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

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Abstract :

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.

Keywords : body temperature ; energy expenditure ; fasting ; heart rate ; physical activity ; seabird ; social density ; stress

48 **Introduction**

49 Energy availability and its efficient use and management constrain many aspects of animal
50 ecology, shaping life-history strategies and evolutionary trade-offs (Drent & Daan 1980;
51 Martin 1987; McNamara & Houston 1996; Green et al. 2009). This is particularly true during
52 reproduction, where parents not only have to allocate energy to their own maintenance but
53 also need to meet the energy requirements of courtship, incubation and chick growth. Trade-
54 offs can be even more challenging when resources become limiting, *e.g.* during prolonged
55 periods of fasting. Accordingly, most seabirds forage at sea but breed while fasting on land
56 (Lack 1968; Ricklefs 1983; Dobson and Jouventin 2007). Hence, their reproductive success is
57 expected to rely on the efficient management of energy stores ashore, and critical depletion of
58 these stores may result in breeding failure (Olsson 1997; Ancel, Fetter & Groscolas 1998;
59 Gauthier-Clerc et al. 2001). Whereas the physiology of fasting seabirds (especially penguins)
60 has been well studied under captive and non-breeding conditions (Cherel et al. 1988a; Cherel,
61 Leloup & Le Maho 1988b; Cherel et al. 1994; reviewed in Groscolas & Robin 2001),
62 relatively little is known on the energetic adjustments of freely-breeding individuals.

63 To ensure breeding success, fasting seabirds are expected to minimize EE through
64 behavioral and/or metabolic adaptations, as for instance by minimizing the energy cost of
65 physical activities (Viera et al. 2011) or thermoregulation (Dewasmes et al. 1980; Gilbert et
66 al. 2007). In addition, breeding seabirds typically crowd into large colonies (*e.g.* Guinet,
67 Jouventin & Malacamp 1995) where nest sites are aggressively defended from territorial
68 conspecifics (Côté 2000; Stokes & Dee Boersma 2000; Kokko et al. 2004). As social stimuli
69 may strongly modulate stress levels (Boonstra & Boag 1992; Kotrschal et al. 1998; Wascher
70 et al. 2008; Dantzer et al. 2013; reviewed in Creel et al. 2013) and metabolic rates (Sloman et
71 al. 2000; Fuuchs & Flügge 2002; Cao & Dornhaus 2008), seabirds also offer an ideal
72 opportunity to examine how EE may be affected by social factors (*e.g.* conspecific density).

73 For fasting seabirds, documenting energy costs linked to coloniality is especially relevant as
74 they have no immediate means of compensation, by adjusting their daily energy intake for
75 instance.

76 When breeding, colonial king penguins (*Aptenodytes patagonicus*) (Fig. 1) lay a single
77 egg, build no nest, but defend a small territory on which they settle after pairing. Breeders
78 display high rates of aggressiveness towards neighbours (Côté 2000) and are highly sensitive
79 to their social surroundings (Viblanç et al. 2012). Due to the prolonged period required to rear
80 a chick (11-12 months; Weimerskirch et al. 1992), reproduction in king penguins is
81 asynchronous and breeding onset ranges from early November to March (Stonehouse 1960;
82 Weimerskirch et al. 1992). Early breeders start reproducing at low and fluctuating densities,
83 and the number of breeders will progressively increase during the breeding season due to
84 colony replenishment until stabilizing at high density. As a result, late breeders reproduce
85 under steady but high breeding densities.

86 Using colonial king penguins as a study system, the objectives of this study were (1)
87 to determine how EE was modulated during the course of long-term fasting in naturally
88 breeding seabirds and (2) to examine how changes in the social environment, *i.e.* colony
89 density, might affect EE. For this, we specifically focused on males that fast on average for
90 one month between the start of courtship (~13-18 days from arrival at the colony to egg
91 laying) to the end of their first incubation shift (~17 days from laying to relief by the partner)
92 (Descamps, Gauthier-Clerc et al. 2002).

93 To assess changes in EE during courtship and incubation, we monitored heart rate
94 (HR) as a proxy to EE (Butler et al. 2004; Green 2011). We also monitored changes in
95 physical activity (ACTI) or body temperature (T_b) over the same period to determine their
96 potential contribution to changes in HR and energy savings. In addition, monitoring HR in
97 early and late breeders that were of identical breeding status but differed in the colonial

98 environment they experienced was expected to provide some information on a potential
99 energy cost of breeding at high density. Finally, to examine adjustments in HR related to
100 fasting but independent of the breeding process and changes in colonial density, we
101 continuously monitored HR and body mass in long-term fasting males that were caught at the
102 onset of breeding but kept captive out of the colony (thus non-breeding) under natural weather
103 conditions.

104

105 **Material and Methods**

106 FIELD PROCEDURE

107 This study was conducted during the 2008-2009 breeding season in the king penguin colony
108 of 'La Baie du Marin' (*ca.* 22,000 breeding pairs), Possession Island, Crozet Archipelago
109 (46°25'S, 51°45'E). The study area was a sub-colony located centrally in the colony and
110 occupied by up to 5000 breeding pairs.

111

112 *Freely breeding birds*

113 Male king penguins were caught soon after arriving ashore for breeding, identified using a
114 non-permanent animal dye (Porcimarck®, Kruuse, Germany) and flipper bands (semi-rigid
115 P.V.C. Darvic bands; 25.8 mm wide, 1.9 mm thick, 7.4 g), and fitted with data-loggers (see
116 below). Sex was determined based on courtship behaviour and later confirmed according to
117 sex-specific breeding cycle chronology (males are the first to incubate the egg, Stonehouse
118 1960). After marking and logger attachment, birds were checked from a distance at least twice
119 a day and breeding phenology (courtship duration, settlement on territory, egg-laying, and
120 incubation) was established from field observations at ± 1 day.

121 Based on the date of courtship onset, we distinguished two groups of courting-
122 incubating males: (1) early-breeding birds (courtship onset range = 11 Nov – 2 Dec 2008, N =

123 14); and (2) late-breeding birds (27 Jan – 8 Feb 2009, N = 10). Early birds thus bred in a
124 colony of initially low but rapidly increasing social density, whereas late-breeders
125 experienced high but stable density conditions (Fig. 2). Early breeders were monitored for an
126 average duration of 27.6 ± 0.9 days (range = 21 – 33 days): 12.5 ± 1.3 days (range = 2 – 21
127 days) before egg laying (courtship) and 15.1 ± 0.9 days (range = 7 – 19 days) afterwards
128 (incubation). Late breeders were monitored for an average duration of 16.5 ± 1.6 days (range
129 = 9 – 25 days): 4.9 ± 0.8 days (range = 1 – 9 days) before egg laying and 11.6 ± 0.9 days
130 (range = 8 – 16 days) afterwards (Fig. 2). Whereas early breeders could be easily and rapidly
131 (1-2 days) identified when coming ashore to breed, this was more complicated in late breeders
132 because of colony crowding. Thus, in our study late breeders were likely marked and fitted
133 with loggers after having been in the colony for several days, explaining why the monitoring
134 duration of the pre-laying period was shorter in those birds. Data collection for early breeders
135 ended when birds were relieved by their partner. For late breeders however, due to time
136 constraints with fieldwork, we retrieved loggers a few days before expected relief by the
137 partner to avoid losing data-loggers at sea. This constraint, and the fact that late-breeders
138 present accelerated phases of the breeding cycle compared to early breeders (Gauthier-Clerc
139 et al. 2002), explains why late breeders were also monitored for a slightly shorter period
140 during incubation. At the end of the study, all loggers and flipper-bands were retrieved. No
141 bird abandoned reproduction during the monitoring period.

142

143 *Captive birds*

144 To disentangle the effects of fasting (and acclimatation to captivity) from breeding activity
145 and/or changes in bird density on HR, we also investigated changes in HR and body mass in 8
146 early males caught in the colony shortly (1-3 days) after courtship onset (early December).
147 These birds were kept in wooden pens (3 m x 3 m), close to the colony and under natural

148 weather conditions, at a density of 3 birds per pen (0.33 bird.m^{-2}). This density was similar to
149 the lowest density observed in freely breeding birds (see Fig. 2). In one of the three pens, only
150 two birds had a HR logger. Pinned birds fasted for a total duration (25 days), which is within
151 the range of fasting duration of free-living incubating birds (see above).

152 The body mass of captive birds was measured ($\pm 10\text{g}$) using a platform scale. Body
153 mass was obtained daily during the first 4 days of captivity. Subsequently, to avoid
154 unnecessary disturbance, body mass was measured when re-setting HR loggers (at days 6, 9,
155 13, 17, 21, 25). The time duration between consecutive body mass measurements was
156 recorded, and daily body mass loss (dm/dt in g.day^{-1}) between mass measurements (*e.g.* from
157 day 1 to day 2 or day 6 to day 9) was calculated.

158

159 HEART RATE, ACTIVITY AND BODY TEMPERATURE MONITORING

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

162

163 *Heart rate*

164 Heart rate was monitored using external cardio-frequency meters (Polar® model RS800,
165 Polar Electro Oy, Kempele, Finland) as previously described in Groscolas et al. (2010),
166 including details on logger-attachment, technology and accuracy of HR measurement. The
167 logger transmitter was attached to the dorsal feathers of the animals using adhesive tape
168 (Tesa®) and the receptor was secured to a flipper-band. Loggers did not appear to interfere
169 with the usual routine of the birds, as individuals soon resumed normal activity (courting,
170 preening, stretching, sleeping, and fighting) after handling, could not be distinguished in
171 behaviour the subsequent day, and successfully paired and engaged in breeding. HR was

172 sampled continuously at 5 second interval. Because of the limitation of logger memory, birds
173 were caught for a few minutes every four days for logger reset, directly on the bird.

174 Although HR has been used as a proxy to metabolic rates in field studies (Butler et al.
175 2004; Green 2011), a number of issues complicate its conversion to actual EE (typically
176 measured as oxygen consumption, VO_2). Variability in the HR- VO_2 relationship within and
177 between individuals increase the errors around the estimates produced when using calibration
178 equations from different individuals and/or under different conditions than the ones under
179 study (Green 2011). For instance, the HR- VO_2 relationship may be affected by gender,
180 physical activity, physiological or nutritional status, and stress (Froget et al. 2001, Green et al.
181 2001, Froget et al. 2002, Fahlman et al. 2004, Groscolas et al. 2010). In particular, previous
182 HR- VO_2 calibrations in king penguins may have been affected by measurement error
183 (Groscolas et al. 2010) or captivity stress (Fahlman et al. 2004). In addition birds' physical
184 activity, environment or breeding status in those calibrations differed from those in the
185 present study. For those reasons, we deliberately choose to present raw HR data as a
186 qualitative, rather than quantitative, index of metabolic rate.

187

188 *Physical activity*

189 A measure of physical activity during courtship and incubation was obtained for 8 early
190 breeders and 6 late breeders using externally attached physical activity monitoring systems
191 (Actical®, Phillips Respironics Inc. Co., OR 97701 USA). The omnidirectional
192 accelerometer sensors monitored the occurrence and intensity of motion. Actical® devices
193 stored the sampled information in the form of activity counts. Acceleration was sampled at 32
194 Hz. The highest of the 32 values recorded each second were summed over 30 seconds and
195 archived on the data logger, allowing us to monitor the animals for 21 days. To avoid
196 disturbing the animals unnecessarily, devices were changed prior to memory saturation at the

197 same time as HR loggers were reset. Actical® devices (28 mm x 27 mm x 10 mm; 16 g) were
198 attached beneath HR loggers, along the dorsal mid-line of the animal, halfway between the
199 armpit and hip lines. Loggers were tightly glued on dorsal feathers, close to the skin, to detect
200 body movements. We checked that this set up integrated movements both from the upper
201 (head, flippers) and lower (legs) body, yielding an index of overall bird activity.

202

203 *Body Temperature*

204 Changes in core body temperature were obtained in 9 early breeders and 9 late breeders by
205 continuously monitoring stomach temperature (Eichhorn et al. 2011). Temperature loggers
206 (Thermochron i-buttons®, model DS1922L: 5.0 mm width, 17.35 mm diameter, 3.2 g, Dallas
207 Semiconductor, Dallas, Texas, 75244 USA) were coated in epoxy resin and forced-fed to the
208 birds (Eichhorn et al. 2011). Loggers were later retrieved, easing them back up the
209 oesophagus by pulling on a string from which one of the extremities was embedded in the
210 resin of the logger, and the other glued amongst the feathers beneath the bill. This relatively
211 non-invasive set-up allowed us to monitor stomach temperature, deploying and recovering the
212 temperature loggers without requiring heavy procedures such as anaesthesia and surgical
213 implantation of temperature sensors. Loggers were set to sample stomach temperature every
214 10 minutes with a resolution of 0.01°C. Temperature sensors were calibrated against a range
215 of fixed temperatures using a hot water bath (range = 30 to 43 °C, temperature resolution +/-
216 0.01°C, 1°C increments, step duration = 20 min., sample rate = 1/5 sec.). Temperature
217 recording during the calibration process proved highly accurate (correlation with set
218 temperatures; $r = 0.996$, $n = 73623$).

219

220 CHANGES IN COLONY DENSITY

221 Two different estimates of colony density were obtained. The first, a quantitative index of
222 daily changes in numbers of reproductive birds (hereafter ‘colony density index’) was
223 determined by monitoring the presence of electronically tagged individuals in a sub-colony
224 located in the vicinity of the study area. In this sub-colony, where approximately 8000 pairs
225 breed every year, 6000 birds have been tagged (mostly as chicks) with passive electronic
226 chips since 1998 (Gendner et al. 2005). In 2008-2009, approximately 650-tagged adults bred
227 in this sub-colony. From mid-September 2008 to mid-April 2009, radio-frequency antennas
228 buried under the usual transit pathways in and out of the sub-colony allowed automatic
229 detection of bird entry and departure from the colony (see Gendner et al. 2005 for details).
230 Birds greater than 4 years in age whose movements in and out of the sub-colony matched the
231 cycle of reproductive king penguins were considered as breeders (Saraux et al. 2011), and
232 used to calculate the ‘colony density index’. In addition, an index of breeding density in the
233 study area (hereafter ‘breeding density index’) was estimated starting on 1 Nov 2008. Every
234 second week, the distance between breeding birds (incubating birds or territorial pairs) was
235 estimated visually at $\pm 10\text{cm}$, in 10 different locations regularly spread over the study area.
236 For each location, we randomly estimated 20 distances between breeding birds (amounting to
237 200 distances in total) which we averaged. As a general rule, each breeder was surrounded by
238 six neighbours. Thus, the smallest unit that could be identified comprised seven birds. The
239 area occupied by these seven birds corresponded to the circle centered on one bird and of
240 radius 1.5 times the average distance between breeders, to account for the entire area of
241 neighbours. Breeding density index (number of breeders per m^2) was then calculated as
242 $7/(\pi(1.5d)^2)$, where d is the mean interbreeder distance.

243

244 WEATHER CONDITIONS

245 Air temperature (at ± 0.1 °C), wind speed (at ± 0.1 m/s) and relative humidity (at $\pm 1\%$) were
246 sampled every minute throughout the study using a Vantage PRO 2 weather station (Davis
247 Instruments, Hayward, CA 94545, DAVIS, USA) installed on the colony site.

248

249 CALCULATIONS AND STATISTICS

250 Analyses were performed using R v.2.10.1 (R Development Core Team 2011). Data collected
251 within an hour of bird capture were systematically discarded to eliminate biases from animal
252 handling. Mean daily HR (dHR) was calculated by averaging all 5-second measurements over
253 24-hour periods. We calculated daily resting HR (rHR) using moving averages to determine
254 the 10 consecutive minutes (120 consecutive HR measurements) where HR was lowest over
255 24-hour periods. Mean body temperature was averaged, and total activity counts summed,
256 over 24-hour periods. Breakpoints in the time-course of physiological parameters during
257 courtship and incubation were identified from segmented regression analysis using the
258 ‘strucchange’ and ‘segmented’ packages from R (Zeileis et al. 2002; Muggeo 2008). Separate
259 Generalized Estimating Equations (GEEs) were used for pre- and post-breakpoint temporal
260 analysis (‘geepack’ package in R; Højsgaard et al. 2005). Bird identity was included as a
261 random factor to account for repeated measures on an individual. To standardize and compare
262 birds at similar breeding dates, data are presented relatively to the day of egg laying. Due to
263 individual variations in courtship and incubation duration, transitory malfunctioning of data-
264 loggers, and logistic reasons (logger removal 0-4 days before bird departure to sea), sample
265 sizes vary across monitoring periods. Days with less than 3 individuals were not considered.
266 Depending on the distribution of model residuals, normal or gamma distributions were used
267 to investigate changes in heart rate and body temperature. Changes in daily activity counts
268 were investigated using Poisson distributions. Values are reported as means \pm SE and results
269 are considered significant for $P < 0.05$.

270

271 ETHICAL NOTE

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

279

280 **Results**

281 COLONY DENSITY

282 Our two indices of bird density demonstrated marked and consistent changes throughout the
283 reproductive season (Fig. 2). Both ‘colony’ and ‘breeding’ density indices were low at the
284 start of breeding (30 tagged birds and 0.3 breeders/m² on the surveyed area at the time of first
285 courtships). Then, both indices markedly increased to reach a maximum level by mid- to late-
286 January (see Fig. 2). This maximum density was approximately 12 (‘colony density index’) to
287 15 (‘breeding density index’) times higher than at the start of breeding. Specifically, the
288 ‘colony density index’ increased by a factor 5 during early breeder monitoring, but was stable
289 during the period of late breeder monitoring.

290

291 WEATHER CONDITIONS

292 Daily climate conditions were similar for early and late breeders. Average wind speed and
293 humidity were similar for early (wind speed = 4.9 ± 0.4 m/s; relative humidity = 80.6 ± 1.1
294 %) and late breeders (wind speed = 4.7 ± 0.4 m/s; relative humidity = 84.0 ± 1.2 %)

295 (Wilcoxon tests: all $P > 0.06$). In early breeders, mean daily wind speed increased slightly
296 from late November to late December ($F_{1,28} = 6.84$, $P = 0.01$). Ambient temperature was
297 slightly higher for late than for early breeders (8.9 ± 0.3 °C vs. 7.6 ± 0.3 °C; t-test; $t = -3.05$,
298 $df = 58.0$, $P = 0.003$). For late breeders, it showed a slight increase from late January to late
299 February ($F_{1,28} = 4.22$, $P = 0.049$).

300

301 CHANGES IN HEART RATE IN FREELY BREEDING BIRDS

302 *Changes in heart rate*

303 In early breeders, dHR decreased rapidly (-5.3 ± 1.3 bpm/day) during the first 6 days of
304 courtship, then progressively (-1.7 ± 0.7 bpm/day) until ~3 days before egg-laying (Table 1,
305 Fig. 3A). Starting on average on November 28 and 3 days before the onset of incubation, dHR
306 started to increase by 0.6 ± 0.2 bpm/day. Eventually, dHR stabilized at 56.5 ± 1.4 bpm from
307 day 11 (on average on December 12) of incubation onwards. Changes in rHR (Fig. 3A)
308 paralleled those of dHR, with the same breakpoints. Resting HR reached a minimum value of
309 35.5 ± 1.6 bpm three days before egg-laying. It subsequently increased by 33%, stabilizing at
310 47.1 ± 1.3 bpm within the final days of the incubation shift. Because the difference between
311 dHR and rHR (*i.e.* HR due to changes in physical activity) remained constant during the
312 period of HR increase (slope parameter not significantly different from zero; GEE; $Wald =$
313 0.25 , $P = 0.62$, $n = 177$, $N = 14$ birds), this suggested that the increase in dHR was essentially
314 driven by an increase in rHR.

315 In late breeders, dHR was stable throughout the recording period (GEE; $Wald = 0.3$, P
316 $= 0.60$, $n = 137$, $N = 10$ birds) and averaged 58.8 ± 0.8 bpm (Fig. 3B). Resting HR followed
317 the exact same trend (Fig. 3B), remaining stable at the average level of 48.4 ± 0.6 bpm ($Wald$
318 $= 0.3$, $P = 0.60$). These average dHR and rHR values were not significantly different from
319 those observed at the end of incubation in early breeders when dHR and rHR were stabilized
320 ($Wald = 0.48$ and 0.18 , $P = 0.49$ and 0.67 , for dHR and rHR, respectively).

321

322 *Relationships to colony density*

323 In early breeders, when dHR and rHR were at their minimum values (44.0 ± 1.9 bpm and 35.5
324 ± 1.6 bpm, respectively) the ‘breeding density index’ was close to 1 breeder/m² and the
325 ‘colony density index’ indicated that ~20% of tagged birds were present in the colony. Thus,
326 dHR and rHR started to increase when colony density reached ~30% of its maximum level
327 (25% to 35% depending on whether the ‘breeding density index’ or the ‘colony density index’
328 was considered). When dHR and rHR stopped increasing, colony density was ~43%
329 (‘breeding density index’) to 65% (‘colony density index’) of its maximum. We investigated
330 whether the observed increase in HR in early breeders could be linked to an increase in
331 colony density. For each day onwards from day -3 (Fig. 3A), we calculated average rHR and
332 ‘colony density index’. We found that rHR was highly and significantly related to the ‘colony
333 density index’ ($R^2 = 0.88$, $F_{1,18} = 136$, $P < 0.001$, $n = 20$ days; Fig. 4). Further, we accounted
334 for potential climate effects causing rHR to increase independently of colony density over this
335 period. Because T_a highly correlated with wind speed ($r = 0.77$, $P < 0.001$) and relative
336 humidity ($r = 0.65$, $P = 0.002$), we used the first axis of a principal component analyses (PC1
337 $= 0.64 T_a + 0.57$ wind + 0.51 humidity; this component explained 73.5% of the variance) to
338 investigate the effects of climate variables on rHR. During the period of HR increase, we
339 found that rHR was indeed positively associated with PC1 ($R^2 = 0.35$, $F_{1,18} = 9.58$, $P = 0.006$,
340 $n = 20$ days) and thus potentially affected by climate. Interestingly however, using the
341 residuals of this regression as a dependent variable to remove climate effects on rHR, we
342 found that colony density remained highly significant and explained a substantial part of the
343 residual variation in rHR ($R^2 = 0.46$, $F_{1,18} = 15.3$, $P = 0.001$, $n = 20$ days). In contrast, rHR
344 was not correlated with colony density ($F_{1,16} = 0.5$, $P = 0.50$, $n = 18$ days; Fig. 4) in late
345 breeders.

346

347 CHANGES IN HEART RATE AND BODY MASS IN CAPTIVE BIRDS

348 *Changes in heart rate*

349 In non-breeding males kept captive at low density, both dHR and rHR continuously decreased
350 over the 25 days of the fast (Fig. 5). Decreases were rapid over the first 5 days of fasting, on
351 average by -6.4 ± 1.5 (dHR) and -5.9 ± 1.1 (rHR) bpm/day, and then stabilized at a slower
352 rate of -1.0 ± 0.3 (dHR) and -0.7 ± 0.2 (rHR) bpm/day for the remaining of the fast.
353 Although, the difference between dHR and rHR slightly reduced over the 25 days of fasting ($-$
354 0.26 ± 0.09 bpm/day; GEE; *Wald* = 8.12, *P* = 0.004, *n* = 25, *N* = 8 birds) (Fig. 5), there was
355 only a marginal decrease in dHR–rHR detectable before the 5 day breakpoint (-0.23 ± 0.13
356 bpm/day; *P* = 0.06) and no substantial change afterwards (-0.45 ± 0.50 bpm/day; *P* = 0.37).

357

358 *Changes in body mass*

359 Over the 25 monitoring days, captive birds lost as much as 26% of their initial body mass, *i.e.*
360 from 13.8 ± 0.1 kg to 10.2 ± 0.1 kg (Fig. 6A). Body mass initially decreased rapidly ($-370 \pm$
361 23 g/day) until a breakpoint of 3.5 days whereupon it was lost at a slower rate (-129 ± 23
362 g/day) (GEEs; all *P* < 0.001). This is well illustrated by the initially high, but rapidly
363 decreasing *dm/dt* values observed during the first days of the fast (Fig. 6B). Both dHR and
364 rHR significantly decreased with decreasing body mass (Fig. 6C) and decreasing daily body
365 mass loss (Fig 6D) (GEEs; all *P* < 0.001).

366

367 BODY ACTIVITY AND BODY TEMPERATURE IN FREE-BREEDING BIRDS

368 *Body activity*

369 Daily body activity decreased progressively over the course of courtship and incubation both
370 in early and late breeders. In early breeders, breakpoint analysis revealed a rapid (slope = -0.5
371 ± 0.09 ; Table 2) initial decrease in body activity over the period of strong HR decline (from

372 days -16 to days -10 prior to egg-laying). Afterwards, body activity decreased at a slow
373 constant rate (Table 2; slope = -0.1 ± 0.05), until the end of the monitoring period (Fig. 7A.).
374 Breakpoint analysis did not reveal any change in slope for late breeders (Table 2, Fig. 7A).

375

376 *Body temperature*

377 No significant change in stomach temperature occurred either in early or late breeders (GEEs;
378 $Wald = 1.8$ and 0.9 , $n = 224$ and 144 , $N = 9$ and 9 birds, $P = 0.20$ and 0.34 , for early and late
379 breeders, respectively) (see Fig. 7B). In addition, we found no significant difference in body
380 temperature between early and late breeding birds. Average body temperature was 38.1 ± 0.03
381 °C for early breeders vs. 38.3 ± 0.04 °C for late breeders (GEE; $Wald = 0.57$, $P = 0.45$, $n =$
382 368 , $N = 18$ birds).

383

384 **Discussion**

385 ENERGETIC ADJUSTMENTS IN CAPTIVE PENGUINS

386 In captive males, both dHR and rHR decreased daily during fasting, paralleling a decrease in
387 body mass. Rapid initial changes in HR within the first five days in the pen could reflect
388 habituation to captivity and a shift from phase I to phase II fasting, a metabolic transition
389 during which animals adjust to a preferential utilization of lipid stores to fuel their
390 metabolism. This is confirmed by the observed changes in body mass loss (dm/dt)
391 characteristic of this transition (Cherel, Robin & Le Maho 1988c; Groscolas 1990). Our
392 results are similar to previous findings in king penguin (Fahlman et al. 2004) and support the
393 view that during long-term fasting the rate of EE is decreased by reducing basal metabolic
394 rate (Cherel et al. 1988c) and the energy expended for physical activity. Although marginal (P
395 = 0.06), we observed a decrease in HR due to physical activity (dHR-rHR) during the initial 5
396 days of fasting. Subsequently, dHR-rHR remained constant suggesting that the main

397 adaptation to long-term fasting was a decrease in rHR (and by extension resting metabolic
398 rate) with increasingly efficient energy sparing as the fast progressed (Cherel et al. 1988c)
399 (see below for a discussion of potential mechanisms).

400

401 ENERGETIC ADJUSTMENTS IN FREELY BREEDING PENGUINS

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

407 In early breeders, the observed decrease in dHR was partly explained by a strong
408 concurrent decrease in physical activity related to breeding activities, as revealed by activity
409 loggers. After selection of a breeding site (Stonehouse 1960), pairs rapidly decreased their
410 physical activity mainly resumed to comfort behaviour and territorial defence (Viblanco et al.
411 2011; Viera et al. 2011). Could our HR-loggers have misidentified muscle artifact as HR
412 activity during peaks of physical activity in early courtship? The fact that similar HR values
413 were observed in captive birds caught at the onset of courtship that could only display
414 minimal movement in the pen (*i.e.* no courting, waddling around the colony or fights for
415 breeding territory establishment) suggests this is unlikely. Actually, the marked decrease in
416 rHR over the same period, points to physiological adaptations affecting resting metabolic rate.
417 Although thermoregulatory adaptations such as transient declines in body temperature may
418 contribute to substantial energy savings in large birds (Butler & Woakes 2001; Handrich et al.
419 1997, Gilbert et al. 2007, Eichhorn et al. 2011), we did not observe marked changes in the
420 core T_b of early and late breeders in the present study. Given the temperature requirements of
421 incubation (around 36 °C for king penguin; Groscolas et al. 2000), one explanation is that

422 departures from normothermia may be limited in breeding birds (Vehrencamp 1982, Csada &
423 Brigham 1994, Gilbert et al. 2007). However, we did not examine peripheral decreases in T_b
424 that would affect the volume of the body normally regulated at high and constant T_b . Yet, by
425 decreasing the energy cost of thermoregulation, changes in core volume during long-term
426 fasting may affect resting metabolic rate (Cherel et al. 1988c, see Eichhorn et al. 2011 for a
427 recent example in king penguin chicks). Whether such changes occur in freely-breeding
428 penguins remains to be examined. For instance, it would be interesting to understand whether
429 and to what extent changes in core volume contribute to decreasing rHR during courtship, and
430 whether the increase in rHR observed shortly before the start of incubation may partly be
431 linked to an increase of core volume and peripheral T_b to warm the egg.

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

443

444 ENERGETICS OF COLONIAL SEABIRDS: A ROLE FOR SOCIAL DENSITY?

445 The increase in dHR starting shortly before incubation in early (but not late) breeders is
446 intriguing. As physical activity (loggers) decreased over this period and dHR-rHR remained

447 constant, the pattern was driven by a significant increase in rHR. Interestingly, we found a
448 strong positive association ($R^2 = 0.88$) between colony density and rHR in early breeders, that
449 remained significant ($R^2 = 0.46$) after controlling for climate-induced effects on HR. In
450 contrast, no such relationship was observed for late breeders at stabilized colony density. In
451 early breeders, rHR eventually reached a plateau at a level similar to the rHR of late breeders
452 during incubation, suggesting a maximum threshold to the effects of colony density on rHR.

453 Is the hypothesis of a conspecific-density effect on the energy budgets (as reflected in the
454 rHR) of breeding seabirds reasonable? Previous studies have documented links between
455 social stimuli, stress levels and metabolic rates (Sloman et al. 2000, Fuchs & Flügge 2002,
456 Cao & Dornhaus 2010). King penguins can be highly sensitive to their social environment
457 (Viblanç et al. 2012), and current data suggests that birds incubating at high density exhibit
458 higher basal stress hormone levels than birds at low density (Viblanç 2011). Thus the
459 possibility that increased social density may affect stress levels (Boonstra & Boag 1992;
460 Rogovin et al. 2003; McCormick 2006, Creel et al. 2013, Dantzer et al. 2013) and HR at rest
461 should be considered. Changes in rHR levels in breeding penguins may then only partly
462 reflect changes in metabolic rate, because of the proportion of HR modulation attributable to
463 stress (activation of the sympathetic nervous system) (Groscolas et al. 2010).

464 Experimental manipulations of local colony density are needed to yield further
465 information on the links between social density, HR and EE expenditure at rest in breeding
466 penguins. First insight is provided by considering the relative increase in rHR for early birds,
467 from 35.5 ± 1.6 bpm at low colony density (3 days before egg-laying) to 47.1 ± 1.3 bpm at
468 high colony density (when rHR stabilized). This corresponded to a 33% increase in rHR
469 potentially due in part to an increase in colony density. In fact, this increase was probably
470 underestimated, as the colony was not empty when rHR started increasing, but rather not
471 quite half of maximum density (116/371 birds). Moreover, our results in captive birds suggest

472 that rHR in incubating birds may have further decreased under the influence of the fast if they
473 had been breeding out of the colonial context. Again, this could have contributed to
474 underestimate the increase in rHR possibly due to the increase in colony density. If converted
475 to VO_2 (Eq. 1, Fahlman et al. 2004), the observed increase in rHR may have resulted in as
476 much as a 21% increase in resting metabolic rate. However, because of the uncertainty of
477 estimates produced using calibration equations from birds that differ from those under study
478 (Green 2011), caution should be advocated and further investigations are required to
479 determine whether the observed increase in rHR actually translates into a significant increase
480 in resting metabolic rate. Those studies are urgently needed to determine to what extent
481 breeding-fasting seabirds may have to support an extra energy cost imposed by their social
482 environment, and to better appreciate the nature of social stimuli in conditioning important
483 life-history variables such as metabolic rates, breeding timing, breeding territory location, or
484 reproductive success in colonial breeders. Finally, regardless of the relationship between HR
485 and EE, our study suggests that long-term monitoring of HR in field studies may yield
486 important insights on individual exposure to stress in natural populations. We hope the
487 present results will stimulate the inclusion of HR monitoring in future research on
488 bioenergetics and stress in free living animal populations.

489

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502

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666 **Figure captions**

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

672 **Fig. 2. Changes in colony density over the 2008-2009 reproductive season for king**
673 **penguins breeding in the Baie du Marin, Possession Island, Crozet Archipelago.**
674 Breeding density (stars): number of breeding birds per m² in the study area. Colony density
675 index (continuous line): number of transponder-tagged breeding birds present in a nearby sub-
676 colony of fixed area (see text). Shadowed zones under the density index curve indicate time
677 periods during which early and late breeders were followed, with arrows showing the
678 respective average laying dates. The date of the first observed laid egg in the study area was
679 November 10.

680 **Fig. 3. Daily changes in mean heart rate (squares) and mean resting heart rate**
681 **(triangles) during courtship and first incubation shift in male king penguins. (A)**
682 Changes occurring in HR for early breeders relative to laying date. Breakpoints (bk1, bk2 and
683 bk3) indicating changes in HR slopes are figured by black dashed lines (see text). Values are
684 given as means ± SE (N = 3-14 birds). **(B)** Changes occurring in HR for late breeders relative
685 to laying date. Values are given as means ± SE (N = 3-10 birds). For both A and B, colony
686 density index appears as the grey area and the arrow shows the laying date.

687 **Fig. 4. Influence of colony density (number of birds) on resting heart rate (rHR, bpm) in**
688 **breeding male king penguins.** Significant regression line (solid line) and 95% confidence

689 intervals (dashed lines) are given for early breeders ($rHR = 24.0 \pm 1.8 + 0.1 \pm 0.01 \times \text{density}$).
690 Values are means for 3 to 14 (early breeders) or for 3 to 10 (late breeders) birds for each day
691 from 3 days before egg laying onwards (early breeders, $n = 20$ days), or throughout the whole
692 monitoring period (late breeders, $n = 18$ days). For clarity, SE (bars) are presented only for
693 early breeders.

694 **Fig. 5. Daily changes in mean heart rate (dHR; squares), mean resting heart rate (rHR;**
695 **triangles) and heart rate relating to activity (dHR-rHR; circles) during fasting in early**
696 **male king penguins caught at courtship and kept captive in a pen under natural weather**
697 **conditions at a density of 0.3 birds/m².** Values are means \pm SE for 8 birds. The dotted
698 vertical line indicates a change in the slope of HR decrease. The equations for pre- and post-
699 breakpoint regressions in dHR are: $dHR = 106.5 \pm 8.3 - 6.4 \pm 1.5 \text{ days}$ and $dHR = 79.0 \pm 7.7 -$
700 $1.0 \pm 0.3 \text{ days}$, respectively. The equations for pre- and post-breakpoint regressions in rHR are:
701 $rHR = 86.6 \pm 6.3 - 5.9 \pm 1.1 \text{ days}$ and $rHR = 60.1 \pm 5.2 - 0.7 \pm 0.2 \text{ days}$, respectively.

702 **Fig. 6. Daily changes in (A) body mass and (B) body mass loss during fasting in early**
703 **male king penguins caught at courtship and kept captive in a pen under natural weather**
704 **conditions at a density of 0.3 birds/m².** Values are means \pm SE for 8 birds. The relationships
705 between body mass and heart rate (C) and body mass loss and heart rate (D) are given.

706
707 **Fig. 7. Daily changes in (A) body activity and (B) body temperature during courtship**
708 **and incubation in early (filled circles) and late (open circles) breeding male king**
709 **penguins.** Values are given as means \pm SE ($N = 3-14$ birds for daily activity and $N = 3-9$ birds
710 for stomach temperature). For comparison, breakpoints identifying changes in the time course
711 of heart rate for early breeders (see Fig. 5) are indicated as dashed lines.

712

713

714 **Tables**

715 **Table 1. Intercept and slope coefficients (\pm SE) for the generalized estimating equations**
 716 **characterizing the time-course of dHR changes during courtship and incubation in early**
 717 **breeding male king penguins.** Significant slope coefficients are given in bold. Sample size is given
 718 as n and number of individual birds is given in brackets (N).

	<i>Intercept</i>	<i>Wald</i>	<i>P</i>	<i>Slope</i>	<i>Wald</i>	<i>P</i>	<i>n (N)</i>
(1) Rapid decrease	4.5 \pm 15.5	0.08	0.77	-5.3\pm1.3	16.4	< 0.001	48 (10)
(2) Slow decrease	39.1 \pm 3.2	146.0	< 0.001	-1.7\pm0.7	5.8	0.02	77 (13)
(3) Increase	46.6 \pm 1.5	941.7	< 0.001	0.6\pm0.2	12.0	0.001	177 (14)
(4) Stabilization	38.6 \pm 11.7	10.8	< 0.001	1.3 \pm 0.9	2.2	0.13	36 (11)

719

720

721 **Table 2. Intercept and slope coefficients (\pm SE) for the generalized estimating equations**
 722 **characterizing the time-course of daily body activity during courtship and incubation in early**
 723 **(EB) and late (LB) breeding male king penguins.** Significant slope coefficients are given in bold.
 724 Sample size is given as n and number of individual birds is given in brackets (N).

	<i>Intercept</i>	<i>Wald</i>	<i>P</i>	<i>Slope</i>	<i>Wald</i>	<i>P</i>	<i>n (N)</i>
EB (1) Rapid decrease	3.7 \pm 0.9	15.4	< 0.001	-0.5\pm0.09	36.0	< 0.001	15 (3)
EB (2) Slow decrease	7.7 \pm 0.3	509.5	< 0.001	-0.1\pm0.05	6.8	0.009	113 (8)
LB (1) Slow decrease	8.2 \pm 0.3	1043.7	< 0.001	-0.2\pm0.04	18.5	< 0.001	60 (6)

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733 **Figures**

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736 **Fig. 1**

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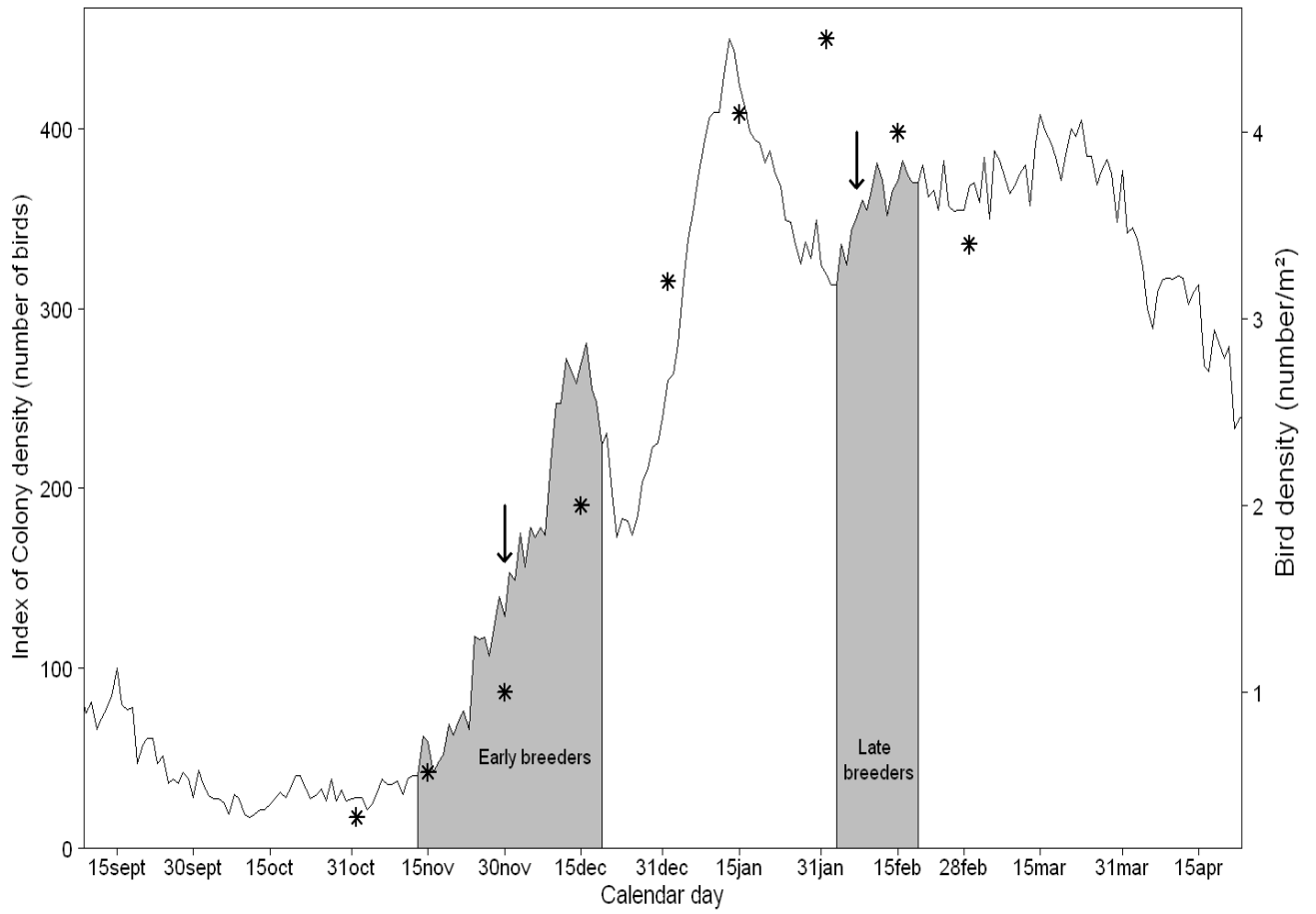
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749 **Fig. 2**

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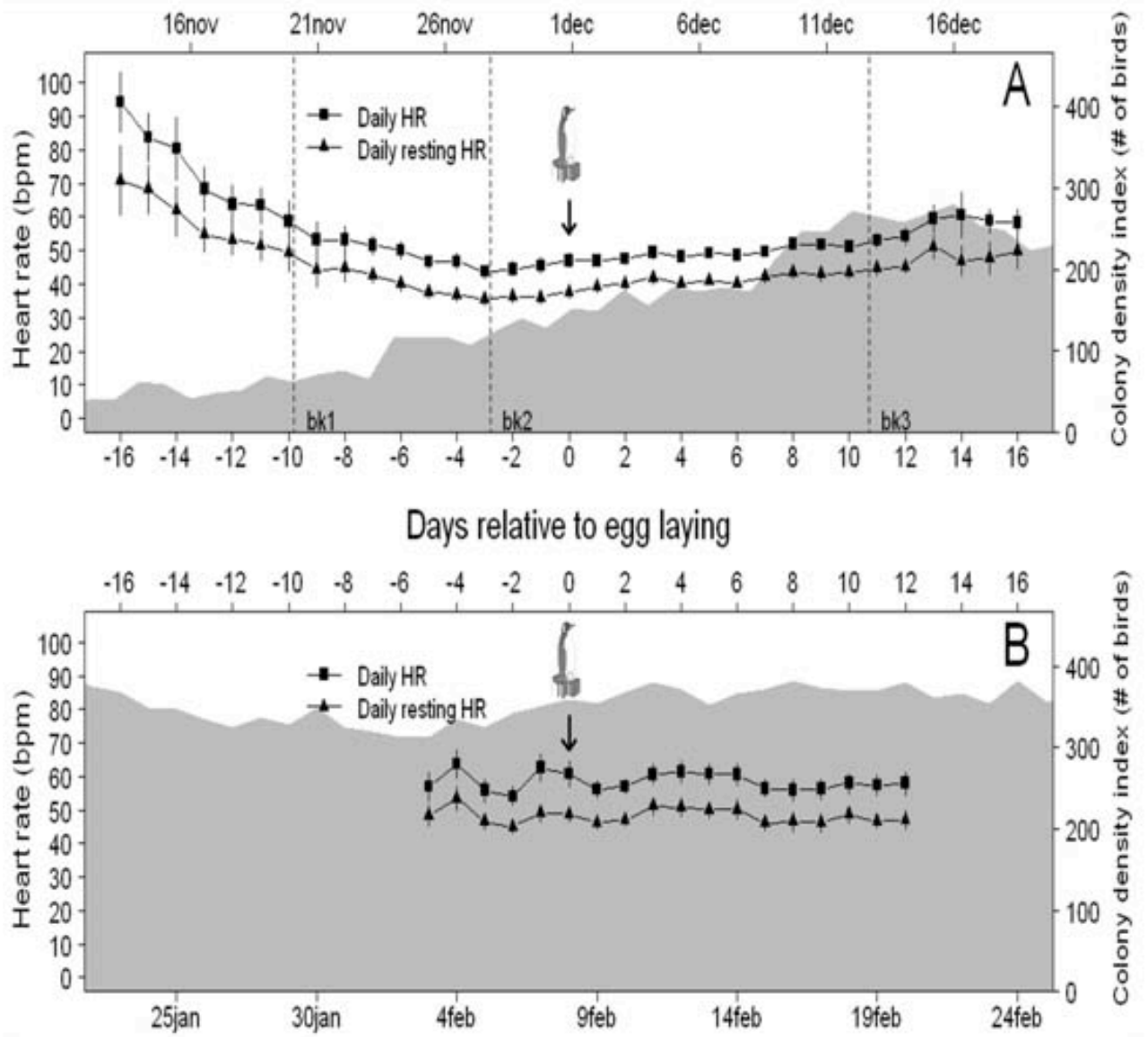
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759 **Fig. 3**

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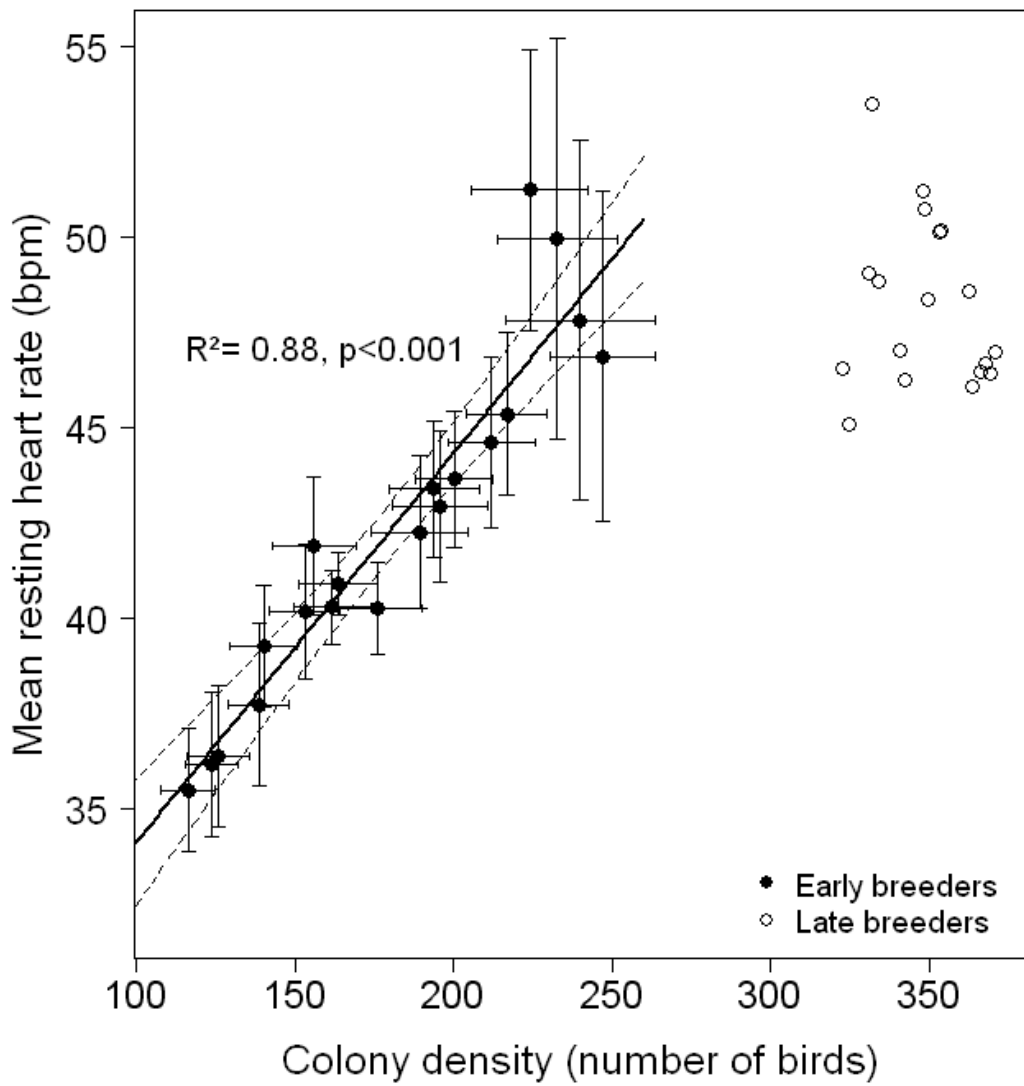
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771 **Fig. 4**

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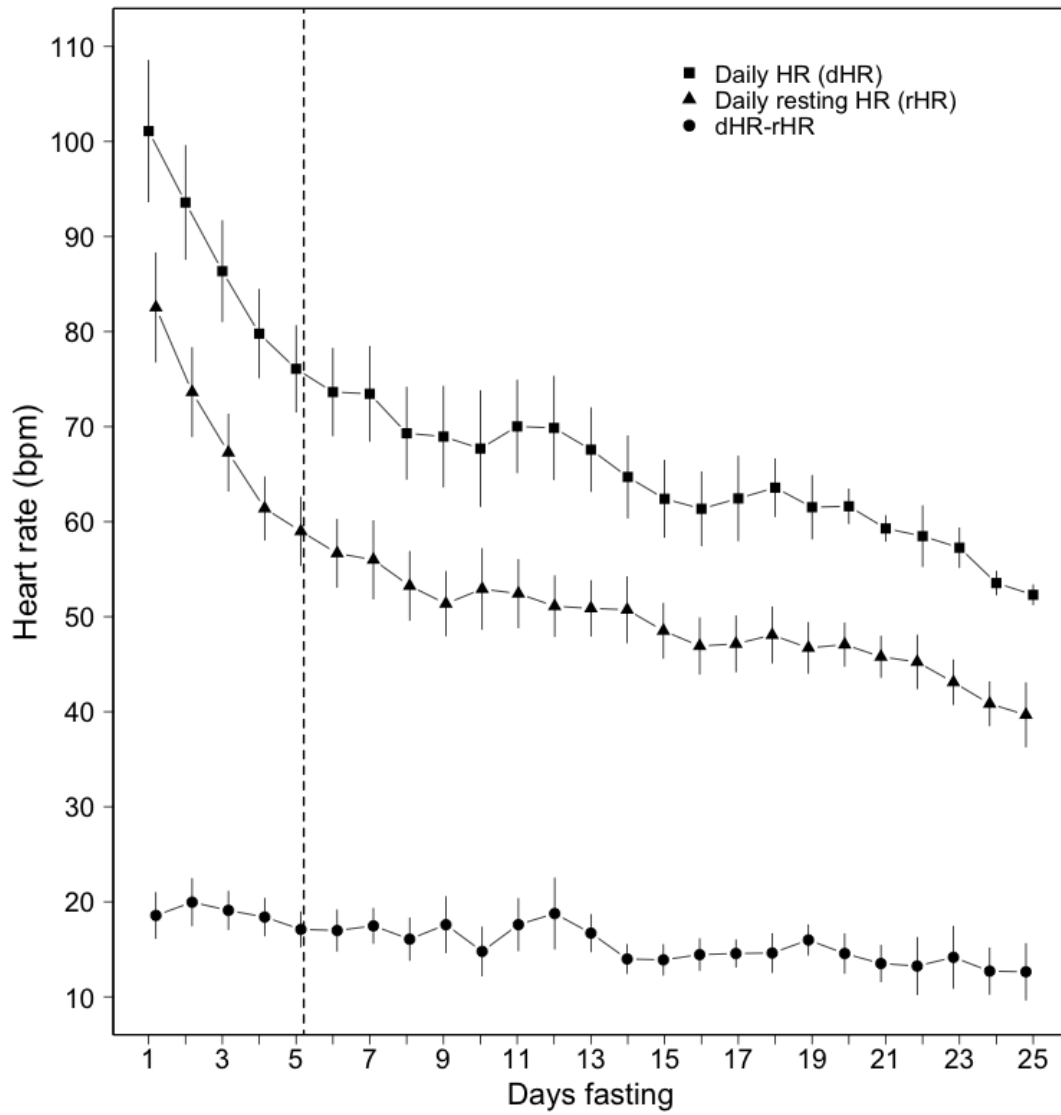
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783 **Fig. 5**

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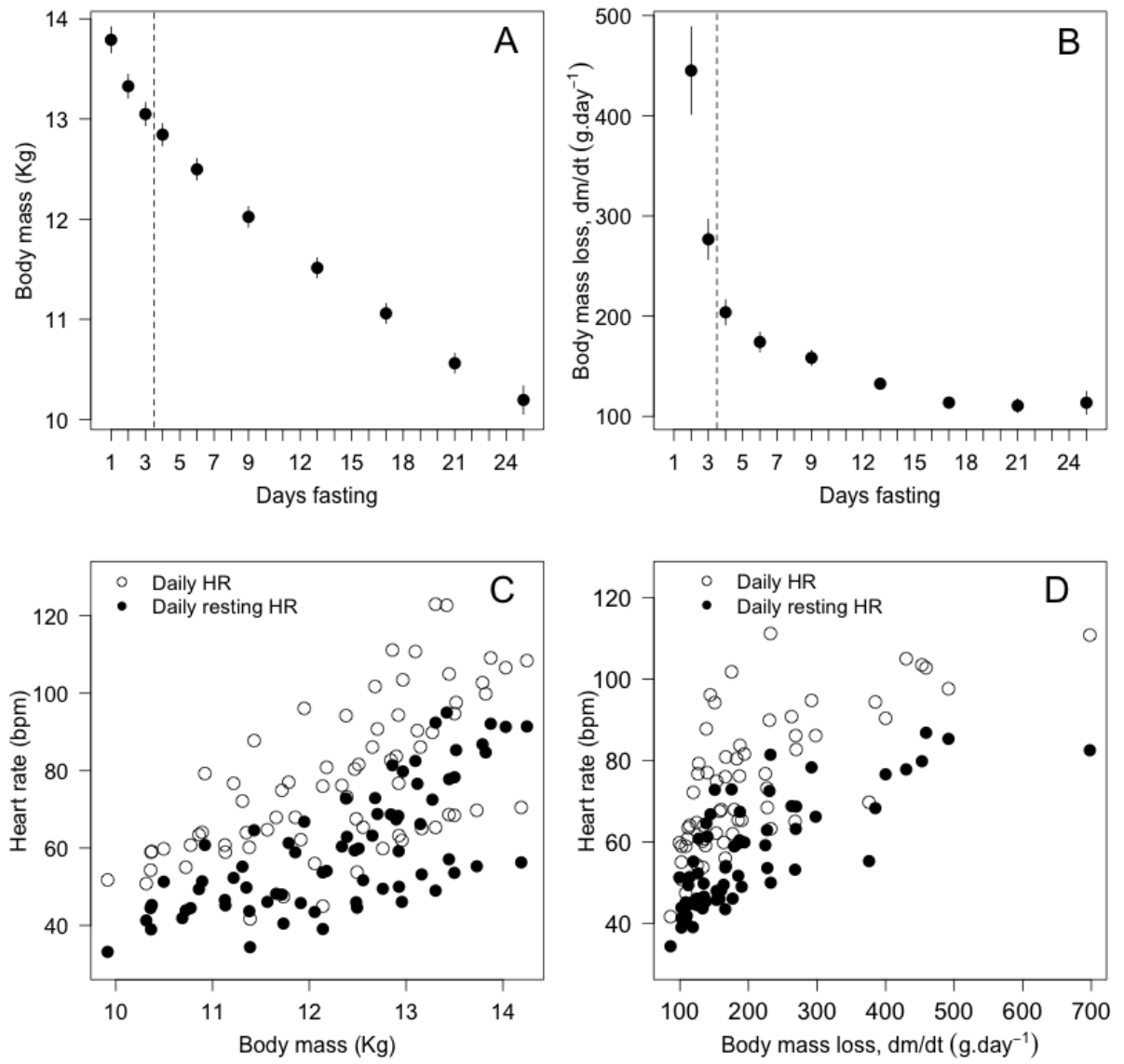
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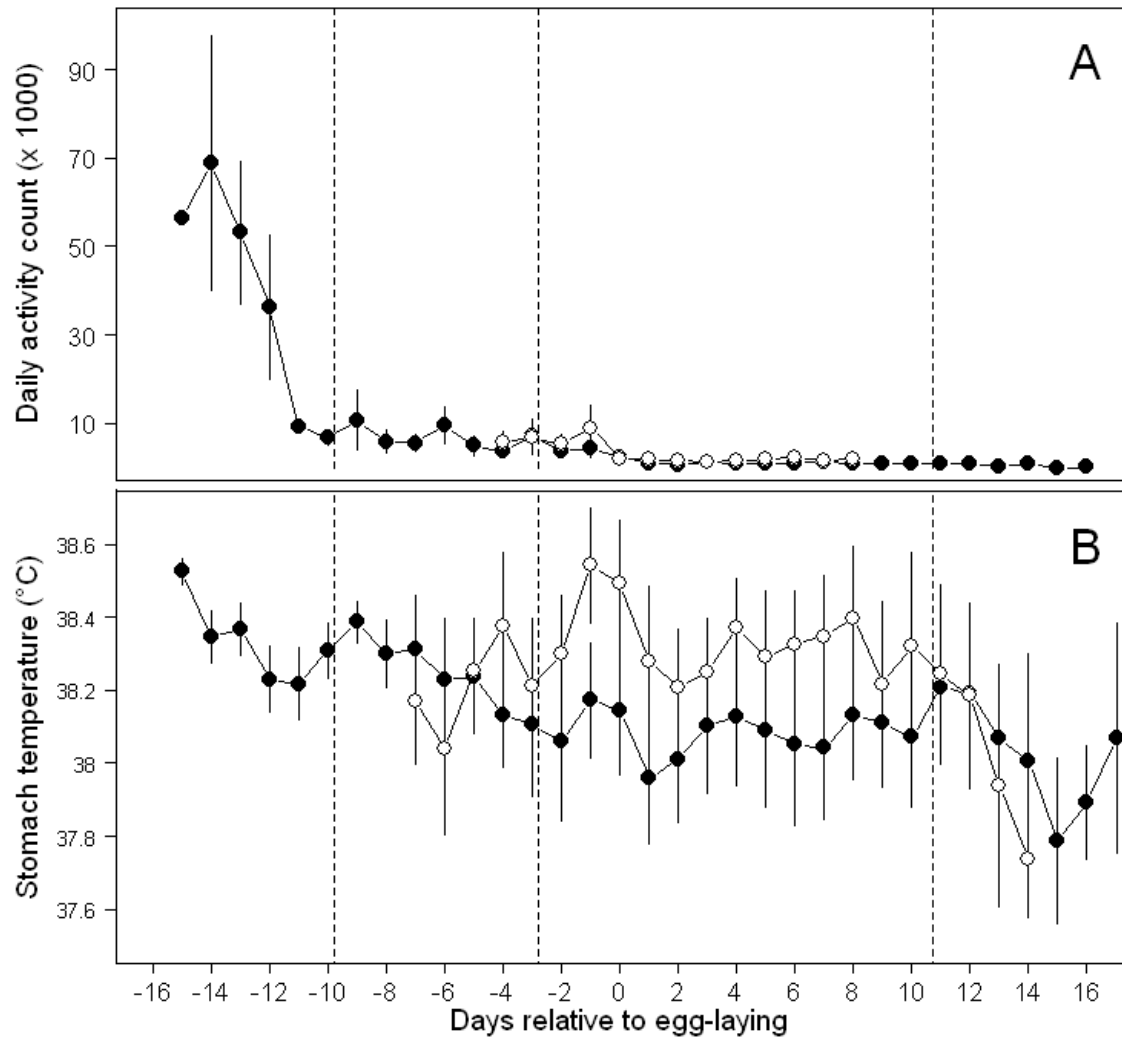
792 **Fig. 6**

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798 **Fig. 7**

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