

Article (refereed) - postprint

Salvante, Katrina G; Dawson, Alistair; Aldredge, Robert A.; Sharp, Peter J.; Sockman, Keith W.. 2013 Prior experience with photostimulation enhances photo-induced reproductive response in female house finches. *Journal of Biological Rhythms*, 28 (1). 38-50. [10.1177/0748730412468087](https://doi.org/10.1177/0748730412468087)

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1 **Prior Experience with Photostimulation Enhances Photo-Induced**
2 **Reproductive Response in Female House Finches: A Potential Basis**
3 **for Age-Related Increase in Reproductive Output**

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20 **Running title:** Photoexperience and reproductive development

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24 **ABSTRACT**

25 In many vertebrates parental age is related to reproductive output with older individuals
26 often performing better (e.g., advanced timing, more offspring) than younger ones. First-
27 year birds differ from older birds in that they lack previous experience with the
28 reproductively-stimulatory effects of long day lengths (photostimulation). The goal of this
29 study was to examine whether this age-related increase in reproductive output can be
30 attributed, at least in part, to previous experience with photostimulation in a
31 photoperiodic bird, the female house finch (*Carpodacus mexicanus*). Specifically, we
32 investigated whether previous experience with photostimulation influences the early
33 stages of reproductive development by quantifying plasma luteinizing hormone (LH),
34 plasma vitellogenin, ovarian follicle size, and immunoreactivity of hypothalamic
35 gonadotropin-releasing hormone (GnRH-I) and vasoactive intestinal polypeptide (VIP).
36 By differentially manipulating photoperiod, we generated two groups of first-year female
37 finches: a photo-experienced group that had been through one photoperiodically-
38 induced cycle of gonadal development and regression, and a photo-naïve group
39 exposed to long days since hatch. Both groups were then transferred from long to short
40 days for nine weeks, to ensure full photoperiodic responsiveness, and then
41 photostimulated for four weeks and exposed to conspecific or heterospecific male song
42 starting 90 minutes before sacrifice. Following photostimulation, although photo-
43 experienced and photo-naïve groups exhibited similar surges in plasma LH
44 concentrations, circulating vitellogenin levels increased in photo-experienced, but not in
45 photo-naïve birds. After four weeks of photostimulation, egg yolk deposition was
46 observed in two of six photo-experienced birds but in none of the photo-naïve birds.

47 After four weeks of photostimulation and exposure to conspecific or heterospecific male
48 song, more GnRH-I-ir cells were seen in the septo-preoptic hypothalamus of photo-
49 experienced than of photo-naïve birds. In contrast, there were no differences between
50 the photo-experienced and photo-naïve birds, irrespective of the song type they were
51 exposed to, in numbers of visible VIP-ir cells in the mediobasal hypothalamus. Our
52 results demonstrate that previous photo-experience enhances some of the early stages
53 of photo-induced reproductive development, and that the reproductive neuroendocrine
54 system of photo-experienced, photoperiodic birds is primed to respond rapidly to
55 reproductively-stimulatory environmental and social cues.

56 **KEYWORDS**

57 Bird song, gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), parental
58 age, photoperiodic history, seasonal breeding, reproduction, vasoactive intestinal
59 polypeptide (VIP), vitellogenin

60

61 INTRODUCTION

62 In most iteroparous animals reproductive output is related to parental age with older
63 individuals performing better than younger ones (Clutton-Brock, 1988; Stearns, 1992).
64 This is most apparent when comparing first-time and reproductively-experienced
65 breeders. For example, in second-year, male European starlings (*Sturnus vulgaris*)
66 testicular maturation is three to four weeks earlier and testicular regression
67 approximately two weeks later than in the same birds in their first year (Dawson, 2003).
68 This results in a longer period of spermatogenic activity and presumably in an earlier
69 seasonal increase in circulating testosterone levels in the older birds. This age-related
70 difference in reproductive function is likely to contribute to the earlier establishment of
71 territories and occupation of nesting cavities by reproductively-experienced males than
72 by first-year males (Feare, 1984). An age-related difference in reproductive function is
73 also seen in female birds, with reproductively-experienced females generally initiating
74 egg production earlier and subsequently laying more, and sometimes larger eggs, and
75 producing more fledglings than first-year females (Saether, 1990; Fowler, 1995).
76 Furthermore, the reproductive output of individual females improves between their first
77 and second breeding attempts (Newton et al., 1981; Hannan and Cooke, 1987;
78 Forslund and Pärt, 1995; Newton and Rothery, 1998).

79 These observations can be explained by two hypotheses (Forslund and Pärt, 1995).
80 The “constraint” hypothesis suggests that individuals breeding for the first time are
81 limited by deficiencies in general life skills, by a slow development of the reproductive
82 system (i.e., age per se), or by lack of breeding experience needed to perfect

83 reproductive behaviors and “prime” the reproductive system to develop more rapidly.
84 This hypothesis predicts that age-related differences in reproductive output are due to
85 the inability of first-year females to match older females’ reproductive physiology and/or
86 behaviours. The “restraint” hypothesis predicts that the resources an individual can
87 afford to allocate to reproduction should increase with age as a consequence of an
88 increase in the rate of the seasonal maturation of the reproductive system, experience
89 of reproductive behavior, and improved life skills. According to this hypothesis, age-
90 related differences in reproductive output are due to first-year females investing fewer
91 resources into their first reproductive attempt than birds that are two years or older. Both
92 of these hypotheses predict that the reproductive potential of first-year females must be
93 limited by factors that do not limit the reproductive potential of second-year and older
94 females.

95 Reproduction in seasonally breeding animals is scheduled to coincide with favorable
96 environmental requirements, such as an ample food supply or nest site availability, to
97 produce offspring (Perrins, 1970). For most temperate zone species, optimal conditions
98 for breeding vary somewhat predictably with season (Wingfield et al., 1992), and
99 consequently, the most reliable cue for initiating the breeding season is the annual cycle
100 of changes in day length (i.e., photoperiod) (Wingfield, 1980, 1983). Temperate-zone
101 birds hatched in the spring and summer are unresponsive to the reproductively-
102 stimulatory effects of long photoperiods (i.e., photorefractory), which prevents
103 premature development of the reproductive system (Farner et al., 1983; Williams et al.,
104 1987a, 1987b, 1989; McNaughton et al., 1992). Exposure to short day lengths during
105 fall and winter dissipates photorefractoriness resulting in the hypothalamus becoming

106 responsive to reproductively-stimulatory photoperiodic and social cues. Increasing
107 daylength in spring accelerates reproductive development (Farner et al., 1983; Follett,
108 1984; Nicholls et al., 1988) by stimulating the release of gonadotropin-releasing
109 hormone (GnRH-I) from the hypothalamus and the consequent increase in secretion of
110 luteinizing hormone (LH) and follicle-stimulating hormone from the anterior pituitary
111 (Sharp and Ciccone, 2005). In females, a photo-induced increase in gonadotropin
112 secretion stimulates ovarian development and production of 17 β -estradiol (E2) and
113 progesterone (Williams, 1998). Elevated circulating E2 stimulates the liver to synthesize
114 and secrete very-low density lipoprotein (VLDL_y), the yolk lipid precursor, and
115 vitellogenin, the yolk protein precursor, which are then taken up by developing ovarian
116 follicles (Bergink et al., 1974; Deeley et al., 1975; Stifani et al., 1988; Walzem, 1996;
117 Williams, 1998).

118 Exposure of photo-sensitive birds to long day lengths also increases the secretion of
119 vasoactive intestinal polypeptide (VIP) from the hypothalamus (Mauro et al., 1992;
120 Chaiseha et al., 1998) to stimulate the production and release of prolactin from the
121 anterior pituitary gland (Mauro et al., 1989; El Halawani et al., 1996; Tong et al., 1997,
122 1998). Increased plasma prolactin plays a role in the onset and maintenance of
123 incubation and parental care (Haywood, 1993; Sockman et al., 2006; Angelier and
124 Chastel, 2009) and in the onset of photorefractoriness, gonadal regression and
125 postnuptial molt at the end of the breeding season (Farner et al., 1983; Nicholls et al.,
126 1988; El Halawani et al., 1997; Dawson and Sharp, 1998; Kuenzel, 2003).

127 A major difference between first-year and older birds is that older birds have had
128 previous experience with photostimulation (photo-experienced) and at least one cycle of

129 photo-induced gonadal development and regression, whereas birds breeding for the
130 first time are at that moment experiencing photostimulation for the first time (photo-
131 naïve).

132 The goal of this study was to examine whether prior experience with photostimulation
133 affects early reproductive development and responses to reproductive cues and thus
134 contributes to the age-related differences observed in reproductive output. Using a
135 photoperiodic bird, the female house finch (*Carpodacus mexicanus*), we investigated
136 whether previous photoexperience, rather than age, per se, influences the rate at which
137 stimulatory environmental cues are integrated into the neuroendocrine signaling
138 pathways that regulate the early stages of photo-induced reproductive development. We
139 designed the experiment based on a similar study examining the contribution of
140 photoperiodic experience to age-related differences in early reproductive development
141 in female European starlings (Sockman et al., 2004). In that study all birds were initially
142 exposed to a short daylength (8h L: 16h D) for 12 weeks to ensure they were fully
143 photosensitive. The photo-naïve group was maintained on short days for an additional
144 20 weeks while the photo-experienced group was transferred to long days for 12 weeks
145 and then back to short days for 8 weeks to induce photosensitivity for the second time.
146 Both groups were then photostimulated. The initial photo-induced increase in plasma
147 LH in the photo-naïve group was 3-fold less than the increase in the photo-experienced
148 group, while the photo-induced increase in plasma vitellogenin in the photo-naïve group
149 was more rapid than in the photo-experienced group. It is possible that the differences
150 in these photoinduced responses may have been a consequence of prolonged
151 photosensitivity of the photo-naïve group. This may have 1) desensitized the pituitary of

152 the photo-naïve group to GnRH, thus dampening the LH response to photostimulation,
153 and 2) increased hepatic storage of vitellogenin, resulting in the more rapid increase in
154 circulating vitellogenin levels after two weeks of photostimulation (Sockman et al.,
155 2004). We designed the present study to avoid these possible problems by maintaining
156 a photo-naïve group in a reproductively quiescent state from hatch by exposure to long
157 days to maintain photorefractoriness. After the induction of photosensitivity by exposure
158 to short days, the first photoperiodic response of the photo-naive group was, therefore,
159 more physiologically comparable to the photo-experienced group than in the earlier
160 starling study. As conspecific song and availability of mates are “supplementary” cues
161 that female songbirds use to fine-tune the timing of early reproductive development
162 (Wingfield, 1980, 1983), we housed all females with males, and during the last day of
163 the study, isolated the females and exposed them to conspecific male song in an effort
164 to maximize reproductive development, using heterospecific male song as a control.

165 We predicted that after photostimulation and exposure to conspecific male song, photo-
166 experienced females would have higher circulating LH and vitellogenin levels, larger or
167 more developed reproductive organs, and more immunocytochemically visible
168 hypothalamic GnRH and VIP neurons than photo-naïve birds. If these predictions are
169 correct, they would be consistent with the view that second year and older finches lay
170 earlier than first year finches, in part, because of their photoperiodic history rather than
171 age per se.

172 **MATERIALS AND METHODS**

173 **Animals and housing**

174 We captured house finches between June and July of 2006 in Chapel Hill, North
175 Carolina (35.91°N 79.05°W) and transferred them into large, outdoor flight cages at the
176 University of North Carolina at Chapel Hill, NC, where we conducted the study. This
177 study was approved by the University's Institutional Animal Care and Use committee
178 (protocol 07-260). For the entire study we provided the birds with food (Daily
179 Maintenance, Roudybush; Woodland, CA) and water ad libitum. We identified hatch-
180 year birds (i.e., new fledglings) by the presence of feather tufts on the head and new,
181 unworn wing feathers (Hill, 2002). On 24 July 2006, we moved all hatch-year birds into
182 large indoor cages on a photoperiod (16h L: 8h D, referred to as long days) that
183 maintained them in a non-reproductive, photorefractory state (Nicholls et al., 1988).
184 Following completion of their annual molt, we identified males and females by the
185 presence or absence, respectively, of yellow plumage on the head and later confirmed
186 *post mortem*.

187 **Photoperiod manipulation**

188 On 21 November 2006 (week 0), we randomly assigned and transferred two females
189 and one male to each of ten light-proof, foam-lined, sound-attenuation chambers
190 located together in one room. Each chamber had a cage with three perches, an air
191 intake and fan-driven exhaust, and a fluorescent light that maintained the chamber-
192 specific photoperiod. We changed the photoperiod to 8h L: 16h D (referred to as short
193 days) in five chambers in order to begin the process of instating sensitivity to

194 reproductive stimuli (Fig. 1). On 16 January 2007 (week 8), we changed the photoperiod
195 in these five chambers to 16h L: 8h D, driving these birds first through a reproductive-
196 like state, and then into a non-reproductive (photorefractory) state (Nicholls et al., 1988)
197 (hereafter referred to as the photo-experienced group) (Fig. 1). Throughout this time, we
198 maintained the original 16h L: 8h D photoperiod in the other five chambers, thereby
199 maintaining the non-reproductive (photorefractory) status of these birds (Nicholls et al.,
200 1988; Williams et al., 1989) (hereafter referred to as the photo-naïve group) (Fig. 1). We
201 spatially interspersed the replicate chambers of both treatments to control for location
202 effects. We exposed the birds to this long-day photoperiod until all of the birds in the
203 photo-experienced group initiated molt, resulting in the photo-experienced group's being
204 exposed to long days for 17 weeks, and the photo-naïve group for 25 weeks. On 16
205 May 2007 (week 25), we moved each triplet group of birds into each of ten cages
206 located together in one room and changed the photoperiod in the room to 8h L: 16h D,
207 thereby beginning the process of instating sensitivity to reproductive stimuli for the first
208 time in the photo-naïve group and for the second time in the photo-experienced group
209 (Fig. 1). On 18 July 2007 (week 34), after exposing all birds to the 8h L: 16h D
210 photoperiod for 9 weeks, we changed the photoperiod in the room to 16h L: 8h D,
211 driving the photo-naïve birds into a reproductive-like state for the first time, and the
212 photo-experienced birds for the second time (Fig. 1).

213 **Body mass measurements and blood sampling**

214 Starting on 21 November 2006 (week 0), we measured the body mass of each bird
215 once every week during the 38-week photoperiod manipulation. We took a blood
216 sample (~150 µl) from each bird at pre-determined time points throughout the study

217 (Fig. 1) to measure temporal variation in circulating levels of LH and vitellogenin. We
218 centrifuged the blood samples to separate the plasma and stored the plasma samples
219 at -20°C until analysis. Only the data for female house finches will be presented here.
220 Some mortality occurred during the 38-week photoperiod manipulation (see Fig. 2).

221 **Song exposure, sacrifice and tissue collection**

222 On the afternoon of 13 August 2007, at week 38 of the study, we weighed seven
223 females (n = 2 photo-experienced; n = 5 photo-naïve) from five cages, moved them
224 individually into each of seven light-proof, sound attenuation chambers (58 x 41 x 36
225 cm, Industrial Acoustics Company, New York, NY, USA) located together in one room,
226 and isolated the birds for one full day. Each chamber was equipped with a cage
227 containing two perches, a food cup, and a water bottle; a fan-driven ventilation system;
228 a light that we used to maintain the 16h L: 8h D photoperiod within the chamber; and a
229 speaker (Pioneer Corp. TS-G1040R, Tokyo, Japan). We powered the speakers by a
230 daisy chain of four mono-block amplifiers interfaced with a computer.

231 Beginning 1 h after the onset of the photophase on 15 August 2007, we exposed one
232 bird to a song set recorded from either male house finches or male northern cardinals
233 (*Cardinalis cardinalis*) (see 'Song recordings used for playback') for 30 min through the
234 chamber's speaker (hereafter termed song treatment). We played the song at
235 approximately 80 dB at 5 cm from the speaker to approximate the amplitude of songs
236 that a free-living bird would experience from a nearby male. Each female heard a
237 unique set of songs from a unique set of male singers (i.e., no male's song was played

238 to more than one female). We staggered exposure to the song treatment by 30 min
239 between females.

240 At 90 min after the onset of the song treatment, we weighed the birds, and after taking a
241 blood sample (~150 μ l) from a brachial vein, rapidly decapitated them and removed
242 their brains. Using previously described protocols (Sockman and Salvante, 2008), we
243 halved each brain using a mid line sagittal cut, fixed one hemisphere (5% acrolein for
244 4.5 hours; alternating left and right hemispheres from successive birds), and stored the
245 fixed hemispheres at -80°C after cryoprotection in 30% sucrose. We recorded the color
246 and diameter of the three largest ovarian follicles from each bird.

247 We repeated these procedures for the remaining females (n = 4 photo-experienced; n =
248 4 photo-naïve) after moving them individually into each of 8 light- and sound-proof
249 chambers on the afternoon of 15 August 2007. By balancing the song treatment levels
250 between subjects from the same photoperiod experience group, we generated four
251 female treatment groups: (1) photo-experienced, conspecific song (n = 3); (2) photo-
252 experienced, heterospecific song (n = 3); (3) photo-naïve, conspecific song (n = 5); and
253 (4) photo-naïve, heterospecific song (n = 4).

254 **Song recordings used for playbacks**

255 We recorded the songs used for playback from free-living male house finches and
256 Northern cardinals in the area surrounding the UNC-Chapel Hill campus using a short-
257 shotgun microphone (Sennheiser ME-66/K6, Wedemark, Germany), connected to a
258 digital recorder (Marantz PMD 660, Mahwah, NJ, USA) set to record uncompressed
259 files sampled at 44.1 kHz. We then selected two songs from each of 24 male house

260 finches and 24 male cardinals using Raven software (v.1.2.1, Cornell Lab of
261 Ornithology). We matched conspecific and heterospecific songs based on individual
262 song duration and created eight sets of duration-matched house finch and northern
263 cardinal songs composed of six songs (two songs from three different males) arranged
264 in a random order such that all six songs were repeated the same number of times
265 within the 30 minutes. All song sets were 30 minutes long and contained a total of 15
266 minutes of song and 15 minutes of silence.

267 **GnRH and VIP immunocytochemistry and quantification**

268 We sectioned the fixed brain hemispheres in the sagittal plane at 40 μm on a cryostat
269 and performed immunocytochemistry (ICC) for GnRH on every third section as
270 previously described by Sockman and colleagues (Sockman et al., 2004). As part of
271 another study, we initially labeled the tissue for ZENK immunoreactivity using a different
272 chromogen. We quenched the tissue with 0.5% H_2O_2 before incubating with a 1:10000
273 dilution of GnRH primary antibody (HU60 bleed H, provided by H.F. Urbanski, Division
274 of Neuroscience, Oregon Regional Primate Center, Beaverton, Oregon). The details of
275 the GnRH antibody have been described previously (Urbanski, 1992). The rabbit-raised
276 GnRH antibody recognizes intact, but not fragmented, forms of GnRH-I and GnRH-II
277 found in birds (Sharp et al., 1990; Sharp and Ciccone, 2005). We processed all of the
278 tissue in two ICC batches. Given the uneven mortality between treatment groups, we
279 counterbalanced the four photoexperience-song treatment groups as much as possible
280 within each ICC batch.

281 We performed ICC for VIP on an alternate set of every third section, as previously
282 described for the transcription factor ZENK (Sockman et al., 2002) except we incubated
283 the sections with VIP primary antibody (Immunostar, Hudson, WI, USA) at 1:10,000
284 dilution for 48 hours at 4°C. We processed all of the sections in two ICC batches,
285 counterbalancing the four photoexperience-song treatment groups within each batch.

286 We conducted all quantification procedures blind to the experimental condition of each
287 subject. Using a Leica DM4000 digital research microscope, we summed the number of
288 GnRH-immunoreactive (GnRH-ir) cells with visible nuclei in the septo-preoptic area
289 between the anterior commissure and the supraoptic decussation of every third-cut
290 section (one or two sections were quantified per subject) under 200x magnification and
291 Köhler illumination. While GnRH-ir cell bodies were not seen in both sections from some
292 birds, GnRH-ir fibers were always present. Although the GnRH antibody recognizes
293 both GnRH-I and -II, only GnRH-I and not GnRH-II cell bodies are present in the septo-
294 preoptic area (Millam et al., 1993; van Gils et al., 1993; Sharp, 2005). Previous studies
295 have found that this region is innervated by central photoreceptors (Saldanha et al.,
296 1994, 2001) and responds to photostimulation with increased fos-like immunoreactivity
297 (Meddle and Follett, 1995, 1997; Millam et al., 2003) and increased GnRH-
298 immunoreactivity (Dawson and Goldsmith, 1997; Péczely and Kovács, 2000; Sockman
299 et al., 2004; Teriuyama and Beck, 2000). We quantified VIP-immunoreactivity (VIP-ir) in
300 every third section of tissue medially from the medial edge of the occipitomesencephalic
301 tract under 400x magnification and Köhler illumination. We counted the number of VIP-
302 ir cell bodies with visible nuclei in four sections through the infundibular nuclear complex
303 (INF) and the ventromedial nucleus (VMN). Previous studies have shown that these

304 areas of the hypothalamus contain dense concentrations of VIP-ir cells and fibers
305 (Yamada et al., 1982; Péczely and Kiss, 1988; Mauro et al., 1989, 1992).

306 **LH and vitellogenin assays**

307 We assayed plasma LH concentrations using a micromodification (Caro et al., 2006) of
308 a homologous chicken LH radioimmunoassay (Sharp et al., 1987) using LH antiserum
309 1/8 at 1:24000 dilution and LH, code AE1a run 4, as iodinated label and standard. The
310 sensitivity of the assay was 0.45 ng/ml at 80% displacement and 1.55 ng/ml at 50%
311 displacement of the iodinated label from the LH antibody. All samples were analyzed in
312 one assay.

313 Plasma samples were assayed for vitellogenin using the zinc method developed for the
314 domestic hen (Zinc kit – Wako Chemicals, Virginia, USA) (Mitchell and Carlisle, 1991)
315 and validated for passerines (Williams and Christians, 1997). This method measures
316 total plasma zinc, and then separates the zinc bound to serum albumen from that bound
317 to vitellogenin and very-low density lipoprotein (VLDL) by depletion of vitellogenin and
318 VLDL from the plasma sample by precipitation with dextran sulfate. The depleted
319 plasma sample is then assayed for zinc. Vitellogenic zinc is equal to the difference
320 between total and depleted zinc; VLDL accounts for only 2% of total plasma zinc
321 (Mitchell and Carlisle 1991). The concentration of vitellogenic zinc is proportional to the
322 plasma concentration of plasma vitellogenin (Mitchell and Carlisle, 1991). Intra- and
323 inter-assay coefficients of variation determined for a laying hen plasma pool were 3% (n
324 = 15 sample replicates) and 7% (N = 16 assays), respectively.

325 **Statistical analyses**

326 Our data consisted of a combination of fixed (e.g., photoexperience, week) and
327 hierarchically-structured random (e.g., individual nested within triplet) effects, each of
328 which may differ from the others in its correlation structure. In addition, some mortality
329 occurred during the 38-week study, rendering our dataset unbalanced. Therefore, we
330 analyzed these data in a mixed, multilevel modeling framework using the software Stata
331 IC 10.0 for the Macintosh (Stata Corporation, College Station, TX), which readily
332 accommodates unbalanced, hierarchically-structured combinations of fixed and random
333 effects (Burton et al., 1998; Goldstein et al., 2002; Rabe-Hesketh and Skrondal, 2005).
334 We used Stata's command for multilevel mixed-effects linear regression but, for the
335 GnRH-ir and VIP-ir cell count data, we instead used the command for multilevel mixed-
336 effects Poisson regression because count data tend to follow Poisson distributions.
337 These models estimated parameters with restricted maximum likelihood and used z-
338 tests to test the null hypothesis that a coefficient equaled zero. For more information on
339 the rationale for and approach to mixed, multilevel modeling frameworks, see Sockman
340 et al. (2008).

341 For analyses of GnRH-ir and VIP-ir, we counted the number of GnRH-ir or VIP-ir cell
342 bodies and used photoexperience, song treatment, and their interaction as fixed factors,
343 with observation (individual bird) nested within triplet as a random intercept and as a
344 random coefficient for song treatment. For analyses of body mass and circulating LH
345 and vitellogenin levels, we used photoexperience, week and their interaction as fixed
346 factors and nested observation (the individual bird's measurement that week) within
347 female as a random coefficient for week and nested female within triplet as a random

348 intercept. For ovarian follicle size, we used photoexperience, female body mass, follicle
349 order (from most to least developed) and the interaction between photoexperience and
350 follicle order as fixed factors and nested observation (the individual bird's measurement
351 of an individual follicle) within female as a random coefficient for follicle order and
352 nested female within triplet as a random intercept. As female body mass differed
353 between photoexperience groups at the end of the study, it was included as a covariate
354 to control for differences in ovarian follicle size due to body mass alone (Sockman et al.,
355 2004). For comparison of circulating LH levels in the two groups during the different
356 rounds of photostimulation (photo-experienced: first and second rounds of
357 photostimulation; photo-naïve: first round of photostimulation), we used
358 photoexperience, number of weeks exposed to long days, their interaction and the
359 interaction between photoexperience and round of photostimulation (i.e., first or second)
360 as fixed factors and nested observation (the individual bird's measurement that week)
361 within female as a random coefficient for week and nested female within triplet as a
362 random intercept. For comparison of circulating vitellogenin levels in the two groups
363 during their first rounds of photostimulation, we used photoexperience, number of
364 weeks exposed to long days and their interaction as fixed factors and nested
365 observation (the individual bird's measurement that week) within female as a random
366 coefficient for week and nested female within triplet as a random intercept. The nesting
367 structure we used for random effects follows the approach recommended by Schielzeth
368 and Forstmeier (2009).

369 **RESULTS**

370 **Body mass**

371 At the start of the study the two groups of female house finches had the same body
372 mass (week 0: $p > 0.5$; Fig. 2), but this changed during the initial 8 week photoperiodic
373 treatment period (weeks 2-8: photoperiodic treatment \times week: $z = -2.05$, $p < 0.05$; Fig.
374 2). While the body mass of birds that had experienced changing photoperiod did not
375 change during the 8 week exposure to short days (weeks 2-8: week: $p > 0.4$), it
376 increased in photo-naïve females retained on long days (weeks 2-8: week: $z = 2.38$, $p <$
377 0.02) (Fig. 2). However, body mass did not differ between photo-experienced and
378 photo-naïve females during the following photoperiodic treatment period when both
379 groups were exposed to long days (weeks 9-12: all $p > 0.1$; weeks 9-25: all $p > 0.2$) and
380 subsequently exposed to short days (weeks 26-34: all $p > 0.1$) (Fig. 2). After both
381 groups were returned to long days, photoperiodic treatment affected body mass over
382 the last four weeks of the study (weeks 35-38: photoperiodic treatment \times week: $z = 2.31$,
383 $p < 0.03$; Fig. 2). During this period, the body mass of photo-experienced females
384 increased (weeks 35-38: week: $z = 2.49$, $p < 0.02$) while that of photo-naïve females,
385 which were being photostimulated for the first time, did not change (weeks 35-38: week:
386 $p > 0.7$; Fig. 2).

387 **GnRH and VIP immunoreactivity**

388 Photoperiodic experience influenced the way in which song treatment affected GnRH-ir
389 in the hypothalamic septo-preoptic area of female house finches (photoexperience \times
390 song treatment: $z = 3.73$, $p < 0.001$; Fig. 3). Within the photo-experienced group,

391 females exposed to conspecific song had more GnRH-ir cells than females exposed to
392 heterospecific song (song treatment: $z = 3.35$, $p < 0.001$; Fig. 3). The opposite was true
393 for the photo-naïve group; females exposed to conspecific song had fewer GnRH-ir
394 cells than females exposed to heterospecific song (song treatment: $z = -5.17$, $p < 0.001$;
395 Fig. 3). Furthermore, within the group of females exposed to conspecific song, the
396 photo-experienced females had more GnRH-ir cells than photo-naïve females
397 (photoexperience: $z = 3.95$, $p < 0.001$; Fig. 3). Again, the opposite was true for the
398 females exposed to heterospecific song; photo-experienced females had fewer GnRH-ir
399 cells than photo-naïve females (photoexperience: $z = -2.08$, $p < 0.04$; Fig. 3).

400 Neither photoexperience nor song treatment nor their interaction influenced the number
401 of VIP-ir cells in the INF (Fig. 4a) or the VMN (Fig. 4b) of the hypothalamus (all $p > 0.3$).
402 Even when song treatment and the interaction between photoexperience and song
403 treatment were removed from the model, VIP-ir cell count in the INF and VMN were not
404 related to photoexperience (both $p > 0.1$).

405 **Plasma LH and vitellogenin**

406 At the beginning of the study and at the end of the 8-week short day photoexperience
407 treatment, both groups of females had similar, low levels of circulating LH (weeks 0 and
408 8: both $p > 0.5$; Fig. 5). Photoexperience determined the way circulating LH levels
409 changed during the four weeks immediately following photoexperience treatment
410 (weeks 9-12: photoexperience \times week: $z = -10.34$, $p < 0.001$; Fig. 5). During this time
411 plasma LH levels remained low in photo-naïve females, who maintained their non-
412 reproductive state (weeks 9-12: week: $p > 0.8$; Fig. 5). In contrast, plasma LH levels in

413 photo-experienced females photostimulated for the first time increased almost 15-fold
414 after one week of exposure to long days and declined to levels that were still 5-fold
415 higher than those of photo-naïve females after four weeks on long days (weeks 9-12:
416 week: $z = -10.55$, $p < 0.001$; Fig. 5). Plasma LH levels were low in both groups at week
417 25 (photoexperience: $p > 0.3$) and week 34 (photoexperience: $p > 0.7$) of the study,
418 when both groups of females were in a non-reproductive state (Fig. 5).

419 During the last four weeks of the study, both groups were photostimulated, and
420 circulating LH levels in both groups increased almost 15-fold after exposure to long
421 days for one week, and then declined approximately 3-fold by the end of the study
422 (weeks 35-38: week: $z = 7.51$, $p < 0.001$; photoexperience and photoexperience x
423 week: $p > 0.9$; Fig. 5). This photo-induced surge in LH was similar to the LH surge
424 observed in photo-experienced females undergoing photo-induced early reproductive
425 development for the first time (photo-experienced weeks 9-12 vs. photo-experienced
426 weeks 35-38 vs. photo-naïve weeks 35-38: weeks exposed to long days: $z = -7.76$, $p <$
427 0.001 ; photoexperience and photoexperience x weeks exposed to long days (1 through
428 4): $p > 0.5$, photoexperience x round of photostimulation: $p > 0.8$; Fig. 5).

429 Both groups of females had similar, low levels of circulating vitellogenin at the beginning
430 of the study (Week 0: $z = -1.53$, $p > 0.1$) and at the end of the 8-week photoperiodic
431 treatment (Week 8: $z = 1.00$, $p > 0.3$) (Fig. 6). Plasma vitellogenin levels also did not
432 differ between the groups in the four weeks following photoperiodic treatment, despite
433 the fact that photo-experienced females were undergoing photostimulation for the first
434 time and the photo-naïve females remained in a non-reproductive state (Weeks 9-12: all
435 $p > 0.3$; Fig. 6). There was, however, one photo-experienced female that had elevated

436 vitellogenin levels after one week of exposure to reproductively-stimulatory long days,
437 but her vitellogenin levels were undetectable in the following week (Fig. 6). Similar to
438 plasma LH, circulating vitellogenin levels were low in both groups at week 25
439 (photoexperience: $p > 0.6$) and week 34 (photoexperience: $p > 0.2$) of the study, when
440 both groups of females were in a non-reproductive state (Fig. 6).

441 When both groups were returned to long days, photoexperience affected how plasma
442 vitellogenin changed over the last four weeks of the study (weeks 35-38:
443 photoexperience \times week: $z = 2.00$, $p < 0.05$; Fig. 6). Photo-experienced females
444 undergoing photo-induced early reproductive development for the second time exhibited
445 an increase in circulating vitellogenin levels over the last four weeks of the study
446 (Weeks 35-38: week: $z = 2.18$, $p < 0.03$; Fig. 6). In contrast, the vitellogenin levels of
447 females in the photo-naïve group, who were undergoing photostimulation for the first
448 time, did not change during this time (Weeks 35-38: week: $p > 0.5$; Fig. 6). This pattern
449 was similar to that of photo-experienced females during their first round of
450 photostimulation (photo-experienced weeks 9-12 vs. photo-naïve weeks 35-38:
451 photoexperience: $p > 0.1$; weeks exposed to long days: $p > 0.8$; interaction $p > 0.1$; Fig.
452 6).

453 The effect of photoexperience on changes in vitellogenin levels was also apparent
454 within individual females. The number of times photo-experienced females were
455 photostimulated affected how vitellogenin changed over the first four weeks on long
456 days (photo-experienced: weeks 9-12 vs. weeks 35-38: round of photostimulation: $z = -$
457 2.05 , $p < 0.05$; number of weeks exposed to long days: $z = -2.06$, $p < 0.05$; interaction: z
458 $= 2.06$, $p < 0.04$; Fig. 6). As mentioned above, vitellogenin levels were relatively stable

459 and low while photo-experienced females were undergoing photostimulation for the first
460 time and, in contrast, increased steadily during the second round of photostimulation.

461 **Follicular development**

462 Photo-experienced females undergoing photo-induced early reproductive development
463 for the second time had larger ovarian follicles than photo-naïve females being
464 photostimulated for the first time (photoexperience: $z = 1.97$, $p < 0.05$; Fig. 7a). The
465 photo-experienced group exhibited large inter-individual variation in ovarian follicle
466 diameter that can be explained by variation in female body mass (body mass: $z = 3.91$,
467 $p < 0.001$). When ovarian follicle diameters were adjusted for body mass, the marked
468 inter-individual variation in the follicle diameter of photo-experienced females decreased
469 (Fig. 7b). By the end of the study, following four weeks of photostimulation, two of the
470 six photo-experienced females had yellow ovarian follicles that had begun to take up
471 yolk, and one of these females laid an egg on the last day of the study. In contrast, none
472 of the nine photo-naïve females had any yellow, yolky follicles.

473 **DISCUSSION**

474 When we exposed female house finches to reproductively-stimulatory long day lengths,
475 we found that females with previous experience with photostimulation (photo-
476 experienced females) had greater increases in body mass, more GnRH-ir cells in the
477 septo-preoptic hypothalamus , greater increases in plasma vitellogenin levels and more
478 pronounced growth and development of ovarian follicles than age-matched females that
479 were photostimulated for the first time (photo-naïve females). In contrast, we did not

480 observe an effect of prior experience with photostimulation on photo-induced circulating
481 LH levels nor VIP immunoreactivity in the hypothalamic INF or VMN . Additionally,
482 photoexperience influenced the effects of song exposure on GnRH-ir in the
483 hypothalamic septo-preoptic area, with conspecific song elevating GnRH-ir cell count in
484 photo-experienced but reducing GnRH-ir cell count in photo-naïve females. Our results
485 suggest that previous photoexperience sensitizes the neuroendocrine system to the
486 reproductively-stimulatory effects of increasing photoperiod and changes the way the
487 neuroendocrine system responds to the supplementary cue of male song. These effects
488 of photoexperience may be responsible, at least in part, for the age-related advance in
489 the early stages of reproductive development.

490 **Body mass**

491 The photo-induced increase in the body mass of photo-experienced female house
492 finches observed over the last four weeks of the study is consistent with the increase in
493 body mass associated with early reproductive development and egg production.
494 Sockman and colleagues observed similar photoexperience-dependent patterns of
495 changes in body mass in female European starlings (Sockman et al., 2004). The one
496 gram difference in body mass between photo-experienced and photo-naïve females in
497 our study was likely due to the additional mass of the newly re-grown ovary, the larger
498 and more developed ovarian follicles of photo-experienced females, the yolk deposited
499 into the largest of the follicles in two of the photo-experienced females, and the recently
500 re-grown oviduct of the photo-experienced female that laid an egg on the last day of the
501 study (Vézina and Salvante, 2010). Egg-producing female passerines of similar size to
502 house finches display similar gains in body mass above non-breeding values (e.g.,

503 great tits, *Parus major* (Silverin, 1978); pied flycatchers, *Ficedula hypoleuca* (Ojanen,
504 1983); zebra finches, *Taeniopygia guttata* (Salvante et al., 2010)).

505 **VIP**

506 We did not find an effect of photoexperience on VIP-ir in the INF or the VMN of the
507 hypothalamus following four weeks of concurrent photostimulation. Similarly, compared
508 to levels measured while exposed to short days, previously photo-naïve, male European
509 starlings showed no change in basal hypothalamic VIP levels in response to
510 photostimulation (measured every two weeks through week 8, then every four weeks
511 through week 24 of exposure to long days) (Dawson et al., 2002). It is possible that the
512 four weeks of photostimulation in our study was long enough for any photoexperience-
513 related differences in the timing of up-regulation of VIP expression to disappear. Only
514 two weeks of photostimulation was enough to trigger a significant increase in VIP-ir cell
515 count in the INF of turkeys in their second reproductive season (Mauro et al., 1989,
516 1992). However, as VIP and prolactin play regulatory roles later in the breeding season
517 during incubation and chick rearing, it is also possible that four weeks of
518 photostimulation was not sufficient to detect significant photo-induced increases in VIP
519 or photoexperience-related differences in VIP expression. VIP-ir cell count in the INF of
520 the reproductively-experienced turkeys mentioned above, continued to increase after 10
521 days of photostimulation through egg laying, incubation and into photorefractoriness
522 (Mauro et al., 1989, 1992). Similarly, while ten days of exposure to a 16h L: 8h D
523 photoperiod was sufficient to stimulate an increase in nascent VIP mRNA in the
524 hypothalamus of female turkeys, significant increases in both steady-state cytoplasmic
525 VIP mRNA levels and VIP levels in the median eminence were not apparent until the

526 birds laid their first eggs (Chaiseha et al., 1998). Therefore, we may have measured
527 VIP-ir too late to observe any influence that photoexperience may have had on the
528 timing of the onset of VIP expression, but too early to detect any photoexperience-
529 related differences in the extent of VIP expression. In contrast, the photo-experienced
530 male house finches in the present study had more VIP-ir cells in the INF than the photo-
531 naïve males (unpublished data; K.G. Salvante, R.A. Aldredge, K.W. Sockman). This sex
532 difference supports previous research showing early up-regulation of reproductive
533 hormones in male birds (Caro et al., 2006), in contrast to more fine-tuned
534 synchronization between local breeding schedules (i.e., timing of egg production and
535 laying) and the up-regulation of reproductive hormones in female birds (Caro et al.,
536 2009).

537 **GnRH**

538 The elevated photo- and conspecific song-induced GnRH expression observed in
539 photo-experienced females suggests that previous experience with photostimulation
540 “primes” the brain to increase its responsiveness to socially-relevant environmental
541 stimuli during subsequent breeding seasons. In photostimulated European starlings
542 prior photoexperience increases the responsiveness of specific brain regions involved in
543 song perception (Sockman and Ball, 2009). Similarly, photorefractory adult European
544 starlings with prior photoexperience, and thus prior exposure to elevated levels of
545 GnRH, exhibited a larger LH response following exogenous GnRH administration than
546 photorefractory juveniles that have not been previously exposed to elevated GnRH
547 levels (McNaughton et al., 1995). Moreover, there is evidence in mammals that GnRH
548 up-regulates its own receptors (Clayton, 1989). Therefore, if the priming effect of GnRH

549 on its own receptors persists until the next breeding season, the age-related advance in
550 laying date observed in many birds (Saether, 1990; Fowler, 1995) may be due, at least
551 in part, to previous experience with photostimulation and the associated exposure to
552 elevated GnRH levels.

553 While the priming effect of previous exposure to elevated GnRH explains the patterns of
554 GnRH-ir observed in photo-experienced versus photo-naïve females and females
555 exposed to conspecific song, it does not explain the other effects of photoexperience
556 and song treatment on GnRH-ir. Why did photo-naïve females exposed to
557 heterospecific song have more GnRH-ir cells than photo-experienced birds exposed to
558 heterospecific song? During the four-week period of simultaneous photostimulation of
559 the photo-experienced and photo-naïve females (weeks 35-38), both groups of females
560 were exposed to conspecific song from the co-housed male house finches. As the
561 photo-experienced females exhibited more advanced early reproductive development at
562 the end of the study than the photo-naïve females, the photo-experienced females were
563 temporally closer to having to choose a mate, and thus may have been more sensitive
564 to changes in social cues conveying information about the availability or quality of
565 potential mates. Therefore, it is possible that the removal of the more socially-relevant
566 conspecific song and/or the introduction of the less socially-relevant heterospecific song
567 as the females' only auditory signal triggered the down-regulation of GnRH levels within
568 the septo-preoptic area of the more reproductively-advanced photo-experienced
569 females compared to the photo-naïve females. European starling females exposed to
570 one week of preferred long-bout conspecific male song and then subsequently exposed
571 to a 30 minute song stimulus of less-preferred, short-bout, conspecific song exhibited a

572 decrease in expression of the immediate early gene ZENK (the avian homolog of and
573 an acronym for zif-268, egr-1, NGFI-A, and Krox-24) in the auditory telencephalon
574 compared to females that were exposed to a 30 minute song stimulus of preferred long-
575 bout conspecific song (Sockman et al., 2005). The decrease in GnRH and ZENK activity
576 in these instances, respectively, may represent a decrease in attraction to or preference
577 for the secondary auditory signals to which these females were exposed (Sockman,
578 2007).

579 **Circulating LH**

580 Following nine weeks on short photoperiods, exposure to long day lengths for only one
581 week induced a similar surge in circulating LH levels in both photo-naïve females
582 undergoing photostimulation for the first time and photo-experienced females
583 undergoing photostimulation for the second time. This surge was similar to the LH surge
584 observed in photo-experienced females undergoing photostimulation for the first time at
585 the beginning of this study and in photo-experienced European starlings undergoing
586 photostimulation for the second time (Sockman et al., 2004). Photo-experienced
587 European starlings undergoing photostimulation for the first time also had similar levels
588 of circulating LH after one week on long days as all of the photostimulated females in
589 our study (Sockman et al., 2004). Interestingly, the marked photo-induced LH surge was
590 absent in photo-naïve starlings exposed to reproductively-stimulatory long days for the
591 first time (Sockman et al., 2004). That study suggested that the lack of LH response
592 may have been due to desensitization of the pituitary to GnRH by negative feedback of
593 chronic, low-level gonadal steroid activity associated with the prolonged time (32 weeks)
594 that photo-naïve females spent on a 8h L: 16h D photoperiod. In contrast, our photo-

595 naïve females only spent nine weeks exposed to short day lengths and did respond to
596 photostimulation with a surge in LH. Therefore, the lack of an effect of photoexperience
597 on the LH response to photostimulation suggests that LH may not play a direct role in
598 the physiological mechanisms underlying age-related variation in early reproductive
599 development. However, as the up-regulation of LH receptors is hormone-dependent
600 (Piquette et al., 1991; Segaloff et al., 1991; You et al., 2000; Johnson and Bridgham,
601 2001; Johnson and Woods, 2009), if the expression of these regulatory hormones is
602 dependent on photoexperience, then LH activity may also vary with photoexperience
603 and potentially contribute to the age-related variation observed in early reproductive
604 development.

605 **Circulating vitellogenin and follicular development**

606 Photoexperience influenced both circulating levels of the egg yolk precursor,
607 vitellogenin, and the timing of yolk deposition into developing ovarian follicles. In
608 passerine birds, the onset of vitellogenin production is tightly coupled with the onset of
609 follicular yolk deposition (Challenger et al., 2001; Salvante and Williams, 2002). As both
610 groups of females had relatively stable and low vitellogenin levels during the first four
611 weeks of their first round of photostimulation, this relationship suggests that neither
612 group had begun follicular yolk deposition during this time. This, together with the
613 elevated plasma vitellogenin levels and the presence of an egg and yolky follicles in
614 photo-experienced females, but not in age-matched photo-naïve females by the end of
615 the study, is consistent with the hypothesis that photoexperience influences the age-
616 related advancement of egg production and laying date.

617 One major difference between the two groups at the end of the study was that the
618 photo-experienced females had previously been exposed to elevated circulating levels
619 of E2 during their first experience with photostimulation and gonadal development.
620 Primary exposure of the avian liver to E2 induces genomic changes to the regulatory
621 sites of the genes coding for vitellogenin and apoVLDL-II, the VLDL_y-specific surface
622 protein, including demethylation of the E2-receptor complex binding site at the 5' end of
623 the vitellogenin gene and changes to the chromatin of the vitellogenin and apoVLDL-II
624 genes resulting in nuclease-hypersensitive sites (Wilks et al., 1982; Burch and
625 Weintraub, 1983; Kok et al., 1985). These and other E2-induced genomic changes may
626 contribute to the earlier induction and more rapid synthesis of vitellogenin and
627 apoVLDL-II mRNA and circulating vitellogenin and VLDL_y following secondary estrogen
628 exposure (Bergink et al., 1973, 1974; Jost et al., 1978; Codina-Salada et al., 1983;
629 Wang and Williams, 1983; Jost et al., 1986). Previous exposure to elevated levels of E2
630 may also contribute to the advance in egg formation in photo-experienced females via
631 the stimulatory effect that E2 has on the synthesis of its own receptors (Sutherland and
632 Baulieu, 1976; Cidlowski and Muldoon, 1978) and DNA polymerase activity (Sutherland
633 et al., 1977) in the avian oviduct. Secondary estrogen administration has also been
634 associated with rapid increases in both nuclear binding of progesterone receptor (Boyd-
635 Leinen et al., 1984) and ovalbumin mRNA transcription (Swanek et al., 1979) in the
636 avian oviduct. If these priming effects of E2 persist until the next breeding season, they
637 could contribute to the advancement of oviduct growth and development, egg formation
638 and laying date in photo-experienced females.

639 We have found that female house finches with prior photoexperience exhibited
640 advanced early reproductive development in comparison with age-matched birds with
641 no prior experience with reproductively-stimulatory long days. Yet, our results do not
642 favor one or the other of Forslung and Pärt's (1995) "constraint" or "restraint"
643 hypotheses. The hormonal mechanisms underlying these differences, including the
644 potential priming effects of GnRH and E2, suggest that first-time breeders may indeed
645 be constrained by their lack of previous exposure to these reproductive hormones, thus
646 supporting Forslund and Pärt's "constraint" hypothesis (Forslund and Pärt, 1995).
647 However, our results do not disprove Forslund and Pärt's "restraint" hypothesis, as we
648 do not know whether first-year females have decided to invest fewer resources into their
649 first breeding attempt to offset the lower reproductive potential associated with initiating
650 early reproductive development and egg laying later than in future breeding attempts.
651 While the lower plasma vitellogenin levels, decreased neural responsiveness to socially-
652 relevant environmental stimuli, and delayed early reproductive development we
653 observed in photo-naïve females photostimulated for the first time may reflect
654 physiological and neural constraints, they may also be components of the mechanisms
655 underlying the females' decision to invest fewer resources into their first reproductive
656 attempt. Regardless of whether the constraint, restraint or both hypotheses are true, our
657 results suggest that photoexperience, and not age, per se, may, at least in part, explain
658 the advancement in laying date and enhanced reproductive output observed in older
659 seasonally-breeding birds compared to first-year females (Saether, 1990; Fowler,
660 1995).

661 **ACKNOWLEDGEMENTS**

662 We thank Kendra B. Sewall, Danielle M. Racke and C. Ryan Campbell for their help
663 with data collection; Sachi Vora, Katie Suppler, Kristina Simmons, and Adam Byerly for
664 their help with bird care; and Tony D. Williams for logistical support. This study was
665 supported by NIH R01 NS055125 to K.W.S.

666 **REFERENCES**

- 667 Angelier F and Chastel O (2009) Stress, prolactin and parental investment in birds: A
668 review. *Gen Comp Endocrinol* 163:142-148.
- 669 Bergink EW, Kloosterboer HJ, Gruber M and Geert, AB (1973) Estrogen-induced
670 phosphoprotein synthesis in roosters: Kinetics of induction. *Biochim Biophys Acta*
671 294:497-506.
- 672 Bergink EW, Wallace RA, Van de Berg JA, Bos ES, Gruber M and Geert, AB (1974)
673 Estrogen-induced synthesis of yolk proteins in roosters. *Amer Zool*, 14:1177-
674 1193.
- 675 Boyd-Leinen P, Gosse B, Rasmussen K, Martin-Dani G and Spelsberg TC (1984)
676 Regulation of nuclear binding of the avian oviduct progesterone receptor. *J Biol*
677 *Chem* 259:2411-2421.
- 678 Burch JBE and Weintraub H (1983) Temporal order of chromatin structural changes
679 associated with activation of the major chicken vitellogenin gene. *Cell* 33:65-76.

680 Burton P, Gurrin L and Sly P (1998) Extending the simple linear regression model to
681 account for correlated responses: An introduction to generalized estimating
682 equations and multi-level mixed modeling. *Stat Med* 17:1261-1291.

683 Caro SP, Charmantier A, Lambrechts MM, Blondel J, Balthazart J and Williams TD
684 (2009) Local adaptation of timing of reproduction: females are in the driver's seat.
685 *Funct Ecol* 23:172-179.

686 Caro SP, Lambrechts MM, Chastel O, Sharp PJ, Thomas DW and Balthazart J (2006)
687 Simultaneous pituitary-gonadal recrudescence in two Corsican populations of
688 male blue tits with asynchronous breeding dates. *Horm Behav* 50:347-360.

689 Chaiseha Y, Tong Z, Youngren OM and El Halawani ME (1998) Transcriptional
690 changes in hypothalamic vasoactive intestinal peptide during a photo-induced
691 reproductive cycle in the turkey. *J Molec Endocrinol* 21:267-275.

692 Challenger WO, Williams TD, Christians JK and Vézina F (2001) Follicular development
693 and plasma yolk precursor dynamics through the laying cycle in the European
694 starling (*Sturnus vulgaris*). *Physiol Biochem Zool* 74:356-365.

695 Cidlowski JA and Muldoon TG (1978) The dynamics of intracellular estrogen receptor
696 regulation as influenced by 17beta-estradiol. *Biol Reprod* 18:234-246.

697 Clayton RN (1989) Gonadotrophin-releasing hormone: Its actions and receptors. *J*
698 *Endocrinol* 120:11-19.

699 Clutton-Brock TH (1988) *Reproductive Success: Studies of Individual Variation in*
700 *Contrasting Breeding Systems*. University of Chicago Press, Chicago.

701 Codina-Salada J, Moore JP and Chan L (1983) Kinetics of primary and secondary
702 stimulation of the mRNA for apoVLDL-II, a major yolk protein, in the cockerel liver
703 by estrogen. *Endocrinology* 113:1158-1160.

704 Dawson A (2003) A comparison of the annual cycles in testicular size and moult in
705 captive European starlings *Sturnus vulgaris* during their first and second years. *J*
706 *Avian Biol* 34:119-123.

707 Dawson A and Goldsmith AR (1997) Changes in gonadotrophin-releasing hormone
708 (GnRH-I) in the pre-optic area and median eminence of starlings (*Sturnus*
709 *vulgaris*) during the recovery of photosensitivity and during photostimulation. *J*
710 *Reprod Fertil* 111:1-6.

711 Dawson A and Sharp PJ (1998) The role of prolactin in the development of reproductive
712 photorefractoriness and post-nuptial molt in the European Starling (*Sturnus*
713 *vulgaris*). *Endocrinology* 139:485-490.

714 Dawson A, Talbot RT, Dunn IC and Sharp PJ (2002) Changes in basal hypothalamic
715 chicken gonadotropin-releasing hormone-I and vasoactive intestinal polypeptide
716 associated with a photo-induced cycle in gonadal maturation and prolactin
717 secretion in intact and thyroidectomized starlings (*Sturnus vulgaris*). *J*
718 *Neuroendocrinol* 14:533-539.

719 Deeley RG, Mullinix KP, Wetekam W, Kronenberg HM, Meyers M, Eldridge JD and
720 Goldberger RF (1975) Vitellogenin synthesis in the avian liver. *J Biol Chem*
721 250:9060-9066.

- 722 El Halawani ME, Pitts GR, Sun S, Silsby JL and Sivanandan V (1996) Active
723 immunization against vasoactive intestinal peptide prevents photo-induced
724 prolactin secretion in turkeys. *Gen Comp Endocrinol* 104:76-83.
- 725 El Halawani ME, Youngren OM and Pitts GR (1997) Vasoactive intestinal peptide as the
726 avian prolactin releasing factor. In *Prospectives in Avian Endocrinology*, S
727 Harvey and R Etches, eds, pp 403-416, The Society of Endocrinology, Bristol.
- 728 Farner DS, Donham RS, Matt KS, Mattocks PW, Moore MC and Wingfield JC (1983)
729 The nature of photorefractoriness. In *Avian Endocrinology: Environmental and*
730 *Ecological Perspectives*, S Mikami, K Homma and M Wada, eds, pp 149-166,
731 Japan Scientific Press and Springer-Verlag, Tokyo and Berlin.
- 732 Feare CJ (1984) *The starling*. Oxford University Press, Oxford.
- 733 Follett BK (1984) Birds. In *Marshall's Physiology of Reproduction*, GE Lamming, ed, pp
734 283-350, Churchill Livingstone, Edinburgh.
- 735 Forslund P and Pärt T (1995) Age and reproduction in birds – hypotheses and tests.
736 *Trends Ecol Evolut* 10:374-378.
- 737 Fowler GS (1995) Stages of age-related reproductive success in birds: Simultaneous
738 effects of age, pair-bond duration, and reproductive experience. *Amer Zool*
739 35:318-328.
- 740 Goldstein H, Brown W and Rasbash J (2002) Multilevel modeling of medical data. *Stat*
741 *Med* 21:3291-3315.
- 742 Hannan J and Cooke FE (1987) Age effects on clutch size and laying dates of individual
743 female lesser snow geese *Anser caerulescens*. *Ibis*, 129, 527-532.

- 744 Haywood, S (1993) Sensory and hormonal control of clutch size in birds. *Q Rev Biol*
745 68:33-60.
- 746 Hill, GE (2002) *A Red Bird in a Brown Bag*. Oxford University Press, Oxford.
- 747 Johnson AL and Bridgham JT (2001) Regulation of steroidogenic acute regulatory
748 protein and luteinizing hormone receptor messenger ribonucleic acid in hen
749 granulosa cells. *Endocrinology* 142:3116-3124.
- 750 Johnson AL and Woods DC (2009) Dynamics of avian ovarian follicle development:
751 Cellular mechanisms of granulosa cell differentiation. *Gen Comp Endocrinol*
752 163:12-17.
- 753 Jost J-P, Moncharmont B, Jiricny J, Saluz H and Hertner T (1986) In vitro secondary
754 activation (memory effect) of avian vitellogenin II gene in isolated liver nuclei.
755 *Proc Natl Acad Sci U S A* 83:43-47.
- 756 Jost J-P, Ohno T, Panyim S and Schueerch AR (1978) Appearance of vitellogenin
757 mRNA sequences and rate of vitellogenin synthesis in chicken liver following
758 primary and secondary stimulation by 17-beta-estradiol. *Eur J Biochem* 84:355-
759 361.
- 760 Kok K, Snippe L, Geert AB and Gruber M (1985) Nuclease-hypersensitive sites in
761 chromatin of the estrogen-inducible apoVLDL-II gene of chicken. *Nucleic Acids*
762 *Res* 13:5189-5202.
- 763 Kuenzel WJ (2003) Neurobiology of molt in avian species. *Poult Sci* 82:981-991.
- 764 Mauro LJ, Elde RP, Youngren OM, Phillips RE and El Halawani ME (1989) Alterations
765 in the hypothalamic vasoactive intestinal peptide-like immunoreactivity are

766 associated with reproduction and prolactin release in the female turkey
767 (*Meleagris gallopavo*). Endocrinology 125:1795-1804.

768 Mauro LJ, Youngren OM, Proudman JA, Phillips RE and El Halawani ME (1992) Effects
769 of reproductive status, ovariectomy, and photoperiod on vasoactive intestinal
770 peptide in the female turkey hypothalamus. Gen Comp Endocrinol 97:481-493.

771 McNaughton FJ, Dawson A and Goldsmith AR (1992) Juvenile photorefractoriness in
772 starlings, *Sturnus vulgaris*, is not caused by long days after hatching. Proc R Soc
773 Lond [Biol] 248:123-128.

774 McNaughton FJ, Dawson A and Goldsmith AR (1995) A comparison of the response to
775 gonadotropin-releasing hormone of adult and juvenile, and photosensitive and
776 photorefractory European starlings, *Sturnus vulgaris*. Gen Comp Endocrinol
777 97:135-144.

778 Meddle S and Follett BK (1995) Photoperiodic activation of Fos-like immunoreactive
779 protein in neurons within the tuberal hypothalamus of Japanese quail. J Comp
780 Physiol A 176:79-89.

781 Meddle S and Follett BK (1997) Photoperiodically driven changes in Fos expression
782 within the basal tuberal hypothalamus and median eminence of Japanese Quail.
783 J Neurosci 17:8909-8918.

784 Millam JR, Craig-Veit CB and Siopes TD (2003) Photostimulated fos-like
785 immunoreactivity in tuberal hypothalamus of photosensitive vs. photorefractory
786 turkey hens. Gen Comp Endocrinol 134:175-181.

- 787 Millam JR, Faris PL, Youngren OM, El Halawani ME and Martman BK (1993)
788 Immunohistochemical localization of chicken gonadotropin-releasing hormones I
789 and II (cGnRH I and II) in turkey hen brain. *J Comp Neurol* 333:68-82.
- 790 Mitchell MA and Carlisle AJ (1991) Plasma zinc as an index of vitellogenin production
791 and reproductive status in the domestic fowl. *Comp Biochem Physiol A* 100:719-
792 724.
- 793 Newton I, Marquiss M and Moss D (1981) Age and breeding in sparrowhawks. *J Anim*
794 *Ecol* 50:839-853.
- 795 Newton I and Rothery P (1998) Age-related trends in the breeding success of individual
796 female sparrowhawks *Accipiter nisus*. *Ardea* 86:21-31.
- 797 Nicholls TJ, Goldsmith AR and Dawson A (1988) Photorefractoriness in birds and
798 comparison with mammals. *Physiol Rev* 68:133-176.
- 799 Ojanen M (1983) Egg development and the related nutrient reserve depletion in the pied
800 flucatcher *Ficedula hypoleuca*. *Ann Zool Fennici* 20:293-300.
- 801 Péczely P and Kiss JZ (1988) Immunoreactivity to vasoactive intestinal polypeptide
802 (VIP) and thyrotropin-releasing hormone (TRH) in hypothalamic neurons of the
803 domesticated pigeon (*Columba livia*): alterations following lactation and exposure
804 to cold. *Cell Tissue Res* 251:485-494.
- 805 Péczely P and Kovács KJ (2000) Photostimulation affects gonadotropin-releasing
806 hormone immunoreactivity and activates a distinct neuron population in the
807 hypothalamus of the mallard. *Neurosci Lett* 290:205-208.
- 808 Perrins CM (1970) The timing of birds' breeding seasons. *Ibis* 112:242-255.

809 Piquette GN, Lapolt PS, Oikawa M and Hsueh AJW (1991) Regulation of luteinizing
810 hormone receptor messenger ribonucleic acid levels by gonadotropins, growth
811 factors, and gonadotropin-releasing hormone in cultured rat granulosa cells.
812 *Endocrinology* 128:2449-2456.

813 Rabe-Hesketh S and Skrondal A (2005) *Multilevel and Longitudinal Modeling Using*
814 *Stata*. Stata Press, College Station.

815 Saether BE (1990) Age-specific variation in reproductive performance of birds. In
816 *Current Ornithology*, D Power, ed, pp 251-283, Plenum Press, New York.

817 Saldanha CJ, Leak RK and Silver R (1994) Detection and transduction of daylength in
818 birds. *Psychoneuroendocrinology* 19:641-656.

819 Saldanha CJ, Silverman AJ and Silver R (2001) Direct innervation of GnRH neurons by
820 encephalic photoreceptors in birds. *J Biol Rhythms* 16:39-49.

821 Salvante KG, Vézina F and Williams TD (2010) Evidence for within-individual energy
822 reallocation in cold-challenged, egg-producing birds. *J Exp Biol* 213:1991-2000.

823 Salvante KG and Williams TD (2002) Vitellogenin dynamics during egg laying: daily
824 variation, repeatability and relationship with egg size. *J Avian Biol* 33:391-398.

825 Schielzeth H and Forstmeier W (2009) Conclusions beyond support: Overconfident
826 estimates in mixed models. *Behav Ecol* 20:416-420.

827 Segaloff DL, Wang H and Richards JS (1991) Hormonal regulation of luteinizing
828 hormone/chorionic gonadotropin receptor mRNA in rat ovarian cells during
829 follicular development and luteinization. *Molec Endocrinol* 4:1856-1865.

830 Sharp PJ and Ciccone N (2005) The gonadotrophin releasing hormone neurone: key to
831 avian reproductive function. In *Functional Avian Endocrinology*, A Dawson and
832 PJ Sharp, eds, pp 59-72, Narosa Publishing House, New Delhi.

833 Sharp PJ, Dunn IC, and Talbot RT (1987) Sex differences in the LH responses to
834 chicken LHRH-I and II in the domestic fowl. *J Endocrinol* 115:323-331

835 Sharp PJ, Talbot RT, Main GM, Dunn IC, Fraser HM and Huskisson NS (1990)
836 Physiological roles of chicken LHRH-I and -II in the control of gonadotrophin
837 release in the domestic chicken. *J Endocrinol* 124:291-299.

838 Silverin B (1978) Circannual rhythms in gonads and endocrine organs of the great tit
839 *Parus major* in south-west Sweden. *Ornis Scand* 9:207-213.

840 Sockman KW (2007) Neural orchestration of mate-choice plasticity in songbirds. *J*
841 *Ornithol* 148:S225-S230.

842 Sockman KW and Ball GF (2009) Independent effects of song quality and experience
843 with photostimulation on expression of the immediate early gene ZENK (EGR-1)
844 in the auditory telencephalon of female European starlings. *Dev Neurobiol*
845 69:339-349.

846 Sockman KW, Gentner TQ and Ball GF (2002) Recent experience modulates forebrain
847 gene-expression in response to mate-choice cues in European starlings. *Proc*
848 *Roy Soc Lond B* 269:2479-2485.

849 Sockman KW, Gentner TQ and Ball GF (2005) Complementary neural systems for the
850 experience-dependent integration of mate-choice cues in European starlings. *J*
851 *Neurobiol* 62:72-81.

852 Sockman KW and Salvante KG (2008) The integration of song environment by
853 catecholaminergic systems innervating the auditory telencephalon of adult
854 female European starlings. *Dev Neurobiol* 68:656-668.

855 Sockman KW, Sharp PJ and Schwabl H (2006) Orchestration of avian reproductive
856 effort: an integration of the ultimate and proximate bases for flexibility in clutch
857 size, incubation behaviour, and yolk androgen deposition. *Biol Rev* 81:629-666.

858 Sockman KW, Weiss J, Webster MS, Talbott V and Schwabl H (2008) Sex-specific
859 effects of yolk-androgens on growth of American kestrels. *Behav Ecol Sociobiol*
860 62:617-625.

861 Sockman KW, Williams TD, Dawson A and Ball GF (2004) Prior experience with
862 photostimulation enhances photo-induced reproductive development in female
863 European Starlings: A possible basis for the age-related increase in avian
864 reproductive performance. *Biol Reprod* 71:979-986.

865 Stearns SC (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

866 Stifani S, George R and Schneider WJ (1988) Solubilization and characterization of the
867 chicken oocyte vitellogenin receptor. *Biochem J* 250:467-475.

868 Sutherland RL and Baulieu EE (1976) Quantitative estimates of cytoplasmic and
869 nuclear oestrogen receptors in chick oviduct: Effect of oestrogen on receptor
870 concentration and subcellular distribution. *Eur J Biochem* 70:531-541.

871 Sutherland RL, Lebeau MC, Schmelck PH and Baulieu EE (1977) Synergistic and
872 antagonistic effects of progesterone and oestrogen on oestrogen receptor

873 concentration and DNA polymerase activity in chick oviduct. FEBS Lett 79:253-
874 257.

875 Swaneck GE, Nordstrom JL, Kreusaler F, Tsai MJ and O'Malley BW (1979) Effect of
876 estrogen on gene expression in chicken oviduct: Evidence for transcriptional
877 control of ovalbumin gene. Proc Natl Acad Sci U S A 76:1049-1053.

878 Teriuyama R and Beck MM (2000) Changes in immunoreactivity to anti-cGNRH-I and –
879 II are associated with photostimulated sexual status in male quail. Cell Tissue
880 Res 300:413-426.

881 Tong Z, Pitts GR, Foster DN and El Halawani M (1997) Transcriptional and post-
882 transcriptional regulation of prolactin during the turkey reproductive cycle. J
883 Molec Endocrinol 18:223-231.

884 Tong Z, Pitts GR, You S, Foster DN and El Halawani M (1998) Vasoactive intestinal
885 peptide stimulates turkey prolactin gene expression by increasing transcription
886 rate and enhancing mRNA stability. J Molec Endocrinol 21:259-266.

887 Urbanski HF (1992) Photoperiodic modulation of luteinizing hormone secretion in
888 orchidectomized Syrian hamsters and the influence of excitatory amino acids.
889 Endocrinology 131:1665-1669

890 van Gils J, Absil P, Grauwels L, Moons L, Vandesande F and Balthazart J (1993)
891 Distribution of luteinizing hormone-releasing hormones I and II (LHRH-I and -II) in
892 the quail and chicken brain as demonstrated with antibodies directed against
893 synthetic peptides. J Comp Neurol 334:304–323.

894 Vézina F and Salvante KG (2010) Behavioral and physiological flexibility are used by
895 birds to manage and support investment in the early stages of reproduction. *Curr*
896 *Zool* 56:767-792.

897 Walzem RL (1996) Lipoproteins and the laying hen: Form follows function. *Poult Avian*
898 *Biol Rev* 7:31-64.

899 Wang S and Williams DL (1983) Differential responsiveness of avian vitellogenin I and
900 vitellogenin II during primary and secondary stimulation with estrogen. *Biochem*
901 *Biophys Res Commun* 112:1049-1055.

902 Wilks AJ, Cato ACB, Cozens PJ, Mattaj JW and Jost JP (1982) Estrogen induces a
903 demethylation at the 5' end region of the chicken vitellogenin gene. *Proc Natl*
904 *Acad Sci U S A* 79:4252-4255.

905 Williams TD (1998) Avian reproduction, overview. In *Encyclopedia of Reproduction*, E
906 Knobil and JD Neil, eds, pp 325-336, Academic Press, San Diego.

907 Williams TD and Christians JK (1997) Female reproductive effort and individual
908 variation: Neglected topics in environmental endocrinology? In *Thirteenth*
909 *International Congress of Comparative Endocrinology*, S Kawashima and S
910 Kikuyama, eds, pp 1669-1675, Monduzzi Editore, Yokohama.

911 Williams TD, Dawson A and Nicholls TJ (1989) Sexual maturation and moult in juvenile
912 starlings *Sturnus vulgaris* in response to different daylengths. *Ibis* 131:135-140.

913 Williams TD, Dawson A, Nicholls TJ and Goldsmith AR (1987a) Reproductive
914 endocrinology of free-living nestling and juvenile starlings, *Sturnus vulgaris*: an
915 altricial species. *J Zool* 212:619-628.

- 916 Williams TD, Dawson A, Nicholls TJ and Goldsmith AR (1987b) Short days induce
917 premature reproductive maturation in juvenile starlings, *Sturnus vulgaris*. J
918 Reprod Fertil 80:327-333.
- 919 Wingfield JC (1980) Fine temporal adjustment of reproductive functions. In Avian
920 Endocrinology, A Epple and MH Stetson, eds, pp 367-389, Academic Press, New
921 York.
- 922 Wingfield JC (1983) Environmental and endocrine control of avian reproduction: An
923 ecological approach. In Avian Endocrinology: Environmental and Ecological
924 Perspectives, S Mikami, K Homma and M Wada, eds, pp 265-288, Japan
925 Scientific Press and Springer-Verlag, Tokyo and Berlin.
- 926 Wingfield JC, Hahn TP, Levin R and Honey P (1992) Environmental predictability and
927 control of gonadal cycles in birds. J Exp Zool 261:214-231.
- 928 Yamada S, Mikami S and Yanaihara N (1982) Immunohistochemical localization of
929 vasoactive intestinal polypeptide (VIP)-containing neurons in the hypothalamus
930 of the Japanese quail (*Coturnix coturnix*). Cell and Tissue Res 226:13-26.
- 931 You S, Kim H, El Halawani ME and Foster DN (2000) Three different turkey luteinizing
932 hormone receptor (TLH-R) isoforms II: Characterization of differentially regulated
933 tLH-R messenger ribonucleic acid isoforms in the ovary. Biol Reprod 62:117-124.
934

935 **FIGURE LEGENDS**

936 **Figure 1. Experimental design.** The photoperiod treatment is depicted at the top of the
937 figure as white bands for long days (16 L: 8 D) and as black bands for short days
938 (8 L: 16 D). At Week 0 we exposed all females to a 16 L: 8 D photoperiod. From
939 Weeks 1 to 8, we exposed the females in the photo-experienced group
940 (Experienced; ■) to short days, and we maintained the females in the photo-
941 naïve group (Naïve; □) on long days. We exposed all females to long days from
942 Weeks 9 thru 25, to short days from Weeks 26 to 34, and then finally to long
943 days for the last four weeks (Weeks 35-38) of the study. Downward arrows
944 indicate the weeks during which we took a blood sample from each bird.

945 **Figure 2. Body mass.** Body mass (mean \pm SEM) of photo-experienced (Experienced
946 ■) and photo-naïve (Naïve; □) female house finches throughout the study. See
947 Figure 1 legend for description of photoperiod treatments. Sample sizes, with
948 number of triplicate groups and number of females in parentheses, are listed
949 above the corresponding weeks for each photoexperience group. We compared
950 the body masses of the photoexperience groups at the beginning of the study
951 (Week 0), throughout the photoperiod treatment (Weeks 2-8), during the first four
952 weeks following photoperiod treatment (Weeks 9-12), throughout the common
953 long-day exposure (Weeks 9-25), during the common short-day exposure
954 (Weeks 26-34), and throughout the final common long-day exposure (Weeks 35-
955 38).

956 **Figure 3. Gonadotropin releasing hormone immunoreactivity.** Number of
957 gonadotropin releasing hormone immunoreactive (GnRH-ir) cells (mean \pm SEM)
958 in the hypothalamic septo-preoptic area of photo-experienced and photo-naïve
959 female house finches following exposure to either conspecific male house finch
960 song (gray columns) or heterospecific male northern cardinal song (white
961 columns). Sample sizes (number of independent females) are shown at the
962 bases of the columns corresponding to each photoexperience-song treatment
963 group.

964 **Figure 4. Vasoactive intestinal polypeptide immunoreactivity.** Number of
965 vasoactive intestinal polypeptide immunoreactive (VIP-ir) cells (mean \pm SEM) in
966 a) the infundibular nuclear complex (INF) and b) the ventromedial nucleus (VMN)
967 of the hypothalamus of photo-experienced and photo-naïve female house finches
968 following exposure to either conspecific male house finch song (gray columns) or
969 heterospecific male northern cardinal song (white columns). Sample sizes
970 (number of independent females) are shown at the bases of the columns
971 corresponding to each photoexperience-song treatment group.

972 **Figure 5. Luteinizing hormone.** Plasma luteinizing hormone (LH) levels (mean \pm SEM)
973 in photo-experienced (Experienced; ■) and photo-naïve (Naïve; □) female house
974 finches throughout the study. See Figure 1 legend for description of photoperiod
975 treatments. Sample sizes, with number of triplicate groups and number of
976 females in parentheses, are listed above the corresponding weeks for each
977 photoexperience group. We compared the circulating LH levels of the
978 photoexperience groups at the beginning of the study (Week 0), at the end of the

979 photoperiod treatment (Week 8), during the first four weeks following photoperiod
980 treatment (Weeks 9-12), at the end of the common long-day exposure (Week
981 25), at the end of the common short-day exposure (Week 34), and throughout
982 the final common long-day exposure (Weeks 35-38).

983 **Figure 6. Vitellogenin.** Plasma vitellogenin levels (mean \pm SEM) in photo-experienced
984 (Experienced; ■) and photo-naïve (Naïve; □) female house finches throughout
985 the study. See Figure 1 legend for description of photoperiod treatments. Sample
986 sizes, with number of triplicate groups and number of individuals in parentheses,
987 are listed above the corresponding weeks for each photoexperience group. We
988 compared the circulating vitellogenin levels of the photoexperience groups at the
989 beginning of the study (Week 0), at the end of the photoperiod treatment (Week
990 8), during the first four weeks following photoperiod treatment (Weeks 9-12), at
991 the end of the common long-day exposure (Week 25), at the end of the common
992 short-day exposure (Week 34), and throughout the final common long-day
993 exposure (Weeks 35-38).

994 **Figure 7. Ovarian follicle size.** Effect of photoexperience on the average diameter
995 (mean \pm SEM) of the three largest ovarian follicles a) before and b) after
996 adjustment for body mass, following four weeks of photostimulation. Sample
997 sizes (number of independent females) are shown at the base of each column.
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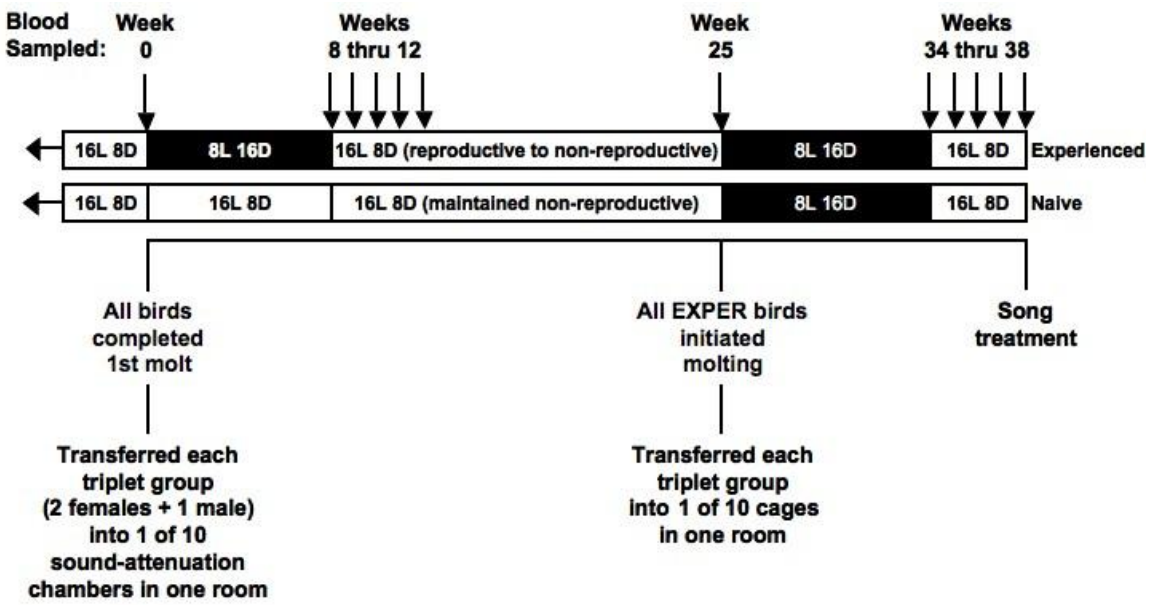
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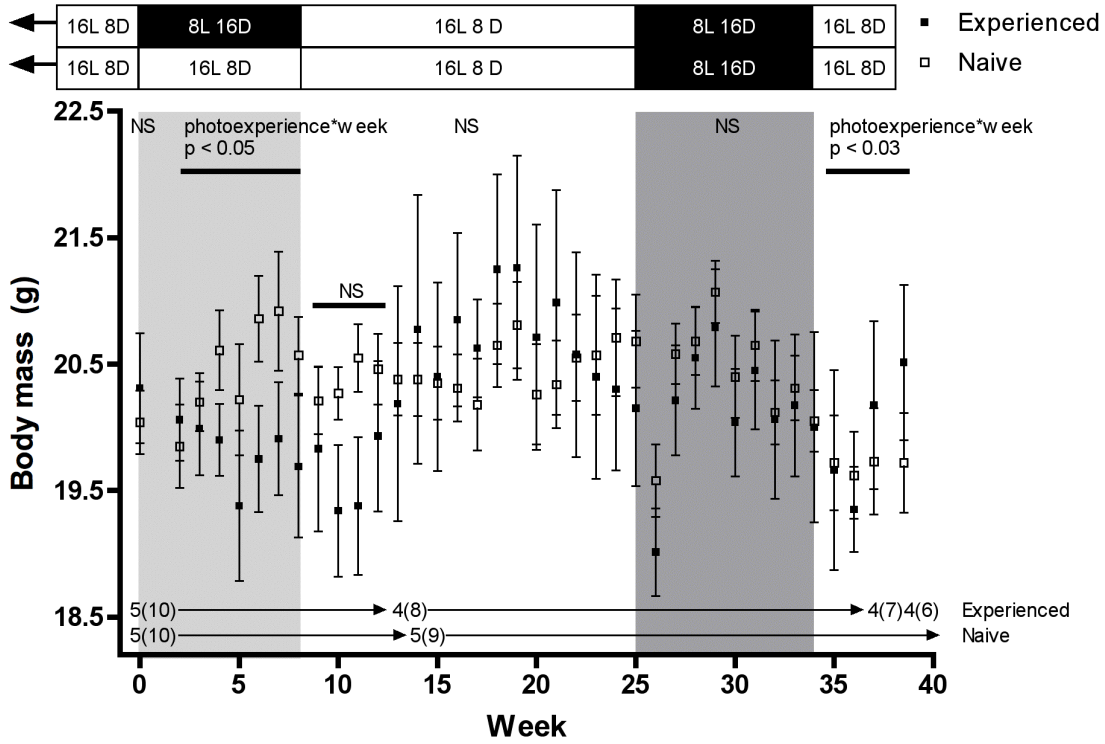
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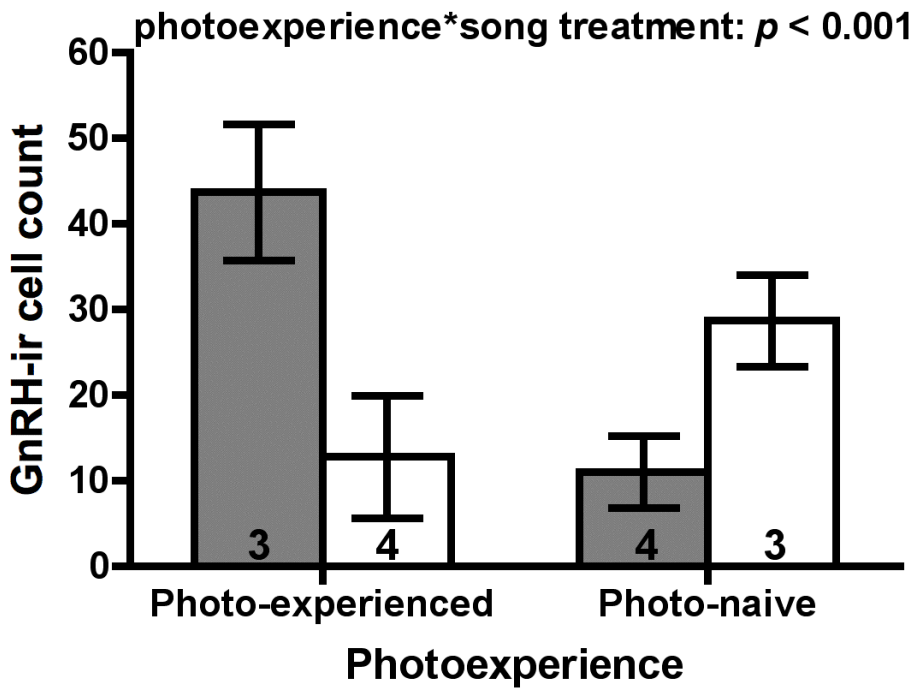
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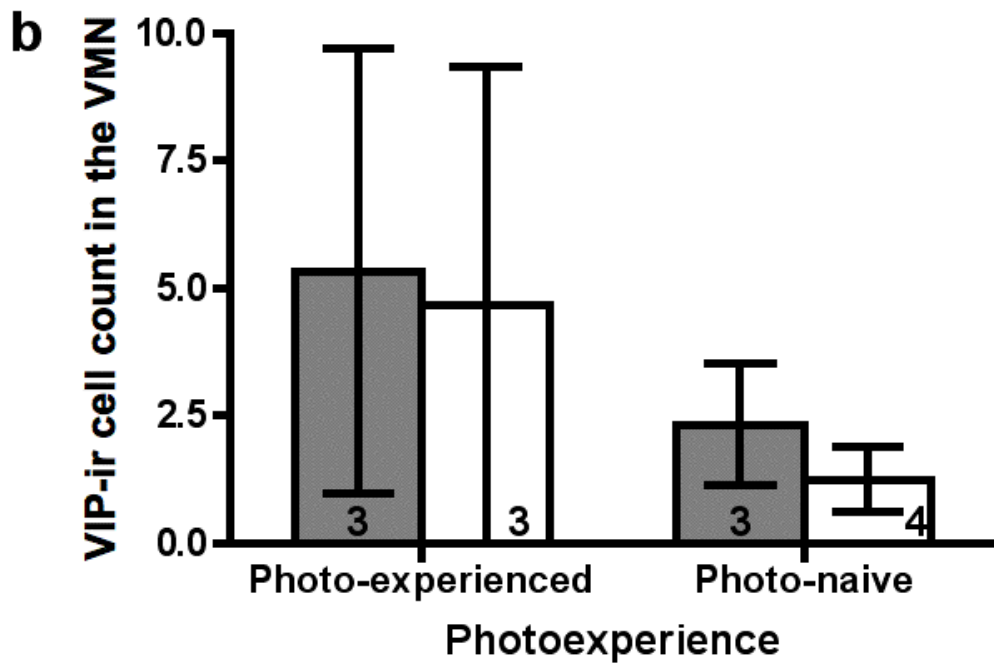
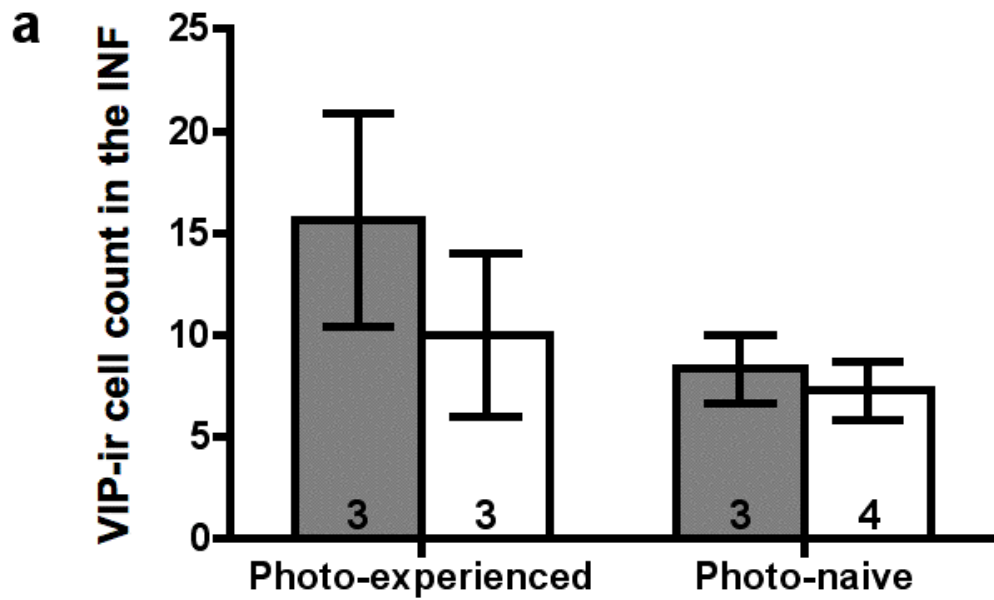
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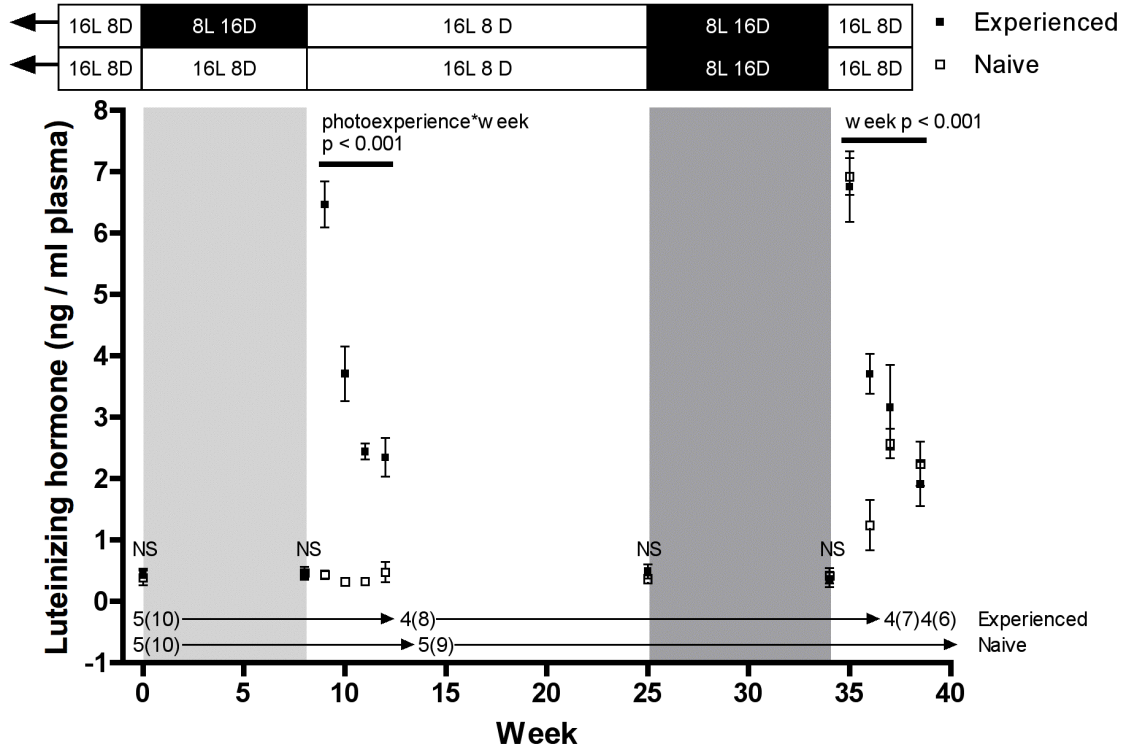


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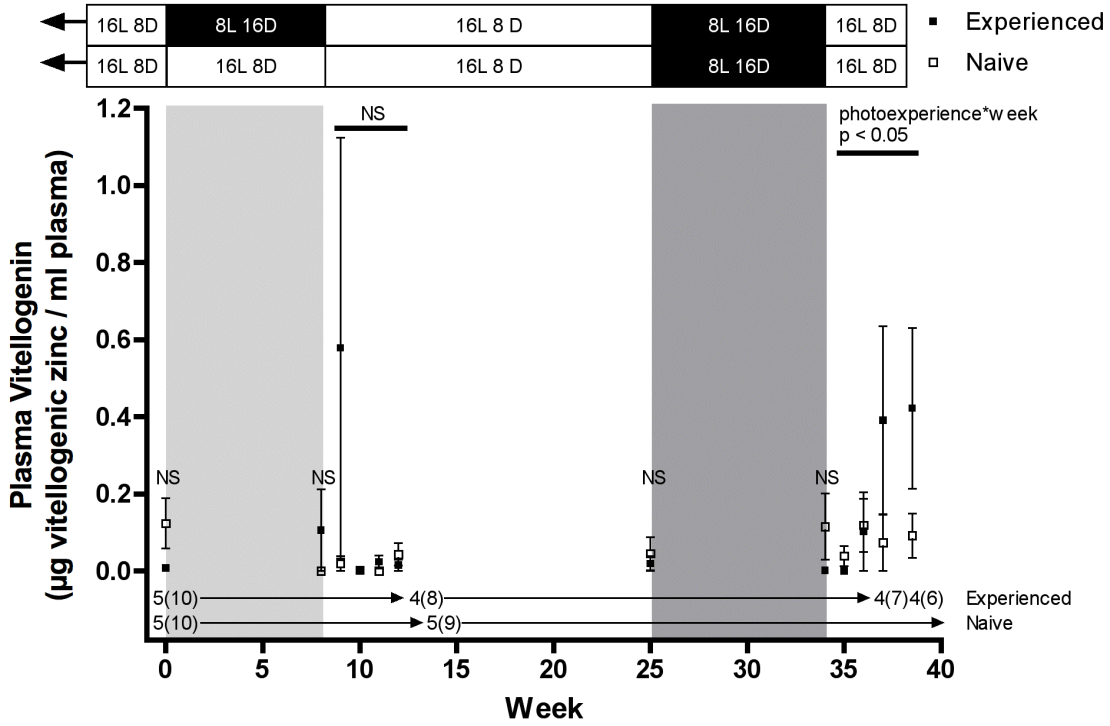
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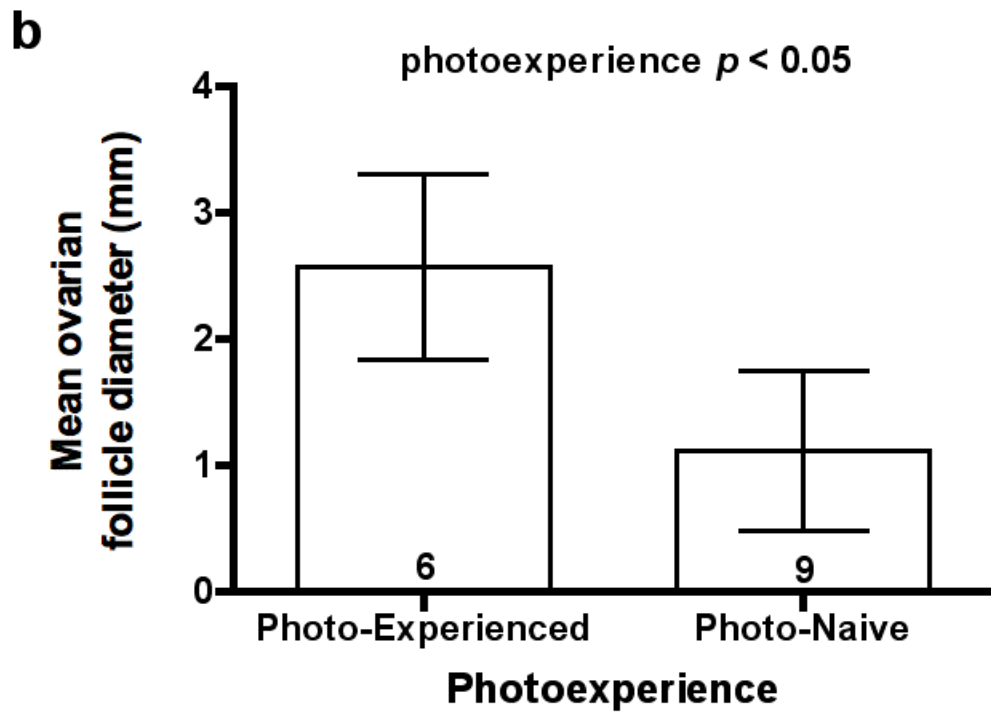
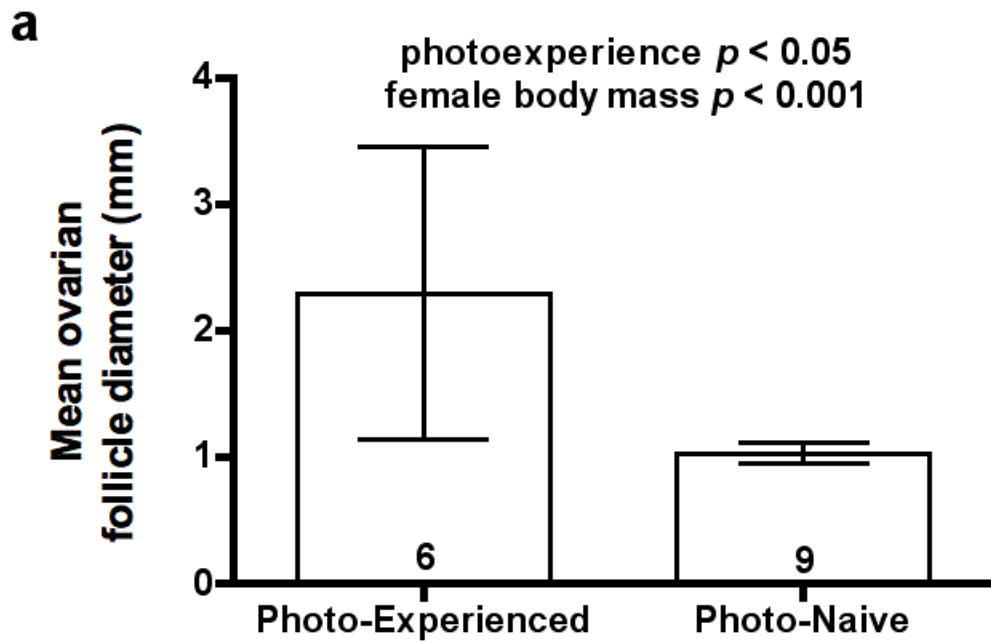
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1027 Figure 6.



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1029 Figure 7.