

Energetic adjustments in freely breeding-fasting king penguins: does colony density matter?

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Summary

1. For seabirds that forage at sea but breed while fasting on land, successful reproduction depends on the effective management of energy stores. Additionally, breeding often means aggregating in dense colonies where social stress may affect energy budgets.
2. Male king penguins (*Aptenodytes patagonicus*) fast for remarkably long periods (up to 1.5 months) while courting and incubating ashore. Although their fasting capacities have been well investigated in captivity, we still know very little about the energetics of freely breeding birds.
3. We monitored heart rate (HR, a proxy to energy expenditure), body temperature and physical activity of male king penguins during their courtship and first incubation shift in a colony of some 24 000 freely breeding pairs. Males were breeding either under low but increasing colony density (early breeders) or at high and stable density (late breeders).
4. In early breeders, daily mean and resting HR decreased during courtship but increased again 3 days before egg laying and during incubation. In late breeders, HR remained stable throughout this same breeding period. Interestingly, the daily increase in resting HR we observed in early breeders was strongly associated with a marked increase in colony density over time. This finding remained significant even after controlling for climate effects.
5. In both early and late breeders, courtship and incubation were associated with a progressive decrease in physical activity, whereas core body temperature remained unchanged.
6. We discuss the roles of decreased physical activity and thermoregulatory strategies in sustaining the long courtship–incubation fast of male king penguins. We also draw attention to a potential role of conspecific density in affecting the energetics of breeding-fasting seabirds, that is, a potential energy cost to coloniality.

Key-words: body temperature, energy expenditure, fasting, heart rate, physical activity, seabird, social density, stress

Introduction

Energy availability and its efficient use and management constrain many aspects of animal ecology, shaping life-history strategies and evolutionary trade-offs (Drent & Daan 1980; Martin 1987; McNamara & Houston 1996; Green *et al.* 2009). This is particularly true during reproduction, where parents not only have to allocate energy to their own maintenance but also need to meet the energy requirements of courtship, incubation and chick growth.

Trade-offs can be even more challenging when resources become limiting, for example during prolonged periods of fasting. Accordingly, most seabirds forage at sea but breed while fasting on land (Lack 1968; Ricklefs 1983; Dobson & Jouventin 2007). Hence, their reproductive success is expected to rely on the efficient management of energy stores ashore, and critical depletion of these stores may result in breeding failure (Olsson 1997; Ancel, Fetter & Groscolas 1998; Gauthier-Clerc *et al.* 2001). Whereas the physiology of fasting seabirds (especially penguins) has been well studied under captive and non-breeding conditions (Cherel *et al.* 1988a; Cherel, Leloup & Le Maho

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1988b; Cherel *et al.* 1994; reviewed in Groscolas & Robin 2001), relatively little is known on the energetic adjustments of freely breeding individuals.

To ensure breeding success, fasting seabirds are expected to minimize energy expenditure (EE) through behavioural and/or metabolic adaptations, as for instance by minimizing the energy cost of physical activities (Viera *et al.* 2011) or thermoregulation (Dewasmes *et al.* 1980; Gilbert *et al.* 2007). In addition, breeding seabirds typically crowd into large colonies (e.g. Guinet, Jouventin & Malacamp 1995) where nest sites are aggressively defended from territorial conspecifics (Côté 2000; Stokes & Boersma 2000; Kokko, Harris & Wanless 2004). As social stimuli may strongly modulate stress levels (Boonstra & Boag 1992; Kotrschal, Hirschenhauser & Mostl 1998; Wascher, Scheiber & Kotrschal 2008; Dantzer *et al.* 2013; reviewed in Creel *et al.* 2013) and metabolic rates (Sloman *et al.* 2000; Fuchs & Flügge 2002; Cao & Dornhaus 2008), seabirds also offer an ideal opportunity to examine how EE may be affected by social factors (e.g. conspecific density). For fasting seabirds, documenting energy costs linked to coloniality is especially relevant as they have no immediate means of compensation, by adjusting their daily energy intake for instance.

When breeding, colonial king penguins (*Aptenodytes patagonicus*; Fig. 1) lay a single egg, build no nest, but defend a small territory on which they settle after pairing. Breeders display high rates of aggressiveness towards neighbours (Côté 2000) and are highly sensitive to their social surroundings (Viblanc *et al.* 2012). Due to the prolonged period required to rear a chick (11–12 months; Weimerskirch, Stahl & Jouventin 1992), reproduction in king penguins is asynchronous and breeding onset ranges from early November to March (Stonehouse 1960; Weimerskirch, Stahl & Jouventin 1992). Early breeders start reproducing at low and fluctuating densities, and the number of breeders will progressively increase during the

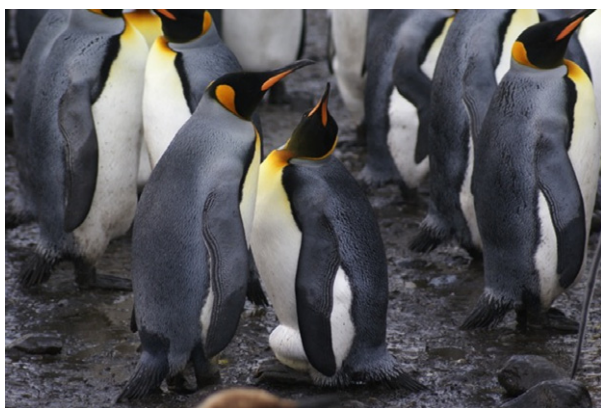


Fig. 1. Early king penguin (*Aptenodytes patagonicus*) pair on its breeding territory in the midst of a crowded colony. Once the female has laid the egg, the male takes duty of the first incubation shift for c. 17 days on average. Including the courtship period (c. 15 days on average), males therefore fast for approximately a month at the beginning of the breeding cycle. Photograph Copyright: N. Malosse.

breeding season due to colony replenishment until stabilizing at high density. As a result, late breeders reproduce under steady but high breeding densities.

Using colonial king penguins as a study system, the objectives of this study were (i) to determine how EE was modulated during the course of long-term fasting in naturally breeding seabirds and (ii) to examine how changes in the social environment, namely colony density, might affect EE. For this, we specifically focused on males that fast on average for 1 month between the start of courtship (c. 13–18 days from arrival at the colony to egg laying) and the end of their first incubation shift (c. 17 days from laying to relief by the partner; Descamps *et al.* 2002).

To assess changes in EE during courtship and incubation, we monitored heart rate (HR) as a proxy to EE (Butler *et al.* 2004; Green 2011). We also monitored changes in physical activity (ACTI) or body temperature (T_b) over the same period to determine their potential contribution to changes in HR and energy savings. In addition, monitoring HR in early and late breeders that were of identical breeding status but differed in the colonial environment they experienced was expected to provide some information on a potential energy cost of breeding at high density. Finally, to examine adjustments in HR related to fasting but independent of the breeding process and changes in colonial density, we continuously monitored HR and body mass in long-term fasting males that were caught at the onset of breeding but kept captive out of the colony (thus non-breeding) under natural weather conditions.

Materials and methods

FIELD PROCEDURE

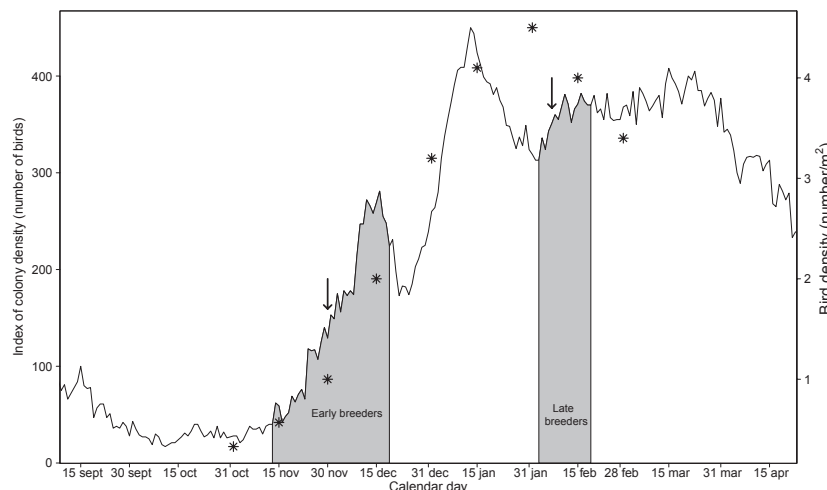
This study was conducted during the 2008–2009 breeding season in the king penguin colony of ‘La Baie du Marin’ (c. 24 000 breeding pairs), Possession Island, Crozet Archipelago (46°25'S, 51°45'E). The study area was a subcolony located centrally in the colony and occupied by up to 5000 breeding pairs.

Freely breeding birds

Male king penguins were caught soon after arriving ashore for breeding, identified using a non-permanent animal dye (Porcimar[®]; Kruuse, Langeskov, Denmark) and flipper bands (semi-rigid P.V.C. Darvic bands; 25.8 mm wide, 1.9 mm thick, 7.4 g) and fitted with data loggers. Sex was determined based on courtship behaviour and later confirmed according to sex-specific breeding cycle chronology (males are the first to incubate the egg, Stonehouse 1960). After marking and logger attachment, birds were checked from a distance at least twice a day, and breeding phenology (courtship duration, settlement on territory, egg laying and incubation) was established from field observations at ± 1 day.

Based on the date of courtship onset, we distinguished two groups of courting-incubating males: (i) early-breeding birds (courtship onset range = 11 November–2 December 2008, $N = 14$) and (ii) late-breeding birds (27 January–8 February 2009, $N = 10$). Early birds bred in a colony of initially low but rapidly increasing social density, whereas late breeders experienced high but stable density conditions (Fig. 2). Early breeders were monitored for an

Fig. 2. Changes in colony density over the 2008–2009 reproductive season for king penguins breeding in the Baie du Marin, Possession Island, Crozet Archipelago. Breeding density (stars): number of breeding birds per square meter in the study area. Colony density index (continuous line): number of transponder-tagged breeding birds present in a nearby subcolony of fixed area (see text). Shaded zones under the density index curve indicate time periods during which early and late breeders were followed, with arrows showing the respective average laying dates. The date of the first observed laid egg in the study area was November 10.



average duration of 27.6 ± 0.9 days (range = 21–33 days): 12.5 ± 1.3 days (range = 2–21 days) before egg laying (courtship) and 15.1 ± 0.9 days (range = 7–19 days) afterwards (incubation). Late breeders were monitored for an average duration of 16.5 ± 1.6 days (range = 9–25 days): 4.9 ± 0.8 days (range = 1–9 days) before egg laying and 11.6 ± 0.9 days (range = 8–16 days) afterwards (Fig. 2). Whereas early breeders could be easily and rapidly (1–2 days) identified when coming ashore to breed, this was more complicated in late breeders because of colony crowding. Thus, in our study, late breeders were likely marked and fitted with loggers after having been in the colony for several days, explaining why the monitoring duration of the prelaying period was shorter in those birds. Data collection for early breeders ended when birds were relieved by their partner. For late breeders, however, due to time constraints with fieldwork, we retrieved loggers a few days before expected relief by the partner to avoid losing data loggers at sea. This constraint, as well as the fact that late breeders present accelerated phases of the breeding cycle compared with early breeders (Gauthier-Clerc *et al.* 2002), explains why late breeders were also monitored for a slightly shorter period during incubation. At the end of the study, all loggers and flipper bands were retrieved. No bird abandoned reproduction during the monitoring period.

Captive birds

To disentangle the effects of fasting (and acclimatization to captivity) from breeding activity and/or changes in bird density on HR, we also investigated changes in HR and body mass in eight early males caught in the colony shortly (1–3 days) after courtship onset (early December). These birds were kept in wooden pens ($3 \text{ m} \times 3 \text{ m}$), close to the colony and under natural weather conditions, at a density of three birds per pen (0.33 bird m^{-2}). This density was similar to the lowest density observed in freely breeding birds (see Fig. 2). In one of the three pens, only two birds had a HR logger. Penned birds fasted for a total duration of 25 days, which is within the range of fasting duration of free-living incubating birds.

The body mass of captive birds was measured ($\pm 10 \text{ g}$) using a platform scale. Body mass was obtained daily during the first 4 days of captivity. Subsequently, to avoid unnecessary disturbance, body mass was measured when resetting HR loggers (at days 6, 9, 13, 17, 21 and 25). The time duration between consecutive body mass measurements was recorded, and daily body mass loss (dm/dt in g day^{-1}) between mass measurements (e.g. from day 1 to day 2 or day 6 to day 9) was calculated.

HR, ACTIVITY AND BODY TEMPERATURE MONITORING

Free-living birds were fitted with data loggers measuring HR, core body (stomach) temperature and overall physical activity. Captive birds were only fitted with HR loggers.

Heart rate

Heart rate was monitored using external cardio-frequency meters (Polar® model RS800; Polar Electro Oy, Kempele, Finland) as previously described in Groscolas *et al.* (2010), including details on logger attachment, technology and accuracy of HR measurement. The logger transmitter was attached to the dorsal feathers of the animals using adhesive tape (Tesa®, Tesa SE, Hamburg, Germany) and the receptor was secured to a flipper band. Loggers did not appear to interfere with the usual routine of the birds, as individuals soon resumed normal activity (courting, preening, stretching, sleeping and fighting) after handling, could not be distinguished in behaviour the subsequent day and successfully paired and engaged in breeding. HR was sampled continuously at 5-s intervals. Because of the limitation of logger memory, birds were caught for a few minutes every 4 days for logger reset, directly on the bird.

Although HR has been used as a proxy to metabolic rates in field studies (Butler *et al.* 2004; Green 2011), a number of issues complicate its conversion to actual EE (typically measured as oxygen consumption, VO_2). Variability in the HR– VO_2 relationship within and between individuals increases the errors around the estimates produced when using calibration equations from different individuals and/or under different conditions than the ones under study (Green 2011). For instance, the HR– VO_2 relationship may be affected by gender, physical activity, physiological or nutritional status, and stress (Froget *et al.* 2001, 2002; Green *et al.* 2001; Fahlman *et al.* 2004; Groscolas *et al.* 2010). In particular, previous HR– VO_2 calibrations in king penguins may have been affected by measurement error (Groscolas *et al.* 2010) or captivity stress (Fahlman *et al.* 2004). In addition, birds' physical activity, environment or breeding status in those calibrations differed from those in the present study. For those reasons, we deliberately chose to present raw HR data as a qualitative, rather than quantitative, index of metabolic rate.

Physical activity

A measure of physical activity during courtship and incubation was obtained for eight early breeders and six late breeders using externally attached physical activity monitoring systems (Actical®;

Philips Respironics, Bend, OR, USA). The omni-directional accelerometer sensors monitored the occurrence and intensity of motion. Actical[®] devices stored the sampled information in the form of activity counts. Acceleration was sampled at 32 Hz. The highest of the 32 values recorded each second were summed over 30 s and archived on the data logger, allowing us to monitor the animals for 21 days. To avoid disturbing the animals unnecessarily, devices were changed prior to memory saturation at the same time as HR loggers were reset. Actical[®] devices (28 mm × 27 mm × 10 mm; 16 g) were attached beneath HR loggers, along the dorsal mid-line of the animal, halfway between the armpit and hip lines. Loggers were tightly glued on dorsal feathers, close to the skin, to detect body movements. We checked that this set-up integrated movements from both the upper (head, flippers) and lower (legs) body, yielding an index of overall bird activity.

Body temperature

Changes in core body temperature were recorded in nine early breeders and nine late breeders by continuously monitoring stomach temperature (Eichhorn *et al.* 2011). Temperature loggers (Thermochron i-buttons[®], model DS1922L: 5.0 mm width, 17.35 mm diameter, 3.2 g; Dallas Semiconductor, Dallas, TX, USA) were coated in epoxy resin and force-fed to the birds (Eichhorn *et al.* 2011). Loggers were later retrieved, easing them back up the oesophagus by pulling on a string from which one of the extremities was embedded in the resin of the logger and the other glued among the feathers beneath the bill. This relatively non-invasive set-up allowed us to monitor stomach temperature, deploying and recovering the temperature loggers without requiring heavy procedures such as anaesthesia and surgical implantation of temperature sensors. Loggers were set to sample stomach temperature every 10 min with a resolution of 0.01 °C. Temperature sensors were calibrated against a range of fixed temperatures using a hot water bath (range = 30–43 °C, temperature resolution ±0.01 °C, 1 °C increments, step duration = 20 min, sample rate = 1/5 s). Temperature recording during the calibration process proved highly accurate (correlation with set temperatures, $r = 0.996$, $n = 73$ 623).

CHANGES IN COLONY DENSITY

Two different estimates of colony density were obtained. First, a quantitative index of daily changes in numbers of reproductive birds (hereafter 'colony density index') was determined by monitoring the presence of electronically tagged individuals in a subcolony located in the vicinity of the study area. In this subcolony, where c. 8000 pairs breed every year, 6000 birds have been tagged (mostly as chicks) with passive electronic chips since 1998 (Gendner *et al.* 2005). In 2008–2009, c. 650 tagged adults bred in this subcolony. From mid-September 2008 to mid-April 2009, radio-frequency antennas buried under the usual transit pathways in and out of the subcolony allowed automatic detection of bird entry and departure from the colony (see Gendner *et al.* 2005 for details). Birds >4 years of age whose movements in and out of the subcolony matched the cycle of reproductive king penguins were considered as breeders (Saraux *et al.* 2011) and used to calculate the 'colony density index'. In addition, an index of breeding density in the study area (hereafter 'breeding density index') was estimated starting on 1 November 2008. Every second week, the distance between breeding birds (incubating birds or territorial pairs) was estimated visually at ±10 cm, in 10 different locations regularly spread over the study area. For each location, we randomly estimated 20 distances between breeding birds (amounting to 200 distances in total) which we averaged. As a general rule, each breeder was surrounded by six neighbours. Thus, the smallest unit that could be identified comprised seven birds. The area occupied by these seven birds corresponded to the circle centred on

one bird and of radius 1.5 times the average distance between breeders, to account for the entire area of neighbours. Breeding density index (number of breeders per square meter) was then calculated as $7/(\pi(1.5d)^2)$, where d is the mean interbreeder distance.

WEATHER CONDITIONS

Air temperature (at ±0.1 °C), wind speed (at ±0.1 m s⁻¹) and relative humidity (at ±1%) were sampled every minute throughout the study using a Vantage PRO 2 weather station (Davis Instruments, Hayward, Davis, CA, USA) installed on the colony site.

CALCULATIONS AND STATISTICS

Analyses were performed using R version 2.10.1 (R Development Core Team 2011). Data collected within an hour of bird capture were systematically discarded to eliminate biases from animal handling. Mean daily HR (dHR) was calculated by averaging all 5-s measurements over 24-h periods. We calculated daily resting HR (rHR) using moving averages to determine the 10 consecutive minutes (120 consecutive HR measurements) where HR was lowest over 24-h periods. Mean body temperature was averaged, and total activity counts summed, over 24-h periods. Breakpoints in the time course of physiological parameters during courtship and incubation were identified from segmented regression analysis using the 'strucchange' and 'segmented' packages from R (Zeileis *et al.* 2002; Muggeo 2008). Separate generalized estimating equations (GEEs) were used for pre- and post-breakpoint temporal analysis ('geepack' package in R; Højsgaard, Halekoh & Yan 2005). Bird identity was included as a random factor to account for repeated measures on an individual. To standardize and compare birds at similar breeding dates, data are presented relatively to the day of egg laying. Due to individual variations in courtship and incubation duration, transitory malfunctioning of data loggers and logistic reasons (logger removal 0–4 days before bird departure to sea), sample sizes varied across monitoring periods. Days with <3 individuals were not considered. Depending on the distribution of model residuals, normal or gamma distributions were used to investigate changes in HR and body temperature. Changes in daily activity counts were investigated using Poisson distributions. Values are reported as means ± SE and results are considered significant for $P < 0.05$.

ETHICAL NOTE

Procedures employed during the fieldwork were approved by the Ethical Committee of the French Polar Institute (Institut Polaire Français Paul Emile Victor, IPEV) and comply with current French laws. Authorizations to enter the breeding colony and handle birds were delivered by Terres Australes et Antarctiques Françaises. During manipulations (between 5 and 20 min), animals were hooded to keep them calm and reduce disturbance to neighbouring birds. HR and activity logger packages were <1% of adult body mass. Flipper bands were removed at the end of the study.

Results

COLONY DENSITY

Our two indices of bird density demonstrated marked and consistent changes throughout the reproductive season (Fig. 2). Both 'colony' and 'breeding' density indices were low at the start of breeding (30 tagged birds and 0.3 breeders m⁻² on the surveyed area at the time of first courtships). Then, both indices markedly increased to reach a

maximum level by mid- to late January (see Fig. 2). This maximum density was *c.* 12 ('colony density index') to 15 ('breeding density index') times higher than at the start of breeding. Specifically, the 'colony density index' increased by a factor 5 during early breeder monitoring, but was stable during the period of late breeder monitoring.

WEATHER CONDITIONS

Daily climate conditions were similar for early and late breeders. Average wind speed and humidity were similar for early (wind speed = 4.9 ± 0.4 m s⁻¹; relative humidity = $80.6 \pm 1.1\%$) and late breeders (wind speed = 4.7 ± 0.4 m s⁻¹; relative humidity = $84.0 \pm 1.2\%$; Wilcoxon tests: all $P > 0.06$). In early breeders, mean daily wind speed increased slightly from late November to late December ($F_{1,28} = 6.84$, $P = 0.01$). Ambient temperature was slightly higher for late than for early breeders (8.9 ± 0.3 °C vs. 7.6 ± 0.3 °C; t -test; $t = -3.05$, d.f. = 58.0, $P = 0.003$). For late breeders, it showed a slight increase from late January to late February ($F_{1,28} = 4.22$, $P = 0.049$).

CHANGES IN HR IN FREELY BREEDING BIRDS

Changes in HR

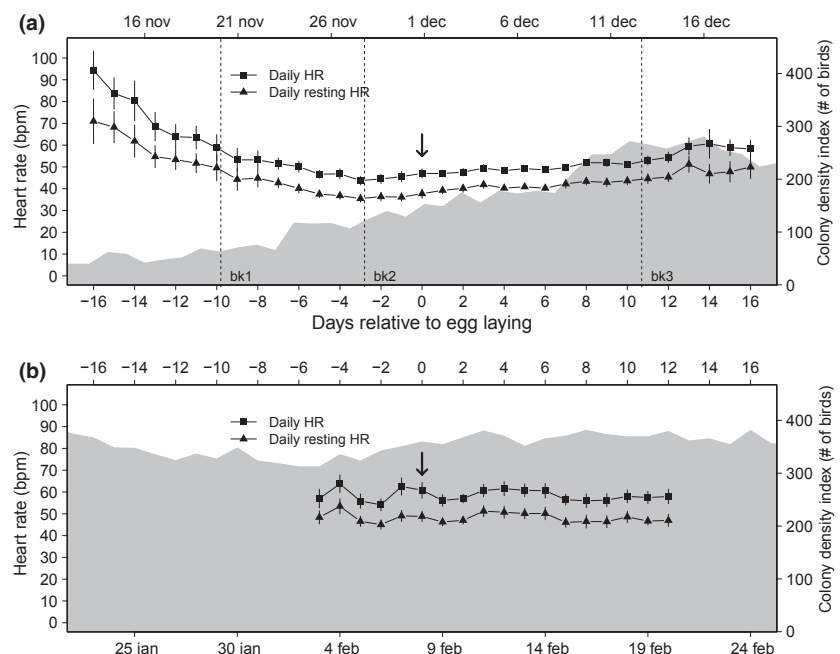
In early breeders, dHR decreased rapidly (-5.3 ± 1.3 bpm day⁻¹) during the first 6 days of courtship and then progressively (-1.7 ± 0.7 bpm day⁻¹) until *c.* 3 days before egg laying (Table 1, Fig. 3a). Starting on average on November 28 and 3 days before the onset of incubation, dHR started to increase by 0.6 ± 0.2 bpm day⁻¹. Eventually, dHR stabilized at 56.5 ± 1.4 bpm from day 11 (on average on December 12) of incubation onwards. Changes in rHR (Fig. 3a) paralleled those of dHR, with the same breakpoints. Resting HR reached a minimum value of 35.5 ± 1.6 bpm three days before egg laying. It subsequently increased by 33%, stabilizing at 47.1 ± 1.3 bpm within the final days of the incubation shift. Because the difference between dHR and rHR (i.e. HR due to changes in physical activity) remained constant during the period of HR increase (slope parameter not significantly different from zero; GEE; Wald = 0.25, $P = 0.62$, $n = 177$, $N = 14$ birds), this suggested that the increase in dHR was essentially driven by an increase in rHR.

Table 1. Intercept and slope coefficients (\pm SE) for the generalized estimating equations characterizing the time course of daily heart rate changes during courtship and incubation in early-breeding male king penguins

	Intercept	Wald	<i>P</i>	Slope	Wald	<i>P</i>	<i>n</i> (<i>N</i>)
Rapid decrease	4.5 ± 15.5	0.08	0.77	-5.3 ± 1.3	16.4	< 0.001	48 (10)
Slow decrease	39.1 ± 3.2	146.0	<0.001	-1.7 ± 0.7	5.8	0.02	77 (13)
Increase	46.6 ± 1.5	941.7	<0.001	0.6 ± 0.2	12.0	0.001	177 (14)
Stabilization	38.6 ± 11.7	10.8	<0.001	1.3 ± 0.9	2.2	0.13	36 (11)

Significant slope coefficients are bold-faced. Sample size is given as *n* and number of individual birds is given in parentheses (*N*).

Fig. 3. Daily changes in mean heart rate (dHR; squares) and mean resting HR (rHR; triangles) during courtship and first incubation shift in male king penguins. (a) Changes occurring in HR for early breeders relative to laying date. Breakpoints (bk1, bk2 and bk3) indicating changes in HR slopes are figured by black dashed lines (see text). Values are given as means \pm SE ($N = 3$ –14 birds). (b) Changes occurring in HR for late breeders relative to laying date. Values are given as means \pm SE ($N = 3$ –10 birds). For both (a) and (b), colony density index appears as the grey area and the arrow shows the laying date.



In late breeders, dHR was stable throughout the recording period (GEE; Wald = 0.3, $P = 0.60$, $n = 137$, $N = 10$ birds) and averaged 58.8 ± 0.8 bpm (Fig. 3b). Resting HR followed the exact same trend (Fig. 3b), remaining stable at the average level of 48.4 ± 0.6 bpm (Wald = 0.3, $P = 0.60$). These average dHR and rHR values were not significantly different from those observed at the end of incubation in early breeders when dHR and rHR were stabilized (Wald = 0.48 and 0.18, $P = 0.49$ and 0.67, for dHR and rHR, respectively).

Relationships to colony density

In early breeders, when dHR and rHR were at their minimum values (44.0 ± 1.9 and 35.5 ± 1.6 bpm, respectively), the 'breeding density index' was close to 1 breeder m^{-2} and the 'colony density index' indicated that c. 20% of tagged birds were present in the colony. Thus, dHR and rHR started to increase when colony density reached c. 30% of its maximum level (25–35% depending on whether the 'breeding density index' or the 'colony density index' was considered). When dHR and rHR stopped increasing, colony density was c. 43% ('breeding density index') to 65% ('colony density index') of its maximum. We investigated whether the observed increase in HR in early breeders could be linked to an increase in colony density. For each day from day -3 onwards (Fig. 3a), we calculated average rHR and 'colony density index'. We found that rHR was highly and significantly related to the 'colony density index' ($R^2 = 0.88$, $F_{1,18} = 136$, $P < 0.001$, $n = 20$ days; Fig. 4). Further, we accounted for potential climate effects causing rHR to increase independently of colony density over this period. Because T_a highly correlated with wind speed ($r = 0.77$, $P < 0.001$) and relative humidity ($r = 0.65$, $P = 0.002$), we used the first axis of a principal component analyses (PC1 = $0.64 T_a + 0.57$ wind + 0.51 humidity; this component explained 73.5% of the variance) to investigate the effects of climate variables on rHR. During the period of HR increase, we found that rHR was indeed positively associated with PC1 ($R^2 = 0.35$, $F_{1,18} = 9.58$, $P = 0.006$, $n = 20$ days) and thus potentially affected by climate. Interestingly however, using the residuals of this regression as a dependent variable to remove climate effects on rHR, we found that colony density remained highly significant and explained a substantial part of the residual variation in rHR ($R^2 = 0.46$, $F_{1,18} = 15.3$, $P = 0.001$, $n = 20$ days). In contrast, rHR was not correlated with colony density ($F_{1,16} = 0.5$, $P = 0.50$, $n = 18$ days; Fig. 4) in late breeders.

CHANGES IN HR AND BODY MASS IN CAPTIVE BIRDS

Changes in HR

In non-breeding males kept captive at low density, both dHR and rHR continuously decreased over the 25 days of

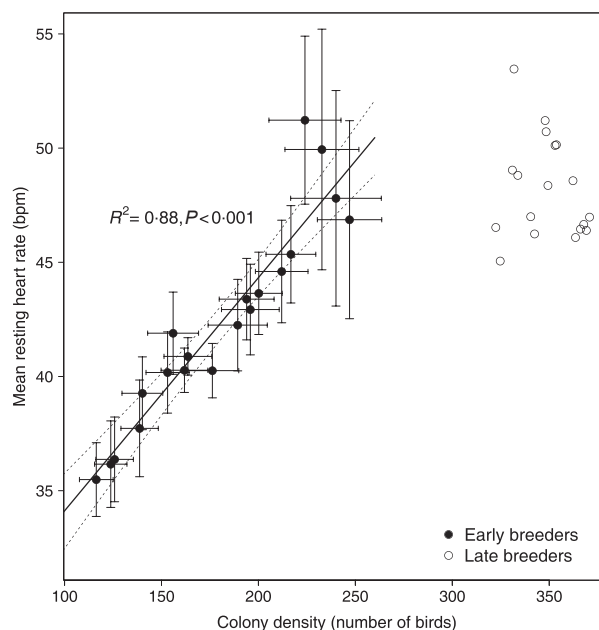


Fig. 4. Influence of colony density (number of birds) on resting heart rate (rHR, bpm) in breeding male king penguins. Significant regression line (solid line) and 95% confidence intervals (dashed lines) are given for early breeders (rHR = $24.0 \pm 1.8 + 0.1 \pm 0.01 \times \text{density}$). Values are means for 3–14 (early breeders) or for 3–10 (late breeders) birds for each day from 3 days before egg laying onwards (early breeders, $n = 20$ days), or throughout the whole monitoring period (late breeders, $n = 18$ days). For clarity, SE (bars) are presented only for early breeders.

the fast (Fig. 5). Decreases were rapid over the first 5 days of fasting, on average by -6.4 ± 1.5 (dHR) and -5.9 ± 1.1 (rHR) bpm day^{-1} , and then stabilized at a slower rate of -1.0 ± 0.3 (dHR) and -0.7 ± 0.2 (rHR) bpm day^{-1} for the remaining of the fast. Although the difference between dHR and rHR slightly reduced over the 25 days of fasting (-0.26 ± 0.09 bpm day^{-1} ; GEE; Wald = 8.12, $P = 0.004$, $n = 25$, $N = 8$ birds; Fig. 5), there was only a marginal decrease in dHR–rHR detectable before the 5-day breakpoint (-0.23 ± 0.13 bpm day^{-1} ; $P = 0.06$) and no substantial change afterwards (-0.45 ± 0.50 bpm day^{-1} ; $P = 0.37$).

Changes in body mass

Over the 25 monitoring days, captive birds lost as much as 26% of their initial body mass, that is, from 13.8 ± 0.1 to 10.2 ± 0.1 kg (Fig. 6a). Body mass initially decreased rapidly (-370 ± 23 g day^{-1}) until a breakpoint of 3.5 days, whereupon it was lost at a slower rate (-129 ± 23 g day^{-1} ; GEEs; all $P < 0.001$). This is well illustrated by the initially high, but rapidly decreasing dm/dt values observed during the first days of the fast (Fig. 6b). Both dHR and rHR significantly decreased with decreasing body mass (Fig. 6c) and decreasing daily body mass loss (Fig. 6d; GEEs; all $P < 0.001$).

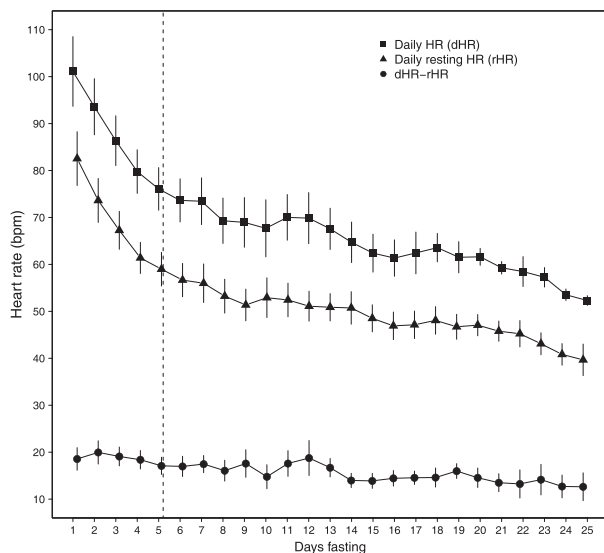


Fig. 5. Daily changes in mean heart rate (dHR; squares), mean resting HR (rHR; triangles) and HR related to activity (dHR–rHR; circles) during fasting in early male king penguins caught at courtship and kept captive in a pen under natural weather conditions at a density of 0.3 birds m^{-2} . Values are means \pm SE for eight birds. The dotted vertical line indicates a change in the slope of HR decrease. The equations for pre- and post-breakpoint regressions in dHR are: $dHR = 106.5 \pm 8.3 - 6.4 \pm 1.5$ days and $dHR = 79.0 \pm 7.7 - 1.0 \pm 0.3$ days, respectively. The equations for pre- and post-breakpoint regressions in rHR are: $rHR = 86.6 \pm 6.3 - 5.9 \pm 1.1$ days and $rHR = 60.1 \pm 5.2 - 0.7 \pm 0.2$ days, respectively.

BODY ACTIVITY AND BODY TEMPERATURE IN FREE-BREEDING BIRDS

Body activity

Daily body activity decreased progressively over the course of courtship and incubation in both early and late breeders. In early breeders, breakpoint analysis revealed a rapid (slope = -0.5 ± 0.09 ; Table 2) initial decrease in body activity over the period of strong HR decline (from days -16 to days -10 prior to egg laying). Afterwards, body activity decreased at a slow constant rate (Table 2, slope = -0.1 ± 0.05) until the end of the monitoring period (Fig. 7a). Breakpoint analysis did not reveal any change in slope for late breeders (Table 2, Fig. 7a).

Body temperature

No significant change in stomach temperature occurred in either early or late breeders (GEEs; Wald = 1.8 and 0.9, $n = 224$ and 144, $N = 9$ and 9 birds, $P = 0.20$ and 0.34, for early and late breeders, respectively; see Fig. 7b). In addition, we found no significant difference in body temperature between early- and late-breeding birds. Average body temperature was 38.1 ± 0.03 °C for early breeders vs. 38.3 ± 0.04 °C for late breeders (GEE; Wald = 0.57, $P = 0.45$, $n = 368$, $N = 18$ birds).

Discussion

ENERGETIC ADJUSTMENTS IN CAPTIVE PENGUINS

In captive males, both dHR and rHR decreased daily during fasting, paralleling a decrease in body mass. Rapid initial changes in HR within the first 5 days in the pen could reflect habituation to captivity and a shift from phase I to phase II fasting, a metabolic transition during which animals adjust to a preferential utilization of lipid stores to fuel their metabolism. This is confirmed by the observed changes in body mass loss (dm/dt) characteristic of this transition (Cherel, Robin & Le Maho 1988c; Groscolas 1990). Our results are similar to previous findings in king penguin (Fahlman *et al.* 2004) and support the view that during long-term fasting, the rate of EE is decreased by reducing basal metabolic rate (Cherel, Robin & Le Maho 1988c) and the energy expended for physical activity. Although marginal ($P = 0.06$), we observed a decrease in HR due to physical activity (dHR–rHR) during the initial 5 days of fasting. Subsequently, dHR–rHR remained constant, suggesting that the main adaptation to long-term fasting was a decrease in rHR (and by extension, resting metabolic rate) with increasingly efficient energy sparing as the fast progressed (Cherel, Robin & Le Maho 1988c; see below for a discussion of potential mechanisms).

ENERGETIC ADJUSTMENTS IN FREELY BREEDING PENGUINS

In freely breeding birds, changes in dHR and rHR during fasting differed markedly from captive individuals and between early and late breeders. In early breeders, both dHR and rHR initially decreased rapidly at the onset of courtship, increased again shortly before incubation and stabilized at the end of incubation. In late breeders, dHR and rHR remained stable throughout courtship/incubation at a value similar to early breeders at the end of incubation.

In early breeders, the observed decrease in dHR was partly explained by a strong concurrent decrease in physical activity related to breeding activities, as revealed by activity loggers. After selection of a breeding site (Stonehouse 1960), pairs rapidly decreased their physical activity mainly resumed to comfort behaviour and territorial defence (Viblanco *et al.* 2011; Viera *et al.* 2011). Could our HR loggers have misidentified muscle artefact as HR activity during peaks of physical activity in early courtship? The fact that similar HR values were observed in captive birds caught at the onset of courtship that could only display minimal movement in the pen (i.e. no courting, waddling around the colony or fights for breeding territory establishment) suggests this is unlikely. Actually, the marked decrease in rHR over the same period points to physiological adaptations affecting resting metabolic rate. Although thermoregulatory adaptations such as transient declines in body temperature may contribute to substantial energy savings in large birds (Handrich *et al.* 1997; Butler

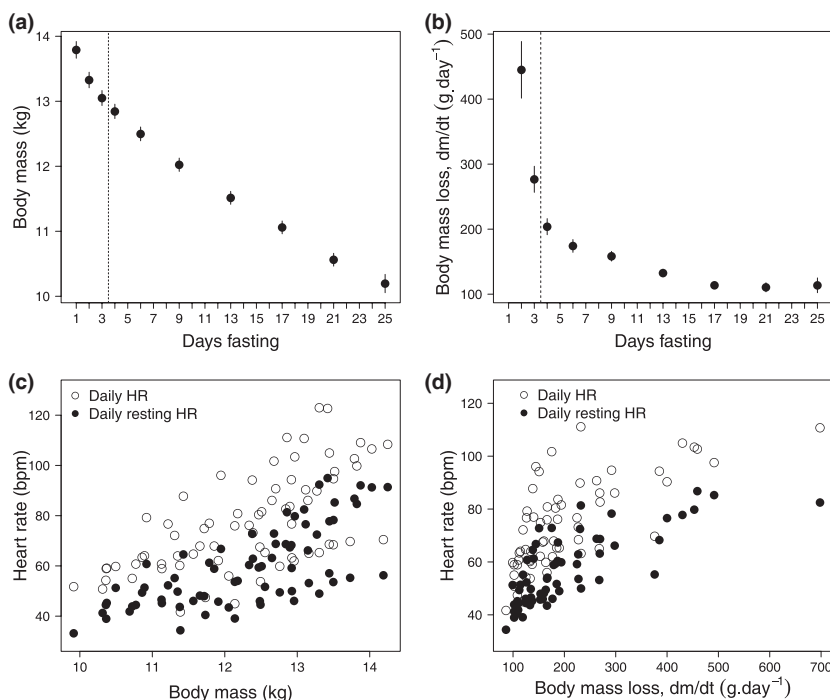


Fig. 6. Daily changes in (a) body mass and (b) body mass loss during fasting in early male king penguins caught at courtship and kept captive in a pen under natural weather conditions at a density of 0.3 birds m⁻². Values are means \pm SE for eight birds. The relationships between body mass and heart rate (HR) (c) and body mass loss and HR (d) are given.

Table 2. Intercept and slope coefficients (\pm SE) for the generalized estimating equations characterizing the time course of daily body activity during courtship and incubation in early (EB)- and late (LB)-breeding male king penguins

	Intercept	Wald	<i>P</i>	Slope	Wald	<i>P</i>	<i>n</i> (<i>N</i>)
EB							
Rapid decrease	3.7 \pm 0.9	15.4	<0.001	-0.5 \pm 0.09	36.0	<0.001	15 (3)
Slow decrease	7.7 \pm 0.3	509.5	<0.001	-0.1 \pm 0.05	6.8	0.009	113 (8)
LB							
Slow decrease	8.2 \pm 0.3	1043.7	<0.001	-0.2 \pm 0.04	18.5	<0.001	60 (6)

Significant slope coefficients are bold-faced. Sample size is given as *n* and number of individual birds is given in parentheses (*N*).

& Woakes 2001; Gilbert *et al.* 2007; Eichhorn *et al.* 2011), we did not observe marked changes in the core T_b of early and late breeders in the present study. Given the temperature requirements of incubation (around 36 °C for king penguin; Groscolas *et al.* 2000), one explanation is that departures from normothermia may be limited in breeding birds (Vehrencamp 1982; Csada & Brigham 1994; Gilbert *et al.* 2007). However, we did not examine peripheral decreases in T_b that would affect the volume of the body normally regulated at high and constant T_b . Yet, by decreasing the energy cost of thermoregulation, changes in core volume during long-term fasting may affect resting metabolic rate (Cherel, Robin & Le Maho 1988c; see Eichhorn *et al.* 2011 for a recent example in king penguin chicks). Whether such changes occur in freely breeding penguins remains to be examined. For instance, it would be interesting to understand whether and to what extent changes in core volume contribute to decreasing rHR during courtship and whether the increase in rHR observed shortly before the start of incubation may partly be linked

to an increase in core volume and peripheral T_b to warm the egg.

In late breeders, we did not observe similar rapid decreases in dHR/rHR nor physical activity during courtship. A likely explanation is that those birds were caught at a more advanced stage of courtship (5 days prior to egg laying), preventing us to detect such changes. Indeed, late breeders tended to establish their territory close to the site of capture and incubated shortly afterwards. For instance, if we consider monitoring days (up to -9 days before egg laying) excluded from our analyses because of low sample size for late breeders (<3 birds), HR values were more than 50% higher than those measured from day -5 onwards. This suggests that, similarly to early breeders and captive birds, an initial decrease in HR was likely during the early stages of fasting. Further, it is interesting to note that body activity decreased slowly during incubation, suggesting that behavioural adjustments in activity may have contributed to energy savings during this period.

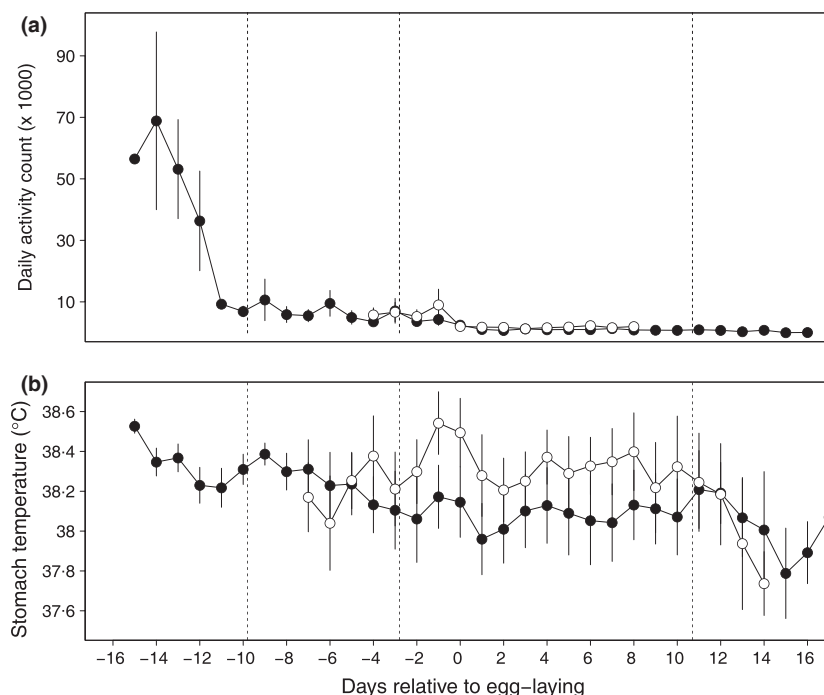


Fig. 7. Daily changes in (a) body activity and (b) body temperature during courtship and incubation in early (filled circles) and late (open circles) breeding male king penguins. Values are given as means \pm SE ($N = 3$ –14 birds for daily activity and $N = 3$ –9 birds for stomach temperature). For comparison, breakpoints identifying changes in the time course of heart rate for early breeders (see Fig. 5) are indicated as dashed lines.

ENERGETICS OF COLONIAL SEABIRDS: A ROLE FOR SOCIAL DENSITY?

The increase in dHR starting shortly before incubation in early (but not late) breeders is intriguing. As physical activity (loggers) decreased over this period and dHR–rHR remained constant, the pattern was driven by a significant increase in rHR. Interestingly, we found a strong positive association ($R^2 = 0.88$) between colony density and rHR in early breeders, which remained significant ($R^2 = 0.46$) after controlling for climate-induced effects on HR. In contrast, no such relationship was observed for late breeders at stabilized colony density. In early breeders, rHR eventually reached a plateau at a level similar to the rHR of late breeders during incubation, suggesting a maximum threshold to the effects of colony density on rHR.

Is the hypothesis of a conspecific density effect on the energy budgets (as reflected in the rHR) of breeding seabirds reasonable? Previous studies have documented links between social stimuli, stress levels and metabolic rates (Sloman *et al.* 2000; Fuchs & Flügge 2002; Cao & Dornhaus 2008). King penguins can be highly sensitive to their social environment (Viblanc *et al.* 2012), and current data suggest that birds incubating at high density exhibit higher basal stress hormone levels than birds at low density (Viblanc 2011). Thus, the possibility that increased social density may affect stress levels (Boonstra & Boag 1992; Rogovin *et al.* 2003; McCormick 2006; Creel *et al.* 2013; Dantzer *et al.* 2013) and HR at rest should be considered. Changes in rHR levels in breeding penguins may then only partly reflect changes in metabolic rate, because of the proportion of HR modulation attributable to stress (activation of the sympathetic nervous system; Groscolas *et al.*

2010).

Experimental manipulations of local colony density are needed to yield further information on the links between social density, HR and EE expenditure at rest in breeding penguins. First insight is provided by considering the relative increase in rHR for early birds, from 35.5 ± 1.6 bpm at low colony density (3 days before egg laying) to 47.1 ± 1.3 bpm at high colony density (when rHR stabilized). This corresponded to a 33% increase in rHR potentially due in part to an increase in colony density. In fact, this increase was probably underestimated, as the colony was not empty when rHR started increasing, but rather not quite half of maximum density (116/371 birds). Moreover, our results in captive birds suggest that rHR in incubating birds may have further decreased under the influence of the fast if they had been breeding out of the colonial context. Again, this could have contributed to underestimating the increase in rHR possibly due to the increase in colony density. If converted to VO_2 (eqn 1, Fahlman *et al.* 2004), the observed increase in rHR may have resulted in as much as a 21% increase in resting metabolic rate. However, because of the uncertainty of estimates produced using calibration equations from birds that differ from those under study (Green 2011), caution should be advocated and further investigations are required to determine whether the observed increase in rHR actually translates into a significant increase in resting metabolic rate. Those studies are urgently needed to determine to what extent breeding-fasting seabirds may have to support an extra energy cost imposed by their social environment, and to better appreciate the nature of social stimuli in conditioning important life-history variables such as

metabolic rates, breeding timing, breeding territory location and reproductive success in colonial breeders. Finally, regardless of the relationship between HR and EE, our study suggests that long-term monitoring of HR in field studies may yield important insights into individual exposure to stress in natural populations. We hope the present results will stimulate the inclusion of HR monitoring in future research on bioenergetics and stress in free-living animal populations.

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