

Age-specific reproductive success in a long-lived bird: do older parents resist stress better?

FREDERIC ANGELIER*, BØRGE MOE†, HENRI WEIMERSKIRCH* and OLIVIER CHASTEL*

*Centre d'Etudes Biologiques de Chizé, Centre National de la Recherche Scientifique, F-79360 Villiers en Bois, Deux-Sèvres, France; and †Department of Biology, Norwegian University of Science and Technology, NO-7491, Trondheim, Norway

Summary

1. In many vertebrates, reproductive performance increases with advancing age but mechanisms involved in such a pattern remain poorly studied. One potential mechanism may be the hormonal stress response, which shifts energy investment away from reproduction and redirects it towards survival. In birds, this stress response is achieved through a release of corticosterone and is also accompanied by a decrease in circulating prolactin, a hormone involved widely in regulating parental cares.

2. It has been predicted that, when the value of the current reproduction is high relative to the value of future reproduction and survival, as it is expected to be in older adults, the stress response should be attenuated to ensure that reproduction is not inhibited.

3. We tested this hypothesis by measuring the corticosterone and prolactin responses of known-age (8–36 years old) incubating snow petrels (*Pagodroma nivea*) to a standardized capture/handling stress protocol. We also investigated whether an attenuation of the stress responses will correlate with a lower occurrence of egg neglect, a frequently observed behaviour in snow petrels.

4. The probability of successfully fledging a chick increased from 6 years to 12 years before stabilizing after 12 years of age. Corticosterone response to stress was unaffected by age. Prolactin response to stress, however, was influenced clearly by age: in both sexes older breeders had higher stress-induced prolactin levels than younger ones. This was due to an increasing attenuation of the prolactin response to stress with advancing age in females, and in males this was due to a probably higher intrinsic capacity of older males to secrete prolactin. Moreover, higher stress-induced prolactin levels were correlated with a lower probability of neglecting the egg.

5. In young breeders, the combination of a robust corticosterone increase with a lower ability to maintain prolactin secretion during acute stress is probably one of the functional causes of their lower incubation commitment. We suggest that the ability to maintain a threshold level of prolactin during a stressful situation may be an important physiological mechanism involved in the improvement of reproductive performance with advancing age in long-lived birds.

Key-words: age, corticosterone, *Pagodroma nivea*, prolactin, snow petrel, stress response.

Journal of Animal Ecology (2007) **76**, 1181–1191

doi: 10.1111/j.1365-2656.2007.01295.x

Introduction

Many long-term studies have described age-specific reproductive performance and the general pattern is that reproductive performance increases through the early years of breeding (Clutton-Brock 1988; Newton 1989; Weimerskirch 1992; Reid *et al.* 2003; Mauck, Huntington & Grubb 2004). Although ultimate factors

Correspondence: Frédéric Angelier, Centre d'Etudes Biologiques de Chizé, Centre National de la Recherche Scientifique, F-79360 Villiers en Bois, Deux-Sèvres, France. Tel: 33 5 49 09 78 37; Fax: 33 5 49 09 65 26; E-mail: angelier@cebc.cnrs.fr

explaining why young breeders reproduce less well than older individuals have been discussed widely (Curio 1983; Forslund & Pärt 1995), proximate factors influencing such patterns remain poorly studied (Angelier *et al.* 2006a, 2007a; Heidinger, Nisbet & Ketterson 2006). In this context physiological mechanisms deserve specific attention because they mediate life-history strategies to environmental stressors and perceived risks (Wingfield *et al.* 1998; Ricklefs & Wikelski 2002).

One potential mechanism underlying age-specific reproductive performance may be the endocrine stress response. In vertebrates, stressors such as food shortage, predators and inclement weather induce a rapid elevation of circulating glucocorticoid hormones, i.e. corticosterone or cortisol (Wingfield *et al.* 1998). This endocrine response promotes the mobilization of energy resources and the occurrence of behavioural and physiological forms that enhance the immediate survival, but also compromises the current reproductive episode by redirecting resources away from reproduction (Wingfield & Sapolsky 2003).

Although the majority of research on the stress response has focused on changes in corticosterone levels other hormones, such as prolactin, may also play a significant role (Chastel *et al.* 2005). Prolactin is a pituitary hormone that is involved widely in regulating parental behaviour and decisions in vertebrates (Buntin 1996), and has been shown to decrease in response to stressors in birds (Chastel *et al.* 2005).

According to life history theory, when the value of the current reproduction is high relative to the value of future reproduction and survival the corticosterone stress response should be attenuated to ensure that reproduction is not inhibited (Wingfield & Sapolsky 2003). Accordingly, the corticosterone stress response has been shown to be attenuated or even suppressed during the stage of the breeding cycle when adults are providing the most care for young (referred to as the 'parental care hypothesis'; Wingfield, O'Reilly & Astheimer 1995; Lendvai, Giraudeau & Chastel 2007), in the sex that provides more care for offspring (O'Reilly & Wingfield 2001) and when breeding opportunities are limited and the probability of reneating is low (referred to as the 'short season hypothesis'; Wingfield *et al.* 1995; Romero, Ramenofsky & Wingfield 1997; Silverin & Wingfield 1998). Similarly, the magnitude of the prolactin decrease has been shown to be modulated in relation to the level of parental effort (Chastel *et al.* 2005). This attenuation of the prolactin response to stress can be considered as a hormonal tactic permitting the maintenance of parental care and maximizing current reproductive efforts during temporary stressful conditions (Chastel *et al.* 2005).

As organisms age, the value of current reproduction is predicted to increase relative to the value of future reproduction and survival because future reproductive opportunities are expected to decline (Stearns 1992). Therefore, the evolutionary logic behind a modulation

of the stress response predicts that the magnitude of the response to stressors (corticosterone and prolactin) should decrease with age (Wingfield & Sapolsky 2003). One study focusing on corticosterone supported this prediction in the common tern, *Sterna hirundo* (Heidinger *et al.* 2006), but empirical data, especially relating to prolactin, are still scarce.

In the present study, we tested the hypothesis that older parents would be better able to resist stress by attenuating their corticosterone and prolactin stress responses in a long-lived bird, the snow petrel (*Pagodroma nivea* Foster). First, we checked whether breeding success improved with age using data from a long-term survey of individual breeding success from 1963 to the present (Chastel, Weimerskirch & Jouventin 1993).

Secondly, we performed a cross-sectional study during one field season to test if older parents are better able to resist the stress response. We examined the relationships between age and corticosterone and prolactin stress responses by using a standardized capture/restraint stress protocol (Wingfield *et al.* 1995). Specifically, we predicted that (1) the rate of corticosterone increase would decrease with age and that stress-induced corticosterone levels would be lower in older birds, and that (2) the rate of prolactin decrease would decrease with age and stress-induced prolactin levels would be higher in older birds.

Finally we tested, for the first time at the individual level, the hypothesis that the strength of the stress responses can mirror parental decisions, by linking the probability of egg neglect to stress-induced corticosterone and prolactin levels. Despite the large amount of published studies on the corticosterone response to acute stress (reviewed in Romero 2002), to our knowledge there are few available data showing that individual variation in the sensibility to acute stress predicts the probability to desert reproduction (Love *et al.* 2004). Snow petrels are excellent subjects for such an investigation because, as many small-sized petrels, they frequently undergo spontaneous egg neglect which delays hatching and lowers the probability for the chick to hatch (Chaurand & Weimerskirch 1994; Olson, Vleck & Vleck 2006). We predicted that (3) elevated stress-induced corticosterone levels and low stress-induced prolactin levels would be associated with a higher probability of egg neglect during the incubation shift following the stress protocol.

Methods

STUDY SITE AND SPECIES

Snow petrels (250–500 g) are long-lived birds with a low annual fecundity (average adult life expectancy is 30 years and only one egg is laid per season, with no replacement clutch in case of failure; Chastel *et al.* 1993; Barbraud & Weimerskirch 2001). Sexual maturity is acquired on average at 10 years of age and birds

breed annually, although a significant proportion of birds skip breeding each year (Chastel *et al.* 1993). Although males undertake slightly longer incubation shifts than females, males and females provide roughly similar amounts of parental care in this species. The age-specific breeding performances were studied on Ile des Pétrels, Pointe Géologie Archipelago, Terre Adélie (66°40' S, 140°01' E), Antarctica. The ongoing mark–recapture programme of this species began in 1963 and has provided yearly information on breeding success of every breeding pair in the surveyed colony. Details on the monitoring methodology are provided in previous studies (Chastel *et al.* 1993; Barbraud & Weimerskirch 2001). All chicks of the monitored colony have been banded prior to fledging every year since 1963. Because some of the fledged chicks have returned to the colony to breed, a substantial proportion of the individuals are of known age (from 6 to 39 years of age). Therefore, with the long-term monitoring data, we were able to investigate the influence of age on breeding performance.

BLOOD SAMPLE, BODY CONDITION AND EGG NEGLECT

The field study was carried out during the austral summer 2005/2006 (16–30 December 2005). During the incubation period, 48 known-age adults (8–36 years old) were captured at their nest in an area situated outside the long-term study plots to reduce handling there, so that breeding experience of the birds was unknown. Only one bird was captured per nest so that male and female data can be considered independently. All birds were bled according to the standardized capture/restraint stress protocol described by Wingfield (1994). Immediately after capture, an initial blood sample was collected from the alar vein with a 1-mL heparinized syringe and a 25-gauge needle. After collection of the initial blood samples birds were placed into cloth bags, and subsequent samples were collected 30 min later. During handling of the adult birds, their eggs were covered with cotton and kept warm. All birds were weighed to the nearest 2 g using a spring balance (Federwaage, Germany) and their skull length (head + bill) was measured to the nearest 0.5 mm. In snow petrels, skull length appears to be a reliable measure of the overall size of a bird (unpublished data). As male and female snow petrels differ greatly in size (Barbraud & Jouventin 1998), their body condition was calculated separately. Because residuals from an ordinary least-squares linear regression of body mass against body size can lead to spurious results (Green 2001), we calculated our residual indices of condition by using the residuals from a reduced major axis regression of body mass against skull length. Petrels were then marked with spots of dye on the forehead and the breast, to distinguish them from their partner, and were released at their nests. After capture and blood sample, each nest

was monitored every 2 days until the manipulated petrel was relieved by its mate. We were therefore able to know whether a bird neglected its egg during the incubation bout following capture/restraint stress protocol. Because temporary egg neglect can last during several days in snow petrels (unpublished data), checking the nests every 2 days appears to be sufficient to monitor egg neglect.

MOLECULAR SEXING AND HORMONE ASSAY

Blood samples were centrifuged and plasma was decanted and stored at –20 °C until they were assayed. After centrifugation, red cells were also kept frozen for molecular sexing. The sex was determined by polymerase chain reaction (PCR) amplification of part of two highly conserved genes (CHD) genes present on the sex chromosomes (Fridolfsson & Ellegren 1999) at the Centre d'Etudes Biologiques de Chizé (CEBC), as detailed in Weimerskirch, Lallemand & Martin (2005). Plasma concentrations of corticosterone were determined first by radioimmunoassay at the CEBC, as described previously (Lormée *et al.* 2003). Because blood samples were collected within 3 min of capture, they were considered to reflect baseline levels of corticosterone (Romero & Reed 2005). Plasma concentrations of prolactin were determined with the remaining plasma by a heterologous radioimmunoassay at the CEBC as detailed in Chérel *et al.* (1994). For one petrel, we did not have enough plasma to assay both baseline and stress-induced prolactin levels. For another petrel, we did not have enough plasma to assay baseline prolactin level. Pooled plasma samples of snow petrels produced a dose–response curve that paralleled chicken prolactin standard curves ('AFP 4444B', source: Dr Parlow, NHPP Harbor-UCLA Medical Center, Torrance, CA, USA), therefore validating our prolactin radioimmunoassay for snow petrels. All samples were run in one assay for both hormones (intra-assay variation, corticosterone: 7.2%, prolactin: 8.3%, $n = 5$ duplicates).

STATISTICAL ANALYSES

All analyses were performed with SAS statistical software (SAS Institute Inc., version 9.1). We used a step-down approach (backward elimination) starting from the most global model. Likelihood ratio statistics and likelihood ratio tests (LRT) were used to select the most parsimonious final model (Burnham & Anderson 2002). Models were therefore simplified by eliminating independent variables with $P \geq 0.15$.

AGE-SPECIFIC REPRODUCTIVE PERFORMANCE

Using data from the long-term study plots, we tested whether age explained a significant proportion of variation in breeding performance among birds whose age was known ($n = 135$ birds, $n = 673$ observations).

Because previous studies on long-lived birds (reviewed in Forslund & Pärt 1995) suggested that breeding performance could increase with age (age variable) before declining in oldest birds, we included the squared term (age^2) in the initial model designed to explain the probability of successfully fledging a chick by age.

Mean fledging success of this population varied among years, so we included a year effect into our models to minimize confounding effects of year of observation on breeding performance patterns. As emphasized by Reid *et al.* (2003), the most rigorous approach would be to model year as a random factor. However, in some years known-age petrels are likely to have been a non-representative subset of the age-structured population and estimates of random-year effect based solely on data from known-age individuals may therefore be biased (Reid *et al.* 2003). It would have been especially true during the first years of the long-term monitoring programme, when only young known-age petrels were monitored. The mean fledging success of the whole monitored population (not only of the birds of known age) in each year was included as a covariate in a generalized linear model (GLM; year variable), as suggested by Reid *et al.* (2003). Most of the snow petrels bred many times during their life, so our fledging success data were not independent. Therefore, we used a generalized linear mixed model (GLMM) by including bird identity as a random factor in our models.

AGE AND HORMONES LEVELS

Because male and female body condition was calculated separately and is known to differ between sexes (Barbraud & Chastel 1999), we could not analyse female and male data together to test an effect of body condition on hormone levels and hormonal stress responses. First, we therefore tested whether baseline hormone levels and hormonal stress responses were influenced by body condition in males and in females by using linear regressions. We then used the whole data set (males and females) to test the influence of sex, age and date of sampling on (1) baseline corticosterone and prolactin levels, (2) stress-induced corticosterone and prolactin levels, (3) the rates of corticosterone increase during the restraint protocol and (4) the rates of prolactin decrease during the restraint protocol. We used GLM with a normal error distribution and an identity link function and we started our analyses from general models including the explanatory variables 'sex', 'age', 'date' and the 'age \times sex' interaction.

HORMONE STRESS RESPONSES AND EGG NEGLECT

First, we tested whether the probability of neglecting the egg was influenced by body condition in males and in females by using logistic regressions. As explained in

the previous paragraph, we could not analyse female and male data together to test an effect of body condition on the probability of neglecting the egg and we therefore used one regression per sex. Then, we used the whole data set (males and females) to test the influence of age and hormone levels on the probability of neglecting the egg. Because hormone levels and age were correlated (see Results section), we had to analyse separately the influence of age and hormone levels on this probability to avoid biased results (Graham 2003). Secondly, we therefore tested whether the probability of neglecting the egg was influenced by age of individuals. We used GLM with a binomial error distribution and a logit link function and we started our analysis from the general model including the explanatory variables 'sex', 'age', 'date' and the 'age \times sex' interaction. Thirdly, we tested whether the probability of neglecting the egg during the incubation bout following the manipulation was linked to (1) baseline corticosterone levels and (2) stress-induced corticosterone levels. We started our analyses from general models including 'sex', '(baseline or stress-induced) corticosterone levels' and 'date' variables and the 'sex \times (baseline or stress-induced) corticosterone levels' interaction. Finally, we tested whether this probability was linked to (1) baseline prolactin levels and (2) stress-induced prolactin levels. Because prolactin levels were influenced by the 'sex' variable (see Results), we faced multicollinearity problems in these analyses and we could not include simultaneously the 'sex' and '(baseline or stress-induced) prolactin levels' variables in a model (Graham 2003). To solve this problem, we standardized (baseline or stress-induced) prolactin levels in each sex separately by subtracting the sexual mean prolactin levels from each observed value, and dividing by the standard deviation. This statistical approach permits avoidance of the potential confounding effects of a factor, i.e. sex, in our study, on the relationship between two parameters, i.e. prolactin levels and the probability of neglecting the egg. Because sex and standardized prolactin levels were no longer correlated, we started our analyses from the general model including the 'sex', '(baseline or stress-induced) prolactin levels' and 'date' variables and the 'sex \times (baseline or stress-induced) prolactin levels' interaction.

Results

AGE AND REPRODUCTIVE PERFORMANCES

Fledging success increased with age among the snow petrels (GLMM, age, $F_{1,534} = 7.15$, $P = 0.008$). The probability of fledging a chick successfully increased from 6 years to 12 years before stabilizing after 12 years of age and was, however, not influenced significantly by the quadratic term (age^2), showing that fledging success did not decline significantly in the oldest petrels of our study (Fig. 1, GLMM, age^2 , $F_{1,534} = 2.25$, $P = 0.134$).

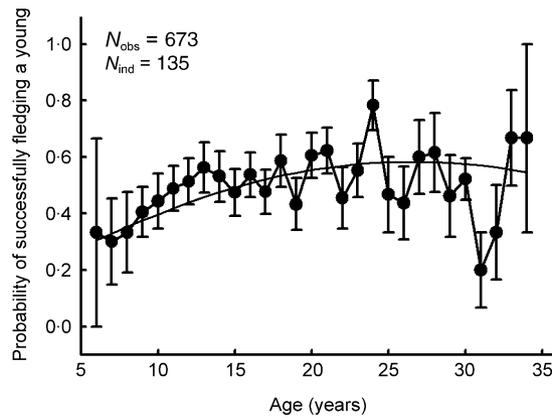


Fig. 1. Relationship between age and the probability of successfully fledging a young (mean \pm SE) among incubating snow petrels. This probability varied significantly with individual age and slightly with individual age². Solid lines represents the relationship described by the best model selected to explain this probability. N_{obs} and N_{ind} mean, respectively, the total number of observations and individuals.

AGE, BODY CONDITION AND BASELINE HORMONAL LEVELS

Baseline corticosterone levels were correlated negatively with body condition in females ($F_{1,21} = 8.12$, $P = 0.010$, $r^2 = 0.279$), but not in males ($F_{1,23} = 0.13$, $P = 0.726$). Baseline prolactin levels were not correlated with body condition in both sexes ($P > 0.60$ for both regressions). Moreover, body condition did not vary with age and

date in either sex (age: $P > 0.35$; date: $P > 0.05$). Baseline corticosterone levels were not influenced by date of sampling, by sex or by the 'age-sex interaction' (Table 1). The selected model that explains baseline corticosterone levels included only the 'age' variable (Table 1). However, the influence of age on baseline corticosterone levels was not significant (Table 1; Fig. 2a,b). Baseline prolactin levels were higher in females than in males and were correlated positively with date of sampling (Table 1). Moreover, there was an effect of 'age' and 'age-sex' interaction on baseline prolactin levels (Table 1). Baseline prolactin levels were correlated positively with age in males, but not in females (parameter estimates of the selected model; females: $\chi^2 = 0.41$, $P = 0.520$; males: $\chi^2 = 5.88$, $P = 0.015$; Fig. 2c,d). Baseline prolactin levels were not related to baseline corticosterone levels ($F_{1,43} = 2.58$, $P = 0.116$).

AGE AND HORMONAL RESPONSE TO STRESS

Males and females responded to the stress of being captured and held by a significant increase in corticosterone levels and a significant decrease in prolactin levels ($P < 0.001$ for all the paired Student's t -tests; Fig. 3a,b). Stress-induced hormonal levels, rates of corticosterone increase and rates of prolactin decrease were not influenced by body condition in both sexes ($P > 0.05$ for all the regressions).

Rates of corticosterone increase were not influenced by sex, date of sampling and age of individuals

Table 1. Model selection and GLM (normal error distribution, identity link function) to test the influence of age on baseline corticosterone and prolactin levels, rates of corticosterone increase, rates of prolactin decrease and stress induced corticosterone and prolactin levels. The best models (in bold type) were selected by using a step-down approach starting from the most global model (models were simplified by eliminating independent variables with $P \geq 0.15$)

Dependent variable	Independent variables	d.f.	<i>F</i>	<i>P</i>
Baseline corticosterone levels (ng mL ⁻¹) (<i>n</i> = 48)	Sex	1,45	0.34	0.565
	Age (years)	1,46	2.32	0.134
	Age \times sex	1,44	1.47	0.232
	Date (Julian day)	1,43	1.43	0.238
Baseline prolactin levels (ng mL ⁻¹) (<i>n</i> = 46)	Sex	1,41	11.10	< 0.001
	Age (years)	1,41	2.22	0.144
	Age \times sex	1,41	6.29	0.016
	Date (Julian day)	1,41	4.26	0.045
Rates of corticosterone increase (ng mL ⁻¹ min ⁻¹) (<i>n</i> = 48)	Sex	1,46	2.22	0.143
	Age (years)	1,45	0.05	0.825
	Age \times sex	1,44	1.93	0.172
	Date (Julian day)	1,43	1.69	0.200
Rates of prolactin decrease (ng mL ⁻¹ min ⁻¹) (<i>n</i> = 46)	Sex	1,42	< 0.01	0.944
	Age (years)	1,42	1.40	0.244
	age \times sex	1,42	11.51	0.001
	Date (Julian day)	1,41	0.06	0.805
Stress-induced corticosterone levels (ng mL ⁻¹) (<i>n</i> = 48)	Sex	1,44	1.71	0.198
	Age (years)	1,44	0.22	0.641
	Age \times sex	1,44	2.39	0.130
	Date (Julian day)	1,43	2.15	0.150
Stress-induced prolactin levels (ng mL ⁻¹) (<i>n</i> = 47)	Sex	1,43	18.63	< 0.001
	Age (years)	1,43	9.70	0.003
	Age \times sex	1,42	0.11	0.737
	Date (Julian day)	1,43	7.06	0.011

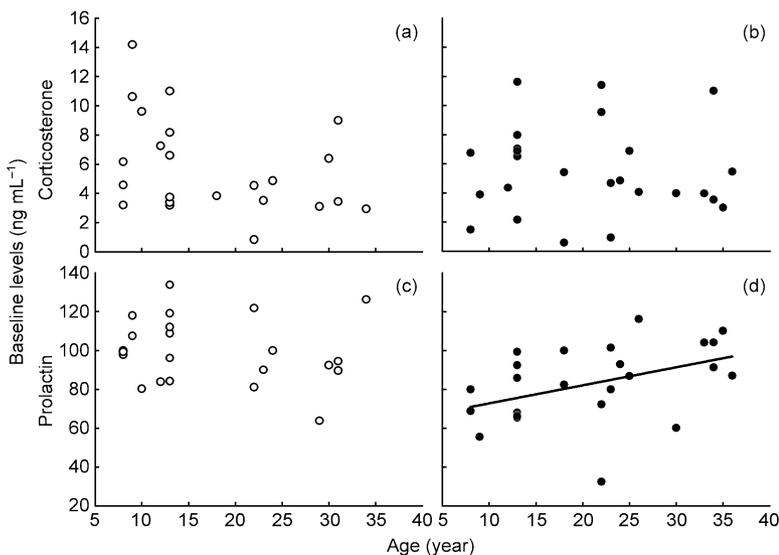


Fig. 2. Relationship between age and baseline corticosterone/prolactin levels among incubating snow petrels. Baseline corticosterone levels were not correlated with individual age. Baseline prolactin levels were significantly positively correlated with individual age in males (solid line), but not in females. Open symbols denote females and filled symbols denote males.

(Table 1). Stress-induced corticosterone levels were not influenced by date of sampling (Table 1). The selected model explaining stress-induced corticosterone levels included the 'sex' and 'age' variables and the 'age-sex' interaction (Table 1). However, parameter estimates of this selected model showed that stress-induced

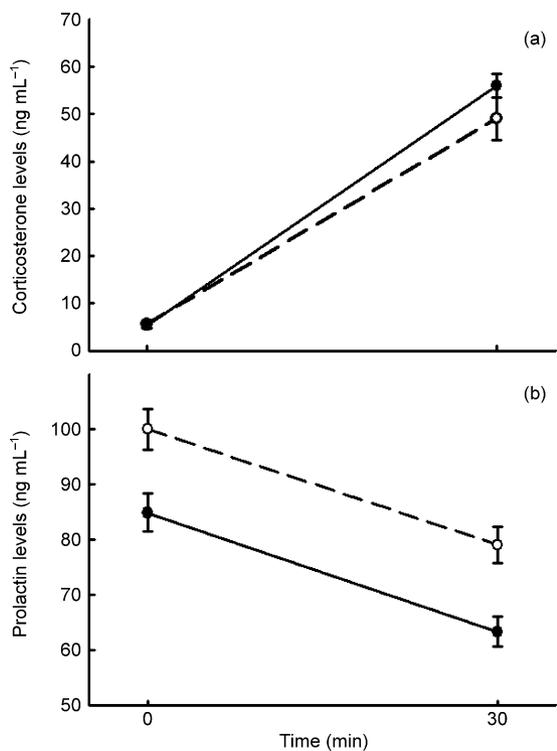


Fig. 3. Changes in plasma corticosterone and prolactin levels in response to the standardized stress protocol (corticosterone: $n = 48$, prolactin, $n = 46$). Open symbols denote females and filled symbols denote males.

corticosterone levels did not vary between sexes and did not vary with age either in females or males (Fig. 4a,b).

Rates of prolactin decrease were not influenced by sex and by date of sampling (Table 1). However, there was a highly significant effect of 'age-sex' interaction on rates of prolactin decrease (Table 1). Rates of prolactin decrease were correlated negatively with age in females but not in males (parameter estimates; females: $\chi^2 = 10.90$, $P = 0.001$; males: $\chi^2 = 2.01$, $P = 0.157$). Stress-induced prolactin levels were higher in females than in males and were correlated positively with date of sampling and age in both sexes (Table 1, Fig. 4c,d). Stress-induced prolactin levels were not related to stress-induced corticosterone levels ($F_{1,44} = 0.40$, $P = 0.531$).

HORMONAL STRESS RESPONSE AND EGG NEGLECT

The probability of neglecting the egg during the incubation bout following the stress protocol was not influenced by sex and by date of sampling (Table 2). However, young birds seem to be more likely to neglect their egg than old ones, although the effect of age was not statistically significant (Table 2). This probability of neglecting the egg was neither influenced by body condition ($P > 0.35$ in both sexes), nor by baseline or stress-induced corticosterone levels (Table 2). However, there was a significant influence of stress-induced prolactin levels on this probability. Incubating petrels with low stress-induced prolactin levels were more likely to neglect their egg than ones with high stress-induced prolactin levels (Fig. 5a,b; Table 2).

Discussion

In the present study we found, as in many other studies (reviewed in Forslund & Pärt 1995), that breeding success improved significantly with advancing age. Such age-specific improvement in breeding performance was not paralleled by a concomitant decrease in the magnitude of the corticosterone response to stress. Specifically, corticosterone response to stress and stress-induced corticosterone levels were not correlated with age. Prolactin response to stress was, however, influenced clearly by age: in females there was an attenuation of the prolactin response to stress with advancing age and in both sexes older breeders had higher stress-induced prolactin levels than younger ones. Moreover, elevated stress-induced prolactin levels were correlated with a lower probability of egg neglect. To our knowledge, this is the first study to show that stress-induced prolactin levels vary with age and can be linked to parental decisions.

AGE AND THE HORMONAL RESPONSE TO STRESS

Because a reduced sensitivity of the hypothalamo-pituitary-adrenal (HPA) system is thought to maximize

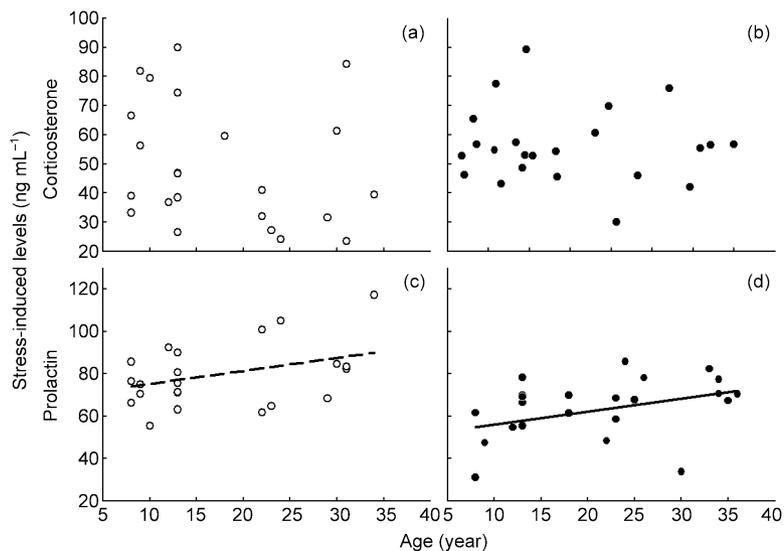


Fig. 4. Relationship between age and stress-induced corticosterone/prolactin levels among incubating snow petrels. Stress-induced corticosterone levels were not correlated with individual age. Stress-induced prolactin levels were significantly positively correlated with individual age in females (dashed line) and males (solid lines). Open symbols denote females and filled symbols denote males.

parental investment by preventing stressors from triggering an adrenocortical stress response (Wingfield *et al.* 1995; O'Reilly & Wingfield 2001; Holberton & Wingfield 2003; Moore & Jessop 2003), we predicted that the sensitivity of the HPA system would decrease with advancing age. Our data show, however, that the magnitude of the adrenocortical response to capture/restraint stress was unaffected by age, as incubating snow petrels showed a robust 14-fold increase in corticosterone levels independently of their age. Because

age did not affect the magnitude of their corticosterone response to stress it suggests, at the physiological level, that young and old snow petrels do not differ in their perception of risk and show a similar functional response of their HPA system over a 30-min capture/restraint protocol. One recent study found that corticosterone response to stress decreased with age in the common tern (Heidinger *et al.* 2006). How to explain such a difference? One possibility is that snow petrels often skip breeding opportunities and show a large variance in their age at first breeding (Chastel *et al.* 1993; Chastel 1995), so that two birds of the same age can differ greatly in their respective breeding experience and probable reproductive residual value. Two recent studies on albatrosses (Angelier *et al.* 2006a, 2007a) have shown that breeding experience was a much better descriptor of corticosterone levels than age *per se*. In our study, we sampled birds of various ages but their breeding experience was unknown. It is then possible that the magnitude of the stress response in snow petrels would be explained better by variations in breeding experience rather than age, whereas in shorter-lived common terns age and experience are probably correlated more highly (Nisbet 2001).

In our study, baseline corticosterone levels and body condition did not vary with age in incubating snow petrels. Baseline corticosterone levels are considered to reflect the energetic status of an individual (Landys, Ramenofsky & Wingfield 2006) and elevated levels have been associated with reduced food availability, low body condition and foraging success (Kitaysky, Wingfield & Piatt 1999; Adams *et al.* 2005; Angelier *et al.* 2007b). Accordingly, we found that elevated corticosterone levels were associated with low body

Table 2. Model selection and GLM (binomial error distribution, logit link function) to test the influence of age, baseline and stress-induced corticosterone, baseline and stress-induced prolactin levels, on the probability of neglecting the egg. The best models (in bold type) were selected by using a step-down approach starting from the most global model (models were simplified by eliminating independent variables with $P \geq 0.15$)

Dependent variable	Independent variables	d.f.	<i>F</i>	<i>P</i>
Probability of neglecting the egg (<i>n</i> = 48)	Sex	1,45	0.68	0.415
	Age (year)	1,46	3.17	0.082
	Age × sex	1,44	0.32	0.573
	Date (Julian day)	1,43	0.03	0.872
Probability of neglecting the egg (<i>n</i> = 48)	Sex	1,45	1.01	0.321
	Baseline corticosterone levels	1,46	2.72	0.106
	Baseline corticosterone levels × sex	1,44	1.93	0.307
Probability of neglecting the egg (<i>n</i> = 48)	Sex	1,45	1.18	0.284
	Stress-induced corticosterone levels	1,46	1.06	0.308
	Stress-induced corticosterone levels × sex	1,44	0.36	0.552
	Date (Julian day)	1,43	0.07	0.790
Probability of neglecting the egg (<i>n</i> = 46)	Sex	1,44	2.12	0.152
	Baseline prolactin levels	1,43	1.83	0.184
	Baseline prolactin levels × sex	1,42	0.04	0.839
	Date (Julian day)	1,41	0.02	0.898
Probability of neglecting the egg (<i>n</i> = 47)	Sex	1,44	1.75	0.193
	Stress-induced prolactin levels	1,45	5.73	0.021
	Stress-induced prolactin levels × sex	1,42	2.05	0.160
	Date (Julian day)	1,43	1.22	0.276

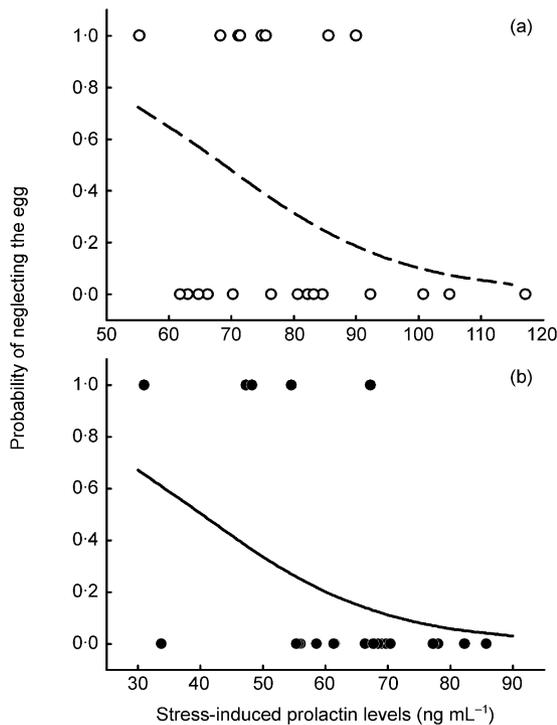


Fig. 5. Relationship between stress-induced prolactin levels and the probability of neglecting the egg during the incubation bout following the manipulation among snow petrels. GLMs with a binomial error distribution and a logit link function were used. The probability of neglecting the egg was significantly negatively correlated with stress-induced prolactin levels in females (dashed line) and males (solid line). Open symbols denote females and filled symbols denote males.

condition in females, but not in males. The absence of relationship between age and either baseline corticosterone levels or body condition suggests that young females were as able as older ones to extract energy from the environment and to maintain their body condition during the incubation period. However, it is also possible that baseline corticosterone levels and body condition varied with breeding experience rather than age in this species, as found previously in procellariiformes species (corticosterone: Angelier *et al.* 2006a, 2007a; body condition: Weimerskirch 1992; Chastel, Weimerskirch & Jouventin 1995). The absence of relationship between baseline corticosterone levels and body condition in males is difficult to interpret. It is possible that males, being significantly larger than females, may be less energetically constrained than females during incubation (Barbraud & Chastel 1999).

As reported previously in another bird species (Chastel *et al.* 2005), we found that prolactin levels decreased in response to an acute stress in incubating snow petrels. The data presented here confirm that birds show a decrease in circulating prolactin when exposed to acute stress (Sharp *et al.* 1989; Chastel *et al.* 2005).

Moreover, according to our prediction, stress-induced prolactin levels reached after 30 min of restraint were correlated positively with age. In the

snow petrel, the way age influenced the prolactin response to stress appears to be sex-dependent. In males, the magnitude of decrease in prolactin levels was similar in young and old individuals. However, older males showed higher baseline prolactin levels than young ones, as found previously in other species (Deviche, Wingfield & Sharp 2000; Pr  ault *et al.* 2005; Angelier *et al.* 2006a, 2007a), therefore explaining their higher stress-induced prolactin levels. Higher stress-induced prolactin levels found in older males may originate from their higher intrinsic capacity to secrete prolactin (Angelier *et al.* 2006a, 2007a).

In females, baseline prolactin levels were not correlated with age, but the magnitude of the decline in prolactin levels was significantly lower in older individuals, resulting therefore in a positive relationship between stress-induced prolactin levels and age. This suggests that older females were better able to maintain prolactin production during the first minutes of the stress episode. Therefore, according to our main prediction, older females seem to actually show a better hormonal resistance to acute stress than younger ones. This better hormonal resistance to stress in older females might result from an acclimation process to handling and capture with advancing age. However, it is unlikely because known-age snow petrels were sampled outside the long-term study plots and these birds have been handled very seldom prior to our study.

AGE, STRESS AND REPRODUCTIVE PERFORMANCES

Prolactin is a hormone involved in parental behaviour (Buntin 1996) and several studies have reported that prolactin enhances the expression of parental (Wang & Buntin 1999; Duckworth, Badyaev & Parlow 2003) and alloparental behaviours (Vleck *et al.* 1991; Schoech, Mumme & Wingfield 1996; Angelier *et al.* 2006b). Supporting this influence of prolactin levels on parental decisions, we found that parents with low stress-induced prolactin levels were more likely to neglect their egg.

Rather than a sole effect of either prolactin or corticosterone on parental decisions, corticosterone and prolactin might act jointly to mediate parental decisions in long-lived birds (Cherel *et al.* 1988, 1994; Chastel *et al.* 2005). The decrease in prolactin levels in response to an acute stress would probably reduce the motivation of young parents to continue incubation, whereas the concomitant increase in corticosterone levels would trigger escape behaviour (Wingfield *et al.* 1998), leading to temporary desertion of the nest (Silverin 1986). On the contrary, older parents may be better able to maintain a threshold level of prolactin during a stress episode, therefore retaining the willingness to incubate the egg and reducing or even suppressing the negative behavioural consequences of elevated corticosterone levels (Chastel *et al.* 2005; Criscuolo *et al.* 2005).

In conclusion, in young snow petrels, the combination of a robust corticosterone increase with a lower ability to maintain prolactin secretion during acute stress (such as inclement weather or temporary food shortage) is probably one of the functional causes of their lower incubation commitment. We suggest that the capacity to maintain a threshold level of prolactin during a stress episode may be an important physiological mechanism involved in the improvement of reproductive performance with advancing age in long-lived birds. To understand fully how modulation of the hormonal stress response governs parental investment, future studies should therefore examine simultaneously corticosterone and prolactin responses to stress in relation to different life-history strategies, contrasting environmental conditions (low/high food availability) and contrasting energetic demands (incubation/brooding).

Can such a mechanism help us to elucidate the main evolutionary theories addressing the effect of age and experience on reproductive success (Curio 1983; Forslund & Pärt 1995)? An attenuation of the stress response in reproducing vertebrates has been interpreted as a mechanism allowing parents to modify reproductive investment flexibly with age (Wingfield & Sapolsky 2003; Heidinger *et al.* 2006). Thus the observed ability of older females to attenuate the prolactin response to stress may favour the 'restraint hypothesis' (Curio 1983), which suggests that breeding investment increases with age as the residual reproductive value declines and breeding events become more valuable (Curio 1983). However, in our study, corticosterone response was unaffected by age and hence the likely perception of the stressor through the central nervous system and the HPA axis. Does this mean that corticosterone response to stress would not be involved as a mechanism permitting reproductive investment to be increased over age whereas the prolactin would? In males, lower stress-induced prolactin levels did not originate from a lower ability to resist stress but rather from a better ability to secrete prolactin, thus suggesting that young males may be more physiologically constrained (the 'constraint hypothesis' Curio 1983). It is also possible that the relationship between age and stress-induced prolactin levels could result from a selection process (Forslund & Pärt 1995; Mauck *et al.* 2004). Hence, parents with elevated stress-induced prolactin levels may be high-quality individuals and might be more likely to survive during several years and therefore to be represented in old age classes. This would imply that the magnitude of the stress response is fixed early in the reproductive career of an individual. However, it is unlikely, as Lendvai *et al.* (2007) showed experimentally in the house sparrow (*Passer domesticus*) that, in a given season, the same individual can modulate its hormonal stress response flexibly according to the value of the reproductive event. Therefore, the decreased prolactin sensitivity with advancing age may not result solely from a selection

process in snow petrels. Despite the difficulty of monitoring longitudinal hormonal data in long-lived birds, future studies should aim to compare hormonal stress responses of the same individual at different ages in order to confirm that hormonal sensitivity to stress decline with advancing age independently of selection process.

Acknowledgements

Fieldwork was supported financially and logistically by the Institut Paul-Emile Victor (IPEV Programme 109). F. Angelier was supported by a BDI grant from CNRS/Région Poitou-Charentes. We are grateful to Dr A. F. Parlow for kindly providing us with a chicken kit for prolactin assay. We thank G. Bouteloup for his assistance in the field. At the CEBC, we thank C. Barbraud and S. A. Shaffer for helpful comments on the manuscript, S. Dano, A. Lacroix and C. Trouvé for their technical assistance in hormones assays and molecular sexing and D. Besson for help in the management of the long-term database.

References

- Adams, N.J., Cockrem, J.F., Taylor, G.A., Candy, E.J. & Bridges, J. (2005) Corticosterone responses of grey-faced petrels (*Pterodroma macroptera gouldi*) are higher during incubation than during other breeding stages. *Physiological and Biochemical Zoology*, **78**, 69–77.
- Angelier, F., Barbraud, C., Lormée, H., Prud'Homme, F. & Chastel, O. (2006b) Kidnapping of chicks in emperor penguins: a hormonal by-product? *Journal of Experimental Biology*, **209**, 1413–1420.
- Angelier, F., Shaffer, S.A., Weimerskirch, H. & Chastel, O. (2006a) Effect of age, breeding experience and senescence on corticosterone and prolactin levels in a long-lived seabird: the wandering albatross. *General and Comparative Endocrinology*, **149**, 1–9.
- Angelier, F., Shaffer, S.A., Weimerskirch, H., Trouvé, C. & Chastel, O. (2007b) Corticosterone and foraging behavior in a pelagic seabird. *Physiological and Biochemical Zoology*, **80**, 283–292.
- Angelier, F., Weimerskirch, H., Dano, S. & Chastel, O. (2007a) Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. *Behavioral Ecology and Sociobiology*, **61**, 611–621.
- Barbraud, C. & Chastel, O. (1999) Early body condition and hatching success in the snow petrel *Pagodroma nivea*. *Polar Biology*, **21**, 1–4.
- Barbraud, C. & Jouventin, P. (1998) What causes body size variation in the snow petrel? *Journal of Avian Biology*, **29**, 161–171.
- Barbraud, C. & Weimerskirch, H. (2001) Contrasting effect of the extent of sea-ice on the breeding performance of an Antarctic top predator, the snow petrel *Pagodroma nivea*. *Journal of Avian Biology*, **21**, 297–302.
- Buntin, J.D. (1996) Neural and hormonal control of parental behaviour in birds. *Advances in the Study of Behavior* (eds J.S. Rosenblatt & C.T. Snowdon), vol. 25, pp. 161–213. Academic Press, New York.
- Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference*. Springer-Verlag, New York.
- Chastel, O. (1995) Influence of reproductive success on breeding frequency in four southern petrels. *Ibis*, **137**, 360–363.

- Chastel, O., Lacroix, A., Weimerskirch, H. & Gabrielsen, G.W. (2005) Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird. *Hormones and Behavior*, **47**, 459–466.
- Chastel, O., Weimerskirch, H. & Jouventin, P. (1993) High annual variability in reproductive success and survival of an Antarctic seabird, the snow petrel, *Pagodroma nivea*. *Oecologia*, **94**, 278–285.
- Chastel, O., Weimerskirch, H. & Jouventin, P. (1995) Influence of body condition on reproductive decision and reproductive success in the blue petrel. *Auk*, **112**, 964–972.
- Chaurand, T. & Weimerskirch, H. (1994) Incubation routine, body mass regulation and egg neglect in the blue petrel *Halobaena caerulea*. *Ibis*, **136**, 285–290.
- Cherel, Y., Mauget, R., Lacroix, A. & Gilles, J. (1994) Seasonal and fasting-related changes in circulatory gonadal steroids and prolactin in king penguins, *Aptenodytes patagonicus*. *Physiological and Biochemical Zoology*, **67**, 1154–1173.
- Cherel, Y., Robin, J.P., Walch, O., Karmann, H., Netchitailo, P. & Le Maho, Y. (1988) Fasting in king penguin. I. Hormonal and metabolic changes during breeding. *American Journal of Physiology*, **254**, R170–R177.
- Clutton-Brock, T.H. (1988) *Reproductive Success*. University of Chicago Press, Chicago.
- Crisuolo, F., Chastel, O., Bertile, F., Gabrielsen, G.W., Le Maho, Y. & Raclot, T. (2005) Corticosterone alone does not trigger a short term behavioural shift in incubating female common eiders, but does modify long term reproductive success. *Journal of Avian Biology*, **36**, 306–312.
- Curio, E. (1983) Why do young birds reproduce less well? *Ibis*, **125**, 400–404.
- Deviche, P., Wingfield, J.C. & Sharp, P. (2000) Year-class differences in the reproductive system, plasma prolactin and corticosterone concentrations, and onset of prebasal molt in male dark-eyed juncos (*Junco hyemalis*) during the breeding period. *General and Comparative Endocrinology*, **118**, 425–435.
- Duckworth, R.A., Badyaev, A.V. & Parlow, A.F. (2003) Elaborately ornamented males avoid costly parental care in the house finch (*Carpodacus mexicanus*): a proximate perspective. *Behavioral Ecology and Sociobiology*, **55**, 176–183.
- Forslund, P. & Pärt, T. (1995) Age and reproduction in birds – hypotheses and tests. *Trends in Ecology and Evolution*, **10**, 374–378.
- Fridolfsson, A.K. & Ellegren, H. (1999) A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology*, **30**, 116–121.
- Graham, M.H. (2003) Confronting multicollinearity in ecological multiple regression. *Ecology*, **84**, 2809–2815.
- Green, A.J. (2001) Mass/length residuals: measures of body condition or generators of spurious results? *Ecology*, **82**, 1473–1483.
- Heidinger, B.J., Nisbet, I.C.T. & Ketterson, E. (2006) Older parents are less responsive to a stressor in a long-lived seabird: a mechanism for increased reproductive performance with age? *Proceedings of the Royal Society of London, Series B*, **273**, 2227–2231.
- Holberton, R.L. & Wingfield, J.C. (2003) Modulating the corticosterone stress response: a mechanism for balancing individual risk and reproductive success in arctic-breeding sparrows? *Auk*, **120**, 1140–1150.
- Kitaysky, A.S., Wingfield, J.C. & Piatt, J.F. (1999) Dynamics of food availability, body condition and physiological response in breeding black-legged kittiwakes. *Functional Ecology*, **13**, 577–585.
- Landys, M.M., Ramenofsky, M. & Wingfield, J.C. (2006) Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology*, **148**, 132–149.
- Lendvai, A.Z., Giraudeau, M. & Chastel, O. (2007) Reproduction and modulation of the stress response: an experimental test in the house sparrow. *Proceedings of the Royal Society of London, Series B*, **274**, 391–397.
- Lormée, H., Jouventin, P., Trouve, C. & Chastel, O. (2003) Sex-specific patterns in baseline corticosterone and body condition changes in breeding red-footed boobies *Sula sula*. *Ibis*, **145**, 212–219.
- Love, O.P., Breuner, C.W., Vézina, F. & Williams, T.D. (2004) Mediation of a corticosterone-induced reproductive conflict. *Hormones and Behavior*, **46**, 59–65.
- Mauck, R.A., Huntington, C.E. & Grubb, T.C. Jr (2004) Age-specific reproductive success: evidence for the selection hypothesis. *Evolution*, **58**, 880–885.
- Moore, I.T. & Jessop, T.S. (2003) Stress, reproduction and adrenocortical modulation in amphibians and reptiles. *Hormones and Behavior*, **43**, 39–47.
- Newton, I. (1989) *Lifetime Reproduction in Birds*. Academic Press, London.
- Nisbet, I.C.T. (2001) Detecting and measuring senescence in wild birds: experience with long-lived seabirds. *Experimental Gerontology*, **36**, 833–843.
- O'Reilly, K.M. & Wingfield, J.C. (2001) Ecological factors underlying the adrenocortical response to capture stress in arctic-breeding shorebirds. *General and Comparative Endocrinology*, **124**, 1–11.
- Olson, C.R., Vleck, C.M. & Vleck, D. (2006) Periodic cooling of bird eggs reduces embryonic growth efficiency. *Physiological and Biochemical Zoology*, **79**, 927–936.
- Préault, M., Chastel, O., Cézilly, F. & Faivre, B. (2005) Bill colour and age are associated with parental abilities and breeding performance in blackbirds. *Behavioral Ecology and Sociobiology*, **58**, 497–505.
- Reid, J.M., Bignal, E.M., Bignal, S., McCracken, D.I. & Monaghan, P. (2003) Age-specific reproductive performance in red-billed choughs *Pyrrhocorax pyrrhocorax*: patterns and processes in a natural population. *Journal of Animal Ecology*, **72**, 765–776.
- Ricklefs, R.E. & Wikelski, M. (2002) The physiology/life-history nexus. *Trends in Ecology and Evolution*, **17**, 462–468.
- Romero, L.M. (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology*, **128**, 1–24.
- Romero, L.M., Ramenofsky, M. & Wingfield, J.C. (1997) Season and migration alters the corticosterone response to capture and handling in an Arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *Comparative Biochemistry and Physiology Part B*, **116**, 171–177.
- Romero, L.M. & Reed, J.M. (2005) Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology Part A*, **140**, 73–79.
- Schoech, S.J., Mumme, R.L. & Wingfield, J.C. (1996) Prolactin and helping behaviour in the cooperatively breeding Florida scrub-jay, *Aphelocoma c. coerulescens*. *Animal Behaviour*, **52**, 445–456.
- Sharp, P.J., Sterling, R.J., Talbot, R.T. & Huskisson, N.S. (1989) The role of the hypothalamic vasoactive intestinal polypeptide in the maintenance of prolactin secretion in incubating bantam hens: observations using passive immunization, radioimmunoassay, and immunocytochemistry. *Journal of Endocrinology*, **122**, 5–13.
- Silverin, B. (1986) Corticosterone-binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period. *General and Comparative Endocrinology*, **64**, 67–74.
- Silverin, B. & Wingfield, J.C. (1998) Adrenocortical responses to stress in breeding willow warblers, *Phylloscopus trochilus*, in Sweden: effects of latitude and gender. *Functional Ecology*, **11**, 376–384.

- Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Vleck, C.M., Mays, N.A., Dawson, J.W. & Goldsmith, A.R. (1991) Hormonal correlates of parental and helping behavior in cooperatively breeding Harris' hawks (*Parabuteo unicinctus*). *Auk*, **108**, 638–648.
- Wang, Q. & Buntin, J.D. (1999) The roles of stimuli from young, previous breeding experience, and prolactin in regulating parental behavior in ring doves (*Streptopelia risoria*). *Hormones and Behavior*, **35**, 241–253.
- Weimerskirch, H. (1992) Reproductive effort in long-lived birds: age-specific patterns of condition, reproduction and survival in the wandering albatross. *Oikos*, **64**, 464–473.
- Weimerskirch, H., Lallemand, J. & Martin, J. (2005) Population sex ratio in a monogamous long-lived bird, the wandering albatross. *Journal of Animal Ecology*, **74**, 285–291.
- Wingfield, J.C. (1994) Modulation of the adrenocortical response to stress in birds. *Perspectives in Comparative Endocrinology* (eds K. G. Davey, R.E. Peter & S.S. Tobe), pp. 520–528. National Research Council of Canada, Ottawa.
- Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S.E., Ramenofsky, M. & Richardson, R.D. (1998) Ecological bases of hormone–behavior interactions: the 'emergency life history stage'. *American Zoologist*, **38**, 191–206.
- Wingfield, J.C., O'Reilly, K.M. & Astheimer, L.B. (1995) Modulation of the adrenocortical response to acute stress in arctic birds: a possible ecological basis. *American Zoologist*, **35**, 285–294.
- Wingfield, J.C. & Sapolsky, R.M. (2003) Reproduction and resistance to stress: when and how? *Journal of Neuroendocrinology*, **15**, 711–724.

Received 6 November 2006; accepted 13 July 2007

Handling Editor: Stuart Bearhop