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Sex-specific Responses to GnRH Challenge, but Not Food Supply, in Kittiwakes: Evidence for the “Sensitivity to Information” Hypothesis

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KEYWORDS

GnRH challenge; HPG axis; hypothalamic–pituitary–gonadal; luteinising hormone releasing hormone; phenology; seabird; testosterone

ABSTRACT

Seasonal timing of breeding is usually considered to be triggered by endogenous responses linked to predictive cues (e.g., photoperiod) and supplementary cues that vary annually (e.g., food supply), but social cues are also important. Females may be more sensitive to supplementary cues because of their greater role in reproductive timing decisions, while males may only require predictive cues. We tested this hypothesis by food-supplementing female and male colonial seabirds (black-legged kittiwakes, *Rissa tridactyla*) during the pre-breeding season. We measured colony attendance via GPS devices, quantified pituitary and gonadal responses to gonadotropin releasing hormone (GnRH) challenge, and observed subsequent laying phenology. Food supplementation advanced laying phenology and increased colony attendance. While female pituitary responses to GnRH were consistent across the pre-breeding season, males showed a peak in pituitary sensitivity at approximately the same time that most females were initiating follicle development. The late peak in male pituitary response to GnRH questions a common assumption that males primarily rely on predictive cues (e.g., photoperiod) while females also rely on supplementary cues (e.g., food availability). Instead, male kittiwakes may integrate synchronising cues from their social environment to adjust their reproductive timing to coincide with female timing.

1. INTRODUCTION

Seasonal timing of reproduction has fitness consequences for both females and males, but the trait itself is usually under greater female control. Variation in timing of reproduction has been linked to intrinsic female difference and extrinsic environmental drivers such as climate and photoperiod (Reale et al. 2003; Nussey et al. 2005ab, Charmantier et al. 2008). Fewer studies test whether males have a role in timing of reproduction and those that do find that, although males can influence timing decisions, females almost always have greater influence on timing (e.g., Caro et al. 2009, Brommer & Rattiste 2008; Whelan et al. 2016; Sauve et al. 2019; Moiron et al. 2020; Whelan et al. 2022; but see Teplitsky et al. 2010). Thus, typically, females are more sensitive to environmental conditions in the pre-breeding season, relative to males (Ball & Ketterson 2008). However, in species with biparental care, the synchrony of reproductive status between partners can be critical and it may be important for males to adjust to female reproductive timing to avoid the costs of maintaining elevated androgen levels for prolonged periods of time (Goymann et al. 2019).

Various types of environmental cues are available to inform timing of reproduction: initial predictive cues (or “primary cues”; e.g., photoperiod) can provide long-term information to initiate gonadal recrudescence well in advance of breeding, supplementary cues provide information for fine-tuning (e.g., food availability), and synchronising/integrating cues can adjust timing of breeding in response to social information (Jacobs & Wingfield 2000). In temperate zone animals sensitive to photoperiod, including birds, increasing day length can initiate gonadal recrudescence in both sexes (Farner & Wilson 1957; Farner et al. 1966; Perfito et al. 2015). Initial

predictive cues alone are sufficient for gonadal maturation in males, but females require both initial predictive and supplementary cues (Perfito et al. 2015). If gonadal recrudescence alone is sufficient for successful reproduction, this could suggest that males only need to be sensitive to initial predictive cues like photoperiod to initiate reproduction. However, Goymann et al. (2019) recently argued that, in species with biparental care, males should be physiologically sensitive to interactions with fertile females. Thus, even if females have a greater role in timing reproduction, male reproductive readiness should be sensitive to synchronising cues up until follicle development and fertilisation.

In both sexes, the hypothalamic–pituitary–gonadal (HPG) endocrine axis regulates reproductive timing. As animals transition into the breeding season from a non-reproductive state, the endocrine system integrates information from the environment and initiates changes in physiology (Jacobs & Wingfield 2000; Ball & Ketterson 2008). Stimulatory cues induce the release of gonadotropin releasing hormone (GnRH) from the hypothalamus, which in turn effects the release of luteinising hormone (LH) and follicle stimulating hormone from the anterior pituitary, which in turn stimulate gametogenesis and the production of gonadal hormones (reviewed in Scanes 2015). Various factors related to physiological state should influence individuals' HPG responses, including age (Goutte et al. 2011), the number of target cells, abundance of hormone receptors (Fudickar et al. 2017; Needham et al. 2019) and inhibitory effects of other hormones (Goutte et al. 2010).

In a recent experiment, we tested the *reproductive readiness hypothesis* (**Fig. 1A**) in female black-legged kittiwakes (*Rissa tridactyla*, hereafter 'kittiwakes') and found

66 that instead of showing continual increases in LH across the pre-breeding period, ,
67 females nearest to laying had the smallest LH releases in response to GnRH challenge
68 (Whelan et al. 2021). We proposed a new hypothesis for interpreting female response
69 to GnRH challenge: the *sensitivity to information hypothesis* (**Fig. 1B**) suggests that the
70 responsiveness of the pituitary to GnRH may peak when females are integrating
71 supplementary cues into breeding decisions via the HPG axis. This hypothesis predicts
72 that individuals will be most sensitive to information when their pituitary and gonadal
73 secretions are highest. Our rationale is that endogenous GnRH is produced by the
74 hypothalamus in response to sensory inputs received at higher brain centers (Williams
75 2012). Thus, environmental cues that indicate favourable conditions for breeding are
76 translated into the hormonal language of the endocrine system via release of GnRH
77 from the hypothalamus, but maximal responses are likely modulated by changes in
78 sensitivity of tissues further down the endocrine axis (i.e., Romero et al. 1998). In
79 particular, the pituitary has been proposed to be a primary "control point" in the HPG
80 axis for regulating reproductive timing in birds (Grieves et al. 2016). Under this
81 hypothesis, pituitary sensitivity to GnRH reflects the period of maximum plasticity in
82 response to either supplementary or synchronizing and integrating cues, rather than
83 reflecting temporal proximity to oviposition (the event that is used almost universally to
84 quantify timing of reproduction in birds).
85 While females should incorporate supplementary cues (e.g., food availability)
86 from their environment into breeding decisions, males are primarily thought to rely on
87 predictive cues (e.g., photoperiod, Ball & Ketterson 2008). However, in species that
88 provide biparental care, males can be sensitive to synchronising cues from females in

89 order to minimise costs of prolonged testosterone elevation (Wingfield et al. 2001) and
90 time their own behavioural and physiological readiness to maximise fitness (Jacobs &
91 Wingfield, 2000; Goymann et al. 2019; **Fig. 1C**). We therefore expand the *sensitivity to*
92 *information hypothesis* to make predictions about male HPG sensitivity in the pre-
93 breeding period. First, the hypothesis predicts that males are less sensitive to the
94 supplementary cue of food availability than females. Second, the hypothesis predicts
95 an increase in male HPG axis responsiveness that lags behind female responsiveness.
96 Here, we used food-supplementation experiments during pre-breeding to test
97 these hypotheses in free-living kittiwakes, colonial seabirds with biparental care and
98 low levels of extra-pair copulations (Helfenstein et al. 2004). This is a follow-up study to
99 Whelan et al. (2021) where we found that food supplementation advanced laying
100 phenology and influenced endocrine responses in female kittiwakes. Food supply also
101 affects the amount of time spent at the colony (Whelan et al. 2021; Kahane-Rapport et
102 al. 2022), and higher food supply during the pre-breeding season should increase time
103 spent at the colony with the mate (and thus social interactions that contain
104 synchronising cues). First, we evaluate the role of food as a supplementary cue for
105 timing of breeding, testing for effects of short-term (10 day) vs long-term (continuous)
106 food supplementation on laying date. Food may affect timing of breeding by acting as
107 a sensory cue with predictive utility (perception of food availability - the “anticipation
108 hypothesis”), or it may affect timing of breeding by altering energy balance (the
109 “constraint hypothesis”, Shultz et al. 2009). If kittiwake physiology and reproductive
110 timing respond similarly to the short- and long-term feeding, we would conclude that
111 food provides predictive information (“anticipation”), while if they responded more

112 strongly to long-term supplementation, we would conclude food availability poses
113 energetic constraints on breeding. Second, we test for sex-specific behavioural and
114 endocrine responses to this food supply. One study found sex-specific responses to
115 stress in pre-breeding Atlantic kittiwakes, where female, but not male, circulating and
116 GnRH-induced LH were negatively associated with circulating stress hormone
117 (corticosterone, Goutte et al. 2010), a physiological marker of food supply (Kitaysky et
118 al. 2007; Riechert et al. 2014). Thus, we predicted that female HPG traits would
119 respond more strongly to a stimulatory environmental cue (food supply) than males.
120 Finally, we examine trends in pituitary and gonadal response to exogenous GnRH over
121 time (both absolute and relative to egg-laying) to test for sex differences in timing of
122 endocrine sensitivity.

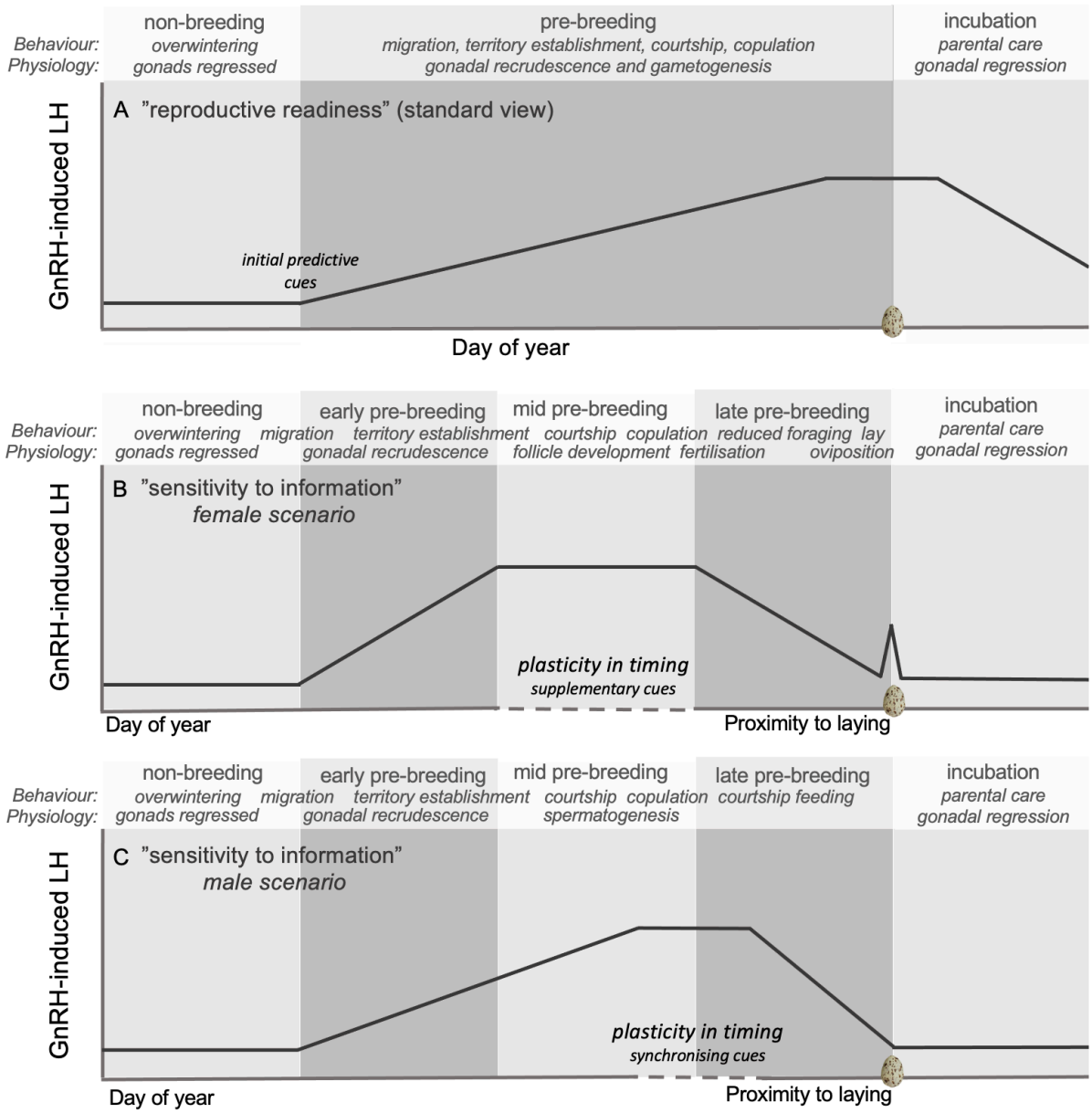

Figure 1 (**A**) The "*reproductive readiness hypothesis*" (standard view) predicts that responsiveness to GnRH should increase steadily over the course of pre-breeding. (**B**, **C**) The "*sensitivity to information hypothesis*" generates different predictions for female and male responsiveness to GnRH (adapted from Whelan et al. 2021).

2. METHODS

2.1 Field methods

We conducted an experiment on adult black-legged kittiwakes breeding on Middleton Island, Alaska, on a modified radar tower (Gill & Hatch 2002). During spring 2019 (April 18 - May 19) we captured banded kittiwakes of known sex (determined by sex-specific behavioural observations, including copulations, which peak 0-18 days before laying; Jodice et al. 2000; Whelan et al. 2021) at their nest sites using a leg hook, obtained a blood sample, and deployed a GPS device; four days later we began recapture efforts, and obtained a series of blood samples as part of a GnRH challenge. In doing so, we replicated the field methods of our previous study, conducted in 2018 (Whelan et al. 2021), with some key differences in experimental design. We began the experiment three weeks earlier (approximately 1.5 months prior to the population's mean laying date; Whelan et al. 2022) to better capture transitions from pre-breeding to breeding life-history stages, and included males. Rather than targeting individuals with already developed nests to standardise proximity to laying (as in the earlier study), we captured most birds before nest development began and when proximity to laying was unknown. We did not include a weight handicap treatment because the treatment had little physiological effect relative to feeding in the previous study. Following experimental manipulations, we monitored nest contents twice per day to obtain laying dates (date the first or single egg appeared).

2.1.1 Short-term food supplementation

We conducted a short-term food supplementation experiment on 22 pairs to evaluate the role of food as a supplementary cue that might influence timing of breeding. To train the birds to accept food, we visited these sites five times per day for the first 2-3 days and provided unlimited capelin (*Mallotus villosus*). Once birds readily accepted

155 fish, we switched to feeding three times per day, as per the regular (long-term) protocol
156 (see below). Feeding was discontinued after birds were recaptured for physiological
157 sampling and GPS retrieval, which was usually 10 days after food supplementation
158 began (**Figure 2**).

159 ***2.1.2 Long-term food supplementation***

160 Since 1996, a subset of kittiwake pairs has been fed an unlimited number of fish three
161 times per day at their nest site in a long-running food supplementation experiment
162 (described in Gill & Hatch 2002; Whelan et al. 2021). From this existing food-
163 supplementation treatment, we captured 48 individuals from unique pairs (i.e., never
164 sampling both the female and male from a pair to reduce disturbance). Kittiwakes
165 exhibit strong breeding philopatry and, in this experiment, we sampled only individuals
166 that were observed attending the same site as in the previous breeding season. Thus,
167 all birds in this group had been fed for at least one prior breeding season. This regular
168 food-supplementation treatment began on May 6, which was approximately mid-way
169 through the experiment and 16 days prior to the onset of egg-laying (first egg date: 22
170 May). However, birds from the long-term feeding treatment were sampled both before
171 and after feeding began (**Figure 2**).

172 ***2.1.3 GPS deployments and physiological sampling***

173 Following the methods in Whelan et al. (2021), we captured one member of each pair
174 of breeding kittiwakes at their nest site between 18 April and 19 May (during daylight
175 hours between 05:17-21:58) and took a baseline blood sample (1 mL). We then
176 deployed a GPS accelerometer (9–11.5 g, AxyTrek, TechnoSmart Europe, GPS fix-rate:
177 3 min) on the tail using marine cloth tape and cable ties, then released the bird. Four

178 days later, we began recapture efforts. Upon recapture, we took another baseline
179 blood sample (1 mL), injected the individual with 0.1 mL of either synthetic GnRH
180 ([Gln8] LHRH (chicken), Phoenix Pharmaceuticals Inc., Lot No. 432694) dissolved in
181 0.9% phosphate buffered saline (Sigma Aldrich) to yield a concentration of 0.6 μ g/0.1
182 mL, or 0.9% saline alone. We took additional blood samples at 10 minutes (0.4 mL)
183 and 30 minutes (0.6 mL) after injection. We then removed the GPS and released the
184 bird.

185 Injection with exogenous GnRH (a "GnRH challenge") is a common method
186 used in endocrinology to assess the reproductive status of an animal via the
187 responsiveness of the HPG endocrine axis, which regulates timing of reproduction by
188 stimulating gonadal growth, gametogenesis, and reproductive behaviours (Wingfield et
189 al. 1979; Schoech et al. 1996). In practice, an animal is injected with dissolved GnRH
190 produced endogenously in the hypothalamus; (sometimes called luteinising hormone
191 releasing hormone, LHRH) and the resultant spike in circulating exogenous GnRH
192 binds to available receptors on the gonadotroph cells in the anterior pituitary, triggering
193 a release of the gonadotropins, luteinising hormone (LH) and follicle-stimulating
194 hormone (FSH) in both sexes. LH and FSH then reach target cells expressing receptors
195 in the gonads: activated LH receptors on the ovarian thecal cells and testicular Leydig
196 cells stimulate the production of testosterone (as well as progesterone and estrogens
197 in females; Porter et al. 1989). Thus, the changes in downstream hormones (e.g., LH,
198 estradiol, testosterone) after a standardised period of time may provide information
199 about an individual's reproductive life-history sub-stage (Jacobs & Wingfield 2000). For
200 example, relatively large increases of LH or sex steroids after injection may indicate

that an individual is further advanced in the sequence of HPG-axis-dependent life-history substages (e.g., temporally closer to folliculogenesis in females and spermatogenesis in males; *reproductive readiness hypothesis*; Schoech et al. 1996; Goutte et al. 2010; **Fig. 1A**). However, this interpretation of individual variation in response to GnRH can be misleading (see below).

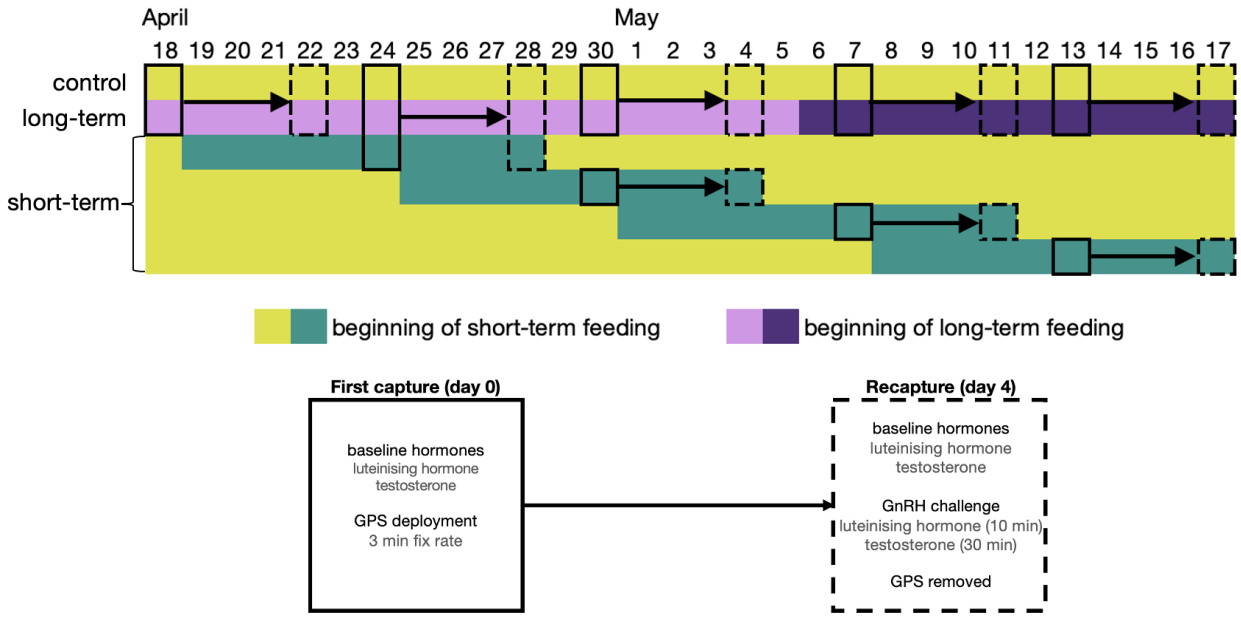

Figure 2 Timeline for food-supplementation experiments, captures, biologging and physiological sampling. Short term food-supplementation occurred for different subsets of nests throughout the study, while long-term food-supplementation began on May 6. Birds were captured on 5 days in April and May (11-36 birds per day), and recapture efforts began 4 days after the individual was first captured (final individual recaptured on May 19). All nests were monitored for date of egg-laying.

2.2 Colony attendance

To quantify exposure to social cues at the colony, we used GPS location data to calculate colony attendance as the proportion of the deployment spent within 1 km of the tower breeding colony. This broad buffer captures the tower colony itself, locations where kittiwakes gather nesting material, and preening/loafing sites.

2.3 Radioimmunoassay

We conducted radioimmunoassay following the methods described in Whelan et al. (2021). We assayed each sample in duplicate for LH (intra-assay CV=7.65%; inter-assay CV=8.35%) and testosterone (intra-assay CV=11.36%; inter-assay CV=20.06%). We were not able to measure estradiol. We excluded two outlier values from statistical analyses (one testosterone 30 min post GnRH-injection value and one baseline LH at first capture value, both >2 SD from female mean). Samples with hormone levels below the detectable limit were given a value 0.01 ng/mL below the lowest detectable level (LH: 0.40 ng/mL, n = 31/356 samples; testosterone: 0.30 ng/mL, n = 82/356 samples). We confirmed that kittiwakes injected with GnRH increased LH and testosterone, relative to saline-injected controls, by calculating and visualising means (± standard error, SE) for each blood sampling timepoint and sex (**Figure S1**).

2.4 Statistical analysis

We completed all movement summaries and statistical analyses in *R* (version 3.6.2, R Core Team 2019). We fitted linear models (LM), generalised linear models (GLM), and generalised additive models (GAMs; *mgcv*, Wood 2011). To test for sex-specific responses to food supplementation, we used a two-way interaction term between sex (female, male) and food treatment (control, short-term, long-term), and assessed significance of fixed effects using Type III ANOVA (*car,* Fox & Weisberg 2019). If the

240 interaction term was non-significant, we removed the interaction term and tested for
241 main effects of sex and food treatment only; we used Type II ANOVA to assess
242 significance of fixed effects in models without interaction terms. We report
243 unstandardized effects sizes ($\pm$ standard error) for all models, standardized effect
244 sizes for LM (partial eta-squared, $\eta 2$), and odds ratios for GLM. For Tukey post-hoc
245 comparisons, we used the package *emmeans* (Lenth 2020) which applied a Bonferroni
246 correction to p-values.

247 ***2.4.1 Effects of food manipulation on laying phenology***

248 To first confirm an effect of food supplementation on timing of reproduction, we
249 modeled laying date (day of year) in response to food treatment (LM). As both females
250 and males were assigned to the food treatment, we did not include sex as a fixed
251 effect in this model.

252 ***2.4.2 Effects of food manipulation and sex on colony attendance and hormones***

253 We ran separate models for the early pre-breeding period (18 April – 5 May) and the
254 late pre-breeding period (6-18 May), coinciding with before and after the regular (long-
255 term) food supplementation began. This allowed us to focus the analysis on hypothesis
256 testing (sex-specific responses to food supply, i.e., response variables ~ sex * food-
257 treatment) without including three-way interactions to facilitate interpretation. We
258 modeled proportion of time spent on-colony (binomial GLM), LH 10 min after GnRH-
259 injection (LM; saline-injected birds excluded), and testosterone 30 min after GnRH
260 injection (LM; saline-injected birds excluded) in response to sex and food treatment.

261 ***2.4.3 Temporal patterns in pituitary and gonadal response to GnRH challenge***

We fitted GAMs to test for temporal shifts in GnRH-induced LH and testosterone. We compared the fit of models with a smoothed fixed effect of absolute day of year vs the number of days until laying (smoothed for each sex) and a parametric effect of sex using R^2. Saline-injected birds and birds that did not ultimately produce an egg were excluded from analyses. If effective degrees of freedom (edf) were greater than two, we interpreted this as evidence for a non-parametric effect (Zuur et al. 2009).

3. RESULTS

We captured 119 adult kittiwakes twice each between 18 April and 19 May 2019. Sample sizes were balanced between sexes within treatment groups, with fewer individuals in the short-term fed group (control: n = 23 females, 26 males; short-term fed: 12 females, 10 males; long-term fed: n = 25 females, 23 males). Four focal females (n = 2 control, 1 short-term fed, 1 long-term fed), and the mates of four focal males (n = 3 control, 1 short-term fed), did not lay an egg during the 2019 breeding season, and were excluded from additional analyses. We retrieved usable GPS data from 116 birds (one device lost during deployment; two devices failed with large data gaps) and obtained plasma for radioimmunoassay for all individual-timepoint combinations except one baseline sample at first capture.

3.1 Effects of food manipulation on laying phenology

Food supplementation advanced laying dates ($\eta^2 = 0.28$, $F_{2,102} = 21.1$, $p < 0.0001$; **Figure 3**), where both fed groups laid earlier than the control group (long-term: -6.5 ± 1.0 d, $p < 0.0001$; short-term:-3.6 ± 1.3 d, $p < 0.05$), and a non-significant trend for the

long-term fed group to lay earliest (–short-term: 2.9 ± 1.3 d, p = 0.07). The long-term fed group laid earliest (29 May ± 1.0 d), followed by the short-term fed group (1 June ± 1.3 d) and control group (5 Jun ± 0.7 d).

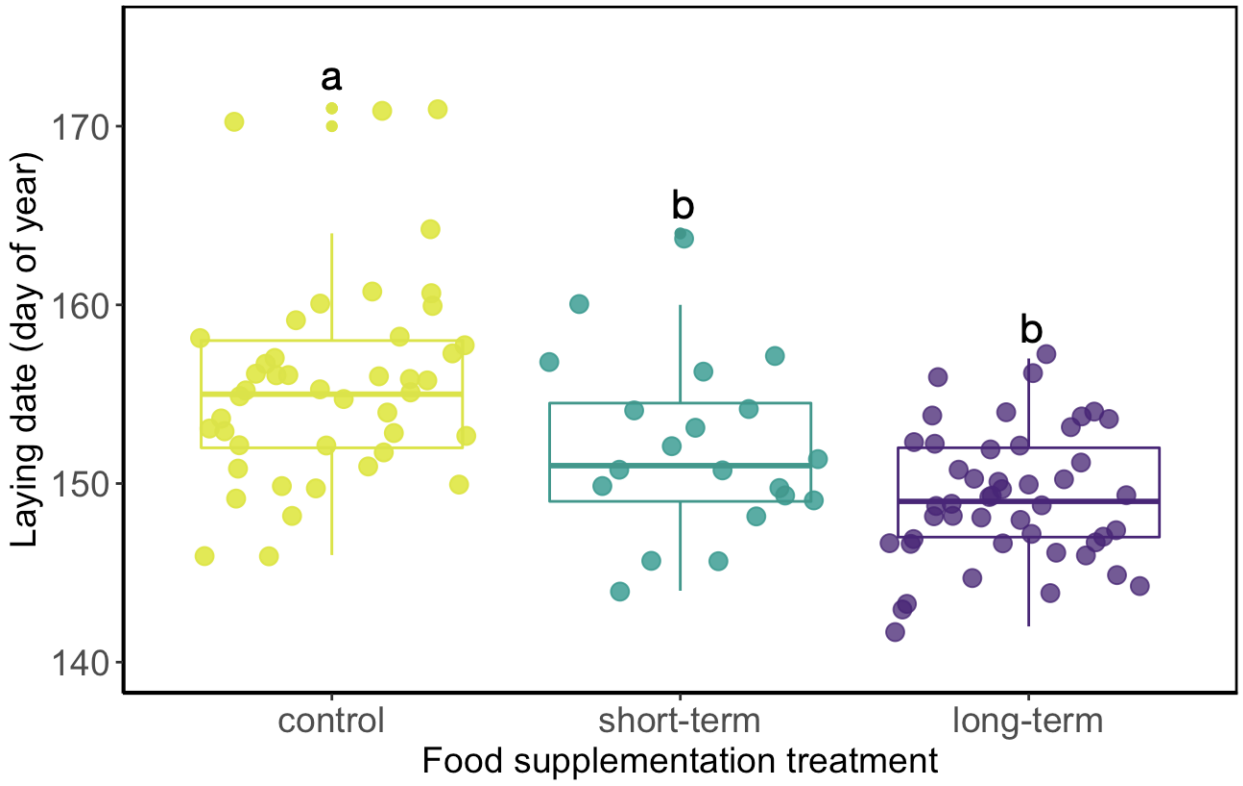

Figure 3 Both short-term and long-term food supplementation treatments advanced laying date in kittiwakes, relative to controls. Small letters indicate significant differences.

3.2 Effects of food manipulation and sex on colony attendance and hormones

Food treatment significantly influenced colony attendance before long-term feeding began, but sex had little effect on colony attendance (**Table 1**). Post-hoc comparisons indicated that the significant effect of food treatment was driven by short-term feeding; while the ANOVA was significant, the post-hoc results were non-significant: control birds had similar colony attendance to the long-term treatment (long-term: -0.15 ± 0.66

298 SE, z = 0.23, p = 0.97) but short-term fed birds tended to attend the colony more than
299 controls (–short-term: 2.1 ± 0.9, z = -2.2, p = 0.075) or the long-term fed birds (short-
300 term – long-term: 2.2 ± 1.0, z = 2.3, p = 0.057; **Figure 4A**). After long-term feeding
301 began, birds from all treatments spent more time at the colony. However, birds in the
302 long-term treatment spent more time at the colony than the control group (control -
303 long-term: -1.8 ± 0.7, z = -2.4, $p < 0.05$) and similar time to the short-term fed group
304 (short-term - long-term: -0.7 ± 0.9, z = -0.78, p = 0.72), but the control and short-term
305 fed groups spent a similar amount of time at the colony (control - short-term: -1.1 ±
306 0.8, z = -1.3, p = 0.39; **Figure 4B**).

307 Food treatment did not significantly influence LH 10 min after GnRH-injection or
308 testosterone 30 min after GnRH-injection at first capture before or after long-term
309 feeding began (**Table 1**). However, LH 10 min after GnRH-injection was lower in males
310 than females before long-term feeding began (-2.7 ± 1.3 ng/mL; **Figure 4C**), but similar
311 after long-term feeding began (0.3 ± 0.9 ng/mL; **Figure 4D**). In contrast, sex had little
312 effect on testosterone 30 min after GnRH-injection before long-term feeding began
313 (0.33 ± 0.22 ng/mL; **Figure 4E**) but was higher among males than females in all
314 treatment groups after the midpoint in the experiment, when long-term feeding began
315 (2.0 ± 0.3 ng/mL; **Figure 4F**).

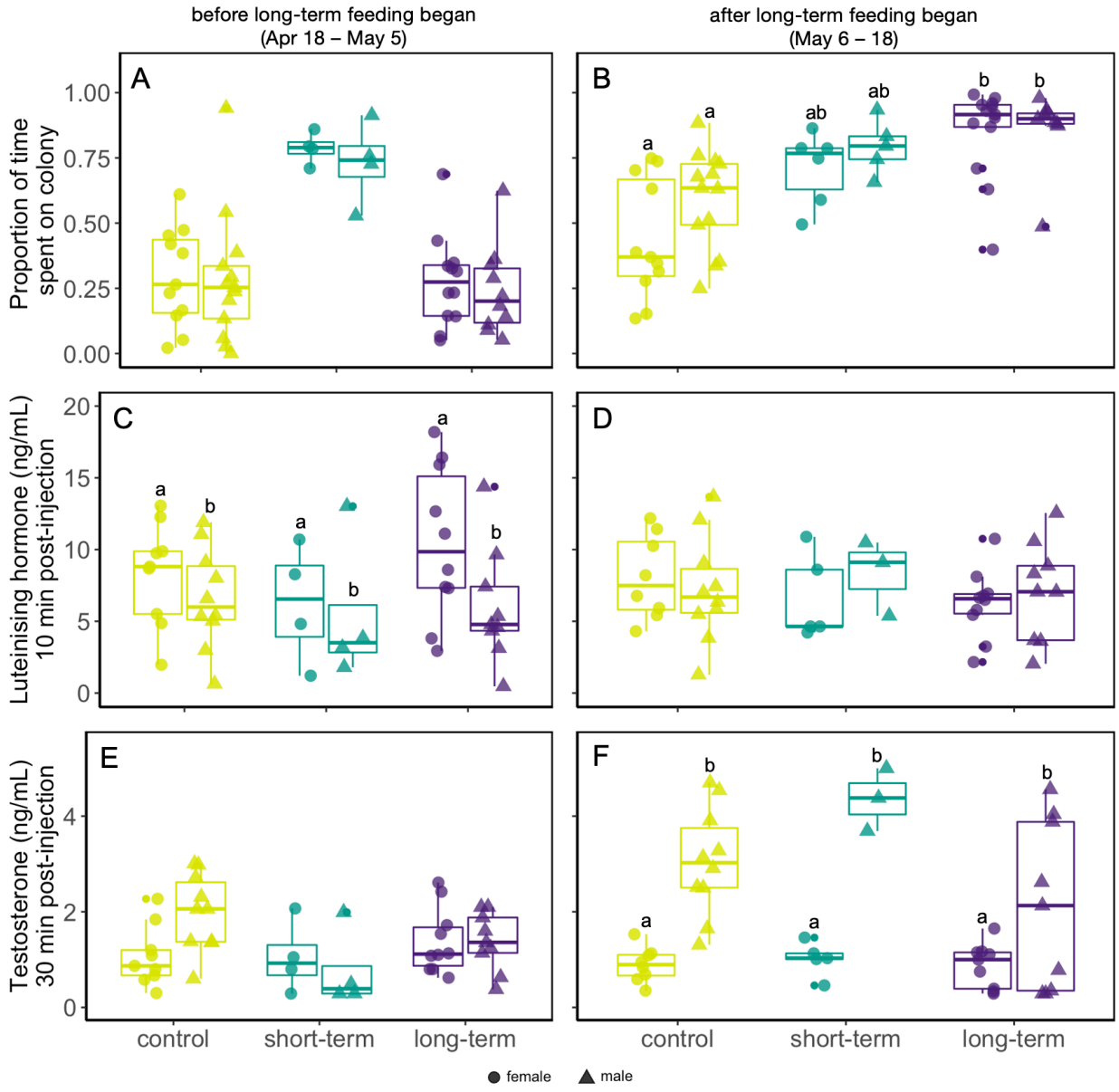

Figure 4 (A) Before the midpoint of the experiment, when long-term feeding had not yet begun, food treatment significantly influenced colony attendance (global effect), but post-hoc comparisons were non-significant before the midpoint of the experiment, when long-term feeding began. **(B)** Long-term food supplementation increased colony attendance after long-term feeding began. **(C)** Females had higher luteinising hormone 10 min post GnRH-injection before long-term feeding began, but **(D)** food treatment and sex did not influence luteinising hormone 10 min post GnRH-injection after long-term

feeding began. **(E)** Food treatment and sex did not influence testosterone 30 min post GnRH-injection before long-term feeding began but, **(F)** relative to females, males had higher testosterone 30 min post GnRH-injection after long-term feeding began. Individuals injected with saline solution were excluded from panels C-F. Small letters indicate significant differences as determined through post-hoc comparisons.

330 **Table 1** Test statistics and significance for models testing for sex-specific responses of food treatment, and/or main effects
331 of sex and food treatment. Intercept set to control (food treatment) and female (sex).

Response variables	Fixed effects	Before long-term feeding (18 Apr – 5 May)						After long-term feeding (6-18 May)					
		X_2	F value	df	p-value	odds ratio	η^2	X_2	F value	df	p-value	odds ratio	η^2
proportion of time on colony	treatment * sex	0.02	-	2	0.98		-	0.12	-	2	0.98		-
	short-term:male					0.77						0.83	
	long-term:male					0.87						0.59	
	treatment	6.83	-	2	**<0.05**		-	6.86	-	2	**<0.05**		-
	short-term					7.8						2.9	
	long-term					0.86						6.0	
	sex	0.05	-	1		0.87	-	0.55	-	1	0.46		-
	male				0.82							1.6	
LH 10 min post-inject	treatment * sex	-	0.71	2,40	0.50	-	0.034	-	0.58	2,39	0.57	-	0.29
	treatment	-	0.91	2,42	0.41	-	0.042	-	0.43	2,41	0.66	-	0.020
	sex	-	4.54	1,42	**<0.05**	-	0.098	-	0.11	1,41	0.74	-	0.0028
testosterone 30 min post-inject	treatment * sex	-	2.94	2,40	0.06		0.13	-	2.90	2,38	0.07	-	0.13
	treatment	-	2.10	2,42	0.13		0.091	-	2.68	2,40	0.08	-	0.12
	sex	-	2.28	1,42	0.14		0.051	-	36.62	1,40	**<0.0001**	-	0.48

332

3.3 Temporal patterns in pituitary and gonadal response to GnRH challenge

Male GnRH-induced LH responses started low, then peaked later in the pre-breeding season than females (**Table 2**; **Figure 6A**). GnRH-induced LH was not significantly associated with the number of days until laying in either sex (**Figure 6B**). When accounting for the sex differences temporal patterns, parametric effects of sex on GnRH-induced LH were not significant (**Table 2**).

GnRH-induced testosterone increased with absolute day of year in males, but not females (**Figure 6C**), and the pattern was similar with the number of days until laying (**Table 2**; **Figure 6D**). Males had higher GnRH-induced testosterone than females (**Table 2**).

343 **Table 2** Test statistics and significance for GAMs testing for sex differences in pituitary
344 and gonadal response to GnRH challenge over time (absolute and relative to laying).
345 Intercept was set to female (sex).

Fixed effects	Model R2	Non-parametric effects				Parametric effects			
		edf	F	DF	p-value	Estimate ± SE	F	DF	p-value
Response: GnRH-induced LH									
Day of year*female	0.26	2.3	3.5	2.8	**<0.05**	-	-	-	-
Day of year*male		3.5	4.9	4.1	**<0.01**	-	-	-	-
Sex (male)		-	-	-	-	-1.1 ± 0.7	2.4	1	0.12
Days until laying*female	0.11	1.6	1.5	2.0	0.20	-	-	-	-
Days until laying*male		3.0	2.3	3.7	0.10	-	-	-	-
Sex (male)		-	-	-	-	-0.8 ± 0.8	1.1	1	0.29
Response: GnRH-induced testosterone									
Day of year*female	0.34	1.0	2.1	1.0	0.15		-	-	-
Day of year*male		2.3	5.9	2.8	**<0.01**		-	-	-
Sex (male)			-	-	-	1.1 ± 0.2	25.6	1	**<0.0001**
Days until laying*female	0.31	1.0	1.7	1.0	0.20	-	-	-	-
Days until laying*male		2.1	4.8	2.6	**<0.01**	-	-	-	-
Sex (male)			-	-	-	1.0 ± 0.2	22.0	1	**<0.0001**

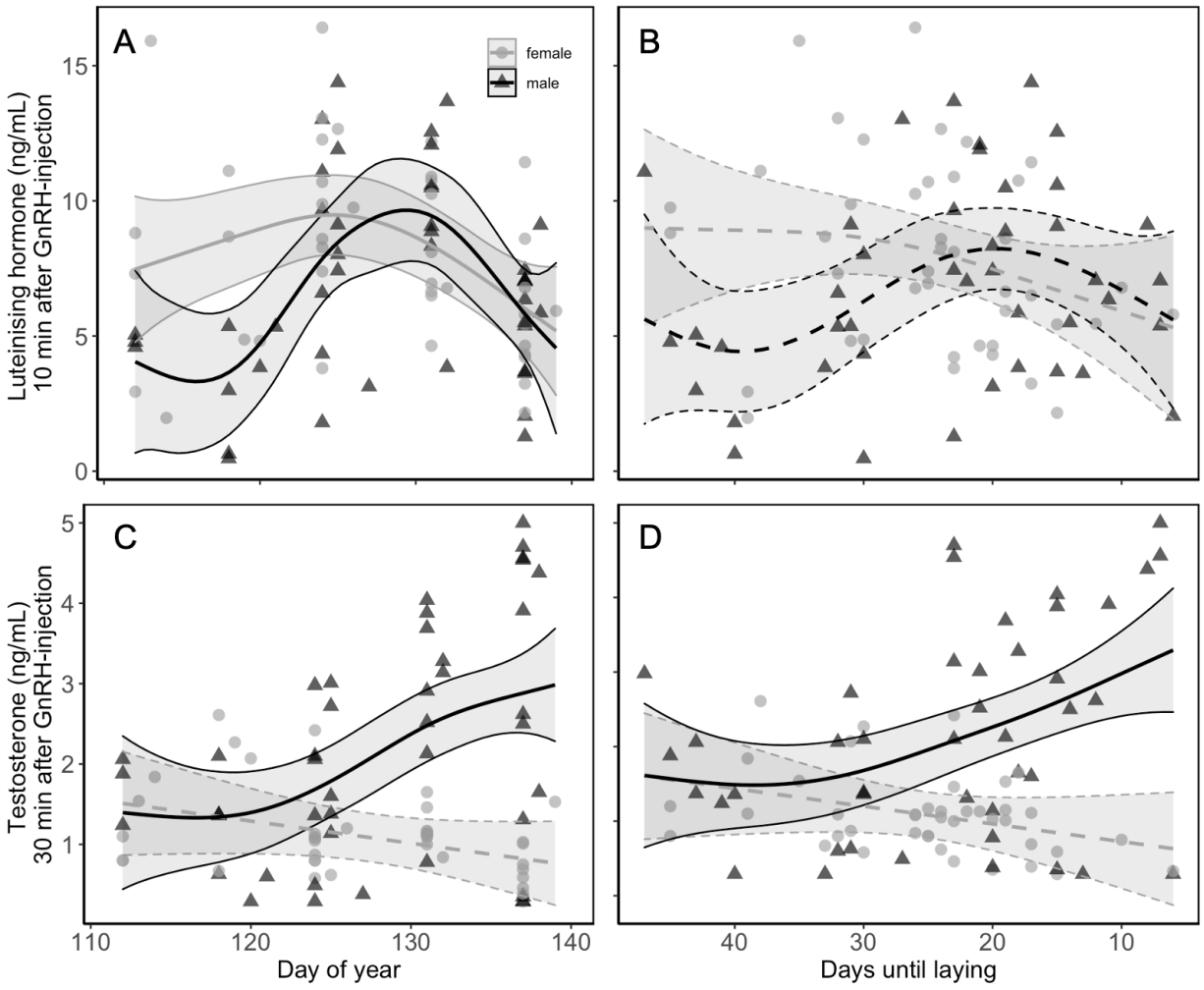

Figure 6 (A) Male GnRH-induced LH peaked later in the pre-breeding season than in females, but (**B**) was not significantly associated with time until laying. (**CD**) Male, but not female, GnRH-induced testosterone increased with time (absolute) and as laying approached. Males had higher GnRH-induced testosterone than females. Lines indicate predictions of GAMs with 95% confidence intervals; dashed lines indicate non-significant GAMs over time.

4. DISCUSSION

Though food supplementation advanced timing of reproduction, we found little evidence of sex-specific responses to food supply but strong sex differences in the

timing of maximal pituitary and gonadal responsiveness. Consistent with the *anticipation hypothesis* (Shultz et al. 2009), food-supplemented kittiwakes advanced laying regardless of whether they received short- or long-term supplementation, suggesting that perception of a stimulatory supplementary cue advanced phenology. Females did not show greater endocrine responses to food supplementation than males, which challenges the assumption that females are more sensitive to supplementary cues than males (Ball & Ketterson 2008), at least at the level of HPG responsiveness. However, we found clear sex differences in pituitary and gonadal responses to GnRH. In particular, the peak in pituitary responses of males was later and shorter in duration, relative to females, which is consistent with the *sensitivity to information hypothesis* and suggests male kittiwakes integrate synchronising cues around the same time females initiate follicle development.

Both short- and long-term feeding experiments increased colony attendance and advanced laying, supporting the idea that a perception of high food availability can advance phenology (i.e., kittiwakes are income breeders; Whelan et al. 2021). However, the endocrine pathway through which feeding advanced laying remains unclear. Under the *reproductive readiness hypothesis* (the current standard view), one might expect larger GnRH-induced LH and testosterone releases by fed individuals because they were closer to reproduction. Under the *sensitivity to information hypothesis*, one might expect earlier declines in GnRH-induced LH and testosterone (i.e., desensitisation of the pituitary and gonads) among fed individuals because they lay earlier (Whelan et al. 2021, this study) and environmental information becomes less relevant to decisions about reproductive timing after follicle development and fertilisation. As both early

laying and greater access to food are associated with greater breeding success (Whelan et al. 2022; Kahane-Rapport et al. 2022), we caution against using the magnitude of pituitary and gonadal response upon GnRH challenge as a metric of individual quality. Similar to Whelan et al. (2021), which found little evidence that feeding increased body condition, our study suggests that indirect perceptual effects rather than metabolic or nutritional effects are at play. However, it is possible that food-supplementation (whether short- or long-term) may meet nutritional thresholds that are not captured by body condition (e.g., micronutrients). Future experiments could evaluate alternative mechanisms to better understand how exactly information about food supply affects timing decisions (Hahn et al. 2005). For example, visual information, tactile information from handling of food items, or increased social interactions via increased colony attendance (e.g., pair bonding behaviours) are potential perception pathways that we did not test here.

We found limited evidence for sex-specific behavioural and hormonal responses to food supply during the early pre-breeding period. While females are expected to respond more strongly to supplementary cues (Ball & Ketterson 2008), both sexes increased colony attendance in response to feeding. Though elevated baseline corticosterone was negatively associated with female, but not male, baseline and GnRH-induced LH in Atlantic kittiwakes (Goutte et al. 2010), we did not find stimulatory effects of feeding on pituitary or gonadal responses in females (or males) in this study. This could suggest that pituitary and gonadal sensitivity is similar across individuals within the population, and variation in laying phenology results from individual differences in environmental information received and processed into downstream

HPG responses, but environmental conditions do not feed back to alter HPG sensitivity itself.

At the pituitary level (LH release), our findings are consistent with the *sensitivity to information hypothesis.* Female and male kittiwakes exhibited different patterns of response to GnRH challenge over the course of the pre-breeding season. These sex differences may reflect sex differences in investment and the lesser influence of males in timing decisions (Ball & Ketterson 2008; Williams et al. 2022). Females sustained relatively high LH in response to GnRH throughout the pre-laying season, while males had lower LH early in the season, then peaked about 20 days before the mean laying date. Though males are thought to be primarily sensitive to photoperiod (Ball & Ketterson 2008), the later peak in male response to GnRH suggests that males are also integrating information important to reproduction late in the pre-breeding period. If male and female LH responses were similar, that would suggest that males integrated the same supplementary cues as females. The peak in male responses was no longer statistically significant when considering proximity to laying, rather than absolute day of year. Thus, male sensitivity may be linked to population-level information (e.g., social cues from the greater colony) rather than individual-specific cues from the mate (e.g., scent indicators of female reproductive status, Caro et al. 2015). Because females laid on different dates (range: 22 May to 20 Jun), chronological date (which may be more related to cues such as photoperiod and food availability at sea but would affect all birds similarly) may not closely capture proximity to oviposition. Thus we analyzed temporal patterns both relative to individuals "days until oviposition" and relative to chronological date. Indeed, social stimulation from neighbouring pairs can influence

timing of reproduction in kittiwakes (Coulson & White 1959; Immer et al. 2021). Alternatively, the response in males could simply be a delayed response to earlier supplementary cues. In either case, our results are consistent with the idea that pituitary responsiveness to GnRH in males occurs after females become responsive, and may be a response to female or colony-wide synchronising cues, rather than supplementary cues about environmental conditions.

At the gonadal level, however, we observed increasing responses over time in males but little temporal effect in females (both absolute and relative to laying). This is in contrast to temporal patterns of response to GnRH in dark-eyed juncos (*Junco hyemalis*), where females showed greatest testosterone releases during follicle development (Jawor et al. 2007) and males showed greatest releases during the early breeding season (Jawor et al. 2006). However, in Atlantic kittiwakes, GnRH-induced testosterone increased with time until laying in males (Goutte et al. 2010). Interestingly, in our study, the shape of gonadal response to GnRH over time did not match the pituitary response in either sex. Male testosterone increased over time (absolute and time until laying) while female testosterone remained low over time despite non-linear patterns in LH in both sexes. Elevated gonadal steroids can inhibit pituitary release of LH (Desjardins & Turek 1977; Grieves et al. 2016) and it is possible that high testosterone responses observed among males late in the experiment were associated with the decline in male LH observed late in the experiment. Testosterone is the final hormone in the HPG cascade for males, and the one responsible for critical male reproductive behaviors and physiological processes such as gametogenesis (reviewed in Hau, 2007). However, testosterone likely plays a less direct role in reproductive

behavior and physiology in females, presumably acting as a precursor to estradiol (but see Smiley et al. 2022). We did not measure estradiol in this study, which might be a better metric of female gonadal sensitivity to gonadotropins.

While we anticipated that females should have a sustained peak in GnRH response during the pre-breeding period, the males' relatively late peak sparks new questions. Under the *sensitivity to information hypothesis*, we expect that males are integrating cues from their mate and/or other individuals in the colony. One possibility is that the males are ready to use information about female reproductive status. For example, male chickens use scent cues from females to determine their reproductive status (Hirao et al. 2009) and this could be an important synchronising cue for reproductive timing (Caro et al. 2015). In kittiwakes, courtship feeding behaviour peaks after pairs have formed and follicle development has already begun, and likely helps females maintain condition as they gain weight and decrease foraging behaviour (Whelan et al. 2021). Alternatively, males may be integrating information necessary for successful copulation. As argued by Goymann et al. (2019), males should benefit from sensitivity to interactions with females for as long as females are fertile. Male pituitary sensitivity peaked about 20 days before the mean laying date but declined during the period when copulation rates peak (0-18 d before laying; Whelan et al. 2021). Male pituitary sensitivity may have declined during this period because of decreasing female fertility (Goymann et al. 2019), or perhaps the peak in gonadal sensitivity observed in males is linked to copulation behaviour.

5. CONCLUSIONS

Seasonal timing of reproduction is often considered a female trait, and environmental drivers of breeding phenology have important consequences in the context of climate change (Ettinger et al. 2022). While many studies have tested environmental drivers of female timing of reproduction (e.g., Nussey et al. 2005ab; Charmantier et al. 2008), drivers of phenology are rarely examined in both sexes (Williams et al. 2022). Further, the mechanisms underlying temporal synchrony between female and male phenology are not well understood. Here, we found little evidence that females were more sensitive to supplementary cues (e.g., food supply) than males, which is a common assumption in animal ecology (Ball & Ketterson 2008). Instead, males became sensitive to information (synchronising cues, Jacobs & Wingfield 2000) later in the pre-breeding period than females, long after predictive cues such as photoperiod initiate gonadal recrudescence. Males may be less reliant on supplementary cues than females, but effectively adjust timing of important reproductive behaviours to variation in the environment by integrating synchronising cues from their social environment. Similar to phenological mismatches between predators and prey, mismatches between sexes due to climate change are an emerging concern (Williams et al. 2022). In species where males integrate synchronising cues from their social environment, this mechanism could reduce the potential for phenological mismatch between sexes.

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REFERENCES

Ball, G. F., & Ketterson, E. D. (2008). Sex differences in the response to environmental cues regulating seasonal reproduction in birds. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1490), 231-246.

Brommer, J. E., & Rattiste, K. (2008). “Hidden” reproductive conflict between mates in a wild bird population. *Evolution: International Journal of Organic Evolution*, *62*(9), 2326-2333.

Caro, S. P., Balthazart, J., & Bonadonna, F. (2015). The perfume of reproduction in birds: chemosignaling in avian social life. *Hormones and Behavior*, *68*, 25-42.

Caro, S. P., Charmantier, A., Lambrechts, M. M., Blondel, J., Balthazart, J., & Williams, T. D. (2009). Local adaptation of timing of reproduction: females are in the driver's seat. *Functional Ecology*, 172-179.

Charmantier, A., McCleery, R. H., Cole, L. R., Perrins, C., Kruuk, L. E., & Sheldon, B. C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *science*, *320*(5877), 800-803.

Coulson, J. C., & White, E. (1959). The effect of age and density of breeding birds on the time of breeding of the kittiwake *Rissa tridactyla*. *Ibis*, *101*(3-4), 496-497.

519 Desjardins, C., & Turek, F. W. (1977). Effects of testosterone on spermatogenesis and
520 luteinizing hormone release in Japanese quail. *General and Comparative*
521 *Endocrinology*, *33*(2), 293-303.
522 Ettinger, A. K., Chamberlain, C. J., & Wolkovich, E. M. (2022). The increasing relevance
523 of phenology to conservation. *Nature Climate Change*, *12*(4), 305-307.
524 Farner, D. S., Follett, B. K., King, J. R., & Morton, M. L. (1966). A quantitative
525 examination of ovarian growth in the white-crowned sparrow. *The Biological*
526 *Bulletin*, *130*(1), 67-75.
527 Farner, D. S., & Wilson, A. C. (1957). A quantitative examination of testicular growth in
528 the white-crowned sparrow. *The Biological Bulletin*, *113*(2), 254-267.
529 Fudickar, A.M., Greives, T.J., Abolins-Abols, M., Atwell, J.W., Meddle, S.L., Friis, G.,
530 Stricker, C.A. and Ketterson, E.D., 2017. Mechanisms associated with an
531 advance in the timing of seasonal reproduction in an urban songbird. *Frontiers in*
532 *Ecology and Evolution*, *5*, p.85.
533 Gill, V. A., & Hatch, S. A. (2002). Components of productivity in black-legged kittiwakes
534 *Rissa tridactyla*: response to supplemental feeding. *Journal of Avian*
535 *Biology*, *33*(2), 113-126.
536 Goutte, A., Angelier, F., Chastel, C. C., Trouvé, C., Moe, B., Bech, C., ... & Chastel, O.
537 (2010). Stress and the timing of breeding: glucocorticoid-luteinizing hormones
538 relationships in an arctic seabird. *General and Comparative*
539 *Endocrinology*, *169*(1), 108-116.
540 Goutte, A., Kriloff, M., Weimerskirch, H., & Chastel, O. (2011). Why do some adult birds

skip breeding? A hormonal investigation in a long-lived bird. *Biology letters*, *7*(5), 790-792.

Goymann, W., Moore, I. T., & Oliveira, R. F. (2019). Challenge hypothesis 2.0: a fresh look at an established idea. *BioScience*, *69*(6), 432-442.

Greives, T. J., Fudickar, A. M., Atwell, J. W., Meddle, S. L., & Ketterson, E. D. (2016). Early spring sex differences in luteinizing hormone response to gonadotropin releasing hormone in co-occurring resident and migrant dark-eyed juncos (*Junco hyemalis*). *General and comparative endocrinology*, *236*, 17-23.

Hahn, T. P., Pereyra, M. E., Katti, M., Ward, G. M., & MacDougall-Shackleton, S. A. (2005). Effects of food availability on the reproductive system. *Functional avian endocrinology*, 167-180.

Hau, M. (2007). Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays*, *29*(2), 133-144.

Hirao, A., Aoyama, M., & Sugita, S. (2009). The role of uropygial gland on sexual behavior in domestic chicken *Gallus gallus domesticus*. *Behavioural Processes*, *80*(2), 115-120.

Immer, A., Merkling, T., Chastel, O., Hatch, S. A., Danchin, E., Blanchard, P., & Leclaire, S. (2021). Spying on your neighbours? Social information affects timing of breeding and stress hormone levels in a colonial seabird. *Evolutionary Ecology*, *35*(3), 463-481.

Jacobs, J.D. and Wingfield, J.C. (2000). Endocrine control of life-cycle stages: a constraint on response to the environment?. *The Condor*, *102*(1), pp.35-51.

Jawor, J. M., McGlothlin, J. W., Casto, J. M., Greives, T. J., Snajdr, E. A., Bentley, G.

564 E., & Ketterson, E. D. (2006). Seasonal and individual variation in response to
565 GnRH challenge in male dark-eyed juncos (*Junco hyemalis*). *General and*
566 *comparative endocrinology*, *149*(2), 182-189.
567 Jawor, J. M., McGlothlin, J. W., Casto, J. M., Greives, T. J., Snajdr, E. A., Bentley, G.
568 E., & Ketterson, E. D. (2007). Testosterone response to GnRH in a female
569 songbird varies with stage of reproduction: implications for adult behaviour and
570 maternal effects. *Functional Ecology*, *21*(4), 767-775.
571 Jodice, P. G. R., Lanctot, R. B., Gill, V. A., Roby, D. D., & Hatch, S. A. (2000). Sexing
572 adult black-legged kittiwakes by DNA, behavior, and morphology. *Waterbirds*,
573 405-415.
574 Kitaysky A.S., Piatt J.F., & Wingfield, J.C. (2007). Stress hormones link food availability
575 and population processes in seabirds. *Marine Ecology Progress Series*, *352*,
576 245–258.
577 Lenth, R. (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means. R
578 package version 1.4.5. https://CRAN.R-project.org/package=emmeans
579 Moiron, M., Araya-Ajoy, Y. G., Teplitsky, C., Bouwhuis, S., & Charmantier, A. (2020).
580 Understanding the social dynamics of breeding phenology: indirect genetic
581 effects and assortative mating in a long-distance migrant. *The American*
582 *Naturalist*, *196*(5), 566-576.
583 Needham, K. B., Burns, C. B., Graham, J. L., Bauer, C. M., Kittilson, J. D., Ketterson, E.
584 D., ... & Greives, T. J. (2019). Changes in processes downstream of the
585 hypothalamus are associated with seasonal follicle development in a songbird,

586 the dark-eyed junco (*Junco hyemalis*). *General and comparative*
587 *endocrinology*, *270*, 103-112.
588 Nussey, D. H., Clutton-Brock, T. H., Elston, D. A., Albon, S. D., & Kruuk, L. E. (2005a).
589 Phenotypic plasticity in a maternal trait in red deer. *Journal of Animal Ecology*,
590 387-396.
591 Nussey, D. H., Postma, E., Gienapp, P., & Visser, M. E. (2005b). Selection on heritable
592 phenotypic plasticity in a wild bird population. *Science*, *310*(5746), 304-306.
593 Perfito, N., Guardado, D., Williams, T. D., & Bentley, G. E. (2015). Social cues regulate
594 reciprocal switching of hypothalamic Dio2/Dio3 and the transition into final
595 follicle maturation in European starlings (*Sturnus vulgaris*). *Endocrinology*,
596 *156*(2), 694-706.
597 Porter, T.R, Hargis, B.N., Silsby, J.L, and Halwani, M. E. E., (1989). Differential steroid
598 production between theca interna and theca externa cells: a three-cell model for
599 follicular steroidogenesis in avian species. *Endocrinology*, *125*(1), pp.109-116.
600 Réale, D., Berteaux, D., McAdam, A. G., & Boutin, S. (2003). Lifetime selection on
601 heritable life-history traits in a natural population of red squirrels. *Evolution*,
602 *57*(10), 2416-2423.
603 Riechert J., Becker P.H., & Chastel O. (2014). Predicting reproductive success from
604 hormone concentrations in the common tern (*Sterna hirundo*) while considering
605 food abundance. *Oecologia*, *176*, 715-727.
606 Romero, L. M., Soma, K. K., & Wingfield, J. C. (1998). Hypothalamic-pituitary-adrenal

axis changes allow seasonal modulation of corticosterone in a bird. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *274*(5), R1338-R1344.

Sauve, D., Divoky, G., & Friesen, V. L. (2019). Phenotypic plasticity or evolutionary change? An examination of the phenological response of an arctic seabird to climate change. *Functional ecology*, *33*(11), 2180-2190.

Scanes, C. G. (2015). Pituitary gland. In *Sturkie's Avian Physiology* (pp. 497-533). Academic Press.

Schoech, S. J., Mumme, R. L., & Wingfield, J. C. (1996). Delayed breeding in the cooperatively breeding Florida scrub-jay (*Aphelocoma coerulescens*): inhibition or the absence of stimulation? *Behavioral Ecology and Sociobiology*, *39*(2), 77-90.

Shultz, M. T., Piatt, J. F., Harding, A. M., Kettle, A. B., & Van Pelt, T. I. (2009). Timing of breeding and reproductive performance in murres and kittiwakes reflect mismatched seasonal prey dynamics. *Marine Ecology Progress Series*, *393*, 247-258.

Smiley, K.O., Lipshutz, S.E., Kimmitt, A.A., DeVries, M.S., Cain, K.E., George, E.M., & Covino, K.M. (2022). Beyond a biased binary: A perspective on the misconceptions, challenges, and implications of studying females in avian behavioral endocrinology. *Frontiers in Physiology, 13*, 970603.

Teplitsky, C., Mills, J.A., Yarrall, J.W., & Merilä, J. (2010). Indirect genetic effects in a sex-limited trait: the case of breeding time in red-billed gulls. *Journal of evolutionary biology*, *23*(5), 935-944.

Whelan, S., Strickland, D., Morand-Ferron, J., & Norris, D. R. (2016). Male experience buffers female laying date plasticity in a winter-breeding, food-storing passerine. *Animal Behaviour*, *121*, 61-70.

Whelan, S., Hatch, S. A., Benowitz-Fredericks, Z. M., Parenteau, C., Chastel, O., & Elliott, K. H. (2021). The effects of food supply on reproductive hormones and timing of reproduction in an income-breeding seabird. *Hormones and Behavior*, *127*, 104874.

Whelan, S., Hatch, S. A., Gaston, A. J., Gilchrist, H. G., & Elliott, K. H. (2022). Opposite, but insufficient, phenological responses to climate in two circumpolar seabirds: relative roles of phenotypic plasticity and selection. *Functional Ecology*.

Williams, C. T., Chmura, H. E., Deal, C. K., & Wilsterman, K. (2022). Sex-differences in Phenology: A Tinbergian Perspective. *Integrative and Comparative Biology*.

Williams, T. D. (2012). *Physiological adaptations for breeding in birds*. Princeton University Press.

Wingfield, J. C., Crim, J. W., Matfocks Jr, P. W., & Farner, D. S. (1979). Responses of photosensitive and photorefractory male white-crowned sparrows (*Zonotrichia leucophrys gambelii*) to synthetic mammalian luteinizing hormone releasing hormone (Syn-LHRH). *Biology of reproduction*, *21*(4), 801-806.

Wingfield, J. C., Lynn, S. E., & Soma, K. K. (2001). Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. *Brain, behavior and evolution*, *57*(5), 239-251.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood

652 estimation of semiparametric generalized linear models. *Journal of the Royal*
653 *Statistical Society: Series B (Statistical Methodology)*, *73*(1), 3-36.
654 Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed*
655 *effects models and extensions in ecology with R* (Vol. 574). New York: Springer.
656