Title

Effect of age, breeding experience and senescence on corticosterone and prolactin levels in a long-lived seabird: The wandering albatross

Permalink

Journal

ISSN

Authors

Angelier, F
Shaffer, Scott A
Weimerskirch, H
et al.

Publication Date

Peer reviewed

3

4   Effect of Age, Breeding Experience and Senescence

5   on Corticosterone and Prolactin Levels in a long-lived seabird:

6   the Wandering Albatross

7

8   Frédéric Angelier[a],*, Scott A. Shaffer[b], Henri Weimerskirch[a], Olivier Chastel[a]

9   [a]Centre d'Etudes Biologiques de Chizé, Centre National de la Recherche Scientifique, F-

10   79360 Villiers en Bois, Deux-Sèvres, France

11   [b]Department of Ecology and Evolutionary Biology, University of California, Santa Cruz,

12   California 95060, USA

13

15

16   * Corresponding author

17   Tel: *33 5 49 09 78 37*

18   Fax: 33 5 49 09 65 26

19

20   E-mail: angelier@cebc.cnrs.fr

21

22   Short title: Hormones and breeding experience in albatrosses

Abstract

Although several studies have described age-specific pattern of breeding performance, physiological and endocrine mechanisms underlying the variation in reproductive success in relation to age, are poorly understood. We described baseline levels of 1) corticosterone, which can trigger nest desertion when secreted at high levels, and 2) prolactin, a hormone known to trigger parental behaviour in incubating known-aged (7 to 39 years old) wandering albatrosses (*Diomedea exulans*). In this long-lived species, reproductive performance increases with age and breeding experience before it stabilizes in middle-aged individuals and declines in senescent individuals. We found that breeding experience was a much better statistical predictor of hormone levels than age. Baseline corticosterone levels slightly increased with advancing experience and reached maximal levels about the sixth breeding attempt. Similarly, prolactin levels were positively correlated with breeding experience in males. No such relationship was found in females, but first-time breeding females had lower prolactin levels than experienced females. This parallel increases in hormonal levels with advancing experience, could result from improvements of skills, development of an endocrine system adapted to energetic constraints of reproduction, or may mirror a higher investment in reproduction. Corticosterone levels decreased in senescent birds but such a decline was not observed for prolactin. Low corticosterone levels in senescent birds could be associated with a lower ability to secrete/sustain elevated corticosterone levels or with a decreased parental expenditure.

Keywords: Age, Breeding experience, Senescence, Parental expenditure, Corticosterone, Prolactin, Long-lived bird, Wandering Albatross, *Diomedea exulans*

Introduction

The influence of age on survival and reproductive performance is crucial in animal life-history because it affects individual reproductive strategies (Stearns, 1992; Roff, 1992) and hence, the dynamics of populations. Variation in these life-history traits with age is well documented for several long-lived animals. Regarding vertebrates, survival and reproductive performance generally increase with age (Clutton-Brock, 1988; Newton, 1989; Forslund and Pärt, 1995). However, for long-lived species, this increase is often followed by stabilization in survival and reproductive performance at middle-age, then a decline in old age (Clutton-Brock, 1988; Weimerskirch, 1992; Forslund and Pärt, 1995; Kirkwood and Austad, 2000; Reid et al., 2003). This decline is called senescence and involves a loss of physiological functions and is accompanied by decreasing fertility and increased risks of mortality with advancing age (Kirkwood and Austad, 2000).

Although several studies have described age-specific pattern of breeding performance (Clutton-Brock, 1988; Pärt, 2001; Mauck et al., 2004), physiological mechanisms underlying the variation in reproductive expenditure in relation to age, are poorly understood. Monitoring hormones levels can offer great insights into the understanding of mechanisms mediating age-specific patterns of life-history traits (Sinervo and Svennson, 1998; Ottinger et al., 2003), but to our knowledge only a few studies have focused on endocrine correlates of ageing in free-living birds (LH, progesterone, oestradiol and androgens, Hector et al., 1990; Nisbet et al., 1999). Moreover, none have simultaneously described baseline levels of corticosterone and prolactin, two hormones involved in reproductive behaviour, in relation to age and breeding experience.

In birds, plasma levels of corticosterone rapidly increase in response to stressful events and maintenance of elevated corticosterone levels during a prolonged period can ultimately trigger nest desertion by altering the allocation of available resources between body

69 maintenance and reproductive processes (Silverin 1986; Wingfield et al. 1998). However, this

70 inhibitory response is generally activated at stress-induced levels, whereas baseline levels of

71 corticosterone have different physiological and behavioural permissive effects (Romero

72 2004). For instance, corticosterone plays a major role in the energy mobilization at baseline

73 levels by activating gluconeogenesis (Sapolsky et al. 2000; Remage-Healey and Romero

74 2001) and elevated baseline corticosterone levels during reproduction could therefore result

75 from the high energetic demand of this life-history stage (Romero 2002). Because depletion

76 of body reserves results in increased baseline corticosterone levels (Cherel et al. 1988;

77 Kitaysky et al. 1999; Love et al. 2005), baseline corticosterone levels can moreover be a

78 witness of the stress imposed by sustained breeding activities. Focusing on corticosterone is

79 thus relevant to studying the relationship between reproductive performance and age because

80 this hormone plays a major role in breeding decision and reproductive expenditure (Wingfield

81 et al., 1998).

82   Reproductive performance also depends on the ability of animals to provide parental

83 care to their progeny (incubation care, guarding for protection against predators, intensive

84 brooding for thermoregulation, etc. review in Clutton-Brock, 1991). In this context, the

85 hormone prolactin is of particular interest. This hormone is involved in the initiation and

86 maintenance of avian parental behaviour (review in Buntin, 1996) and prolactin enhances the

87 expression of several parental behaviours such as feeding behaviour, nest attendance or

88 defensive behaviour (Wang and Buntin, 1999). High prolactin levels also appear necessary to

89 maintain parental care despite stressful or unfavourable conditions for parents (Lormée et al.,

90 2000; Chastel et al., 2005). As a result, examining the relationship between prolactin and age

91 is particularly interesting to assess the effect of age and breeding experience on parental care.

92   Albatrosses and petrels (order Procellariiformes) are a promising model to study the

93 mechanism of ageing because they are long lived seabirds with low annual fecundity (one egg

94   per clutch with no replacement clutch; Lack, 1968; Warham, 1990). In these birds, the risk of

95   increased mortality during a breeding attempt should also be reduced because of their high

96   residual reproductive value (Stearns, 1992; Weimerskirch, 1999). Moreover, survival and

97   breeding performance of these pelagic seabirds are easy to monitor because they are highly

98   philopatric and often mate with the same individual throughout their life (Tickell, 1968,

99   Warham, 1990).

100   The aim of this study is to examine the relationship between baseline corticosterone

101   and prolactin levels in plasma in relation to age and breeding experience in the wandering

102   albatrosses (*Diomedea exulans*). The breeding cycle of this large (up to 12 kg) seabird lasts a

103   complete year and pairs that are successful in fledging a chick breed in alternate years, while

104   those failing during incubation or during the early stages of chick-rearing breed again in the

105   following year (Tickell, 1968). Sexual maturity is acquired on average at 8-9 years

106   (Weimerskirch and Jouventin, 1987). Wandering albatrosses are long-lived birds (40+ years)

107   that show a progressive increase in breeding success in relation to breeding experience

108   between the first and fifth breeding attempts, which on average corresponds to 8-9 to 20 years

109   of age (Weimerskirch, 1992). Thereafter, breeding success decreases after 6-7 breeding

110   attempts, corresponding to birds aged over 20-25 years, suggesting senescence at older ages

111   (Weimerskirch, 1992; Weimerskich et al. 2005). Therefore wandering albatrosses constitute

112   an excellent study system to explore relationships between hormones and age-related

113   breeding performances.

114   Elevated baseline levels of corticosterone are a witness of low foraging success

115   (Kitaysky et al. 1999; Woodley et al. 2003; Angelier et al. submitted) and poor nutritional

116   condition (Cherel et al. 1988; Kitaysky et al. 1999; Love et al. 2005) and could therefore be

117   associated with low breeding capacities and low breeding success (Kitaysky et al. 1999).

118   High levels of prolactin are associated with the maintenance of parental care (review in

119   Buntin, 1996), which plays a critical role in breeding success (Clutton-Brock, 1991).

120   Therefore, we predict that (1) baseline corticosterone levels should be higher in young and

121   older/senescent albatrosses than in middle-aged birds, whereas (2) baseline prolactin levels

122   should be lower in young and older/senescent albatrosses compared to middle-aged birds.

123

124   **Methods**

125   *Study area and birds*

126   Fieldwork was carried out between 3 January and 13 February 1999, and 1 January

127   and 25 February 2004 at Ile de la Possession, Crozet Archipelago, south-western Indian

128   Ocean (46°S, 52°E). The ongoing mark-recapture programme of wandering albatrosses at Ile

129   de la Possession has provided yearly information on the entire population of the island;

130   approximately 400 breeding pairs each year for the last 40 years (Weimerskirch et al., 2005).

131   Banding of chicks prior to fledging has been carried out since 1965. We were thus able to

132   know the age and breeding experience (number of breeding attempts, i.e. 1 refers to the first

133   breeding attempt) of each wandering albatross breeding on the island. However, banding of

134   breeding birds of unknown age started in 1959. For these individuals banded as adults, we

135   used an estimated age calculated from the year of banding and adding 6 years; the minimum

136   age at first breeding (Weimerskirch et al., 1997). Hence, age and breeding experience of these

137   birds are estimates. The sex of each bird was determined by body size and plumage

138   characteristics (Weimerskirch et al., 1989).

139

140   *Blood sampling and hormone assays*

141   A total of 75 albatrosses were captured on the nest and bled during the early incubation period

142   in both years (1999, $n = 22$; 2004, $n = 53$). Data were independent because birds sampled in

143   1999 were not sampled in 2004. One bird per nest was sampled in order to maintain

144   independence of the data. Immediately after capture, a blood sample (maximum: 1 ml) was

145   collected from the tarsus vein with a 1 ml heparinized syringe and 25 gauge needle. Blood

146   samples were centrifuged and plasma was decanted and stored at –20°C until they were

147   assayed. Plasma concentrations of prolactin were first determined by an heterologous

148   radioimmunoassay (RIA) at the Centre d'Etudes Biologiques de Chizé (Cherel et al., 1994).

149   Pooled plasma samples of albatrosses produced a dose response curve that paralleled chicken

150   prolactin standard curves ("AFP 4444B", source: Dr. Parlow, N.H.P.P. Harbor-UCLA

151   Medical Center, Torrance, California, USA, Fig. 1). All samples were run in one assay and

152   the intra assay variation was 4.2%. Time of day for blood sampling (males: $F_{1,38} = 0.375$, $P =$

153   0.55; females: $F_{1,35} < 0.01$, $P = 0.98$) did not influence prolactin levels. Plasma concentration

154   of corticosterone was then determined with the remaining plasma (n = 69) by

155   radioimmunoassay at the Centre d'Etudes Biologiques de Chizé as previously described

156   (Lormée et al., 2003). At Ile de la Possession, Crozet Archipelago Island, wandering

157   albatrosses are loosely colonial and typically nest in clusters of 1–8 nests. To reduce possible

158   disturbance to nearby breeders during the blood sampling of an individual, only one bird was

159   sampled within a cluster. As blood samples were collected within three minutes of capture,

160   they were considered to reflect baseline levels of corticosterone (Wingfield et al., 1982;

161   Kitaysky et al., 1999; Lormée et al., 2003). All samples were run in one assay and the intra

162   assay variation was 6.3%. Time of day for blood sampling ($F_{1,68} = 1.52$, $P = 0.23$) did not

163   influence corticosterone levels. Because body weight is known to influence corticosterone and

164   prolactin levels (Cherel et al. 1988, 1994), a sub-sample of birds was weighed in 1999 to the

165   nearest 50 g using a Salter spring balance (Salter Weightronix Ltd, West Bromwich, UK) to

166   test for an effect of body weight on corticosterone levels (n = 16) and prolactin levels ($n =$

167   22).

168

Statistics

170   We used the information-theoretic approach to identify suitable models for predicting

171 baseline levels of corticosterone and prolactin in breeding albatrosses (Burnham and

172 Anderson, 2002). Candidates models were constructed with explanatory variables

173 hypothesized to influence prolactin and corticosterone levels (e.g. Age, Breeding experience,

174 Year and Date). Where inspection suggested that relationships between hormonal levels and

175 age or breeding experience were non-linear, squared terms (Age^2 and $Experience^2$) were

176 included in models. Because age and breeding experience were strongly correlated in our

177 study ($n = 75$, $r = 0.92$, $P < 0.001$), we did not include both variables in the same model to

178 avoid multi-colinearity problems and we started our analysis from two general models (1/

179 Year, Date, Breeding experience, Breeding experience^2 and interactions; 2/ Year, Date, Age,

180 Age^2 and interactions). We used Generalized Linear Models with normal errors and an

181 identity link function to explain the effect of age, breeding experience, date of sampling and

182 year of sampling on corticosterone and prolactin levels. Model fitting was performed by

183 maximum likelihood with the PROC GENMOD procedure in SAS (SAS Institute Inc., 1999).

184 We used therefore maximum likelihood techniques and Akaike Information Criterion

185 statistics corrected for small sample size (AICc) to compare the extent to which our data fitted

186 each model (Burnham and Anderson, 2002). Because we used the same data set to test an

187 effect of 1/ breeding experience and 2/ age on hormonal levels, we can use AICc to compare

188 the ability of models including age variable or breeding experience variable to describe data

189 even if these models were not nested (Burnham and Anderson, 2002). We calculated the

190 Akaike Information Criterion corrected for small sample size using the formula AICc = -

191 2*log(Likelihood) +2*K + 2*K*(K+1)/(N – K – 1), where N is sample size and K the number

192 of parameters estimated in each model. Values for difference in AICc values (ΔAICc) were

193 computed by subtracting the minimum AICc from all candidate model AICc. Differences

194   between AICc values for different models can be used to determine which provides the most

195   adequate description of the data based on the fewest model parameters. The model with the

196   lowest AICc was considered the best fit that describes the relationship. ΔAICc values > 2 are

197   a good indicator that the model with the lowest AICc is preferable, whereas ΔAICc values < 2

198   indicate that models are fairly similar in their ability to describe the data (Burnham and

199   Anderson, 2002). Previous studies showed that prolactin levels vary between sexes in the

200   wandering albatross (Hector and Goldsmith, 1985), whereas corticosterone does not (Hector

201   and Harvey, 1986). We verified these results with our data set by using generalized linear

202   models. We used one model selection per sex to explain prolactin levels, whereas we pooled

203   data from both sexes to analyse the effect of our explanatory variables on corticosterone

204   levels. We included all combinations of parameters in our models because we had no a priori

205   reason to exclude particular states of any parameter or combination of parameters. Moreover,

206   we used the same method to assess the influence of the baseline levels of corticosterone on

207   baseline levels of prolactin in each sex. We started therefore this last analysis from this

208   general model (Year, Date, baseline levels of corticosterone and interactions). To make our

209   tables easier to read, we represented only the five most parsimonious models and the model

210   without any explanatory variable (the "Intercept model") in each table, and we ranked these

211   models according to their AICc. Because body weight measurements were available only for

212   a sub-sample of albatrosses, we could not incorporate the "body weight" variable in our

213   model selection frameworks. We therefore tested for an effect of body weight on 1)

214   corticosterone levels by using an ANCOVA; 2) prolactin levels by using one linear regression

215   per sex because prolactin levels vary between sexes in albatrosses (Hector and Goldsmith

216   1985).

217

Results

Effect of body condition, sex, year and date of sampling on hormonal levels

There was no significant relationship between body weight and plasma corticosterone or prolactin levels within both sexes (corticosterone, ANCOVA, $F_{2,13} = 0.02$ $P = 0.89$; prolactin, linear regressions, male: $F_{1,13} = 2.329$, $P = 0.15$, female: $F_{1,5} = 0.657$, $P = 0.45$). Baseline corticosterone levels did not vary between sexes because the model including "Sex" as a variable had a larger AICc than the "Intercept model" (ΔAICc = 2.10). For prolactin, the model including "Sex" as a variable had a much smaller AICc than the "Intercept" model (ΔAICc = 19.37), with prolactin levels being significantly higher in females than in males (Fig. 2 a,b). The information-theoretic approach identified two models with similar AICc as the best approximating models to explain baseline levels of corticosterone (Table 1, Model 1 & 2, ΔAICc = 0.74). Both models included "Date" as a variable and the second model included "Year" as a variable. Estimates of parameters of these models demonstrated that baseline levels of corticosterone decreased with date of sampling (Table 1, estimates of parameters) and were slightly higher in 1999 than in 2004, ($n = 69$, $\chi^2 = 1.68$, $P = 0.19$). Similarly, two models with similar AICc were identified as the best approximating models to explain baseline prolactin levels in both sexes (Table 2a, Model 1 & 2, ΔAICc = 1.83; Table 2b, Model 1 & 2, ΔAICc = 1.05). Both included "Year" as a variable and the second model included "Date" as a variable. Estimates of parameters demonstrated that baseline levels of prolactin were higher in 2004 than in 1999 (Table 2a,b, estimates of parameters) but did not vary significantly with the date of sampling, (Males, $n = 39$, $\chi^2 = 0.70$, $P = 0.40$; Females, $n = 36$, $\chi^2 = 1.32$, P = 0.25).

240

241   *Effect of age and breeding experience on hormonal levels*

242   Age and breeding experience distribution were similarly distributed in 1999 and 2004

243   (Age, 1999, mean = 18.50, min = 7, max = 33, 2004, mean = 22.19, min = 7, max = 38;

244   breeding experience, 1999, mean = 5.59, min = 1, max = 13, 2004, mean = 6.68, min = 1, max

245   = 15). Both models identified as the best approximating models to explain variations in

246   baseline corticosterone levels included "Experience" and "Experience^2" as variables,

247   demonstrating a strong effect of breeding experience on baseline levels of corticosterone

248   (Table 1, Model 1 & 2). Baseline levels of corticosterone increased therefore with breeding

249   experience to reach a maximum at the sixth breeding attempt, and then decreased with the

250   increasing breeding experience (Fig. 3, Table 1, estimates of parameters). There was however

251   no effect of age on baseline levels of corticosterone because models including "Age" or

252   "Age^2" variables had much larger AICc than the "Intercept model".

253   Both models identified as the best approximating models for baseline levels of

254   prolactin in males included "Experience" as a variable, demonstrating a strong influence of

255   breeding experience on baseline levels of prolactin in males (Table 2a, Model 1 & 2, ΔAICc =

256   1.83). Consequently, there was a positive linear relationship between breeding experience and

257   baseline levels of prolactin in males (Fig 4a, Table 2a, estimates of parameters). The

258   information-theoretic approach identified two models with similar AICc as the best

259   approximating models for baseline levels of prolactin in females, but none of these models

260   included "Experience" as a variable (Table 2b, Model 1 & 2, ΔAICc = 1.05). Plasma levels of

261   prolactin were therefore not explained by breeding experience in females (Fig 4b). Prolactin

262   levels were however, lower in inexperienced females than in experienced individuals

263   (Kruskall-Wallis test, n = 36, P = 0.023, Fig. 4b). Plasma levels of prolactin were not

264   explained by age in males and females. In both sexes, models including "Age" as a variable

265   were not selected as the best approximating models for baseline levels of prolactin.

266 Furthermore, the baseline levels of prolactin were not explained by baseline levels of

267 corticosterone in both sexes. The information-theoretic approach identified models without

268 baseline levels of corticosterone as the best approximating models for baseline levels of

269 prolactin.

270

271 **Discussion**

272 *Difference in hormonal levels between years, dates, and sex*

273 In our study, we found no relationships between body weight and hormones levels.

274 Wandering albatrosses have a wide safety margin of energy reserves and are able to buffer the

275 costs of fasting for several weeks without neglecting their egg (Weimerskirch, 1995, 1999)

276 and without significant changes in corticosterone and prolactin levels (Hector and Goldsmith,

277 1985; Hector and Harvey, 1986). Corticosterone levels were identical between males and

278 females (Hector & Harvey, 1986; this study), illustrating a similar shared breeding

279 expenditure between sexes in wandering albatrosses (Weimerskirch, 1995). Prolactin levels

280 were higher in females than in males (Hector & Goldsmith, 1985; this study), a pattern

281 commonly observed in several bird species but still not well understood (Ball 1991; Vleck,

282 1998; Lormée et al., 2000) because males and females provide roughly similar amounts of

283 parental care in the great majority of seabirds (Lack, 1968). We also found that baseline levels

284 of corticosterone and prolactin respectively varied with date and year of sampling. This could

285 result either from differences in energetic needs (Love et al. 2004; Chastel et al. 2005) or from

286 differences in environmental conditions/stressors through the study period (Delahanty et al.,

287 1997; Wingfield et al., 1998; Kitaysky et al., 1999; Pravosudov et al., 2001; Wingfield and

288 Kitaysky, 2002; Chastel et al. 2005).

289

290 *Parallel increase in hormonal levels over the first six breeding attempts*

291   We showed for the first time that breeding experience was a much better statistical

292   predictor of corticosterone and prolactin levels than age *per se* in wandering albatrosses.

293   Similarly, Cichoň, (2003) has experimentally demonstrated a larger contribution of previous

294   breeding experience than age on breeding performances. In our study baseline corticosterone

295   and prolactin levels exhibited a concomitant increase over the first six breeding attempts,

296   which shows a striking parallel with the improvement in breeding performance

297   (Weimerskirch, 1992, Weimerskirch et al., 2005).

298   Corticosterone can be seen either as a stimulator of breeding activities (Love et al.

299   2004) and/or as witness of the stress imposed by sustained breeding activities (review in

300   Wingfield et al. 1998). Thus, the corticosterone patterns we found (1) might be explained

301   either by a direct influence of previous breeding experience on the ability to secrete

302   corticosterone or (2) might be elevated in response to the extra parental expenditure that

303   might be expended by experienced successful birds.

304   Because elevated baseline corticosterone levels are traditionally associated with a poor

305   body condition (Kitaysky et al. 1999; Cherel et al. 1988, Kitaysky et al. 1999, Love et al.

306   2005), a lower ability to extract resources from the environment (Woodley et al. 2003,

307   Angelier et al. submitted) and a lower breeding success (Kitaysky et al. 1999), we predicted

308   that inexperienced albatrosses would bear elevated corticosterone levels. The pattern we

309   found was however the reverse. It could however be explained according to several recent

310   studies, which found that plasma corticosterone levels could be moderately elevated during

311   reproductive event without inducing breeding abandonment (Koch et al., 2002; Romero,

312   2002; Moore and Jessop, 2003; Love et al., 2004). Although these moderately elevated

313   corticosterone levels might only result from the stress of an increased foraging expenditure

314   necessary to breed successfully, several experimental studies have shown that small

315   elevations of corticosterone levels trigger foraging activity (Astheimer et al., 1992; Kitaysky

316   et al., 2001; Breuner and Hahn, 2003) and could therefore permit to meet the energetic

317   demands of reproduction (Love et al. 2004). Recent findings suggest even that small

318   elevations in corticosterone are an inherent component of successful reproduction because

319   they may facilitate parental behavior by increasing parental provisioning (Love et al., 2004;

320   Koch et al., 2004; Carlson et al. in press).

321   This increase in baseline corticosterone with advancing experience is paralleled by the

322   concomitant increase in prolactin levels in males. Although prolactin levels did not vary with

323   breeding experience among experienced females, inexperienced birds had the lowest prolactin

324   levels. To our knowledge, this relationship between breeding experience and prolactin levels

325   has only been examined in two species of monkeys: urinary prolactin levels increased with

326   experience in captive cottontop tamarins (*Saguinus oedipus*, Ziegler et al., 1996), but not in

327   captive common marmoset fathers (*Callithrix jacchus,* Schradin and Anzenberger, 2004).

328   Prolactin can be seen either as a stimulator of parental behavior or as the result of parental

329   activity (review in Buntin 1996). Thus, the prolactin patterns we found might be explained

330   either by: (1) a direct influence of previous breeding experience on the ability to secrete

331   prolactin or (2) by the intrinsic quality of birds with higher prolactin which may be more

332   successful breeders and therefore breed more often. In most bird species, stimuli from nest,

333   egg or chick are necessary to maintain elevated prolactin secretion rates and prolactin levels

334   decrease dramatically within few hours when these stimuli are removed (El Halawani et al.

335   1980; Hall, 1987; Book 1991; Wang and Buntin 1999). However, pelagic seabirds such as

336   albatrosses and penguins undergo very long absences from the nest when foraging (weeks or

337   even months; Jouventin and Weimerskirch 1990) and have probably evolved an endogenously

338   scheduled prolactin secretion poorly influenced by stimuli from nest, egg or chick (Hector and

339   Goldsmith 1985; Garcia et al. 1996; Vleck 1998; Lormée et al. 1999; Vleck et al. 2000).

340   Moreover, in the wandering albatrosses, successful breeders will systematically undergo a

341 sabbatical year (Tickell, 1968) entirely spent at sea (Weimerskirch and Wilson 2000),

342 whereas failed breeders will breed again in the following year (Tickell, 1968). All these

343 features strongly suggest a direct influence of previous breeding experience on the ability to

344 secrete prolactin in the wandering albatross. Because elevated prolactin levels are an essential

345 component of reproduction (review in Buntin, 1996), the lower breeding success of

346 inexperienced albatrosses (Weimerskirch 1992) could result from a lower ability to secrete

347 prolactin.

348 The increase in baseline hormonal levels with advancing experience observed in

349 wandering albatrosses would suggest an increase in parental investment over the first six

350 breeding attempts (Forslund and Pärt, 1995; the "restraint" hypothesis, Curio, 1983).

351 According to this hypothesis, hormonal secretion would then be down-regulated in less

352 experienced albatrosses resulting in low hormonal levels which in turn may limit breeding

353 expenditure (Love et al. 2004; Koch et al. 2004) or, stressful breeding activities would then be

354 limited in less experienced birds resulting therefore in low corticosterone levels (Wingfield et

355 al. 1998). Alternatively, the increase in baseline hormonal levels with advancing experience

356 may result from the development of an endocrine system adapted to energetic constraints of

357 reproduction which results in the inability of inexperienced birds to secrete a large amount of

358 hormones or from the lack of experience to sustain stressful breeding activities in less

359 experienced birds resulting then in limited stressful breeding activities and in low

360 corticosterone levels (Forslund and Pärt, 1995; the "constraint" hypothesis, Curio, 1983).

361 To test these hypotheses, we need to explore the causes of these hormonal changes

362 occurring with advancing experience. The "constraint hypothesis" (Curio, 1983) would be

363 validated if inexperienced birds are unable to secrete a large quantity of hormones.

364 Alternatively, the "restraint hypothesis" (Curio, 1983) would be validated if inexperienced

365 birds are physiologically able to secrete a large quantity of hormones, but down-regulate this

366 secretion either by endocrine mechanisms (i.e. regulation of corticosterone secretion by the

367 hypothalamic-pituitary-adrenal axis) or by limiting their stressful breeding activities. For

368 example, the ability to secrete corticosterone could be investigated by focusing on

369 physiological changes occurring in the hypothalamic-pituitary-adrenal axis with advancing

370 experience. Changes in adrenal's sensitivity to ACTH (which control adrenal corticosterone

371 release) with advancing experience could be tested by injecting inexperienced and

372 experienced birds with ACTH (Ait-Chaoui et al., 1995, Wilson and Holberton, 2001).

373 Similarly, testing whether increases in corticosterone secretion with advancing experience

374 results from a higher pituitary's sensitivity to releasing factors controlling ACTH secretion

375 could be evaluated by injecting these releasing factors to known-experienced birds (Romero

376 et al. 1998).

377

378 *Hormonal levels among senescent birds*

379 In our study, we found that corticosterone levels decreased in senescent breeders

380 which shows a striking parallel with the decrease in breeding performance (Weimerskirch,

381 1992, Weimerskirch et al., 2005). The decrease in corticosterone levels we found among

382 senescent albatrosses might be explained either by: (1) a direct influence of the accumulating

383 reproductive costs of previous breeding attempts on the ability to secrete corticosterone

384 (physiological senescence) or (2) by a decreased parental expenditure in senescent birds. The

385 decrease in baseline corticosterone levels that we found among senescent birds resembles the

386 pattern found by Goya et al. (1989) in senescent female rats. This decrease could therefore

387 result from physiological changes in the hypothalamic-pituitary-adrenal axis among senescent

388 birds. For example, this decrease could occur from changes in adrenal's sensitivity to ACTH.

389 Similarly, an alteration of the pituitary's sensitivity to releasing factors controlling ACTH

390 secretion (i.e. corticotropin releasing factor, arginine vasotocin and mesotocin) could result in

391   this decrease in corticosterone levels (Romero et al., 1998). The possible decline in adrenal's

392   or pituitary's sensitivity in senescent birds might be tested by injecting known-experienced

393   birds with ACTH or releasing factors controlling ACTH secretion (Ait-Chaoui et al., 1995;

394   Brudieux et al., 1995).

395   In our study, we did not observe the same decline in prolactin levels. Although we

396   cannot exclude that senescence may be accompanied by a deficit in prolactin ability to

397   stimulate parental care, the lack of a decline in prolactin levels among senescent birds may

398   suggest that their low breeding success does not result from poor quality care during

399   incubation.

400

401   **Conclusion**

402   We showed for the first time that baseline corticosterone and prolactin levels varied with

403   breeding experience in a long-lived species, the wandering albatross. Currently, no data exist

404   on the effects of age, experience and senescence on avian prolactin and corticosterone

405   secretion. Our results underline the need to describe baseline hormones levels and age in more

406   birds species in relation to different life-styles and life-history traits. There is also a need to

407   identify the physiological mechanisms involved in such experience-related hormonal patterns.

408   Future studies should also focus on corticosterone and prolactin responses to acute stress in

409   relation to age and breeding experience because these hormonal responses have been

410   considered as adaptive because they may trigger behavioral and physiological responses

411   which promote fitness of individuals during stressful periods (Wingfield et al., 1998; Chastel

412   et al., 2005).

413

414   **Acknowledgments**

426

427 **References**

428 Ait-Chaoui, A., Rakotondrazafy, J., Brudieux, R., 1995. Age-related changes in plasma

429   corticosterone and aldosterone responses to exogenous ACTH in the rat. Horm. Res. 43,

430   181-187.

431 Angelier, F., Shaffer, S.A., Weimerskirch, H., Trouvé, C., Chastel, O. Corticosterone and

432   foraging behaviour in a pelagic seabird. Submitted

433 Astheimer, L.B, Buttemer, W.A., Wingfield, J.C., 1992. Interactions of corticosterone with

434   feeding, activity and metabolism in passerine birds. Ornis Scand. 23, 355-365.

435 Ball, G.F., 1991. Endocrine mechanisms and the evolution of avian parental care. Acta XX

436 Congressus Internationalis Ornithologici 984-991. New Zealand Ornithological Trust Board,

437 Wellington.

438 Book, C.M., 1991. Brood patch innervation and its role in the onset of incubation in the

439   turkey hen. Phys. Behav. 50, 281-285.

440   Breuner, C.W., Hahn, T.P., 2003. Integrating stress physiology, environmental change, and

441   behavior in free-living sparrows. Horm Behav. 43, 115-123.

442   Brudieux, R., Ait-Chaoui, A., Rakotondrazafy, J., 1995. Age-related decreases in plasma

443   adrenocorticotropic hormone, corticosterone, and aldosterone responses to exogenous

444   corticotropin-releasing hormone in the rat. Gerontology. 41, 308-314.

445   Buntin, J.D., 1996. Neural and hormonal control of parental behaviour in birds. In:

446   Rosenblatt, J.S., Snowdon, C.T. (Eds.), Advances in the Study of Behavior, vol. 25.

447   Academic Press, New York, pp. 161-213.

448   Burnham, K.P., Anderson, D.R., 2002. Model selection and multimodel inference. Springer-

449   Verlag, New-York, USA.

450   Carlson, A.A., Manser, M.B., Young, A.J., Russell, A.F., Jordan, N.R., McNeilly, A.S.,

451   Clutton-Brock, T. Cortisol levels are positively associated with pup-feeding rates in male

452   meerkats. Proc. R. Soc. B. In press.

453   Chastel, O., Lacroix, A., Weimerskirch, H., Gabrielsen, G.W., 2005. Modulation of prolactin

454   but not corticosterone responses to stress in relation to parental effort in a long-lived bird.

455   Horm. Behav. 47, 459-466.

456   Cherel, Y., Robin, J.P., Walch, O., Karmann, H., Netchitailo, P., Le Maho, Y., 1988. Fasting

457   in king penguin. I. Hormonal and metabolic changes during breeding. Am. J. Physiol.

458   Regul. Integr. Comp. Physiol. 254, R170-R177.

459   Cherel, Y., Mauget, R., Lacroix, A., Gilles, J., 1994. Seasonal and fasting-related changes in

460   circulational gonadal steroids and prolactin in king penguins, *Aptenodytes patagonicus*.

461   Physiol. Biochem. Zool. 67, 1154-1173.

462   Cichoñ, M., 2003. Does prior breeding experience improve reproductive success in collared

463   flycatcher females? Oecologia 134, 78-81.

464   Clutton-Brock, T.H., 1988. Reproductive success. University of Chicago Press.

465   Clutton-Brock, T.H., 1991. The evolution of parental care. Princeton University Press.

466   Curio, E., 1983. Why do young birds reproduce less well? Ibis 125, 400-404.

467   Delahanty, D.J., Oring, J.W., Fivizzani, A.J., El Halawani, M.E., 1997. Circulating prolactin

468   of incubating male Wilson's phalaropes corresponds to clutch size and environmental stress.

469   Condor. 99, 397-405.

470   El Halawani, M.E., Burke, W.H., Dennison, P.T., 1980. Effects of p-chlorophenylalanine on

471   the rise in serum prolactin associated with nesting in broody turkeys. Biol. Repro. 35, 59-67.

472   Forslund, P., Pärt, T., 1995. Age and reproduction in birds – hypotheses and tests. Tr. Ecol.

473   Evol. 10, 374-378.

474   Garcia, V., Jouventin, P., Mauget, R., 1996. Parental care and the prolactin secretion pattern

475   in the king penguin: an endogenously timed mechanism? Horm. Behav. 30, 259-265.

476   Goya, R.G., Castro, M.G., Sosa, Y.E., 1989. Diminished diurnal secretion of corticosterone in

477   aging female but not male rats. Gerontology. 35, 181-187.

478   Hall, M.R., 1987. External stimuli affecting incubation behavior and prolactin secretion in the

479   duck (*Anas platyrhynchos*). Horm. Behav. 21, 269-287.

480   Hector, J.A.L., Goldsmith, A.R., 1985. The role of prolactin during incubation: comparative

481   studies of three *Diomedea* albatrosses. Gen. Comp. Endocrinol. 60, 236-243.

482   Hector, J.A.L., Harvey, S., 1986. Corticosterone secretion through long incubation shifts in

483   *Diomedea* albatrosses. Gen. Comp. Endocrinol. 62, 349-352.

484   Hector, J.A.L, Pickering, S.P.C., Croxall, J.P., Follett, B.K., 1990. The endocrine basis of

485   deferred sexual maturity in the wandering albatross, *Diomedea exulans* L. Funct. Ecol. 4,

486   59-66.

487   Jouventin, P., Weimerskirch, H., 1990. Satellite tracking of wandering albatrosses. Nature

488   343, 746-748.

489   Kirkwood, T.B.L., Austad, S.N., 2000. Why do we age? Nature 408, 233-238.

490   Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 1999. Dynamics of food availability, body

491   condition and physiological response in breeding black-legged kittiwakes. Funct. Ecol. 13,

492   577-585.

493   Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 2001. Corticosterone facilitates begging and

494   affects resource allocation in the black-legged kittiwake. Behav. Ecol. 12, 619-625.

495   Koch, K.A., Wingfield, J.C., Buntin, J.D., 2002. Glucocorticoids and parental hyperphagia in

496   ring doves (*Streptopelia risoria*). Horm. Behav. 41, 9-21.

497   Koch, K.A., Wingfield, J.C., Buntin, J.D., 2004. Prolactin-induced parental hyperphagia in

498   ring doves: are glucocorticoids involved? Horm. Behav. 46, 498-505.

499   Lack, D., 1968. Ecological Adaptations for breeding in birds. Methuen Ltd, London.

500   Lormée, H., Jouventin, P., Chastel, O., Mauget, R., 1999. Endocrine correlates of parental

501   care in an Antarctic winter breeding seabird, the emperor penguin, *Aptenodytes forsteri*.

502   Horm. Behav. 35, 9-17

503   Lormée, H., Jouventin, P., Lacroix, A., Chastel, O., 2000. Reproductive endocrinology of

504   tropical seabirds : sex-specific patterns in LH, steroids, and prolactin secretion in relation

505   to parental care. Gen. Comp. Endocrinol. 117, 413-426.

506   Lormée, H., Jouventin, P., Trouve, C., Chastel, O., 2003. Sex-specific patterns in baseline

507   corticosterone and body condition changes in breeding Red-footed Boobies *Sula sula*. Ibis

508   145, 212-219.

509   Love, O.P., Breuner, C.W., Vézina, F., Williams, T.D., 2004. Mediation of a corticosterone-

510   induced reproductive conflict. Horm Behav. 46, 59-65.

511   Love, O.P., Chin, E.H., Wynne-Edwards, K.E., Williams T.D., 2005. Stress hormones: a link

512   between maternal condition and sex-biased reproductive investment. Am. Nat. 166, 751-

513   766.

514   Mauck, R.A., Huntington, C.E., Grubb Jr, T.C., 2004. Age-specific reproductive success:

515   evidence for the selection hypothesis. Evolution 58, 880-885.

516   Moore, I.T., Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation in

517   amphibians and reptiles. Horm. Behav. 43, 39-47.

518   Newton, I., 1989. Lifetime reproduction in birds. Academic Press, London.

519   Nisbet, I.C.T., Finch, C.E., Thompson, N., Russek-Cohen, E., Proudman, J.A., Ottinger,

520   M.A., 1999. Endocrine patterns during aging in the common tern (*Sterna hirundo*). Gen.

521   Comp. Endocrinol. 114, 279-286.

522   Ottinger, M.A., Reed, E., Wu, J., Thompson, N., French Jr. J.B., 2003. Establishing

523   appropriate measures for monitoring in birds: comparing short and long-lived species.

524   Exp. Gerontol. 38, 747-750.

525   Pärt, T., 2001. Experimental evidence of environmental effects on age-specific reproductive

526   success: the importance of resource quality. Proc. R. Soc. Lond. B 268, 2267-2271.

527   Pravosudov, V.V., Kitaysky, A.S., Wingfield, J.C., Clayton, N.S., 2001. Long-term

528   unpredictable foraging conditions and physiological stress response in mountain

529   chickadees (*Poecile gambeli*). Gen. Comp. Endocrinol. 124, 324-331.

530   Reid, J.M., Bignal, E.M., Bignal, S., McCracken, D.I., Monaghan, P., 2003. Age-specific

531   reproductive performance in red-billed choughs Pyrrhocorax: patterns and processes in a

532   natural population. J. Anim. Ecol. 72, 765-776.

533   Remage-Healey, L., Romero, L.M., 2001. Corticosterone and insulin interact to regulate

534   glucose and triglyceride levels during stress in a bird. Am. J. Physiol. Regulatory

535   Integrative Comp. Physiol. 281, R994-R1003.Roff, D.A., 1992. The evolution of life

536   histories: theory and analysis. Chapman and Hall, New-York.

537   Romero, L.M., Soma, K.K., Wingfield, J.C., 1998. Hypothalamic-pituitary-adrenal axis

538   changes allow seasonal modulation of corticosterone release in a bird. Am. J. Physiol.

539   274, R1338-R1344.

540   Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living

541   vertebrates. Gen. Comp. Endocrinol. 128, 1-24.

542   Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Tr.

543   Ecol. Evol. 19, 249-255.

544   Sapolsky, R.M., Romero, L., Munck, A., 2000. How do glucocorticoids influence stress

545   responses? Integrating permissive, suppressive, stimulatory, and preparative actions.

546   Endocr. Rev. 21, 55-89?

547   SAS Institute, 1999. SAS user's guide. Version 8.02. SAS Institute, Cary, North Carolina,

548   USA.

549   Schradin, C., Anzenberger, G., 2004. Development of prolactin levels in marmoset males:

550   from adult son to first-time father. Horm. Behav. 46, 670-677.

551   Silverin, B., 1986. Corticosterone-binding proteins and behavioural effects of high plasma

552   levels of corticosterone during the breeding period. Gen. Comp. Endocrinol. 64, 67-74.

553   Sinervo, N., Svensson, E., 1998. Mechanistic and selective causes of life-history trade-offs

554   and plasticity. Oikos 83, 432-442.

555   Stearns, S.C., 1992. The Evolution of Life Histories. Oxford University press, Oxford.

556   Tickell, W.L.N., 1968. The biology of the great albatrosses, *Diomedea exulans and Diomedea*

557   *epomophora*. Ant. Res. Series 12, 1-55.

558   Vleck, C.M., 1998. Hormonal control of incubation/brooding behavior: lessons from Wild

559   birds. Proceedings of the WSPA 10th European Poultry Conference, Israel, 1998. pp. 163-

560   169.

561 Vleck, C.M., Ross, L., Vleck, D., Bucher, T.L., 2000. Prolactin and parental behavior in

562   Adélie penguins: effect of absence from nest, incubation length, and nest failure. Horm.

563   Behav. 38, 149-158.

564 Wang, Q., Buntin, J.D., 1999. The roles of stimuli from young, previous breeding experience,

565   and prolactin in regulating parental behavior in ring doves (*Streptopelia risoria*). Horm;

566   Behav. 35, 241-253.

567 Warham, J., 1990. The Petrels: Their Ecology And Breeding Systems. Academic Press Inc.,

568   San Diego, CA.

569 Weimerskirch, H. & Jouventin, P., 1987. Population dynamics of the wandering albatross,

570   *Diomedea exulans*, of the Crozet Islands: causes and consequences of the population

571   decline. Oikos 49, 315-322.

572 Weimerskirch, H., Lequette, B., Jouventin, P., 1989. Development and maturation of plumage

573   in the wandering albatross, *Diomedea exulans*. J. Zool. 219, 411-421.

574 Weimerskirch, H. 1992. Reproductive effort in long-lived birds: age-specific patterns of

575   condition, reproduction and survival in the wandering albatross. Oikos 64, 464-473.

576 Weimerskirch, H., 1995. Regulation of foraging trips and incubation routine in male and

577   female wandering albatrosses. Oecologia 102, 37-43.

578 Weimerskirch, H., Brothers, N., Jouventin, P., 1997. Population dynamics of wandering

579   albatrosses *Diomedea exulans* and Amsterdam albatross *D. amsterdamensis* in the Indian

580   ocean and their relationships with longline fisheries; conservation implications. Biol.

581   Cons. 79, 257-270.

582 Weimerskirch, H., 1999. The role of body condition on breeding and foraging decisions in

583   albatrosses and petrels. In: N.J., Adams, R.H., Slotow, (Eds), Proceedings of the XXII

584   International Ornithology Congress. University of Natal, Durban, August 1998, pp. 1179-

585   1189. Birdlife South Africa, Johannesburg.

586   Weimerskirch, H., Wilson, R.P., 2000. Oceanic respite for wandering albatrosses. Birds

587   taking time off from breeding head for their favourite long-haul destinations. Nature 406,

588   955-956.

589   Weimerskirch, H., Lallemand, J., Martin, J., 2005. Population sex ratio in a monogamous

590   long-lived bird, the wandering albatross. J. Anim. Ecol. 74, 285-291.

591   Wilson, C.M., Holberton, R.L., 2001. An alternative method for delivering

592   adrenocorticotropin hormone in birds. Gen. Comp. Endocrinol. 122, 349-353.

593   Wingfield, J.C., Smith, J.P., Farner, D.S., 1982. Endocrine response of white-crowned

594   sparrows to environmental stress. Condor 84, 399-409.

595   Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M.,

596   Richardson, R.D., 1998. Ecological bases of hormone-behavior interactions: the

597   "emergency life history stage." Am. Zool. 38, 191-206.

598   Wingfield, J.C., Kitaysky, A.S., 2002. Endocrine responses to unpredictable environmental

599   events: stress or anti-stress hormones? Integ. Comp. Biol. 42, 600-609.

600   Woodley, S.K., Painter D.L., Moore M.C., Wikelski M., Romero, L.M., 2003. Effect of tidal

601   cycle and food intake on the baseline plasma corticosterone rhythm in intertidally foraging

602   marine iguanas. Gen. Comp. Endocrinol. 132, 216-222.

603   Ziegler, T.E., Wegner, F.H., Snowdon, C., 1996. Hormonal responses to parental and

604   nonparental conditions in male cotton-top tamarins, *Saguinus Oedipus*, a New world

605   primate. Horm. Behav. 30, 287-297.

606   **Table 1.** Model selection using Akaike's Information Criterion (AICc) to explain baseline

607   corticosterone levels measured at the nest ($n = 69$). General linear models with normal

608   distribution and identity link were used to test for variable effect. K refers to the number of

609   parameters. The five most parsimonious models and the model without any explanatory

610   variable (the "Intercept model") are represented and are ranked according to their AICc. The

611   second part of the table includes the parameter estimates for the selected model.

N°	Model	K	Log Likelihood	AICc	ΔAICc
1	Experience; Experience²; Date	4	-168.16	344.95	0
2	Year; Experience; Experience²; Date	5	-167.37	345.69	0.74
3	Year; Experience; Experience; Date*Year	6	-167.20	347.75	2.80
4	Year; Experience*Year; Experience²*Year; Date	7	-166.20	348.24	3.29
5	Date	2	-172.74	349.66	4.71
6	Intercept	1	-178.96	359.98	15.03

Selected Model	Parameter	Estimate ± SE	Chi-Square	p
Experience; Experience²; Date	Intercept	4.61 ± 1.28	12.93	< 0.001
Experience; Experience²; Date	Experience	1.03 ± 0.37	7.73	0.005
Experience; Experience²; Date	Experience²	-0.08 ± 0.03	9.32	0.002
Experience; Experience²; Date	Date	-0.08 ± 0.02	12.67	< 0.001

26

612 **Table 2a.** Model selection using Akaike's Information Criterion (AICc) to explain baseline

613 prolactin levels in males (n = 39). Generalized linear models with normal distribution and

614 identity link were used to test for variable effect. K refers to the number of parameters. The

615 five most parsimonious models and the model without any explanatory variable (the

616 "Intercept model") are represented and are ranked according to their AICc. The second part of

617 the table includes the parameter estimates for the selected model.

N°	Model	K	Log Likelihood	AICc	ΔAICc
1	Year; Experience	3	-107.88	222.45	0
2	Year; Date; Experience	4	-107.55	224.28	1.83
3	Year; Experience; Experience*Year	4	-107.91	225.00	2.55
4	Year	2	-110.55	225.43	2.98
5	Year; Date; Date*Year; Experience	5	-107.27	226.36	3.91
6	Intercept	1	-119.41	240.93	18.48

Selected Model	Parameter	Estimate ± SE	Chi-Square	p
Year; Experience	Intercept	26.29 ± 1.54	291.98	< 0.001
Year; Experience	Year (1999)	-6.47 ± 1.33	23.48	< 0.001
Year; Experience	Year (2004)	.	.	.
Year; Experience	Experience	0.45 ± 0.20	5.34	0.021

618 **Table 2b.** Model selection using Akaike's Information Criterion (AICc) to explain baseline

619 prolactin levels in females ($n = 36$). General linear models with normal distribution and

620 identity link were used to test for variable effect. K refers to the number of parameters. The

621 five most parsimonious models and the model without any explanatory variable (the

622 "Intercept model") are represented and are ranked according to their AICc. The second part of

623 the table includes the parameter estimates for the selected model.

N°	Model	K	Log Likelihood	AICc	ΔAICc
1 Year		2	-101.78	207.92	0
2 Year; Date		3	-101.11	208.97	1.05
3 Year; Experience		3	-101.59	209.93	2.01
4 Year; Age		3	-101.81	210.37	2.45
5 Year; Date; Date*Year		4	-101.11	211.51	3.59
6 Intercept		1	-106.98	216.08	8.16

Selected Model	Parameter	Estimate ± SE	Chi-Square	p
Year	Intercept	33.97 ± 0.79	1828.58	< 0.001
Year	Year (1999)	-5.70 ± 1.69	11.45	< 0.001
Year	Year (2004)	.	.	.

624

28

625 **Fig. 1.** Dose response curve for prolactin in poultry chickens and wandering albatrosses.

626 Prolactin standard is expressed in pg tube^{-1}.

627 **Fig. 2.** Baseline corticosterone (a) and prolactin (b) levels (ng ml^{-1}) of male (white bars) and

628 female (black bars) breeding wandering albatrosses in 1999 and 2004. Data are expressed as

629 mean ± SE. Numbers above bars denote sample sizes. Asterisks ("***") indicate significant

630 differences between groups; "n.s" indicates no significant differences between groups.

631 **Fig. 3.** Baseline corticosterone (ng ml^{-1}) levels and breeding experience in wandering

632 albatrosses (*n* = 69). Statistical analyses treated each hormone measurements as single

633 observations. Data are expressed as mean ± SE. Numbers above bars denote sample sizes.

634 Solid line represents the relationship described by the best model (lowest AICc) selected to

635 explain baseline corticosterone levels (Table 1, Model 1). Males and Females data were

636 pooled

637 **Fig. 4.** Baseline prolactin levels (ng ml^{-1}) and breeding experience in male and female

638 wandering albatrosses (*n* = 75). Statistical analyses treated each hormone measurements as

639 single observations. Data were corrected for a year effect and are expressed as means ± SE.

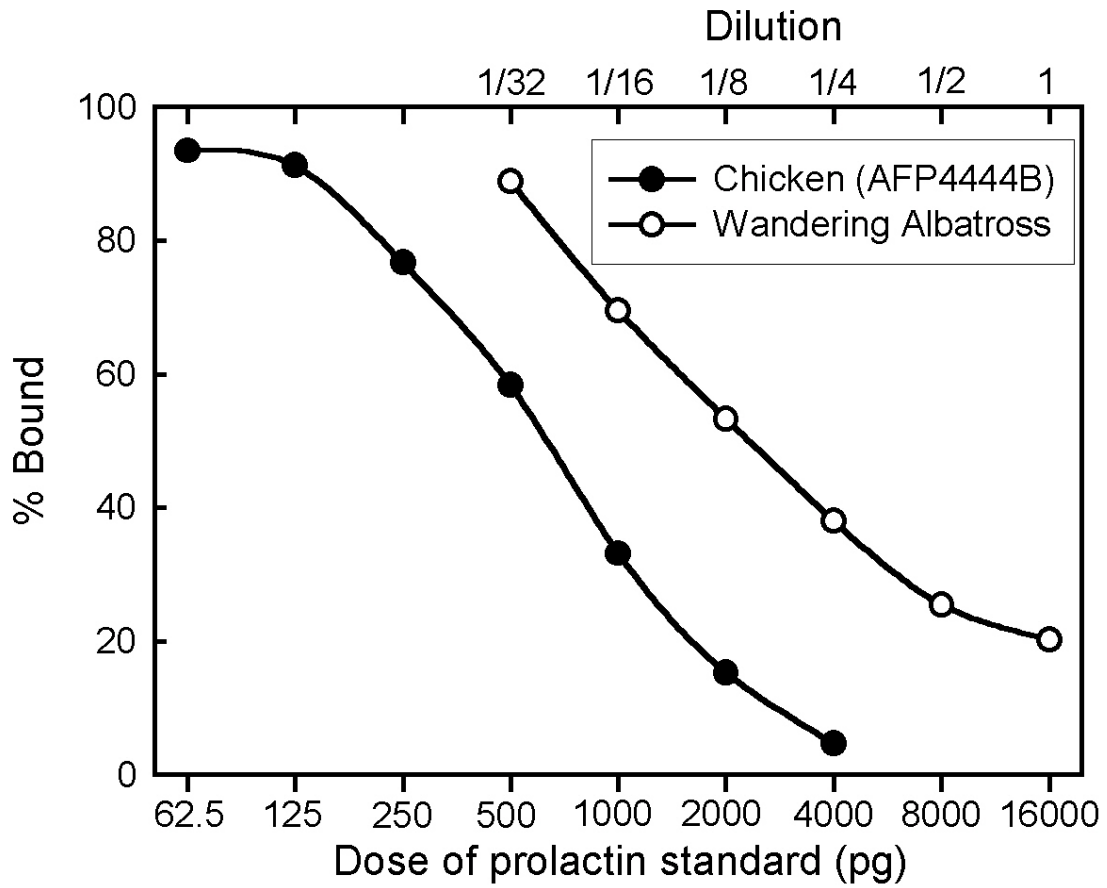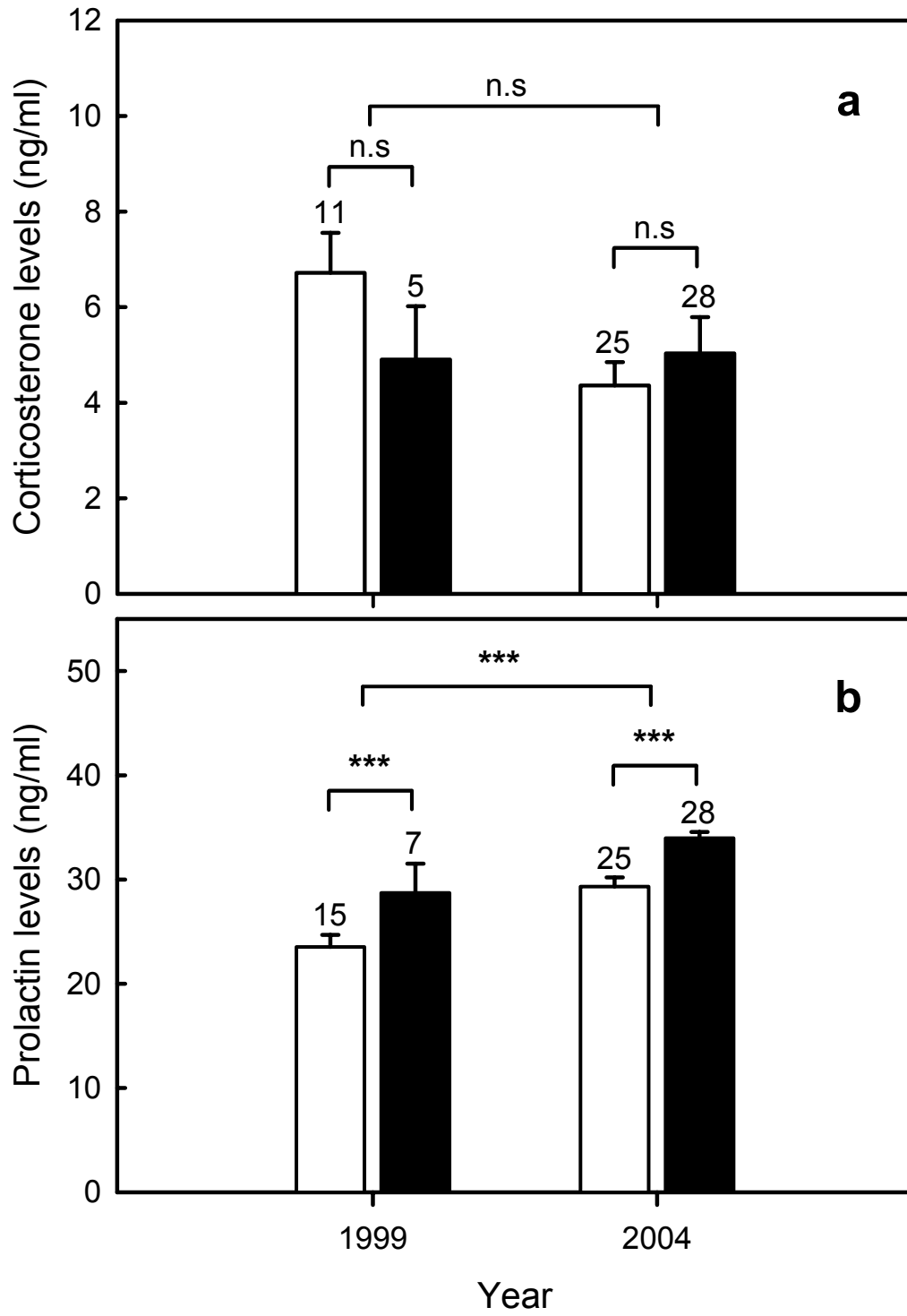640 Numbers above bars denote sample size.

641

642

643

644

645

646

647   Fig. 1., Angelier et al.
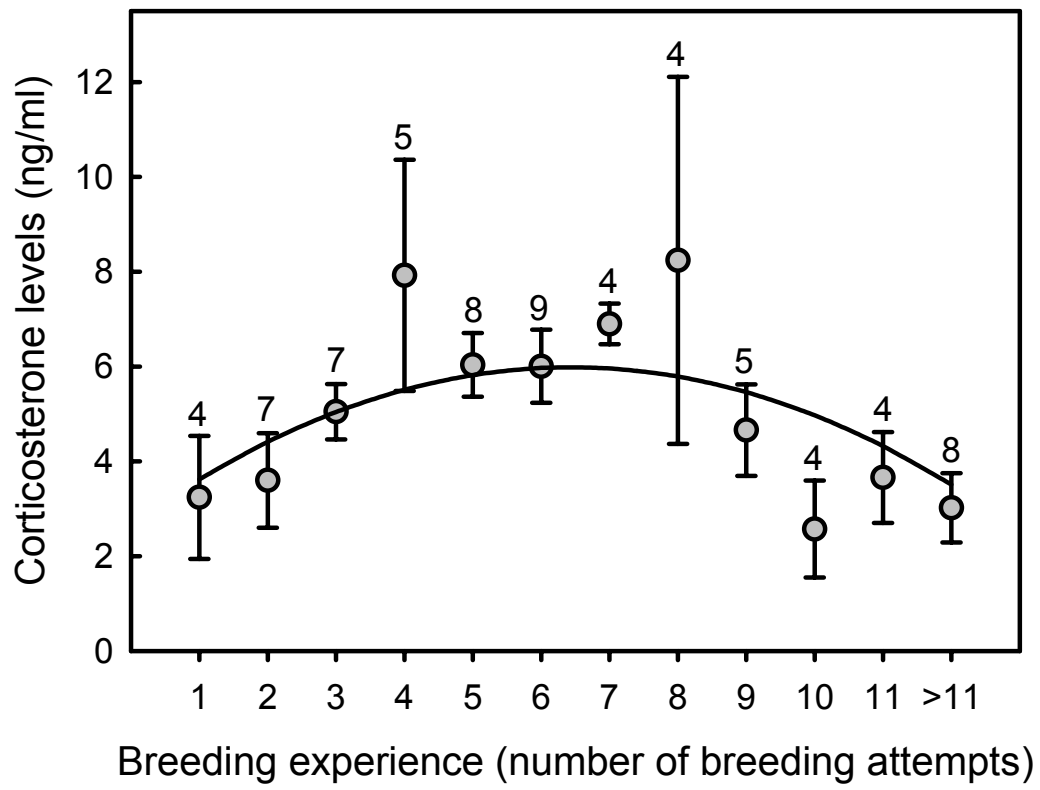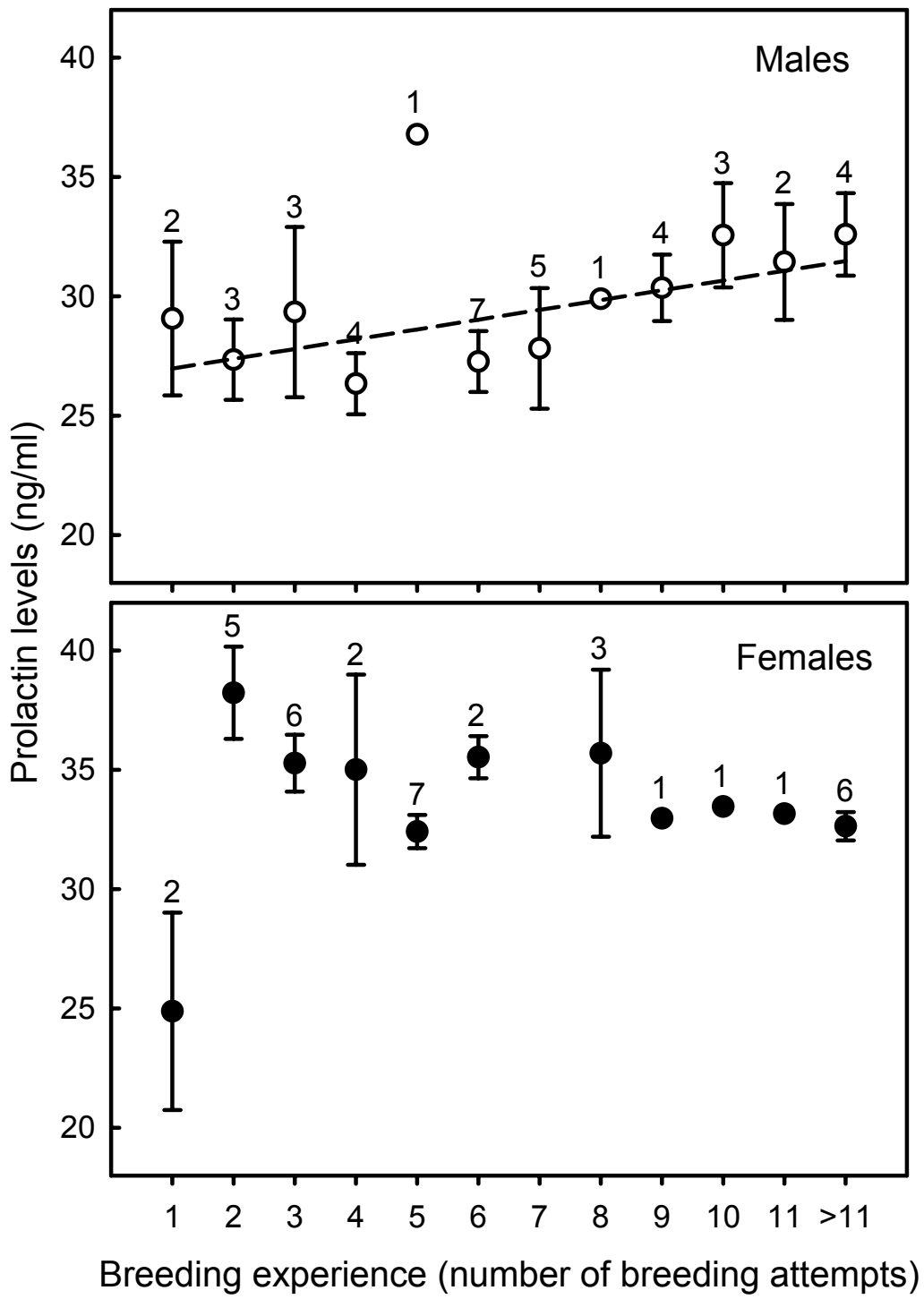
648

649

650

Fig. 2., Angelier et al.

Fig. 4., Angelier et al.