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Environmental influences on Adelie penguin breeding schedules, endocrinology, and chick survival

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ABSTRACT

To understand how the social and physical environment influences behaviour, reproduction and survival, studies of underlying hormonal processes are crucial; in particular, interactions between stress and reproductive responses may have critical influences on breeding schedules. Several authors have examined the timing of breeding in relation to environmental stimuli, while others have independently described endocrine profiles. However, few studies have simultaneously measured endocrine profiles, breeding behaviour, and offspring survival across seasons. We measured sex and stress hormone concentrations (oestrogens, testosterone, and corticosterone), timing of breeding, and chick survival, in Adelie penguins (*Pygoscelis adeliae*) at two colonies in two different years. Clutch initiation at Cape Bird South (CBS; year 1, ~14,000 pairs) occurred later than at Cape Crozier East (CCE; year 2, ~25,000 pairs); however, breeding was more synchronous at CBS. This pattern was probably generated by the persistence of extensive sea ice at CBS (year 1). Higher corticosterone metabolite and lower sex hormone concentrations at CBS correlated with later breeding and lower chick survival compared to at CCE – again, a likely consequence of sea ice conditions. Within colonies, sub-colony size (S, 50–100; M, 200–300; L, 500–600; XL, >1000 pairs) did not influence the onset or synchrony of breeding, chick survival, or hormone concentrations. We showed that the endocrine profiles of breeding Adelie penguins can differ markedly between years and/or colonies, and that combining measures of endocrinology, behaviour, and offspring survival can reveal the mechanisms and consequences that different environmental conditions can have on breeding ecology.

1. Introduction

Penguins and many other species initiate breeding synchronously because individuals respond similarly to seasonal environmental cues, like increasing photoperiod ([24], see also [49]) or rainfall [50,67]. For example, differences in the annual cycle of Adelie penguins, such as time of arrival at breeding grounds, are largely driven by photoperiod, and change along a latitudinal gradient [1]. Studies of Adelie penguins at Cape Bird [51] and at Cape Crozier [3] (very close in latitude) show the timing of the occupation period (averaged across seasons for each study) to be identical [1]. However, inter-annual variation of other environmental conditions may interrupt the initiation of breeding following the primary cue. For example, divergences from the usual timing of breeding of Adelie penguins at either Cape Crozier or Cape Bird have been attributed to seasonal differences in the extent of sea ice [1].

Additionally, following arrival at breeding grounds, reproduction is often more synchronous than would be expected based solely on environmental cues [28]. Darling [18], who was working with herring gulls (*Larus argentatus*) and lesser black-backed gulls (*L. fuscus*), proposed that social stimulation, derived from the presence or activities of conspecifics, synchronised and hastened egg-laying in colonial birds by influencing the reproductive state of females. This phenomenon became known as the 'Darling Effect' [25]. One obvious way to examine this effect was to measure reproductive schedules in breeding assemblages of differing size [18]. Darling suggested that, mediated via endocrine pathways (though he never measured these), individuals in larger

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assemblages would receive greater levels of social stimulation from the numerous neighbours, leading to earlier and more synchronous breeding compared to smaller assemblages. Conversely, exposure to greater levels of agonistic activities in large groups could counteract the Darling Effect [56]; higher levels of agonistic activities could stress birds, affecting reproduction, as reproductive hormones are inhibited by stress [43,47]. Many studies have provided support for the Darling Effect (e.g. [10,48,57], see [25] for review), while other studies have found no evidence of it (e.g. [35,14,54]).

Thus, neuroendocrine changes, in response to both physical and social environmental stimuli, have been hypothesised as the mechanism controlling the timing of breeding in colonial birds ([18], also see [25]). Despite this, while previous studies have examined intra-seasonal endocrine profiles in a single breeding season [34], or timing of breeding across seasons ([1] and references therein), studies have seldom simultaneously examined breeding schedules, social context, and endocrine parameters of free-living birds across seasons (with the notable recent exception of Setiawan et al. [48]). Information on the endocrine profiles of free-living birds in different contexts may reveal whether any differences in breeding schedule are associated with changes in hormone concentrations.

The aim of this study was to describe seasonal changes of baseline testosterone, oestrogen, and corticosterone metabolite concentrations in Adelie penguins (*Pygoscelis adeliae*), across two different colonies in two different years. Baseline hormone concentrations are important to quantify as a reference with which to compare hormone concentrations in experimental studies (e.g. in disturbance studies); hence, knowing how much intra- and inter-seasonal variation endocrine patterns display is valuable [58]. By also measuring the timing of egg laying, and offspring survival, we aimed to investigate the extent to which these are related to neuroendocrine changes. Additionally, as previous studies of other colonial birds have described differences in the timing of egg laying across colonies of differing size, we sampled sub-colonies of differing size, to investigate this as a source of within-colony variation.

2. Materials and methods

2.1. Study colonies

This study was undertaken over two austral summer field seasons (from early November to mid-January, 2004–2006), at two nearby (<80 km) colonies on Ross Island, Antarctica. The first season (year 1; austral summer of 2004/2005) was conducted at Cape Bird South (CBS: ca. 166°28′E, 77°14′S; ca. 14,000 pairs, Lyver and Barton, Landcare Research, unpublished data). CBS is positioned over ice-free rocky substrate at altitudes from 30 to 70 m above mean sea level, and within 500 m of the McMurdo Sound shoreline. The second field season (year 2; austral summer of 2005/2006) was undertaken at Cape Crozier East (CCE: ca. 169°16′E, 77°28′S; ca. 25,000 pairs, Lyver and Barton, Landcare Research, unpublished data). CCE comprises similar habitat to CBS: ice-free rocky substrate at altitudes from 10 to 30 m above mean sea level, and within 300 m of the Ross Sea shoreline.

2.2. Sub-colonies

Within each colony, four discrete sub-colonies were identified: one small (S, ~50–100 pairs), one medium (M, ~200–300 pairs), one large (L, ~500–600 pairs), and one extra-large (XL, >1000 pairs). All of the identified sub-colonies were bordered by at least 5 m of unoccupied space, and none neighboured each other. Following male arrival at the breeding grounds in early November, 20 widely separated nests within each of the four sub-colonies (S, M, L, and XL) were marked, in which a solo male was clearly occupying and maintaining a nest, prior to female arrival.

2.3. Timing of clutch initiation

Subsequent to marking nests within sub-colonies, nest checks were conducted every second day until egg laying had occurred, then every 4 days following. Nests were checked by an observer slowly approaching the nest and gently lifting the front of the bird with a 2 m pole (as described in [19]), or lifting the tail from behind, so the nest contents could be clearly identified. The onset and synchrony of breeding were measured using the date that the first egg was laid (clutch initiation).

2.4. Faecal sample collection

Faecal samples were collected from each sub-colony size class at five stages throughout the breeding season (corresponding to courtship (C), early incubation (E: males' shift), late incubation (L: females' shift), guard (G: post hatching), and pre-crèche (P: just before both parents leave the nest, and chicks form crèches)) to examine temporal variation; samples were collected into cryovials and stored in a cryogenic dry shipper within 2 h of collection. Faecal samples were collected from S, M, L, and XL sub-colonies that were not study sub-colonies being subjected to nest checking, to more accurately quantify undisturbed baseline hormone concentrations. As urinal and faecal excretion are combined in birds, we attempted to collect only the faecal portion, which was distinguishable by colour. Samples were collected at various times of the day (09:00–20:00 h), however, circadian rhythms in hormone concentrations were unlikely to have produced any within-day variation due to continuous daylight ([44] and references therein); furthermore, Vleck and Van Hook [55] found no evidence that circadian rhythms operate in Adelie penguins in this context.

Due to logistical constraints, molecular determination of sex from faecal samples could not be achieved; however, where possible, samples were collected both from suspected males and females, based on observed copulations, occurrence of tread marks on the female's back, and bill size comparisons [2,32]. However, a subset of experimental subjects ($n = 39$) from which faecal samples were obtained were also blood sampled (for other purposes); molecular sexing of these blood samples was undertaken following the protocol used by Ninnes et al. [38]. Using faecal samples from birds that had also been blood sampled (and hence were of known sex), we investigated whether a coefficient based on the proportion of testosterone to oestrogens could be used to infer sex. Significant overlap in the range of concentrations of these hormones across sex meant this was not feasible. Student's *t*-tests were carried out on these 39 faecal samples to investigate sex differences. Differences between the sexes for testosterone and oestrogens were small, and did not approach statistical significance (although males had higher corticosterone metabolite concentrations than females; $t = 3.03$, $p < 0.01$). These results correspond to those reported in Ninnes et al. [38], in which sample sizes were larger, and all birds were of known sex (from molecular sexing); thus, the sexes were pooled in the present study.

2.5. Extraction of hormones and hormone metabolites from faeces

Faecal samples were thawed and allowed to reach room temperature. We then weighed 300 mg (where possible) of wet faeces (wet faeces were used as the weight of faecal samples was relatively consistent: 3.93 g ± 0.06 (SE)) and transferred it to a test tube, where we added 1.5 mL (5× sample weight) of double-distilled water, and the sample was vortexed. We added 2.25 mL (7.5× sample weight) of methanol to the sample (making a 60%

methanol solution), and samples were shaken at 920–980 orbits per minute (opm) for 30 min. Samples were then centrifuged (3000*g*, 20 °C, 10 min) and 300 μL of the supernatant transferred to a 1.5 mL microcentrifuge tube and diluted with 150 μL of assay buffer (trishydroxyaminomethane (Merck 8382) 20 mmol/L; NaCl (Merck 6404) 0.3 mol/L; 1 g/L bovine serum albumin (Sigma A-4503); 1 mL/L Tween 80 (Merck 822187)). Extracts were then subjected to enzyme immunoassays (EIAs) to measure the concentrations of immunoreactive tetrahydrocorticosterone, testosterone, and oestrogens (and their metabolites).

Concentrations of oestrogens were too low to detect using the above extractions. Oestrogens were de-conjugated using glucuronidase/arylsulfatase to test whether a significant proportion of oestrogens were present in a conjugated form, and hence undetected by the EIA. However, this made only a minor difference suggesting most of the oestrogens were already in a de-conjugated form. Extracts could not be further concentrated without danger of interfering molecules (e.g. lipids) affecting the EIA, so an organic extraction was developed for oestrogens. The previous extracts (kept frozen after use in the previous assays) were brought to room temperature, vortexed briefly then centrifuged (3000*g*, 20 °C, 10 min). One millilitre of the supernatant was transferred to a new tube, to which 5 mL of diethyl-ether was added. The methanol and hormones from the previous extract was retained in the diethyl-ether phase, leaving the heavier aqueous phase sitting at the bottom of the tube. The two phases were then chilled to −20 °C, and the organic phase containing the hormones decanted into a new tube, with the aqueous phase left frozen on the bottom. The samples were then dried under nitrogen, and re-suspended in 1 mL of assay buffer.

2.6. Enzyme immunoassays

All assays were group specific, and detected a range of immunoreactive metabolites, as well as the native hormones being examined. Details of cross-reactivity can be found in the reference given for each assay. All samples were assayed in duplicates; the intra-assay variation was calculated from the variation of measurements between duplicates, except when there was a large discrepancy in which case the sample would be assayed again. One sample with low hormone concentrations (low pool) and one sample with high hormone concentrations (high pool) were assayed on every plate, so that inter-assay variation coefficients could be calculated for both the top and the bottom of the standard curve, where variation is greatest. The sensitivity of all assays was below 1 pg/well.

For the measurement of testosterone (and metabolites) from faeces, an EIA for testosterone-3-CMO:BSA was used, as described by Palme and Möstl [40]. Biotinylated 5α-androstane-3ß,17ß-diol-3-HS served as the label. The working dilution of the antibody was 1:75,000, and 1:5,000,000 for the label. The standard curve ranged from 0.33 to 80 pg/well. The intra-assay variation was 8.0% and the inter-assay variation was 19.6% and 11.8% in the low and high pools, respectively.

To measure oestrogens in faeces, an EIA described by Palme and Möstl [40] for 17ß-estradiol-17-HS:BSA was used. Biotinylated 17ß-estradiol-17-glucuronide was used as the label. The working dilution of the antibody was 1:50,000 and 1:5,000,000 for the label. The standard curve ranged from 0.33 to 80 pg/well. The intra-assay variation was 14.5%, and the inter-assay variation was 11.7% and 12.9% in the low and high pools, respectively.

To measure corticosterone metabolites in faeces, an EIA developed by Quillfeldt and Möstl [42] and validated in Adelie penguins by Nakagawa et al. [37] was used to measure the principal metabolite tetrahydrocorticosterone (5β-pregnane-3α,11β,21-triol-20-one). Biotinylated tetrahydrocorticosterone-21-hemisuccinate (HS) was used as the label. The working dilution of the antibody was 1:40,000, and 1:8000 for the label. The standard curve ranged from 0.82 to 200 pg/well. The intra-assay variation was 7.0%, and the inter-assay variation was 13.8% and 6.2% for the low and high pools, respectively.

Apart from the tetrahydrocorticosterone (THCC) assay which was biologically validated for Adelie penguins [37], a lab-based approach was used to validate the assays on the basis of chromatographic mobility of the immunoreactive substances [11]. A faecal sample extract with high hormone concentrations, following a clean up using Sep-pak C18 cartridges, was chromatographed using reverse phase high performance liquid chromatography (HPLC), with a gradient elution and a starting concentration of 50% methanol, increasing to 75%. Fractions of 0.3 mL were collected and the immunoreactive substances were analysed by EIA. For testosterone, two main immunoreactive peaks eluted: one at the same position as authentic testosterone, and one eluting much earlier. Therefore, this immunoreactive compound is much more polar and it is likely that the earlier eluting peak is a conjugated form of testosterone. This provides strong evidence that what was being measured in the testosterone assays was in fact testosterone. For oestrogens, two main peaks also eluted, one at the position of authentic oestradiol and another much earlier, suggesting a conjugated form of oestrogen metabolites; however, there were also many other smaller peaks in the oestrogen HPLC. As oestrogens were present in lower concentrations than the other hormones being examined, there was an increased chance of some lipid interference occurring in the oestrogen assay, as a more concentrated sample had to be used. This must be considered when interpreting the results from the oestrogen assays. Although the THCC assay has been previously validated in Adelie penguins, an HPLC immunogram for it was also undertaken. The dominating immunoreactive peak eluted in the first fractions, with only a small peak eluting at the position of authentic THCC. To verify that this first peak was not caused by interfering substances, a second reverse phase HPLC with a gradient elution and a starting concentration of 20% methanol increasing to 100% was undertaken. As expected, the two peaks eluted later, showing that the early peaks seen in the first immunogram were not due to lipid interference.

2.7. Survival

Survival was taken as the proportion of nests that still had a chick or chicks present just prior to the onset of the crèche period. This corresponded to 1 January ± 1 day, as not all nests in both years were able to be checked on the same day. This measurement of survival was used, as following crèching it became impractical to follow which nest any given chick originated from. Clarke et al. [13] found that at Béchervaise Island, the majority of Adelie penguin chicks that survive to the crèche period survive through to fledging, so it is likely that this measure of survival is reflective of survival at fledging.

2.8. Statistical analysis

Clutch initiation was not normally distributed, so non-parametric tests were employed to examine the timing of clutch initiation. A Mann–Whitney *U* test was used to compare the means across the two colonies (and corresponding years), and a Kruskal–Wallis ANOVA was used to compare the means across sub-colony sizes. To examine differences in variability (i.e. synchrony) between colonies and across sub-colony sizes, Levene's tests were used, as recommended by Waas et al. [57]. Due to the non-normality of these data, they were first rank transformed before applying the Levene's test. These rank transformed data of clutch initiation were also used to investigate the presence of an interaction term between sub-colony size and colony size, using a factorial ANOVA.

Survival data was binomial (presence/absence of chicks at crèching), and as both continuous and categorical predictors needed to be investigated, generalised linear models (with binomial errors) were used. The full model was examined first, which contained all relevant potential explanatory variables (first egg date, colony size, sub-colony size), and their interaction terms, for the response variable 'survival'. Adopting the principle of parsimony, terms were deleted sequentially until a minimum adequate model was resolved. Akaike's information criterion (AIC) [6], which considers model simplicity against the fit of the model, was used to assess the best minimum adequate model. It is a useful tool in model simplification, as a model with a lower AIC is preferred to one with a higher AIC [15].

All hormone data were log transformed (natural log) to achieve normality or near normality. As all relevant potential predictor variables were categorical, factorial ANOVAs were used. Again, the full model was the starting point, followed by stepwise removal of terms until a minimum adequate model was reached, based on AIC.

Although p-values were calculated in the analysis of these data and presented for the reader, discussion of the results attempts to focus more on effect size, and includes discussion of some trends or differences that may not have reached the statistical significance threshold of $p < 0.05$. Effect sizes and associated 95% confidence intervals are presented, to better facilitate an assessment of the biological importance of results, as recommended by Nakagawa and Cuthill [36].

Because this study compared only one sub-colony of each size at each colony, sampling 20 nests from each sub-colony, there was potential for pseudoreplication. To investigate this, a colony 'id' code was created (incorporating colony size, sub-colony size, and period) and included as a random effect in preliminary mixed model ANOVAs. It was found to account for a trivial amount of variation (<1%). Consequently, nests within each colony were regarded as independent and simpler factorial ANOVAs were used. This issue was not a consideration for hormone data, which were collected from different colonies from those being nest checked.

This study was approved by the University of Waikato Animal Ethics Committee and Antarctica New Zealand.

3. Results

3.1. Timing of clutch initiation

Clutch initiation was later at CBS (year 1) compared to CCE (year 2) ($Z = 5.87$, $p < 0.001$; Mann–Whitney U test; Fig. 1); however, pairs at CBS displayed greater synchrony (SD = 2.73 days versus SD = 4.20 days) ($F = 16.40$, $p < 0.001$; Levene's test on rank transformed first egg lay date; Fig. 1). Within colonies, sub-colony size had no significant effect on clutch initiation date (CBS, KW-H (3, $n = 77$) = 0.43, $p = 0.93$; CCE, KW-H (3, $n = 71$) = 4.38, $p = 0.22$; Kruskal–Wallis ANOVA), or synchrony (CBS, $F = 0.43$, $p = 0.74$; CCE, $F = 1.43$, $p = 0.24$; Levene's tests on rank transformed first egg lay date), and no significant sub-colony size * colony size interaction term was present ($F = 0.88$, $p = 0.45$; Factorial ANOVA on rank transformed first egg lay date).

3.2. Hormones

The full model for testosterone showed that sub-colony size, and all interaction terms including sub-colony size, were poor predictors of testosterone concentrations (Table 1, top). The removal of all statistically insignificant terms was found to produce the lowest AIC value following stepwise deletions, and was adopted as the minimum adequate model (Table 1, bottom). Colony (year), breeding stage, and their interaction term were found to be the strongest predicting variables. The birds at CCE (year 2) had higher overall testosterone than those at CBS (year 1) (Fig. 2a). The general trend across breeding stages showed a gradual decrease from courtship to the guard stage, before increasing through to the pre-crèche stage (Fig. 2b). Although the colony (year) * breeding stage differences approached ($p = 0.069$), but did not reach a threshold of statistical significance of $p < 0.05$, it is notable that the courtship concentrations were higher at CCE (where they displayed peak concentrations within that season) than at CBS (Fig. 2b). This difference was significant when the courtship period was analysed independently ($t = -3.005$, $p = 0.008$).

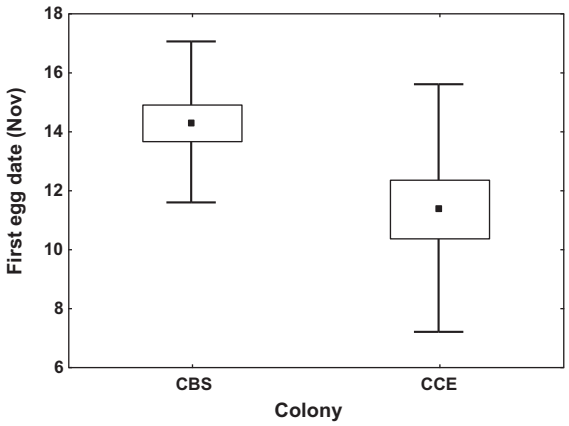

Fig. 1. Mean first egg date (solid squares) across CBS (year 1) and CCE (year 2) colonies ±95% confidence intervals (box) and ±1 SD (whiskers). Outliers and extreme values were included in the analysis, but have been excluded from the graph to more clearly display the differences.

Sub-colony size was also a poor predictor of oestrogen concentrations. The full model for oestrogens (Table 2, top) showed colony (year) and breeding stage to be the only statistically significant predictors, however, the minimum adequate model also included their interaction term (Table 2, bottom). Birds at CCE (year 2) displayed higher overall concentrations of oestrogens than those at CBS (year 1) (Fig. 3a). The trends across breeding stages were similar to those for testosterone, with concentrations gradually decreasing from courtship to the guard stage, before rising through to the pre-crèche stage (Fig. 3b). Although the colony (year) * breeding stage differences did not reach a threshold of statistical significance of $p < 0.05$, it is notable that the courtship concentrations were higher at CCE (where they displayed peak concentrations within that season) than at CBS (Fig. 3b). This dif-

Table 1
Full model (factorial ANOVA) for testosterone, AIC = 223 (top) and minimum adequate model (factorial ANOVA) for testosterone, AIC = 200 (bottom).

Effect	Degrees of freedom	F	p
Sub-colony size	3	0.390	0.760
Colony (year)	1	3.835	0.054
Breeding stage	4	13.262	0.000
Sub-colony size * colony (year)	3	0.310	0.818
Sub-colony size * breeding stage	12	0.714	0.733
Colony (year) * breeding stage	4	1.853	0.128
Sub-colony size * colony (year) * breeding stage	12	0.720	0.727
Colony (year)	1	4.51	0.036
Breeding stage	4	14.63	0.000
Colony (year) * breeding stage	4	2.24	0.069

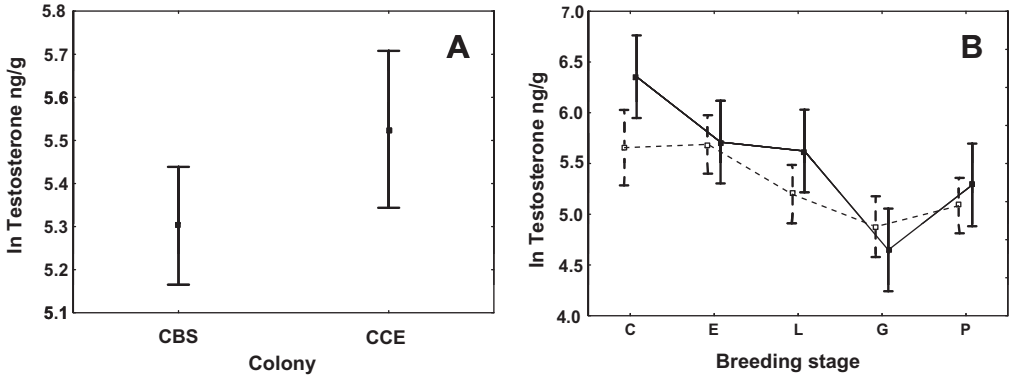

Fig. 2. Mean faecal testosterone concentrations across (A) CBS (year 1) and CCE (year 2) colonies, and (B) breeding stages for each colony (CBS (year 1) = open squares and dashed line; CCE (year 2) = solid squares and solid line). Vertical bars denote 95% confidence intervals. Letters for breeding stage are defined in the text.

Table 2
Full model (factorial ANOVA) for oestrogens, AIC = −29 (top) and minimum adequate model (factorial ANOVA) for oestrogens, AIC = −43 (bottom).

Effect	Degrees of freedom	*F*	*p*
Sub-colony size	3	0.540	0.657
Colony (year)	1	4.574	0.036
Breeding stage	4	7.380	0.000
Sub-colony size * colony (year)	3	1.378	0.256
Sub-colony size * breeding stage	12	1.054	0.410
Colony (year) * breeding stage	4	2.109	0.088
Sub-colony size * colony (year) * breeding stage	12	1.142	0.341
Colony (year)	1	4.528	0.036
Breeding stage	4	7.477	0.000
Colony (year) * breeding stage	4	1.958	0.106

Table 3
Full model (factorial ANOVA) for tetrahydrocorticosterone, AIC = 262 (top) and minimum adequate model (factorial ANOVA) for tetrahydrocorticosterone, AIC = 250 (bottom).

Effect	Degrees of freedom	*F*	*p*
Sub-colony size	3	0.471	0.703
Colony (year)	1	10.470	0.002
Breeding stage	4	1.013	0.406
Sub-colony size * colony (year)	3	3.504	0.019
Sub-colony size * breeding stage	12	1.034	0.427
Colony (year) * breeding stage	4	4.487	0.003
Sub-colony size * colony (year) * breeding stage	12	1.330	0.220
Sub-colony size	3	0.335	0.800
Colony (year)	1	9.665	0.003
Breeding stage	4	1.002	0.411
Sub-colony size * colony (year)	3	3.035	0.033
Colony (year) * breeding stage	4	4.669	0.002

ference bordered significance when the courtship period was analysed independently ($t = -2118$, $p = 0.050$).

THCC varied significantly across colonies (years) (Table 3, bottom), with CBS subjects (year 1) displaying much higher concentrations than those at CCE (year 2) (Fig. 4a). Trends of THCC across breeding stage also differed markedly between colonies (years) (Fig. 4b). Birds at CBS (year 1) had a conspicuous increase in THCC concentrations between courtship and early incubation, following which concentrations remain relatively high. In contrast, birds at CCE (year 2) showed decreasing concentrations from courtship to early incubation, with courtship representing the highest THCC concentrations within any breeding stage. THCC concentrations at CCE (year 2) were particularly low during the guard stage. Sub-colony size was a poor predictor of THCC concentrations within colonies (years) (Table 3, top), however, it was included in the minimum adequate model because of the sub-colony size * colony (year) interaction term, which reached statistical significance. Post-hoc tests (Fisher LSD test) revealed that the significance of this interaction term was driven solely by disparate THCC concentrations of large-sized sub-colonies between colonies

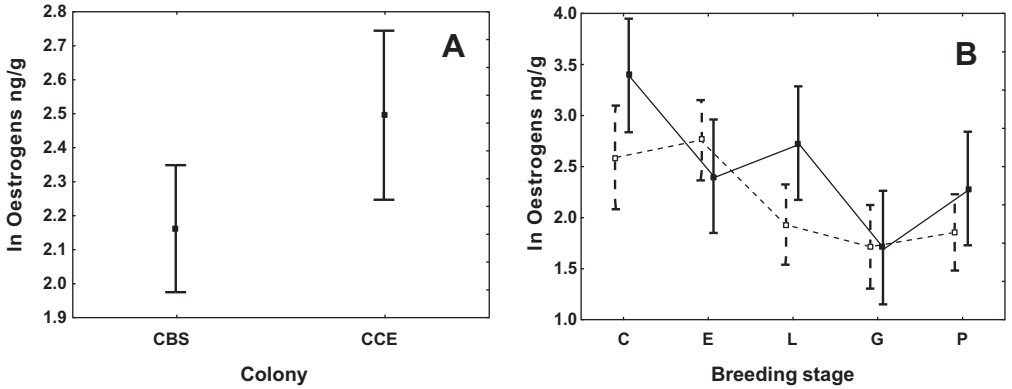

Fig. 3. Mean concentrations of faecal oestrogens across (A) CBS (year 1) and CCE (year 2) colonies, and (B) breeding stages for each colony (CBS (year 1) = open squares and dashed line; CCE (year 2) = solid squares and solid line). Vertical bars denote 95% confidence intervals. Letters for breeding stage are defined in the text.

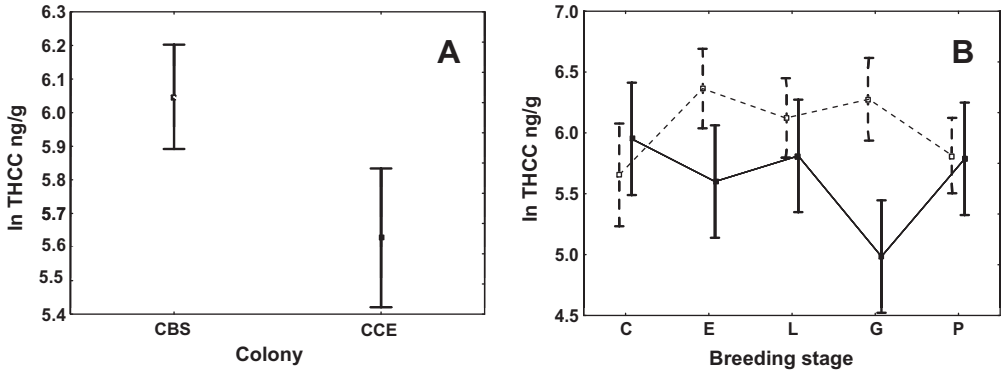

Fig. 4. Mean faecal tetrahydrocorticosterone concentrations across (A) CBS (year 1) and CCE (year 2) colonies, and (B) breeding stages for each colony (CBS (year 1) = open squares and dashed line; CCE (year 2) = solid squares and solid line). Vertical bars denote 95% confidence intervals. Letters for breeding stage are defined in the text.

Table 4
Full model (generalised linear model) for survival with binomial errors, AIC = 196 (top) and minimum adequate model (generalised linear model) for survival with binomial errors, AIC = 187 (bottom).

Effect	Degrees of freedom	Wald stat.	*p*
First egg lay date	1	0.006	0.941
Colony (year)	1	11.420	0.001
Sub-colony size	3	0.665	0.881
Colony (year) * sub-colony size	3	10.486	0.015
Colony (year)	1	13.038	0.000
Sub-colony size	3	0.667	0.881
Colony (year) * sub-colony size	3	10.557	0.014

(years). The minimum adequate model is shown in the lower portion of Table 3.

3.3. Survival

First egg date was not a significant predictor of survival (Table 4, top), and was consequently omitted from the model. The minimum adequate model contained the factors colony (year), sub-colony size, and their interaction term (colony (year) * sub-colony size) (Table 4, bottom). Colony (year) was the most significant predictor of survival (Table 4, bottom), with pairs at CBS (year 1) having lower survival (45% of nests were successful) than those at CCE (year 2) (75%) (Fig. 5a). Sub-colony size alone was not a good predictor of survival, however, the colony (year) * sub-colony size interaction term was significant (Table 4, bottom); survival in M and XL sub-colonies was lower in CBS (year 1) (Fig. 5b).

4. Discussion

4.1. Timing of clutch initiation

4.1.1. Colony level

Clutch initiation was about 3 days later, but more synchronous, at CBS (year 1) than at CCE (year 2). In year 1 of this study, when we were at CBS, a large iceberg (B15a) and a smaller daughter iceberg (B15k) situated between the Drygalski Ice Tongue and Ross Island, blocked off McMurdo Sound, preventing the sea ice from breaking up and dispersing out of the Sound. This meant that instead of walking a short distance between open water and their nesting sites, penguins had to walk much greater distances (~50 km). Travel over sea ice by foot is energetically costly, and time consuming for Adelie penguins [17,64], which can travel rapidly over long distances if swimming [3,13]. Indeed, the importance of sea ice conditions in relation to foraging or breeding success in Adelie penguins is well reported [3,13,16,29,30,39,45,46,51,63,65,66]. Breeding success of Adelie penguins is negatively correlated with the extent of summer sea ice, followed by according fluctuations to the population after a 5 year lag (the age of sexual maturity) [13,29,30].

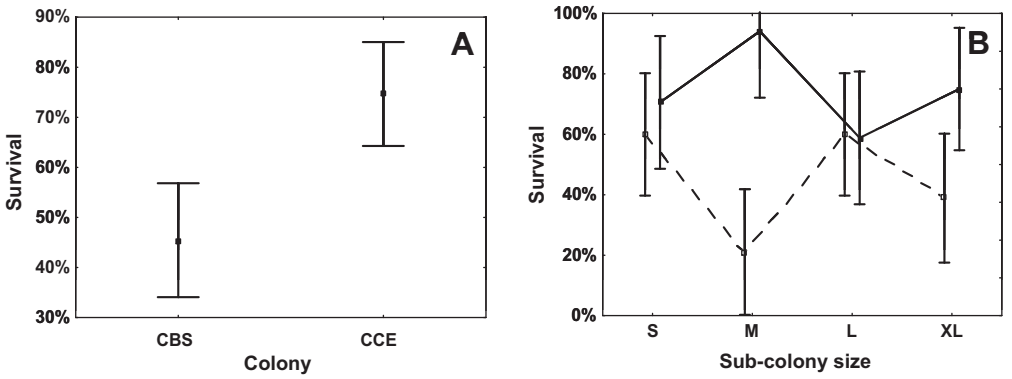

Fig. 5. Mean proportion of nests in which chicks survived through to crèching across (A) CBS (year 1) and CCE (year 2) colonies, and (B) sub-colony size for each colony (CBS (year 1) = open squares and dashed line; CCE (year 2) = solid squares and solid line). Vertical bars denote 95% confidence intervals. Letters for sub-colony size are defined in the text.

The later laying date at CBS (year 1) could simply be due to the increased time taken for the penguins to reach their breeding grounds. Under this scenario, birds that may have laid earlier (i.e. early outliers in the laying distribution) may have laid later. Given the extreme seasonality at these latitudes and consequent narrow breeding window [53], there may be a theoretical 'cut-off date' to egg laying; chicks from eggs laid after a given point may be unable to survive. If Adelie penguins use environmental cues to initiate breeding (e.g. photoperiod, see [1]) and are then delayed, it would compress the laying distribution against this cut-off date, synchronising egg laying. This hypothesis is supported by the findings that when fast ice delayed the arrival of Adelie penguins to breeding grounds at Wilkes Station, it did not affect subsequent stages of the breeding cycle [41], and that at Cape Bird, previously successful breeders that failed to breed had arrived late [51]. Davis and McCaffrey [22] also found that for Adelie penguins to breed successfully they must initiate breeding promptly after arriving at the breeding grounds. This could explain why breeding at CBS (year 1) was more synchronous, but clutch initiation was later, than at CCE (year 2).

4.1.2. Sub-colony level

There were no differences in the mean date of first egg laying, or the synchrony of laying across sub-colonies of different sizes. The hypothesised ultimate and proximate mechanisms for the synchrony/sub-colony size relationship (i.e. the Darling Effect) are increased survival (through predator swamping) and neuroendocrine changes, respectively ([18], also see [25]). That sub-colony size had no significant influence on survival or hormone concentrations within colonies suggests that sub-colony size was unlikely to influence reproductive schedules of Adelie penguins. Hence, the suggestion by Burger [10], that in herring gulls a colony divides into autonomously synchronised sub-colonies after it grows past a given size threshold, appears not to hold for Adelie penguins, with no evidence for the Darling Effect at the sub-colony level detected in this study.

4.2. Hormones

4.2.1. Colony level

Overall hormone levels differed significantly across colonies, with CCE (year 2) displaying higher concentrations of both reproductive hormones, but lower THCC concentrations, than CBS (year 1). The higher THCC and lower reproductive hormone concentrations at CBS (year 1), shows that endocrine profiles of Adelie penguin colonies can differ between years and/or colonies. In this study, we suggest hormone concentrations at CBS (year 1) were likely influenced by persistent sea ice conditions.

Both oestrogens and testosterone varied significantly across breeding stages. Concentrations of oestrogens and testosterone at both colonies gradually declined from courtship to the guard stage before showing an increase at the pre-crèche stage. These findings differ from McQueen et al. [34], who found concentrations of plasma oestrogens and androgens in Adelie penguins to drastically decline from courtship to clutch completion and then remain stable. These differences could be due in part to the different sample types used between the studies (refer to [38] for details on differences between plasma and faecal measurements). Elevations in plasma androgens during late incubation were detected in macaroni penguins (*Eudyptes chrysolophus*) [62] and Fiordland crested penguins (*E. pachyrhynchus*) [50]; the trend is thought to be due to a rise in agonistic interactions, renewed nest defence, and pair bonding [33,62]. It is unclear why no late testosterone rise was found by McQueen et al. [34] in Adelie penguins, as the explanation for the late rise in macaroni and Fiordland crested penguins should also apply. Indeed, the pattern of incubation duties in Fiordland crested penguins is similar to Adelie penguins [20]; females take the first foraging trip, unlike all other eudyptid species [59], so similar endocrine profiles may be expected. No rise in oestrogens was found during late incubation by McQueen et al. [34] in Adelie penguins, or by McQueen et al. [33] in Fiordland crested penguins, suggesting the regression of ovaries after clutch establishment [26,33]. The fact that increases in both oestrogens and testosterone from the guard stage to the pre-crèche stage occurred at both colonies in this study, suggests that Adelie penguins may still be capable of secreting gonadal hormones late in the breeding season. The purpose of such late secretion of gonadal hormones is unclear.

The breeding stage which showed the greatest difference in concentrations of testosterone and oestrogens between colonies (years) was the courtship stage, with courtship concentrations at CCE (year 2) being higher (at CCE courtship concentrations were higher than at any other breeding stage) than at CBS (year 1). Although the colony (year) $*$ breeding stage interaction term was not significant (likely due to the similarity in concentrations between colonies (years) in subsequent breeding stages), the difference in courtship concentrations between colonies is worth pointing out, especially considering that the colony (year) $*$ breeding stage interaction term approached significance for testosterone. Other studies for plasma oestrogen and androgen concentrations in Adelie penguins [21,26,34], and in other penguin species (king penguins (*Aptenodytes patagonicus*) [12]; Fiordland crested penguins [33]; macaroni and gentoo (*P. papua*) penguins [62]), have found concentrations to be highest during the courtship stage. That concentrations of testosterone and oestrogens at CBS (year 1) were not highest during the courtship stage, combined with delayed clutch initiation at CBS (year 1), supports our suggestion that penguin endocrine profiles and breeding behaviour at CBS (year 1) were likely affected by persistent sea ice conditions.

THCC trends throughout the breeding season differed between colonies (years). At CCE (year 2), the highest THCC concentrations occurred during courtship. McQueen et al. [34] also found the highest concentrations of corticosterone occurred during courtship in Adelie penguins, perhaps due to the stress of nest establishment and defence, and the creation and maintenance of pair bonds. THCC concentrations at CCE (year 2) then declined by about 40% from courtship to early incubation, never again approaching courtship concentrations. THCC concentrations might decrease following courtship as the stressors mentioned above may diminish, with incubating birds settling into their incubating duties. Fluctuations observed across the following periods may occur because sampled birds spent differing lengths of time fasting, as elevated corticosterone concentrations during fasting occur in other bird species [27], although the low concentrations of THCC during the guard phase are difficult to explain.

Conversely, courtship THCC concentrations at CBS (year 1) were relatively low, approximately doubling through to early incubation. Lower courtship THCC concentrations might be expected if birds at CBS (year 1) experienced less agonistic interactions, however, they would not be expected to subsequently rise. This trend could be explained by sea ice delaying the arrival of birds at the breeding grounds; if this reduced the duration of the courtship stage, the amount of stress experienced by the birds during courtship may have been reduced, explaining the lower concentrations of THCC at courtship at CBS (year 1). Elevated THCC concentrations observed at the early incubation period at CBS (year 1) may be due to incubating birds being less physiologically prepared for fasting, due to the increased energy expended in reaching the breeding grounds over the extensive sea ice. The consistently high THCC concentrations over late incubation and guard stages may also have reflected the difficulty of the birds to physiologically cope with fasting and chick provisioning, whilst having to spend a

greater proportion of their foraging trips travelling, and less time feeding.

4.2.2. Sub-colony level

Neither oestrogens, testosterone, nor THCC concentrations varied significantly across sub-colonies of differing size. However, there was a sub-colony size * colony size (year) interaction for THCC. This interaction is difficult to interpret, and as only THCC concentrations in large-sized sub-colonies differed significantly between colonies (years), the trend may be an artefact of limited replication and sample sizes. That sub-colony size was a poor predictor of hormone concentrations is consistent with the results for survival, and the timing and synchrony of clutch initiation.

4.3. Survival

4.3.1. Colony level

Survival of chicks through to the crèche period was much lower at CBS (year 1) (45% of nests produced $\geqslant 1$ chick) compared to CCE (year 2) (75%). This compares with 55–66% in normal years and 43% in a bad year which Spurr [51] recorded at Cape Bird (his estimates will be lower than ours as he accounted for 2-egg clutches in which 1 egg did not survive), and 57% and 31% in a 'good' and 'bad' year, respectively, recorded by Ainley and LeResche [3] at Cape Crozier (their estimates will be lower than ours as they only examined birds aged 3–7 years and maximum chick raising ability is reached at 7–8 years of age [4]). The lower survival at CBS (year 1) further supports our suggestion that the presence of the iceberg, and its consequent affect on sea ice conditions, affected the Adelie penguin breeding season at CBS (year 1).

Although higher stress hormone concentrations do not necessarily represent stress or reduced relative fitness [8], elevated THCC concentrations and low offspring survival at CBS supports the Cort-Fitness Hypothesis [9]. The duration of foraging trips of Adelie penguins during the incubation and guard stages increases during seasons of low breeding success [5,31,60], and greater sea ice extent [65]. Years when fast-ice persists longer than normal are often associated with poor breeding performance [5,52], most likely because Adelie penguins spend more of their foraging trips travelling, and consequently return with smaller meals [61]; the success and length of foraging trips and consequent timing of relief of incubating partners determines breeding success to a large extent [19,65]. In this study, poor survival, elevated corticosterone metabolite concentrations, and later clutch initiation at CBS (year 1) is likely due to changes in foraging trip duration and success, and delayed relief of incubating parents.

4.3.2. Sub-colony level

Sub-colony size explained very little variation within and across colonies for laying synchrony, and within colonies for survival, but was a significant predictor of survival across colonies. The pattern of survival across sub-colonies of differing size showed a reverse trend between large and small colonies. This may suggest directional selection, in which medium and extra-large-sized sub-colonies had better survival rates than small and large-sized sub-colonies at CCE (year 2, favourable sea ice conditions), yet suffered relatively greater nest failure rates at CBS (year 1, unfavourable sea ice conditions). Sub-colonies of differing size may be comprised of birds of differing age (see [25]), with younger birds being later ([14] and references therein, [23]) and poorer breeders than older or more experienced birds [7,23,65]. However, this trend may be an artefact of limited replication and sample sizes. Longer-term studies with greater sample sizes are needed to verify this potential phenomenon.

5. Conclusion

No differences in breeding schedule or hormone concentrations were detected across sub-colony sizes in either season of this study, representing little intra-colony variation and suggesting each colony can be considered one breeding unit. Differences in breeding schedule and hormone concentrations were, however, detected at the colony level, with CBS (year 1) displaying later clutch initiation, lower oestrogen and testosterone concentrations, higher THCC concentrations, and lower offspring survival than at CCE (year 2). These differences all align with the suggestion that persistent sea ice conditions had a significant impact on the breeding season at CBS (year 1). This study shows that the endocrine signature of breeding Adelie penguins can show significant variation between years and/or colonies. Furthermore, this study links endocrine profiles with differences in breeding schedule, reproductive success, and environmental variability, providing new insight into the proximate mechanisms that may be mediating differences in breeding behaviour and success.

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