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1 Social, not genetic, programming of development and stress physiology
2 of a colonial seabird

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22 **Author Contributions:**

23 C. Lemonnier analyzed the data and wrote the paper with the supervision of P. Bize, F. Criscuolo and
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25 P. Bize & V.A. Viblanc designed the study.

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27 B. Delehanty, R. Boonstra, and Q. Schull ran the laboratory analyses.

28 All authors contributed critically to the drafts and gave final approval for publication.

29

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31

32

33 **Abstract**

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

49 **Keywords:** behavior, early-life, glucocorticoid, king penguin, phenotype, social stress,
50

51 **Introduction**

52 Phenotypic variability is a key component of adaptation to variable environments. Phenotypic variability
53 can arise from genetic variation (G; the influence of the genotype on the individual's phenotypic value),
54 environmental effects (E), or genotype-by-environment interactions (GxE; environment-dependent
55 expression of genotypes). The ability to match phenotypes to environmental conditions can result in
56 fitness benefits, making individuals better equipped to respond appropriately to environmental stressors
57 (1–3). This is especially the case for social species, where individuals are subject to interactions with,
58 and cues from, conspecifics that can either be amicable or agonistic, making the social environment a
59 potent source of stress. For instance, social interactions have been associated with differences in the
60 secretion of glucocorticoids (often referred to as stress hormones) across a wide range of vertebrates
61 (4,5). Phenotypic plasticity (in its broad morphological, behavioral and physiological dimensions) may
62 be key in modulating adaptive responses to variable social environments, and studies have shown that
63 social phenotypes are plastic and context dependent (6–8). Phenotypes may therefore vary depending

64 on the nature of the social environment individuals are born and raised in, and experience as adults
65 (4,8,9). However, the ontogeny of phenotypic plasticity in the context of variable social environments is
66 unclear. Specifically, it is often difficult to assess whether the phenotypic differences observed in wild
67 species in their natural context are due to differences in genotypes, the environment, or genotype x
68 environment interactions.

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

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

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

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

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

127

128 **Materials and Methods**

129 **Study site and breeding cycle of King penguins**

130 King penguins were monitored during the 2012-13 and 2013-14 breeding seasons in the “Baie
131 du Marin” colony, Possession Island (46°26’S, 51°52’E), home to ~20,000 pairs (37). We monitored 68
132 (2012) and 66 (2013) pairs of unknown age, from courtship (November) to the beginning of the Austral
133 winter (~March). Monitored birds were all early breeders that had laid their egg early in the season
134 (before mid-December) (24) as early and late birds have been shown to substantially differ in terms of
135 physiological state and breeding success (38,39). Laying spanned from November 21st to December 3rd
136 in 2012 and from November 19th to December 6th in 2013. Chicks were monitored until fledging
137 (~November-December, the subsequent year).

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

144

145 **Cross fostering**

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

152 that had laid their egg on the same day, corresponding to 34 and 33 dyads (*i.e.*, associated pairs) in
153 2012 and 2013, respectively. Eggs were swapped between pairs breeding at similar or different social
154 density (2012: HD/HD = 20 pairs, HD/LD = 15, LD/HD = 15, LD/LD = 18; 2013: HD/HD = 16, HD/LD =
155 16, LD/HD = 16, LD/LD = 18). This design allowed exchanging eggs between areas of the same
156 breeding colony, therefore subjected to rather similar overall environmental conditions (HD and LD areas
157 were located roughly 200 meters apart), but with local differences in breeding territories. In particular,
158 chicks were exchanged between colony areas that differed markedly in their density of breeding pairs
159 (see below), making sure that breeding density was one important source of variation potentially
160 influencing chick phenotype.

161

162 **Adult monitoring**

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

172 Blood (~2 mL) was collected from a marginal flipper vein using a 2.5-mL heparinized syringe,
173 for males during the third incubation shift (day 3), and for females during the second incubation shift
174 (day 3), ensuring that both sexes had undergone comparable fasting durations. We specifically chose
175 not to sample males during the first incubation shift, since those had already undergone a 15-day fasting
176 period on-land during courtship, and were thus in different nutritional status. Samples were acquired
177 under 5 minutes and after 30 minutes of handling. Samples taken under 5 minutes (mean \pm SE = 2.43
178 \pm 1.05 minutes) were considered to represent baseline (*i.e.* T₀) corticosterone levels (see (35) and
179 **Supplementary Materials Figure S2 and Table S5** for a validation), while samples at ~30 minutes
180 (31.36 \pm 1.70 minutes) were taken to represent acute corticosterone increases (*i.e.* T₃₀) in response to
181 handling. Samples were kept on ice in the field, and centrifuged (3,000 g for 10 minutes) within 15

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

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

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

197

198 **Chick monitoring**

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

210

211 **Corticosterone analyses**

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

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

230

231 **Statistical analysis**

232 Statistical analyses were performed using R (v.3.5.3). Generalized Linear (GLMM) and Linear
233 (LMM) mixed models were run using the “lme4” package (47). Multinomial Logistic Regression were run
234 with the “mlogit” package (48). As the experiment was carried out in two consecutive breeding seasons
235 (2012 and 2013), Year (2019/2022) was introduced as a cofactor in all models to account for interannual
236 variability. When relevant, Bird ID and experimenter ID were introduced as random factors to account
237 for repeated measures and experimenter effect. When explaining trivial amounts of variance, random
238 factors (typically individual ID and handler ID) were removed from the models to allow convergence.
239 Results are presented as means \pm SE unless otherwise indicated. Effects with a $p < 0.05$ were
240 considered statistically significant, although we also discuss results with regards to effect sizes as
241 recommended for ecological data (49). Model estimates are given with HD (for HD vs LD comparisons)

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

246

247 ***Adult Size and Body Condition***

248 Adults' body size was estimated using flipper length (cm) (41). Adults body condition was defined
249 as the residuals of a linear regression *Body girth*~*Body size* (LMM, slope = +0.73±0.23, t = 6.43, p <
250 0.001 (see validation in (41)). Adult size and body condition were similar for adults breeding at HD and
251 LD (**Table S3 & S4 Supplementary materials**).

252

253 ***Adult Behavior***

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

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

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

270

271 ***Adult and chick stress responses and metabolic rate***

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

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

282

283 ***Chick growth and growth trajectories***

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

$$289 \quad PC1 \sim 0.97 * \text{beak length} + 0.99 * \text{left flipper length} + 0.99 * \text{right flipper length} + 0.98 * \\ 290 \quad \text{right tarsus length} + 0.98 * \text{left tarsus length}$$

291 Structural size and body weight were then analyzed in LMMs with Origin and Fostering breeding density,
292 Stage (10, 35, 105 days and fledging) specified as independent variables. To establish the differences
293 in either size and body weight at each stage, interaction terms (Stage x Origin x Fostering) were
294 originally introduced, but removed if not significant. Then, the slopes of the linear phase of the growth
295 (10 to 105 days; (39)) for both body mass and body size were extracted for each individual chick and
296 analyzed as growth trajectories. The individual slopes were included in LMs with Origin and Fostering
297 breeding density, Year and initial body mass or size (at 10 days) as independent variables.

298

299 ***Chick survival***

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

302 densities. Time was assessed in days since laying (therefore hatching ~54 days). For each chick, 0
303 indicated that the chick was alive, and 1 indicated a death event. The date of last observation was used
304 as the time of event. Year was included as a cofactor in the model accounting for interannual differences
305 in survival probabilities. Hazard ratios indicating mortality risk were computed (decreased mortality risk
306 <1, increased mortality risk >1).

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

318

319 **Results**

320 ***Adult behavior, stress and metabolic rate***

321 Adults spent 26% more time resting at LD than HD (GLMM binomial; odds ratio = 1.26 ± 0.20 , z
322 = 2.61 and $p = 0.009$; **Fig. 1.A, Supplementary Materials Table S7**). Compared to breeders at LD,
323 adults at HD increased time allocated to comfort, aggression, and vigilance behavior, but not to egg or
324 chick-care, relative to resting (Multinomial regression; **Fig. 1.B**).

325 Baseline (T_0) levels of both total ($N = 220$) and free CORT ($N = 120$) were not different for birds
326 at HD and LD ($t = -0.62$, $p = 0.538$; and $t = -0.19$, $p = 0.99$, for total and free CORT respectively; **Fig.**
327 **2.A**). Adult total CORT significantly increased (by 39.47 ± 1.33 ng/mL) over a 30 minutes handling ($t =$
328 29.75 , and $p < 0.001$), and this increase was similar for HD and LD birds (+247% in HD and +257% in
329 LD; interaction Density x Time: $F_{1,214.4} = 0.30$ and $p = 0.58$; **Fig. 2.A**). In contrast, increase in free CORT
330 between T_0 and T_{30} was significantly less pronounced in adults at HD ($+4.6 \pm 1.91$ ng/mL) than LD ($+11.0$
331 ± 2.13 ng/mL) (Density x Time: $F_{1,75.7} = 5.14$ and $p = 0.026$). This difference stemmed from higher levels
332 ($+6.8 \pm 2.0$ ng/mL) of free CORT at T_{30} for birds at LD compared to HD ($t = -3.39$ and $p = 0.001$) while

333 free CORT levels at T_0 were not different ($t = -0.19$ and $p = 0.850$). The lower MCBC levels ($-54.2 \pm$
334 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
335 had a less buffered free CORT response to handling compared to HD birds. MCBC levels did not change
336 significantly from T_0 to T_{30} for both groups (Time: $t = 1.36$ and $p = 0.177$) (**Supplementary Materials**
337 **Table S7**).

338 At peak stress, heart rate ($N = 206$) increased by 70.7 ± 2.5 bpm (+95.3%) due to handling
339 compared to baseline levels ($t = 28.38$ and $p < 0.001$; **Fig. 2.C**). This stress-induced increase in HR
340 tended to be more pronounced, but not significantly so, in birds at LD compared to birds at HD (+104%
341 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
342 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
343 $= 0.023$) while baseline (pre-stress) levels were not different in HD and LD birds ($t = -0.01$, $p = 0.992$)
344 **Supplementary Materials Table S7**).

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

350

351 ***Chick growth trajectories***

352 Chicks reared at LD tended to gain less body mass during their linear growth phase (10 days to
353 105 days) than chicks reared at HD (70.7 ± 3.0 g/day vs. 66.2 ± 3.0 g/day, $t = -1.77$ and $p = 0.080$).

354 Even though this difference in growth rate was not significant, it resulted in significant
355 differences in body mass at later stages of growth (Origin x Fostering x Stage: $F_{3,240.8} = 3.71$ and $p =$
356 0.012). Especially, at day 105, LD/HD chicks were significantly heavier than chicks in the other three
357 groups (HD/HD: $\Delta = 680 \pm 253$ g, $t = 2.68$, $p = 0.041$; HD/LD: $\Delta = 767 \pm 270$ g, $t = 2.84$, $p = 0.025$; and
358 LD/LD: $\Delta = 673 \pm 250$ g, $t = 2.69$, $p = 0.038$). At fledging, LD/HD chicks were significantly heavier than
359 LD/LD chicks only ($\Delta = 1439 \pm 428$ g, $t = 3.36$ and $p = 0.005$) (**Fig. 3.A, Table S8**), and LD/LD chicks
360 were also significantly less heavy than HD/LD chicks ($\Delta = -1338 \pm 514$, $t = -2.61$ and $p = 0.047$).

361 Structural size (PC1) was not influenced by the origin of the chick at any stage ($t = 0.87$ and p
362 $= 0.384$) and was not different for chicks fostered at LD or HD at 10 and 35 days ($t = -0.49$ and $p = 0.625$

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

368

369 ***Chick baseline stress levels, and acute stress responses***

370 Both baseline (T_0) and stress-induced (T_{30}) levels of total CORT at fledging ($N = 71$) were not
371 influenced by either origin or fostering densities (Origin: $t = 0.81$ and $p = 0.42$, Fostering: $t = 0.47$ and p
372 $= 0.64$) (**Fig. 4.A, Table S9**). Chick total CORT ($N = 50$) increased significantly (by 49.1 ± 4.7 ng/mL) in
373 response to handling stress regardless of their origin and fostering environments ($t = 10.38$ and $p <$
374 0.001 , **Fig. 4.A**). Similarly, free CORT levels increased due to handling (by 8.6 ± 2.8 ng/mL, $t = 3.13$
375 and $p = 0.003$). Finally, MCBC levels ($N = 49$, **Fig. 4.B**) were significantly higher in chicks fostered in
376 LD compared to HD ($\Delta = +203.0 \pm 64.4$ nM, $t = 3.15$ and $p = 0.019$), and significantly decreased from T_0
377 to T_{30} in chicks reared at LD only (LD: $\Delta = -141.2 \pm 48.1$, $t = -2.93$ and $p = 0.032$, and HD: $\Delta = +23.6 \pm$
378 61.6 , $t = 0.38$ and $p = 0.980$).

379 HR (at 105 days) increased significantly (by $+160 \pm 4$ bpm) as a response to handling stress (t
380 $= 42.22$ and $p < 0.001$), and this increase in HR was similar regardless of the origin and fostering social
381 environment (similar HR both before and at peak of stress for all chick groups, $0.343 < p < 0.999$) (**Fig**
382 **4.C, Table S9**).

383

384 ***Egg and chick survival***

385 Chick death hazard over the monitoring period nearly doubled (odds ratio = 1.99 ± 0.21) for chicks
386 fostered at LD vs. HD (Cox model; Fostering: $z = 3.36$, $p < 0.001$, **Fig 5.A**). However, chick survival was
387 not significantly influenced by the area of origin (HD or LD) (Origin: odds ratio = 0.82 ± 0.20 , $z = -0.99$
388 and $p = 0.32$). Investigating survival at different stages revealed that, eggs originating from LD showed
389 higher hatching probability (+12%, **Table 1**), while the fostering environment did not influence hatching
390 probability (**Table 1**). Survival probability from hatching to the beginning of winter (105 days) was not
391 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

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

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

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

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

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

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

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

508 Whereas LD and HD rearing environments differed primarily in terms of breeding density (being
509 14% higher in HD than LD), it is likely that other factors varying locally between LD and HD (related or
510 not to social density) may have influenced adult and chick phenotype and survival. For instance, the
511 increased survival of the chicks in HD compared LD from 35 days (emancipation from rearing parents)
512 to fledging (see **Fig 6**) might have been explained by reduced predation risk at HD owing to selfish herd
513 effects and/or reduced adult aggression as chicks clustered into larger “crèches” (70,71). Similarly,
514 chicks aggregating in denser “crèches” may have benefitted from increased protection against harsh
515 climate during the winter period (71). Other factors such as local variation in parasite prevalence, or
516 differences in microclimate (e.g., exposure to wind), might also have influenced chick survival by
517 diverting energy resources from the parents and/or chicks, though these remain to be thoroughly
518 investigated. Our current data suggests that tick loads (*Ixodes uriae*) are higher in LD areas of the colony
519 (Bize et al., *unpublished data*), which may also explain differences in survival between HD and LD areas.
520 The prevalence of ticks on breeding adults has been suggested to affect breeding success (72), though
521 no difference was previously found between tick infested and non-infested areas – and the reproductive
522 costs of parasitism may only manifest during years of particularly high parasite prevalence (72).
523 Regardless of the exact nature of differences between HD and LD areas, marked differences were
524 evident for adults breeding at HD and LD, as were differences in chick phenotype and survival.

525 Overall, the experimental cross-fostering design allowed highlighting predominant roles of the
526 early-life environment (E) compared to genetic/maternal (G) background in shaping offspring phenotype
527 and survival in king penguins. Breeding in areas of the colony of higher social density, likely to covary
528 with lower predation risk, lower exposure to parasites, and higher social aggression conferred clear
529 survival benefits for offspring, independently of their genetic/maternal background. Future work on the
530 at-sea foraging capabilities and food-provisioning strategies of adults breeding at HD or LD, together
531 with a characterization of on-land breeding performances should provide further insights into the
532 importance of parental vs. territory quality in determining chick phenotype and survival in colonial
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534

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| | | <i>odds ratio</i> | <i>95% CI</i> | <i>Statistic</i> | <i>p-value</i> |
|-----------------------------|----------------|-------------------|---------------|------------------|-------------------|
| 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 |

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731 **Table 1:** Output of Generalized Linear Models for the survival of chicks at 3 stages: Hatching, 105 days

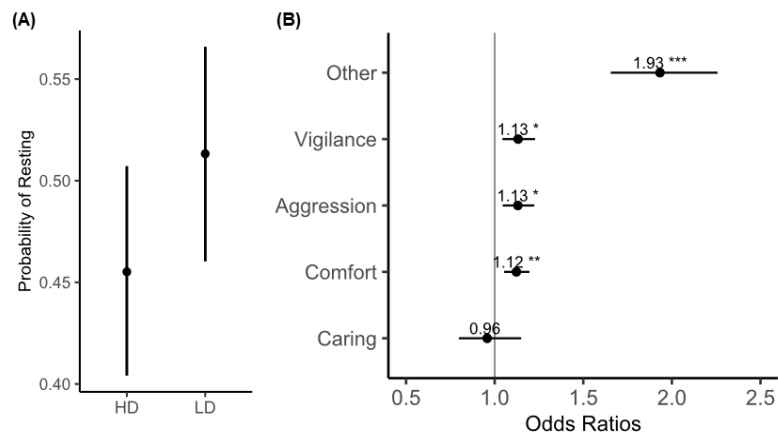
732 (beginning of winter) and fledging. Models were run separately. Origin and Fostering environment were

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, odds736 ratio and 95%CI are given for the ratio $\frac{Survival_{2013}}{Survival_{2012}}$.

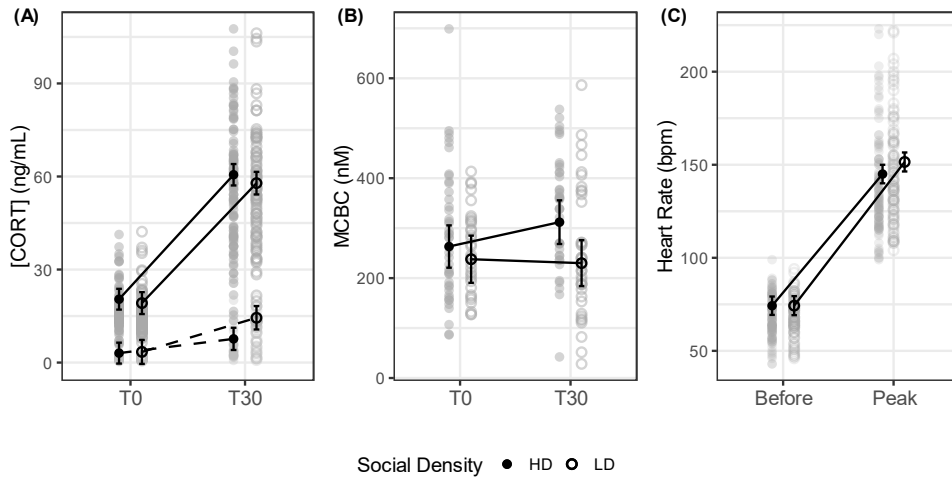
737 **Figures**



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

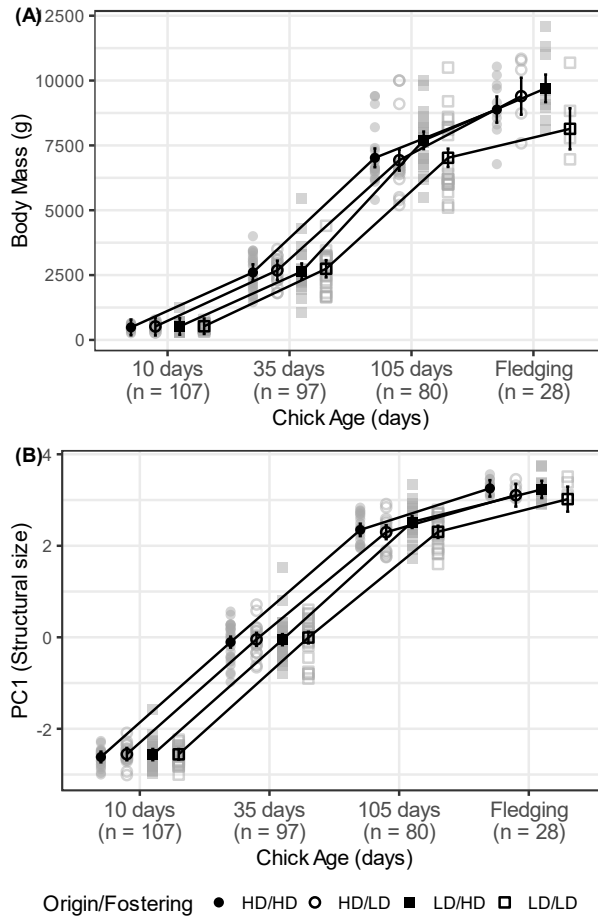
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745 **Figure 2.** Physiological phenotypes of adults breeding at high (HD) and low (LD) density. **(A)** Baseline (T_0) and
 746 stress-induced (T_{30}) levels of Total (solid lines) and Free corticosterone [CORT] (dashed lines). **(B)** Maximum
 747 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.

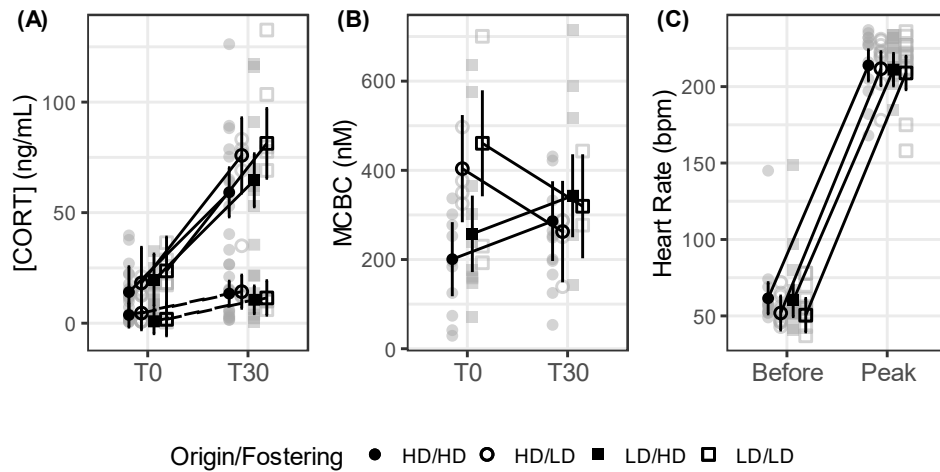
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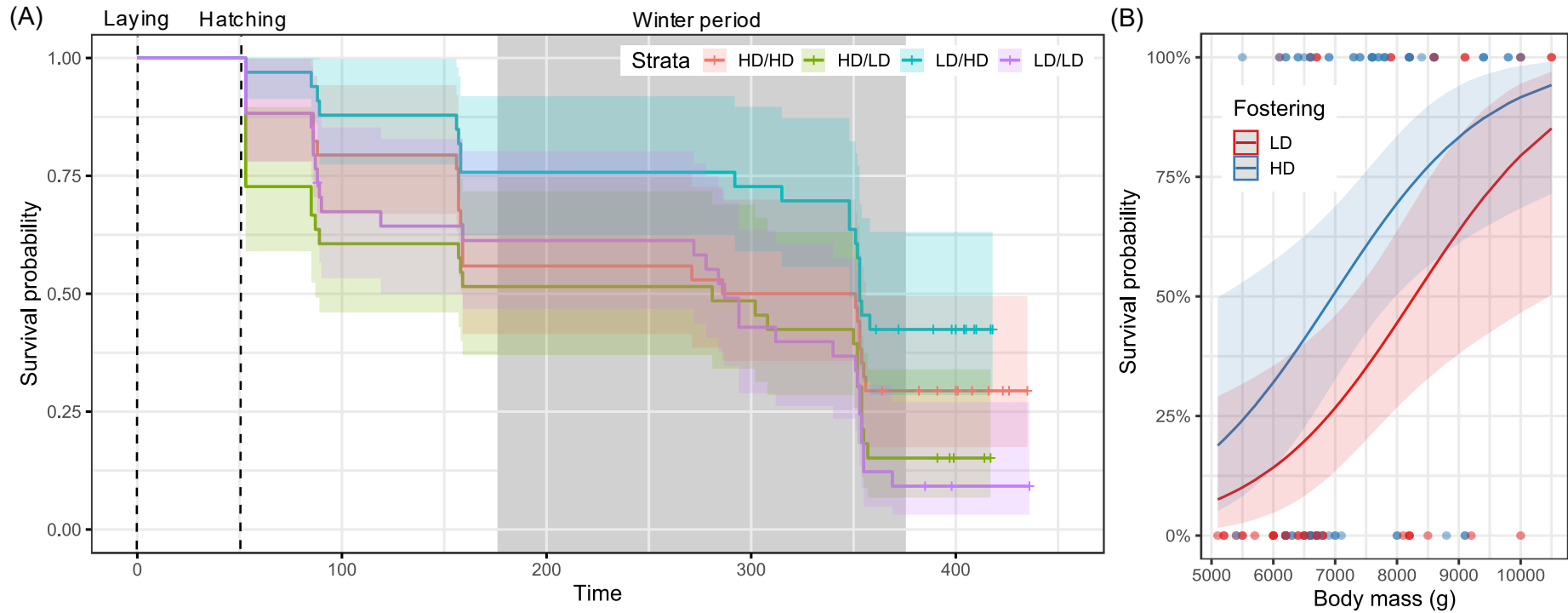
753 **Figure 3.** Changes in chick (A) body mass (g) and (B) structural size (PC1, see methods) from 10 days to fledging
 754 according to the breeding densities (high = HD; low = LD) of their origin and fostering environments. Marginal means
 755 and 95% confidence intervals computed from LMMs are presented 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.

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



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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.

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