

The effects of food supply on reproductive hormones and timing of reproduction in an income-breeding seabird

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ABSTRACT

Current food supply is a major driver of timing of breeding in income-breeding animals, likely because increased net energy balance directly increases reproductive hormones and advances breeding. In capital breeders, increased net energy balance increases energy reserves, which eventually leads to improved reproductive readiness and earlier breeding. To test the hypothesis that phenology of income-breeding birds is independent of energy reserves, we conducted an experiment on food-supplemented (“fed”) and control female black-legged kittiwakes (*Rissa tridactyla*). We temporarily increased energy costs (via weight handicap) in a 2 × 2 design (fed/unfed; handicapped/unhandicapped) during the pre-laying period and observed movement via GPS-accelerometry. We measured body mass, baseline hormones (corticosterone; luteinising hormone) before and after handicap manipulation, and conducted a gonadotropin-releasing hormone challenge. Females from all treatment groups foraged in similar areas, implying that individuals could adjust time spent foraging, but had low flexibility to adjust foraging distance. Consistent with the idea that income breeders do not accumulate reserves in response to increased food supply, fed birds remained within an energy ceiling by reducing time foraging instead of increasing energy reserves. Moreover, body mass remained constant until the onset of follicle development 20 days prior to laying regardless of feeding or handicap, implying that females were using a ‘lean and fit’ approach to body mass rather than accumulating lipid reserves for breeding. Increased food supply advanced endocrine and laying phenology and altered interactions between the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal axis, but higher energy costs (handicap) had little effect. Consistent with our hypothesis, increased food supply (but not net energy balance) advanced endocrine and laying phenology in income-breeding birds without any impact on energy reserves.

1. Introduction

The ability to adjust timing of reproduction according to environmental conditions and internal state optimises fitness in predictable environments (Love et al., 2004; Charmantier et al., 2008). Within a breeding season, reproductive success is often highest for individuals that breed early (Perrins, 1970; de Forest and Gaston, 1996) and align the energetic demands of reproduction with peaks in resources (Verhulst et al., 1995). To optimise timing of breeding and maximise expected reproductive success, environmental cues (e.g. temperature, Schaper et al., 2011) and internal cues (e.g. body fat, Bêty et al., 2003) are integrated at the hypothalamus, producing cascading effects through the endocrine system. Timing of reproduction can also be

influenced by whether resources come from accumulated capital (i.e. energy reserves accumulated prior to breeding that are then invested into breeding) or current income (i.e. energy available in the environment that can be converted into breeding output). Along this capital-income breeding continuum, income-breeding species should be relatively more sensitive to short-term pre-breeding environmental conditions, adjusting timing based on current conditions to predict the optimal investment that maximises future success.

For both income- and capital-breeding species, the endocrine system transforms information about breeding conditions—from environmental conditions and individual state—into physiological and behavioural responses that drive ultimate outcomes. In female birds, the role of the reproductive axis (HPG: hypothalamic-pituitary-gonadal) shifts

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throughout the preoviposition period, at first regulating the onset of reproduction (Dawson et al., 2001; Christians and Williams, 1999) and later controlling follicle development and ovulation (Yang et al., 2000). If sensory information is favourable for reproduction, the hypothalamus releases gonadotropin-releasing hormone (GnRH) that, when bound to receptors at the anterior pituitary, causes the pituitary to release luteinising hormone (LH) and follicle stimulating hormone, which finally act on the gonads, stimulating release of testosterone and estradiol (Hattori et al., 1986; Dawson et al., 2001). Females can have heightened endocrine sensitivity to environmental conditions during the pre-breeding season compared to males, presumably due to greater female reproductive investment via egg formation (Ball and Ketterson, 2008).

Interactions between the hypothalamic-pituitary-adrenal (HPA) and HPG axes may allow individuals to adjust reproductive investment, including via timing of breeding, in response to variable environmental conditions. In some cases, as implied by the negative relationships between corticosterone and reproductive success predicted by the cort-fitness hypothesis (Bonier et al., 2009a), there is abundant evidence that corticosterone can inhibit HPG activity and delay reproduction (Goutte et al., 2010; Deviche et al., 2012; Lynn et al., 2015; Calisi et al., 2018). In other cases, poor environmental conditions inhibit HPG activity without increasing circulating corticosterone (Valle et al., 2015). However, in some contexts, elevated corticosterone is associated with increased reproductive investment (cort-adaptation hypothesis, Bonier et al., 2009b). For example, violet-green swallows (*Tachycineta thalassina*) with experimentally increased flight costs during chick-rearing increased circulating corticosterone but achieved similar breeding success to unmanipulated controls (Rivers et al., 2017). Thus, though corticosterone is likely a mechanism that mediates timing of reproduction via its interaction with the HPG axis (Lattin et al., 2016), the predicted direction of the relationship between circulating corticosterone and timing of reproduction is not always clear and could depend on both the life-history strategy of the animal and timing within the annual cycle (Bonier et al., 2009b; Lattin et al., 2016).

Variation in food supply (which we use as synonymous with ‘food availability’) is an environmental challenge that has strong effects on animal movement, endocrinology, and reproductive phenology, but the underlying mechanisms likely differ along the capital-income breeding continuum. When food supply is high, animals gain more energy per unit time and expend less energy to forage (Jodice et al., 2002), leading to high net energy balance (i.e. energy intake minus energy expenditure). Surplus energy can be harnessed in body fat reserves, but high net energy balance may not translate into high energy reserves for income-breeding animals if there are fitness costs to storing energy (*lean and fit vs fat and fit* strategies, Schultner et al., 2013). For example, decreasing mass during reproduction can be adaptive in birds because reduced flight costs increase foraging efficiency and lower predation risk (Norberg, 1981; Slagsvold and Johansen, 1998). Food supply can influence HPG activity directly (via integration of cues from the environment at the hypothalamus, Hahn et al., 2005) or indirectly (via regulation of HPA axis in response to internal energy reserves). Income breeders may therefore rely more heavily on their perception of cues about food supply in the environment to time reproduction, whereas capital breeders may need to meet a threshold of energy stores before breeding. Ultimately, increased food supply advances timing of reproduction in nearly all species, regardless of position along the capital-income continuum (Boutin, 1990).

Here, we test the hypothesis that food supply during the pre-breeding season can advance timing of reproduction of income breeders without any effect on energy reserves in a long-lived seabird species (Fig. 1). We manipulated energy costs and gains for female black-legged kittiwakes (*Rissa tridactyla*) during their pre-breeding period on Middleton Island, Alaska. Specifically, we used a weight handicap to increase energy expenditure via higher flight costs, and food supplementation to increase energy intake and decrease energy expenditure via reduced foraging effort. Thus, handicapped birds had lower net

energy balance (higher costs and likely lower food intake) and fed birds had higher net energy balance (lower costs due to reduced need to forage and higher food intake). The experiment was integrated into a long-term food supplementation study where individuals have been fed throughout the breeding season for many years, therefore food supplementation is a reliable indicator of continued high food supply. To explore potential mechanisms that might mediate effects of our manipulations on timing of breeding we also measured movement behaviour (via biologging of movement), body mass, baseline and handling-induced corticosterone, baseline and GnRH-induced reproductive hormones (LH, testosterone), and timing of reproductive behaviours and laying (i.e. reproductive phenology).

First, we test several assumptions of the experiment that are necessary to interpret the results of hypothesis tests: (i) kittiwakes are income-breeders, (ii) weight handicaps increase flight costs, (iii) food supplementation and weight handicaps alter movement behaviour and energy expenditure, (iv) reproductive behaviours are associated with a surge in circulating LH in the pre-breeding period, and (v) food supplementation and weight handicaps alter corticosterone and HPA-HPG interactions. Then, we test the overarching hypothesis, which predicts that breeding phenology will be earlier in fed birds than controls, but weight handicap will have little effect on reproductive phenology. Finally, we test predictions of three specific sub-hypotheses regarding the relationships between food supply, reproductive hormones, and timing of breeding (Table 1).

2. Material and methods

2.1. Study system

We studied black-legged kittiwakes that were part of a long-term, individual-based monitoring program that began in 1995 at Middleton Island, AK (59.48N, 146.38W). Breeding adults were banded with a unique combination of colour bands and a stainless steel USFWS band, and sexed via behavioural observations (i.e. courtship feeding, copulations). The study site was uniquely suited to experimental manipulation because kittiwakes nest on the outside of an abandoned radar tower that is equipped with one-way mirrored windows. Adult birds were observed through windows and captured using a leg hook with minimal disturbance to non-target birds. This population of kittiwakes return to their nesting sites two months prior to breeding (Whelan et al., 2020); during that period, the population switches from feeding on pelagic myctophids, which require distant foraging, to eulachon, invertebrates (squid, krill and polychaetes) and forage fish near the coast (Hatch, 2013).

We conducted an experiment on pre-breeding, female kittiwakes from May 6 until June 10, 2018. We observed site occupants daily to determine which breeding pairs were established and used a qualitative scale to describe stage of nest development (0 = no nest material, 1 = some nest material, 2 = structured nest material, 3 = complete platform, and 4 = fully egg-ready nest). To observe copulation events, we recorded video of 177 nest sites for approximately 4 h per day, from 10 May to 12 Jun. An observer blind to treatment watched an average of 100 min per nest site per day. The observer noted the date, nest site, and time at which copulations took place. We opportunistically recorded courtship feeding events throughout the study, recording the date, nest site, male and female identities. We checked nests for new eggs twice daily (9 h, 18 h) from May 5 until Aug 14 and considered laying date as the date that the first or single egg was laid.

2.2. Experimental design

We used a two-by-two experimental design (Fig. 2) to manipulate energy gains (control/fed) and energy costs (control/weight-handicapped) of 119 female kittiwakes. Beginning May 6, we provided unlimited fish (capelin, *Mallotus villosus*) three times each day (9 h, 14 h,

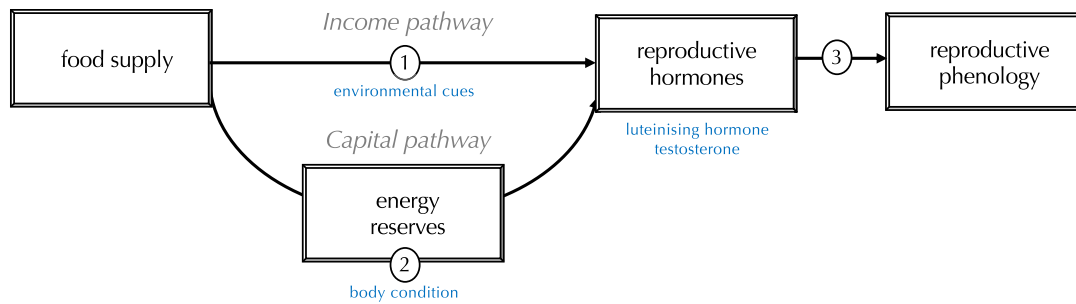


Fig. 1. Conceptual diagram of relationship between food supply and reproductive phenology (numbers refer to hypotheses in Table 1). The “income pathway” shows that environmental cues about food supply can act directly on the HPG axis to influence reproductive hormones and phenology. The “capital pathway” shows that food supply can influence reproductive hormones and phenology via energy reserves.

and 18 h) at a subset of breeding sites. Both the male and female were hand-fed fish through a plastic pipe until both birds refused additional fish. This protocol was part of a long-term food supplementation experiment and continued from pre-breeding until chicks fledged (described in Gill and Hatch, 2002).

To temporarily increase energy costs for half of the females in each food-supplementation treatment (control/fed), we attached a 23 g weight handicap to the back for four days. We conducted five rounds of captures on groups of 14–30 females, staggered 6 days apart (May 12, 18, 24, 30, Jun 5). At each round of captures, we selected females with highly developed nests (i.e. 3–4), with the exception of some females at sites with low nest development (i.e. 1–2) at the final round of captures. Food-supplemented pairs ultimately laid earlier than non-supplemented pairs, thus we did not capture any fed birds in the final round because all females had either been captured or laid eggs already.

2.3. Capture and baseline I samples

Upon first capture, we collected a single blood sample (1 mL) from the alar vein to measure baseline reproductive hormones (LH, testosterone) and corticosterone. We obtained the blood samples within 3 min post-capture to ensure that measurements indicated baseline hormone levels, rather than acute stress response to capture. We used 1 mL heparinised syringes with a 25-gauge needle. Before centrifugation, we ran a small amount of whole blood from the baseline samples through point-of-care devices validated to measure glucose, beta-hydroxybutyrate, and cholesterol (published as Case Study I in Morales et al., 2020). We used principal components analysis to determine whether these metabolites represented energy reserves; the metabolites

loaded orthogonal to body condition index (BCI) and corticosterone (Fig. 3). This pattern indicated that metabolites may play a role in energy management rather than act as energy reserves, so we excluded these variables from subsequent analyses. We centrifuged the remainder of the blood sample for 7 min, separated plasma from red blood cells, and stored samples at -20°C until assay.

After blood sampling, we weighed the bird and attached a GPS-accelerometer (9–11.5 g, AxyTrek, TechnoSmart Europe, GPS fix-rate: 3 min, triaxial acceleration: 25 Hz) to every female included in the experiment. We fastened the device to the top of the two central tail feathers with marine cloth tape (tesa®) and two nylon cable ties. If the female was assigned a handicap treatment, we also attached a weight to the back feathers using marine cloth tape and nylon cable ties.

2.4. Recapture, baseline II samples, and GnRH challenge

We began recapture efforts four days after initial capture and baseline I sampling. Upon recapture, we collected a 1 mL baseline blood sample for an identical set of measurements as during initial capture. After obtaining the baseline sample, we immediately injected 0.1 mL of either GnRH solution ([Gln8] LHRH (chicken), Phoenix Pharmaceuticals Inc., Lot No. 432694) or control solution (0.9% phosphate-buffered saline, Sigma-Aldrich) into the alar vein. We dissolved the GnRH to achieve a dosage of 0.6 $\mu\text{g}/0.1\text{ mL}$ (1.5 $\mu\text{g}/\text{kg}$ body mass in 1 mL of 0.9% saline solution). Following injection, we removed weights and/or GPS-accelerometers and measured body mass, wing chord, tarsus, culmen, and skull length. After measurements, we placed the bird into a cotton bag until 10 min post-injection, then we took a second $\sim 0.4\text{ mL}$ blood sample to measure LH and corticosterone. We returned the bird to the cotton bag

Table 1

Sub-hypotheses within Fig. 1 and predictions that were tested in this study. We designed the experiment assuming that supplemental feeding maximises the opportunity for energy gains (via both increased energy intake and decreased energy expenditure to forage) and weight-handicapping imposes energy costs (via increased energy expenditure during flight).

Hypotheses	Predictions	Findings from this study
<i>Income</i>		
1 Cues of food supply are integrated at the HPG axis	a) Fed females will have higher LH and testosterone (baseline and GnRH-induced), independent of body condition b) Weight handicap will not influence LH and testosterone (baseline and GnRH-induced)	- Supported - Supported
<i>Capital</i>		
2 Energy surplus increases reproductive hormones via energy reserves	a) Fed birds will have higher body condition (regardless of handicap treatment), followed by birds that are not handicapped or fed, then birds that are handicapped and not fed b) Fed birds will have greater increases in LH and testosterone (baseline and GnRH-induced), followed by birds that are not handicapped or fed, then birds that are handicapped and not fed	- Not supported - Not supported
<i>Both</i>		
3 Reproductive hormones increase as females approach egg-laying, and with individual quality	a) LH (baseline and GnRH-induced) will be higher in females closer to laying b) LH (baseline and GnRH-induced) will be higher in fed females and females that lay on earlier absolute dates c) Energy manipulation driven differences in corticosterone (baseline and handling-induced) will correlate negatively with LH and testosterone (baseline and GnRH-induced)	- Equivocal - Not supported - Equivocal

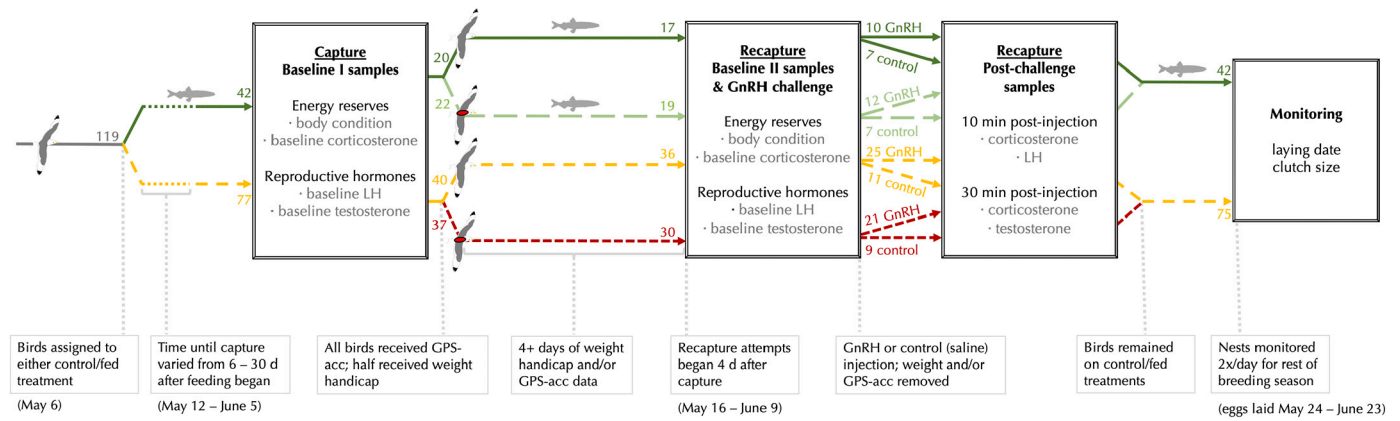


Fig. 2. Schematic of experimental design, sampling, and timeline. Number of females per group is indicated at each timepoint.

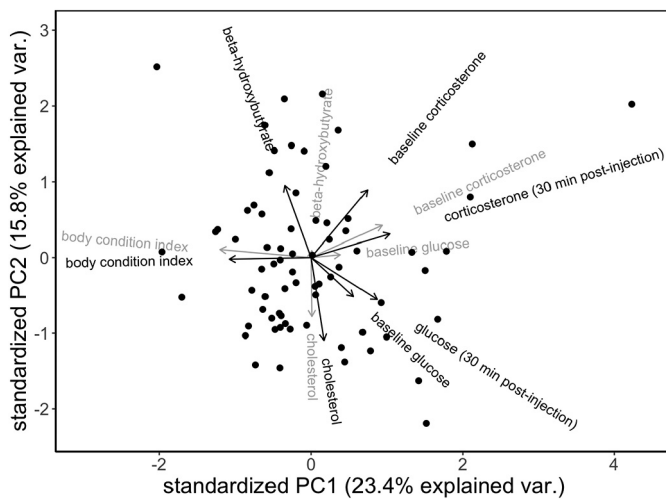


Fig. 3. Principal components analysis of metabolites, baseline and handling-induced corticosterone, and body condition index ($N = 71$ females with complete set of measurements) measured at capture (grey) and recapture (back).

until 30 min post-injection, then took a third ~ 0.6 mL blood sample to measure testosterone and corticosterone.

2.5. Hormone radioimmunoassays

We conducted radioimmunoassays at the Centre d'Études Biologiques de Chizé, following protocols biochemically and biologically validated to measure plasma corticosterone (Lormée et al., 2003), LH (Chastel et al., 2005a), and testosterone (Chastel et al., 2003). We measured testosterone to verify whether changes in LH were having cascading effects on the gonads; while estradiol is important for female birds in the pre-breeding period, we focused on testosterone because we were able to obtain more reliable measurements for testosterone via radioimmunoassay than estradiol. Methods were biochemically validated using several tests: linearity, spiking recovery and precision. The LH radioimmunoassay protocol has been biologically validated specifically for black-legged kittiwakes (Goutte et al., 2010). In this study, we confirmed that GnRH-injected females elevated LH and testosterone post-injection, relative to saline-injected controls (Appendix 1). We calculated sensitivity as two standard deviations from the readings of our blank tubes within the hormone assays (LH sensitivity = 0.54 ng/mL; testosterone sensitivity = 0.3 ng/mL; corticosterone sensitivity = 0.28 ng/mL).

Plasma corticosterone and testosterone were extracted by adding 3 mL of diethyl-ether, vortexing and centrifuging. The diethyl-ether phase containing the steroids was decanted and poured off after snap freezing the tube in an alcohol bath at -30 °C and the residue was then

evaporated. The dried extract was re-dissolved in phosphate buffer and incubated overnight at 4 °C with 3000 CPM of ^3H -corticosterone (Perkin Elmer, US) and a polyclonal rabbit antiserum (Sigma, US). The bound and free fractions were separated by adding dextran-coated charcoal. Activity of the bound fraction was counted in a Tri-carb liquid scintillation counter 2810TR (Perkin Elmer, US).

The LH was measured directly in plasma which was incubated overnight at 4 °C with 8000 CPM of ^{125}I -LH and a polyclonal antiserum. The bound fraction was separated from free fraction by immunoprecipitation with a second antibody against LH antiserum. Activity of the bound fraction was counted in a Wizard Gamma counter 2470 (Perkin Elmer, US).

Samples from each female were analysed in chronological sequence (baseline I and II, 10 min and 30 min post-challenge) within the same assay. We assayed each sample in duplicate for LH (intra-assay CV = 7.04%; inter-assay CV = 10.13%), testosterone (intra-assay CV = 6.33%; inter-assay CV = 18.94%), and corticosterone (intra-assay CV = 10.12%; inter-assay CV = 18.65%). We excluded one outlier corticosterone value from statistical analyses (> 2 SD from mean; 30 min post GnRH-injection).

The experimental design included a long-term food supplementation (> 1 month) and short-term weight handicap (4-day). We therefore tested for effects of food supplementation on baseline hormones at first capture, then also tested for effects of food supplementation and weight handicap on changes in hormones over the 4-day manipulation and in response to GnRH injection and handling. We defined Δ baseline hormones as concentration at recapture (baseline II) minus concentration at capture (baseline I). LH concentrations peak 10 min after GnRH injection, while testosterone and corticosterone concentrations peak 30 min after GnRH injection (Goutte et al., 2010). We therefore defined GnRH-induced LH as concentration 10 min post-injection minus concentration at recapture (baseline II) and GnRH-induced testosterone and handling-induced corticosterone as concentration 30 min post-injection minus concentration at recapture (baseline II).

2.6. Movement data

2.6.1. Foraging locations

Visual inspection of the GPS tracks showed two primary foraging locations: one cluster near the breeding colony (~ 10 km NE), and the second near mainland Alaska (~ 120 km NE). We therefore defined a foraging trip as a departure from the colony (> 0.5 km) where the bird travelled at least 5 km away. We then classified GPS locations within foraging trips as transit flight, area-restricted search, or rest, via Residence in Space and Time models (Torres et al., 2017). Finally, we calculated utilisation distributions (50, 75, 85, 95%) of area-restricted search locations using the R package *adehabitatHR* (Calenge, 2011) to map foraging areas for each treatment group.

2.6.2. Behavioural classification

We classified GPS-accelerometer data from all females into three behavioural categories (flight, on colony, and on water) using hidden Markov models (*momentuHMM*, McClintock and Michelot, 2018). We used a combination of GPS locations (distance from the colony; off-colony > 0.5 km > on-colony) and accelerometer-derived wingbeat frequency as inputs for the hidden Markov model (see Patterson et al., 2019).

2.6.3. Wingbeat frequency

We calculated mean wingbeat frequency during outbound foraging trips with uninterrupted flight between 5 and 10 km from the colony (< 5 km may see warm-up effects, > 10 km may see exhaustion effects). We calculated mean airspeed of the bird based on groundspeeds/bearings from GPS locations and wind speeds/bearings from a weather station on the island (National Oceanic and Atmospheric Administration, Air Force Catalog Station Number: 703430).

2.6.4. Activity budgets and daily energy expenditure

We used the GPS-accelerometer behavioural classification to calculate the proportion of time spent in each behaviour over the entire deployment for each female. Using these activity budgets, we then estimated mean daily energy expenditure (DEE) for each female using activity-specific metabolic rates for black-legged kittiwakes (from Table 2 in Jodice et al., 2003: *on-colony* = “nest attendance” = 1.85 mL CO₂/g/h, *flight* = average of “commuting flight” and “searching flight” = 8.69 mL CO₂/g/h and 7.41 mL CO₂/g/h, *on-water* = “surface feeding” = 2.27 mL CO₂/g/h):

$$DEE = energy_{on-colony} + energy_{flight} + energy_{on-water}$$

where:

$$\begin{aligned} energy_{colony} &= time_{on-colony} (\%) * 1.85 \text{ mL CO}_2/\text{g/h} * 24 \text{ h} \\ energy_{flight} &= time_{flight} (\%) * 8.10 \text{ mL CO}_2/\text{g/h} * 24 \text{ h} \\ energy_{on-water} &= time_{on-water} (\%) * 2.27 \text{ mL CO}_2/\text{g/h} * 24 \text{ h} \end{aligned}$$

We then converted mL CO₂/g/day to mL O₂/g/day through dividing by the respiratory quotient (0.85) and multiplying by 20.1 to convert mL O₂ to J (Elliott et al., 2013). Therefore, DEE represents averaged expenditure per gram of body mass over the entire deployment.

2.7. Statistical analyses

We conducted all analyses in *R* (version 3.5.1, R Core Development Team 2019). Where there was a single response measured for each experimental female (e.g. laying date), we used linear models (LM) and generalised linear models (GLM). When testing for effects of experimental manipulation, we first tested for an interaction between food treatment and handicap treatment using Type 3 ANOVA (or MANOVA if multiple response variables); if the interaction term was non-significant, we removed the interaction term and tested for main effects only using Type 2 ANOVA (or MANOVA if multiple response variables). If there were multiple measurements per female, we fitted linear mixed effects models (LMM) with a random effect of female ID using *lme4* (Bates et al., 2014). We used single-term deletions and the Kenward-Roger approximation for degrees of freedom to test for significance of fixed effects in LMM (*lmerTest*, Kuznetsova et al., 2017). We calculated individual adjusted repeatability for baseline hormones (corticosterone, LH, testosterone; while accounting for food treatment, handicap treatment, and days until laying) using *rptR* (Stoffel et al., 2017). We report unstandardized effects sizes (\pm SE) for all models, and standardized effect sizes for LM (eta-squared, η^2) and GLM (odds ratio).

2.7.1. Tests of assumptions

2.7.1.1. Kittiwakes are income breeders. To better understand and describe patterns of pre-laying weight gain in female kittiwakes, we

used historical data (1995–2017) from pre-breeding captures, behavioural sexing, and laying dates to describe sex-specific patterns in pre-breeding weight gain. We fitted a general additive model to male and female body mass in the 1–60 days before laying their first egg, using only complete records for individuals of known sex. We then modelled body mass of females from the 2018 experiment in response to days until laying, clutch size, and food treatment. We excluded body mass at recapture from females that laid during the 4-day manipulation.

2.7.1.2. Handicaps increased flight costs. To verify that weight handicapping increased flight costs, we modelled mean wingbeat frequency in response to handicap treatment, while controlling for body mass (mean of mass at capture and recapture; g), mean airspeed (m/s), wing chord length (mm), and a random effect of female ID (LMM).

2.7.1.3. Food supplementation and weight handicap altered daily energy expenditure via movement. To test whether energy manipulation affected movement, we first tested whether food and handicap treatment affected whether a female left the colony to forage (binomial GLM; 0 = no, 1 = yes), while controlling for number of days until laying. We excluded females for which we failed to retrieve movement data, females that laid an egg during the 4-day manipulation, and females that did not lay an egg in 2018. We modelled activity budgets (proportion of time spent on colony, in flight, on water) in response to food treatment, handicap treatment, and days until laying (MANOVA; post-hoc ANOVA). Flight was log-transformed to achieve a log-normal distribution. Finally, we modelled DEE in response to food treatment, handicap treatment, days until laying, and all two-way interactions (LM).

2.7.1.4. LH surge is associated with reproductive behaviours. We examined trends in copulation rates, courtship feeding events, and baseline hormones over time (number of days until laying). We excluded data from females that did not lay at least one egg in 2018.

2.7.1.5. Energy manipulation altered corticosterone and HPA-HPG interactions. First, we tested for effects of experimental manipulation on baseline, Δ baseline, and handling-induced corticosterone. After finding significant effects of food treatment on LH (Section 3.3), we also tested whether Δ baseline LH or GnRH-induced LH was associated with an interactive effect between food treatment and corticosterone (Δ baseline and handling-induced). We excluded saline-injected females from models of GnRH-induced LH.

2.7.2. Effects of energy manipulation on laying phenology (overarching hypothesis)

To test whether experimental manipulation shifted laying phenology, we modelled laying date in response to food and handicap treatment (LM), excluding females that never laid an egg, and females that laid an egg during the experiment. We also modelled breeding decision, defined as whether the female laid at least one egg in 2018 (0 = no, 1 = yes) in response to food and handicap treatment (GLM).

2.7.3. Income or capital: effects of energy manipulation on energy reserves and reproductive hormones (alternative hypotheses 1 & 2)

If cues of food supply directly influence the HPG axis, energy manipulation may not influence energy reserves (i.e. body condition index), but food treatment (but not energy reserves) may still influence reproductive hormones (i.e. LH and testosterone).

First, we tested whether experimental manipulation influenced baseline I and Δ baseline LH and testosterone (LM). Then, we tested for experimental effects on GnRH-induced changes in LH and testosterone (LMs), excluding saline-injected females. Then, we tested for experimental effects on energy reserves by modelling changes in corticosterone (baseline and handling-induced) and body condition index (BCI) using LMs. We excluded females that laid an egg during the 4-day manipulation. Residuals from a linear regression relating body mass (g)

to skull length (mm) can be used to measure body condition in kittiwakes (e.g. Chastel et al., 2005b). However, this model fit the data very poorly ($R^2 = 0.02$, 3.6 ± 1.9 g, $F_{1,115} = 3.6$, $p = 0.060$) because mass increased as the female approach laying. Because body mass was primarily driven by proximity to laying date, we calculated BCI as the residuals of a linear regression of body mass in response to days until laying at measurement (LM), excluding body mass at recapture from females that laid during the experiment.

2.7.4. Associations between reproductive hormones, laying phenology and individual quality (hypothesis 3)

We modelled LH (baseline I and GnRH-induced) in response to number of days until laying, absolute laying date, and experimental treatment. We included all females in the model of baseline I LH; we excluded females that laid during the 4-day manipulation and saline-injected females from the model of GnRH-induced LH.

3. Results

We recaptured 117 out of 119 females (Table 2), with an average of 4.3 days (± 0.1) between capture/baseline I sample and recapture/baseline II sample/GnRH challenge. Of the 119 females included in the experiment, 103 laid at least one egg in 2018 (all within one month of first capture). The 16 females that did not lay in 2018 were two females that lost their nest site during the experiment and fourteen additional females that returned to the nest site but did not lay. Fifteen females laid an egg between capture and recapture; upon recapture, we did not collect blood samples from these females and we excluded their recapture data from subsequent analyses (incl. body mass, movement data). We obtained a complete set of hormone measurements for all females at capture, and for all females at recapture that did not lay during the experiment. We failed to retrieve movement data for seven females, so they were excluded from models with movement parameters as predictors or responses.

3.1. Tests of assumptions

3.1.1. Kittiwakes are income breeders

We observed contrasting patterns of pre-breeding body mass between the sexes in the long-term data (1995–2017). Female kittiwakes gained body mass in the ~20 days before laying (Fig. 4A), while male mass remained similar throughout the pre-breeding period (Fig. 4B). During the 2018 experiment, female mass increased by 2.6 g per day (± 0.4 ; $F_{1,179} = 47.5$, $p < 0.0001$; Fig. 4C), gaining approximately 23% of their original body mass during the 30 days prior to laying. Surprisingly, we found no significant effect of clutch size (-4.6 ± 7.5 g, $F_{1,98} = 0.4$, $p = 0.54$) or food treatment (food: 7.3 ± 6.6 g, $F_{1,99} = 1.2$, $p = 0.27$) on body mass.

Table 2

Description of sample sizes per treatment group.

Sample size descriptor	Food No handicap	Food Handicap	No food No handicap	No food Handicap
Assigned to treatment	20	22	40	37
Deployment 1	5	5	5	5
Deployment 2	7	8	8	7
Deployment 3	7	7	8	7
Deployment 4	1	2	12	12
Deployment 5	0	0	7	6
Lost nest site	0	0	0	2
No movement data	0	1	4	2
Saline-injected	7	7	11	9
GnRH-injected	10	12	25	21
Laid during 4-day manipulation	3	3	4	5
Laid egg(s) in 2018	20	22	34	27
Never laid egg(s) in 2018	0	0	6	10
Foraged during 4-day manipulation	5	5	25	15
Overall activity budget/DEE	17	15	27	20
Outbound flights (5–10 km)	4	5	27	12

3.1.2. Handicaps increased flight costs

We obtained 48 outbound, uninterrupted flights from 36 females. Females carrying weight handicaps had higher mean wingbeat frequencies (0.12 ± 0.06 Hz, $F_{1,32} = 4.5$, $p < 0.05$; Fig. 5E), when controlling for body mass (0.0013 ± 0.0009 Hz, $F_{1,31} = 2.0$, $p = 0.16$), mean airspeed (0.0072 ± 0.0053 Hz, $F_{1,15} = 1.7$, $p = 0.20$), and wing chord length (-0.0041 ± 0.0054 Hz, $F_{1,32} = 0.6$, $p = 0.45$).

3.1.3. Food supplementation and weight handicap altered daily energy expenditure via movement

Females in all four treatment groups foraged at similar locations (Fig. 5A–D), but food-supplemented females were less likely to forage at all (food: odds ratio = 0.2, $\chi^2 = 11.8$, $df = 1$, $p < 0.01$) and less likely to forage when close to laying (days until laying: odds ratio = 1.2, $\chi^2 = 11.0$, $df = 1$, $p < 0.01$). Foraging occurred primarily to the north, near the Copper River delta, where abundant eulachon and herring runs attract kittiwakes and other predators in May (Hatch, 2013), and, if they foraged, all kittiwakes appeared constrained to take advantage of this large food source. Handicap and days until laying did not significantly influence probability of foraging (food * handicap: odds ratio = 3.2, $\chi^2 = 1.3$, $df = 1$, $p = 0.26$; handicap: odds ratio = 0.5, $\chi^2 = 2.2$, $df = 1$, $p = 0.14$). Recent foraging behaviour was not associated with changes in baseline corticosterone and glucose, nor with changes in body condition index (Appendix 2).

3.1.3.1. Activity budgets. Across treatment groups, females spent most of their time at the colony, followed by on-water and flying (Fig. S4). Handicap and food treatments did not interactively affect activity budgets; we removed the interaction term and found significant main effects of food treatment, handicap treatment, and number of days until laying (Table 3). Fed females spent more time on colony and less time in flight; handicapped females spent more time on water; females far from laying spent less time at the colony, more time in flight and on water (Table 4).

3.1.3.2. Daily energy expenditure. Non-supplemented females had higher DEE than fed females, especially when far from laying (food * days until laying: 0.025 ± 0.09 kJ/g/day, $\eta^2 = 0.05$, $F_{1,74} = 7.3$, $p < 0.01$; Fig. 5F). However, we found little evidence that handicap influenced DEE (food * handicap: -0.13 ± 0.10 kJ/g/day, $\eta^2 = 0.01$, $F_{1,72} = 1.9$, $p = 0.17$; days until laying * handicap: -0.011 ± 0.008 kJ/g/day, $\eta^2 = 0.09$, $F_{1,72} = 2.0$, $p = 0.17$; handicap: -0.06 ± 0.05 kJ/g/day, $\eta^2 = 0.01$, $F_{1,74} = 1.8$, $p = 0.19$).

3.1.4. LH surge is associated with reproductive behaviours

We observed 231 independent copulation events in 7117 site-hours of video (80 h of video footage). Copulation rates peaked in the 15 days prior

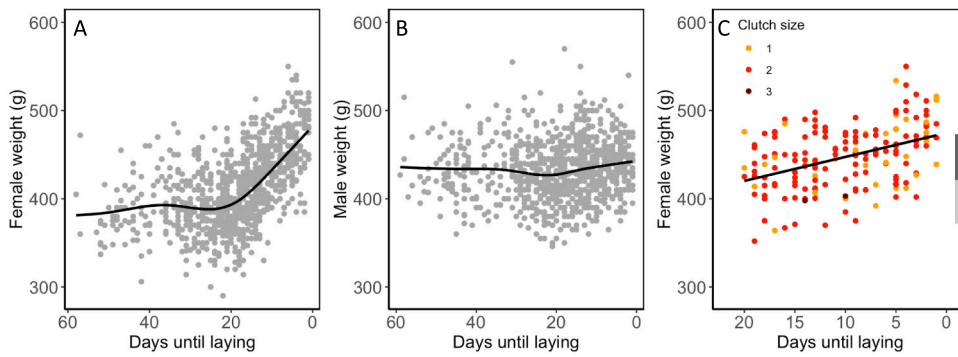


Fig. 4. Relationship between weight and timing of egg laying, using historical capture data (AB: 1995–2017) and experimental capture data (C: 2018). (A) Pre-laying weight of females increases rapidly leading up to egg laying, while (B) male weight remains relatively constant. (C) In 2018, mass was highest among females nearest egg-laying but was not predicted by clutch size. Grey scale bar shows average weight of a single-egg clutch (48 g, dark grey only) or two-egg clutch (101 g, whole bar) in 2018. Most females lay two eggs (red points).

to laying, which coincides with an increase in baseline LH (Fig. 6). We opportunistically observed 83 courtship feeding behaviours from pairs that ultimately laid eggs. Most copulations and courtship feedings occurred in the 20 days before females laid eggs, peaking approximately 10 days before laying.

3.1.5. Energy manipulations did not influence corticosterone, but altered HPA-HPG interactions

Neither food treatment nor weight handicap influenced baseline, Δ baseline, or handling-induced corticosterone (Table 5). Regarding HPA-HPG interactions, Δ baseline corticosterone was not associated with Δ baseline LH (Δ baseline cort * food: -0.01 ± 0.13 ng/mL, $\eta^2 = 9.3 \times 10^{-5}$, $F_{1,98} = 0.01$, $p = 0.92$; Δ baseline cort: -0.02 ± 0.06 ng/mL, $\eta^2 = 0.001$, $F_{1,99} = 0.14$, $p = 0.71$, Fig. 7A), but fed females increased baseline LH more over the 4-day period than non-supplemented females (-1.8 ± 0.7 ng/mL, $\eta^2 = 0.06$, $F_{1,99} = 6.9$, $p < 0.05$, Fig. 7A). However, the two food treatments exhibited contrasting LH-corticosterone relationships (Fig. 7). Δ baseline corticosterone and GnRH-induced LH were associated, where greatest LH releases were observed in non-supplemented females with reduced Δ baseline corticosterone (Δ baseline cort * food: -0.4 ± 0.2 ng/mL, $\eta^2 = 0.08$, $F_{1,64} = 5.7$, $p < 0.05$, Fig. 7B). In contrast, handling-induced corticosterone and GnRH-induced LH were also associated, but the greatest LH releases were observed in non-supplemented females with greater handling-induced corticosterone (Δ challenged cort * food: 0.2 ± 0.1 ng/mL, $\eta^2 = 0.08$, $F_{1,63} = 5.6$, $p < 0.05$, Fig. 7C). These trends persisted when we ran the regressions with the residuals from a regression of days until laying on GnRH-induced LH, indicating the patterns were not driven by proximity to laying (Fig. S5).

3.2. Effects of energy manipulation on reproductive phenology (overarching hypothesis)

On average, fed females laid their first egg 4.3 days earlier than non-supplemented females (± 1.3 days, $\eta^2 = 0.12$, $F_{1,85} = 11.7$, $p < 0.001$; Fig. 8), but handicapped females did not lay eggs later than control females (food * handicap: 0.04 ± 2.6 days, $\eta^2 = 3.0 \times 10^{-6}$, $F_{1,84} = 0.0003$, $p = 0.99$; handicap: 0.5 ± 1.3 days, $\eta^2 = 0.001$, $F_{1,85} = 0.1$, $p = 0.71$). Food-supplemented birds had higher laying success (100% vs. 79%; food: odds ratio = 8.54×10^7 , $\chi^2 = 15.7$, $df = 1$, $p < 0.0001$) with no effect of handicap (85% vs 73%; food * handicap: odds ratio = 2.1, $\chi^2 = 1.0$, $df = 1$, $p = 1.0$; handicap: odds ratio = 0.5, $\chi^2 = 1.7$, $df = 1$, $p = 0.19$). Interestingly, all fed birds laid, with rate of laying lowest for handicapped, non-supplemented females (Table 2). Food supplementation also advanced nest-building phenology (Appendix 3).

3.3. Income without capital: evidence for direct effects of food supply on reproductive hormones (alternative hypotheses 1 & 2)

Food treatment increased baseline LH at deployment and increased change in LH over the deployment but had no effect on testosterone; handicapping did not affect initial or changes in baseline LH or

testosterone (Table 6). Food treatment did not influence initial BCI, and neither food nor handicap treatment influenced change in BCI over the 4-day manipulation (Table 6).

3.4. Associations between reproductive hormones, laying phenology and individual quality (hypothesis 3)

Baseline LH at first capture was higher among females closer to laying (-0.18 ± 0.05 ng/mL, $\eta^2 = 0.10$, $F_{1,99} = 12.2$, $p < 0.001$, Fig. 9A) and non-supplemented females (1.4 ± 0.7 ng/mL, $\eta^2 = 0.03$, $F_{1,99} = 4.2$, $p < 0.05$, Fig. 9A, B), but was not associated with absolute laying date (0.1 ± 0.1 ng/mL, $\eta^2 = 0.02$, $F_{1,99} = 3.0$, $p = 0.09$, Fig. 9B). In contrast, GnRH-induced LH was lower among females closer to laying (0.29 ± 0.08 ng/mL, $\eta^2 = 0.19$, $F_{1,54} = 13.0$, $p < 0.001$, Fig. 9C), but not associated with food treatment (1.0 ± 1.0 ng/mL, $\eta^2 = 0.02$, $F_{1,54} = 1.2$, $p = 0.28$, Fig. 9C, D) or absolute laying date (-0.1 ± 0.1 ng/mL, $\eta^2 = 0.04$, $F_{1,54} = 2.7$, $p = 0.11$, Fig. 9D).

Changes in LH (Δ baseline and GnRH-induced) were positively associated with changes in testosterone (Δ baseline and GnRH-induced; Fig. S6). Baseline corticosterone and baseline LH were repeatable, but we found non-significant repeatability for baseline testosterone (Table S1, repeatability and mean for each hormone). This repeatability over the four days between measurements (i) reassures us that our manipulations did not have unintended effects on all captured individuals, and (ii) confirms that changes observed in LH are not due to random fluctuations in hormone titres or sampling error (Williams, 2008).

4. Discussion

4.1. Effects of energy manipulation on reproductive phenology (overarching hypothesis)

As hypothesized, food supply advanced timing of reproduction—but did not increase energy reserves—in an income-breeding seabird. Increased energy gains (food supplementation) advanced endocrine and laying phenology and increased the likelihood a female would lay, but increased energy costs (weight handicap) did not decrease energy reserves (i.e. body condition) or influence reproductive hormones, laying phenology, or breeding decision of female kittiwakes. GPS tracking confirmed that females adjusted movement in response to experimental energy manipulations; most of the flexibility occurred in terms of time budgets rather than foraging locations, with pre-breeding birds constrained to forage in the coastal environment for herring and eulachon (Hatch, 2013). Overall, we conclude that food supply had direct effects on reproductive phenology, independent of energy reserves.

4.2. Support for income pathway: direct effects of food supply on reproductive hormones (hypothesis 1)

Our results are consistent with the hypothesis that food supply affects the HPG axis directly, rather than via energy reserves, in income breeders. First and foremost, we observed effects of food

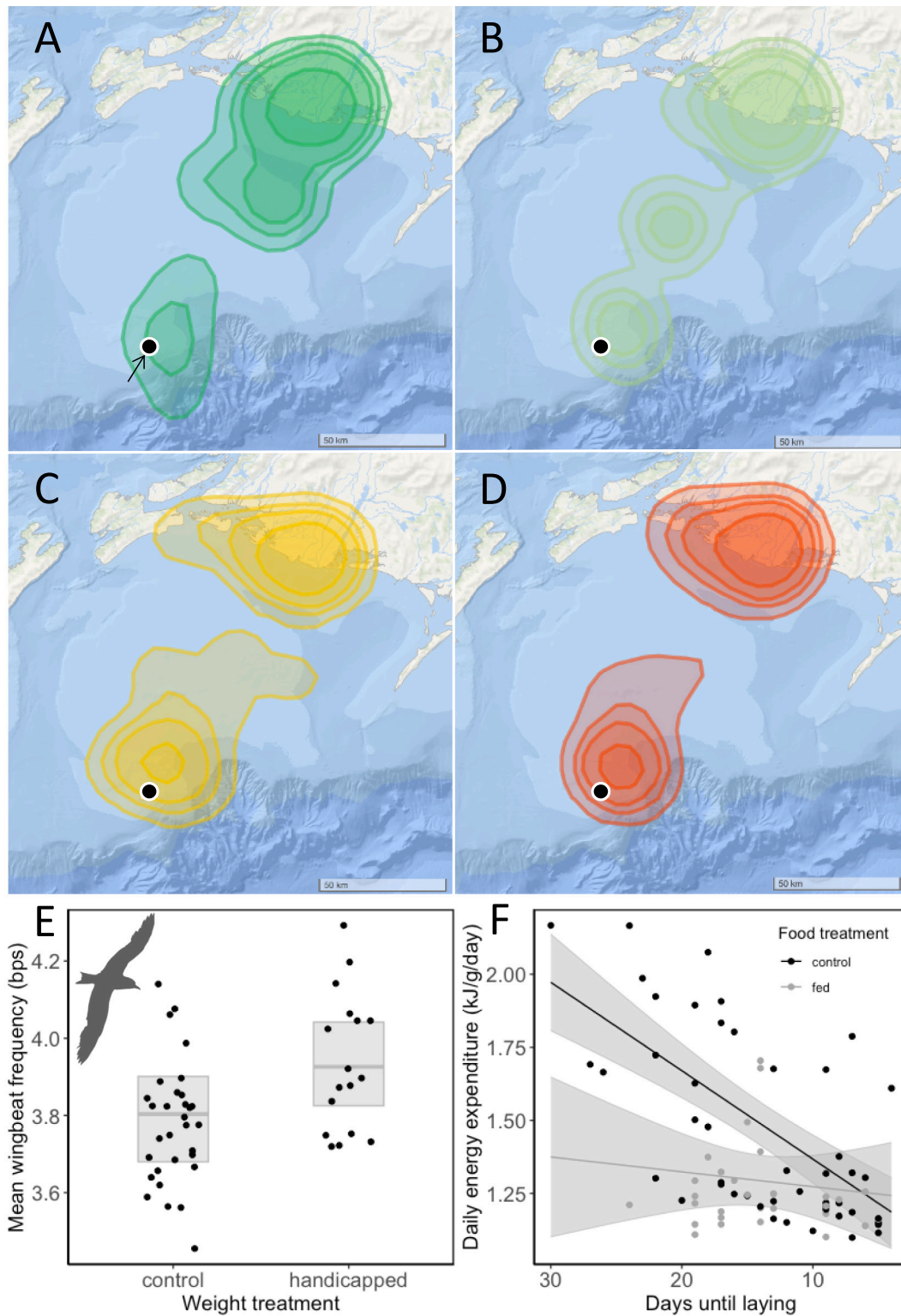


Fig. 5. (A–D) Females foraged in similar locations, regardless of treatment group. Polygons show 50, 75, 85, and 95% utilisation distributions of foraging locations (i.e. area-restricted search) for the four treatment groups: (A) food, no handicap; (B) food, handicap; (C) no food, no handicap; (D) no food, handicap. (E) Weight handicap increased mean wingbeat frequency during outbound flight 5–10 km from the colony. Grey lines indicate model predictions for control and handicapped females, assuming mean body mass, airspeed, and wing chord. Grey box shows 95% confidence intervals of LMM produced via bootstrap. (F) Non-supplemented females had higher daily energy expenditure than fed females, especially early in pre-laying. Lines show model predictions for the interactive effect between food treatment and number of days until laying (grey shading represents 95% confidence intervals; intercept set to non-handicapped female).

supplementation on laying phenology, but no effects of food supplementation or weight handicap on energy reserves (i.e. body condition). Second, increased food supply advanced the timing of pulses in

reproductive hormones and pre-laying weight gain, relative to controls. However, when accounting for time until laying, eventual clutch size and food treatment were not associated with female body mass. Instead,

Table 3

MANOVA results for overall activity budgets (three responses: proportion of time spent on colony, in flight, on-water).

Predictors	Pillai's trace	F	df	p-Value	η^2
Food * handicap	0.03	0.8	3, 73	0.50	0.03
Food	0.17	4.8	3, 73	0.003	0.17
Handicap	0.13	3.7	3, 73	0.02	0.13
Days until laying	0.31	10.7	3, 73	6.3×10^{-6}	0.31

Bold font indicates statistically significant relationship.

it appears that all females gained weight on a similar trajectory regardless of food supply and, in favourable conditions, advanced laying date rather than increasing body condition. Fed females were not more responsive to GnRH challenge, though their baseline LH increased more over the 4-day manipulation, relative to controls. Thus, after maintaining a constant body mass during 1–2 months pre-laying, females increased body mass (presumably representing egg development), copulation rate and courtship feedings in the 20 days before laying, coincident with an LH surge—implying that reproductive readiness was modulated by food supply in ~20 days pre-laying.

4.3. No support for capital pathway: energy balance did not influence reproductive hormones via energy reserves (hypothesis 2)

We did not find evidence that our energy manipulations caused changes in energy reserves. Despite unlimited access to food, reduced foraging activity and low energy expenditure, fed females had similar body condition to controls. This suggests that even in the pre-breeding period with ad libitum food supply, females followed a ‘lean-and-fit’ strategy (Schultner et al., 2013). The cort-adaptation hypotheses predicts that handicapped, non-supplemented females should increase baseline corticosterone (due to the “decline” in environmental conditions and the increased energetic demands of reproduction), as observed in violet green swallows (Rivers et al., 2017). In contrast, the cort-fitness hypothesis (Bonier et al., 2009a) predicts that handicapped females might enter an energy deficit relative to non-supplemented controls (but that fed, handicapped individuals would be unaffected). Entering energy deficit would increase corticosterone (Harding et al., 2009), which can inhibit HPG sensitivity to GnRH (Goutte et al., 2010). However, handicapping did not increase baseline corticosterone in this experiment; instead, females buffered effects of energy manipulation by adjusting foraging behaviour. Despite increased flight costs (as evidenced by higher wingbeat frequency), females compensated by spending more time on water (presumably feeding and resting). Thus, we conclude that behavioural adjustments prevented females from entering energy deficit because, while both energy manipulations altered movement behaviour, handicapping did not decrease body condition or increase baseline corticosterone and feeding did not increase

Table 4

Post-hoc ANOVA results for proportion of time spent on colony, in flight, and on water (overall activity budgets).

Response	Predictors	Estimate \pm SE	F	df	p-Value	η^2
On colony	Food * handicap	−0.05 \pm 0.07	0.6	1, 74	0.44	0.006
	Food (fed)	0.09 \pm 0.03	6.0	1, 75	0.02	0.07
	Handicap (weighted)	−0.03 \pm 0.03	0.9	1, 75	0.36	0.01
	Days until laying	−0.01 \pm 0.00	17.2	1, 75	8.7×10^{-5}	0.17
Log(flight)	Food * handicap	0.3 \pm 0.3	0.63	1, 74	0.43	0.005
	Food (fed)	−0.6 \pm 0.2	11.6	1, 75	0.001	0.10
	Handicap (weighted)	−0.2 \pm 0.2	2.2	1, 75	0.14	0.02
	Days until laying	0.07 \pm 0.01	28.5	1, 75	9.6×10^{-7}	0.24
On water	Food * handicap	0.02 \pm 0.06	0.11	1, 74	0.74	0.001
	Food (fed)	−0.04 \pm 0.03	1.4	1, 75	0.24	0.02
	Handicap (weighted)	0.06 \pm 0.03	4.3	1, 75	0.04	0.05
	Days until laying	0.005 \pm 0.002	5.8	1, 75	0.02	0.07

Bold font indicates statistically significant relationship.

body condition or decrease baseline corticosterone.

Similar to corticosterone, we found remarkably little effect of weight handicapping on circulating LH and testosterone. At first glance, this is a surprising result because many studies have shown that short-term stressors influence circulating corticosterone and reproductive hormones (e.g. Lynn et al., 2015). Furthermore, energy reserves can influence the HPG axis without affecting baseline corticosterone; for example, Valle et al. (2015) found no effects of food treatment on circulating corticosterone, but did find differences in LH and GnRH-induced testosterone. Taken together, our endocrine and movement results suggest that females had the capacity to buffer our short-term challenge through behavioural adjustment, preventing changes in reproductive hormones.

The lack of weight handicap-driven variation in reproductive hormones and laying phenology may be because some females had entered the post-follicular period. Many females in this study had begun pre-laying weight gain and therefore could have lost phenotypic flexibility in laying date (and thus LH or testosterone); some females may have already committed to breeding and were only able to upregulate or downregulate the rate of progress. Alternatively, the weight handicap (~6% of body mass) might not have been heavy enough to alter energy status (but see Chivers et al., 2015). Weight handicap may not be a strong challenge for pre-laying females because increasing body mass is natural during egg development—pre-laying females gained approximately 20% of their weight during follicle development. However, we detected effects of weight handicap on wingbeat frequency and activity budgets. Nonetheless, we cannot rule out the possibility that a longer handicap duration or heavier weight might have affected endocrine and laying phenology.

4.4. Associations between reproductive hormones, laying phenology and individual quality (hypothesis 3)

Although pituitary sensitivity to GnRH declined as females approached laying, this coincided with an increase in circulating LH, implying that birds always had the capacity to upregulate LH release but only did so close to laying. Response to GnRH-challenge prior to breeding is often interpreted as a measure of ‘reproductive readiness’ (e.g. Schoech et al., 1996; Goutte et al., 2010; Covino et al., 2018; Fig. 10A), but we found the opposite pattern—females closest to laying (and therefore presumably more “ready”) had the smallest LH releases. After the onset of reproduction and follicle development, there is likely a shift towards a less responsive HPG-axis (Fig. 10B). This shift could be linked to the initiation of follicle development, which can occur very quickly. For example, female Eurasian starlings (*Sturnus vulgaris*) developed a preovulatory follicle with one week of exposure to social cue (male presence), which was accompanied by rapid shifts in female behaviour and HPG-liver physiology (Perfito et al., 2015). Distinct substages of the pre-breeding period that correspond to different levels

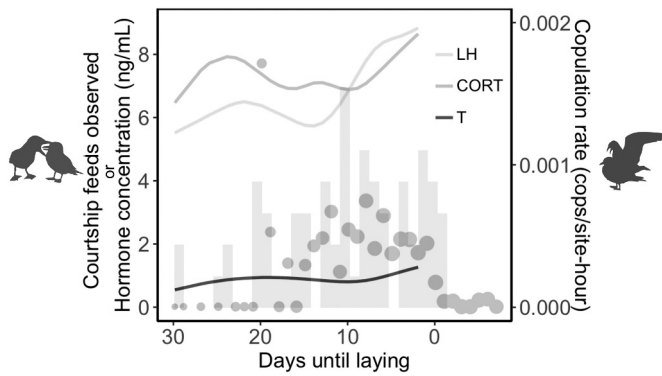


Fig. 6. Associations between copulation rates (points), circulating hormone concentrations (lines), and courtship feed observations (histogram) over time until laying. Copulation rates were extracted from video, where point size corresponds to number of sites watched at given day until laying. Lines show generalised additive models for baseline LH ($p = 0.15$), baseline corticosterone ($p = 0.42$), baseline testosterone ($p = 0.09$) from all females in study. Histogram shows frequency of courtship feeding observations by number of days until laying ($N = 83$). Breaks in left y-axis apply to both courtship feeds observed (counts) and hormone concentrations (ng/mL).

of pituitary sensitivity to GnRH may be unique to females. Compared to males, females play a larger role in aligning the timing of reproduction to match environmental conditions (Ball and Ketterson, 2008) and rely more heavily on variation in HPG axis activity to regulate sequential reproductive processes (e.g. follicle selection and development, ovulation, oviposition).

4.5. Lean and fit: income-breeding kittiwakes maintained low mass to reduce flight costs

Both males and females 20 days prior to laying showed little variation in body mass, implying that mass is tightly regulated to optimise flight costs. Mass during chick-rearing is often reduced to minimise flight costs (Norberg, 1981; Moreno, 1989), and we show clear evidence that this is also likely the case for pre-breeding kittiwakes. Females with developing eggs are an exception, and they spent less time flying as they approach laying—especially when relieved of the need to fly by supplemental feeding—perhaps due to a coincident increase in courtship feeding by males. Presumably, the flight costs associated with carrying a heavy egg meant that females preferred to stay at the colony; females that were experimentally fed seldom left. In Atlantic black-legged kittiwakes, male foraging distances are greater than females during the pre-laying period (Goutte et al., 2014). This suggests kittiwake pairs might behaviourally buffer the effects of reproductive burden and declines in female locomotor performance through courtship feeding. A functional role of courtship feeding in maintaining energy balance of gravid females may also explain the surprising lack of relationships between time since foraging and various measures of energy reserves (e.g. increases in body condition among non-supplemented females that did not leave the colony to forage).

Table 5

Effects of food supplementation and weight handicap on corticosterone. Model intercept is control group for both food and handicap treatments.

Response	Predictors	Estimate \pm SE	F	df	p-Value	η^2
Baseline I corticosterone Δ baseline corticosterone	Food	-0.8 ± 0.9	0.8	1117	0.38	0.007
	Food * handicap	-0.02 ± 2.37	0.00	1,98	0.99	4.9×10^{-7}
	Food	-1.5 ± 1.2	1.5	1,99	0.22	0.02
Handling-induced corticosterone (30 min)	Handicap	0.1 ± 1.1	0.01	1,99	0.93	8.7×10^{-5}
	Food * handicap	3.6 ± 5.0	0.5	1,97	0.47	0.005
	Food	-2.3 ± 2.5	0.8	1,98	0.36	0.008
	Handicap	-1.1 ± 2.4	0.2	1,98	0.65	0.002

4.6. Food supply altered HPA-HPG interactions, but not circulating corticosterone

Despite little evidence that energy manipulations altered circulating corticosterone, food supplementation altered HPA-HPG interactions. We focused primarily on relative changes in hormone levels within individuals, as variation in receptor and binding dynamics among individuals causes large inter-individual variation in absolute levels (Williams, 2008). In non-supplemented females, LH capacity decreased as baseline corticosterone increased within individuals. This relationship did not occur in fed birds. One possible explanation is that fed birds perceived constant and high food availability, regardless of information from circulating corticosterone; the brain may have overridden any cue from circulating corticosterone.

In contrast to baseline levels, which are often cited as indicative of nutritional stress (Kitaysky et al., 2001), corticosterone levels in response to handling may be more indicative of recent exposure to cumulative chronic stress (Kitaysky et al., 2001) or acute stress such as presence of a predator (Breuner et al., 2008). For instance, bald eagles were a regular predator that harassed pre-breeding kittiwakes at this colony, although they were unable to access birds directly breeding on the tower. In this case, fed birds showed the expected decrease in LH capacity with acute corticosterone response, implying that those birds that had the largest stress response suppressed their LH production and presumably their propensity to breed, which seems consistent with the cort-fitness hypothesis (Bonier et al., 2009a; Breuner et al., 2008). It is important to note that food supplementation likely reduced variation in “individual quality” associated with access to food for birds in the fed treatment, but they were as likely to be impacted by predators as non-supplemented birds. In contrast, non-supplemented birds showed an increase in LH capacity with corticosterone response, implying that those birds that had the largest stress response also had the greatest sensitivity to information about when to breed. While inconsistent with inhibitory effects of corticosterone on HPG activity, this may be due to the ability of higher quality individuals with higher investment in reproduction to secrete more corticosterone, as predicted by the cort-adaptation hypothesis—although Bonier et al. (2009b) restricted application of the cort-adaptation hypothesis to baseline levels and made no specific predictions about the pre-breeding period. While not tested here, food supplementation consistently increases reproductive output in this population (Gill and Hatch, 2002; Whelan et al., 2020); thus, fed kittiwakes appear to have both higher fitness and lower integration of HPA activity in reproductive decisions.

5. Conclusions

We tested the effects of food supply and energy status on female reproductive physiology and phenology in a wild, free-living population. As expected for an income-breeding species, female kittiwakes advanced laying date slightly instead of accumulating reserves in response to increased food supply. Thus, although some authors have concluded that seabirds are phenologically insensitive to oceanographic conditions (Keogan et al., 2018), kittiwakes are clearly phenologically sensitive to environmental food supply. This aligns with the evidence for the ‘constraint hypothesis’ previously found in kittiwakes (Shultz et al., 2009), suggesting that energetic limitations prior to egg laying constrain the

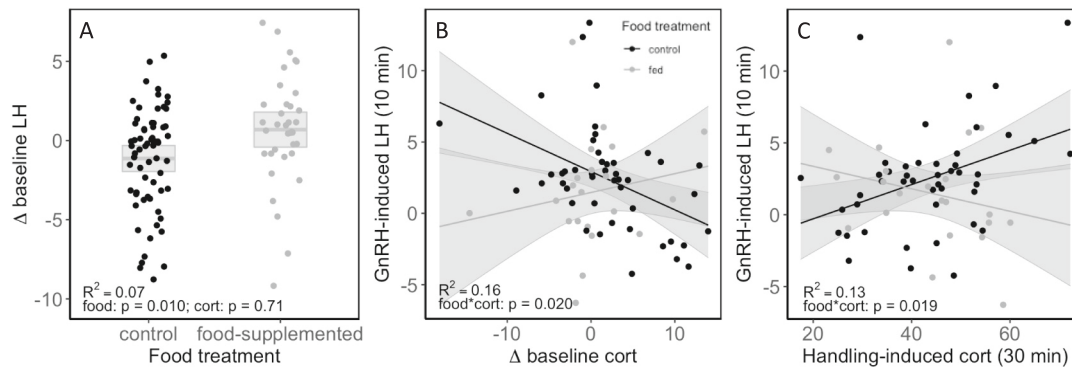


Fig. 7. (A) Baseline LH increased more among fed birds over the 4-day manipulation but was not associated with Δ baseline corticosterone. (B) GnRH-induced LH was associated with Δ baseline corticosterone and food treatment. (C) GnRH-induced LH was associated with handling-induced corticosterone and food treatment. Units are in ng/mL. Lines indicate model predictions (shading represents 95% confidence intervals) for fed (grey) and non-supplemented (black) treatments (model predictions for A generated assuming mean Δ baseline corticosterone; saline-injected birds excluded from panels B and C).

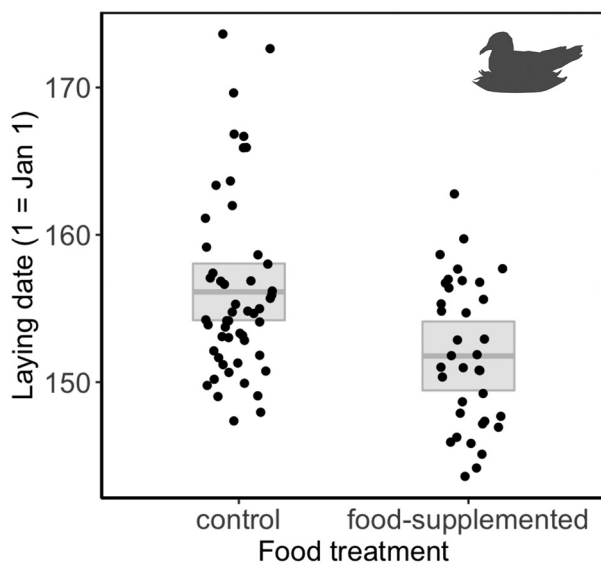


Fig. 8. Food-supplementation advanced laying date by 4.3 days. Grey lines indicate model predictions for control and food supplemented females, where grey box shows 95% confidence intervals (assuming non-handicapped treatment).

timing of egg laying. The experiment fell within the pre-laying period (< 30 days before laying) and many females had presumably initiated follicle development (yolk formation spans 9–15 days in kittiwakes; Roudybush et al., 1979; Astheimer and Grau, 1990). Interestingly, food-supplementation advanced the timing of reproductive phenology (i.e. baseline hormones, behaviour, weight gain), but fed birds were not in better body condition and did not have greater gonadotroph sensitivity. Pre-laying females were able to buffer the energy costs of weight handicapping by adjusting behaviour (increasing resting). Indeed, heavy females near lay, burdened by carrying an egg, foraged seldom during the week leading up to laying, relying partially on male courtship feeding. Future research might examine the role of the male response to food supply in timing of breeding (Whelan et al., 2016), as well as social stimulation beyond the pair (Coulson and White, 1959).

Overall, our findings suggest that for females, late pre-laying may be a physiologically and behaviourally distinct life stage from early pre-laying. Females closest to laying foraged less often, reducing energy expenditure despite increasing body mass, and relied on courtship feeding from males. At the same time, baseline reproductive hormones were highest and pituitary responsiveness to GnRH lowest among female kittiwakes closest to laying. Thus, female sensitivity to cues in the environment likely increases leading up to the breeding season, plateaus as females gather information about current environmental conditions, then declines in late pre-laying once follicle development begins. These windows of high and low environmental sensitivity could be

Table 6

Effects of food supplementation and weight handicapping on reproductive hormones and body condition index (BCI). Estimates are for deviation of non-supplemented from fed (food) and handicapped from control (handicap).

Response	Predictors	Estimate \pm SE	F	df	p-Value	η^2
Baseline I LH	Food	-1.7 \pm 0.6	6.9	1117	0.01	0.06
Δ baseline LH	Food * handicap	-0.7 \pm 1.4	0.3	1,98	0.60	0.003
	Food	1.9 \pm 0.7	8.0	1,99	0.006	0.07
	Handicap	-1.1 \pm 0.7	2.6	1,99	0.11	0.02
GnRH-induced LH (10 min)	Food * handicap	-0.8 \pm 2.0	0.2	1,64	0.70	0.002
	Food	-0.9 \pm 1.0	0.8	1,65	0.37	0.01
	Handicap	-0.3 \pm 0.9	0.1	1,65	0.78	0.001
Baseline I testosterone	Food	0.04 \pm 0.11	0.1	1117	0.71	0.001
Δ baseline testosterone	Food * handicap	0.4 \pm 0.3	1.3	1,98	0.26	0.01
	Food	-0.1 \pm 0.1	0.5	1,99	0.50	0.005
	Handicap	-0.1 \pm 0.1	0.9	1,99	0.34	0.009
GnRH-induced testosterone (30 min)	Food * handicap	0.6 \pm 0.3	4.5	1,64	0.04	0.06
Initial BCI	Food	5.9 \pm 6.6	10.8	1101	0.37	0.008
Δ BCI	Food * handicap	11.2 \pm 11.1	1.0	1,82	0.31	0.01
	Food	1.9 \pm 5.6	0.1	1,83	0.73	0.001
	Handicap	-3.9 \pm 5.4	0.6	1,83	0.43	0.007

Bold font indicates statistically significant relationship.

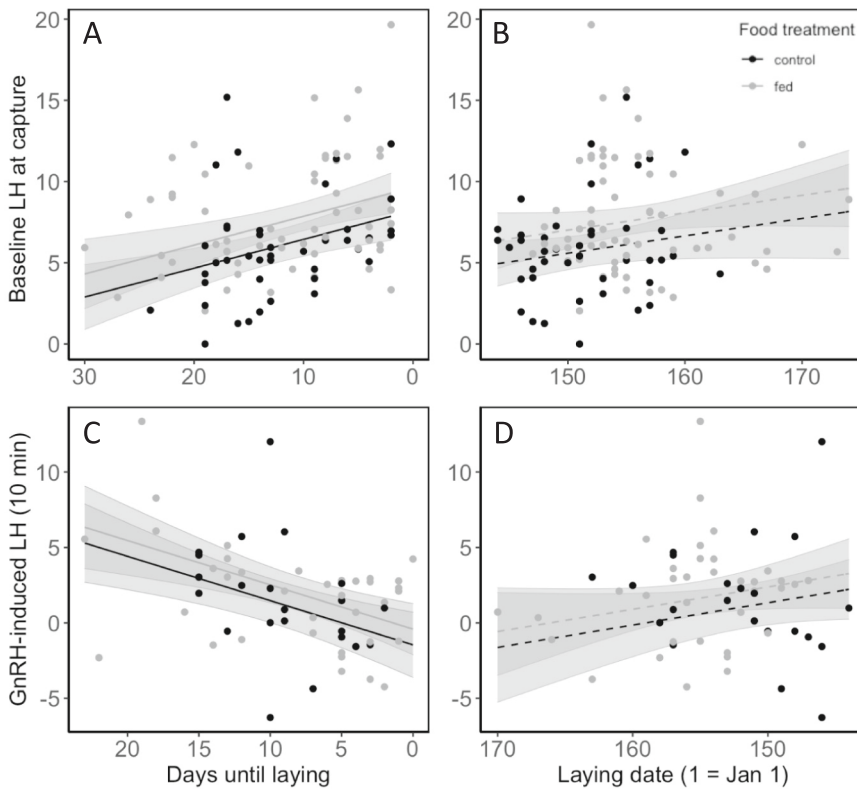


Fig. 9. Effect of days until laying and absolute laying date on (A, B) baseline LH at capture and (C, D) GnRH-induced LH at recapture. Fed birds had lower baseline LH at capture, relative to controls, but supplementation had no significant effect on GnRH-induced LH. Lines indicate model predictions (shading shows 95% confidence intervals) for fed (grey) and non-supplemented (black) treatments (saline-injected birds excluded from panels C and D).

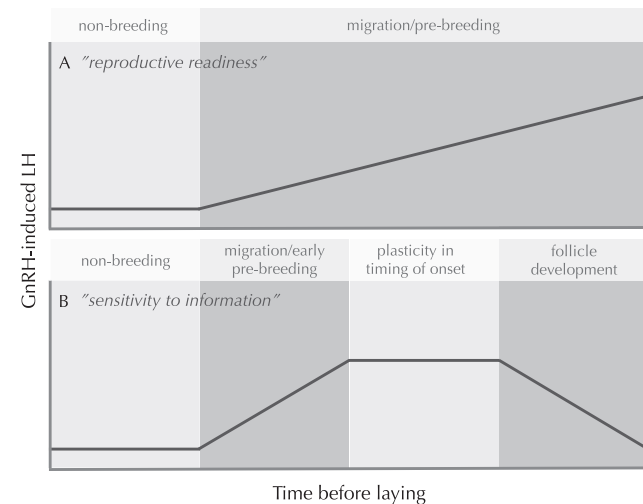


Fig. 10. While GnRH-induced LH release is often interpreted as an indicator of reproductive readiness, generating the prediction that responsiveness to GnRH should increase steadily over the course of pre-breeding (A), we found a decline in GnRH-induced LH as females approached laying. This suggests that responsiveness to GnRH injection may instead be an indicator of sensitivity to information from the environment (e.g. supplementary cues) or intrinsic state (e.g. individual condition). If so, pituitary responses to GnRH injection should be strongest during the period of plasticity, when individuals are gathering information before initiating follicle development (B).

important for understanding how, whether, and when animals are able to adjust phenology in response to environmental change.

CRediT authorship contribution statement

SW and KHE developed the ideas and methodology; ZMBF and OC contributed to study design and protocols; SW and SAH collected the

data; SW and CP conducted radioimmune assay; SW analysed the data and led writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability statement

Data presented in analyses (hormones, phenology, movement summaries) and code are available as supplementary files. Raw GPS-accelerometer data are publicly available on Dryad (doi:<https://doi.org/10.5061/dryad.2v6wwpzjv>).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yhbeh.2020.104874>.

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