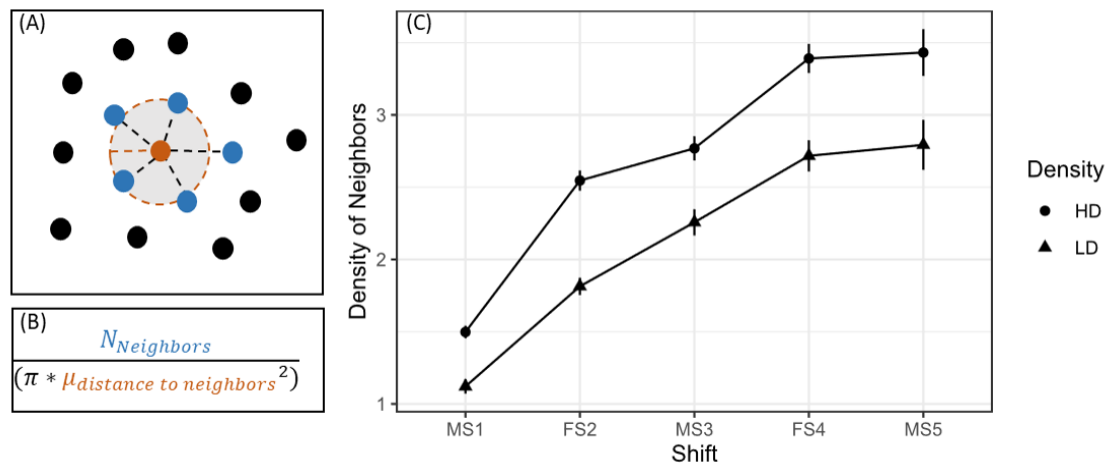


## Supplementary Material for

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

#### S1. Breeding environment



**Figure S1.** Characterization of breeding densities in king penguins (*Aptenodytes patagonicus*) (A) Representation of field methodology for counting neighbors and evaluating distances around focal birds (see Methods). (B) Formula for the computation of Neighbor Density index. (C) Changes in neighbor density (neighbor density index) throughout incubation and early chick brooding at high density (HD) and low density (LD) respectively (mean $\pm$ se), MS = Male Shift (the male is incubating the egg/guarding the chick), FS = Female Shift, and the number represent the number of shifts since the egg was laid. HD = high density and LD = low density.

<i>Predictors</i>	<b>Neighbor Density</b>		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>
(Intercept)	-1341.61	-1887.19 – -796.03	<0.001
Density [LD]	-0.57	-0.71 – -0.43	<0.001
Shift [FS2]	0.94	0.77 – 1.10	<0.001
Shift [MS3]	1.26	1.09 – 1.43	<0.001
Shift [FS4]	1.72	1.55 – 1.89	<0.001
Shift [MS5]	1.78	1.57 – 1.98	<0.001
Year	0.67	0.40 – 0.94	<0.001
<b>Random Effects</b>			
$\sigma^2$	0.94		
$\tau_{00}$ Pair_ID	0.10		
$\tau_{00}$ Experimenter	0.08		
ICC	0.16		
N Experimenter	4		
N Pair_ID	134		
Observations	1549		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.323 / 0.431		

**Table S1:** Linear Mixed Model output for Neighbor Density. Density and Shift were introduced as explanatory variable, and Year as a cofactor to account for interannual variability in the data. Pair ID was introduced as a random factor to account for repeated measurements on breeding pairs (the 2 partners of one pair experiencing similar social conditions), experimenter ID was introduced as a random factor to account for heterogeneity in measurements.

**Wilcoxon test**

HD vs LD	HD		LD		W	p
	mean	se	mean	se		
<b>Sun</b>	0.72	0.02	0.77	0.02	371987	0.183
<b>Wind</b>	0.81	0.01	0.87	0.01	<b>380405</b>	<b>0.002</b>
<b>Rain</b>	0.23	0.02	0.23	0.02	357699	0.874
<b>Temperature</b>	7.41	0.13	7.55	0.14	345212	0.719

**Table S2:** Mean and se of the scoring values for climate parameters (Sun, Wind, Rain) and Temperature in HD and LD areas of the colony. Wilcoxon non-parametric tests were used to compare the mean values for each parameter in both groups (HD and LD). Sun, Wind and Rain are score data (0-4, 0 = No sun/rain/wind and 4 = Blue sky/strong continuous wind/heavy rain) noted before each capture and each behavioral scan (N = 1728). Outside temperature was recorded with a thermometer. HD = high density and LD = low density.

**Text S1.** The social environment was assessed from a distance before each capture. Specifically, we assessed the number of neighbors (in blue) and the distance to neighbors (black dashed lines) within the first circle of neighbors (grey area) around focal birds (**Fig. S1A**). Neighbor density was then calculated 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 (red dashed-line) (**Fig. S1B**). Neighbor density was tested in a linear mixed model (LMM) with social density (HD or LD) and breeding shift as explanatory variables, year as a covariate, and pair and observer IDs as random factors (to account for the non-independence of birds within breeding pairs, and potential observer effects) (**Table S2**). Neighbor density increased with the advancing breeding season, as the colony filled with early, then late breeders: from Male Shift 1 (MS1, mid-November) to Female Shift 2 (FS2, beginning of December), the number of neighbors increased by mean  $\pm$  SE =  $0.94 \pm 0.08$  ( $t = 11.24$  and  $p < 0.001$ ). Breeder density in HD averaged  $0.57 \pm 0.07$  more neighbors per unit area – *i.e.*, 14% more – than adults in LD over the entire monitoring period (incubation and early chick brooding,  $t = 7.75$  and  $p < 0.001$ ), confirming the *a priori* characterization of the breeding locations as HD and LD (**Fig. S1C**). Overall, HD and LD areas did not differ in terms of exposure to sun, rain and temperature. However, LD area was significantly more exposed to wind, probably due to the topology of the area (**Table S3**).

## S2. Adults size and body condition

<b>Mean flipper length (cm)</b>				
<i>Predictors</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
(Intercept)	321.83	320.26 – 323.41	401.10	<b>&lt;0.001</b>
Density [LD]	-0.39	-1.99 – 1.21	-0.48	0.634
Sexe [M]	7.19	5.59 – 8.79	8.82	<b>&lt;0.001</b>
Year [2013]	-1.72	-3.32 – -0.12	-2.11	<b>0.035</b>
Observations	511			
R <sup>2</sup> / R <sup>2</sup> adjusted	0.140 / 0.135			

**Table S3:** Linear Mixed Model output for Flipper Length of adults. Density and Sex were introduced as explanatory factors, and Year as a cofactor to account for interannual variability in the data. Bird ID was first introduced as a random factor to account for repeated measurements on birds, but subsequently removed to allow convergence.

<b>Body Condition</b>				
<i>Predictors</i>	<i>Estimates</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
(Intercept)	3.81	-0.09 – 7.70	1.92	0.056
Density [LD]	2.21	-1.87 – 6.28	1.06	0.288
Sexe [M]	4.15	0.00 – 8.30	1.97	<b>0.050</b>
Year [2013]	-15.91	-20.08 – -11.74	-7.49	<b>&lt;0.001</b>
Observations	444			
R <sup>2</sup> / R <sup>2</sup> adjusted	0.132 / 0.126			

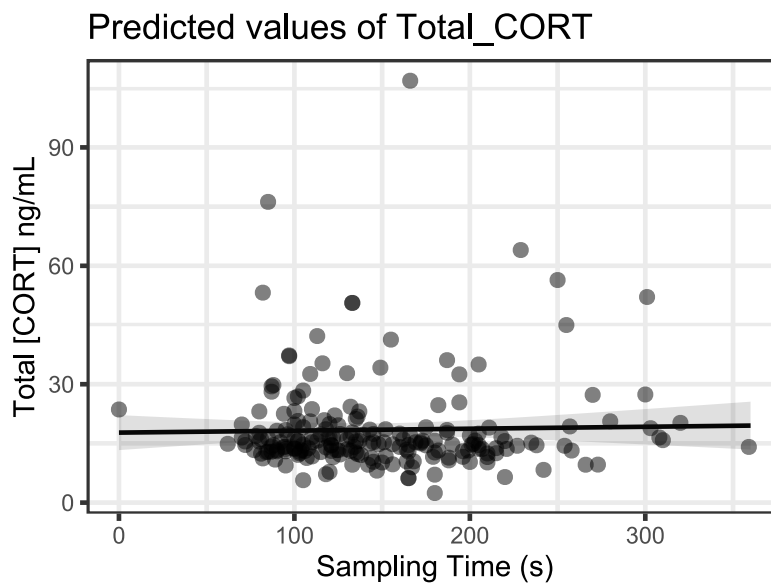
**Table S4:** LMM output for Body Condition of adults throughout the monitoring period. Density and Sex were introduced as explanatory factors, and Year as a cofactor to account for interannual variability in the data. Bird ID was first introduced as a random factor to account for repeated measurements on birds, but subsequently removed to allow convergence.

There was a significant effect of the Year on the body condition of adults, with adults from 2013 breeding season showing decreased body condition compared to adults from the 2012 breeding season (Table 2). Therefore, Year was introduced as a cofactor in the models to account for interannual variability. However, there was no significant difference between adults in LD and HD, allowing comparison of the two groups.

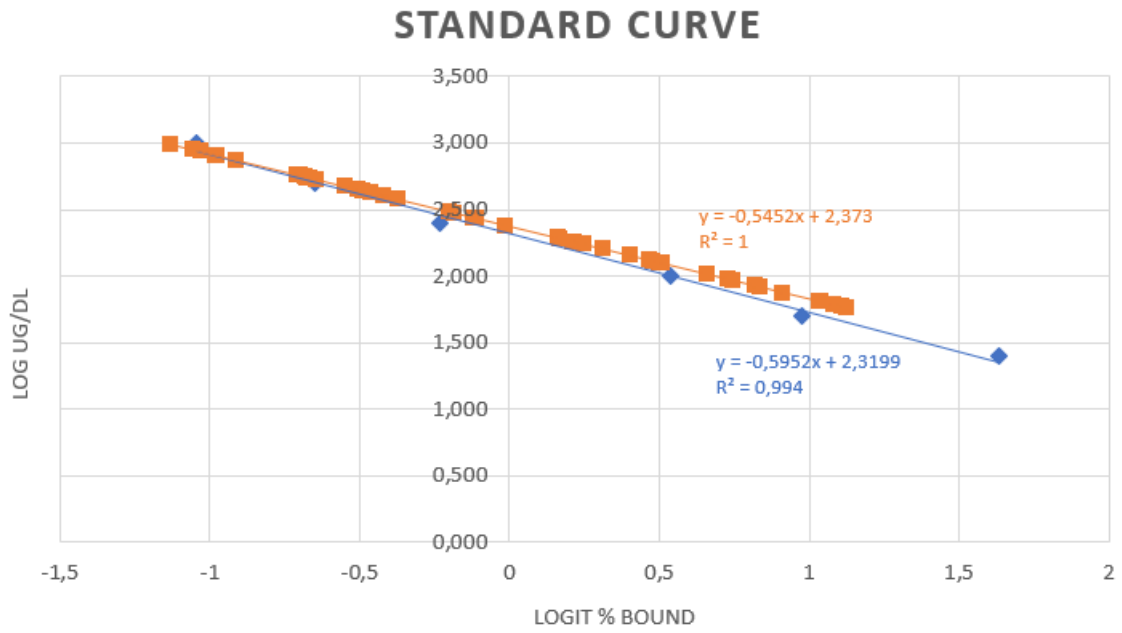
### S3. Baseline Corticosterone level and Corticosterone assay method

<i>Predictors</i>	<b>Total_CORT</b>				
	<i>Estimates</i>	<i>std. Error</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
(Intercept)	17.74	2.26	13.27 – 22.20	7.84	<0.001
Time	0.00	0.01	-0.02 – 0.03	0.35	0.727
Observations	206				
R <sup>2</sup> / R <sup>2</sup> adjusted	0.001 / -0.004				

**Table S5:** LM output for Baseline ( $T_0$ ) corticosterone level in plasma of adults of blood sample taken under 5 minutes ( $2.43 \pm 1.05$  min). Time (in s) represents the amount of time between the focal bird spotted the handler and the blood sample was taken.



**Figure S2:** Baseline ( $T_0$ ) plasma levels of corticosterone as a function of sampling time for adults king penguins sampled under 5 minutes ( $2.43 \pm 1.05$  min on average). Time (in s) represents the amount of time between the focal bird spotted the handler and the blood sample was taken. Consistent with previous studies on king penguin, we found no significant elevation in CORT levels with time between first sighting of the bird and sampling time for all samples taken under 5 minutes. Therefore, samples taken under 5 minutes were considered representative of near-baseline levels.



**Figure S3.** Verification of parallelism over a range of dilutions for king penguin plasma samples compared to a standard curve. We tested the corticosterone assay method for parallelism over a range of dilutions for king penguin plasma and found the dilutions paralleled the standard curve. Hence the assay was robust for this measure. There was no departure from Parallelism. We also ran low- and high-quality controls in every assay and these were varied only 5.5% and 3.7%, respectively.

**S4. Adult behavioral scans**

Behavior category	Code	Description	
Sleeping	S	the bird is sleeping with its head under the flipper	
Resting	R	eyes closed, not moving	
Vigilance	V	eyes open, slight head movement	
Aggressive	Flipper blow	FB	
	Gapping	G	beak open towards neighbors, vocalization
	Menace	M	beak closed towards neighbors, bent posture
	Pecking	P	pecks neighbor with the beak
Comfort	Grooming	T	
	Stretching	St	Stretches neck, back and flipper, often followed by body shaking and tail wagging
	Tail wagging	TW	
	Nodding	N	movement of the head up and down
	Locomotion	L	
	Head scratching	HS	scratches head with foot
	Head rubbing	HR	rubs head on flipper
Head back and forth	HBF	moves head back and forth	
Parental care	Egg care	EC	uses beak to turn or move the egg
	Chick grooming	CT	uses beak to groom the chick
	Chick feeding	CF	

**Table S6:** Full ethogram for behavioral scans of breeding adult king penguins.

**S5. Model output for behavioral & physiological parameters of adults & chicks**

	est	se	Num DF	Den DF	stat	p	Model
<b>Response</b>	<b>Resting (vs. Not-Resting)</b>						GLMER (Binomial)
Intercept	-0.15	0.10			-1.47	0.142	
Density [LD]	0.18	0.08			2.37	0.018	
Stage [Incubation]	0.47	0.03			13.65	<0.001	
Year [2013]	0.06	0.11			0.56	0.574	
Random effect	ID	8.6 %					
	Observateur	0.6 %					
<b>Response</b>	<b>Total CORT (ng/mL)</b>						LMER
Intercept	20.75	1.60			12.97	<0.001	
Density [LD]	-1.97	1.53			-1.29	0.199	
Sampling Time [T30]	39.47	1.33			29.75	<0.001	
Sexe [M]	0.13	1.55			0.08	0.933	
Year [2013]	-8.59	1.54			-5.56	<0.001	
Random effect	ID	15.8%					
<b>Response</b>	<b>MCBC (nM)</b>						LMER
Intercept	278.71	34.85			8.00	<0.001	
Density [LD]	23.48	17.25			1.36	0.177	
Sampling Time [T30]	-54.18	20.87			-2.60	0.012	
Sexe [M]	42.69	21.95			1.95	0.056	
Year [2013]	-3.31	35.72			-0.09	0.927	
Random effect	ID	19.6 %					
<b>Response</b>	<b>Free CORT (ng/mL)</b>						LMER
Intercept	-7211.79	4848.94			-1.49	0.142	
Density [LD]	0.39	2.04			0.19	0.849	
Sampling Time [T30]	4.59	1.91			2.41	0.019	
Sexe [M]	-2.43	1.66			-1.47	0.148	
Year [2013]	3.58	2.41			1.49	0.141	
Density : Sampling Time			1	81.778	5.14	0.026	
Random effect	ID	0.1 %					
<b>Response</b>	<b>HR response (bpm)</b>						LMER
Intercept	74.26	2.52			29.45	<0.001	
Density [LD]	0.03	2.84			0.01	0.992	
Sampling Time [stress peak]	70.74	2.49			28.38	<0.001	
Sexe [M]	-6.38	2.19			-2.91	0.004	
Year [2013]	-5.00	2.18			-2.29	0.023	
Density : Sampling Time			1	203.84	3.18	0.076	
Random effect	ID	18.7 %					
<b>Response</b>	<b>Resting HR (bpm)</b>						LMER
Intercept	91.20	1.38			65.96	<0.001	
Density [LD]	-2.45	1.50			-1.64	0.102	
Fasting days	-2.33	0.14			-16.29	<0.001	
Day vs Night	-2.07	0.24			-8.57	<0.001	
Time recorded	-0.45	0.03			-15.75	<0.001	
Sexe [M]	-6.03	1.22			-4.96	<0.001	
Year [2013]	-7.43	1.21			-6.12	<0.001	
Density : Fasting days			1	1321.40	7.23	0.007	
Random effect	ID	84.2 %					

**Table S7.** Results of mixed effects model output for behavioral & physiological parameters of adult king penguins.



	est	se	Num DF	Den DF	stat	p	Model
<b>Response</b>	<b>Structural size (PC1)</b>						
Intercept	207.31	100.21			2.07	0.041	LMER
Origin [LD]	0.04	0.05			0.87	0.384	
Fostering [LD]	0.03	0.06			0.49	0.625	
Year [2013]	-0.10	0.05			-2.10	0.039	
Stage			3	216.31	7279.33	<0.001	
Stage : Fostering			3	217.79	3.30	0.021	
Random effect	ID	42.5 %					
<b>Response</b>	<b>Body mass (g)</b>						
Intercept	620726.78	225832.38			2.75	0.007	LMER
Origin [LD]	35.65	224.56			0.16	0.874	
Fostering [LD]	35.54	235.20			0.15	0.880	
Year [2013]	-308.20	112.22			-2.75	0.007	
Origin:Fostering			1	155.18	6.88	0.010	
Origin:Stage			3	234.59	1.37	0.254	
Fostering:Stage			3	234.39	2.20	0.089	
Origin:Fostering:Stage			3	234.62	3.40	0.018	
Random effect	ID	17.7 %					
<b>Response</b>	<b>Body mass gain (g/day)</b>						
Intercept	50.43	7.37			6.85	<0.001	LMER
Origin [LD]	3.75	2.52			1.49	0.141	
Fostering [LD]	-4.48	2.53			-1.77	0.080	
Initial body mass (10d)	0.04	0.01			3.30	0.002	
Year [2013]	-1.64	5.65			-0.29	0.772	
Random effect	ID	9.2 %					
<b>Response</b>	<b>Survival through winter (0/1)</b>						
Intercept	0.72	0.37			1.93	0.054	GLM (Binomial)
Origin [LD]	-0.15	0.47			-0.32	0.746	
Fostering [LD]	-1.04	0.46			-2.29	0.022	
Year [2013]	-0.89	0.50			-1.78	0.075	
Body mass (105 days)	1.01	0.36			2.80	0.005	
Structural size	0.14	0.33			0.41	0.680	

**Table S8.** Results of mixed effects model output (LMER and GLM) for growth and survival of chicks.

	est	se	Num DF	Den DF	stat	p	Model
<b>Response</b>	<b>Total CORT (ng/mL)</b>						
Intercept	10.44	5.98			1.75	0.090	LMER
Sampling Time [T30]	49.14	4.73			10.38	<0.001	
Fostering [LD]	10.07	7.52			1.34	0.192	
Origin [LD]	5.87	6.73			0.87	0.391	
Year [2013]	4.94	7.67			0.64	0.525	
Random effect	ID	30.5 %					
<b>Response</b>	<b>MCBC (nM)</b>						
Intercept	178.37	44.37			4.02	<0.001	LMER
Sampling Time [T30]	85.40	40.37			2.12	0.043	
Fostering [LD]	203.01	61.55			3.30	0.003	
Origin [LD]	56.83	50.76			1.12	0.278	
Year [2013]	68.56	52.83			1.30	0.213	
PS:Fostering			1	12.764	22.84	<0.001	
Random effect	ID	34.0%					
	MCBC vial	61.0%					
<b>Response</b>	<b>Free CORT (ng/mL)</b>						
Intercept	3.40	2.89			1.18	0.249	LMER
PS [T30]	8.64	2.76			3.13	0.003	
Fostering [LD]	0.97	3.62			0.27	0.789	
Origin [LD]	-2.48	3.36			-0.74	0.465	
Year [2013]	1.30	3.61			0.36	0.721	
Random effect	ID	14.0%					
<b>Response</b>	<b>HR response (bpm)</b>						
(Intercept)	60.22	4.48			13.45	<0.001	LMER
Sampling Time [stress peak]	154.98	3.67			42.22	<0.001	
Origin [LD]	-2.10	4.58			-0.46	0.648	
Fostering [LD]	-5.92	4.41			-1.34	0.186	
Year [2013]	9.63	4.54			2.12	0.040	
Random effect	ID	17.6%					

**Table S9.** Results of mixed effects model output for physiological parameters of chicks.