



Stress and parental care: Prolactin responses to acute stress throughout the breeding cycle in a long-lived bird

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ABSTRACT

While the role of corticosterone in mediating the response of birds to acute stress is well established, it has recently been proposed that a decrease in prolactin levels following stress may complement corticosterone in redirecting resources away from breeding activities and towards behaviors promoting immediate survival. Here, for the first time, we detail changes in the prolactin stress response of birds throughout the breeding cycle. We then discuss the modulation of the corticosterone and prolactin stress responses over successive stages of breeding, differing in reproductive value and parental effort. In a long-lived Procellariiform seabird, the Manx shearwater *Puffinus puffinus*, we found that prolactin levels decreased in response to acute stress during incubation and mid chick-rearing but increased in response to stress during late chick-rearing and in non parenting birds, a pattern similar to that previously described for mammals. The high corticosterone stress response in pre-breeders was consistent with predictions based on reproductive value, but a similar response during late chick-rearing was not. This probably reflected foraging effort and a heightened importance of the parents' own nutritional status at this stage of the season, in advance of post-breeding migration. We also found that baseline prolactin levels were maintained at high levels during chick-rearing and were only slightly lower during late chick-rearing and in failed breeders and non-breeders. These data suggest that prolactin may play a role in nestling care long beyond the brooding phase, that this is not due to birds spending long periods away from the colony and that prolactin secretion may be necessary for nest-guarding behavior.

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1. Introduction

Throughout the course of the breeding cycle, parents of wild birds can be exposed to sudden unpredictable stressful conditions, such as facing a predator. They also experience predictable changes in energy demand associated with the varying degrees of parental effort required to incubate eggs and feed growing chicks. Physiological and behavioral mechanisms have evolved to maintain parents at a physiological equilibrium in the face of these sudden stressful events and changing energetic constraints (McEwen and Wingfield, 2003; Landys et al., 2006). Activation of the hypothalamic–pituitary–adrenal (HPA) axis under stress, resulting in a rapid increase of circulating corticosterone levels (known as the corticosterone stress response), is thought to be one of the essential mechanisms that vertebrates use to cope with these stressors (Wingfield et al., 1998; McEwen and Wingfield, 2003). This sharp increase in corticosterone leads to a redirection of resources away from non-essential activities (e.g. parental care) and towards behaviors that promote immediate survival (Wingfield and Farner,

1993; Romero, 2002). While the role of corticosterone is well-established, it has been proposed that another hormone, prolactin, may play an integral part in the stress response because of its direct relationship with parental activities (Chastel et al., 2005; Angelier and Chastel, 2009). Yet few data are available concerning the prolactin stress response and it is not known how this response changes through the breeding cycle.

The peptide hormone prolactin, produced by the pituitary gland, invariably rises during incubation in response to a poorly understood combination of changes in photoperiod and tactile or visual stimuli from the nest. Prolactin is thought to play a central role in mediating parental care (e.g. Riddle et al., 1935; El Halawani et al., 1986; Buntin et al., 1991), but substantial differences among species in patterns of prolactin secretion during the breeding cycle suggests a complex relationship between this hormone, parental effort and the stimuli that promote prolactin secretion (reviewed in Buntin, 1996). In seabirds alone, prolactin levels are highly variable during breeding. Levels are high from laying to hatching in black-browed albatrosses *Thalassarche melanophrys* and grey-headed albatrosses *T. chrysostoma* but only increase midway through incubation in the wandering albatross *Diomedea exulans* (Hector and Goldsmith, 1985). Prolactin levels fall during chick

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rearing but the timing varies across species, for example levels fall shortly after hatching in albatrosses whereas they are maintained until fledging in penguins (Hector and Goldsmith, 1985; Garcia et al., 1996; Lormée et al., 1999, 2000). Similarly, whereas breeding failure leads to a reduction in prolactin levels in most birds (Chastel and Lormée, 2002; Angelier and Chastel, 2009), this is not true for some penguins (Lormée et al., 1999). It has been suggested therefore that elevated prolactin levels may be maintained throughout chick-rearing in pelagic seabirds such as penguins, not by stimuli from the nest but rather by an endogenous rhythm enabling the continuity of parental effort during prolonged periods of foraging away from the colony (Garcia et al., 1996; Lormée et al., 1999; Angelier et al., 2006). This hypothesis is supported almost exclusively, however, by studies of penguins and it is not known how it may apply to other pelagic seabirds.

The role of prolactin during stress is poorly understood. In mammals, plasma prolactin has been reported to increase during acute stress, possibly in accordance with a role in attenuating the deleterious effects of high corticosterone levels (Neill, 1970; De Vlaming, 1979; Torner and Neumann, 2002; Tilbrook et al., 2006). This response may, however, be stage-dependent because prolactin levels have also been reported to remain stable or even decrease during stress in lactating females (Walker et al., 1992; Bánky et al., 1994; Hill et al., 2003). The limited data available for birds suggest that in contrast to mammals, prolactin levels decrease during a stressful event in both breeding and non-breeding individuals (Opel and Proudman, 1986; Sharp et al., 1989; Maney et al., 1999; Chastel et al., 2005; Angelier et al., 2007). This decrease, in conjunction with an increase in corticosterone levels, may promote a transient reduction of parental behavior, required to overcome the stressor (Angelier and Chastel, 2009).

The magnitude of the stress response is thought to reflect the outcome of the life-history trade-off between reproduction and survival: when the fitness value of current reproduction is high relative to future breeding prospects, the stress response should be dampened (Wingfield and Sapolsky, 2003; Lendvai et al., 2007; Bokony et al., 2009). Chicks have higher value than eggs in these terms, and supporting the above hypothesis, a reduced corticosterone stress response has been found during chick-rearing, as compared to incubation, in grey-faced petrels *Pterodroma macroptera* (Adams et al., 2005). Older parents, for which the value of current reproduction relative to survival is high, have also shown a reduced corticosterone stress response in a seabird species, the common tern *Sterna hirundo* (Heidinger et al., 2006). A similar hypothesis has been suggested for prolactin, i.e. when the value of current reproduction is high, the decline in prolactin levels under acute stress should be less pronounced (Chastel et al., 2005; Angelier and Chastel, 2009). No data are available, however, to compare the prolactin stress response between different breeding stages.

The main goal of this paper is to determine whether the prolactin response to stress changes through successive stages of the avian breeding cycle, using the Manx shearwater *Puffinus puffinus*, a long-lived and sexually monomorphic pelagic seabird, as a model. We also compare the prolactin and corticosterone stress responses among breeding stages and discuss the modulation of the stress response in relation to the trade-off between reproduction and survival. We test the prediction that the stress response is down-regulated during chick-rearing compared with incubation and in breeding birds compared with non-breeding and pre-breeding birds.

2. Methods

Fieldwork took place in spring and summer 2008 at the large colony of Manx shearwaters on Skomer Island, South-west Wales (51.73°N, 5.28°W). Manx shearwaters are long-lived pelagic sea-

birds breeding in colonies around the Northeast Atlantic and they exhibit the typical Procellariiform characteristics of delayed sexual maturity and low reproductive rate. They are burrow nesters and therefore, all birds with the exception of pre-laying and non-breeding individuals were caught from within their nest chambers via short access tunnels (typically 10–15 cm long) in the roof of each burrow, built for the monitoring of breeding activity throughout the season (Brooke, 1990; Hamer and Hill, 1997). Pre-laying individuals were caught by hand in the colony between 2 and 4 April, a time at which mainly future breeders visit the colony (Brooke, 1990). Incubating birds were caught during two periods: early incubation (17–20 May) and late incubation (10–23 July; mean hatching date was 10 July). Laying dates were estimated from hatching dates using an incubation period of 51 days (Brooke, 1990); birds in these two samples were estimated to be 1–14 days and 40–48 days through incubation, respectively, when sampled. Chick-rearing birds were caught between July and September, after they had returned from the sea and fed their chick. Failed breeders were sampled during August from among pairs occupying empty burrows that had previously contained an egg. Non-breeders were caught by hand during early July, when most non-breeders visit the colony. They were identified as single birds moving between burrows and spending considerable time on the ground (Brooke, 1990; Bourgeois et al., 2008).

Most birds arrive at the colony within an hour of nautical dusk (Riou and Hamer, 2008). Blood sampling thus took place in a short time window (2–3 h) following nautical dusk. To obtain baseline and stress-induced hormone levels, we sampled blood from the brachial vein using a syringe and a 25 gauge needle following a standardized procedure of capture and restraint (Wingfield, 1994). The first sample (max 200 µl) was collected immediately after capture (<3 min) and taken to represent baseline levels (Romero and Reed, 2005). Birds were then weighed to the nearest 5 g using a spring balance and their tarsus was measured to the nearest 0.1 mm with digital calipers. They were kept in a cloth bag and blood sampled again at 30 min, before being released. Samples were stored on ice, centrifuged within 2 h and plasma and blood cells were stored at –20 °C until analysis. Genomic DNA was extracted from the blood cells and individuals molecularly sexed using a sexually dimorphic locus, amplified using primer sets Z-002 B, C and D, with the amplified products separated on an ABI3730 DNA Analyzer, as described in Dawson (2007). This method was validated on a set of 30 individuals of known sex, using sex differences in vocalizations (Brooke, 1990).

Plasma concentrations of hormones were measured in duplicate by radioimmunoassay at the CEBC following protocols described in Lormée et al. (2003) for corticosterone and Cherel et al. (1994) for prolactin. The detection limit for corticosterone was 0.3 ng/ml and the intra-assay coefficient of variation was 0.05 ($N = 6$ duplicates). Pooled plasma samples taken from Manx shearwaters resulted in dose–response curves that paralleled the chicken prolactin standard curves (Fig. 1). Thus, the cross-reactivity of the chicken prolactin antibody with prolactin was equivalent in both species and this heterologous assay could be used to assess relative levels of Manx Shearwater prolactin. The detection limit of the assay was 2.8 ng/ml and intra-assay variation 0.05.

Hormone levels were examined in relation to breeding status, sex and body condition (BC) using linear modeling in R (R, 2007). BC was calculated separately for males and females as the residuals of a linear regression of mass on tarsus length; in both cases a polynomial function did not improve the fit of the model ($F_1 = 0.02$, $P = 0.9$ and $F_1 = 1.05$, $P = 0.3$, respectively), and the linear relationship between mass and tarsus length was significant ($F_{1,80} = 5.8$, $P < 0.02$ and $F_{1,84} = 6.4$, $P = 0.01$, respectively). Blood samples collected during chick-rearing were divided into two periods: mid chick-rearing (chick aged 25–44 days post-hatching, a period when

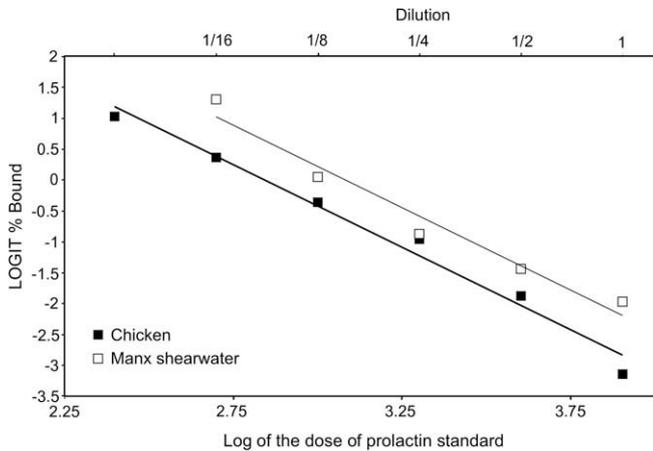


Fig. 1. Dose response curve for chicken (AFP4444B) and Manx shearwater prolactin.

parents provision their chick at the highest rate) and late chick-rearing (chick between 45 days and fledging (ca. 70 days), a period during which parents reduce their provisioning effort). Our standard statistical model described hormone levels as a function of sex, BC, breeding status and all two-way interactions. Two blood samples were taken per bird as part of the capture and restraint protocol, but aside from that, all samples were independent. Thus models were simple linear models (lm function in R). Assumptions of normality and homoscedasticity were checked using diagnostic plots of fitted and observed values and residuals. Corticosterone levels were log transformed to fit these assumptions. Terms were sequentially removed from the models if their effect was not significant (as determined by F -tests). Single-degree-of-freedom orthogonal contrasts (Crawley, 2007) were used to compare failed-vs. non-breeders, parental vs. non parental breeding stages, and late chick-rearing with earlier breeding stages.

3. Results

3.1. Baseline prolactin

Baseline prolactin levels were higher in females than in males ($F_{1,116} = 53.5$, $P < 0.0001$) and were strongly affected by breeding

status ($F_{6,116} = 84.2$, $P < 0.0001$, Fig. 2): prolactin levels were lowest in pre-laying birds, highest during the first days of incubation and maintained at high levels until late chick-rearing, when they were significantly reduced ($t = 8.9$, $P < 0.0001$). Prolactin levels were slightly but significantly lower in failed breeders and non-breeders compared to late chick-rearing birds ($t = 2.04$, $P = 0.04$) but they were more than ten fold higher in the former than in pre-laying birds. BC had no effect on prolactin levels ($F_{1,81} = 1.0$, $P = 0.3$).

3.2. Stress-induced prolactin

In a model assessing the change in prolactin levels with stress (stress-induced prolactin levels as a function of baseline prolactin \times sex, baseline prolactin \times BC, baseline prolactin \times breeding status, and all three way interactions with prolactin) only the interaction between baseline prolactin and breeding status remained in the minimum model and its effect was highly significant ($F_{6,73} = 3.5$, $P < 0.005$; baseline prolactin \times BC: $P > 0.2$; baseline prolactin \times sex: $P > 0.1$; higher level interactions: $P > 0.2$): levels decreased in response to acute stress in incubating and mid chick-rearing birds but increased in all other birds (Figs. 2 and 3). There was no difference in this respect between incubating and mid chick-rearing birds ($t = 1.2$, $P = 0.2$) or between non-breeders and failed breeders ($t = 0.6$, $P = 0.5$).

3.3. Corticosterone

There was a strong effect of breeding status on corticosterone levels (baseline: $F_{6,118} = 10.9$, $P < 0.0001$, Fig. 4A; stress-induced (i.e. at 30 min): $F_{6,77} = 2.8$, $P = 0.016$, Fig. 4B), which was due to higher levels during pre-laying and late chick-rearing stages than at any other stage. Levels did not differ between failed breeders and non-breeders, although sample size was low (baseline: $t = 0.2$, $P = 0.9$; stress-induced: $t = -1.1$, $P = 0.3$). There was no effect of body condition (baseline: $F_{1,81} = 0.2$, $P = 0.7$; stress-induced: $F_{1,62} = 0$, $P = 0.96$) and although there was a tendency for males to show slightly higher levels than females, this was not significant (baseline: $F_{1,115} = 2.5$, $P = 0.1$; stress-induced: $F_{1,75} = 2.9$, $P = 0.09$). Omitting pre-laying and late chick-rearing birds (exhibiting reduced parental care), baseline levels were slightly but significantly higher in breeding individuals than in birds showing no parental

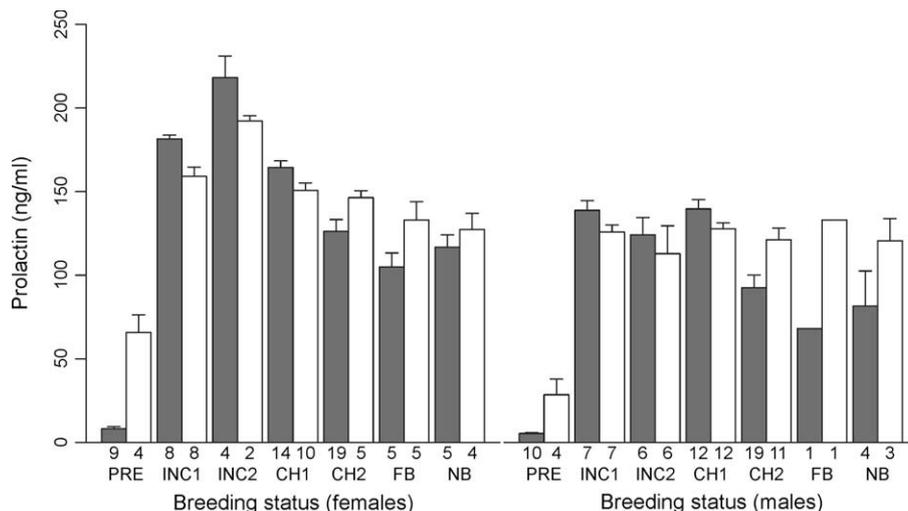


Fig. 2. Baseline (filled bars) and stress-induced (unfilled bars) prolactin levels (+ SE) in females (left) and males (right) throughout the breeding cycle (PRE: pre-laying, INC1: early incubation, INC2: late incubation, CH1: mid chick-rearing, CH2: late chick-rearing, FB: failed breeders, NB: non-breeders; see methods for details). Sample sizes shown in grey.

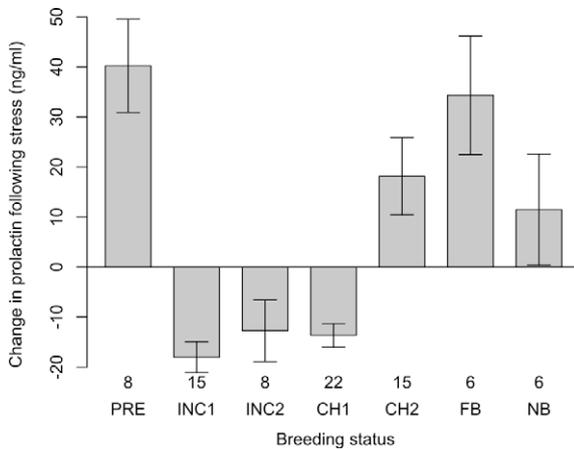


Fig. 3. Change in prolactin levels from 3 to 30 min (\pm SE) across stages of the breeding cycle.

activity ($t = -2.4$, $P = 0.02$), whereas there was no significant difference for stress-induced levels ($t = 0.1$, $P = 0.9$).

4. Discussion

4.1. Baseline prolactin levels

Baseline prolactin levels were high during incubation and chick rearing, but gradually decreased towards the end of chick-rearing and were low in failed breeders and non-breeders. The drop in prolactin levels from breeders to failed breeders was, however, very slight compared to that seen in other species (El Halawani et al., 1980; Hall, 1986; Sharp et al., 1988; Chastel and Lormée, 2002). A similar pattern to ours in king penguins *Aptenodytes patagonicus* and emperor penguins *A. forsteri* led authors to suggest that prolactin levels might be maintained at high levels in the absence of stimuli from the chick in order to maintain reproductive behaviors in environments where adults need to forage for long periods away from the colony (Garcia et al., 1996; Lormée et al., 1999). However, our findings, as well as those from two other seabird studies (Chastel et al., 2005; Angelier et al., 2009b), show that prolactin levels

can be maintained following reproductive failure in seabirds that visit their chick much more frequently than penguins. Therefore, the above hypothesis may not apply generally. Rather, maintenance of prolactin secretion in non-breeders and failed breeders may be related to nest-guarding activity, since both failed breeders and non-breeders guard a burrow throughout the breeding season to secure breeding opportunities in subsequent years (Brooke, 2004; Angelier et al., 2009a).

It is known that prolactin secretion remains high during the chick-brooding phase in seabirds, at a time when the chick is thermally dependent on its parents (Hector and Goldsmith, 1985; Lormée et al., 2000). In this study we show that plasma prolactin remains elevated long beyond this phase (Fig. 2), brooding lasting only a few days post-hatching in this species. Prolactin levels were only slightly lower during mid chick rearing than during incubation (and they were equally high in males in both periods); it was only towards the end of chick rearing that levels showed a marked decline. The extent to which prolactin influences chick-provisioning remains uncertain but our finding that prolactin secretion is maintained during chick rearing supports claims of a role for this hormone in mediating chick-rearing behavior (Buntin et al., 1991; Schoech et al., 1996; Duckworth et al., 2003).

4.2. The prolactin stress response

Our data suggest there may be less contrast than previously thought between birds (at least pelagic seabirds) and mammals in the change in plasma prolactin levels following stress. In Manx shearwaters, plasma prolactin levels decreased in response to acute stress during incubation and mid chick-rearing, as found for other birds (Angelier and Chastel, 2009) and lactating mammals (Walker et al., 1992; Bánky et al., 1994). These data are in accord with the findings of previous studies carried out during incubation, where a consistent decline in prolactin levels following a stressful event has been interpreted as an integral part of the stress response, acting in conjunction with corticosterone secretion to guide the behavior of individuals according to the fitness benefit of sustaining the current reproductive effort (Chastel et al., 2005; Angelier et al., 2007, 2009b; Verreault et al., 2008).

In contrast to the response above, plasma prolactin levels of Manx shearwaters increased in response to acute stress under con-

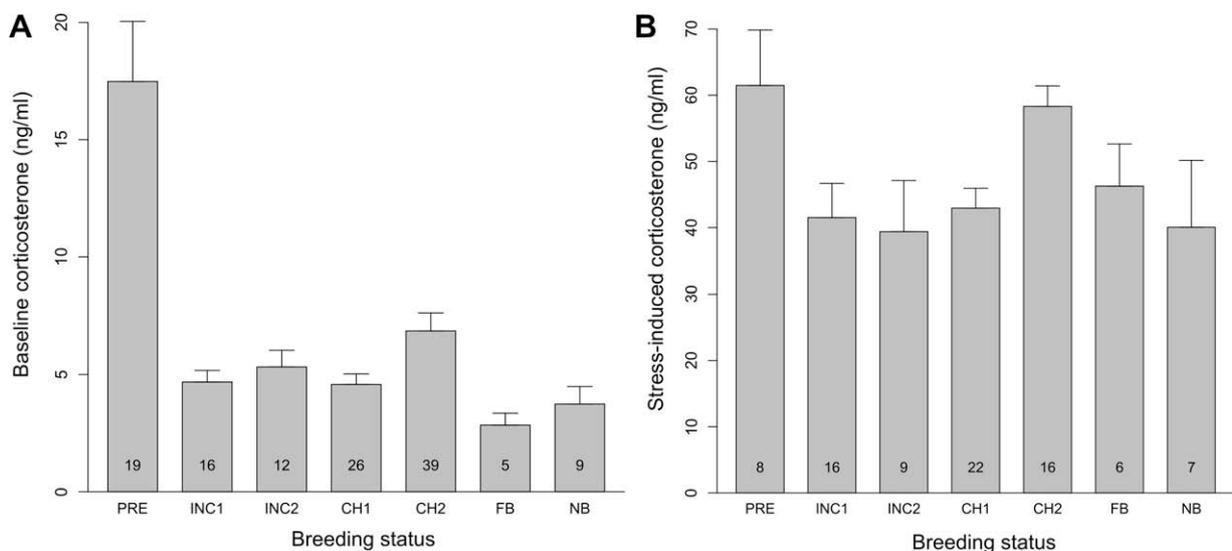


Fig. 4. Baseline (A) and stress-induced (B) corticosterone levels (\pm SE) throughout the breeding cycle.

ditions where there was relatively little parental care (during late chick-rearing) or no parental care (in pre-breeders, non-breeders and failed breeders), a pattern that closely resembles the well established one for non-lactating mammals (De Vlaming, 1979). These data suggest that stress-induced levels of prolactin are under the control of predominantly different mechanisms during and outside the main period of parental activities. For instance, if prolactin attenuates the deleterious effects of high corticosterone levels (Törner and Neumann, 2002; Kitaysky et al., 2003; Tilbrook et al., 2006), an increase in prolactin secretion may be needed in periods when adults do not exhibit parental behavior and when levels are generally lower. Alternatively, an increase in prolactin may be triggered by a decline in testosterone following stress (Cernak et al., 1997).

4.3. Modulation of the stress response

We expected a down-regulation of the stress response in breeders compared to other birds (Bokony et al., 2009). In practice, however, stress-induced corticosterone levels of breeders were no different to those of failed breeders or non-breeders, which does not support predictions based on the current reproductive value of offspring. However, the higher response of pre-laying birds was in accordance with these predictions.

There were no differences in the corticosterone or prolactin stress responses of birds incubating and those midway through chick rearing, which contrasts with another Procellariiform seabird where, as expected, the corticosterone stress response was higher during incubation than during chick rearing (Adams et al., 2005). Inter-annual variation in corticosterone levels may be associated with variation in prey abundance (Kitaysky et al., 2007; Williams et al., 2008) and Manx shearwaters on Skomer displayed poor reproductive performance in 2008 (Riou, 2009). Thus high baseline and stress-induced corticosterone during chick rearing in our study may have been related to low food availability (Kitaysky et al., 1999; Lormée et al., 2003).

Stress-induced corticosterone levels during breeding were at their highest in late chick-rearing, when the reproductive value of offspring is highest. This again did not match our predictions. In Manx shearwaters, parents spend more time at sea in the weeks preceding fledging but this heightened foraging effort does not result in increased food delivery to the chick (Riou and Hamer, 2010). Rather, it seems to be used by adults to replenish their nutritional reserves ahead of post-breeding migration (Brooke, 1990). Thus increased corticosterone levels towards the end of chick-rearing may reflect the greater importance of the parents' own nutritional status at this stage of the season. High baseline corticosterone levels during pre-breeding might reflect the intense activity of birds at the colony at that time (e.g. courting, digging burrows and agonistic behavior). Finally, the fact that male and female Manx shearwaters showed similar levels of baseline and stress-induced corticosterone is in accordance with predictions (Wingfield and Sapolsky, 2003) and observations (Bokony et al., 2009) for species having overall comparable levels of parental care and energy expenditure through the breeding cycle.

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