establishment, mating, and mate guarding affect testosterone titre in other avian species
330 (Moore 1982; Wingfield et al. 1990). The experience of mating in our study may therefore
331 have caused a hormonal change in males that subsequently elevated alarm call rates. Note,
332 however, that crowing is also testosterone dependent (Marler et al. 1962), yet this was
333 unaffected (Fig. 3*b*). Second, increased alarm calling might reflect cognitive processes. Adult
334 fowl are capable of individual discrimination (Guhl & Ortman 1953; Hauser & Huber-Eicher
335 2004). If males recalled their previous mating experiences, it would be possible for them to
336 invest selectively in individuals with which they had mated. Further experiments will be
337 required to distinguish between these two putative mechanisms.

338

339 The increase in alarm calling effort observed during the breeding season in this study
340 is consistent with the mate investment hypothesis. During the breeding season, dominant
341 males establish territories from which they exclude other males (McBride et al. 1969).
342 Broody females (i.e. those incubating eggs or those accompanied by chicks) reside within
343 these territories, but are unlikely alarm call recipients, as they remain solitary and distant
344 from other adults. In contrast, sexually receptive females are each accompanied closely by a
345 male throughout their nest-building and egg-laying periods, and are hence the probable
346 beneficiaries of mating-induced alarm calls (McBride et al. 1969).

347

348 Our results emphasize the importance of direct benefits in the evolution of alarm
349 signalling (see also Blumstein et al. 1997) and of apparently altruistic behaviour more
350 generally. We acknowledge the possibility that alarm calling in fowl has multiple functions;
351 continued calling by control males suggests strongly that it does. Nevertheless, we believe

352 this to be the first demonstration of a causal relationship between mating success and alarm
353 calling effort in any species.

354

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356

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365

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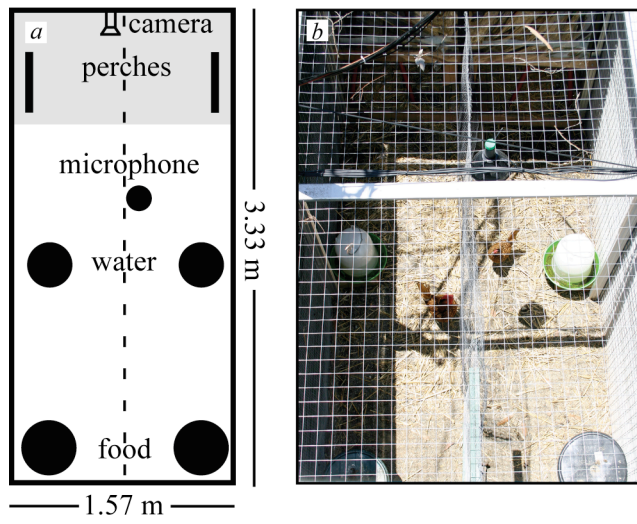
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511

Figure 1.



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514 Figure 1.

515 (a) Schematic and (b) photograph of an experimental pen used to manipulate male mating

516 success. (c) Six adjacent pens were used to test each of five cohorts of birds.

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baseline

treatment

post-treatment

b)

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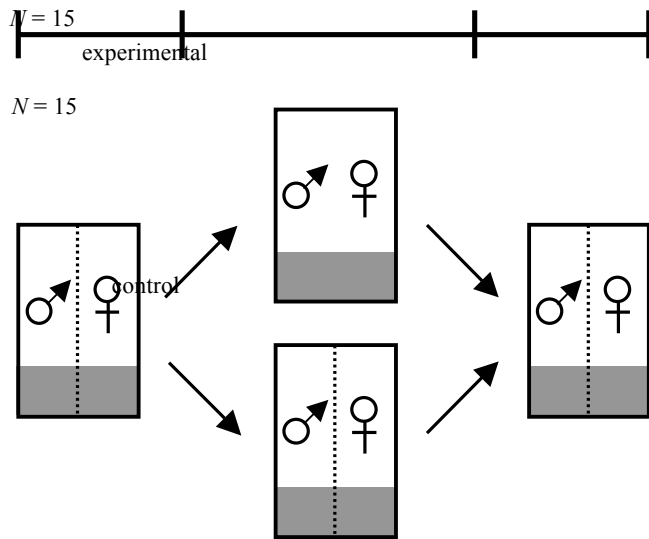
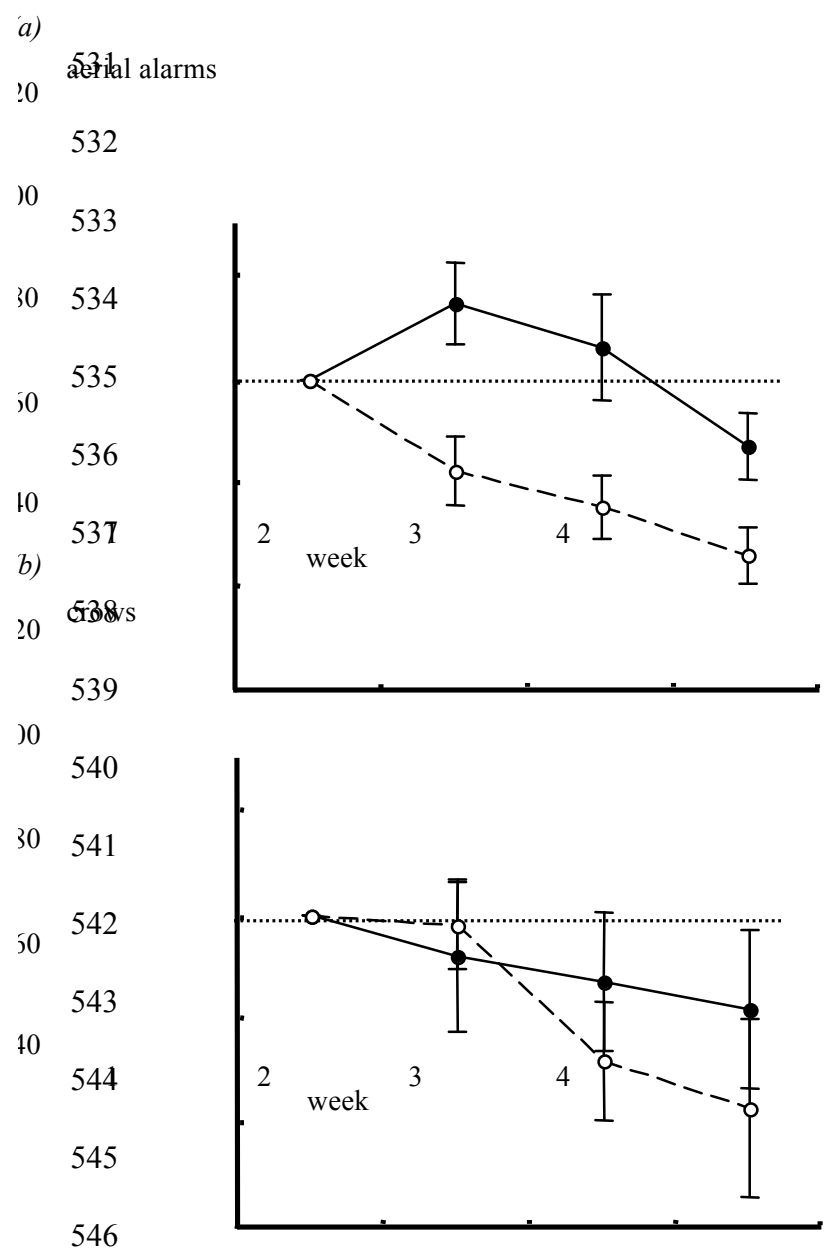


Figure 2.

(a) Timeline and (b) experimental design used to test the male investment hypothesis.



550 Figure 3.

551 Effects of mating on (a) alarm calling and (b) crowing. Shown are both the experimental
 552 treatment (filled circles) and the control treatment (open circles) during the baseline (week
 553 1), treatment (weeks 2 and 3), and post-treatment (week 4) periods.