Title: Corticosterone elevation is associated with rapid, sex-specific increases in food-acquisition behaviors in free-living seabird chicks

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ABSTRACT.

The energetic consequences of behaviors may rapidly alter glucocorticoid levels, and glucocorticoids are assumed to redirect behavioral responses to stimuli in ways that increase fitness. We investigated these relationships in young seabirds (Black-legged kittiwakes, *Rissa tridactyla*) with facultative siblicide and robust HPA-axis activity, considering environmental context and genetic sex. First, we assessed potential impacts of behavior on corticosterone by quantifying behavior of first-hatched chicks in nests containing two chicks for 60 min before taking a baseline blood sample, restraining for 15 min, then bleeding again. The only behavior that predicted subsequent corticosterone levels was feeding (fewer feeds = higher restraint-induced corticosterone). However, there was evidence for consequences of increased corticosterone secretion on behavior: post-restraint corticosterone levels were positively correlated with increases in begging and aggression in the subsequent hour, primarily in males. Larger corticosterone response to restraint was also associated with more rapid brood reduction. Corticosterone:behavior relationships were eliminated in chicks from food-supplemented nests. To evaluate potential causality of corticosterone in redirecting behavior, we isolated it from other components of the endogenous stress response using minimally-disruptive exogenous topical treatments, and confirmed a positive relationship between corticosterone and aggression (stronger in males), but not begging or feeding. We conclude that elevation of endogenous corticosterone is negatively correlated with recent feeding rates, and is subsequently associated with rapid, sex- and context-specific changes in kittiwake chick behaviors, at least some of which are causally driven by elevated corticosterone and associated with fitness-relevant consequences.

Keywords (max 10): birds, food availability, glucocorticoids, ontogeny, stress response, survival

1. INTRODUCTION

Hormones can change behavior rates by altering the probability that an animal will respond to a particular context and set of stimuli with appropriate behaviors, while behavior itself can also alter hormone levels (Adkins-Regan, 2005; Hau and Goymann, 2015; Myers et al., 2014). These bidirectional relationships have been well-studied in the context of competitive or reproductive behaviors and androgens in vertebrates (Casto and Edwards, 2016; George and Rosvall, 2022; Goymann, 2009; Olivera, 2004; Pradhan et al., 2015, Rosvall et al., 2020), where they often differ by sex (Jennings and DeLecia, 2020; Smiley et al., 2022). However, glucocorticoid hormone levels can also influence and be influenced by behavior (Haller, 2014; Horton and Hollberton, 2009; Myers et al., 2014). Glucocorticoids' effects are usually framed in terms of context-appropriate redirection of behaviors towards those that will help an animal survive a threat and re-establish homeostasis (Orchinik, 1998, Sapolsky, 2000, Wingfield and Kitaysky, 2002). Simultaneously, behaviors require energy expenditure and may thereby affect the activity of the hypothalamo-pituitary-adrenal (HPA) axis, the endocrine cascade culminating in glucocorticoid release (Falson et al., 2009; Grunst et al., 2024; Hackney and Waltz, 2013; Mateos, 2005). Elucidation of the functional role of glucocorticoids with respect to behavior in free-living animals requires experiments that test for causal, context-appropriate relationships, which are rare.

In birds, several factors may modulate relationships between corticosterone (the primary avian glucocorticoid) and behavior. First, because animals may have sex-specific strategies for coping with challenges (Ouyang et al., 2012; Wingfield, 1985), relationships between behavior and corticosterone may also differ by genetic sex. Second, the relationships between physiology and behavior may be altered by the environmental context (Killen et al., 2013; Pradhan et al., 2015), particularly food availability. Food availability has consistently been shown to affect both behavior (Golabek et al., 2012; Quillfelt and Masello, 2004;) and HPA activity (reviewed in Busch and Hayward, 2009; Kitaysky et al., 2007; Sorensen et al., 2017) in birds. The behavioral and metabolic responses to elevated glucocorticoids may differ in individuals not constrained by their energy budgets, thus by altering the amount of resources available to allocate across competing demands, variation in food availability may either obscure or reveal underlying relationships between physiology and behavior (Benowitz-Fredericks et al., 2022; Killen, et al. 2013). Finally, temporal scale is likely to be important. Despite animals' capacity to increase glucocorticoids on the scale of minutes (Romero and Reed, 2005), most studies that measure or manipulate glucocorticoids evaluate its relationship with behavior on the scale of days, potentially missing an important time-window when glucocorticoids and behavior may interact. In this study, we assessed the effects of food availability and genetic sex on glucocorticoid:behavior relationships over a short time scale in young chicks with robust adrenocortical activity and intense sibling competition.

Black-legged kittiwakes. We conducted two studies to investigate relationships between corticosterone and a set of focal behaviors related to food acquisition (begging, aggression and feeding) on a short time scale (an hour). We studied 5 day old black-legged kittiwakes (*Rissa tridactyla)*, nest-bound seabird chicks that engage in intense sibling competition and have adult-like hypothalamo-pituitary-adrenal (HPA) axis activity (Benowitz-Fredericks et al., 2024). Kittiwakes are

long-lived seabirds with broods of 1-3 chicks who engage in facultative siblicide, which peaks in the first 10 days post-hatch (White et al., 2010). Food availability is a probable link between glucocorticoids and behaviors in kittiwake chicks because low food availability leads to increased secretion of corticosterone (Kitaysky et al., 1999; Benowitz-Fredericks et al., 2024), and increased rates of brood reduction (Vincenzi et al., 2015; White et al., 2010; Gill et al., 2002; Suryan et al., 2002). When brood reduction due to siblicide occurs, it is most often because the older "A" chick eliminates its younger siblings ("B" or "C" chick) by physically attacking them, either killing them outright, preventing them from acquiring food, or driving them from the nest (Dickins, 2021, Braun and Hunt, 1983, White et al., 2010). Multiple studies have quantified sources of variation in kittiwake chick corticosterone (reviewed in Benowitz-Fredericks et al., 2024), and several have manipulated corticosterone in older chicks, finding that exogenous corticosterone from subcutaneous implants increases begging and aggression on the scale of days (Kitaysky et al., 2001, 2003). This foundation, combined with the fact that by 5 days post-hatch chicks engage in intensive begging and aggression (White et al. 2010), have sexually dimorphic developmental trajectories (Merkling et al., 2012), and have highly responsive HPA axes that are sensitive to food availability (Benowitz-Fredericks et al., 2024), make them good candidates for testing hypotheses about sex-specific relationships among food, corticosterone and behavior on short time-scales.

Manipulating endogenous and exogenous corticosterone levels in seabird chicks.

To investigate hormone:behavior interactions and the functional role of corticosterone release in young kittiwake chicks, we conducted two experiments. The first experiment tested relationships between behavior and endogenous corticosterone levels over two hours. Because engaging in behavior expends energy, and corticosterone levels can reflect metabolic activity (Jimeno and Verhulst, 2023), we evaluated the relationships between behavior over the course of an hour and subsequent baseline corticosterone levels, measured at the end of that hour. Next, to test the basic assumption that elevated corticosterone redirects behavior, we evoked endogenous elevations in corticosterone via 15 min of handling and restraint, and tested relationships between baseline and elevated corticosterone levels, and changes in behavior in the subsequent hour. Because food availability can alter relationships between hormones and behavior (Cote et al., 2010, Killen et al., 2013, Benowitz-Fredericks et al., 2022), and corticosterone in kittiwake chicks rises when food availability is low (Kitaysky et al., 1999, Kitaysky et al., 2001, Benowitz-Fredericks et al., 2024), we also tested the hypothesis that relationships between corticosterone and behavior are stronger when food availability is limited by comparing hormone:behavior relationships in both control and experimentally food-supplemented nests.

In the second experiment, we addressed the possibility that correlative relationships between behavior and corticosterone levels evoked by restraint may be driven by components of the endogenous stress response other than corticosterone. We experimentally decoupled the endogenous stress response from corticosterone elevation by quantifying behavior in chicks for one hour before and one hour after minimally-disruptive application of exogenous corticosterone. To the best of our knowledge, this is the first study to manipulate both endogenous and exogenous corticosterone to simultaneously test assumptions about context-dependent hormone:behavior relationships in birds, on a time scale that is relevant but often overlooked.

2. METHODS

2.1 Field site and focal chicks.

All research was conducted at a colony on Middleton Island, AK (59.433552, -146.341008), where free-living kittiwakes nest on the face of a modified radar tower. Each nest site is equipped with a sliding, one-way mirrored glass window that allows nest observation and access from inside the tower with minimal disturbance to nesting birds, and PVC tubes that facilitate delivery of whole thawed fish (capelin) 3 times per day at a subset of sites; see Gill and Hatch (2002) for details. All adults are banded, and nests are checked twice daily from initiation of nest building through fledging. Consequently, precise dates of egg laying, chick hatching and death/disappearance dates are known. The tower structure precludes predation by terrestrial and aerial predators, removing a major extrinsic source of variation in chick survival. On hatch day and every 5 days afterwards, all chicks are weighed, measured and marked on the head with a colored pen to indicate hatch order (facilitating identification in videos).

All nests used in this experiment contained two chicks and were tested in the morning when the first-hatched (hereafter "A chick") was 5 days post-hatch. All second-hatched (hereafter "B chicks") were 2 – 5 days post-hatch. All data were collected in June and July of 2021 (experiment 1) and 2022 (experiment 2), between 0545 and 0900 hrs which was prior to any handling or research-related disturbance to the colony such as supplemental feeding or chick-measuring.

2.2 Supplemental feeding. Kittiwake chicks eat semi-digested food regurgitated by their parents. As part of a long-term experiment, a subset of tower nests were provided with supplemental food for the duration of the breeding season (hereafter "food supplemented" nests). Each food supplemented nest was equipped with a PVC pipe that allowed minimally-disruptive delivery of fish from inside the tower. Occupants of food supplemented nests were offered whole fish ad libitum, 3x per day, from the start of nest establishment through fledging, to augment the food they acquired at sea. Behavioral experiments were conducted when chicks were 5 days old and too small to consume the supplemental fish directly. However, by day 10 post-hatch they regularly consume the supplemental fish. "Control" chicks came from nests in which no supplemental food was provided, such that parents were entirely dependent on their own at-sea foraging to feed themselves and their chicks. Food supplementation successfully alleviates many nutritional constraints, more than doubling reproductive success in 2021 (control nests: ~0.41 chicks per nest, versus ~1.0 in both food supplemented nests and control nests in a "good" year; Ferris and Zador, 2021).

2.3 EXPERIMENT 1. Endogenous corticosterone (Figure 1A).

In 2021, we collected video and blood samples from food supplemented nests (n = 16) , and control nests (n = 17). GoPro Hero Session 5 cameras were mounted on tripods inside the tower, and used to record each nest through the one-way mirrored glass for 62 ± 4.2 min. Blood sampling methods have been described previously (Benowitz-Fredericks et al., 2024) but in brief: At the end of the recording period, a timer was started, parents were alerted via a tap on the window, and the A chick was taken from the nest and blood-sampled from the alar vein using a sterile 26G needle and heparinized microhematocrit tubes. All samples were collected within 3 min of initial tap (range 46 - 120 sec,

mean 93 ± 35 sec). We then placed the chick in a breathable cloth bag that we suspended 12 inches from a space heater to minimize thermoregulatory costs. Approximately 15 minutes (14 ± 1.8 min) from the time of original disturbance, the A chick was bled a second time and returned to the nest, followed by an additional 62 ± 6.5 min of video recording.

2.4 EXPERIMENT 2. Exogenous corticosterone (Figure 1B).

The second experiment was conducted in 2022 using only 5 day old A chicks from control (non-food supplemented) nests containing two chicks. The experimental design was similar to that in Experiment 1, however after 60 min of video recording we administered minimally-invasive topical treatments with the veterinary solvent dimethyl sulfoxide ("DMSO", n = 14), or with DMSO containing corticosterone (hereafter "CORT" treatment, n = 14), instead of capturing chicks for blood sampling. Corticosterone doses were ~46 µg corticosterone per chick, or 0.62 µg corticosterone/g body mass. Detailed methods for creating and administering the DMSO solutions as well as the consequences for circulating corticosterone at 15 and 60 min following this topical treatment for kittiwake chicks have been previously described (Benowitz-Fredericks et al., 2024). Briefly, within 15 min, this topical corticosterone treatment elevated circulating corticosterone in 5 day old kittiwake chicks to levels similar to those evoked by the endogenous response to 15 min of handling and restraint, and this elevation persisted 60 min after treatment. Corticosterone in the DMSO-treated control group did not differ from baseline levels in undisturbed chicks 15 or 60 min later, confirming that the process of administering topical treatments did not itself evoke endogenous corticosterone elevation.

Following corticosterone administration, nests were video recorded for a second hour, then at the end of the experiment (60 min after CORT or DMSO treatment), a single blood sample was taken from each A chick < 3 min after recapture from the nest (for genetic sexing and to measure corticosterone levels generated by treatments).

2.5 Hormone assays.

After collection, blood was refrigerated until centrifugation for 5 minutes at ~2000 rpm, after which plasma was separated from erythrocytes and both were frozen and transported at -20□ for storage in the lab at -80□. Samples from the same chick were run in the same assay; assays were conducted blind to chick sex or feeding treatment. Corticosterone was extracted after adding tritiated corticosterone to calculate percent recovered (96% ± 3% (SD) and 92% ± 3% (SD)) for experiments 1 and 2, respectively)). 20 µL plasma samples were run in duplicate in two radioimmunoassays for experiment 1, and a single assay for experiment 2, using an Esoterix (#B3-163) corticosterone antibody (lowest detectable limit: 7.8 pg/sample) following protocols described in Kitaysky et al. (2007). Final values were corrected for sample-specific percent recovery. Each assay included an internal corticosterone standard (inter assay CV for standard across three assays: 0.11%; intra assay CV for duplicates: 1%).

2.6 Genetic sexing.

To genetically sex chicks, we used DNA that was extracted from frozen erythrocytes using Qiagen DNeasy kits, and PCR and gel electrophoresis to determine chromosomal sex of chicks using the primers and protocol described in Merkling et al. (2012).

2.7 Behavior analysis.

Behavior of A chicks was coded using the software BORIS (Friard and Gamba, 2016) and a kittiwake chick ethogram (Appendix Table A.1). Focal behaviors were aggression towards the B chick, begging, and feeding (calculated as rates - number of behavior bouts or events divided by total video time). To correct for variation among individuals in undisturbed behavior levels in both experiments, we quantified changes in behavior from the first to the second hour using residuals of the regression of behavior rates in the second hour on behavior rates in the first hour.

2.8 Data analysis.

In the first experiment, we compared relationships between behavior and endogenous corticosterone to ask whether chick sex or food supplementation altered answers to the following questions 1) Do recent behaviors influence HPA-axis activity? Specifically, how are rates of behavior over the course of an hour related to baseline and restraint-induced corticosterone at the end of the hour. 2) Is elevated corticosterone associated with redirection of behavior? I.e. is the magnitude of corticosterone elevation elicited by 15 min handling and restraint (endogenous stress response) related to changes in behavior using residuals of behavior rates from the hour prior to the hour following handling and restraint. Finally, to evaluate potential fitness correlates of HPA activity, we asked 3) whether A chick's HPA-axis activity was associated with survival of the focal A chicks and the survival duration of B chicks.

In the second experiment, we compared changes in each behavior using residuals of behavior rates from the hour prior to treatment to the hour following treatment in both corticosterone-treated (hereafter "CORT") and control chicks (hereafter "DMSO") from non-supplemented nests only.

To meet statistical assumptions for parametric analyses we log-transformed corticosterone concentrations if it improved normality. Thus corticosterone values were log-transformed for experiment 1, but not for experiment 2. We also tested for equal variance between feeding treatment groups using the var.test function in R and accounted for unequal variance between feeding treatments in post-restraint corticosterone concentrations in all cases except when doing AICc model selection (see below), in which case we verified that the model output was the same for both approaches. We used generalized linear models with a gaussian link except in the case of modeling survival in which case we used a binomial link and untransformed corticosterone concentrations.

In experiment 1, feeding treatment was a significant predictor of baseline corticosterone ($t_{30} = 6.23$, $p < 0.001$, higher in control nests) and our previous study showed that, as an interaction term with sex, it was an informative predictor of the HPA function (Benowitz-Fredericks et al. 2024). Therefore, we first ran the analysis with feeding treatment, sex, and their interactions included (Appendix 2), then re-ran the model selection analyses on control and supplementally fed chicks separately to disentangle

the effects of feeding treatment on the results, given the number of predictor variables, our sample size, and our explicit interest in testing whether supplemental feeding weakened relationships between corticosterone levels and response variables. We conducted separate model selection analyses to examine whether changes in each of the focal behaviors (aggression bout rate, begging bout rate and feeding rate) were associated with sex, baseline corticosterone or post-restraint corticosterone at the start of behavior measurements (i.e. whether behavior predicted hormone levels). Finally, we checked for potential collinearity among closely related variables. Baseline and post-restraint corticosterone were not correlated (t_{32} = -0.12, p = 0.90), neither were aggression and begging in either the first (t_{35} = -0.83, p = 0.41) or second (t_{35} = 1.43, p =0.16) hour of behavior.

For model outputs we report the effect size, a standardized coefficient with its 95% confidence interval (*effectsize*). To determine what variable or combination of variables best described the response we used Akaike's Information Criteria corrected for small sample sizes (AICc) to rank models from an a priori model set. All models with a deltaAICc less than 2 were considered top performing and those with significant predictors are reported in the results. If the null model was included in the top performing model set, we considered the evidence for models with lower AICc than the null as more viable than those AICc values larger than the null, but considered the evidence for all models with deltaAICc <2 if the null model was not included in the top model set. A priori model sets were built according to our study questions as described above.

3. RESULTS

3.1 Experiment 1.

3.1.1 Analysis 1 – Can behavior affect HPA-axis activity?

In both supplemented and control nests we found no evidence for behavior, sex, or recent feeding experience as informative predictors of baseline corticosterone (Table 1, but see Appendix 2 Table A2.1, for feeding treatment effect). For both feeding conditions the null model was the top performing model with AICc values were > 2 higher than the next best model (Table 1). We found evidence that post-restraint corticosterone in control, but not food supplemented nests was correlated with the feeding rate in the previous hour alone (standard coefficient = 0.02 [0.01,0.03]; Table 1; Fig 2) and in an additive model with chick sex (sex effect: standard coefficient = -0.02 [-0.04, 0.00]). In control nests, post-restraint corticosterone was lower in chicks that had fed more often in the previous hour, and was generally higher in male chicks than females. In food supplemented nests, the null model was the top performing model (Table 1).

3.1.2 Analysis 2: Does elevated corticosterone redirect behavior?

In control nests, we found strong evidence that sex and post-restraint corticosterone affected changes in aggression, with both variables occurring in 3 of the 4 top-ranked models (Table 1). The two best-performing models were sex and the additive model of sex + post-restraint corticosterone. Males had a larger increase in bouts of aggression post-restraint than females following handling and restraint (standard coefficient = 0.16 [0.35, 1.96]). Higher levels of post-restraint corticosterone were positively correlated with increased aggression over the course of the experiment (standard coefficient = 0.52 [0.08, 0.95]; Fig 3A), this relationship was stronger in males (standard coefficient = 0.71 [0.10, 1.33]) than in females (standard coefficient = 0.26 [-0.41, 0.93]). In food supplemented nests, the null model was the top-ranked model (Table 1).

In control nests, changes in begging bout rate were best described by post-restraint corticosterone, with the null as the next best model. Higher post-restraint corticosterone was positively correlated with an increase in begging bout rate (standard coefficient = 0.49 [0.05, 0.93]; Table 1, Fig 3B). The null model was the highest ranked model for chicks from food supplemented nests (Table 1).

Changes in feeding rate were best described by chick sex, with the null model as the second-best model, in both control (standard coefficient = 1.02 [0.17, 1.88]) and food supplemented nests (standard coefficient = 0.84 [-0.08, 1.77], Table 1). Males had larger increases in their feeding rates than females (Fig 3C). Post-restraint corticosterone appeared in top models when feeding treatments were combined (Appendix Table A2.5).

3.1.3 Analysis 3 – Are there fitness-related implications of HPA-axis activity?

Survival of A chicks was best explained by post-restraint corticosterone and feeding treatment. The null model was also included in the top model set (Table 2). A chicks with higher baseline

corticosterone were more likely to die than those with lower levels of baseline corticosterone; for each 1 ng/ml increase in baseline corticosterone the probability of chick survival decreased by 9% (odds ratio: 0.92, 95% confidence interval [0.81, 1.00]). A chicks that survived had, on average, lower baseline corticosterone (8.04+/-8.01 (SD) vs 19.83+/-16.2 (SD) for live vs dead, correspondingly, beta (ß)=-0.415 [-0.743, -0.087]). Five out of 18 control A chicks didn't survive to fledging, while all 16 food supplemented A chicks survived; the ratio of surviving A chicks tended to be lower in control treatment (Yates corrected Chi-square=3.23, 1 d.f., p= 0.072).

The length of time B chicks survived post-experiment was best described by a single model (Table 2), which included A chick post-restraint corticosterone, feeding treatment and their interaction (standard coefficient = -1.11 [-1.9, -0.032]). In control nests, post-restraint corticosterone was negatively correlated with the length of time B chicks survived post experiment (Fig. 4; standard coefficient = -0.64 [-1.10, -0.18]); there was no correlation in supplementally fed nests (standard coefficient = 0.43 [-0.61, 1.47]). 15 out of 18 control B chicks and 7 out of 16 food supplemented B chicks did not survive to fledging; the ratio of surviving B chicks was lower in control treatment (Yates corrected Chi-square=4.21, 1 d.f., p=0.04).

3.2 Experiment 2. Exogenous CORT affects behavior

When we looked at the effect of experimentally elevated corticosterone on behavior in chicks from non-supplemented nests, we found no evidence that exogenously applied corticosterone induced changes in begging bout rates or feeding rates - none of our models for begging or feeding rate were ranked above the null model (Table 3).

We found strong evidence that exogenous corticosterone treatment induced an increase in aggression: i.e. changes in aggression bout rates were lower in chicks treated with DMSO alone compared to those treated with exogenous corticosterone (standard coefficient = -0.73 [-1.44, -0.02]; Table 3). Sex and post-treatment corticosterone levels also contributed to explaining changes in aggression, with both variables occurring in 3 of the 4 top-ranked models (Table 3). However, one of these models was the interactive model of sex x post-treatment corticosterone (Table 3, Fig. 5), revealing that corticosterone levels were positively correlated with an increase of aggression bout rates in males (standard coefficient = 0.67, [0.24,1.11]) but not in females (standard coefficient = 0.12, [-0.44, 0.68]).

4. DISCUSSION

4.1 Overview

We did not find strong evidence that behavior drives baseline HPA-axis activity in young kittiwakes, which we previously reported to be affected by food supplementation and chick body condition (Benowitz-Fredericks et al. 2024). However, five day old kittiwake chicks are capable of rapidly secreting high levels of corticosterone in response to 15 min of handling and restraint, comparable to adults (Benowitz-Fredericks et al. 2024), and we found that these post-restraint corticosterone levels were higher in chicks who had received fewer feeds in the previous hour. In addition, post-restraint

corticosterone levels were positively associated with subsequent changes in behavior (particularly increases in begging and aggression in males) and negatively associated with survival of younger siblings. Importantly, most of these hormone:behavior relationships were eliminated in nests where food limitation was alleviated via supplemental feeding. The second experiment, in which we administered topical corticosterone, revealed that the corticosterone:aggression relationship is the one most likely to be causal. The increase in begging was not retained when corticosterone was elevated in isolation, and may have been caused or modulated by other components of the stress response (such as catecholamines; see section 4.5 below). Together, these results support the hypothesis that elevation of endogenous corticosterone can rapidly influence behavior in context- and sex- specific ways.

Understanding why such young, nest-bound chicks are capable of rapidly and dramatically elevating corticosterone is important because many studies have indicated that there are costs to chicks of exposure to elevated glucocorticoids during development, presumed to reflect unavoidable trade-offs (Herborn et al., 2014; Kitaysky et al., 2003; reviewed et Schoech et al., 2011), while benefits are often assumed. No clear pattern has been identified to explain why the HPA-axes of some species are hyporesponsive early in development while others can mount robust responses at very young ages (Benowitz-Fredericks et al., 2024), but behavioral demands may play a role. Increases in begging and aggression have been identified as potential benefits of elevated corticosterone (Kitaysky et al., 2001; Kitaysky et al., 2003; Wada et al. 2008). While this is consistent with our general understanding of glucocorticoids as hormones that, during challenges, redirect physiology and behavior in ways that maximize fitness (in this case, behaviors associated with increasing food acquisition), most prior studies evaluated effects of chronic or repeated exposure to elevated corticosterone rather than short-term effects, and few have considered chick sex or food availability.

4.2 Sex differences

Though chromosomal sex imperfectly captures sources of interindividual variation (Smiley et al. 2024), in this study it better explained variation in hormone:behavior relationships than factors that may co-vary with it, such as body condition. There are sex differences in kittiwake chick growth rates and survival, often attributed to preferential parental allocation (Merkling et al., 2012; Young et al., 2016), however sex differences in the relationships between chick behavior and HPA axis activity have not been studied. In fact, we were only able to identify one study that considered (but did not find) sex-specific relationships between corticosterone and chick behavior in any bird (the white-crowned sparrows; Wada and Breuner, 2008). Our study suggests that behavioral strategies for coping with stressors may be sex-specific in kittiwake chicks, despite a lack of consistent dimorphism in morphology or HPA activity at this age (Benowitz-Fredericks et al., 2024). While chicks are not consistently sexually dimorphic at 5 days post hatch, males have higher growth rates, and body size diverges by 15 days post hatch (Merkling et al., 2012), post-restraint and exogenously elevated corticosterone levels were positively correlated with changes in aggression in males but not females. We previously showed that sex did not affect corticosterone levels at baseline or in response to 15 min of handling and restraint in 5 day old kittiwake chicks in the years of this study (Benowitz-Fredericks et al., 2024). However, there may be underlying sex differences in molecular substrates like glucocorticoid receptor expression (Jimeno et al., 2023), which can vary without concurrent

differences in HPA axis activity (Cornelius et al., 2018), such that relationships between corticosterone and behavior may still vary by sex even if circulating levels of corticosterone do not. Given that sex-specific strategies for maximizing fitness can include differences in both developmental rates (Richner, 1991; Weimersckirch et al., 2000) and somatic state at the end of development (Metcalfe and Monaghan, 2001), our results suggest that female chicks experiencing elevated corticosterone may be able to maximize their fitness without escalating competitive behaviors.

4.3 Focal behaviors

We focused on behaviors that we considered relevant to food acquisition (and therefore glucocorticoid secretion) in young chicks: begging (a nest-bound chick's primary mode of 'foraging'), aggression (acquiring a larger share of food by suppressing or eliminating competition), and feeding (an integration of both chick and parent behaviors). Most studies of corticosterone and chick behavior have focused on begging, but chicks of siblicidal species like kittiwakes have larger behavioral repertoires than those who compete only through begging, and behaviors like aggression may also interact with HPA axis activity on acute time scales (Kitaysky et al., 2003). Our study supports the overarching hypothesis that elevations in corticosterone can rapidly increase food acquisition behaviors in chicks.

4.3.1 Aggression

We found a positive relationship between aggression and elevated corticosterone (though not baseline corticosterone) that was consistently stronger in male chicks compared to females. To our knowledge, a single study has looked at relationships between corticosterone and aggression on acute time scales. Consistent with our results that recent aggression did not affect baseline corticosterone, a study of siblicidal Nazca booby chicks did not find any changes in corticosterone immediately following major aggression bouts (Ferree et al. 2004), though they did find that testosterone was higher. Indeed, the transient elevation of testosterone as a consequence of aggressive behavior has been extensively studied in many taxa using the "Challenge Hypothesis" framework (Wingfield et al., 1990), and is one the classical examples of behaviors driving changes in hormone levels. The effects of glucocorticoids on aggression have been better-studied in mammals than in birds, and such studies generally conclude that the relationship depends on the social context and the nature of glucocorticoid elevation (acute, chronic, or "toxic"; Haller, 2020, Yohe et al., 2012). Our study provides strong evidence that corticosterone may have a causal role in rapidly increasing context-appropriate aggression in very young kittiwake chicks.

4.3.2 Begging

There is variation among species, and among studies within species in the amount of energy expended during begging, but there is a general agreement that begging does not contribute substantially to most nestlings' daily energy budget (Morena-Rueda, 2007). The relatively low metabolic cost of begging may explain the lack of relationship between begging rates and subsequent baseline corticosterone levels. A handful of studies have tested whether elevated corticosterone is associated with variation in begging behavior in free-living passerine chicks on the scale of minutes, usually administering a series of corticosterone treatments over multiple days. Most of these found either no

clear effects (Elderbrock et al., 2017), subtle changes in begging such as increased latency to beg (Wada and Breuner, 2008), or inconsistent, transient increases in begging (i.e. in the first 5 minutes on one of several days of treatment; Bowers et al., 2019). We found stronger positive relationships between increases in begging and endogenous corticosterone elevation than exogenous corticosterone, which may be due to other components of the endogenous stress response that correlate with corticosterone (see section 4.5), or a dose-dependent effect of corticosterone(see section 4.3.3).

4.3.3 Feeding

Feeding rates by parents had no effect on baseline corticosterone. While frequent or recent feeding might be predicted to reduce corticosterone levels as net energy balance improves, postprandial elevations in glucocorticoids (presumably associated with changes in intermediary metabolism) have been well-documented on the scale of 30-60 min in humans (Slag et al., 1981, Anderson et al., 2019) and in chicks of at least one species of bird (the Florida scrub jay; Elderbrock et al., 2018). In contrast to baseline levels, post-restraint corticosterone levels were negatively correlated with feeds in the previous hour, suggesting increased reactivity of the HPA axes of chicks who had been receiving less food. Whether this was due to the events of the past hour, or lower long-term feeding rates for chicks who were fed less in our first hour of observation is unclear, though changes in HPA axis responsiveness are thought to reflect an individual's recent history of HPA axis activation (Dallman, 1993). Post-restraint corticosterone levels were not related to subsequent feeding when control and food supplemented nests were analyzed separately, potentially because feeding is moderated by parental responsiveness to chick behavior, rather than being controlled by chicks. However, when feeding treatments were combined for model analysis, post-restraint corticosterone emerged in two of the three top models (Appendix 2, Table A.2.5), suggesting that the relationship is weaker than for aggression or begging, but present.

4.4 Rapid behavioral effects of corticosterone

Hormones can have different effects over long- and short- time scales. In seabirds, prolonged elevation of exogenous corticosterone in chicks tends to increase begging rates on the scale of days (Kidawa et al., 2017; Kitaysky et al., 2001; Kitaysky et al., 2003; Loiseau et al., 2007; Vallarino et al. 2006). However, the HPA axis is also highly responsive in young chicks of many species, capable of substantially elevating glucocorticoids on the scale of minutes (Bebus et al., 2020) and putative membrane glucocorticoid receptors in birds may allow rapid non-genomic effects of corticosterone on this same short time scale (Breuner and Orchinik, 2009; Gao and Deviche, 2018). Rapid effects of glucocorticoids on behavior are not consistent with classical genomic mechanisms of action for glucocorticoid receptors. While the existence of a non-genomic (membrane) glucocorticoid receptor in birds was proposed long ago, with strong evidence in the form of rapid changes in locomotor activity in response to oral corticosterone administration (Breuner et al., 1998), and some evidence for a membrane-localized corticosterone binding site (Breuner and Orchinik, 2009), little subsequent biochemical evidence for such a receptor in birds has emerged since (Romero and Beattie, 2021). However, there is also evidence in mammals that "classical" nuclear steroid receptors can be found outside the nucleus, where they evoke non-genomic responses (Ordóñez-Morán and Muñoz 2009; Wilkenfeld et al. 2018). The accumulating body of evidence that corticosterone can evoke rapid

effects in birds and the conserved steroid hormone related pathways across vertebrates suggest that birds are likely to share such mechanisms of action for glucocorticoids. This evidence, combined with growing interest in rapid increases in corticosterone and their consequences (Taff et al., 2022), suggests that the phenomenon warrants continued investigation across species and ages.

4.5 Other components of the stress response?

The endogenous response to stressors include a suite of physiological changes (Gaidica & Dantzer, 2020; Gormally & Romero, 2020), of which elevated glucocorticoids is just one. The inability to separate the effects of corticosterone from the effects of other components of the stress response like catecholamines makes it challenging to interpret relationships between experimentally-induced increases in endogenous corticosterone and other physiological and behavioral variables of interest. Our experimental manipulation using minimally disruptive delivery of exogenous corticosterone isolated potential effects of corticosterone, allowing us to attribute causality to the relationship between corticosterone levels and aggression. Our results do not necessarily exclude the possibility that begging and feeding are also driven by elevated corticosterone for two reasons. First, experiment 1 and 2 were conducted in two different years, and interannual variability in chick or parental physiological state could generate variation in responses to elevated corticosterone. Second, behavioral responses may change along a continuum of corticosterone levels, triggered by dose dependent activation of different receptors (Mifsud & Ruel, 2018; Proszkowiec-Weglarz & Porter, 2010), with aggression being facilitated by lower levels of corticosterone than begging and feeding. While topical treatment significantly elevated corticosterone, average levels after 15 min were lower in the exogenous CORT treatment than those generated by the endogenous response to 15 min of restraint (Benowitz-Fredericks et al. 2024).

4.6 Challenges reveal relationships

Our study provided two lines of evidence that, as seen in adult kittiwakes (Benowitz-Fredericks et al., 2022), relationships between behavior and corticosterone in chicks emerge in response to challenges. First, baseline corticosterone levels were not strongly related to behaviors before or after the sample was taken - hormone:behavior relationships emerged after chicks were exposed to a stressor (handling and bag restraint) that elevated corticosterone. Killen et al. (2013) described the potential role of environmental challenges in shaping the emergence of relationships between physiology and behavior, proposing that they can either attenuate or mask them, or amplify them, as we found in this study. Second, supplemental feeding eliminated most of the relationships between endogenous corticosterone and behavior. Interestingly, chicks from food supplemented nests reached the same average levels of corticosterone as chicks from non-supplemented nests after 15 min of bag restraint (secreting more corticosterone to do so, since they started at lower baseline levels; Benowitz-Fredericks et al. 2024). Thus, the elimination of hormone:behavior relationships in food-supplemented nests was not due to a reduction in HPA sensitivity or capacity. Instead, our results are consistent with the basic tenet of hormone:behavior relationships that hormones do not cause behaviors, but increase the likelihood of their expression in response to appropriate stimuli. Chicks from food supplemented nests were less likely to be in a hungry state, therefore it was less likely that an elevation in corticosterone would evoke increases in behaviors associated with hunger (begging and aggression).

4.7 Chick physiology predicts sibling survival

The endocrine physiology of A chicks weakly predicted their own survival, and strongly predicted survival duration of their younger siblings. In experiment 1, nearly all of the A chicks survived, though those that died had higher baseline corticosterone than those that survived. Most of the B chicks died, which is normal for non-supplemented nests at this site, where average fledging success is <1 chick per nest (Ferris and Zador, 2021). However, in nests where B chicks died, they did so more quickly when they had A chick siblings who mounted larger corticosterone responses to restraint. More rapid death of B chicks with A siblings who had greater HPA responsiveness could be directly related to the phenotype of the A chick (since chicks with more responsive HPA axes were more aggressive), though proximate cause of death can rarely be determined in the field. Alternatively, it could be due to the fact that in nests where parents were low quality or otherwise struggling to sufficiently provision chicks, both A chick HPA axis reactivity and B chick survival reflect lower provisioning rates.

The fitness impacts of B chick deaths for A chicks are nuanced, since siblicide reduces indirect fitness but, presumably, increases direct fitness. Elimination (and more rapid elimination) of competition for parental provisioning should increase a chick's energy budget both by increasing food intake and by reducing expenditure for competitive behaviors like aggression. Brood-size manipulations in kittiwakes support this assumption, finding increased growth and survival of A chicks in reduced broods compared to control or enlarged broods (Jacobsen et al., 1995; Young et al., 2017). Our study indicates that siblicidal "decisions" in this species with facultative siblicide species may be facilitated by corticosterone.

4.8 Conclusion

The ability of very young chicks to release large quantities of corticosterone despite the potential costs of exposure to corticosterone during development suggests that such an early onset of HPA-axis responsiveness to nutritional state and external stimuli should increase their fitness despite their somewhat limited ability to change their environment. Our finding that the magnitude of corticosterone released in response to a challenge is correlated with subsequent increases in a repertoire of behaviors that promotes resource acquisition and brood reduction in contexts where food is limited is consistent with this hypothesis. In addition, the emergence of sex-specific relationships between corticosterone release and behavior at an age where other sex differences are undetectable suggests the existence of sex-specific differences in proximate molecular mechanisms (such as the location, density, and/or downstream paths for glucocorticoid receptors) and ultimate differences in male and female behavioral strategies during development. To better understand the selection pressures shaping patterns of HPA axis ontogeny across species, we encourage future research about relationships between glucocorticoids and behavior in free-living animals to consider: a) effects on short time-scales, b) the potential for sex-specific strategies, and c) the role of environmental challenges in masking or revealing potential relationships among them. Finally, glucocorticoids are fundamentally metabolic hormones, so understanding the rapid metabolic correlates and

consequences of corticosterone elevation in young chicks will further elucidate the function of HPA axis activity in this context.

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Table 1. Model selection results for experiment 1 (behavior 1 hour before and 1 hour after endogenous corticosterone elevation via 15 min of handling and restraint). We ran separate model comparisons for chicks from control and food-supplemented nests. All models contributing to a cumulative weight of 95% are included. Models within 2AIC units of the best performing model are indicated in bold, except for models with delta AICc values greater than the null model, which we did not consider.

		K	AICc	Delta AICc	AICc Wt
Baseline corticosterone					
Control					
	null	**2**	**11.21**	**0**	**0.46**
	sex	3	13.54	2.33	0.14
	begging previous hour	3	14.18	2.97	0.1
	feeding previous hour	3	14.19	2.98	0.1
	aggression previous hour	3	14.20	2.99	0.1
	sex + begging previous hour	4	17.01	5.8	0.03
Food supplemented					
	null	**2**	**7.02**	**0**	**0.49**
	begging first hour	3	9.97	2.95	0.11
	aggression previous hour	3	10.05	3.03	0.11
	feeding previous hour	3	10.07	3.04	0.11
	sex	3	10.07	3.05	0.11
	sex + begging previous hour	4	13.56	6.53	0.02
Post-restraint corticosterone					
Control					
	feeding previous hour	**3**	**-36.11**	**0**	**0.46**
	sex + feeding previous hour	**4**	**-35.92**	**0.19**	**0.42**
	sex * feeding previous hour	5	-31.99	4.12	0.06
Food supplemented					
	null	**2**	**-6.46**	**0**	**0.44**
	aggression previous hour	3	-4.15	2.32	0.14
	begging previous hour	3	-3.84	2.62	0.12
	feeding previous hour	3	-3.80	2.66	0.12
	sex	3	-3.48	2.98	0.1
	sex + aggression previous hour	4	-0.54	5.92	0.02
Change in aggression	-				
Control					
	sex	**3**	**-170.90**	**0**	**0.35**
	log post-restraint corticosterone + sex	**4**	**-169.59**	**1.31**	**0.18**
	log post-restraint corticosterone * sex	**5**	**-169.16**	**1.74**	**0.14**
	log post-restraint corticosterone	**3**	**-168.97**	**1.93**	**0.13**
	log baseline corticosterone + sex	4	-167.74	3.16	0.07
	null	2	-166.71	4.19	0.04
	log baseline corticosterone + log post-restraint corticosterone	4	-165.79	5.11	0.03
Food supplemented					
	null	**2**	**-160.46**	**0**	**0.32**
	log baseline corticosterone	3	-160.00	0.46	0.26
	log baseline corticosterone + log post-restraint corticosterone	4	-159.28	1.18	0.18

	sex	3	-157.76	2.7	0.08
	log post-restraint corticosterone	3	-157.40	3.07	0.07
Change in begging	-				
Control					
	log post-restraint corticosterone	**3**	**-160.07**	**0**	**0.43**
	null	**2**	**-158.43**	**1.64**	**0.19**
	sex	3	-156.66	3.41	0.08
	log baseline corticosterone + log post-restraint corticosterone	4	-156.64	3.43	0.08
	log post-restraint corticosterone + sex	4	-156.64	3.44	0.08
	log post-restraint corticosterone * sex	5	-155.95	4.12	0.06
Food supplemented					
	null	**2**	**-153.11**	**0**	**0.48**
	sex	3	-150.97	2.14	0.16
	log post-restraint corticosterone	3	-150.73	2.38	0.14
	log baseline corticosterone	3	-150.12	2.99	0.11
	log post-restraint corticosterone + sex	4	-148.22	4.9	0.04
Change in feeding	-				
Control					
	sex	**3**	**-187.87**	**0**	**0.44**
	null	2	-185.52	2.35	0.14
	log post-restraint corticosterone	3	-185.17	2.7	0.11
	log post-restraint corticosterone + sex	4	-185.01	2.86	0.11
	log baseline corticosterone + sex	4	-184.38	3.48	0.08
	log post-restraint corticosterone * sex	5	-183.47	4.4	0.05
Food supplemented					
	sex	**3**	**-173.51**	**0**	**0.31**
	null	**2**	**-173.29**	**0.23**	**0.28**
	log post-restraint corticosterone + sex	4	-171.55	1.96	0.12
	log post-restraint corticosterone	3	-171.24	2.28	0.1
	log baseline corticosterone	3	-170.32	3.2	0.06
	log baseline corticosterone + sex	4	-169.95	3.56	0.05

Table 2. Model selection results for survival of A and B chicks, considering endogenous corticosterone levels of the A chick. Models with delta AICc < 2 are in bold. All models contributing to a cumulative weight of 95% are included.

A chick survival				
A chick baseline corticosterone	2	21.86	0	0.36
feeding treatment	2	23.12	1.26	0.19
null	1	23.81	1.95	0.13
sex	2	23.99	2.13	0.12
A chick baseline corticosterone * sex	4	25.37	3.51	0.06
A chick post-restraint corticosterone	2	25.47	3.61	0.06
B chick days until death (survival duration)				
post-restraint corticosterone * feeding treatment	**5**	**162.11**	**0**	**0.45**
post-restraint corticosterone	**3**	**164.33**	**2.23**	**0.15**
post-restraint corticosterone + sex	**4**	**165.15**	**3.04**	**0.1**
baseline corticosterone	**3**	**165.46**	**3.35**	**0.08**
post-restraint corticosterone * feeding treatment + sex	**6**	**165.65**	**3.54**	**0.08**
baseline corticosterone + sex	**4**	**166.52**	**4.41**	**0.05**
post-restraint corticosterone + feeding treatment	**4**	**167.34**	**5.23**	**0.03**
baseline + feeding treatment	**4**	**167.53**	**5.43**	**0.03**

Table 3. Model selection results for experiment 2 (changes in behavior from 1 hour before to 1 hour after exogenous corticosterone elevation using topical treatments). Corticosterone treatments were topical DMSO (control), or corticosterone in DMSO; chick corticosterone was measured once at the end of the experiment, 1 hour after topical treatments. All chicks were from non-food supplemented nests. All models contributing to a cumulative weight of 95% are included. Models within 2 AIC units of the best performing model are indicated in bold.

	K	AICc	Delta AICc	AICc Wt
Change in aggression				
corticosterone treatment	**3**	**-254.2**	**0**	**0.24**
post experiment corticosterone * sex	**5**	**-252.9**	**1.3**	**0.12**
post experiment corticosterone	**3**	**-252.87**	**1.33**	**0.12**
sex + corticosterone treatment	**4**	**-252.73**	**1.47**	**0.11**
null	**2**	**-252.73**	**1.47**	**0.11**
post experiment corticosterone + sex	4	-251.93	2.26	0.08
post experiment corticosterone * corticosterone treatment	4	-251.61	2.59	0.07
sex	3	-251.47	2.72	0.06
sex + corticosterone treatment + post experiment corticosterone	5	-250.09	4.11	0.03
Change in begging				
null	**2**	**-258.55**	**0**	**0.34**
post experiment corticosterone	**3**	**-257.81**	**0.74**	**0.23**
corticosterone treatment	3	-256.25	2.29	0.11
sex	3	-256.01	2.54	0.09
post experiment corticosterone + corticosterone treatment	4	-255.34	3.21	0.07
post experiment corticosterone + sex	4	-255.04	3.5	0.06
post experiment corticosterone * corticosterone treatment	5	-253.66	4.89	0.03
Change in feeding				
null	**2**	**-292.82**	**0**	**0.39**
post experiment corticosterone	3	-290.71	2.11	0.13
corticosterone treatment	3	-290.56	2.26	0.12
sex	3	-290.4	2.42	0.12
post experiment corticosterone + corticosterone treatment	4	-289.76	3.06	0.08
post experiment corticosterone + sex	4	-288.11	4.71	0.04
sex + corticosterone treatment	4	-287.93	4.89	0.03
post experiment corticosterone * corticosterone treatment	5	-287.65	5.17	0.03

FIGURE CAPTIONS

Figure 1. Experimental timelines. Timeline for behavioral recordings, treatments and blood sampling from 5-day old first-hatched chicks ("A-chicks") in black-legged kittiwake nests containing two chicks. A) Experiment 1 (endogenous corticosterone). Undisturbed nests were video recorded for 60 min. Chicks were then bled in < 3min of nest disturbance, measured then restrained; the post-restraint bleed was taken 15 min after initial disturbance. Chicks were returned to the nest and recorded for another hour. B) Experiment 2 (exogenous corticosterone). Chicks were recorded for 60 min undisturbed, then briefly disturbed but not handled for topical treatment with DMSO or corticosterone in DMSO, recorded for another 60 min undisturbed, then captured for blood sampling at the end of the experiment. Some visual elements from BioRender.com

Figure 2. Feeding and subsequent restraint-induced corticosterone. In control nests where parents were not supplementally fed, corticosterone levels after 15 min of handling and restraint were higher in chicks who had received fewer feeds in the previous hour, and in male chicks. Solid line = control (non-supplemented); dashed line = food-supplemented.

Figure 3. Restraint-induced corticosterone and subsequent changes in behavior. First-hatched kittiwake chicks with higher restraint-induced corticosterone levels subsequently showed larger increases in all three behaviors, calculated as residuals from the regression of behavior bout rates the hour before against behavior bout rates the hour after 15 min of handling and restraint (to control for interindividual variation in baseline behavior rates), but these relationships were eliminated in experimentally food-supplemented nests. Changes in A. begging, B. feeding, and C. aggression. Solid line = control (non-supplemented); dashed line = food-supplemented.

Figure 4. A chick corticosterone and B chick survival duration. Relationship between endogenous corticosterone levels of 5 day old A chicks after 15 min of restraint and the number of days until death for their younger sibling (B chick) who did not survive. Solid line = all chicks.

Figure 5. Exogenously-elevated corticosterone levels and changes in aggression. Male chicks with higher corticosterone levels 60 min after treatment with topical corticosterone (at the end of behavioral observations) had larger increases in aggression, calculated as residuals from the regression of aggression bout rates the hour after treatment on aggression bout rates the hour before treatment (to control for interindividual variation in baseline behavior rates). Solid line = male; dashed line = female.

Figure 1

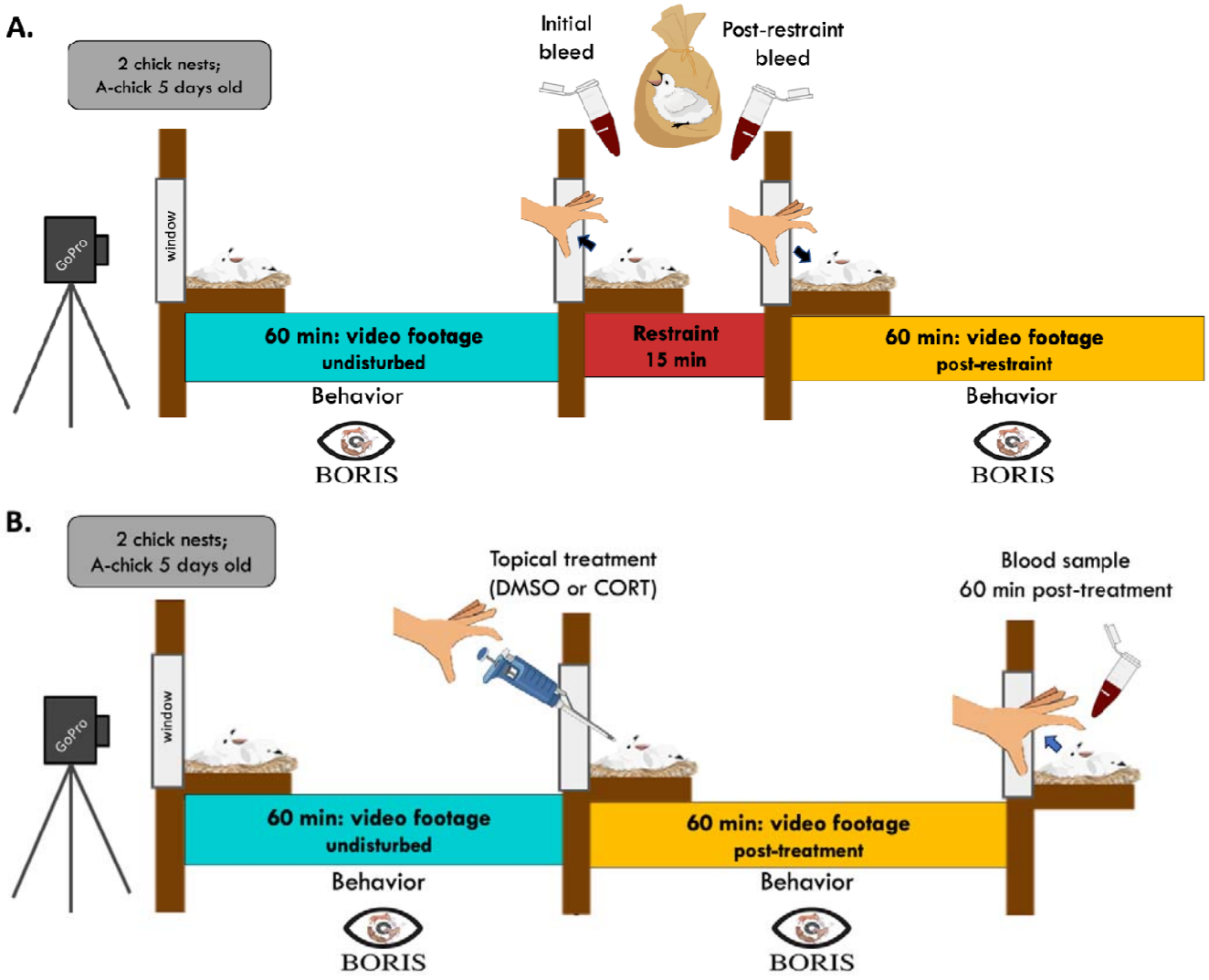
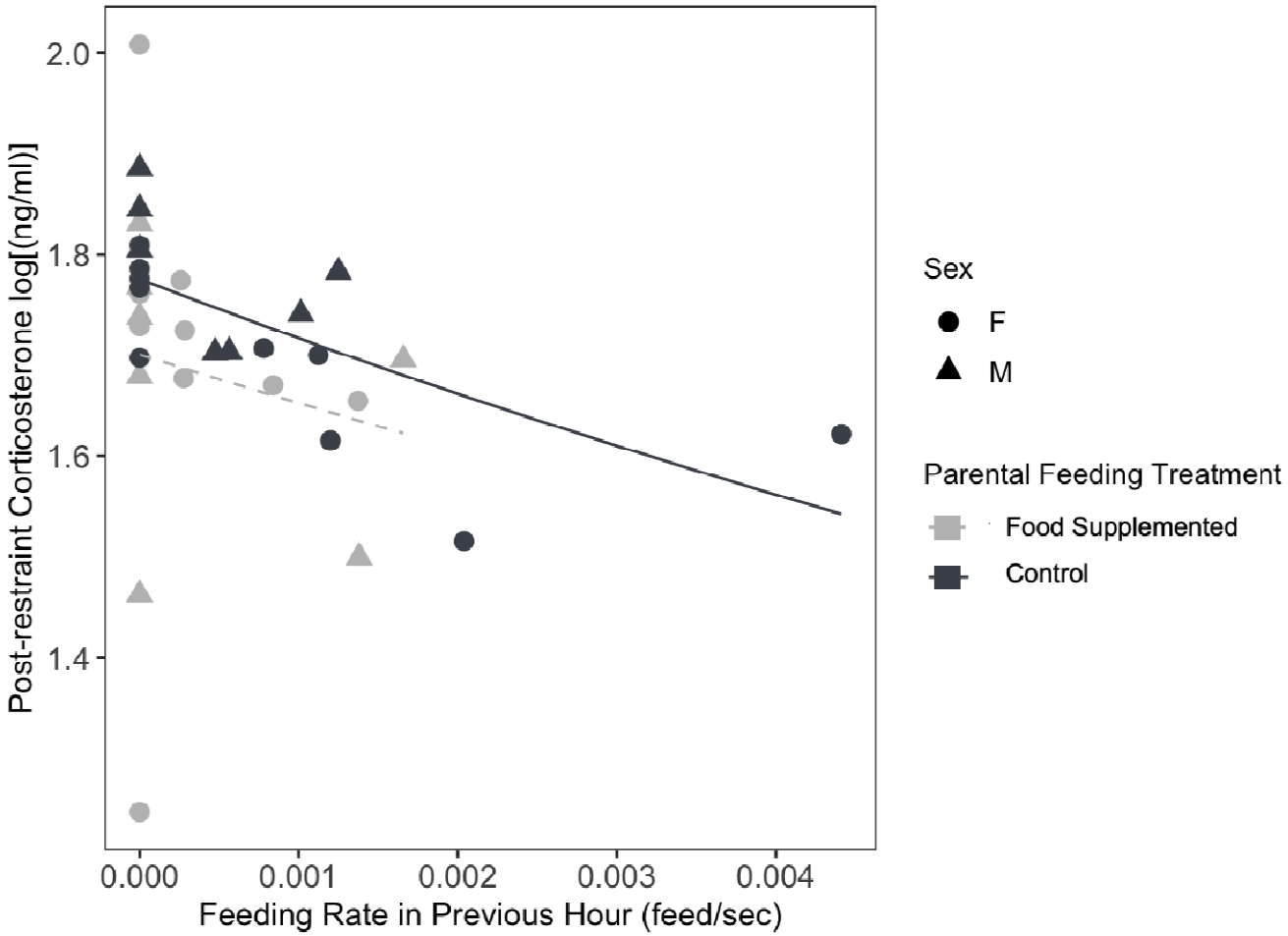

A.
2 chick nests;
A-chick 5 days old
Initial
bleed
Post-restraint
bleed
GoPro
window
60 min: video footage
undisturbed
Restraint
15 min
60 min: video footage
post-restraint
Behavior
BORIS
Behavior
BORIS
B.
2 chick nests;
A-chick 5 days old
Topical treatment
(DMSO or CORT)
Blood sample
60 min post-treatment
GoPro
window
60 min: video footage
undisturbed
60 min: video footage
post-treatment
Behavior
BORIS
Behavior
BORIS

Figure 2


2.0
1.8
1.6
1.4
Post-restraint Corticosterone log[(ng/ml)]
0.000
0.001
0.002
0.003
0.004
Feeding Rate in Previous Hour (feed/sec)
Sex
F
M
Parental Feeding Treatment
Food Supplemented
Control

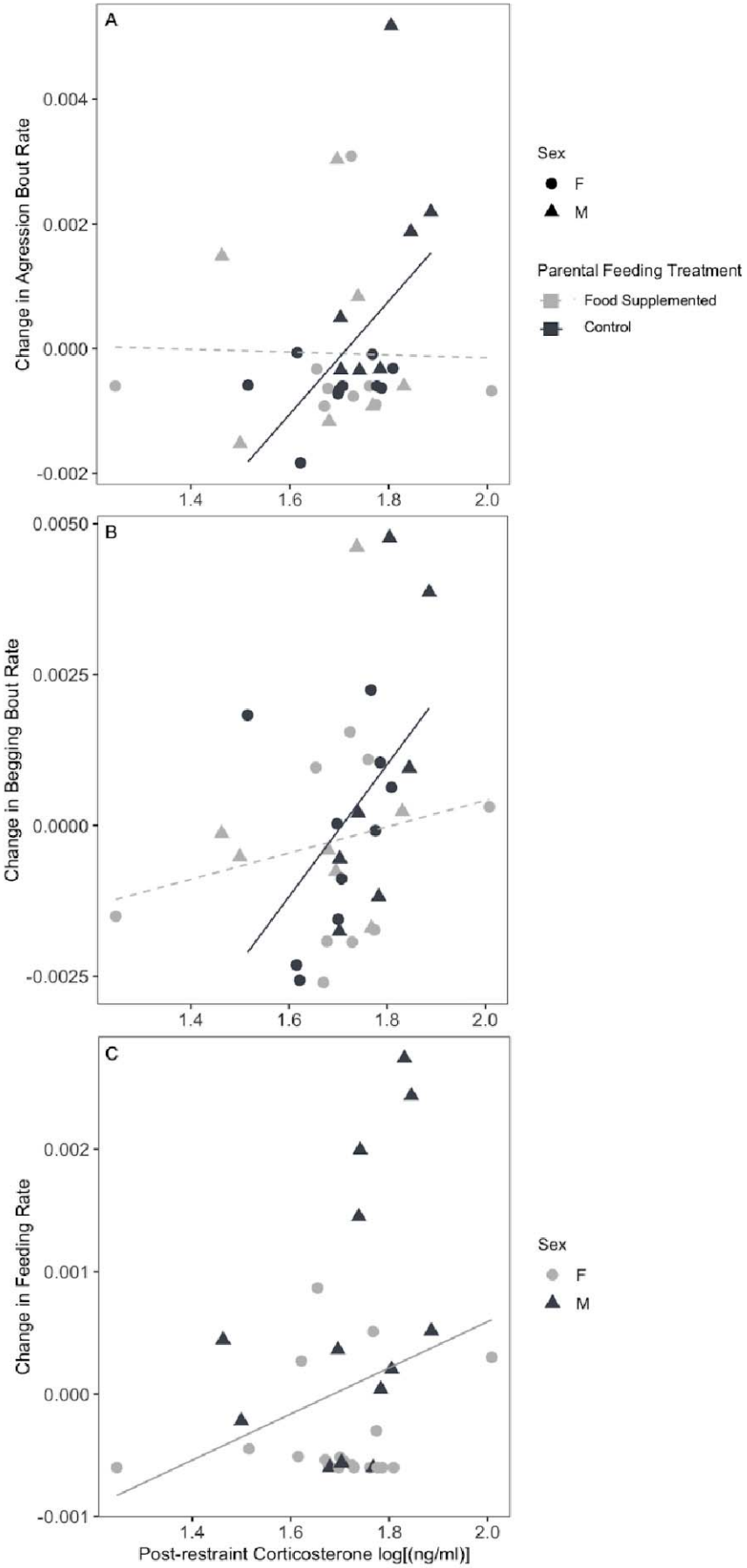

A
Change in Agression Bout Rate
0.004
0.002
0.000
-0.002
1.4
1.6
1.8
2.0
Sex
F
M
Parental Feeding Treatment
Food Supplemented
Control
B
Change in Begging Bout Rate
0.0050
0.0025
0.0000
-0.0025
1.4
1.6
1.8
2.0
C
Change in Feeding Rate
0.002
0.001
0.000
-0.001
1.4
1.6
1.8
2.0
Post-restraint Corticosterone log[(ng/ml)]
Sex
F
M

Figure 3

Figure 4

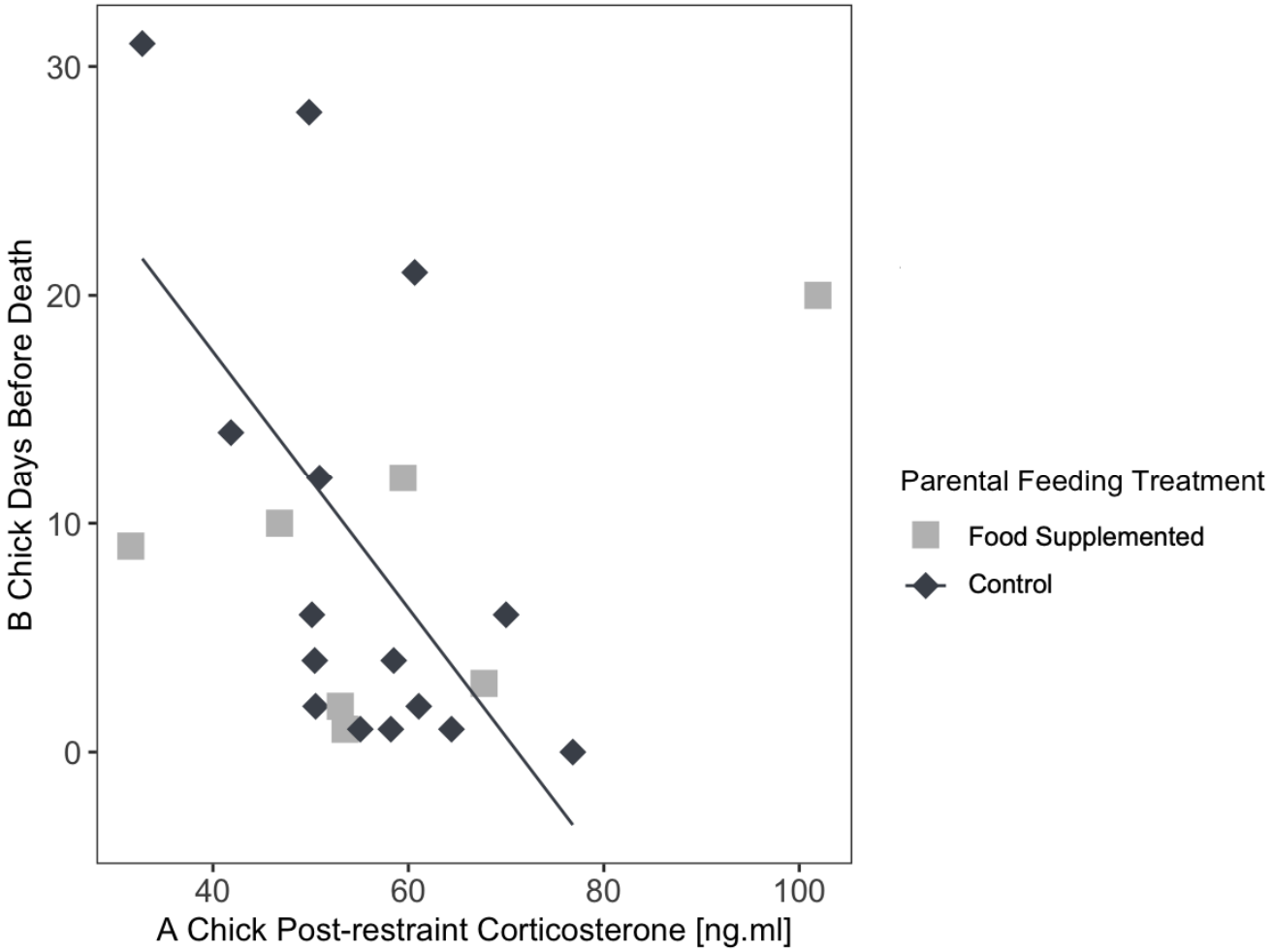
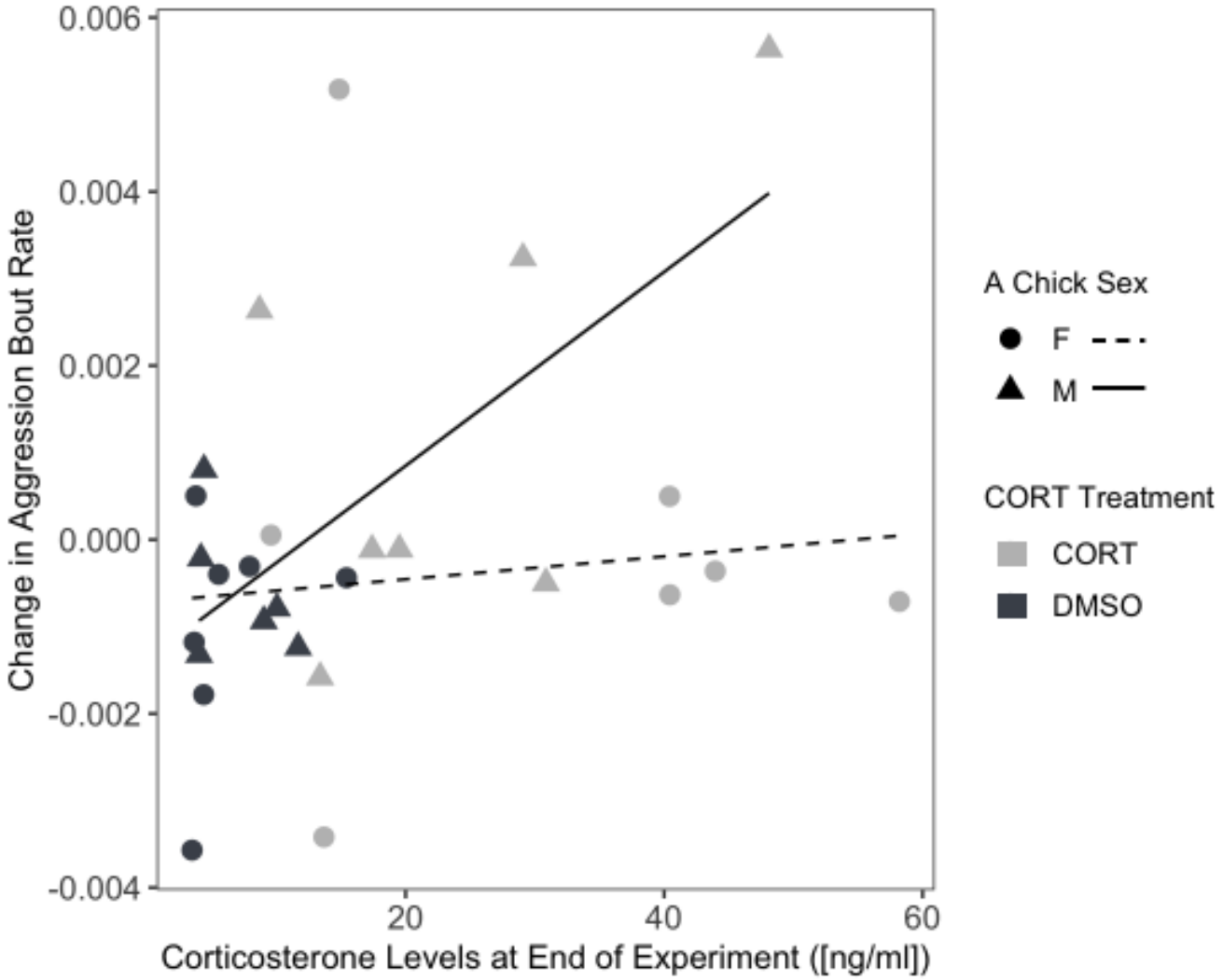

30
20
10
0
B Chick Days Before Death
40
60
80
100
A Chick Post-restraint Corticosterone [ng.ml]
Parental Feeding Treatment
Food Supplemented
Control

Figure 5


0.006
0.004
0.002
0.000
-0.002
-0.004
Change in Aggression Bout Rate
20
40
60
Corticosterone Levels at End of Experiment ([ng/ml])
A Chick Sex
F
M
CORT Treatment
CORT
DMSO