Low heritability and high phenotypic plasticity of cortisol in response to environmental heterogeneity in fur seals

Running header: Response of the cortisol phenotype to environmental heterogeneity

Rebecca Nagel[1,†], Sylvia Kaiser[2], Claire Stainfield[3], Camille Toscani[3], Cameron Fox-Clarke[3], Anneke J. Paijmans[1], Camila Costa Castro[1], David L. J. Vendrami[1], Jaume Forcada[3,*], Joseph I. Hoffman [1,3,*]

1. Department of Animal Behaviour, Bielefeld University, 33501 Bielefeld, Germany
2. Department of Behavioural Biology, University of Münster, 48149 Münster, Germany
3. British Antarctic Survey, Cambridge CB3 OET, UK

* Joint senior authors

† Corresponding author: rebecca.nagel@uni-bielefeld.de

Lay summary

Stress is ubiquitous, but individuals differ. In Antarctic fur seals, individual stress levels can be explained by sex, weight, day, density and food availability. Stress can influence how an individual behaves and interacts with its environment, so gaining a better understanding of those factors that explain variation in the stress response is important for population monitoring and conservation.

Title

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Abstract

Individuals are unique in how they interact with and respond to their environment. Correspondingly, unpredictable challenges or stressors often produce an individualized response of the hypothalamic-pituitary-adrenal axis and its downstream effector cortisol. We used a fully crossed, repeated measures design to investigate the factors shaping individual variation in baseline cortisol and cortisol responsiveness in Antarctic fur seal pups and their mothers. Saliva samples were collected from focal individuals at two breeding colonies, one with low and the other with high population density, during two consecutive years of contrasting food availability. Mothers and pups were sampled concurrently at birth and shortly before weaning, while pups were additionally sampled every 20 days. We found that heritability was low for both baseline cortisol and cortisol

responsiveness, while within-individual repeatability and among-individual variability were high. A substantial proportion of the variation in baseline cortisol could be explained in pups and mothers by a combination of intrinsic and extrinsic factors including sex, weight, day, season, and colony of birth. However, the same variables explained little of the variation in cortisol responsiveness. Our findings provide detailed insights into the individualization of endocrine phenotypes in a wild pinniped.

Keywords

Antarctic fur seal, baseline cortisol, cortisol responsiveness, individualization, phenotypic plasticity, pinniped

Introduction

Despite similarities in age, sex, or social status, individuals often differ in how they interact with their environment (Réale and Dingemanse 2010; Dall et al. 2012). Once perceived as a statistical nuisance, an ever growing body of evidence now suggests that these individual differences are often consistent and stable across time and contexts, with profound implications for understanding phenotypic variation, niche specialization, and animal personality (Sih et al. 2012; Wolf and Weissing 2012). The past decade has thus witnessed an increased awareness of individualization as a fundamentally important and compelling aspect of evolutionary biology, ecology, and animal behavior (Bolnick et al. 2003; Trillmich et al. 2018; Krüger et al. 2021).

Consistent differences among individuals are likely to be mediated by a combination of intrinsic and extrinsic factors and can be understood as the interaction between an individual's phenotype, genotype, and its ecological context such that its fitness is maximized (Dingemanse and Réale 2005; Vessey and Drickamer 2010; Lihoreau et al. 2021). At the proximate level, individualized phenotypic adjustments to environmental factors may be governed by, among other things, variation in the concentrations of circulating hormones (Müller et al. 2020). In particular, individual variation in cortisol, commonly known as one of the most important stress hormones, appears to play a major role in shaping individual responses to environmental conditions (Wingfield and Romero 2011).

Cortisol is a steroid hormone that belongs to the class of glucocorticoids. Its release is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoids

play an essential role in maintaining metabolic and homeostatic functions (Kuo et al. 2015). Under predictable conditions, cortisol is released continuously at baseline levels that vary naturally throughout the day and over an individual's lifetime (Lightman and Conway-Campbell 2010). In the face of unpredictable challenges, however, activation of the HPA axis results in increased levels of secreted cortisol for the duration of the stressor, before levels return to baseline (Bellavance and Rivest 2014).

While the physiological effects of circulating cortisol are conserved across individuals and species (Wingfield and Romero 2011), empirical studies have documented significant differences in baseline cortisol and cortisol responsiveness among individuals (Schoenemann and Bonier 2018; Taff et al. 2018). These differences could potentially facilitate adaptation to different habitats and environmental conditions (Sih et al. 2012). For example, cortisol levels have been shown to vary significantly among individuals experiencing different population densities (Meise et al. 2016) and levels of nutritional stress (Kitaysky et al. 2007). Variation in cortisol among individuals has also been linked to intrinsic factors such as age (Pavitt et al. 2015), sex (Azevedo et al. 2019), and weight (Jeanniard du Dot et al. 2009). Furthermore, when such factors are accounted for, empirical studies have shown that cortisol levels are often highly repeatable within individuals (Schoenemann and Bonier 2018; Taff et al. 2018). In guinea pigs, for example, cortisol responsiveness and to a lesser extent baseline cortisol levels are highly repeatable from late adolescence to adulthood (Mutwill et al. 2021).

Given that cortisol levels often exhibit high among-individual variability and within-individual repeatability, it has been argued that genetic rather than environmental

factors could explain much of the observed phenotypic variance, which would imply a high evolvability of this endocrine trait (Boake 1989; Jenkins et al. 2014). Correspondingly, several empirical studies have reported moderate to high levels of cortisol heritability in free-living vertebrate populations (Jenkins et al. 2014; Stedman et al. 2017; Bairos-Novak et al. 2018). However, persistent intrinsic and / or extrinsic factors might also produce repeatable phenotypes regardless of the underlying genotype (Taff et al. 2018), a possibility that has often been overlooked in the literature (Bonier and Martin 2016). Such examples may include variation in the endocrine phenotype in response to current internal and environmental stimuli (contextual plasticity) or stimuli from the past (developmental plasticity) (Stamps and Biro 2016).

Pinnipeds, and otariids in particular, are ideally suited to investigate the effects of internal and environmental factors on cortisol levels. First, otariids are colonially breeding, with males competing to establish and maintain harems on densely packed breeding beaches (Forcada and Staniland 2018). Cortisol may play an important role in how individuals adapt to this dynamic environment by restoring homeostasis after unpredictable challenges such as territorial bouts or unwanted mating attempts. Second, while many breeding beaches do not differ appreciably in qualities such as substrate type or topology, the density of individuals often varies from one place to another, setting up a spatial dynamic that tends to remain stable over time (Cassini 1999). Consequently, as pups are born on land and remain ashore throughout much of their early ontogeny (Payne 1979; McCafferty et al. 1998), cortisol might play an important role in mediating individual responses to variation in density. Finally, baseline cortisol levels and cortisol

responsiveness have been investigated in several pinniped species in relation to ontogeny (Ortiz et al. 2003; Atkinson et al. 2011), environmental conditions (DeRango et al. 2019), and handling regimes (Engelhard et al. 2002; Harcourt et al. 2010; Bennett et al. 2012; Champagne et al. 2012). Methodologies for collecting and assessing baseline cortisol and cortisol responsiveness in pinnipeds are therefore well established in the literature.

Our model otariid species, the Antarctic fur seal (*Arctocephalus gazella*), has been extensively studied by the British Antarctic Survey (BAS) on Bird Island, South Georgia since the 1980s. Two breeding colonies on the island provide a unique "natural experiment" for investigating individual responses to population density. Freshwater Beach (FWB) and Special Study Beach (SSB) are situated less than 200 meters apart (Figure 1a), meaning they are exposed to comparable climatic conditions. Breeding females from both locations also likely forage in the same areas (Hunt et al. 1992) and do not differ significantly in quality traits such as body size and condition (Nagel et al. 2021). Despite these similarities, the two colonies differ in the density of conspecifics. Direct counts of individuals ashore suggest that the density of breeding females is almost four times higher at SSB than FWB (Meise et al. 2016) and the modal local density of focal pups across the entire breeding season is also higher for pups born at SSB (Nagel et al. 2021).

We took advantage of this unique natural setup to investigate the intrinsic and extrinsic factors shaping individual variation in baseline cortisol and cortisol responsiveness in Antarctic fur seal pups and their mothers. We used a fully crossed, repeated measures design (Figure 1b, c) comprising longitudinal data from mother-

offspring pairs from the two colonies over two consecutive breeding seasons, the first of which was coincidentally one of the worst years on record with respect to food availability (Forcada and Hoffman 2014; Nagel et al. 2021). Specifically, we collected saliva samples and accompanying biometric data from 25 randomly selected focal pairs from each colony in both seasons. To quantify baseline and cortisol responsiveness levels, saliva was collected once immediately after capture and again after approximately ten minutes of “capture stress”, a procedure known to elicit a response of the HPA axis (reviewed in (Wingfield and Romero 2011)). Mothers were sampled twice during the breeding season, while pups were sampled every 20 days from birth until just before molting at around 60 days of age.

We used animal models to obtain heritability estimates for baseline cortisol and cortisol responsiveness using both a simple pedigree and a genomic relatedness matrix obtained from a high density single nucleotide polymorphism (SNP) array (Humble et al. 2020). We then used linear mixed models to evaluate the within- and among-individual variability of cortisol levels in pups and their mothers. Included in each model as explanatory variables were multiple intrinsic (sex, weight, body condition, and days after initial sampling) and extrinsic (density and year) variables. In line with previous studies of wild vertebrate populations, we hypothesized that cortisol levels would be heritable and repeatable within individuals. We further hypothesized that baseline cortisol and cortisol responsiveness would be higher in mothers and pups from the high-density colony and in the season of low food availability.

Methods

Field study

This study was conducted during the Antarctic fur seal breeding seasons (December to March) of 2018–19 (hereafter 2019) and 2019–20 (hereafter 2020) at Bird Island, South Georgia (54°00’24.8 S, 38°03’04.1 W). Each season, we sampled 25 unique mother-pup pairs from two neighboring breeding colonies, one of low (FWB) and the other of high density (SSB) (Figure 1a). Sampling at both locations was randomized with respect to pup sex, resulting in a final sample size of 51 male and 49 female pups. Pup mortality was 25.6% averaged over the two colonies and seasons.

Each mother and her pup were captured concurrently on two separate occasions: 2–3 days postpartum (December) and again as the pups began to moult shortly before weaning (March). Pups were additionally recaptured every 20 days. For the capture, restraint, and sampling of individuals, we employed protocols that have been established and refined over 30 consecutive years of the BAS long-term monitoring and survey program. Briefly, adult females were captured with a noosing pole and held on a restraint board during processing. Pups were captured with a slip noose or by hand and were restrained by hand. After sampling, individuals were released as close to their capture site as possible and, when present, pups were reunited with their mothers.

At the first sampling, focal individuals were fitted with VHF transmitters to the dorsal side of the neck between the shoulder blades with epoxy glue (mothers: Sirtrack core marine glue-on V2G 154C; pups: Sirtrack core marine glue-on V2G 152A). Transmitter signals were monitored throughout the season using a hand-held VHF

receiver (AOR LTD., AR8200). Focal individuals were also given cattle ear tags (Dalton Supplies, Henley on Thames, UK) in the trailing edge of each foreflipper (Gentry and Holt 1982) for identification. Tissue plugs were collected and stored in 20% dimethyl sulfoxide (DMSO) saturated with salt at -20°C for subsequent genetic analysis.

At every capture, weight and length measurements were taken from which a scaled mass index was calculated according to (Peig and Green 2009). This condition metric serves as a reliable indicator of overall fitness as it has been correlated with, among other things, offspring survival (Milenkaya et al. 2015; Gélin et al. 2016) and mating success (Gastón and Vaira 2020). Two saliva samples were collected at every capture, the first of which was taken within three minutes of capture to provide data on baseline cortisol levels (Bozovic et al. 2013). The second saliva sample was taken 10 ± 2 minutes thereafter to provide data on cortisol responsiveness. Saliva was collected by rotating sterile cotton tip applicators fitted in polypropylene tubes (ROTH, Art. No. XC10.2) in the cheek pouch and under the tongue. Samples were centrifuged and stored at -20°C for subsequent cortisol analysis.

Hormone quantification

Saliva samples were thawed and centrifuged for ten minutes to separate the mucins. The clear supernatant was then used for the determination of cortisol concentrations. Samples contaminated with blood (reddish supernatant) were discarded (n = 30 in 2019 and n = 31 in 2020), as cortisol values are often falsely elevated in such samples. Cortisol concentrations were determined in duplicate using an enzyme-linked

immunosorbent assays (cortisol free in saliva DES6611, Demeditec Diagnostics GmbH, Kiel, Germany). The intra-assay variances were on average CV = 5.2% and the inter-assay variances were on average CV = 6.9%. The antibody showed the following ross-reactivities: cortisol 100%, 11-desoxycortisol 50%, corticosterone 6.2%, 11-Deoxycorticosterone 2.6%, 17α-oh-progesterone 1.3%, cortisone and prednisone < 1%, testosterone, estradiol, and androstendione < 0.1%.

SNP genotyping and genomic relatedness matrix construction

For the 96 focal individuals sampled in 2019, we extracted total genomic DNA from tissue samples using a standard chloroform-isoamylalcohol protocol (for a description of the full protocol, see the Supplementary methods). SNP genotyping was performed on these samples from 2019 using a custom Affymetrix SNP array as described by (Humble et al. 2020). Quality control of the raw output data and genotyping were implemented using the Axiom Analysis Suite (5.0.1.38, Affymetrix) based on parameter thresholds set to their default values for diploid organisms. SNPs that were initially classified as “off target variants” (OTV) were recovered by using the “Run OTV caller” function. Of the 85,359 SNPs tiled on the array, 77,873 were retained for further analysis representing SNPs classified as “PolyHighResolution” (SNPs that passed all of the Axiom Analysis Suite quality controls) and “NoMinorHomozygote” (SNPs that passed all quality controls but no homozygote genotypes for the minor allele were found). An additional 3,423 SNPs with minor allele frequencies below 0.01 and 2,096 SNPs that departed significantly from Hardy Weinberg equilibrium (HWE) were removed using

PLINK version 1.9 (Purcell et al. 2007). Departures from HWE were identified based on an alpha level of 0.01 after implementing mid-*p* adjustment (Graffelman and Moreno 2013). After filtering, a total of 72,354 SNPs were retained and used to produce a genomic relatedness matrix using the --make-grm option in GCTA version 1.93.1 (Yang et al. 2011).

Heritability of cortisol levels

To quantify the proportion of the total variance in baseline cortisol attributable to genetic differences among individuals, we fitted two multivariate generalized linear mixed models (GLMMs) in *MCMCglmm* (Hadfield 2010) with baseline cortisol as the dependent variable and individual ID and relatedness as random effects. For the first model, a simple pedigree (comprising mother-offspring pairs) was built for the full dataset, while the second model incorporated the SNP relatedness matrix from individuals sampled only in the first season (2019). We used weak but informative priors (0.05 of the observed phenotypic variance) in both models. Markov chains were run for 9,000,000 iterations and we retained every 8,500th value after removing 150,000 iterations of burn-in to generate posterior distributions of the random parameters. The posterior distribution of the model intercept and autocorrelation were checked to assess model fit. We obtained estimates of baseline cortisol heritability by dividing the additive genetic variance by the total phenotypic variance ($h^2 = V_A/V_P$) for each sample of the posterior distribution. We used the same approach to estimate the proportion of variance

in cortisol responsiveness, calculated as the difference between baseline and response cortisol concentrations.

Estimating intrinsic and extrinsic factors influencing baseline cortisol

To determine whether weight, body condition, age, sex, season, colony, and an interaction between season and colony explained a significant proportion of the variation in baseline cortisol among pups, we fitted a GLMM with a log-link gamma error distribution in *lme4* (Bates et al. 2015). A second GLMM with maternal cortisol as the dependent variable was used to determine the proportion of variation explained by weight, body condition, the number of days postpartum, season, colony, and an interaction between season and colony. To account for both structural and data multicollinearity among weight, condition, and age (pups) / days postpartum (mothers), these variables were rescaled and centered by subtracting the mean from all observed values. In preliminary analyses, we tested for the presence of heterogeneous variance by allowing individual slopes to vary by age (pups) or days postpartum (mothers). For both models, random intercepts were included for each individual to account for repeated measures. The residuals of the full models were inspected for linearity and equality of error variances (using plots of residuals versus fits), normality (using Q – Q plots), and homogeneity of variance (using Levene's test). A backward elimination based on the chi-squared statistic was implemented to simplify the full models. The best fitting models were selected based on AIC values. The statistical significance of fixed predictors was assessed using Wald tests. We determined the marginal R^2 (variance explained by fixed

effects) and conditional R^2 (variance explained by fixed and random effects) according to (Nakagawa and Schielzeth 2013).

Estimating intrinsic and extrinsic factors influencing cortisol responsiveness

To determine whether weight, body condition, age, sex, season, colony, and an interaction between season and colony explained a significant proportion of the variation in cortisol responsiveness among pups, we fitted a generalized additive model (GAM) in *mgcv* (Wood et al. 2016). A second GAM with maternal cortisol responsiveness as the dependent variable was fitted to determine the proportion of variation explained by weight, body condition, days postpartum, season, colony, and an interaction between season and colony. In our dataset, cortisol responsiveness was "heavy-tailed" compared to a Normal distribution so we used a scaled *t*-distribution. To account for both structural and data multicollinearity, weight, body condition, and age (pups) / days postpartum (mothers) were rescaled and centered. Since cortisol response samples were collected between 8 and 12 minutes after the baseline, in preliminary analyses we tested if accounting for variation in the amount of time between sampling significantly improved model fit. In both models, random intercepts were included for each individual to account for repeated measures. The residuals of the full models were inspected for linearity and equality of error variances (using plots of residuals versus fits), normality (using Q – Q plots), and homogeneity of variance (using Levene's test). A backward elimination based on the *z*-values of explanatory variables was implemented to simplify the models. The best fitting model for pups and mothers was determined based on AIC values. The

statistical significance of fixed predictors was assessed using Wald tests. Conditional R^2 was calculated according to (Nakagawa and Schielzeth 2013). All analyses and visualizations were implemented in R version 4.0.2 (R Core Team 2020) using the integrated development environment RStudio version 1.3.1093 (RStudio Team 2020).

Animal handling, ethics and permits

Sampling was carried out by the BAS under permits from the Government of South Georgia and the South Sandwich Islands (Wildlife and Protected Areas Ordinance (2011), RAP permit numbers 2018/024 and 2019/032). The samples were imported into the UK under permits from the Department for Environment, Food, and Rural Affairs (Animal Health Act, import license number ITIMP18.1397) and from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (import numbers 578938/01-15 and 590196/01-18). All procedures used were approved by the BAS Animal Welfare and Ethics Review Body (AWERB applications 2018/1050 and 2019/1058).

Results

We used a fully crossed, repeated measures design incorporating saliva samples from 98 unique pups and 93 unique mothers from two colonies of contrasting density across two consecutive years of contrasting food availability (Figure 1). Sample sizes were balanced between the colonies (n = 94 from FWB and n = 97 from SSB) and seasons (n = 96 from 2019 and n = 95 from 2020). Each season, pups were sampled every 20 days from birth until weaning, amounting to a total of 551 analyzed saliva samples (290 baseline and 221 response samples) or an average of six samples per individual. Mothers were sampled twice each season, once shortly after birth and again shortly before molting, which amounted to a total of 240 analyzed saliva samples (145 baseline and 95 response samples) or an average of three samples per individual.

Cortisol heritability estimates

We estimated heritability using two animal models, the first incorporating known pedigree relationships (i.e. mother-offspring pairs from both years) and the second incorporating a SNP relatedness matrix, which was only available for the first year of the study. For baseline cortisol, narrow-sense heritability (h^2) estimates from both models were low with overlapping 95% credible intervals (pedigree model: h^2 = 0.013, 95% highest posterior density 0.004 – 0.045; SNP relatedness model: h^2 = 0.018, 95% highest posterior density 0.004 – 0.062) (Figure 2). The additive genetic (V_A) and residual (V_R) variance estimates of the two models were also comparable (pedigree model: 95% highest posterior density of V_A = 0.1 – 1.6 and V_R = 30.6 – 39.9; SNP relatedness model:

95% highest posterior density of V_A = 0.1 – 2.3 and V_R = 28.5 – 41.7). We obtained similarly low h^2 estimates for cortisol responsiveness, but the 95% credible intervals were somewhat broader (pedigree model: h^2 = 0.015, 95% highest posterior density 0.004 – 0.101; SNP relatedness model: h^2 = 0.035, 95% highest posterior density 0.010 – 0.225) (Figure 2). The V_A and V_R variance estimates of the two models were also comparable (pedigree model: 95% highest posterior density of V_A = 0.1 – 1.9 and V_R = 16.0 – 22.3; SNP relatedness model: 95% highest posterior density of V_A = 0.1 – 3.5 and V_R = 9.7 – 17.8).

Intrinsic and extrinsic factors influencing baseline cortisol

The best supported model of pup baseline cortisol contained age ($p < 0.001$), weight ($p < 0.001$), sex ($p = 0.004$), season ($p = 0.003$), body condition ($p = 0.093$), and colony ($p = 0.107$) as fixed effects (Table 1a, Figure 3a). The total amount of variance explained by this model was high (conditional R^2 = 0.657), as was the repeatability of baseline cortisol values across individuals (ICC = 0.39). Including ID as a random effect significantly improved the fit of the model, indicating appreciable among-individual variability in baseline cortisol ($p < 0.001$; Supplementary Table S1a). Allowing individual slopes to vary between age groups also significantly improved model fit ($p < 0.001$; Supplementary Table S1a) suggesting that individuals responded to the covariates differently depending on their age. Overall, baseline cortisol decreased with increasing pup age (Figure 3b) and was lower among pups born in 2020, the year of higher food availability (Figure 3c). Baseline cortisol decreased significantly as pup weight increased

(Figure 3d), although the slope of the regression between cortisol and weight approached zero as pups approached their moult. Finally, baseline cortisol tended to be higher in males than females (Figure 3e).

The best-supported model of maternal baseline cortisol contained days postpartum ($p < 0.001$), season ($p = 0.004$), and colony ($p = 0.068$) as fixed effects (Table 1b, Figure 3f). Neither weight nor body condition were retained in the model. The total variance explained by the model was again high (conditional $R^2 = 0.774$), as was repeatability of baseline cortisol within individuals (ICC = 0.43). Including ID as a random effect significantly improved model fit, indicating appreciable among-individual variability in baseline cortisol ($p < 0.001$; Supplementary Table S1b). The concentration of cortisol in maternal saliva decreased as the season progressed (Figure 3g) and tended to be higher in 2020, the year of higher food availability (Figure 3h).

Intrinsic and extrinsic factors influencing cortisol responsiveness

For the majority of observations (80.0% in pups and 89.5% in mothers), individual cortisol levels increased from the first to the second sampling time point, suggesting that the capture and handling procedure induced a measurable stress response (Figure 4a, e). Including the amount of time between samples as a random intercept did not significantly improve the fit of the model (Supplementary Table S2). The best-supported model of pup cortisol responsiveness contained age ($p < 0.001$) and sex ($p = 0.010$) as fixed effects (Table 2a, Figure 4b). Compared to the baseline cortisol model, the total amount of explained variance was small (conditional $R^2 = 0.080$). The fixed

effects of the cortisol responsiveness model were also in the opposite direction to the equivalent fixed effects in the baseline cortisol model, with cortisol responsiveness increasing with pup age and being higher in female pups (Figure 4c, d). For maternal cortisol responsiveness, the best-supported model contained only days postpartum (p = 0.001) as a fixed effect (Table 2b, Figure 4f). The total amount of explained variance was again small (conditional R^2 = 0.056) and maternal cortisol responsiveness decreased as the season progressed (Figure 4g).

Discussion

We used a fully crossed, repeated measures design to characterize individual variation in baseline cortisol levels and their response to capture stress in Antarctic fur seals. We found that baseline cortisol was only marginally explained by genetic factors, while high within-individual repeatability and among-individual variability in both pups and mothers could be largely explained by a combination of intrinsic and extrinsic factors. For cortisol responsiveness, we found that neither genetic nor environmental factors explained a substantial proportion of the phenotypic variation. Our results provide detailed insights into the individualization of an endocrine phenotype in a wild pinniped population.

Baseline cortisol

We quantified the narrow sense heritability of baseline cortisol using animal models based on a simple pedigree and a SNP array. The former approach assumes that individuals of unknown parentage are unrelated to all other individuals in the population, which can lead to heritability being underestimated (Kruuk 2004). By contrast, genomic approaches are capable of quantifying unbiased relatedness for all sampled individuals, but can be time-consuming and costly to produce (Frentiu et al. 2008). Despite these differences, we found that both approaches produced consistently low heritability estimates for baseline cortisol in Antarctic fur seals. Heritability can be low because of low additive genetic variation, high environmental variance, or cross-environment genetic correlations (i.e. the genetic basis of the trait varies between different

environments) (Charmantier and Garant 2005). Our low estimates, which stand in contrast to our expectations and previously published empirical results (Jenkins et al. 2014; Stedman et al. 2017; Bairos-Novak et al. 2018), might therefore be reflective of the extreme heterogeneity of the environmental conditions encountered by the study population at Bird Island.

For both pups and mothers, we were able to explain a substantial proportion of the total phenotypic variance in baseline cortisol by including individual-based and environmental variables in our models (conditional R^2 = 0.66 for pups and R^2 = 0.77 for mothers). Similar results have been obtained for a variety of species (e.g. (Joly and Cameron 2018; Uchida et al. 2021), but see (Azevedo et al. 2019)), suggesting that contextual and developmental phenotypic plasticity in response to environmental heterogeneity may be a widespread phenomenon. In addition, we found that baseline cortisol levels were highly consistent within individuals, suggesting that individualized endocrine phenotypes become established during early ontogeny and persist at least until nutritional independence. Baseline cortisol might therefore represent a stable attribute by which fur seals adapt to spatial or temporal heterogeneity in their environment (Réale and Dingemanse 2010).

Our models uncovered a strong influence of age and days postpartum on baseline cortisol levels in pups and mothers, respectively, with salivary cortisol decreasing from birth to moult. One explanation for this may be the shifting environmental conditions that individuals experience as the season progresses. Pregnant females arrive ashore in December and give birth on crowded breeding beaches. Mothers continue to suckle their

pups on the beach until about 30 days postpartum, when most females transition into the tussock grass that covers most of the island's interior (Doidge et al. 1984). Also during this time, adult males begin to abandon their territories and migrate to higher latitudes around the Antarctic ice shelf (Forcada and Staniland 2018). Consequently, the frequency of unpredictable challenges for both pups and mothers likely declines as the season progresses.

We detected significantly higher cortisol concentrations among pups and mothers sampled in 2020, the year of higher food availability. This was surprising given the many empirical studies that have linked elevated cortisol concentrations to food shortages and periods of nutritional stress (e.g. (Kitaysky et al. 2007; Behie et al. 2010; Bryan et al. 2013; Garber et al. 2020)). We can think of two possible explanations for this pattern. On the one hand, our results could be explained by higher population densities in the second year of our study, as significantly more females bred in 2020 compared to 2019 (Nagel et al. 2021). This would be in line with the small, albeit non-significant, effect of colony on baseline cortisol, with hormone concentrations being marginally higher in both pups and mothers at SSB compared to FWB. On the other hand, circulating levels of cortisol are essential for the maintenance of metabolic functions (Kuo et al. 2015), and food-induced cortisol secretions have been documented in the literature (Quigley and Yen 1979; Gibson et al. 1999; Stimson et al. 2014). Shorter foraging trip durations (Nagel et al. 2021) and consequently more frequent meals for pups and mothers in 2020 may have resulted in higher average baseline cortisol concentrations, which facilitate protein and carbohydrate metabolism.

More indicative of the hypothesized correlation between cortisol and nutritional stress, we found a significant negative relationship between baseline cortisol levels and weight in pups. Elevated cortisol levels stimulate gluconeogenesis and thereby fat oxidation, which provides individuals with a source of energy (Wingfield and Romero 2011). Given that pups must tolerate bouts of starvation lasting up to 11 days while their mothers forage at sea (Forcada and Staniland 2018), our results may reflect a physiological response to prolonged periods of natural fasting (Ortiz et al. 2001; Jeanniard du Dot et al. 2009). In other words, fasting pups may increase baseline cortisol to release energy, resulting in a reduction of absolute body fat and overall weight. This would also explain why we do not see a similar relationship in mothers, who remain ashore between foraging trips for as little as 24 hours and on average only two days (Boyd et al. 1991). Alternatively, pups may be more susceptible to environmental stressors than adult females, with lighter pups requiring more energy to maintain homeostasis under, for example, extreme climatic conditions.

In pups, we also uncovered a significant association between baseline cortisol and sex, with hormone concentrations being moderately higher in males than females. Previous studies of the sex-specific secretion of cortisol have produced diverse results, reporting higher concentrations in males (Egyptian mongoose: Azevedo et al. 2019), females (caribou: Joly and Cameron 2018; marmoset: Garber et al. 2020), or comparable concentrations in both sexes (grizzly bear: Macbeth et al. 2010). These contrasting findings highlight the complexity of interactions between cortisol and the sex hormones, which can vary with the reproductive system, phase, and cycle (Levine 2002). In

addition, the social and environmental stressors associated with growth and reproduction in each sex are likely to vary among species. For example, male Antarctic fur seal pups engage more often in social interactions and risk prone behaviors (Jones et al. 2020) such that the number of "stressful" events encountered may be higher in males than females.

Contrary to our initial expectations, colony only had a marginal, non-significant effect on baseline salivary cortisol in pups and their mothers. This is in contrast to a previous study where cortisol concentrations, measured from hair, were higher in mothers (but not offspring) from the high density colony (Meise et al. 2016). We can think of two non-mutually exclusive explanations for this discrepancy. First, hair cortisol concentrations are thought to reflect events in the recent past (hours or days) (Kalliokoski et al. 2019), whereas salivary cortisol captures circulating hormone levels at the time of sampling (Lewis 2006). Consequently, hair concentrations of baseline cortisol might integrate a larger number of stressful events, allowing density-dependent differences to more readily accumulate. Second, the first study was conducted in 2011 when population densities were much higher at SSB (Forcada and Hoffman 2014), which may have accentuated differences between the two colonies.

Cortisol responsiveness

When measuring baseline cortisol in the wild, there is typically little control over the stimuli that focal individuals experience prior to sampling. By contrast, "capture stress" protocols elicit a repeatable and uniform acute stress response, providing a measure of the activity of the HPA axis that can be compared across individuals,

populations, and time points (Wingfield and Romero 2011). Cortisol responsiveness, measured here as the difference between cortisol concentrations recorded at baseline and after ten minutes of induced handling stress, thus represents an individual's secretory capacity and potential to respond to an immediate stressor.

Given its important role in initiating appropriate physiological and behavioral responses to unpredictable challenges, it has been hypothesized that cortisol responsiveness may be under stronger selection than baseline cortisol (Jenkins et al. 2014; Bairos-Novak et al. 2018). However, our heritability estimates for cortisol responsiveness were of a similar magnitude to those obtained for baseline cortisol, although the associated credible intervals were broader. One factor that may contribute towards this finding is our experimental design. While our ten-minute capture stress protocol did elicit a measurable HPA response, peak cortisol concentrations in pinnipeds may occur anywhere from 30 to 60 minutes after the cessation of stress (Engelhard et al. 2002; Champagne et al. 2012). Consequently, if we could have applied a longer period of capture stress, we might have obtained more accurate estimates of the HPA response. This was not possible in the current study due to both logistic constraints and ethical concerns (Richter and Hintze 2019), but could potentially be investigated in the future.

Although our models did not explain a large proportion of the variation in cortisol responsiveness, they did reveal significant effects of age and days postpartum, which were in the opposite direction for pups and mothers. While responsiveness increased from birth to moult in pups, it decreased over the same period in mothers. For pups, an increase in cortisol responsiveness over time is likely related to the corresponding

decrease in baseline concentrations. Additionally, pups may be more sensitive to HPA axis activation or mount a stronger HPA response as the adrenocortical system develops with age (Walker and Scribner 1991; Engelhard et al. 2002). For mothers, a decrease in cortisol responsiveness over time might be a reflection of acclimation to capture stress. However, mothers were captured only twice during the season, whereas pups were captured on average five times, so we would expect to observe greater habituation in pups than mothers. Alternatively, exposure to chronic stress has been shown to downregulate cortisol responses to an acute stressor (Rich and Romero 2005; DeRango et al. 2019). Maternal provisioning is costly, with offspring survival and growth often being traded off against parental condition (Smiseth et al. 2013). Prolonged energetic investment in offspring care might therefore have depressed the ability of mothers to mount a strong cortisol response to capture and handling stress.

Finally, we also uncovered a significant association between cortisol responsiveness and pup sex, with the magnitude of the stress response being moderately higher in females than males. This may be linked to elevated levels of testosterone in males, which have been shown in other mammals to reduce cortisol responsiveness (e.g. (Lürzel et al. 2011)). For example, artificially increased testosterone levels in female rats resulted in decreased activity of the HPA axis in response to stress (Seale et al. 2005). Consequently, although males have higher baseline levels, cortisol secretion in response to a stressor may be depressed due to the inhibiting effect of testosterone on the HPA axis.

Conclusions

The past decade has witnessed a shift in our understanding of individual differences from a perceived statistical nuisance to a fundamental and compelling aspect of behavioral ecology and evolutionary biology (Bolnick et al. 2003; Trillmich et al. 2018; Krüger et al. 2021). Our study contributes towards this narrative by decomposing individual variation in endocrine phenotypes across different life-history stages and environments in Antarctic fur seal pups and their mothers. Our results are indicative of substantial contextual and developmental plasticity in the HPA axis, which may help to facilitate the match between an individual's phenotype and the environment. However, further research is needed to elucidate both the fitness consequences of endocrine variability and how this may respond to environmental heterogeneity over longer timescales.

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Figure Legends

Figure 1: Location and sampling protocol for the study. (a) Map of Bird Island, South Georgia, a sub-Antarctic island in the southern Atlantic Ocean. The inset shows an enlarged view of the two study colonies from which mother-pup pairs were sampled. Freshwater Beach (FWB, shown in blue) and Special Study Beach (SSB, shown in red) are separated by approximately 200 meters. (b) We employed a fully crossed sampling scheme involving the collection of saliva samples from a total of 100 pairs from the two colonies in two successive breeding seasons, the first of which was coincidentally a year of particularly low food availability. (c) Each focal mother was sampled twice in a season while pups were sampled every 20 days from birth until weaning.

Figure 2: Posterior distributions of heritability (h^2) estimates for baseline cortisol and cortisol responsiveness. A simple pedigree (mother-offspring pairs) for the entire dataset and a custom 85K SNP array for the 96 individuals sampled in the 2019 season were used to calculate the relatedness matrix. The modes and highest posterior density intervals of the posterior distributions are shown in yellow (baseline) and green (responsiveness).

Figure 3: Generalized linear mixed models for pup (a – e) and mother (f – h) baseline cortisol values. Estimates ± 95% confidence intervals for all fixed effects included in the best fit models for pups and mothers are shown in panels a and f, respectively. Significant main effects for both models are shown in panels b – e and panels g – h, respectively.

Boxes show median values ± 75% percentiles with the vertical lines indicating 95% confidence intervals. Further details of the model output can be found in Table 1.

Figure 4: Generalized linear mixed models for pup (a – d) and mother (e – g) cortisol responsiveness. The change in an individuals' cortisol level from baseline (t = 0 minutes) to response (t = 10 ± 2 minutes) sampling is shown for pups and mothers in panels a and e, respectively. Estimates ± 95% confidence intervals for all fixed effects included in the best fit models are respectively shown in panels b and f. Significant main effects are also shown for pups (panels c, d) and mothers (panel g). Boxes show median values ± 75% percentiles, with the vertical lines indicating 95% confidence intervals. Further details of the model output can be found in Table 2.

Tables

Table 1: Parameter estimates from the best fit generalized linear mixed models of (a) pup and (b) maternal baseline cortisol. Random intercepts were included for each individual to account for repeated measures. Estimates together with their 95% confidence intervals (CI) are presented. Significant *p*-values are in bold. The mean squared error (σ^2), between group variance ($\tau 00$), Intraclass Correlation Coefficient (ICC; the consistency within an individual across multiple measurements), the sample size (*n*) and total number of observations, as well as the variance explained by the fixed effects (marginal R^2) and variance explained by both fixed and random effects (conditional R^2) are given.

(a)	**Pup baseline cortisol**		
Fixed effects	Estimates	CI	*p*
(Intercept)	1.58	1.40 – 1.75	**<0.001**
Age	-0.29	-0.44 – -0.14	**<0.001**
Season [2020]	0.25	0.09 – 0.41	**0.003**
Weight	-0.36	-0.52 – -0.20	**<0.001**
Sex [male]	0.24	0.08 – 0.41	**0.004**
Body condition	0.14	-0.02 – 0.31	0.093
Colony [SSB]	0.13	-0.03 – 0.29	0.107
Random effect			
σ^2	0.18		
$\tau_{00\ ID}$	0.05		

ICC	0.39		
n_{ID}	96		
Observations	290		
Marginal R^2 / conditional R^2	0.440 / 0.657		
(b)	**Maternal baseline cortisol**		
Fixed effects	Estimates	CI	*p*
(Intercept)	1.39	1.18 – 1.60	**<0.001**
Days postpartum	-0.66	-0.73 – -0.59	**<0.001**
Season [2020]	0.35	0.11 – 0.59	**0.004**
Colony [SSB]	0.22	-0.02 – 0.47	0.068
Random effect			
σ^2	0.16		
$\tau_{00\ ID}$	0.12		
ICC	0.43		
n_{ID}	92		
Observations	145		
Marginal R^2 / conditional R^2	0.603 / 0.774		

Table 2: Parameter estimates from the best fit generalized additive models of (a) pup and (b) maternal cortisol responsiveness. Random intercepts were included for each individual to account for repeated measures. Estimates together with their 95% confidence intervals (CI) are presented. Significant *p*-values are in bold. The sample size (*n*) and total number of observations, as well as the variance explained by the fixed and random effects in the model (conditional R^2) are given.

(a)	**Pup cortisol responsiveness**		
Fixed effects	Estimates	CI	*p*
(Intercept)	2.91	2.26 – 3.55	**<0.001**
Age	0.88	0.41 – 1.35	**<0.001**
Sex [male]	-1.18	-2.07 – -0.28	**0.010**
Random effect	13.42		**0.032**
n_{ID}	91		
Observations	221		
Conditional R^2	0.080		
(b)	**Maternal cortisol responsiveness**		
Fixed effects	Estimates	CI	*p*
(Intercept)	3.33	2.62 – 4.04	**<0.001**
Days postpartum	-0.96	-1.54 – -0.38	**0.001**
Random effect	0.00		0.660
n_{ID}	71		
Observations	95		

Conditional R^2	0.056

Figures

Figure 1

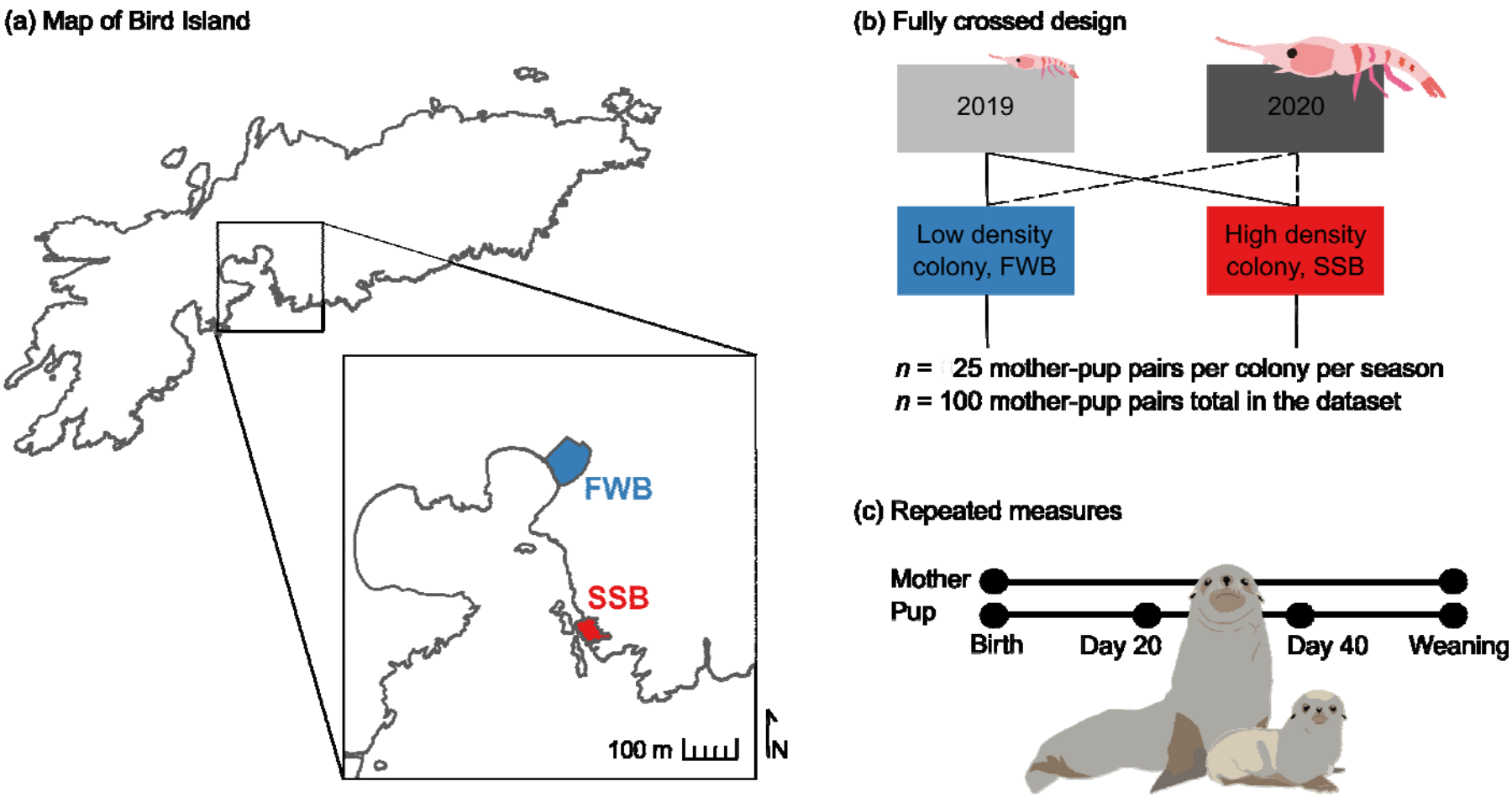


Figure 2

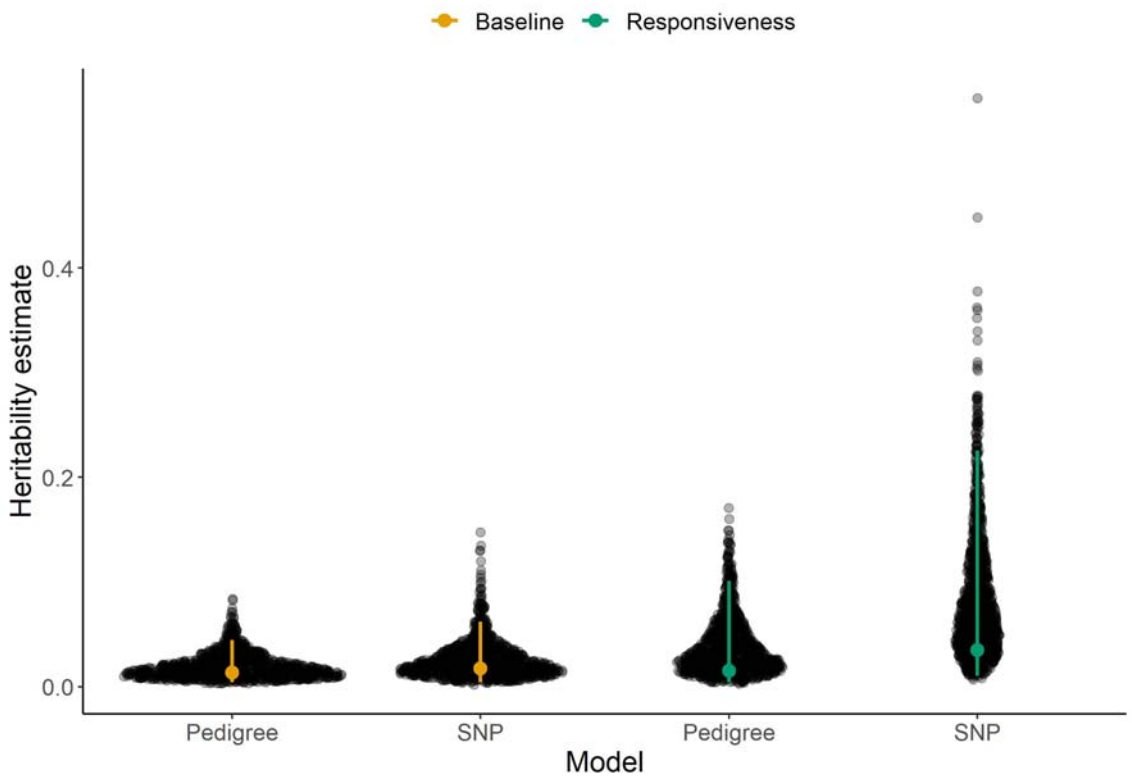

Baseline
Responsiveness
Heritability estimate
0.4
0.2
0.0
Pedigree
SNP
Pedigree
SNP
Model

Figure 3

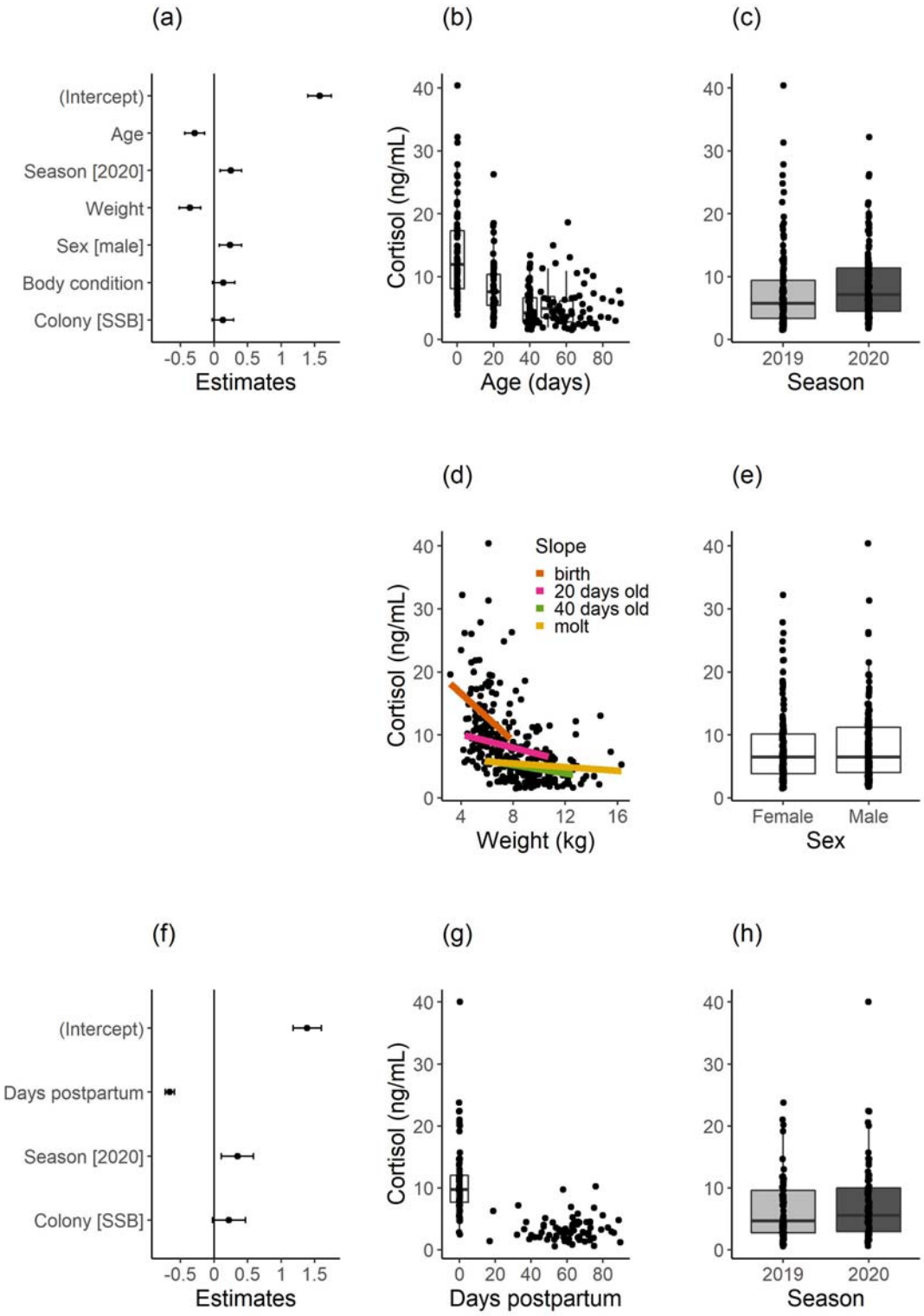
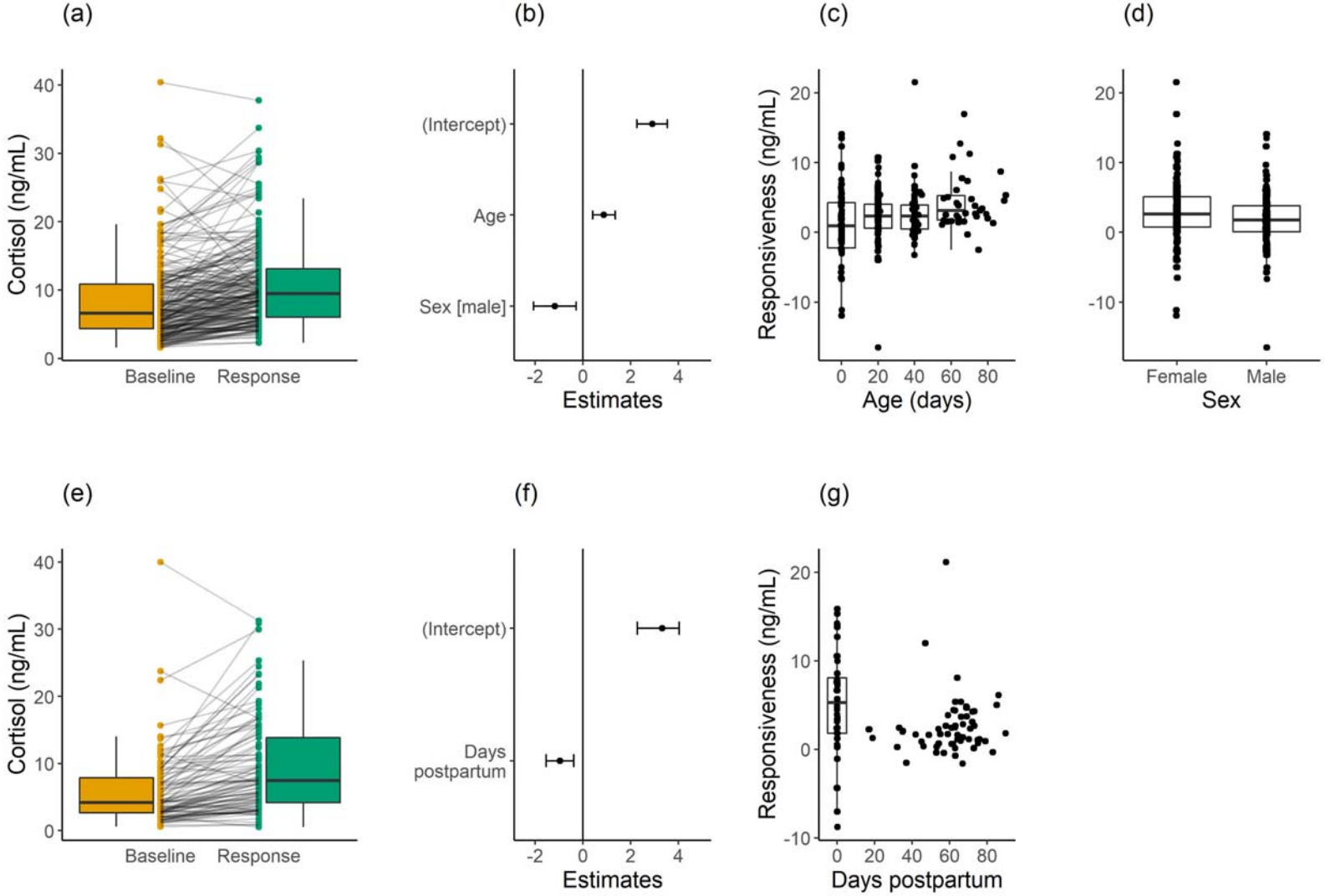

(a)
(Intercept)
Age
Season [2020]
Weight
Sex [male]
Body condition
Colony [SSB]
-0.5 0 0.5 1 1.5
Estimates
(b)
Cortisol (ng/mL)
0 20 40 60 80
Age (days)
(c)
2019 2020
Season
(d)
Slope
birth
20 days old
40 days old
molt
Cortisol (ng/mL)
4 8 12 16
Weight (kg)
(e)
Female Male
Sex
(f)
(Intercept)
Days postpartum
Season [2020]
Colony [SSB]
-0.5 0 0.5 1 1.5
Estimates
(g)
Cortisol (ng/mL)
0 20 40 60 80
Days postpartum
(h)
2019 2020
Season

Figure 4


(a)
Cortisol (ng/mL)
40
30
20
10
0
Baseline
Response
(b)
(Intercept)
Age
Sex [male]
-2
0
2
4
Estimates
(c)
Responsiveness (ng/mL)
20
10
0
-10
0
20
40
60
80
Age (days)
(d)
20
10
0
-10
Female
Male
Sex
(e)
Cortisol (ng/mL)
40
30
20
10
0
Baseline
Response
(f)
(Intercept)
Days postpartum
-2
0
2
4
Estimates
(g)
Responsiveness (ng/mL)
20
10
0
-10
0
20
40
60
80
Days postpartum

Data Availability Statement

Raw data will be uploaded to an open-access data repository upon acceptance of this manuscript. R code is available as a Markdown file and included in the Supplementary Material.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author contributions

RN, CS, CT, and CF-C collected the data. SK advised on field protocols and coordinated cortisol quantification. RN analyzed the data. AJP generated the SNP genotype dataset and DLJV built the genomic relatedness matrix. RN and CC performed the molecular laboratory work. JIH, SK, and JF conceived and developed the project. RN drafted the manuscript. All of the authors commented on and approved the final manuscript.