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Abstract

Phenotypic differences often stem from genetic/maternal differences and/or early-life adaptations to local environmental conditions. In colonial animals, little is known on how variation in the social environment is embedded into individual phenotypes, nor what the consequences are on individual fitness. We conducted an experimental cross-fostering study on king penguins (*Aptenodytes patagonicus*), exchanging eggs among 134 pairs breeding in high-density (67 pairs) or low-density (67 pairs) areas of the same breeding colony. We investigated differences in parent and chick phenotypes and survival in relation to the density of their origin and foster environment. Adults breeding in colony areas of high density exhibited decreased resting behavior and increased aggression and vigilance, increased hypometabolism during incubation fasts, and more moderate CORT responses to chronic stressors (*e.g.*, constant aggression by neighbors). Chick phenotypes were more influenced by the environment in which they were reared than their genetic/maternal origin. Chicks reared in high-density colonial environments showed enhanced weight gain and survival rates regardless of the density of their genetic parents' breeding areas. Our study experimentally shows advantages to breeding in colonial areas of higher breeder densities in king penguins, and highlights the importance of social settings in shaping phenotype expression in colonial seabirds.

Keywords: behavior, early-life, glucocorticoid, king penguin, phenotype, social stress,

Introduction

Phenotypic variability is a key component of adaptation to variable environments. Phenotypic variability can arise from genetic variation (G; the influence of the genotype on the individual's phenotypic value), environmental effects (E), or genotype-by-environment interactions (GxE; environment-dependent expression of genotypes). The ability to match phenotypes to environmental conditions can result in fitness benefits, making individuals better equipped to respond appropriately to environmental stressors (1–3). This is especially the case for social species, where individuals are subject to interactions with, and cues from, conspecifics that can either be amicable or agonistic, making the social environment a potent source of stress. For instance, social interactions have been associated with differences in the secretion of glucocorticoids (often referred to as stress hormones) across a wide range of vertebrates (4,5). Phenotypic plasticity (in its broad morphological, behavioral and physiological dimensions) may be key in modulating adaptive responses to variable social environments, and studies have shown that social phenotypes are plastic and context dependent (6–8). Phenotypes may therefore vary depending

on the nature of the social environment individuals are born and raised in, and experience as adults (4,8,9). However, the ontogeny of phenotypic plasticity in the context of variable social environments is unclear. Specifically, it is often difficult to assess whether the phenotypic differences observed in wild species in their natural context are due to differences in genotypes, the environment, or genotype x environment interactions.

Early-life experiences, encountered in the womb or egg via parental interactions with conspecifics, or encountered by the offspring themselves at an early age when they are nursed (mammals) or fed (birds), are likely critical in determining phenotypic expression. For example, in red squirrels (*Tamiascius hudsonicus*), offspring growth rates are adaptively shaped by maternal cortisol in response to increased social competition experienced by mothers (7). Environmental (including social) stressors experienced by parents can thus be passed on to offspring, affecting their phenotypes (6) through parental care, transmission of maternal hormones, and/or epigenetic inheritance (10). Such intergenerational effects appear to be ubiquitous in nature (reviewed in (11)), however, their adaptive value is context dependent and their relevance to natural systems is still debated (12,13).

Seabirds are ideal models for studying early environmental effects on parental and offspring phenotypes and fitness. Most seabirds breed in large colonies (14), a lifestyle that, besides known reproductive advantages in the form of increased mate availability, reduced predation risks, and social facilitation (for food or mate acquisition), also incurs costs including aggression from territorial conspecifics, the attraction of predators, and the transmission of parasites or diseases (15). Although macro-environmental conditions (such as climate and habitat) may be relatively similar within seabird colonies, these pressures can vary locally between breeding territories. For instance, individuals breeding in central parts of the colony often benefit from reduced predation risk (16), but pay costs related to higher social densities and aggression from territorial conspecifics (17,18), and increased parasitism (19). Territorial conflicts are known to cause individual stress (e.g. as measured by heart increases in response to conflicts, even in bystanders; 21), and individual stress levels should be shaped by trade-offs between the benefits and costs stemming from different environmental pressures due to the heterogeneity of colony structure. This, in turn, may account for the frequently reported phenotypic differences between individuals living in different social environments within the same colony (21–23).

King penguins (*Aptenodytes patagonicus*) are colonial seabirds of the Southern Ocean that aggregate in large colonies of several thousands of pairs during reproduction (24). From November to March, pairs aggressively defend a small breeding territory (25) on which they lay a single egg (26). Both parents cooperate to guard the egg/chick, taking turns guarding ashore or foraging at sea. Spatial heterogeneity in colony density markedly affects adult behavior and physiology: aggression between breeders is high (up to > 100 interactions/hour) and increases with conspecific density in the colony (27). Increased conspecific density is associated with increased levels of baseline corticosterone (CORT) in incubating and brooding birds (28), and increased resting energy expenditure in breeding males (29,30). However, little is known about the determinants of such phenotypic differences, and their consequences in terms of reproductive success. Specifically, more aggressive birds are known to monopolize central breeding areas of higher social density in the colony (31,32), that are thought to be of higher breeding quality (22,25). However, whether individuals in high-density areas are of higher individual quality allowing them to occupy these areas, or whether high-density areas confer higher reproductive success (i.e. offspring survival) despite greater physiological costs for adults, is unknown. Simply put, what are the relative contributions of genetic and environmental factors in shaping offspring phenotype and survival – and therefore adult fitness – in king penguins?

We used a cross-fostering experiment in wild king penguin to test for genetic/maternal vs. early-life environmental influences on offspring phenotypes and fitness. First, we tested for phenotypic differences between adults breeding in colony areas of high (HD) and low (LD) social density. We focused on adult behavioral time budgets, glucocorticoid hormones and metabolic rate. We measured individual baseline corticosterone (CORT) levels, and both CORT and heart rate (HR a proxy of metabolic rate) increase in response to a standardized capture (33–35). Second, we tested whether differences in chick phenotype and survival were associated with breeding at HD or LD. To do so, we swapped the single egg of penguin pairs three days after egg-laying within and between HD and LD locations in the same breeding colony, and tested if the phenotypic differences (growth, metabolic rate, CORT response) between offspring were primarily explained by their environment of origin, or by the environment in which they were raised. If phenotypic differences were explained by genetic/prenatal maternal effects (G), we expected chick phenotypes to be primarily explained by the density of their genetic parents' environment. If phenotypic differences were induced by the rearing environment itself (E), we expected chick phenotypes to be primarily explained by the density of their foster environment.

Furthermore, if phenotypic differences depended on the interaction between both origin and foster environments (GxE), we hypothesized that chicks cross-fostered in similar conditions (HD/HD or LD/LD) should show optimal growth and survival prospects compared to chicks cross-fostered between mismatched environments (HD/LD or LD/HD) as matching phenotypes to environmental conditions should theoretically result in fitness benefits (36).

Materials and Methods

Study site and breeding cycle of King penguins

King penguins were monitored during the 2012-13 and 2013-14 breeding seasons in the "Baie du Marin" colony, Possession Island (46°26′S, 51°52′E), home to ~20,000 pairs (37). We monitored 68 (2012) and 66 (2013) pairs of unknown age, from courtship (November) to the beginning of the Austral winter (~March). Monitored birds were all early breeders that had laid their egg early in the season (before mid-December) (24) as early and late birds have been shown to substantially differ in terms of physiological state and breeding success (38,39). Laying spanned from November 21st to December 3rd in 2012 and from November 19th to December 6th in 2013. Chicks were monitored until fledging (~November-December, the subsequent year).

After ~15 days of courtship, females lay a single egg. Males and females then alternate caring for the egg/chick during incubation/brooding shifts while their partner forages at sea (26). Males are the first to incubate. The egg hatches after ~54 days of incubation. Chicks are brooded on their parents' feet until thermal emancipation (~30 days), then gather in creches while both parents forage at sea. Chicks are fed by both parents and grow over 10–11 months before their first molt, and fledging at sea (November-December) (24).

Cross fostering

We randomly selected breeding pairs shortly before egg-laying in areas of low (LD) and high (HD) breeding density within the same breeding colony. Males and females were marked on the chest using animal spray dye (Porcimark®, Kruuse, Langeskov, Denmark) and fitted temporarily with a plastic flipper band (PVC Darvic flipper bands) when first captured, for subsequent monitoring during the study. All bands were removed at the end of the monitoring period, as long-term banding was reported to have negative effects in this species (see (40)). On incubation day 3, we cross-fostered eggs between pairs

that had laid their egg on the same day, corresponding to 34 and 33 dyads (*i.e*., associated pairs) in 2012 and 2013, respectively. Eggs were swapped between pairs breeding at similar or different social density (2012: HD/HD = 20 pairs, HD/LD = 15, LD/HD = 15, LD/LD = 18; 2013: HD/HD = 16, HD/LD = 16, LD/HD = 16, LD/LD = 18). This design allowed exchanging eggs between areas of the same breeding colony, therefore subjected to rather similar overall environmental conditions (HD and LD areas were located roughly 200 meters apart), but with local differences in breeding territories. In particular, chicks were exchanged between colony areas that differed markedly in their density of breeding pairs (see below), making sure that breeding density was one important source of variation potentially influencing chick phenotype.

Adult monitoring

Adults were captured in the breeding colony on the third day of two successive incubation shifts (incubation shifts 1 and 3 for males and shifts 2 and 4 for females). Birds' heads were covered with hoods to keep them calm during handling, and eggs temporarily replaced with warm dummy eggs to avoid breakage. Beak length, flipper length, and body girth were measured to the nearest millimeter (as described in 38) on the first capture of each adult. The density of breeding birds surrounding each individual was assessed from a distance before each capture as the number of neighbors within the area of the first circle around a focal bird with circle radius taken as the mean distance to neighbors (see **Section S1 Supplementary Materials**)**.** This confirmed the *a priori* characterization of HD breeding locations being 14% more dense than LD areas.

Blood (~2 mL) was collected from a marginal flipper vein using a 2.5-mL heparinized syringe, for males during the third incubation shift (day 3), and for females during the second incubation shift (day 3), ensuring that both sexes had undergone comparable fasting durations. We specifically chose not to sample males during the first incubation shift, since those had already undergone a 15-day fasting period on-land during courtship, and were thus in different nutritional status. Samples were acquired under 5 minutes and after 30 minutes of handling. Samples taken under 5 minutes (mean $\pm$ SE = 2.43 $\pm$ 1.05 minutes) were considered to represent baseline (*i.e*. T_0) corticosterone levels (see (35) and **Supplementary Materials Figure S2 and Table S5** for a validation), while samples at ~30 minutes (31.36 $\pm$ 1.70 minutes) were taken to represent acute corticosterone increases (*i.e*. T_{30}) in response to handling. Samples were kept on ice in the field, and centrifuged (3,000 g for 10 minutes) within 15

minutes of sampling. Plasma and blood cells were immediately frozen separately at -20°C and moved at the end of the day to a -80°C freezer until assayed.

Heart rate (HR) was monitored using external cardio-frequency meters adapted for use in king penguins, as detailed in (29). Individuals were equipped with HR loggers during the second incubation shift (day 3) for females, and the third incubation shift (day 3) for males. HR was recorded every 5 seconds for 4 days. When removing loggers, we measured birds' heart rate response to a standardized capture: the bird was observed resting from a distance for at least 3 minutes. It was then approached at constant speed (following (42)), captured and held for 3 minutes, before removing the logger.

We recorded adult behavior using scan sampling throughout the incubation and chick-brooding periods (mean ± SE = 4.6 ± 1.3 scans/bird; *i.e.,* 2.24 ± 0.62 hours of observation/bird). Scans were performed throughout the day from 8 am to 6 pm to capture daily behavioral variation. Scans consisted in 30 minutes of observation of the monitored birds from a distance of at least 10 meters outside the colony using binoculars, and the behavior of each individual was recorded every 2 minutes. We recorded comfort, aggressive, parental care, vigilance and resting behaviors (full ethogram in **Supplementary Materials Table S6**).

Chick monitoring

Chicks were captured at day 10, 35, 105 after hatching and fledging (end of first molt soon before fledging). At 10 days, chicks were identified using color-coded tags (Floy Tag and MFG, Seattle, USA) attached subcutaneously to their upper-back (39). At each stage, flipper, beak, and tarsus length were measured (to the nearest millimeter), and chicks were weighed (to the nearest 5 grams) using a spring-slide Pesola scale. At fledging, two blood samples (~1.5mL each) were taken (under 5 and after 30 minutes). At 105 days, two blood samples were also taken and chicks were equipped with HR recorders for 4 days (mean ± SE = 3.64 ± 0.14 days, sampling every 5 seconds). Loggers were removed following the same de-equipment protocol as adults (see above). Missing or dead chicks were recorded throughout the monitoring period, and dates were noted as the last day chicks were sighted alive or confirmed dead in the colony. Chick survival throughout the monitoring period was then used as a proxy of reproductive success of parents.

Corticosterone analyses

Plasma total corticosterone (CORT) concentrations were measured using an MP Biomedicals RIA kit (MP Biomedicals Cat. No. 07120103) as described in (43). Samples were run in duplicates, including control samples with low and high CORT values in every assay (coefficient of variation of 5.5% and 3.7%, respectively). A test for parallelism insured the dilution series paralleled the standard curve (**Supplementary Materials Fig S2).** The free hormone hypothesis states that only free CORT is active and can bind to glucocorticoid receptors to trigger cellular signaling pathways and modifications in gene transcription and cell functioning (44). Because about 90% of blood glucocorticoids are bound to corticosterone binding globulin (CBG) in the vast majority of vertebrates (43,45,46), we also chose to estimate free CORT concentrations (*i.e*. not bound by CBG) by measuring the maximum corticosterone binding capacity (MCBC) of individual plasma samples using a 96-well microdialysis plate (HTDialysis, Gales Ferry, CT, USA) as described in (45). We calculated the binding coefficient needed for the calculation of MCBC as 20.0 nM (95% CI 19.0 – 21.0) (the mean K_d of three runs at 37°C).

The measurement of MCBC not only provides us with relevant information on the biologically active fraction of CORT (the free CORT) and level of activation of the Hypothalamic – Pituitary Adrenal (HPA) axis in response to breeding density, it further allows us to assess the organism's capacity to buffer elevated levels of circulating CORT by binding to corticosterone binding globulin (CBG). Such information is functionally important to understand how organisms faced with chronic stressors (e.g., social aggression) can maintain physiologically acceptable levels of active CORT in the organism.

Statistical analysis

Statistical analyses were performed using R (v.3.5.3). Generalized Linear (GLMM) and Linear (LMM) mixed models were run using the "lme4" package (47). Multinomial Logistic Regression were run with the "mlogit" package (48). As the experiment was carried out in two consecutive breeding seasons (2012 and 2013), Year (2019/2022) was introduced as a cofactor in all models to account for interannual variability. When relevant, Bird ID and experimenter ID were introduced as random factors to account for repeated measures and experimenter effect. When explaining trivial amounts of variance, random factors (typically individual ID and handler ID) were removed from the models to allow convergence. Results are presented as means ± SE unless otherwise indicated. Effects with a $p < 0.05$ were considered statistically significant, although we also discuss results with regards to effect sizes as recommended for ecological data (49). Model estimates are given with HD (for HD vs LD comparisons)

and 2012 (for year comparisons) as reference categories, unless stated otherwise. When relevant (*i.e.*, in the case of significant interaction term), *post-hoc* contrasts were between groups were assessed using the *emmeans* package (50). When needed (*i.e.*, multiple comparisons) p-value were adjusted using Tukey adjustment method.

Adult Size and Body Condition

Adults' body size was estimated using flipper length (cm) (41). Adults body condition was defined as the residuals of a linear regression $Body\ girth \sim Body\ size$ (LMM, slope = +0.73$\pm$0.23, t = 6.43, $p < 0.001$ (see validation in (41)). Adult size and body condition were similar for adults breeding at HD and LD (**Table S3 & S4 Supplementary materials**).

Adult Behavior

Single behaviors (see ethogram **Table S6 Supplementary materials**) were grouped as aggression (10.8%), comfort (18.0%), chick or egg-care (1.8%), vigilance (9.9%), resting (56.9%) and other behaviors (2.6%, *i.e.,* rarely observed behavior as sleeping, calling, exploration), and summed over 30 minutes scans (N = 1178 scans).

First, we compared the time budget allocated to resting by parents at HD vs LD. The proportion of time spent resting relative to other behavior ($\frac{N_{Resting}}{N_{Total} - N_{Resting}}$) was specified as the dependent variable in a GLMM (binomial distribution), breeding density (HD or LD) as the independent variable, and year and breeding stage specified as covariates to control for differences in time budgets between breeding stages (51), and potential interannual effects on adult behavior.

Second, we analyzed behavioral tradeoffs using a Multinomial Logistic Regression to simultaneously analyze the 6 behavioral categories (52). The multinomial response outcomes (dependent variable) included aggression, comfort, egg/chick-care, vigilance and other behaviors. We tested if adult behaviors changed in proportion relative to resting (set as reference category) at HD *vs.* LD. Results are given as odds ratios with 95% confidence intervals. Odds ratios significantly > 1 (or < 1) (*i.e.,* confidence intervals not overlapping 1) indicate the odds of increasing (or decreasing) the behavior of interest relative to resting for a transition from LD to HD.

Adult and chick stress responses and metabolic rate

Individual baseline stress level and response to stress were estimated via plasma CORT levels (both total and free CORT), and bird HR increase during approach and handling. We used Linear Mixed Models (LMMs) to test for the effect of sampling time (T_0 and T_{30} for hormones, and times of $T_{baseline}$ and T_{peak} HR levels) to describe baseline CORT and HR levels, and responses to acute captures.

Birds' daily and resting metabolic rate were assessed *via* HR over 4 days. Mean resting HR and daily HR were calculated over "day" and "night" periods based on sunrise and sunset times over the study (R package "Suncalc" (53)). Resting HR was calculated using moving averages to identify the 10 consecutive minutes of lowest HR over the recording periods (30). As not all birds were recorded for 4 days exactly (3.68 ± 0.04 days), and because "day" and "night" periods varied during the study, we controlled for recording duration as a covariate in the LMMs.

Chick growth and growth trajectories

Chick growth was analyzed through temporal increase in structural size, and body weight. Structural size was computed as the first component of a Principal Component Analysis comprising flipper, tarsus and beak lengths for all chicks at the four stages of development (10 days, 35 days, 105 days and fledging) (41,54). PC1 explained 96% of the total variance in the data and increased with increasing body size, with

$$PC1 \sim 0.97 * beak\ length + 0.99 * left\ flipper\ length\ +\ 0.99 * right\ flipper\ length + 0.98 * right\ tarsus\ length\ +\ 0.98 * left\ tarsus\ length$$

Structural size and body weight were then analyzed in LMMs with Origin and Fostering breeding density, Stage (10, 35, 105 days and fledging) specified as independent variables. To establish the differences in either size and body weight at each stage, interaction terms (Stage x Origin x Fostering) were originally introduced, but removed if not significant. Then, the slopes of the linear phase of the growth (10 to 105 days; (39)) for both body mass and body size were extracted for each individual chick and analyzed as growth trajectories. The individual slopes were included in LMs with Origin and Fostering breeding density, Year and initial body mass or size (at 10 days) as independent variables.

Chick survival

Chick survival was analyzed using COX Proportional hazards models (R package "survival" (55)). We modelled how chick survival was influenced by Origin (HD/LD) and Fostering (HD/LD) breeding

densities. Time was assessed in days since laying (therefore hatching ~54 days). For each chick, 0 indicated that the chick was alive, and 1 indicated a death event. The date of last observation was used as the time of event. Year was included as a cofactor in the model accounting for interannual differences in survival probabilities. Hazard ratios indicating mortality risk were computed (decreased mortality risk <1, increased mortality risk >1).

We used separate GLMs (binomial distribution) to assess chick survival probability (0/1) at hatching, 105 days and fledging with Origin and Fostering breeding density and year as independent factors. This investigated the probability of chicks surviving to a specific stage, *if* they had survived the preceding one. Further, in order to fine-tune our survival analyses, we focused on the winter period. For king penguin chicks, the winter period following their first summer of growth constitutes a strong energy bottleneck (24,56,57) during which mortality is high (i.e. over 30%; (24,39); see Results). We tested if chick survival probability (0/1) over this critical period was influenced by the breeding density of Origin and Fostering social environments (independent variables), as well as chick body mass, structural size and baseline CORT levels at the entry of winter (105 days). This allowed us to test whether chicks of differing body condition and stress levels benefitted differently from HD or LD environments during the winter.

Results

Adult behavior, stress and metabolic rate

Adults spent 26% more time resting at LD than HD (GLMM binomial; odds ratio = 1.26±0.20, z = 2.61 and p = 0.009; **Fig. 1.A, Supplementary Materials Table S7**). Compared to breeders at LD, adults at HD increased time allocated to comfort, aggression, and vigilance behavior, but not to egg or chick-care, relative to resting (Multinomial regression; **Fig. 1.B**).

Baseline (T_0) levels of both total (N = 220) and free CORT (N = 120) were not different for birds at HD and LD (t = -0.62, p = 0.538; and t = -0.19, p = 0.99, for total and free CORT respectively; **Fig. 2.A**). Adult total CORT significantly increased (by 39.47±1.33 ng/mL) over a 30 minutes handling (t = 29.75, and p < 0.001), and this increase was similar for HD and LD birds (+247% in HD and +257% in LD; interaction Density x Time: $F_{1,214.4}$ = 0.30 and p = 0.58; **Fig. 2.A**). In contrast, increase in free CORT between T_0 and T_{30} was significantly less pronounced in adults at HD (+4.6±1.91 ng/mL) than LD (+11.0 ± 2.13 ng/mL) (Density x Time: $F_{1,75.7}$ = 5.14 and p = 0.026). This difference stemmed from higher levels (+6.8 ± 2.0 ng/mL) of free CORT at T_{30} for birds at LD compared to HD (t = -3.39 and p = 0.001) while

free CORT levels at T_0 were not different (t = -0.19 and p = 0.850). The lower MCBC levels (-54.2 ± 20.9 nM, t = 2.59 and p = 0.012), for birds at LD both at T_0 and T_{30} (**Fig. 2.B**) may explain why LD birds had a less buffered free CORT response to handling compared to HD birds. MCBC levels did not change significantly from T_0 to T_{30} for both groups (Time: t = 1.36 and p = 0.177) (**Supplementary Materials Table S7**).

At peak stress, heart rate (N = 206) increased by 70.7 ± 2.5 bpm (+95.3%) due to handling compared to baseline levels (t = 28.38 and p < 0.001; **Fig. 2.C**). This stress-induced increase in HR tended to be more pronounced, but not significantly so, in birds at LD compared to birds at HD (+104% for LD, +95% for HD, Density x Time, $F_{1,103.8}$ = 5.31 and p = 0.076) with peak HR levels being higher for LD birds (145.8± 2.1 bpm) compared to HD (139.3± 1.9 bpm) (+4.7%, -6.5 ± 2.8 bpm, t = -2.28 and p = 0.023) while baseline (pre-stress) levels were not different in HD and LD birds (t = -0.01, p = 0.992) **Supplementary Materials Table S7**).

Daily resting HR (N = 206) was not different between HD (72.1 ± 0.9 bpm) and LD (70.8 ± 0.9 bpm) birds at the start of the monitoring period (day 3; t = 1.02 and p = 0.307). Resting HR subsequently decreased by -2.33 ± 0.14 bpm/day as the incubation shift progressed (t = -16.28 and p < 0.001), and this decrease was significantly more pronounced in adults at HD (Density x Days fasting: $F_{1,1320.8}$ = 7.21 and p = 0.007; HD = -2.55 bpm/day vs. LD = -1.78 bpm/day) (**Supplementary Materials Table S7**).

Chick growth trajectories

Chicks reared at LD tended to gain less body mass during their linear growth phase (10 days to 105 days) than chicks reared at HD (70.7 ± 3.0 g/day *vs.* 66.2 ± 3.0 g/day, t = -1.77 and p = 0.080).

Even though this difference in growth rate was not significant, it resulted in significant differences in body mass at later stages of growth (Origin x Fostering x Stage: $F_{3,240.8}$ = 3.71 and p = 0.012). Especially, at day 105, LD/HD chicks were significantly heavier than chicks in the other three groups (HD/HD: Δ = 680 ± 253 g, t = 2.68, p = 0.041; HD/LD: Δ = 767 ± 270 g, t = 2.84, p = 0.025; and LD/LD: Δ = 673 ± 250 g, t = 2.69, p = 0.038). At fledging, LD/HD chicks were significantly heavier than LD/LD chicks only (Δ = 1439 ± 428 g, t = 3.36 and p = 0.005) (**Fig. 3.A, Table S8**), and LD/LD chicks were also significantly less heavy than HD/LD chicks (Δ = -1338 ± 514, t = -2.61 and p = 0.047).

Structural size (PC1) was not influenced by the origin of the chick at any stage (t = 0.87 and p = 0.384) and was not different for chicks fostered at LD or HD at 10 and 35 days (t = -0.49 and p = 0.625

and t = -0.83 and p = 0.410 respectively) (**Fig 3.B, Table S8**). However, chicks fostered in LD tended to show reduced structural size at later stages of growth, significantly at 105 days (-0.14 $\pm$ 0.07, t = 1.99 and p = 0.048), but no longer significantly at fledging (-0.17 $\pm$ 0.11, t = 1.56 and p = 0.121). These changes in structural size during the different developmental stages of chicks foster in LD explain the significant interaction term Fostering x Stage ($F_{3,217.8}$ = 3.30 and p = 0.021).

Chick baseline stress levels, and acute stress responses

Both baseline (T_0) and stress-induced (T_{30}) levels of total CORT at fledging (N = 71) were not influenced by either origin or fostering densities (Origin: t = 0.81 and p = 0.42, Fostering: t = 0.47 and p = 0.64) (**Fig. 4.A, Table S9**). Chick total CORT (N = 50) increased significantly (by 49.1 $\pm$ 4.7 ng/mL) in response to handling stress regardless of their origin and fostering environments (t = 10.38 and p < 0.001, **Fig. 4.A**). Similarly, free CORT levels increased due to handling (by 8.6 $\pm$ 2.8 ng/mL, t = 3.13 and p = 0.003). Finally, MCBC levels (N = 49, **Fig. 4.B**) were significantly higher in chicks fostered in LD compared to HD (Δ = +203.0 $\pm$64.4 nM, t = 3.15 and p = 0.019), and significantly decreased from T_0 to T_{30} in chicks reared at LD only (LD: Δ = -141.2 $\pm$ 48.1, t = -2.93 and p = 0.032, and HD: Δ = +23.6 $\pm$ 61.6, t = 0.38 and p = 0.980).

HR (at 105 days) increased significantly (by +160 $\pm$ 4 bpm) as a response to handling stress (t = 42.22 and p < 0.001), and this increase in HR was similar regardless of the origin and fostering social environment (similar HR both before and at peak of stress for all chick groups, 0.343 < p < 0.999) (**Fig 4.C, Table S9**).

Egg and chick survival

Chick death hazard over the monitoring period nearly doubled (odds ratio = 1.99$\pm$0.21) for chicks fostered at LD *vs*. HD (Cox model; Fostering: z = 3.36, p < 0.001, **Fig 5.A**). However, chick survival was not significantly influenced by the area of origin (HD or LD) (Origin: odds ratio = 0.82$\pm$0.20, z = -0.99 and p = 0.32). Investigating survival at different stages revealed that, eggs originating from LD showed higher hatching probability (+12%, **Table 1**), while the fostering environment did not influence hatching probability (**Table 1**). Survival probability from hatching to the beginning of winter (105 days) was not explained by either the origin or fostering environment (**Table 1**). However, the winter period constituted

392 a critical survival bottleneck with only 30.2% survival. Winter survival in LD was 25% of the survival in
393 HD (GLM: odd ratio = 0.25±0.16, z = -2.82 and p = 0.005).

394 We assessed whether chick survival through winter could be explained by chick body mass,
395 structural size and/or levels of total CORT before winter (105 days). When accounting for all three
396 variables (N = 76) we found no evidence for an effect of either structural size or total CORT (PC1: z =
397 0.04 and p = 0.969, Total CORT: z = -0.24 and p = 0.810). However, chicks with increased body mass
398 at 105 days had increased survival probability over the winter (z = 2.31 and p = 0.021). In the model
399 accounting for all three variables, neither origin nor fostering densities significantly affected chick
400 survival (z = -0.90 and p = 0.368, z = -0.76 and p = 0.449 respectively). When including chicks without
401 CORT measurements (N = 116, **Fig. 5.B, Table S8**) we found a positive effect of chick body mass at
402 105 days on surviving the first winter (z = 2.80 and p = 0.005), and foster chicks reared at HD having a
403 significantly higher survival probability through the winter than foster chicks reared at LD (32% in LD
404 and 54% in HD, z = 2.29 and p = 0.022).

405

406 **Discussion**

407 For animals breeding in densely packed colonies, such as seabirds, the social density
408 experienced by individuals can either increase their stress, e.g. due to frequent aggressive interactions
409 with conspecifics or increased risk of exposure to parasites, or reduce it, e.g. by reducing predation risk.
410 Ultimately, these changes in exposure to stressors can impact both adult and chick phenotypes and
411 breeding success. Accordingly, our study shows that adult king penguins breeding at HD showed
412 reduced resting behavior and increased aggression and vigilance, more pronounced hypometabolism
413 (higher daily decline in resting HR), and lower free corticosterone (but not HR) increases in response to
414 capture stress compared to adults breeding at LD. Reduced resting time and increased vigilance,
415 comfort and aggression are known to increase energy expenditure in king penguins (27,58), likely
416 decreasing the fasting capacities of adults breeding at HD. The sharper decrease in metabolic rate
417 during incubation shifts and lower responsiveness to acute stressors (attenuated free CORT response),
418 could then be viewed as an adaptation geared towards energy savings. Lower CORT increases resulting
419 from higher buffering effects of corticosterone binding globulin (higher MCBC), and possibly more
420 efficient negative feedback loops, would indeed prevent an overactivation of stress responses and

mobilization of stored energy reserves (see also (34,59)) for adults subject to chronic social aggression by neighbors at HD during incubation and chick-rearing (51). Our study also shows that chick phenotypes were foremost influenced by the environment in which they were reared than their genetic/maternal origin. Chicks reared in high-density colonial environments showed enhanced weight gain and increased survival rates, especially during the winter period which constitutes a strong energy bottleneck (60). Body mass and survival benefits were strongest for chicks originating from low-density and reared in high-density. However, these differences in chick growth and survival were not reflected in measures of stress physiology.

Whether the phenotypic differences observed between adults or chicks at HD *vs*. LD results from (i) breeding territories differing in quality, (ii) from the aggregation of parents of different phenotypes selecting different breeding areas in the colony, or (iii) from a mix of both may be hard to disentangle. For instance, aggregating in high breeding densities may increase protection against detrimental weather and predators, positively affecting chick survival (61). At the same time, more competitive individuals that are better equipped to withstand the energetic demands of defending a territory in an area subject to high competition, may be expected to remain in these areas, while less competitive phenotypes may be expected to relocate to an environment better suited to their phenotype, *i.e.* the "matching habitat choice" hypothesis (62). For instance, in king penguins, more aggressive phenotypes are known to adorn larger auricular feather patches ("badges of status") (32), and individuals with larger patches occupy more central areas in the breeding colony (31), suggesting habitat selection by specific phenotypes. Following individuals over multiple seasons, and in different behavioral contexts, may help understand if a correlation exists between individual characteristics, territory location (31) and reproductive success. Specifically, the higher aggression rates of breeders observed at HD may be part of a general coping style (*i.e.* behavioral changes that result from the integrated effect of individual's specific external/internal stressors,(63,64)) correlating with lower HPA activity, bolder behavior, better foraging capacities, and increased vigilance and protection of chicks. Aggression and boldness often covary in animals (65), and boldness has been associated with foraging behavior and foraging site fidelity in other seabird species (66). Thus, it is conceivable that differences in foraging and provisioning strategies between individuals in HD and LD exist, in turn causing differences in offspring mass gain and survival in these environments (see below).

We found that hatching probability was more influenced by the origin of the egg than by the foster environment in which it was incubated. Eggs originating from LD had significantly higher hatching probabilities than eggs originating from HD. This indicates that genetic/maternal effects were more important than the quality of incubation in determining hatching success and offspring survival at very early stages of development. Once the egg had hatched however, chicks reared at HD had increased survival probabilities to fledging, regardless of their genetic/maternal (HD or LD) origin, highlighting a greater importance of parental/environment effects over genetic/maternal effects. One important consideration is whether differential offspring survival between HD and LD environments may have been confounded by differential costs of raising male or female offspring. Unfortunately, we did not sex chicks from HD and LD environments in this study. However, given that no evidence for a systematic sex-ratio bias as a function of colony density over a 6-year period has been found previously (67), it is unlikely that our results can be explained by the sex of the chicks.

Chick survival through winter, while gathering in large “crèche” (see below), was significantly explained by chick body mass at the pre-winter stage (integrating the influence of both foster parents and social environment from hatching to 105 days). As chicks rely entirely on their parents for food until fledging, but are seldom fed by during winter (56), mass gain before winter is critical to cope with the energetic demands of prolonged fasting and thermoregulation (56). While chicks in HD/LD gained body mass at a similar rate compared to chicks in HD/HD and LD/HD, they exhibited significantly lower survival probabilities through winter. This suggests that parents in LD may have allocated more energy towards their own survival rather than towards chick provisioning during the winter period. Better food provisioning from parents at HD, even marginal, would result in increased over-winter survival probabilities in chicks reared in those areas, regardless of their genetic background. Also, chicks reared at HD tended to be slightly taller, making them both less easy targets for predators known to target the smallest chicks (61), and more conspicuous/competitive when adults return to the colony overwinter to feed them (including allofeeding from unrelated parents, (68)). Whereas king penguin parents are known to resort to different chick-provisioning strategies over winter (from little to regular chick-provisioning), with direct consequences on reproductive success (69), nothing is known as to how adult foraging/provisioning strategies differ in relation to HD/LD breeding environments. As provisioning strategies are thought to be plastic responses to fluctuating environmental and intrinsic (individual) conditions, studies focusing on parental foraging behavior using GPS or GLS loggers and 3-D

accelerometers would provide comprehensive insights into the mechanisms underlying differences in parental behavior and chick survival, which may differ depending on breeding environments.

Phenotypic differences related to the HPA (stress) axis were found between HD and LD-reared chicks at fledging for their MCBC response to handling stress, but not for measures of total or free CORT. Baselines MCBC levels were generally higher in LD chicks, and more importantly declined significantly with handling stress in LD, but not HD chicks. This may translate two different mechanisms. First, the chicks able to survive until fledging in LD might be those with elevated baseline MCBC levels (survivor effect). The steep decrease in MCBC levels in response to acute (handling) stress might be a mechanism for releasing active CORT in the blood stream enabling the mobilization of energy substrates, for example, in the case of predation, but this was not detected in the free CORT response to handling in our study. Chicks able to adequately respond to environmental stressors might have been better at surviving in LD, while this selection might have been less intense in HD where chicks benefitted from lower predation risk in a denser social environment (selfish herd effect;(70,71)). Second, as for adults, lower free CORT increases (though marginal) in HD may buffer negative effects of chronically elevated CORT due to higher adult aggression in denser environments.

We found no clear evidence for genotype-by-environment interactions (GxE) shaping chick phenotypes and survival in our study. Phenotypic matching (here for HD/HD and LD/LD chicks) resulting in increased survival prospects would be expected if offspring phenotypic traits were predictively shaped to conform to their rearing environment (36). Rather, we found that chick phenotype depended mostly on the rearing environment, with those rearing effects becoming gradually more important as chicks aged, while genetic/maternal effects remained negligible throughout development up to fledging. There are strong energetical constraints on parents and their chicks during early rearing (from hatching to fledging) leading to strong selection pressures (adult aggression, land-based predation, over-winter-related mortality) on chick survival. Hence it is not surprising that influences of the fostering environment are of greater importance up to fledging. It would be interesting to test for the relative effects of environmental vs. genetic/maternal effects on later performances (e.g. post-fledging traits and survival), especially since return rates of juvenile king penguins to their natal colony after fledging have been shown to depend on pre-fledging traits such as body condition (54).

Whereas LD and HD rearing environments differed primarily in terms of breeding density (being 14% higher in HD than LD), it is likely that other factors varying locally between LD and HD (related or not to social density) may have influenced adult and chick phenotype and survival. For instance, the increased survival of the chicks in HD compared LD from 35 days (emancipation from rearing parents) to fledging (see **Fig 6**) might have been explained by reduced predation risk at HD owing to selfish herd effects and/or reduced adult aggression as chicks clustered into larger "crèches" (70,71). Similarly, chicks aggregating in denser "crèches" may have benefitted from increased protection against harsh climate during the winter period (71). Other factors such as local variation in parasite prevalence, or differences in microclimate (e.g., exposure to wind), might also have influenced chick survival by diverting energy resources from the parents and/or chicks, though these remain to be thoroughly investigated. Our current data suggests that tick loads (*Ixodes uriae*) are higher in LD areas of the colony (Bize et al., *unpublished data*), which may also explain differences in survival between HD and LD areas. The prevalence of ticks on breeding adults has been suggested to affect breeding success (72), though no difference was previously found between tick infested and non-infested areas – and the reproductive costs of parasitism may only manifest during years of particularly high parasite prevalence (72). Regardless of the exact nature of differences between HD and LD areas, marked differences were evident for adults breeding at HD and LD, as were differences in chick phenotype and survival.

Overall, the experimental cross-fostering design allowed highlighting predominant roles of the early-life environment (E) compared to genetic/maternal (G) background in shaping offspring phenotype and survival in king penguins. Breeding in areas of the colony of higher social density, likely to covary with lower predation risk, lower exposure to parasites, and higher social aggression conferred clear survival benefits for offspring, independently of their genetic/maternal background. Future work on the at-sea foraging capabilities and food-provisioning strategies of adults breeding at HD or LD, together with a characterization of on-land breeding performances should provide further insights into the importance of parental *vs.* territory quality in determining chick phenotype and survival in colonial seabirds.

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729 **Tables**

		odds ratio	*95% CI*	*Statistic*	*p-value*
Hatching probability	Intercept	7.80	3.01 - 24.49	3.89	**< 0.001**
	Origin [LD]	3.29	1.14 - 10.98	2.10	**0.036**
	Fostering [LD]	0.41	0.13 - 1.15	-1.64	0.100
	Year [2013]	0.74	0.26 - 2.07	-0.57	0.566
Survival at 105 days	Intercept	2.67	1.25 - 6.07	2.46	**0.014**
	Origin [LD]	1.15	0.51 - 2.57	0.34	0.736
	Fostering [LD]	0.92	0.41 - 2.05	-0.21	0.832
	Year [2013]	0.70	0.31 - 1.56	-0.87	0.386
Survival at fledging	Intercept	1.93	0.81 - 4.86	1.45	0.148
	Origin [LD]	1.17	0.45 - 3.13	0.32	0.746
	Fostering [LD]	0.25	0.09 - 0.64	-2.82	**0.005**
	Year [2013]	0.41	0.15 - 1.06	-1.81	0.071

730

731 **Table 1:** Output of Generalized Linear Models for the survival of chicks at 3 stages: Hatching, 105 days
732 (beginning of winter) and fledging. Models were run separately. Origin and Fostering environment were
733 included as explanatory factors and Year and a co-factor to account for inter-annual variability in survival
734 probability. For Origin and Fostering, odds ratio and 95% Confidence Interval (CI) are given for the ratio
735 $\frac{Survival_{LD}}{Survival_{HD}}$, odds ratio > 1 (< 1) indicate that LD chicks had higher (lower) survival probability. For Year, odds
736 ratio and 95%CI are given for the ratio $\frac{Survival_{2013}}{Survival_{2012}}$.

Figures

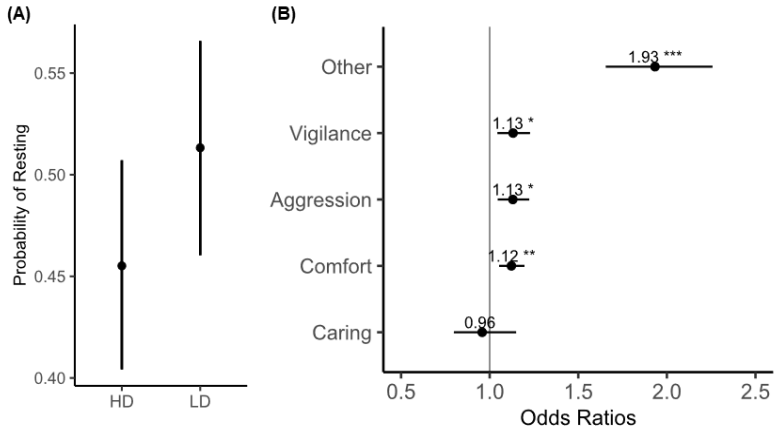
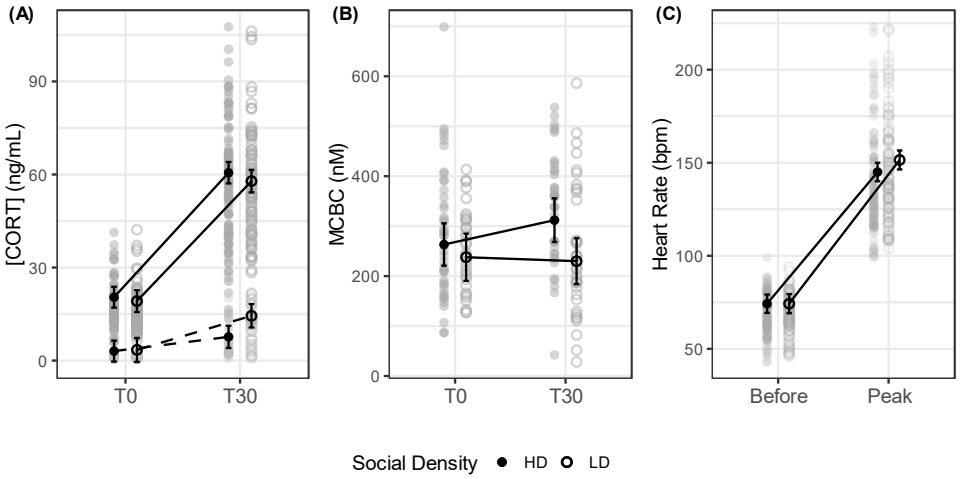


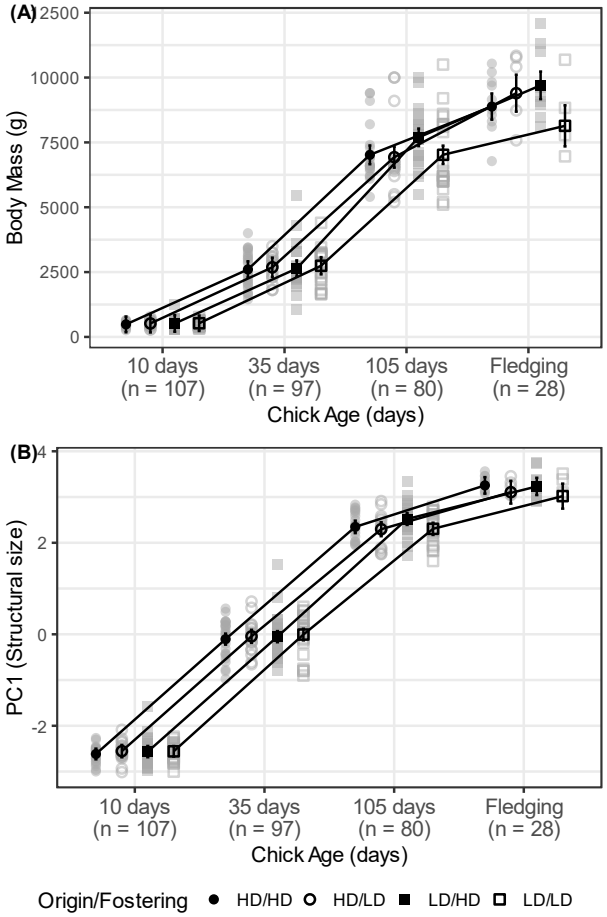
Figure 1. Time-budget allocated to 6 behavioral categories in adult king penguins breeding at high (HD) and low (LD) density. (A) Probabilities of resting for adults breeding at HD and LD obtained from the fitted LMM. (B) Odds ratios relative to resting (reference category) at LD compared to HD (Multinomial Logistic Regression model, significance: * P < 0.05, ** P < 0.01, *** P < 0.001). HD = high density and LD = low density.



744

745 **Figure 2.** Physiological phenotypes of adults breeding at high (HD) and low (LD) density. **(A)** Baseline (T_0) and
746 stress-induced (T_{30}) levels of Total (solid lines) and Free corticosterone [CORT] (dashed lines). **(B)** Maximum
747 corticosterone binding capacity (MCBC) at baseline (T_0) and at stress-induced (T_{30}) levels **(C)** Baseline heart rate
748 (HR, bpm) measured before the beginning of an approach (Before) and maximal HR reached during the approach
749 (Peak). For all panels, marginal means and confidence intervals computed from LMMs are presented (black and
750 white dots), along with raw data in grey. HD = high density and LD = low density.

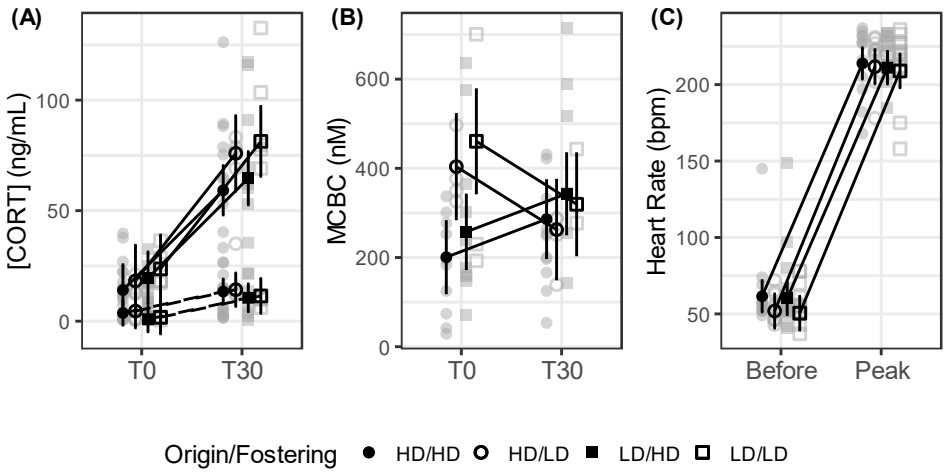
751



752

753 **Figure 3.** Changes in chick **(A)** body mass (g) and **(B)** structural size (PC1, see methods) from 10 days to fledging
754 according to the breeding densities (high = HD; low = LD) of their origin and fostering environments. Marginal means
755 and 95% confidence intervals computed from LMMs are presented in black. Raw data is presented in grey. The
756 sample size for each age is given (*n*). Point shapes indicate the environment of origin (circle: HD or square: LD),
757 filled shapes indicate chicks reared at HD and open shapes at LD. HD = high density and LD = low density, and
758 groups are specified as Origin/Fostering environment.

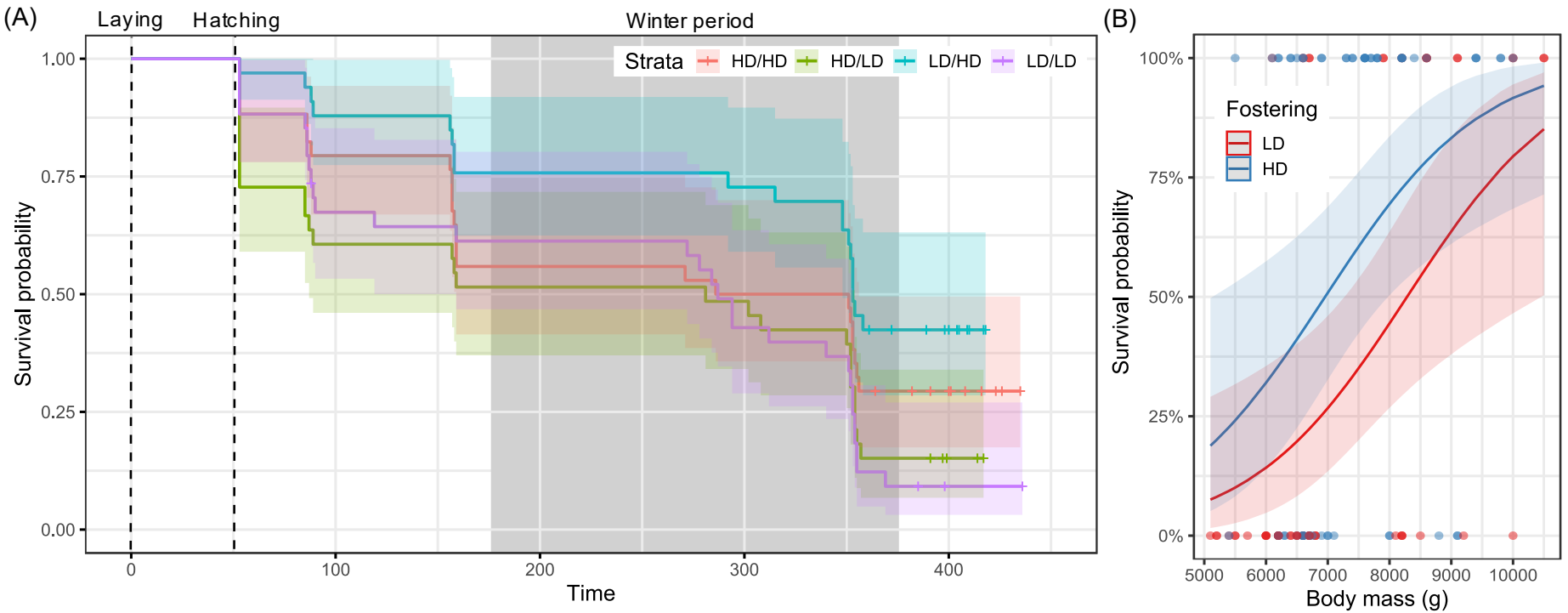
759



760

761 **Figure 4. (A)** Chick baseline (T_0) and stress-induced (T_{30}) levels of Total (plain lines) and Free corticosterone
762 [CORT] (dashed lines) at **fledging**. **(B)** Maximum corticosterone binding capacity (MCBC) at baseline (T_0) and at
763 stress-induced (T_{30}) levels at fledging. **(C)** Heart Rate (bpm) measured during the de-equipment of chicks **at 105**
764 **days** before the beginning of approach (Before) and at the first peak of stress during the approach (Peak). For all
765 panels, marginal means and confidence intervals computed from LMMs are presented (black and white dots), along
766 with raw data in grey. HD = high density and LD = low density, and groups are specified as Origin/Fostering
767 environment.

768



769

770 **Figure 5. (A)** Survival curves of chicks from laying (day 0) to fledging (>400 days) from a COX hazard model for the four group of chicks (Strata = HD/HD, HD/LD, LD/LD, LD/HD).

771 The cross-shaped dots correspond to missing chicks (their status alive/dead was unknown). Filled areas correspond to 95% confidence intervals computed from the COX hazard

772 model. **(B)** Results from GLM model for survival probability of chicks through winter (105 days to fledging). Model accounting for chick body mass and structural size (N = 116)

773 as explanatory variables, with Origin and Fostering, and Year as co-factors to account for interannual variability in the data. HD = high density and LD = low density, and groups

774 are specified as Origin/Fostering environment.

775