

Spontaneous egg or chick abandonment in energy-depleted king penguins: A role for corticosterone and prolactin?

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Abstract

Various exogenous or endogenous factors may induce an emergency response in birds, redirecting current activity towards survival. In fasting, breeding penguins, the achievement of a critical energy depletion was suggested to induce egg abandonment and departure to sea for re-feeding. How such a behavioral shift is hormonally controlled remains unknown. The possible involvement of corticosterone and prolactin was examined by characterizing the nutritional and hormonal states of king penguins at egg abandonment. Further, we tested if these states differ according to whether an egg or a chick is abandoned, and according to the timing of breeding. In every case of abandonment, birds were in phase III fasting characterized by accelerated protein catabolism. However, body condition at egg abandonment was lower in early than in late breeders, suggesting that king penguins are willing to tolerate a larger energy depletion when their potential breeding success is high. At egg and chick abandonment, plasma corticosterone levels were, respectively, increased by 2- and 4-fold, whereas plasma prolactin levels were, respectively, depressed by 3- and 1.4-fold. The increase in plasma corticosterone and the decrease in plasma prolactin could be involved in the control of abandonment by, respectively, stimulating the drive to re-feed and diminishing the drive to incubate or brood. The smaller decrease in prolactin levels and the greater increase in corticosterone levels observed at chick vs egg abandonment suggest that, in addition to nutritionally-related stimuli, tactile or audible stimuli from the egg or chick could intervene in the endocrine control of abandonment.

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Introduction

Strong evidence indicates that under some circumstances an emergency response operates in birds, which involves interruption of the life history cycle and re-direction of behavior and physiology towards survival (Wingfield et al., 1998). This survival strategy notably evolves when birds have to face unpredictable events such as bad weather or food shortage. It includes seeking refuge or an alternate habitat, and searching for food. During breeding, the abandonment of territories, nests or offspring in response to factors which have a detrimental effect on energy balance has been documented (Wingfield, 1988; Wingfield and Ramenofsky, 1997). Although in this case

immediate breeding success becomes zero, temporary suspension of breeding activity may enhance lifetime reproductive success by allowing an individual to survive the period of perturbation and breed again. Unravelling the physiological and hormonal mechanisms underlying the emergency response is therefore of great interest in trying to understand how birds adjust their breeding activity in response to variations in their energy state.

Amongst factors that can stimulate the redirection of behavior from breeding to searching for food, and thus egg or chick abandonment, critical energy depletion has been suggested for those birds such as penguins and petrels that fast for prolonged periods during incubation and chick brooding (Chaurand and Weimerskirch, 1994; Olsson, 1997; Ancel et al., 1998; Robin et al., 1998; Groscolas et al., 2000; Gauthier-Clerc et al., 2001). Because in these seabirds both males and females alternate

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incubation and brooding duties, a critical depletion of energy stores ashore can result from delayed relief by the partner, perhaps due to food shortage at sea. It has been observed that below a threshold body mass and adiposity the sparing of protein that characterizes phase II of fasting is no longer possible (Cherel et al., 1988; Robin et al., 1988). At this stage, penguins enter phase III of fasting, a period of accelerated protein catabolism that remains reversible provided its duration is limited. In prolonged fasting, non-breeding, captive emperor penguins, the transition from phase II to phase III is accompanied by a rise in locomotor activity and by attempts to escape the pen (Robin et al., 1998). These behavioral changes were interpreted as reflecting an increase in the drive to re-feed. Further, in captive-incubating king penguins, the drive to incubate progressively decreases after reaching the threshold body mass, the egg being eventually abandoned a few days later (Groscolas et al., 2000). The hypothesis that these behavioral changes correspond to an emergency response and are directed towards survival at the expense of current breeding was supported by the finding that all abandoning birds rapidly restore their energy reserves after departing to re-feed at sea (Robin et al., 2001).

Although it is possible that in penguins, egg abandonment is related to a re-feeding signal and that this signal is linked to a metabolic shift (rise in the catabolism of body protein at the phase II–phase III transition), measurements of the nutritional state of egg abandoning birds has not been examined except for body mass (Olsson, 1997; Groscolas et al., 2000; Gauthier-Clerc et al., 2001) and plasma uric acid (Robin et al., 2001). Moreover, what the components of the signal are and how it works is unknown. From the observation that in fasting but non-breeding emperor and king penguins, entrance into phase III is concomitant to a rise in the plasma level of corticosterone (Cherel et al., 1988; Robin et al., 1998), it has been suggested that this hormone might be involved in the redirection of behavior from incubation to searching for food. This suggestion is supported by the findings that in birds, corticosterone stimulates protein catabolism and gluconeogenesis (Holmes and Philips, 1976; Gray et al., 1990) whereas elevated plasma levels of this hormone promote food searching in response to nutritional stress in the Japanese quail (Bray, 1993), dark-eyed junco (Gray et al., 1990) and white-crowned sparrow (Astheimer et al., 1992). Also, administration of corticosterone caused breeding birds to redirect their behavior away from reproductive activities and appeared to promote increased foraging (Wingfield, 1988; Silverin, 1986; Wingfield and Silverin, 1986). Whether the same applies in fasting penguins, and notably whether egg or chick abandonment at critical energy depletion is related to increased levels of plasma corticosterone has not been investigated.

In birds, an increase in prolactin secretion plays a role in the induction and maintenance of incubation behavior (Sharp et al., 1988; Buntin, 1996). In most species, stimuli from the nest, egg or chick are necessary to maintain elevated prolactin secretion rates and prolactin decreases within the few hours or days after these stimuli are removed (Hall, 1987; Book, 1991; Wang and Buntin, 1999). Several studies suggest that in pelagic seabirds such as penguins, prolactin secretion is endogenously scheduled

over the long-term (Garcia et al., 1996; Lormée et al., 1999; Vleck et al., 2000). However, this does not preclude the possibility that in the short-term prolactin secretion might be modulated by various stimuli, including nutritional ones. A decrease in the secretion of prolactin as a consequence of critical exhaustion of energy stores could therefore be an endocrine component of the signal that triggers egg desertion and re-feeding in the king penguin. Low levels of prolactin have actually been measured in emaciated king penguins (Cherel et al., 1994a). However, whether they were breeders that had deserted, and whether they were caring for an egg or a young chick was unknown. Similarly, it is unknown whether other hormones such as LH could be involved in breeding abandonment. Such a possibility cannot be discarded since it has been suggested that in the emperor penguin the maintenance of high plasma concentration of LH might be related to brooding and territorial behavior (Groscolas et al., 1986; Lormée et al., 1999).

The major aim of the present study was to further characterize the nutritional state of king penguins at egg abandonment and to determine whether this abandonment could be related to changes in the secretion of corticosterone, prolactin or LH. Given that a chick might be a stronger stimulus than an egg, and/or that a parent might be more reluctant to abandon the former than the latter, a second aim was to compare the nutritional and endocrine states at chick vs egg abandonment. In the king penguin, the onset of breeding is spread over several months, from November to February, and only early breeders have a chance to successfully raise a chick (Stonehouse, 1960; Weimerskirch et al., 1992). Consequently, a third aim was to test the possibility that early breeders are more reluctant to abandon their egg, and thus accept a greater deterioration of their nutritional state before abandoning. The endocrine and nutritional states of king penguins were determined in freely-living birds at egg or chick abandonment and compared to those of incubating or brooding penguins who had experienced less severe energy depletion. In addition, these states were determined at egg abandonment in captive incubating birds in comparison to those of captive non-breeding birds at a similar fasting stage, and examined in relation to the timing of breeding. Some of the results on plasma metabolites and hormones obtained at egg abandonment in captive birds were briefly discussed and cited as unpublished data in a previous review (Groscolas and Robin, 2001).

Materials and methods

The study was conducted in the breeding colony of Baie du Marin, Possession Island, Crozet Archipelago (46°26'S, 51°52'E, Indian Ocean) during two consecutive breeding seasons. This colony represents about 25,000 pairs of king penguins habituated to human presence.

Species

King penguins (*Aptenodytes patagonicus*) are large-sized seabirds breeding in dense colonies in the sub Antarctic region. They do not make nests, the single egg being kept on the feet under the brood pouch. The egg is laid after 10–15 days of courting ashore and left for the male to incubate, usually within hours of laying (Stonehouse, 1960). The duration of the first incubation shift is 16 ± 0.5 days

(Robin et al., 2001) and the total fasting duration of males at the onset of breeding is therefore close to 1 month. Thereafter, the pair alternates incubation shifts of 2 weeks each until hatching (Weimerskirch et al., 1992), which occurs after 53 days of incubation (Stonehouse, 1960). Usually, the egg hatches during shift 4 and the newly hatched chick is fed with food preserved by the parent in its stomach (Gauthier-Clerc et al., 2000). Thus, this fasting shift includes incubation and then brooding duties. The chick abandonments observed in this study occurred at the end of this shift (see Results). During the first month of its life the chick is kept under the brood pouch and fed by each parent in alternating bouts averaging 1 week (Weimerskirch et al., 1992). Both males and females are known to abandon their egg when the return of the partner is delayed (Olsson, 1997; Gauthier-Clerc et al., 2001). The proportion of breeding failures due to egg abandonment by lean birds averages 8–13%, reaching 61% in unfavourable years (Olsson, 1995; Gauthier-Clerc et al., 2001). Other breeding failures are mostly due to bad weather (flooding), accidental egg loss or predation.

Sampling states

Freely incubating and brooding birds

The metabolic and hormonal states of free-living king penguins were determined at six stages of breeding: 1) beginning of an incubation shift (BI), 2) day 12 of an incubation shift (D12), 3) relief at the end of a normal (egg not abandoned) incubation shift (R), 4) relief at the end of a normal (chick not abandoned) brooding shift (EBS), 5) egg abandonment (EA), and 6) chick abandonment (CA).

Most of the sampled birds had been banded at the onset of incubation and then observed daily. From December 2002 to February 2003, 110 males were banded at the flipper at the onset of the first incubation shift, within 24 h following laying. They were sexed based on the observation that males always perform the first incubation shift (Stonehouse, 1960). The females of 30 of these males were banded the day after they relieved their mate at the end of the first incubation shift. At the beginning of the first incubation shift (day 3 of incubation), a blood sample was taken from 32 males, followed by the weighing of them. Since our previous observations (Groscolas, unpublished data) have shown that in males the minimum duration of the first incubation shift is 12 days, blood sampling was performed at this stage on 18 other birds. Daily observations of banded birds allowed detection of the return of the female and thus relief of the male from incubating in 25 pairs. The males of these pairs were sampled and then weighed within 1 h following relief by the female. At this stage birds had been incubating for 12 to 21 days. A blood sample and the weight were also taken from five unsexed birds at the point of relief by the partner at the end of a brooding shift. Given the small size of their chicks, these birds were likely sampled at the end of the first brooding shift. The latter sampling was performed because this part of the study was mostly based on the comparison of relieved vs abandoning birds and because at relief the metabolic and hormonal states of brooding birds could be different from those of incubating ones.

The regular checking of the colony by several people from a distance, including watching the banded birds whose breeding state was well known, allowed the observation of 14 spontaneous abandonments, six of eggs (egg abandonment, EA) and eight of very young chicks (chick abandonment, CA). These abandonments occurred between February 8 and March 15 for eggs and between February 14 and March 26 for chicks. Only abandonments preceded by the changes in behavior previously described in energy depleted and abandoning birds were considered. These changes include transitory abandonments lasting a few minutes and during which the parent moved a few meters away from the egg or chick (Groscolas et al., 2000). Comfort activities (e.g. stretching, flipper flapping) and calls were also behaviors usually observed before abandonment. Following definitive abandonment, the bird was watched from distance until it had left the colony. Then it was caught and a blood sample was immediately taken, followed by weighing and release at the edge of the colony. Blood sampling was performed within the hour following abandonment (at an average of 20 min for the 9 birds, the abandonment and capture of which were accurately timed).

Captive incubating birds

Captive, incubating birds were used to further characterize the metabolic and hormonal states of egg abandoning birds in comparison to those at the beginning of incubation, but using a far larger number of birds than under free-living

conditions. Further, captive-incubating birds allowed the testing for an effect of the timing of breeding on the body condition and metabolic and endocrine states at egg abandonment.

To overcome the difficulty of sighting abandoning birds within the colony, egg abandonment was experimentally induced by preventing relief by the partner. This was done by penning incubating birds in groups of 5–10 under natural weather conditions, knowing that in this situation king penguins continue to incubate and behave normally (Groscolas et al., 2000). The method of penning and the conditions of captivity were as previously described (Groscolas et al., 2000; Robin et al., 2001). During the 2003–2004 breeding season, a total of 37 males at day 3 of the first shift of incubation were caught in the colony and kept in pens (3 × 3 × 1.7 m high) made of wood and located 50 m from the colony. This precluded the captive birds from sighting birds within the colony or from hearing calls from relieving mates. To limit the impact of the experiment on the colony, birds breeding in unfavourable locations where natural egg mortality is known to be close to 100% (e.g. within an area submitted to frequent flooding) were used. Males categorized as very early (average laying date = 21 November, $n=7$), early (average laying date = 14 December, $n=13$), late (average laying date = 16 January, $n=5$) and very late (average laying date = 7 February, $n=12$) breeders were successively penned. They were divided into six groups of 5–7 individuals, the individuals within a group being penned within 3 days. Before penning, 20 of the birds were sampled and weighed to characterize their initial (beginning of the first incubation shift) metabolic and hormonal states. Once in pens, all birds were marked on the chest with a dye to allow further identification during continuous video recording (see Groscolas et al., 2000 for technical details). Thereafter, penguins were left undisturbed until an abandonment was observed. Video recording was used to check that abandonment behavior was normal, i.e. that egg abandonment was preceded by short transitory periods (maximum duration of 30 min) during which the egg was not being incubated (Groscolas et al., 2000). The egg was considered as definitively abandoned when it had not been incubated for 2 h. At this point, the pen door was opened allowing the abandoning bird to egress the pen spontaneously, usually within 1 h. A blood sample was immediately taken followed by weighing and the recording of morphometric measurements and then the bird was released at the edge of the colony. The bird subsequently departed to re-feed at sea within the following 24 h. It has been shown that under such circumstances all the birds survived and came back to the colony after having restored their energy reserves (Robin et al., 2001). The duration of incubation at egg abandonment ranged from 12 to 43 days.

Captive non-incubating birds

Captive fasting but non-incubating male and female penguins were used to compare the metabolic and endocrine states of captive birds at egg abandonment to those of non-breeding birds at fasting durations and body masses known from previous studies to correspond to phase III fasting. To date, the metabolic and hormonal states of phase III fasting females has not been examined. In January–February 2004, 13 males and 7 females were caught in the breeding colony while courting, which is about 10 days before the onset of incubation (Stonehouse, 1960). The studied fasting period was therefore exactly the same as for captive- and freely-incubating birds, i.e. the first fasting period of the breeding cycle including courtship and the first incubation shift. Birds were marked and penned under conditions strictly identical to those for incubating birds in the egg abandonment experiment. Repeated weighing and calculation of daily body mass loss allowed the estimation of the time at which captive penguins would reach approximately the same body mass as those at egg abandonment. On that day, birds were sampled, weighed and released at the edge of the colony from which they departed to re-feed at sea within the following 24 h. The duration of captivity reached 25.7 ± 1.8 days in males and 21.7 ± 1.0 days in females vs 19.1 ± 1.4 days in penned incubating males.

All procedures were approved by the Ethical Committee of the Institut Polaire Français Paul-Emile Victor (IPEV) and by the Polar Environment Committee of the Terres Australes et Antarctiques Françaises. They comply with current French laws. Bands were removed at the end of the study.

Blood sampling, weighing and morphometric measurements

Blood samples (3–5 ml) were taken from a flipper marginal vein using a 5 ml heparinized syringe fitted with a 21G needle. Incubating birds were sampled while standing up whereas non-incubating birds were sampled while lying on their back, the head being covered with a hood in both cases to limit

agitation. The duration of blood sampling, starting from the point of bird capture, was precisely timed. No sampling period lasted longer than 5 min, i.e. considerably less than the handling duration (10 min) at which a significant stress-induced increase in plasma corticosterone is observed in king penguins (Ménard, 1998). According to the breeding or fasting states considered in the present study ($n=10$), the average duration of blood sampling ranged from 1.6 ± 0.2 to 3.3 ± 0.3 min. Consequently, all data on corticosterone levels were considered in calculations. Following collection, blood was kept at $+4^\circ\text{C}$ for less than 1 h until centrifugation for 10 min at 4500 rpm. Plasma was kept frozen into 0.3 ml aliquots at -20°C and analyzed within 6 months.

Following blood sampling, birds were weighed to the nearest 20 g on a platform balance. To determine the body condition of captive incubating males at egg abandonment, and to relate this condition to the timing of breeding (early vs late), body size was determined from the measurement of the length of the flipper and of the beak (upper jaw, from the feather limit to the extremity) to the nearest mm using a metal rule.

Plasma analysis

The plasma concentration of uric acid (UA) and non-esterified fatty acids (NEFA) were measured by enzymatic colorimetric methods using commercial kits (UA: SIGMA Diagnostics; NEFA: Wako chemicals). The measurement was performed on whole plasma (UA: 25 μl ; NEFA: 10 μl). In fasting penguins, plasma UA is known to reflect the intensity of body protein catabolism (Robin et al., 1988). It remains at a steady and low level during phase II of fasting and progressively increases during phase III (Cherel et al., 1988; Robin et al., 1988), thus providing a reliable index of the severity of the depletion of energy stores. In penguins, plasma NEFA also remains steady during phase II, then it increases at the transition from phase II to phase III and eventually decreases when fat stores approach total exhaustion (Groscolas, 1986; Cherel et al., 1988).

Radioimmunoassay (RIA) was used to measure the plasma concentration of corticosterone and prolactin. Corticosterone was measured in duplicate using a double-antibody ^{125}I RIA kit (MP Biomedicals, cat. no. 07-120103) designed for mouse and rats and previously validated in-house for king penguins (Bernard et al., 2002). Plasma (20 μl) was diluted with steroid diluent at a 1:40 ratio instead of the 1:200 recommended ratio. Intra- and inter-assay coefficients of variation were 6% and 9%, respectively, and sensitivity of the assay was 1.0 ng ml^{-1} . Prolactin was measured in triplicate using a double-antibody method with an anti-chicken prolactin serum, as previously described and validated for king penguins (Cherel et al., 1994a). The intra-assay variance was 2.8% and all samples were measured in a single radioimmunoassay to exclude inter-assay variation. The plasma concentration of LH was determined in triplicate in free-living birds using a radioimmunoassay, as described and validated previously for king penguins (Mauget et al., 1994). The intra-assay variance was 5.2%. Prolactin and LH concentrations were determined at CEBC in Chizé (France).

Statistics

Values are expressed as means \pm S.E.M. Data were checked for normality of distribution and homogeneity of variance, and those data that were not normally distributed were analyzed using nonparametric tests. In free-living birds, we made comparisons between the four stages of incubation, between the two stages of brooding and also between egg and chick abandoning birds. Comparisons of two means (duration of incubation, body mass, plasma concentrations) were performed using paired or unpaired Student's *t*-tests, as appropriate, or the Mann–Whitney *U* test. Multiple comparisons of means (body mass and plasma concentrations at the four stages of incubation in free-living birds, at the four stages considered for captive birds and at the four breeding times) were made using one-way ANOVA or the Kruskal–Wallis test. Post-hoc comparisons were performed using the Student–Newman–Keuls or the Dunn's test, respectively. In captive, egg abandoning birds, a body size index (BSI) was derived from size measurements by performing a principal component analysis including log-transformed lengths of beak and flipper (Lafi and Kaneene, 1992; Green, 2001). The first principal component (PC1) had the highest degree of correlation with the two variables and explained 60.2% of the total variance in bird size. The factor score from the PC1 was used as our BSI. An index of body condition (IBC) was obtained by computing residuals of a regression analysis with log body mass (to obtain homogeneity of variance) as the dependent variable and BSI as the independent variable ($R^2=0.202$, $P=0.005$, $n=37$; Schulte-Hostedde et al.,

2005). Least-squares linear regression analyses with *F* tests were performed to investigate whether at egg abandonment in captive birds the plasma concentration of metabolites and hormones were related, and whether these concentrations depended on body condition. The relationship between body condition at egg abandonment and the timing of breeding was similarly tested in these birds. All tests were two-tailed and performed using SigmaPlot 10.0 software, the level of significance being set at $P<0.05$.

Results

Freely incubating and brooding birds

Characteristics of abandonments

Amongst egg abandonments in free-living birds, four were from males at the end of shift 1 of incubation and two were from unsexed birds during unknown incubation shifts. Amongst chick abandonments, two were from males, two from females and four from unsexed birds. Maximum chick age at abandonment was visually estimated at 10 days. The four chick abandonments from sexed birds occurred at the end of the shift during which the chick hatched, that is after the parent had been incubating and then brooding. It is likely that this was also the case for the four chick abandonments from unsexed birds given the small size of abandoned chicks.

For sexed and regularly checked birds, the duration of the incubation shift at egg abandonment (21.7 ± 2.4 days, $n=4$) was the same as the duration of the incubation plus brooding shift at chick abandonment (21.8 ± 3.5 days, $n=4$; $t_6=0.081$, $P=0.938$). These durations were 6 days more than for birds at the point of relief by the partner at the end of an incubation shift (15.8 ± 0.6 days, $n=25$), although the differences were not significant ($U<86.0$, $P>0.100$).

Body mass and plasma metabolites

For incubating birds, BM at egg abandonment was 1 kg (10%) lower than at relief ($F_{2,59}=48.298$, $P<0.001$; post-hoc: $P=0.007$, Fig. 1). The plasma UA concentration was 1.5-fold higher at egg abandonment than at the onset of incubation ($H_3=10.674$, $P=0.014$; post-hoc: $P<0.05$) whereas the plasma NEFA concentration was 1.6-fold higher at egg abandonment than at other stages, notably relief ($F_{3,76}=5.893$, $P=0.001$; post-hoc: $P<0.001$).

For brooding birds, BM and plasma NEFA concentration were, respectively, 0.8 kg (7.5%) lower ($t_{11}=-3.355$, $P=0.006$) and 2.5-fold higher ($U=15.0$, $P=0.002$) at chick abandonment than at relief at the end of brooding shift (Fig. 1). Plasma UA concentration at chick abandonment tended to be higher than at relief at the end of a brooding shift, although not significantly ($U=23.5$, $P=0.093$).

BM ($t_{12}=-1.661$, $P=0.123$), plasma UA concentration ($U=34.5$, $P=0.181$) and plasma NEFA ($t_{12}=-0.203$, $P=0.842$) were not significantly different in egg vs chick abandoning birds.

Plasma hormones

For freely incubating birds, plasma CORT and PRL concentrations at egg abandonment were, respectively, 2.2-fold higher ($H_3=29.262$, $P<0.001$; post-hoc, $P<0.05$) and 3-fold

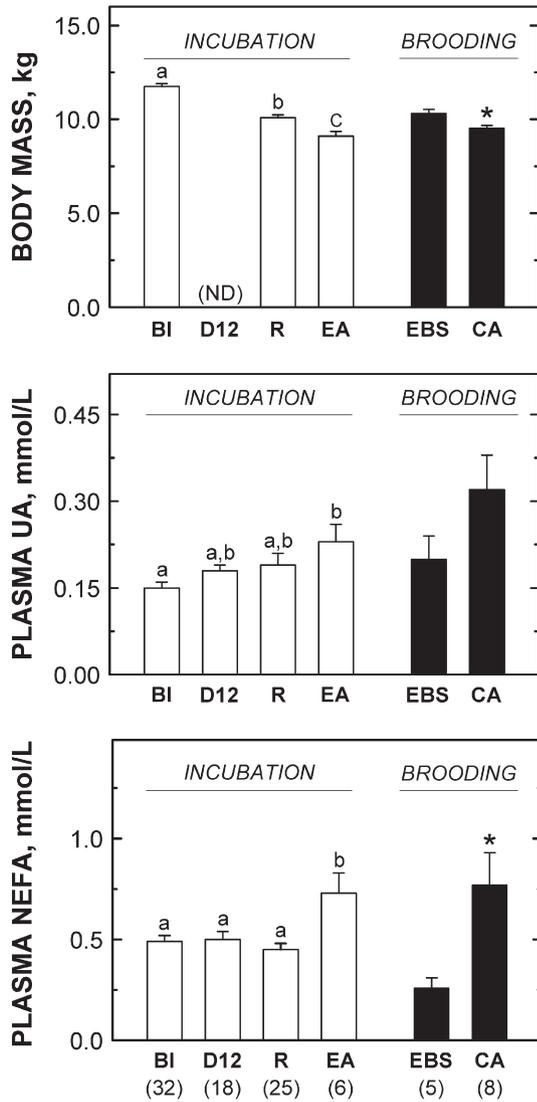


Fig. 1. Body mass and plasma levels of uric acid and NEFA at different stages of incubation and chick brooding in free-living king penguins. Values are means±S.E.M. The sample size is in brackets and for a given stage it was the same for the three parameters. ND, not determined. Within incubating birds, values not sharing the same superscript letter are significantly different ($P<0.05$). Within brooding birds, an asterisk denotes a significant difference between abandoning and relieved birds. BI, beginning of incubation; D12, day 12 of the incubation shift; R, relief by partner at the end of incubation shift; EA, egg abandonment; EBS, relief by partner at the end of brooding shift; CA, chick abandonment.

lower ($F_{3,76}=25.181$, $P<0.001$; post-hoc: $P<0.001$) than at relief (Fig. 2). At relief, plasma CORT ($P<0.05$) and PRL ($P<0.001$) were higher than at the beginning of incubation. The plasma LH concentration of egg abandoning birds did not differ from that of birds at relief or at the beginning of incubation ($P>0.05$).

For brooding birds, plasma CORT, PRL and LH concentrations were, respectively, 4.4-fold higher ($U=16.0$, $P=0.003$), 1.4-fold lower ($U=55.0$, $P=0.002$) and not significantly different ($t_{11}=0.222$, $P=0.828$) at chick abandonment compared to relief at the end of a brooding shift.

The plasma concentration of CORT and PRL were, respectively, 2.3- ($U=26.0$, $P=0.013$) and 2.2-fold higher ($t_{12}=-3.560$,

$P=0.004$) at chick than at egg abandonment, the LH concentration not being significantly different ($t_{12}=1.164$, $P=0.267$) in the two situations.

Captive birds

Metabolic and hormonal states

Captive incubating birds abandoned their egg after a duration of incubation (22.1 ± 1.4 days, $n=37$) similar to that in free-living birds ($U=83.5$, $P=0.999$). BM ($t_{41}=-0.296$, $P=0.769$), plasma PRL, UA and NEFA levels ($U<168.0$, $P>0.201$) at egg

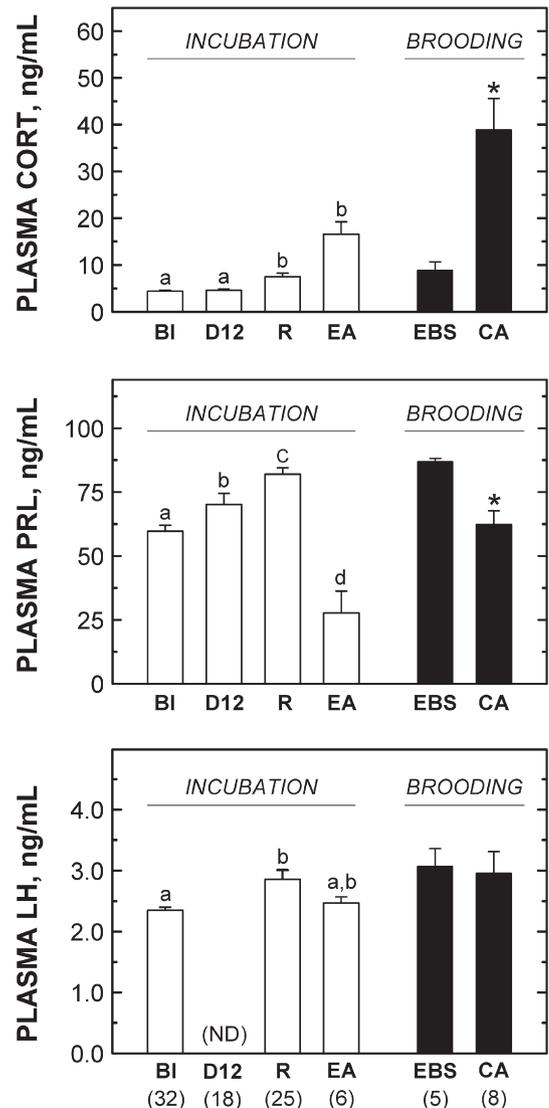


Fig. 2. Plasma level of corticosterone, prolactin and LH at different stages of incubation and chick brooding in free-living king penguins. Values are means±S.E.M. The sample size is in brackets and for a given stage it was the same for the three hormones. ND, not determined. Within incubating birds, values not sharing the same superscript letter are significantly different ($P<0.05$). Within brooding birds, an asterisk denotes a significant difference between abandoning and relieved birds. BI, beginning of incubation; D12, day 12 of the incubation shift; R, relief by partner at the end of incubation shift; EA, egg abandonment; EBS, relief by partner at the end of brooding shift; CA, chick abandonment.

Table 1
Body mass (BM), and plasma levels of uric acid (UA), NEFA, corticosterone (CORT) and prolactin (PRL) in captive, incubating and captive, non-incubating king penguins

State	(n)	BM, kg	UA, mmol/l	NEFA, mmol/l	CORT, ng/ml	PRL, ng/ml
Beginning of incubation	(20)	11.37±0.15	0.15±0.01	0.51±0.03	4.3±0.2	58.4±2.2
Egg abandonment	(37)	8.99*±0.13	0.33*±0.03	0.92*±0.08	26.4*±2.0	14.7*±1.3
Non-incubating, males	(13)	8.99±0.11	0.65 ^a ±0.08	0.74±0.10	26.5±2.7	5.2 ^a ±0.4
Non-incubating, females	(7)	8.57±0.26	0.76 ^a ±0.09	0.66±0.09	32.9±4.4	8.4 ^a ±1.0

Values are means±S.E.M.

*Significantly different from the beginning of incubation when comparing the birds ($n=20$) which were sampled at both stages (paired t -test, $P<0.001$). ^aSignificantly different from egg abandonment, $P<0.001$.

abandonment were also similar for captive and free-living birds, plasma CORT levels being 1.6-fold higher ($U=75.0$, $P=0.048$) in the captive ones. At egg abandonment, captive birds had a 2.4-kg lower BM, 2-fold higher plasma UA and NEFA levels, a 6-fold higher plasma CORT and a 4-fold lower plasma PRL than at the beginning of incubation (paired t -test, $t_{19}>3.785$, $P<0.001$; Table 1). There was not a significant relationship between plasma CORT, PRL, UA and NEFA levels of captive birds at egg abandonment ($F_{1,35}<2.941$, $R^2<0.081$, $P>0.096$).

For similar BM ($F_{2,54}=1.080$, $P=0.347$), captive incubating birds at egg abandonment and captive non-incubating males and females penguins had similar plasma NEFA ($H_2=2.093$, $P=0.351$) and CORT ($F_{2,54}=0.959$, $P=0.390$) levels (Table 1). Plasma UA was 2-fold higher in non-incubating males and females compared to incubating birds at egg abandonment ($F_{2,54}=17.689$, $P<0.001$) whereas plasma PRL was 2- to 3-fold lower ($H_2=23.447$, $P<0.001$).

Effect of the timing of breeding

Table 2 shows that in captive incubating birds BM at egg abandonment was significantly higher in early, late and very late breeders compared to very early breeders ($F_{3,33}=3.304$, $P=0.032$; post-hoc: $P<0.05$) whereas plasma metabolites and hormones concentrations were not affected ($F_{3,33}<2.111$, $P>0.118$). Since BM at abandonment was related to flipper ($F_{1,35}=6.406$, $R^2=0.155$, $P=0.016$) and beak length ($F_{1,35}=4.271$, $R^2=0.109$, $P=0.046$), differences in BM could be partly related to differences in body size. A body size index was calculated from beak and flipper length, allowing for the calculation of an index of body condition (IBC, see "Materials and methods"). IBC at egg

Table 2
Effect of the timing of breeding on body mass (BM), plasma levels of uric acid (UA), NEFA, corticosterone (CORT) and prolactin (PRL) at egg abandonment in captive, incubating king penguins

Breeding timing	(n)	BM, kg	UA, mmol/l	NEFA, mmol/l	CORT, ng/ml	PRL, ng/ml
Very early	(7)	8.23 ^a ±0.21	0.45±0.10	0.87±0.12	21.5±3.5	12.1±2.3
Early	(13)	9.08 ^b ±0.21	0.33±0.06	1.09±0.18	22.7±2.7	17.1±2.6
Late	(5)	9.36 ^b ±0.44	0.30±0.10	0.72±0.22	34.6±8.4	14.0±3.2
Very late	(12)	9.21 ^b ±0.20	0.26±0.03	0.85±0.08	30.0±3.1	14.1±2.0

Values are means±S.E.M.

Within a column, values not sharing the same superscript letter are significantly different, $P<0.05$.

abandonment increased as breeding was delayed ($F_{1,35}=7.443$, $R^2=0.175$, $P=0.010$; Fig. 3). At abandonment, IBC was directly related to plasma UA ($F_{1,35}=8.446$, $R^2=0.194$, $P=0.006$), but not to plasma NEFA, CORT and PRL levels ($F_{1,35}<1.780$, $R^2<0.048$, $P>0.191$).

Discussion

The nutritional state at egg or chick abandonment

The first aim of the present study was to further characterize the nutritional state of king penguins at spontaneous egg or chick abandonment. Our results show that abandoning birds *i.* had been fasting for 6 days longer than birds that did not abandon and were relieved by their partners, *i.i.* had body masses lower than the 9.6–10.0 kg threshold body mass considered as corresponding to the phase II–phase III transition in male king penguins (Cherel et al., 1988, 1994b), and *i.i.i.* had increased plasma uric acid and NEFA level, as observed in the present and in previous studies in captive–non breeding birds at the phase III stage of fasting. The present results further show that in the latter birds, nutritional state was similar in males and females (previous studies have only considered males), and that the nutritional state of egg abandoning birds was comparable whether the birds were free-living or captive. Lastly, the direct relationship found between plasma levels of uric acid and the

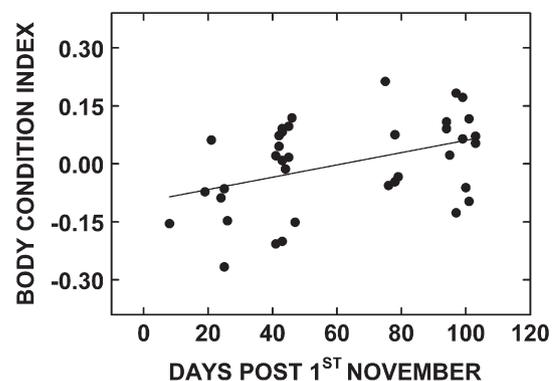


Fig. 3. Relationship between body condition at egg abandonment and the timing of breeding in captive-incubating king penguins. The timing of breeding is expressed as the duration (days) between the onset of incubation and the 1st November. $IBC = -0.090 + 0.00159 * \text{Days}$, $n = 37$.

index of body condition at egg abandonment in captive birds confirms that this level is a very good index of body condition in fasting penguins. Based on previous findings in fasting but non-breeding penguins (Cherel et al., 1988; Robin et al., 1988), these results collectively demonstrate that egg abandonment occurs when king penguins are in phase III of fasting, where there is an increase in body protein breakdown after the achievement of a critical depletion of fat stores. Given that body mass and plasma levels of uric acid and NEFA in chick abandoning birds were similar to those in egg abandoning penguins (free-living and captive), the same conclusion applies for chick abandonment. The latter results further demonstrate that the intensity of energy depletion at abandonment is comparable in incubating and brooding penguins. This suggests that the tolerated depletion is not dependent on whether an egg or a chick is abandoned or whether birds have been repeatedly fasting or not during the breeding season.

Another objective of the study was to investigate the possibility that the nutritional state of king penguins at egg abandonment depends on the timing of breeding. The rationale for such a hypothesis was that parents would be more reluctant to abandon their egg if laid early because the egg is of greater value (will yield a chick with a higher survival probability) than if laid late in the season. Our results show that body condition at egg abandonment actually increases throughout the breeding season, being the lowest in very early breeders. This finding supports the view that breeding king penguins are able to “sense” the value of their egg in terms of predicted chick survival, and/or that early breeders have a greater drive to incubate and are willing to tolerate a larger energy depletion than do late breeders. Perhaps, the amount of energy depletion that can be tolerated depends on reproductive experience and on foraging proficiency, as previously suggested for male king penguins (Olsson, 1997).

Corticosterone and the control of egg or chick abandonment

Whether corticosterone and prolactin could intervene in the control of egg or chick abandonment was examined through the measurement of their circulating levels. King penguins which were abandoning were characterized by high levels of plasma corticosterone, these levels being comparable to those measured during a handling stress protocol in non-breeding adults (Ménard, 1998). Notably, at chick abandonment, plasma corticosterone levels were as high as the plateau level (40 ng/ml) measured during immobilization stress, suggesting that chicks were abandoned when corticosterone secretion was maximally stimulated. The main metabolic effect of corticosterone is an increase in net protein degradation, mainly through a suppression of synthesis but also via an increase in breakdown (Challet et al., 1995). An increase in the plasma level of this hormone in response to various stressors and as a means to increase the availability of energy substrates has been repeatedly demonstrated, including in birds during food deprivation (Harvey et al., 1984). It has also been suggested that the perception of food deprivation as a stressor appears to be dependent upon an individual's assessment of its energy reserves, probably in the

form of depot fat (Astheimer et al., 1992). Thus, it is very likely that in abandoning king penguins, reaching critical exhaustion of the fat depot was perceived as stressful and that the increase in protein breakdown, as reflected by high plasma levels of uric acid, was stimulated by high circulating levels of corticosterone. How a limited availability of fat depots stimulates corticosterone secretion remains to be determined. One possibility is that hepatic fatty acid oxidation decreases when fat stores become critically depleted and that this decrease is sensed by hepatic receptors connected to the CNS via vagal afferents, as suggested in mammals (Langhans and Scharrer, 1987). Activation of the HPA axis would then stimulate corticosterone secretion by the adrenals. Such a mechanism is supported by the observation that in king penguins the experimental blockade of fatty acid oxidation induces an increase in circulating corticosterone (Bernard et al., 2002).

In addition to its metabolic role, corticosterone has behavioral effects. In phase III fasting rats, its involvement in the rise in locomotor activity, interpreted as an increased drive for re-feeding, together with an increase in protein breakdown, has been demonstrated (Challet et al., 1995). In nutritionally stressed birds, elevated plasma levels of corticosterone promote behaviors related to food searching (Gray et al., 1990; Astheimer et al., 1992; Bray, 1993) whereas administration of corticosterone in breeding birds promote increased foraging (Silverin, 1986; Wingfield, 1988). It has also been hypothesized that the increase of locomotor activity, the beginning of foraging activity and the propensity to disperse is promoted by elevated plasma levels of corticosterone in independent juvenile screech-owls (Belthoff and Dufty, 1998). Similarly, a transient elevation of circulating corticosterone around fledging has been reported in nestlings of several bird species, suggesting that an endogenous elevation of the secretion of this hormone could facilitate the decision to depart from the nest (Heath, 1997; Schwabl, 1999; Simms and Holberton, 2000). Lastly, a contribution of corticosterone to hyperphagia has been demonstrated in a bird species, the ring dove, these orexigenic effects being mediated in part by CNS receptors (Koch et al., 2002). Altogether, these results strongly suggest that in birds, corticosterone is involved in the control of feeding behavior and/or of associated locomotor activity. We suggest that the same applies in king penguins, and that this hormone plays a major role in the decision to abandon the egg or chick by stimulating the drive to re-feed.

Prolactin and the control of egg or chick abandonment

Plasma prolactin levels were markedly depressed at egg abandonment, whether penguins were free-living or captive and whatever the timing of breeding. Given that in birds prolactin plays a role in the induction and maintenance of incubation behavior (Sharp et al., 1988; Buntin, 1996), this decrease could have caused a decrease in the drive to incubate. Thus, egg abandonment could be induced by a simultaneous increase in plasma corticosterone and decrease in plasma prolactin. Previous studies have provided evidence that in penguins prolactin secretion is mostly endogenously controlled (Mauget et al., 1994;

Vleck et al., 2000). It could therefore be suggested that this secretion was depressed at the point of critical fat store exhaustion, which characterizes egg abandonment. The finding that there was no relationship between plasma prolactin levels and IBC at egg abandonment argues against a direct effect of body condition. An inhibitory effect of corticosterone on prolactin secretion, as demonstrated in mammals (Freeman et al., 2000) and in a bird species that fasts while incubating, the common eider *Somateria mollissima* (Criscuolo et al., 2005), could be postulated. However, the finding that at chick abandonment, plasma prolactin levels remained elevated in the face of very high corticosterone levels and also that prolactin was high at relief in freely incubating birds whereas corticosterone was slightly increased argue against such a direct dependence of prolactin secretion on corticosterone secretion. The interactions between the two hormones are actually complex, as illustrated for example by the observation that in doves, intracranial injections of prolactin increases plasma corticosterone (Koch et al., 2004). Thus, our results did not allow us to discriminate whether the secretion of the two hormones was inter-related or whether it was independently controlled.

Prolactin can be seen either as a stimulator of parental behavior or as a result of parental activity, stimuli from the nest, egg or chick being necessary to maintain elevated rates of secretion (Buntin, 1996). The possibility that at egg abandonment the plasma prolactin levels partly reflected the intensity of such stimuli must therefore be considered. It has been observed that within the 48 h following accidental egg loss plasma prolactin levels did not decrease significantly in king penguins kept in individual enclosures (Jouventin and Mauget, 1996), whereas nest failure has no (females) or limited (males) effect on prolactin levels in Adélie penguins (Vleck et al., 2000). Thus, prolactin secretion would be relatively independent of tactile output in penguins. This suggestion is not supported by the present finding that plasma prolactin levels were slightly but significantly higher at the end of an incubation shift (relief) than at its onset, and that for a similar nutritional state plasma levels of prolactin were higher in captive birds that have been incubating (egg abandoning males) compared to the non-incubating ones (males and females). Thus, the egg would to some extent stimulate prolactin secretion while the reduced egg attentiveness observed within the 2 days preceding egg abandonment (Groscolas et al., 2000) could have contributed to reduced prolactin levels. In the king penguin, the chick is kept in the brood pouch during the month following hatching, as was the egg during the entirety of incubation. It is therefore likely that in this species a chick is a greater stimulus than an egg because it can stimulate parents not only visually and tactilely but also audibly through calls for food. In mammals, of the many inputs controlling prolactin secretion, the effect of specific sounds is one of the most responsive and robust (Freeman et al., 2000). The finding that at chick abandonment, plasma prolactin levels were only slightly depressed could therefore be explained by the high stimulatory effect of a chick on prolactin secretion. As a way to overcome the limited reduction in the drive to brood associated with the moderate decrease in plasma prolactin, a very strong increase in the drive to re-feed should be

required to induce chick abandonment. Such a compensatory mechanism is suggested by the observation that in free-living birds, plasma corticosterone was more than two-fold higher at chick than at egg abandonment, the corticosterone/prolactin ratio being close to 0.6 in both cases. The comparison of egg and chick abandoning penguins thus indicates that a massive reduction in the concentration of circulating prolactin is not a pre-requisite for abandonment. Further, the finding that body mass and plasma uric acid level were similar at egg and chick abandonment whereas plasma prolactin levels differed by more than two-fold supports the view that, as suggested above, in fasting-breeding king penguins prolactin secretion is not under the control of nutritional state alone.

A marked decrease in plasma prolactin levels at egg abandonment could be adaptative through reducing the antagonist action of prolactin on LH and gonadal hormones (Sharp et al., 1998), and thus allowing rebreeding. Rebreeding has been observed in king penguins provided the egg is lost early in the season, thus leaving time for a new breeding attempt (Robin et al., 2001; Viera et al., 2006). Thus, if the drop in plasma prolactin at egg abandonment was a mechanism facilitating rebreeding, this drop could be expected to be higher in early than in late breeders. This was not the case, plasma prolactin being similarly depressed whatever the timing of breeding. In addition, an increase in plasma LH was not observed in association with the decrease in plasma prolactin, in contrast to observations of other bird species within the hours following nest loss or egg removal (see Vleck et al., 2000 for references). Although there are numerous pieces of evidence showing that in birds the activity of the hypothalamo–pituitary–gonadal axis is reduced under conditions of negative energy state, such an increased secretion of LH has been reported in fasting king penguins after pharmacological depression of prolactin levels (Jouventin and Mauget, 1996). Thus, the drop in plasma prolactin observed here at egg abandonment does not seem to be related to the possibility of starting a new breeding attempt. The finding that plasma LH levels were the same in abandoning and normally relieved birds, and the same as basal levels measured previously during incubation, brooding and molt (Mauget et al., 1994; Jouventin and Mauget, 1996) also indicates that severe energy depletion has no effect on LH secretion, and that this hormone is probably not involved in the process of egg or chick abandonment.

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