Experimentally delayed hatching triggers a magnified stress response in a long-lived bird

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ABSTRACT

In birds, the timing of breeding is a key life-history trait with crucial fitness consequences. We predicted that parents may value a brood less if it hatched later than expected, thereby decreasing their parental effort. In addition, breeding effort would be further modulated by the age-specific decline of future breeding opportunities. We experimentally investigated whether snow petrels, *Pagodroma nivea*, were less committed to care for a chick that hatched later than expected. The timing of hatching was manipulated by swapping eggs between early and late known-age pairs (7–44 years old), and investigations on hormonal and behavioral adjustments were conducted. As a hormonal gauge of parental commitment to the brood, we measured the corticosterone stress response of guarding adults. Indeed, an acute stress response mediates energy allocation towards survival at the expense of current reproduction and is magnified when the current brood value is low, as it is expected to be in young and/or delayed parents. As predicted, egg desertion and the magnitude of the stress response was stronger in delayed pairs compared to control ones. However, the treatment did not decrease the length of the guarding period, chick condition and chick survival. In addition, old parents resisted stress better (lower stress-induced corticosterone levels) than young ones. Our study provides evidence that snow petrels, as prudent parents, may value a brood less if it hatched later than expected. Thus, in long-lived birds, the responsiveness to stressors appeared to be adjusted according to the individual prospect of future breeding opportunities (age) and to the current brood value (timing of breeding).

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Introduction

The timing of breeding is a key life-history trait (Lyon et al., 2008; Reed et al., 2009), which greatly influences final reproductive success (Lack, 1950; Perrins, 1970). A brood that hatched later than expected may suffer from higher environmental deterioration, such as resource depletion, competition, and/or predation risk for the offspring ('date hypothesis,' Lack, 1968; reviewed by Verhulst and Nilsson, 2008). Moreover, experimental manipulations of the breeding schedule have provided evidence that delayed breeding may not only penalize the brood, but may also induce long-term costs for parents, due to the overlap with post-breeding activities, such as moult (Nilsson and Svensson, 1996; Brinkhof et al., 2002; Verhulst and Nilsson, 2008; McNamara and Houston, 2008). Hence, it is conceivable that long-lived parents would be less committed to care for a brood that hatched later than expected, thereby favouring their own survival and future breeding opportunities ("prudent parent hypothesis," Drent and Daan, 1980). In addition, as future breeding prospects decline with advancing age, old breeders are expected to invest more in current reproduction than young ones (Stearns, 1992; Isaac and

Johnson, 2005; but see McNamara et al., 2009). Consequently, the modulation of parental effort should be adjusted according to both the timing of current breeding and the age of the breeder. Such adaptive parental strategies should be regulated in a flexible manner, via the integration of fluctuating environmental conditions and the individual perception of the risks (Wingfield et al., 1998; Ricklefs and Wikelski, 2002).

One potential mechanism underlying the adjustment of parental effort may be the endocrine stress response (Wingfield and Sapolsky, 2003). Vertebrates release glucocorticoid hormones (corticosterone in birds) in response to stressors, such as food scarcity, predator attacks, and harsh weather. This adrenocortical stress response is thought to promote the immediate survival through the mobilization of stored energy and the adoption of emergency behaviors at the expense of current breeding activities (Wingfield and Sapolsky, 2003; but see Breuner et al., 2008). Therefore, the stress response is seen as an adaptive mechanism that reallocates energy toward survival when individual and/or environmental conditions could not sustain the current reproductive effort (Landys et al., 2006). However, when the value of current reproduction is high relative to the value of future breeding opportunities, stress response should be attenuated or even suppressed to ensure that the current breeding success is not threatened (Silverin and Wingfield, 1998; Wingfield and Sapolsky, 2003). This 'brood value hypothesis' is highly supported by recent

correlative, comparative and experimental studies (Wingfield et al., 1995; Heidinger et al., 2006; Lendvai et al., 2007; Lendvai and Chastel, 2008; Bokony et al., 2009). Thus, the magnitude of the stress response appears to be a good proxy for the level of parental commitment to the brood. It is crucial to distinguish the stress response (that is, the stress-induced corticosterone levels) from the baseline corticosterone levels, which are a marker of activities, energetic state and/or food availability (Kitaysky et al., 1999; reviewed by Landys et al., 2006). Increased baseline corticosterone levels would provide information on the levels of sustained reproductive activities/work (Kitaysky et al., 2001; Love et al., 2004). If the brood hatches later than expected and is less valuable, parents are predicted to mount a stronger adrenocortical stress response (stress-induced corticosterone levels) and to be less committed to care for the offspring. In contrast, if they work harder, delayed parents are predicted to exhibit higher baseline corticosterone levels than controls. In addition, older adults should respond less strongly to stressors (lower stress-induced corticosterone levels) than younger ones (Heidinger et al., 2006, 2008; Angelier et al., 2007a). In summary, stress response is likely to reflect the relative value of a brood that hatched earlier/later than expected, for young/old parents.

In this study, we tested whether parental effort declined with delaying breeding through the measure of parental behavior and stress response in a long-lived Antarctic seabird, the snow petrel (*Pagodroma nivea*, Forster 1777). Snow petrels are excellent models for such an investigation because, like all Procellariiformes, they lay only one egg during the extremely short Antarctic breeding season. The breeding schedule is thus of high importance for an optimal allocation of energy to the single chick and thus for reproductive success. Furthermore, snow petrel, as a very long-lived bird (up to 46 years old), should modulate the stress response in relation to the current brood value and to their own age (Angelier et al., 2007a; Bokony et al., 2009).

By swapping eggs between early and late breeding pairs, we manipulated the hatching date. We then measured breeding success, incubating and chick-rearing behavior and the corticosterone stress response of chick-rearing snow petrels through a capture/restraint stress protocol (Wingfield, 1994). Parental behavior and breeding success were expected to be reduced in delayed pairs and enhanced in advanced pairs compared to controls. According to the brood value hypothesis, delayed breeders should exhibit a higher stress-induced corticosterone release and advanced breeders, a lower stress-induced corticosterone release than controls. In addition, stress-induced corticosterone levels are expected to be higher in young than in old parents, as an additional effect of elevated future breeding expectancies.

Methods

Study site and species

The study was carried out on Ile des Pétrels, Adélie Land (66°40′S, 140°01′E), Antarctica. Snow petrels (250–500 g) are long-lived seabirds (>40 years old) that lay one egg per season with no replacement clutch in case of failure (Warham, 1990). In Adélie Land, the laying period ranges from late November to mid-December (Barbraud et al., 2000; Goutte et al., 2010) and incubation lasts 44.2 ± 0.3 days ($n = 34$). Although males undertake slightly longer incubation shifts than females, males and females provide roughly similar amounts of parental care in this species (Warham, 1990). Nest desertion is a common behavior in snow petrels (Angelier et al., 2007a). At the individual level, reproductive success significantly decreases with increasing egg-laying date during the 2007/2008 season (GLM, $n = 50$, $\chi^2 = 4.910$, $p = 0.027$). A significant part of the population on Ile des Pétrels is of known age because of intensive yearly banding of chicks since 1963. Some adults were also ringed during their first breeding attempt and a minimum age could be

attributed to them by adding the minimum age at first breeding in snow petrels (i.e., 5 years, Chastel et al., 1993) to the time elapsed since ringing.

The treatment: Manipulation of hatching date

To manipulate hatching dates, a cross-fostering protocol was applied between paired nests, on the 7th and 8th of January 2008, which was during the second half of the incubating period. The authors attest to possess the legal authorized use of wild animals 'Certificat d'autorisation d'expérimenter sur animaux vivants N°79-2′ delivered to O. Chastel by 'Services Vétérinaires des Deux Sèvres.' In order to avoid egg unviability, we selected nests that were not deserted prior to the treatment and we did not switch eggs with hairlines cracks on their shell. All the eggs were measured (length and breadth) to the nearest 0.5 mm using a caliper, and were swapped with eggs of similar volume (difference in egg volume between swapped eggs: $4.0\% \pm 0.7$ [SE]) to reduce potential egg volume effects (Barbraud et al., 2000). During egg transfer (less than 10 min), the egg in the first nest was temporarily replaced with warmed dummy egg that was readily accepted by the incubating parent. Eggs were manipulated with care and transported from one nest to the other in a box covered by cotton. Eggs were swapped between two nests whose egg-laying date differed from 5 days. Egg-laying dates were precisely known by daily nest checking during the egg-laying period (i.e., 3–17 December 2007). Pairs receiving an egg that was laid 5 days earlier than their own were called "advanced pairs" ($n = 20$ nests), whereas pairs receiving an egg that was laid 5 days later than their own were called "delayed pairs" ($n = 20$ nests). Two of the advanced pairs received a cracked egg and were thus removed from the analyses. We only considered 18 advanced pairs. Pairs with unchanged hatching date constituted the control group ($n = 37$ nests). Among the 75 studied nests, 63 parents were of known age (banded as chicks) and 12 parents had a minimum estimated age (number of years since banding + 5 years). Groups did not differ in the age of the parents ($F_{2,72} = 0.042$, $p = 0.959$, advanced: 20.0 ± 2.4 [SE], 10–40 (range) years old; control: 20.0 ± 1.3 [SE], 7–44 (range) years old and delayed: 20.7 ± 2.0 [SE], 11–37 (range) years old).

Effect of the treatment on hatching success and brooding effort

The 75 nests were checked every day following the treatment and until the end of the chick-rearing period to monitor parental behavior, hatching success, hatching date, the length of the guarding period and chick survival. The duration of the guarding period (mean ± SE: 10.2 ± 0.4 days, $n = 62$ in 2007/2008 and in 2008/2009) is of high importance because young chicks are not thermally independent and require constant attendance by their parents. The opportunities of breeders to forage for themselves are thus constrained (Catry et al., 2006). Male and female snow petrels provide roughly similar amounts of parental care and the shifts during brooding last between 2 and 3 days (Warham, 1990). During early guarding (chick was 4.9 ± 0.1 days old), body mass and skull length (head + bill) of chicks were recorded to the nearest 0.5 g using a spring balance and to the nearest 0.5 mm using a caliper. Chick body condition was then calculated from a least-squares linear regression of body mass against skull length ($F_{1,56} = 13.699$, $p < 0.001$).

Measuring the stress response

During the guarding period (chick was 4.9 ± 0.1 days old), one known-age parent of each experimental pair was captured by hand at its nest. An initial blood sample (for baseline corticosterone levels) was collected from the alar vein immediately after capture (mean ± SE: 119 ± 5 s) using a 1-mL heparinized syringe and a 25-gauge needle. For two of them, bleeding time exceeded 3 min (i.e., the time

required for baseline corticosterone levels, Romero and Reed, 2005), so these two values were not used for the analysis. After the first bleeding, the bird was placed into cloth bags and a second blood sample was collected 30 min later (for stress-induced corticosterone levels) according to the standardized capture/restraint stress protocol (Wingfield, 1994). Adults were weighted to the nearest 2 g using a spring balance and their skull length (head + bill) was measured to the nearest 0.5 mm using a caliper. In snow petrels, skull length appears to be a reliable measure of the overall size of a bird (see Angelier et al., 2007a). Adult body condition was then calculated from a least-squares linear regression of body mass against skull length ($F_{1,57} = 39.476$, $p < 0.001$). Petrels were marked with spots of dye on the forehead to distinguish them from their partner. During handling of adults, chicks were placed in a box covered with cotton to keep them warm. At the end of the sampling, first chicks then adults were put back onto their nest.

Molecular sexing and hormone assay

Within 6 h from sampling, blood samples were centrifuged and plasma was decanted and stored at −20 °C until assayed. Red cells were also kept frozen for molecular sexing in our laboratory (Centre d'Etudes Biologiques de Chizé, CEBC). The sex of adults was determined by polymerase chain reaction (PCR) amplification of part of two highly conserved genes (CHD) present on the sex chromosomes (Fridolfsson and Ellegren, 1999), as detailed by Weimerskirch et al. (2005). Plasma concentrations of corticosterone were determined by radioimmunoassay at the CEBC (Lormée et al., 2003). Corticosterone levels were determined by radioimmunoassay at the CEBC. Total plasma corticosterone was measured in samples (50 μL) after ethyl ether extraction by radioimmunoassays using a commercial antiserum, raised in rabbits against corticosterone-3-(Ocarboxy-methyl) oxime bovine serum albumin conjugate (Biogenesis, UK). Cross-reaction was 9% with 1-desoxycorticosterone and less than 0.1% with other plasma steroids. Duplicate aliquots (100 μL) of the extracts were incubated overnight at 4 °C with 8000 cpm of H^3-corticosterone (Amersham Pharmacia Biotech-France) and antiserum. The bound and free corticosterones were separated by adding dextran-coated charcoal. After centrifugation, the bound fraction was counted in a liquid scintillation counter. H^3-corticosterone recovery from the extraction was 93.7%. Pooled plasma of different snow petrels produced a dose–response curve that paralleled the corticosterone standard curve. The lowest detectable concentration was 0.52 ng/mL. Mean recovery of added corticosterone was 108%. All samples were run in one assay and intra-assay variation was 7.9% ($n = 6$ duplicates).

Data processing and analysis

All analyses were conducted using R 2.8.0 (R Development Core Team 2008). The corticosterone stress response was expressed as the stress-induced levels of corticosterone. Baseline and stress-induced corticosterone levels were not correlated in our sample size ($r = 0.212$, $p = 0.104$, $n = 60$). We first used generalized additive models (GAM) and considered age of adults as a smoothed variable. However, significant effects of age were only linear so we moved to generalized linear model (GLM). GLM with binomial error distribution and a logit link function was used to examine hatching success as a function of treatment (advanced, delayed and control groups) and age of adults. The interaction treatment * age was not included in the analysis, due to the low number of eggs that failed to hatch ($n = 6$). Chick survival was tested as a function of treatment, adult body condition and adult age. The interactions treatment * adult body condition and treatment * age were not included in the analysis, due to the low number of dead chicks ($n = 6$). We used GLM with normal errors and an identity link function to examine the length of the guarding period and chick body condition as a function of treatment, adult body condition, adult age and the interactions treatment * adult body condition and treatment * adult age. The magnitude of stress response was expressed as the stress-induced levels of corticosterone. We also calculated the amplitude of corticosterone release (i.e., the difference between stress-induced and baseline levels) and the statistical analyses of this variable gave nearly identical results; hence, we report only the results for stress-induced corticosterone levels to facilitate the comparison with other published results. Baseline corticosterone and stress-induced corticosterone levels were tested as a function of treatment, sex, adult body condition, adult age and the interactions treatment * adult body condition, treatment * adult age. Dependent continuous variables were log-transformed if necessary. We used a step-down approach starting from the most global models and therefore simplified them by eliminating step by step non-significant independent variables. When treatment significantly affects one of the dependant variables, a post hoc pairwise *t*-test with Bonferroni correction was used to compare the three groups. The effect of intrinsic breeding schedule was not included in the above models, since the expected hatching date differed between groups (post hoc Bonferroni correction: delayed-control: $p = 0.012$, advanced-delayed: $p < 0.001$, advanced-control: $p = 0.265$, Fig. 1).

Results

Expected hatching date (own egg) significantly differed from the manipulated hatching date (foster egg) in advanced pairs (paired *t*-test: df = 13, $t = 13.769$, $p < 0.001$, Fig. 1) and in delayed pairs (paired
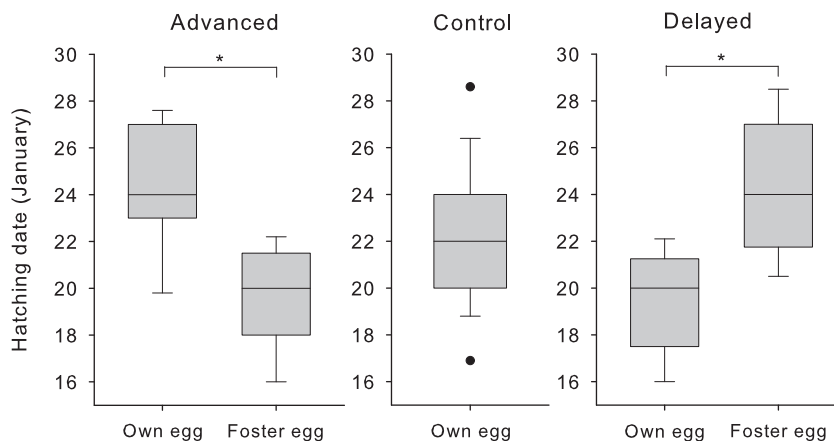

Fig. 1. Expected (own egg) and manipulated (foster egg) hatching date in the advanced and the delayed groups, and controls hatching date.

t-test: df $= 12$, $t = -14.506$, $p < 0.001$, Fig. 1). Moreover, the hatching date (January) differed among groups ($F_{2,65} = 9.086$, $p < 0.001$).

Effect of the treatment on reproductive success and brooding effort

Hatching success differed significantly between experimental groups ($n = 75$, $\chi^2 = 12.150$, $p = 0.003$) and was lower in delayed pairs (post hoc Bonferroni correction: advanced-control: $p = 1$; advanced-delayed: $p = 0.011$, delayed-control: $p = 0.007$, Fig. 2A). Indeed incubation behavior was reduced in the delayed group: five delayed pairs deserted their nest 3.6 ± 2.6 days after the expected hatching date, while only one control pair and no advanced pairs deserted their nest. There was no effect of adult age on hatching success ($n = 75$, $\chi^2 = 1.175$, $p = 0.278$). The treatment and the age of adults did not influence the length of the guarding period and the chick body condition (Table 1a and b). Chick survival was neither influenced by the treatment ($n = 68$, $\chi^2 = 0.398$, $p = 0.820$, Fig. 2B), nor by parents age ($n = 68$, $\chi^2 = 0.053$, $p = 0.819$) and nor by parents body condition ($n = 68$, $\chi^2 = 0.003$, $p = 0.960$).

Effect of treatment on adult stress response

Adult body condition was not affected by the treatment ($F_{2,56} = 0.584$, $p = 0.561$). Baseline corticosterone levels were not affected by the treatment (Table 2a, Fig. 3A) but decreased significantly with increasing adult age (Table 2a, Fig. 4A). Circulating plasma levels of corticosterone increased significantly following capture and handling ($p < 0.001$ for paired Student's t-tests). There was a significant effect of treatment on stress-induced corticosterone levels (Table 2b, Fig. 3B): delayed birds responded more strongly to the acute stress protocol than control ones (post hoc Bonferroni correction: delayed-control: $p = 0.015$, advanced-delayed, $p = 0.143$, advanced-control, $p = 1.000$). In addition, stress-induced corticosterone significantly decreased with increasing adult age (Fig. 4B) and with improving adult body condition
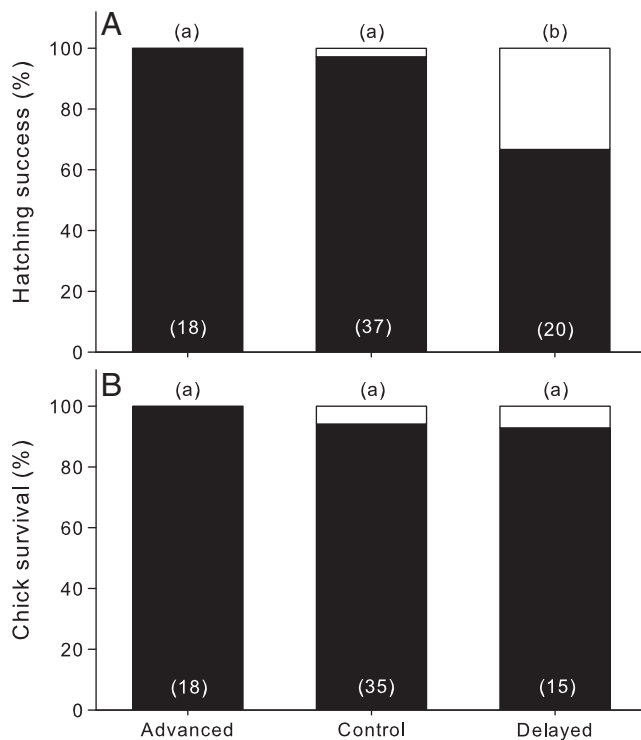
Table 1
Modelling (a) length of the guarding period and (b) chick body condition at the end of the guarding period according to the treatment, adult body condition and the interactions using GLMs (normal error distribution, identity link function). A step-down approach was used and variables are ordered according to the elimination process.

Dependent variable	Independent variables	df	F-value	p-value
(a) Length of the guarding period (log)	Adult body condition	1,60	2.986	0.089
	Treatment	2,58	1.523	0.227
	Adult age	1,57	0.468	0.497
	Treatment * Body condition	2,55	0.959	0.390
	Treatment * Age	2,53	<0.001	0.999
(b) Chick body condition	Adult body condition	1,59	2.794	0.099
	Treatment	2,57	0.326	0.723
	Adult age	1,56	0.470	0.496
	Treatment * Age	2,54	1.513	0.229
	Treatment * Body condition	2,52	0.960	0.390

(estimate of the slope: -0.080 ± 0.044), without interactions effect treatment * adult age and/or treatment * adult body condition (Table 2b).

Discussion

In this experimental study, we tested whether parental effort is modulated when hatching occurs earlier or later than expected. According to our hypothesis, we found that incubation behavior and hatching success were reduced in delayed snow petrels. Moreover they mounted a stronger stress response during early chick-rearing period compared to control snow petrels. To our knowledge, this is the first study to show that an experimental manipulation of the timing of breeding can influence the magnitude of stress response and the parental commitment to reproduction. In addition to this treatment effect, the magnitude of the stress response declined with older age and with better body condition.

Fitness consequences of the treatment and parental behavior

Our manipulation had clear fitness consequences, since the overall breeding success of the delayed group was significantly reduced compared to advanced and control pairs. In seabirds, the decline in

Fig. 2. (A) Hatching success in relation to experimental manipulation of hatching dates. (B) Chick survival in relation to experimental manipulation of hatching dates. Sample sizes are given for each group.

Table 2
Modelling baseline (a) and stress-induced (b) corticosterone levels according to the treatment, adult age, sex, adult body condition and the interactions using GLMs (normal error distribution, identity link function). The best models (in bold type) were selected by using a step-down approach starting from the most global model. In this table, variables are ordered according to the elimination process.

| Dependent variable	Independent variables	df	F-value	p-value
(a) Baseline corticosterone levels (log)	Adult age	1,56	4.586	0.037
	Treatment	2,54	0.582	0.562
	Treatment * Adult age	2,52	2.726	0.075
	Sex	1,49	0.071	0.791
	Treatment * Sex	2,47	1.684	0.197
	Adult Body condition (BC)	1,45	0.208	0.651
	Treatment * Adult BC	2,43	0.865	0.428
(b) Stress-induced corticosterone levels	Treatment	1,54	4.733	0.013
	Adult body condition (BC)	1,54	4.136	0.047
	Adult age	1,54	4.756	0.034
	Treatment * Adult BC	2,52	1.945	0.153
	Treatment * Adult age	2,50	1.003	0.374
	Sex	1,49	0.014	0.905
	Treatment * Sex	2,47	0.048	0.954
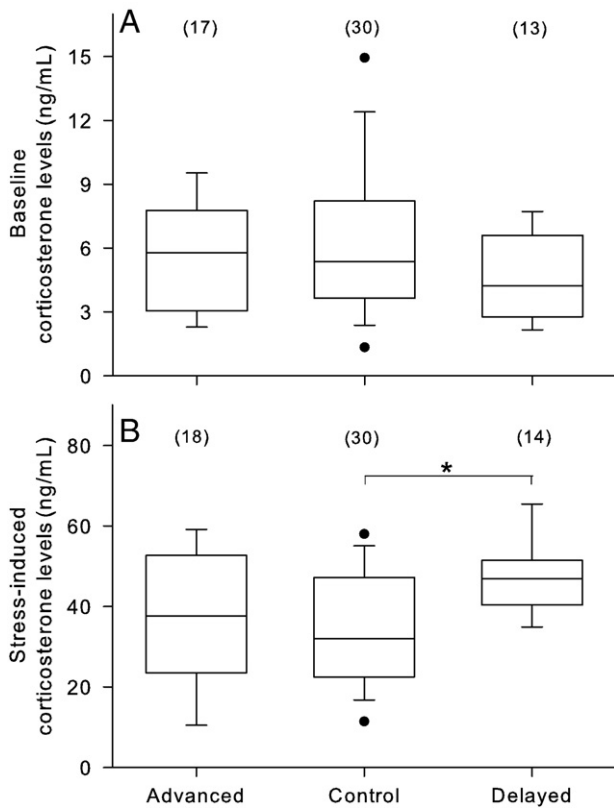
Fig. 3. Effect of experimental manipulation of hatching dates on plasma levels of baseline (A) and of stress-induced (B) corticosterone (ng/mL) measured in brooding adults within each experimental group. Sample sizes are given for each group.

reproductive success within the season is mainly due to hatching failure, rather than chick mortality (DeForest and Gaston, 1996; Minguez, 1998). Moreover in petrels, the release of corticosterone in response to stress is higher during incubation than during brooding period (Adams et al., 2005; Angelier et al., 2009), possibly because of the lower fitness value of an egg relative to chick's one. Our treatment clearly reflected this pattern, since hatching success, but not chick survival, was compromised by the experimentally postponed hatching date. This hatching failure was entirely due to egg desertion, the main source of failure in snow petrels (Angelier et al., 2007a) and this low nest attendance was observed only at the end of the incubation period: delayed snow petrels were more susceptible to desert an egg after the expected hatching date. In addition to this incubation behavior, the hypothesis of reduced parental effort in delayed pairs was supported by a magnified stress response during the early chick-rearing period.

Alternatively, as incubation requires fasting at the nest (Heaney and Monaghan, 1996), delayed birds could have been exhausted by the experimentally prolonged incubation time. However, the adult body condition and/or baseline corticosterone levels (markers of physiological state and activities, Wingfield et al., 1998; Landys et al., 2006) did not differ between delayed petrels and control ones. This suggests that stored energy was not depleted in delayed birds and that the extended incubation effort did not require a significantly harder work, compared to controls. However, we could not exclude the possibility that delayed and failed birds were not able to cope with energetic constraints of extended incubation effort, since body condition and baseline corticosterone levels were not measured during incubation to avoid extra disturbance, and were thus only monitored in birds that did not desert the nest. It is therefore possible that baseline levels might have been affected by the treatment (Doody et al., 2008).

In contrast with the incubation period, we did not detect any observable behavioral consequences of the treatment, during the brooding period. Contrary to our expectations, chicks' body condition and survival were not reduced in the delayed groups. However, it is important to notice that the 2007/2008 breeding season was characterized by a high breeding success (68% of successfully fledged chicks, $n = 204$ eggs in the long-term study plots), well above the average 50% recorded for this species in Adélie Land (Chastel et al., 1993). Since adult baseline corticosterone levels and body condition did not differ between groups, delayed birds seemed to have provided the same levels of sustained activities (Landys et al., 2006) and brooding effort compared to control and advanced ones. These results support the ideas that the fitness value of the brood, once hatched, did not differ between delayed, advanced and control pairs. To go further, we investigated the stress response of brooding parents, which are known to be actively modulated according to the brood value (Lendvai et al., 2007).

Timing of breeding and parental stress response

According to our prediction, delayed snow petrels responded stronger to acute stress than control ones. An elevated stress response redirects energetic resource away from parental duties and towards survival ('emergency stage,' Wingfield et al., 1998). According to the brood value hypothesis, stress response is attenuated or even suppressed, when the value of reproduction is high relative to the value of future breeding opportunities, to ensure that the current
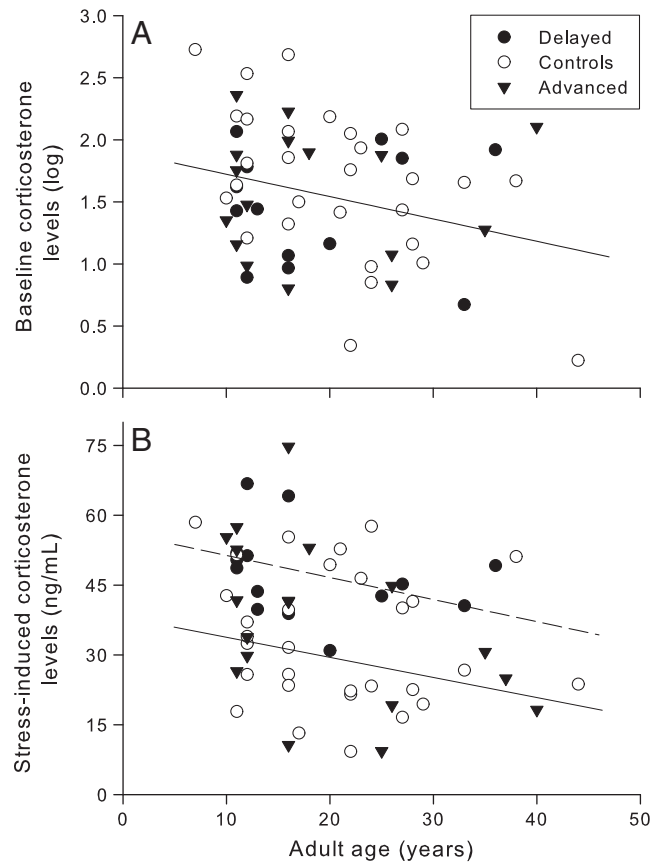

Fig. 4. Relationships between age and plasma levels of baseline (A) and (B) stress-induced corticosterone (ng/mL) measured in brooding adults within each experimental group. Open circles denotes controls birds; filled circles, delayed birds; and triangles, advanced birds. The decrease of baseline corticosterone with age (A) did not differ between groups (solid line). The decrease of stress-induced corticosterone levels with age (B) is represented by a solid line for control and advanced birds and by a dashed line for delayed birds.

breeding success is not threatened (Silverin and Wingfield, 1998; Wingfield and Sapolsky, 2003; Lendvai et al., 2007; Lendvai and Chastel, 2008). Hence, our findings support the hypothesis that parents value the brood less if it hatched later than expected. Alternatively, we could not exclude that delayed snow petrels exhibited elevated stress-induced corticosterone levels as a reaction to the delayed hatching date.

Parental behavior during the guarding period was not affected by the treatment. Despite high stress-induced corticosterone levels, the length of guarding period, the chick's body condition, and the chick's survival were not reduced in delayed pairs. Thus, how can we interpret the stronger release of corticosterone in response to acute stress in experimentally delayed pairs compared to controls? First, our results are consistent with studies in house sparrows (*Passer domesticus*, Lendvai et al., 2007; Lendvai and Chastel, 2008): an experimentally increased brood value (increased clutch size) did not change adult body condition and baseline corticosterone levels but clearly attenuated the stress-induced corticosterone release. Second, it is important to keep in mind that the stress-induced corticosterone levels reflect the parental motivation in a stressful context (Wingfield and Sapolsky, 2003). In case of unpredictable adverse conditions (e.g., snow storm which causes significant chick mortality in snow petrels; Chastel et al., 1993), delayed pairs would have mounted a higher acute release of corticosterone than controls. In turn, they would likely have provided poorer brooding effort, thereby promoting their own survival in case of a life-threatening perturbation.

Interestingly, advanced pairs did not attenuate their stress response compared to control ones, suggesting that an earlier-hatching chick was not value more than a control one. This contrasts with the results obtained in the short-lived house sparrow (Lendvai et al., 2007): parents with experimentally enhanced brood value (increased clutch size) were able to actively attenuate their adrenocortical response to stress. Our result suggest that an advanced brood was not valued more than the expected one. However, snow petrels, as very long-lived organisms (up to 46 years old) show a stronger and probably less flexible response to stress than short-lived species (Bokony et al., 2009). In this study, the very long-lived snow petrels tended to behave as prudent parents (Drent and Daan, 1980), and would have not jeopardized their own survival by suppressing their stress response, even for a highly valuable chick. This also highlights possible survival costs of early breeding (Brinkhof et al., 2002; reviewed by Drent, 2006) and the need for long-lived species to adjust parental care for future breeding prospects. It would be interesting to conduct a similar study in short-lived birds to compare the flexibility of the adult stress response in regard to an advanced/delayed brood.

Regarding the age of adult snow petrels, we did not find any effect of age on parental behavior, through hatching success, length of the guarding period, chick body condition and chick survival. At a proximate level, young snow petrels exhibited higher baseline corticosterone levels than old ones, suggesting higher energetic constraints (Angelier et al., 2007b). Since birds were sampled just after the extended or shortened incubation period, it is possible that baseline levels might have been also affected by the treatment (Doody et al., 2008). During the brooding period, older snow petrels exhibited lower stress-induced corticosterone levels than younger ones. A decline of the stress response over age has been also found in incubating common terns (*Sterna hirundo*, Heidinger et al., 2006; Heidinger et al., 2008). Similarly, prolactin, a hormone involved widely in regulating parental cares, decreased after a stressor in a age-specific manner: old snow petrels maintained higher stress-induced prolactin levels than young ones (Angelier et al., 2007a). Such age-related stress responses have been interpreted as a mechanism underlying the well-known improvement of reproductive performance over age (Heidinger et al., 2006; Angelier et al., 2007a). Alternatively, our transversal study could not exclude the selection hypothesis: phenotypes excessively sensitive to stress could have progressively disappeared. This implies that the magnitude of the stress response is fixed early in the reproductive life of an individual.

However, individuals appear to modulate their hormonal stress response flexibly according to the value of the reproductive event (Lendvai et al., 2007). Stress resistance may have also arisen from a habituation process to handling with age. However known-age snow petrels have been seldom handled prior to this study and a similar study found no effect of recapture history on stress-hormone levels (Heidinger et al., 2006). Such age-related resistance to acute stress was independent of the treatment which only magnified the stress response in delayed birds. Hence, the degree of flexibility in response to reduced brood value did not differ across age. This strengthens the hypothesis that the responsiveness to stressors is adjusted according to the current brood value (timing of breeding) and additionally, to the individual prospect of future breeding opportunities (age of breeders).

Our study provides evidence that snow petrels value the brood less if it hatched later than expected. This adjustment in parental effort, combined with individual (age, body condition) and environmental factors, might give new insights into the tight link between breeding schedule and reproductive success.

Acknowledgments

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. Physiol. Biochem. Zool. 78, 69–77.

Angelier, F., Moe, B., Weimerskirch, H., Chastel, O., 2007a. Age-specific reproductive success in a long-lived bird: do older parents resist stress better? J. Anim. Ecol. 76, 1181–1191.

Angelier, F., Weimerskirch, H., Dano, S., Chastel, O., 2007b. Age, experience and reproductive performance in a long-lived bird: a hormonal perspective. Behav. Ecol. Sociobiol. 61, 611–621.

Angelier, F., Moe, B., Blanc, S., Chastel, O., 2009. What factors drive prolactin and corticosterone responses to stress in a long-lived bird species (Snow Petrel *Pagodroma nivea*)? Physiol. Biochem. Zool. 82, 590–602.

Barbraud, C., Lormee, H., LeNeve, A., 2000. Body size and determinants of laying date variation in the Snow Petrel *Pagodroma nivea*. J. Avian Biol. 31, 295–302.

Bokony, V., Lendvai, A.Z., Liker, A., Angelier, F., Wingfield, J.C., Chastel, O., 2009. Stress response and the value of reproduction: are birds prudent parents? Am. Nat. 173, 589–598.

Breuner, C.W., Patterson, S.H., Hahn, T.P., 2008. In search of relationships between the acute adrenocortical response and fitness. Gen. Comp. Endocrinol. 157, 288–295.

Brinkhof, M.W.G., Cave, A.J., Daan, S., Perdeck, A.C., 2002. Timing of current reproduction directly affects future reproductive output in European coots. Evolution 56, 400–411.

Catry, P., Phillips, R., Forcada, J., Croxall, J.P., 2006. Factors affecting the solution of a parental dilemma in albatrosses: at what age should chicks be left unattended? Anim. Behav. 72, 383–391.

Chastel, O., Weimerskirch, H., Jouventin, P., 1993. High annual variability in reproductive success and survival of an antarctic seabird, the Snow petrel *Pagodroma-nivea*: a 27-year study. Oecologia 94, 278–285.

DeForest, L.N., Gaston, A.J., 1996. The effect of age on timing of breeding and reproductive success in the thick-billed Murre. Ecology 77, 1501–1511.

Doody, L.M., Whilelm, S.I., McKay, D.W., Walsh, C.J., Storey, A.E., 2008. The effects of variable foraging conditions on common murre (*Uria aalge*) corticosterone concentrations and parental provisioning. Horm. Behav. 53, 140–148.

Drent, R.H., 2006. The timing of birds' breeding seasons: the Perrins hypothesis revisited especially for migrants. Ardea 94, 305–322.

Drent, R.H., Daan, S., 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68, 225–252.

Fridolfsson, A.K., Ellegren, H., 1999. A simple and universal method for molecular sexing of non-ratite birds. J. Avian Biol. 30, 116–121.

Goutte, A., Antoine, E., Weimerskirch, H., Chastel, O., 2010. Age and the timing of breeding in a long-lived bird: a role for stress hormones? Funct. Ecol. 24, 1007–1016.

Heaney, V., Monaghan, P., 1996. Optimal allocation of effort between reproductive phases: the trade-off between incubation costs and subsequent brood rearing capacity. Proc. R. Soc. B Biol. Sci. 263, 1719–1724.

Heidinger, B.J., Nisbet, I.C.T., Ketterson, E.D., 2006. Older parents are less responsive to a stressor in a long-lived seabird: a mechanism for increased reproductive performance with age? Proc. R. Soc. B Biol. Sci. 273, 2227–2231.

Heidinger, B.J., Nisbet, I.C.T., Ketterson, E.D., 2008. Changes in adrenal capacity contribute to a decline in the stress response with age in a long-lived seabird. Gen. Comp. Endocrinol. 156, 564–568.

Isaac, J.L., Johnson, C.N., 2005. Terminal reproductive effort in a marsupial. Biol. Lett. 1, 271–275.

Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 1999. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. Funct. Ecol. 13, 577–584.

Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 2001. Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. Behav. Ecol. 12, 619–625.

Lack, D., 1950. The breeding seasons of European birds. Ibis 92, 288–316.

Lack, D., 1968. Ecological adaptations for breeding in birds. Methuen, London.

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. Gen. Comp. Endocrinol. 148, 132–149.

Lendvai, A.Z., Chastel, O., 2008. Experimental mate-removal increases the stress response of female house sparrows: the effects of offspring value? Horm. Behav. 53, 395–401.

Lendvai, A.Z., Giraudeau, M., Chastel, O., 2007. Reproduction and modulation of the stress response: an experimental test in the house sparrow. Proc. R. Soc. B Biol. Sci. 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., Vezina, F., Williams, T.D., 2004. Mediation of a corticosterone-induced reproductive conflict. Horm. Behav. 46, 59–65.

Lyon, B.E., Chaine, A.S., Winkler, D.W., 2008. Ecology: a matter of timing. Science 321, 1051–1052.

McNamara, J.M., Houston, A.I., 2008. Optimal annual routines: behaviour in the context of physiology and ecology. Philos. Trans. R. Soc. B 363, 301–319.

McNamara, J.M., Houston, A.I., Barta, Z., Scheuerlein, A., Fromhage, L., 2009. Deterioration, death and the evolution of reproductive restraint in late life. Proc. R. Soc. B Biol. Sci. 276, 4061–4066.

Minguez, E., 1998. The costs of incubation in the British Storm petrel: an experimental study in a single-egg layer. J. Avian Biol. 29, 183–189.

Nilsson, J.A., Svensson, E., 1996. The cost of reproduction: a new link between current reproductive effort and future reproductive success. Proc. R. Soc. B Biol. Sci. 263, 711–714.

Perrins, C.M., 1970. Timing of birds breeding seasons. Ibis 112, 242–255.

Reed, T.E., Warzybok, P., Wilson, A.J., Bradley, R.W., Wanless, S., Sydeman, W.J., 2009. Timing is everything: flexible phenology and shifting selection in a colonial seabird. J. Anim. Ecol. 78, 376–387.

Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. Trends Ecol. Evol. 17, 462–468.

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. A Mol. Integr. Physiol. 140, 73–79.

Silverin, B., Wingfield, J.C., 1998. Adrenocortical responses to stress in breeding Pied Flycatchers Ficedula hypoleuca: relation to latitude, sex and mating status. J. Avian Biol. 29, 228–234.

Stearns, S.C., 1992. The evolution of life histories. Oxford University Press, New York.

Verhulst, S., Nilsson, J.A., 2008. The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. Philos. Trans. R. Soc. B Biol. Sci. 363, 399–410.

Warham, J., 1990. The petrels: their ecology and breeding systems. Academic Press, London.

Weimerskirch, H., Lallemand, J., Martin, J., 2005. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. J. Anim. Ecol. 74, 285–291.

Wingfield, J.C., 1994. Modulation of adrenocortical response to stress in birds. In: Davey, K.G., Peter, R.E., Tobe, S.S. (Eds.), Perspectives in comparative endocrinology. National Research Council of Canada, Ottawa, pp. 520–528.

Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. J. Neuroendocrinol. 15, 711–724.

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. Am. Zool. 35, 285–294.

Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone-behavior interactions: the "emergency life history stage". Am. Zool. 38, 191–206.