



Sex- and breeding stage-specific hormonal stress response of seabird parents

Katarzyna Wojczulanis-Jakubas^{a,*}, Dariusz Jakubas^a, Monika Kulpińska-Chamera^a, Olivier Chastel^b

^a University of Gdańsk, Faculty of Biology, Department of Vertebrate Ecology and Zoology, Wita Stwosza 59, 80-308 Gdańsk, Poland

^b Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 – CNRS & Université de la Rochelle, 79360 Villiers-en-Bois, France

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ABSTRACT

Changes in corticosterone (CORT) and prolactin (PRL) levels are thought to provide complementary information on parental decisions in birds in the context of stressful situations. However, these endocrine mechanisms have yet to be fully elucidated, appearing to vary among avian species without any clear pattern. Here, we examined CORT and PRL stress responses in a small Arctic seabird, the little auk (*Alle alle*). We analysed the levels of these hormones (baseline, and stress response, i.e. the change in the baseline in response to stress) with respect to the breeding phase (mid incubation and mid chick rearing) and the sex of the birds. Baseline CORT concentrations were similar during both breeding phases but baseline PRL levels were higher during incubation than chick rearing. The CORT and PRL stress responses were stronger during incubation than chick rearing (although with respect to CORT the effect was only marginally significant). There were also some sex-specific baseline levels and stress responses for both hormones (during the incubation period males compared to females exhibited higher CORT stress response and lower baseline PRL; during the chick rearing period males exhibited higher PRL stress response). Our results suggest that in the case of the little auk, both the incubation and the chick rearing periods may represent similar levels of physiological stress. However, the birds may be more sensitive to stress during incubation than during chick rearing, possibly because of inter-phase differences in predation pressure. The sex differences suggest differential exposure of males and females to stressors.

1. Introduction

Endocrine stress response is one possible mechanism regulating avian reproductive effort. Potentially deleterious stressors experienced by the parents, e.g. predators, extreme weather events, food shortages, activate neuro-hormonal pathways that change their behaviour (Wingfield and Sapolsky, 2003). The most important of these pathways involves activation of the hypothalamic–pituitary–adrenal axis (HPA), which leads to rapid and dramatic increase in the circulating level of corticosterone (CORT). According to the “emergency life history-stage” hypothesis, a sharp increase in the CORT level redirects resources away from activities inessential at a given moment, such as parental care, towards the immediate survival of the parent (Landys et al., 2006; Wingfield et al., 1998). When the stressor disappears, the CORT level returns to a low concentration and reproductive activities are resumed. But, when the stressor is chronic, CORT levels remain high, and this leads to a dramatic reduction in parental care, including brood abandonment (e.g. Ouyang et al., 2012).

The CORT stress response is a well understood mechanism regulating parental investment. But there is another hormone, that could

also play a part in regulating reproductive effort as it affects parental activities directly: this is prolactin (PRL) (Angelier and Chastel, 2009; Chastel et al., 2005). CORT and PRL levels often appear to provide complementary information regarding parental decisions in the context of stressful situations: CORT is primarily related to the parents' survival, while PRL is associated with benefits to the offspring (Angelier et al., 2009, 2016; Angelier and Chastel, 2009; Chastel et al., 2005; Heidinger et al., 2006). Although the pattern of changes in baseline PRL levels over the breeding season differs among species, depending on the offspring developmental mode, PRL secretion is dramatically accentuated in all species when individuals enter the parental phase (Angelier et al., 2016; Dawson and Goldsmith, 1983). Some experimental studies have clearly demonstrated casual role of PRL in post-hatching care (Smiley and Adkins-Regan, 2018; Wang and Buntin, 1999). Be that as it may, the PRL stress response is still not well understood. A growing, though still limited, body of data for birds suggests that the PRL concentration in breeders decreases during stressful events (Angelier et al., 2016; Angelier and Chastel, 2009; Chastel et al., 2005; Opel and Proudman, 1986; Sharp et al., 1989). If PRL level drops below a certain threshold, as a result of acute or chronic stress, parental activities cease (Angelier

* Corresponding author.

E-mail address: biokwj@univ.gda.pl (K. Wojczulanis-Jakubas).

et al., 2016; Angelier and Chastel, 2009; Chastel et al., 2005). The direction and magnitude of this response may, however, be stage- or concentration-dependent, because the PRL level has also been reported to remain stable or even increase in response to acute stress, with no effect on parental performance (reviewed in Angelier et al., 2016).

Numerous studies have demonstrated that the effect of a stressor on endocrine response is not always the same, and depends on current circumstances. According to the “brood value hypothesis” (reviewed in Bókony et al., 2009), baseline CORT and PRL levels and hormonal stress response should vary depending on the relative importance of the current reproductive effort. If the value of this is relatively high, the baseline level of CORT should be reduced, and that of PRL elevated, and the stress response of both hormones should be attenuated; this ensures that the current breeding attempt is not compromised (Bókony et al., 2009). Consequently, parents in short-lived birds, which have just a small number of reproductive attempts at their disposal, are expected to have higher CORT and PRL baseline concentrations, and to mitigate the stress response of both hormones, all in order to maximize reproduction during the current breeding attempt. In contrast, parents in long-lived, iteroparous species (i.e. with multiple reproductive cycles over the course of the lifetime), are expected to exhibit the opposite pattern of baseline levels of both hormones and to sustain hormonal stress responses at a high level, thus preventing their own survival from being jeopardized (Angelier et al., 2016; Angelier and Chastel, 2009; Bókony et al., 2009; Drent and Daan, 1980). This is because the lifetime reproductive success of long-lived species is primarily a function of adult survival rather than of seasonal fecundity (Williams, 1966). Following the same line of reasoning, the brood value is believed to increase as the breeding season progresses. When opportunities for replacement breeding in the current season are limited, the importance of current reproduction increases considerably (e.g. Breuner et al., 2003; O’Reilly and Wingfield, 2001; Silverin et al., 1997). Hence, the hormonal stress response is expected to attenuate with the progress of the breeding season (Bókony et al., 2009). The result of the few studies to have investigated the hormonal stress response over the breeding season are contradictory: on the one hand, they support the brood value hypothesis in that they reveal a lower stress response in the late phase of breeding compared to earlier phases (e.g. the grey-face-petrel *Pterodroma macroptera gouldi*; Adams et al., 2005), while on the other they do not support it, demonstrating no differences in hormonal levels between the breeding phases (e.g. the Manx shearwater *Puffinus puffinus*; Riou et al., 2010). Finally, the brood value and hormonal levels may be differently modulated in males and females, depending on the amount of parental care provided by each sex. It has been demonstrated for several species that the sex investing more in offspring care exhibits higher baseline CORT and PRL levels, and a weaker hormonal stress response (Holberton and Wingfield, 2003; O’Reilly and Wingfield, 2001; Wingfield et al., 1995).

In this study we examined CORT and PRL stress response in the little auk (or dovekie, *Alle alle*), a small pelagic seabird, breeding exclusively in the high Arctic. Being a high-latitude species, the little auk is expected to exhibit a weak stress response when breeding, as it is adapted to the extreme, stressful weather events that are relatively frequent in the polar zone (Breuner et al., 2003; O’Reilly and Wingfield, 2001; Silverin et al., 1997, but see also Bókony et al., 2009). Moreover, because of the little auk’s long nesting period and the short time-window of the Arctic summer with favorable environmental conditions (both ca 2 months), only one brood is completed annually, and opportunities for replacement breeding are extremely limited. Presumably, only breeders re-nesting early in the season stand any chance of successfully rearing a replacement brood, even though their chicks are usually in a worse body condition than those hatched earlier in the year (Jakubas and Wojczulanis-Jakubas, 2013). In such time-constrained breeding circumstances, selection is expected to attenuate the breeders’ hormonal stress responses. On the other hand, the little auk is a typical seabird: it is long-lived, with a life-span of at least ten - twenty years, (own

unpublished data), and a prolonged phase of bi-parental care for a single chick. Given these life-history traits, little auk parents are expected to respond strongly to stressful situations, prioritizing their self-maintenance and survival over the wellbeing of their offspring. Contrasting expectations regarding the magnitude of the stress response in the little auk make the species particularly interesting, especially that avian stress responses have been little studied in high-latitude seabirds.

We examined CORT and PRL stress response in the little auk with respect to the breeding phase (mid incubation and mid chick rearing) and sex. Basically, we focused on changes in the levels of these hormones in response to stress; but in order to obtain a fuller picture, we also analysed their baseline levels, and the relationship between the baseline and stress response levels of both. Given the time-constraints imposed by the Arctic summer (Moe et al., 2009), we assumed that the brood value did not change for little auk parents across the breeding phases. Thus, we expected no differences in hormonal stress responses between the mid-incubation and mid-chick rearing periods; neither did we expect any differences in CORT baseline levels. Although the parental care provided during the two breeding phases takes fundamentally different forms, previous inter-phases comparisons of baseline CORT levels in the little auk did not yield significant differences (Wojczulanis-Jakubas et al., 2015). We did expect differences in baseline PRL levels, however, with higher values during mid-incubation than mid-chick rearing. This is because baseline PRL generally increases rapidly with the onset of incubation in semi-precocial species like the little auk and remains at stable high level throughout the incubation period (Angelier et al., 2016; Massaro et al., 2007; Vleck et al., 2000). After hatching, it drops slightly, thereafter decreasing gradually (Angelier et al., 2016). The baseline PRL level in the mid-chick rearing period in the little auk should thus be much lower than during the mid-incubation period. This is consistent with previous inter-phase comparisons of baseline PRL levels in this species (Wojczulanis-Jakubas et al., 2015). With regard to sex, we did not expect any differences in hormone levels and stress responses during the two breeding phases between males and females they make similar contributions to parental care during both incubation and chick rearing (Harding et al., 2004; Wojczulanis-Jakubas et al., 2009). There are no apparent differences between the parents in the amounts of time they spend at the nest during the incubation period (Wojczulanis-Jakubas et al., 2009). Moreover, for most of the chick rearing phase, both parents brood and feed their offspring with a similar frequency (Wojczulanis-Jakubas et al., 2012). The only differences in parental care occur at the end of the chick rearing phase, when the female ceases feeding but the male continues to care for the chick and escorts it during its departure from the colony. For this reason, little auk males have been hypothesized to be less sensitive to stressors than females: escorting the chick during its first flight to sea is expected to be a stressful event because of the greater predation pressure in that period (Wojczulanis-Jakubas et al., 2005). However, a study comparing baseline and stress-induced hormone concentrations between the two sexes during the chick rearing period did not reveal significant differences (Wojczulanis-Jakubas et al., 2013).

2. Materials and methods

2.1. Fieldwork

We carried out the study in a large little auk breeding colony on the Arikammen slopes in Hornsund (SW Spitsbergen, Norway; 77°00’ N, 15°33’ E). We considered only active breeders and used two capture methods, both of which effectively minimized the handling time to < 3 min (crucial for the standardized capture-restraint protocol (Wingfield et al., 1994, see below). During the incubation period (season 2014), we captured the birds by hand while they were incubating on the nest. The breeding status of the incubating birds was self-evident, and the individuals were sampled on day 9–12 of the

incubation period (established on the basis of daily inspections of the focal nests during the egg-laying period). During the chick rearing period (seasons 2011 and 2015) we captured the birds delivering food to their offspring using noose-carpet deployed over the colony area. The gular pouches full of food in the captured birds indicated chick provisioning. Although we did not know the exact chick hatching dates for those individuals, we assumed, given the high hatching synchrony in the monitored nests in the colony, that chick rearing had reached a similar stage (day 10–13 in 2011 and day 9–12 in 2015, in relation to the median hatching date in the season). We sampled a total of 45 birds (season 2014) during the incubation period, and 89 birds during the chick rearing period (two seasons: 2011, $n = 59$, 2015, $n = 30$).

To establish the birds' hormonal stress responses, we applied the standardized capture-restraint protocol (Wingfield et al., 1994). In brief, the protocol requires double blood-sampling of the same individual:

- 1) immediately after capture, before the hormones released due to the stress associated with capturing appear in the blood stream at a detectable level (hereafter, baseline level);
- 2) 30 min after capture, when the hormones released are assumed to have reached a peak level in response to stress. Although we did not determine this time interval for the little auk, we assumed it to be appropriate for the species by analogy to other relevant studies, e.g. (Angelier et al., 2007). Even if we would did not capture the exact instant of the peak stress response, we could still treat the change as stress-induced and compare the values among the breeding phases and sexes. This is because all the birds were sampled within the same time interval, and considerable changes were recorded in the hormone concentrations compared to the baseline levels in all cases.

We collected the first blood sample from individuals immediately after capture, timing the duration of this first sampling precisely. On average, each such sampling lasted 1.7 min, and no such event took longer than 3 min. In addition, there was no correlation between the duration of the first sampling and the baseline concentration of either CORT or baseline PRL (Pearson correlation coefficient, $P \geq 0.62$ for both hormones). We kept the birds in separate, opaque cloth bags for 30 min before the second sampling. During the whole sampling procedure during the incubation period, we kept the egg of the captured birds in warm. We ringed all the birds before releasing them (individual metal ring, Stavanger, Norway), to prevent double sampling of the same individual. We returned the birds to the nest during incubation, or released them into the air during the chick rearing period. We sampled all the birds within a time window of 9 h (“day” hours), to avoid any potential effect of diurnal cycles on the hormone levels (e.g. Rich and Romero, 2001). Indeed, baseline and stress-induced PRL and CORT levels were not correlated with time of day (in all cases $P > 0.05$).

We collected the blood samples from the brachial vein of each individual using disposable heparinized capillaries. Our intention was to collect 100 μ L per sample, i.e. a total of 200 μ L per bird, which is $\sim 1\%$ of the total body mass (average 159–171 g). This volume of blood is considered safe for birds (Voss et al., 2010), and we did not observe any apparent changes in the birds' behaviour. In fact, in some cases we were unable to collect even this amount, as the blood clotted rapidly; this sometimes turned out to be insufficient for the hormone analysis, so the sample sizes for particular groups in the analyses varied slightly from the initial number of birds sampled (Table 1). In the field, we kept the samples in a coolbox ($+4$ °C; for 2–3 h); they were subsequently centrifuged for 10 min at 6000 rpm. We stored the plasma (for hormones analysis; frozen at -20 °C) and red blood cells (for molecular sexing; preserved in 90% ethanol) separately for several months prior to the analyses.

Table 1

Final sample size for particular sampling groups.

Season	Breeding phase	Sex	CORT baseline	CORT stress-induced	PRL baseline	PRL stress-induced
2011	Chick rearing	Female	29	26	25	21
		Male	31	29	23	25
2014	Incubation	Female	21	20	21	20
		Male	23	24	24	24
2015	Chick rearing	Female	12	10	–	–
		Male	18	15	–	–

2.2. Laboratory analyses

2.2.1. Hormone assay

We measured the baseline and stress-induced concentrations of CORT and PRL by radio-immunoassay at the Centre d'Etudes Biologiques de Chizé, France. We measured the total plasma CORT in the samples after ethyl ether extraction using a commercial antiserum, raised in rabbits against corticosterone 3-(*O*-carboxymethyl) oxime bovine serum albumin conjugate (Biogenesis, UK). Cross-reaction was 9% with 1-desoxycorticosterone and $< 0.1\%$ with other plasma steroids. We incubated duplicate aliquots of the extracts (100 μ L) overnight at 4 °C with 8000 cpm of 3H-corticosterone (Amersham Pharmacia Biotech-France) and antiserum. We separated the free and bound fractions of CORT by adding dextran-coated charcoal. After centrifugation, we counted the bound fraction in a liquid scintillation counter. Minimal detectable CORT levels were 0.3 ng. We determined the plasma levels of PRL twice in each sample using the heterologous radio-immunoassay (RIA) described by Cherel et al. (1994). The PRL samples from the little auks produced a dose-dependent response curve that paralleled the one of the standard chicken plasma (Parlow, UCLA Medical Center, Los Angeles). All the samples were run in one assay for both hormones. To measure intra-assay variation, the same sample was included four times in the corticosterone and prolactin assays. The intra-assay variation for total corticosterone and prolactin levels was within the 5–12% range.

2.2.2. Molecular sexing

We performed molecular sexing at the University of Gdańsk, Poland. We extracted DNA from the frozen red cells using Blood Mini Kit (A&A Biotechnology, Gdynia, Poland). We amplified introns on the CHD-W and CHD-Zs genes located on the avian sex chromosomes using the primers F2550 and R2718 (Griffiths et al., 1998) in PCR with an annealing temperature of 50 °C. The sex differences in the PCR products were clearly visible in UV-light on 1% agarose gel stained in Advanced Midori Green (Nippon Genetics Europe, Germany), with one band for male (i.e. ZZ) and two bands for female (i.e. ZW).

2.3. Data analysis

We collected some of the data used in the present study during another project (data from the chick rearing period in 2011, $n = 59$, Wojczulanis-Jakubas et al., 2013). Those data were obtained using exactly the same methods (both in the field and the laboratory) as in the present study; hence, the two sets were fully compatible and could be analysed together. The present study, analysing hormonal stress-response per se (see below), provides new values in regard to the results given in Wojczulanis-Jakubas et al. (2013), where only a simple comparison of male and female differences in baseline and stress-induced levels of the hormones in a single breeding period was made.

We analysed two proxies of the hormonal stress response:

- 1) the baseline hormone concentration – the hormone level governed by the stress associated with the metabolic adjustment related to the

current stage of the annual cycle (Kitaysky et al., 2007, 2010); this was established on the basis of samples collected within a three minute time interval following the moment of capture (Wingfield et al., 1994);

- 2) the change in the hormone level induced by the stress associated with capturing and handling, related to the baseline level, hereafter, the stress response; this was established on the basis of samples collected 30 min after the bird had been captured, during which time it was restrained (Pakkala et al., 2013; Wingfield et al., 1994).

Since we found no evident relationship between baseline CORT and PRL levels in any season, period, or sexes, we investigated the two hormones separately (all $P > 0.26$, Table A.1). Basically, we analysed the two hormones following the same approach, but because we had sampled the birds for CORT data during the chick rearing period in two seasons, we modified some of the procedures in the CORT analysis. We evaluated assumptions of linearity, normality and homoscedasticity using diagnostic plots of residuals and log-transformed the response variables when necessary.

2.3.1. Corticosterone

Since we had data on CORT from two seasons for the chick rearing phase, we used season-phase variable (resulting in three levels: incubation, chick rearing '11 and chick rearing '15) for all models where breeding phase was considered. To analyse the baseline level of the hormone, we initially modelled it with respect to the phase of the breeding period, sex and all their interactions using a general linear model (LM). However, this full model explained very little variance (adjusted $R^2 = 0.03$) and was not significant ($F_{5,117} = 1.71$, $P = 0.14$). We therefore examined all possible combinations of the explanatory variables using the *dredge* function from the *MuMIN* R package (Bartoń, 2017; Table A.2). Based on the weight of the model and AIC criteria ($\Delta AIC_c < 2$; Burnham and Anderson, 2000), we then averaged the first two models and identified the importance of each variable (Bartoń, 2017). The averaged model included only the season-phase variable (0.34 importance). To analyse the CORT stress response, we modelled it using LM with sex, season-phase and their interaction. The full model explained 18% of the variance and was significant ($F_{5,117} = 6.38$, $P < 0.001$). To calculate the overall significance of the season-phase factor, we used the Wald-statistic (*aod* package, Lesnoff and Lancelot, 2012). Because there were some season-phase differences (see Results) in the CORT stress response, we analysed the relationship between the stress response and the baseline CORT level separately for each phase of breeding using LM, with the CORT stress response as the response variable and baseline CORT level, sex and the interaction of baseline CORT level and sex (due to the sex-specific stress response) as predictors. We calculated separate models for particular phases of breeding. They all explained high amount of variance and were significant (incubation: 64%, $F_{2,40} = 38.74$, $P < 0.001$, chick rearing '11: 62%, $F_{2,52} = 45.79$, $P < 0.001$ and chick rearing '15: 72%, $F_{2,22} = 31.38$, $P < 0.001$).

2.3.2. Prolactin

Firstly, we used the LM approach to analyse the baseline level of this hormone with regard to the breeding phase, sex and their interaction. This model explained 81% of the variance and was significant ($F_{3,76} = 111.6$, $P < 0.001$). Secondly, using the same approach, we modelled the stress response with respect to the breeding phase, sex and their interaction. This model explained 34% of the variance and was also significant ($F_{3,76} = 14.76$, $P < 0.001$). Since PRL stress response exhibited negative values during the chick rearing phase, to analyse solely magnitude of the response we performed a separate analysis on the absolute values of the stress response. This model explained 23% of the variance and was significant ($F_{3,76} = 8.94$, $P < 0.001$). Finally, we modelled the stress response (response variable) with respect to the baseline PRL level using LM model with PRL stress response as response

Table 2
Modelling output of the corticosterone (CORT) analyses (general linear models).

Model formula	Parameter	Estimate	SE	/t	P	
CORT	Intercept	2.26	0.23	9.95	< 0.001	
	baseline ~ season-phase (conditional averaged model)	Incubation	-0.56	0.41	1.34	0.18
	Chick rearing '15	0.22	0.48	0.45	0.65	
log(CORT_stress re- sponse) ~ season-phase * sex	Intercept	2.54	0.17	14.79	< 0.001	
	Incubation	0.51	0.26	1.97	0.05	
	Chick rearing '15	0.1	0.33	0.32	0.75	
	Sex (male)	-0.17	0.24	-0.71	0.48	
	Incubation × sex (male)	0.75	0.36	2.09	0.04	
	Chick rearing '15 × sex (male)	0.43	0.43	1.00	0.32	
Incubation: log(CORT_stress response) ~ CORT baseline + CORT baseline: sex	Intercept	4.11	0.12	34.12	< 0.001	
	CORT baseline	-0.37	0.05	-8.04	< 0.001	
	CORT	-0.10	0.07	-1.38	0.18	
Chick rearing '11: log(CORT_stress response) ~ CORT baseline + CORT baseline: sex	Intercept	3.34	0.12	26.86	< 0.001	
	CORT baseline	-0.46	0.07	-6.54	< 0.001	
	CORT	0.13	0.06	2.13	0.04	
Chick rearing '15: log(CORT_stress response) ~ CORT baseline + CORT baseline: sex	Intercept	3.76	0.14	26.03	< 0.001	
	CORT baseline	-0.35	0.05	-6.80	< 0.001	
	CORT	-0.04	0.05	-0.84	0.41	

variable and baseline PRL level, sex, and interaction of baseline PRL level and sex (due to the sex difference in the hormone levels) as predictors. Due to significant effects of the breeding phase on hormone levels, we calculated separate models for each breeding phase. The model for the incubation phase explained 34% of variance and was significant ($F_{3,76} = 14.76$, $P < 0.001$). The model for the chick rearing explained 27% of variance and was also significant ($F_{2,34} = 7.53$, $P = 0.002$).

2.3.3. Ethical note

We performed all the fieldwork by permission of the Norwegian Animal Research Committee and the Governor of Svalbard. All the birds were handled with the utmost care and released without any harm after the sampling.

3. Results

3.1.1. Corticosterone

Baseline CORT was similar in all the season-phases (Table 2; Fig. 1). Similarly, the season-phase was not significant (Wald test, $\chi^2 = 4.0$, $df = 2$, $P = 0.13$) for the CORT stress response, although during the incubation phase the stress response was slightly higher compared to the chick rearing phase 2011 (marginally significant, Table 2; Fig. 2). Neither sex differed in its stress response, although the males had a tendency to exhibit a stronger response than females during the incubation phase (interaction of phase × sex; Table 2, Fig. 2). The CORT stress response was significantly related to the baseline CORT level in all the season-phases, with the response being stronger with increasing baseline concentration (Table 2; Fig. 3). This relationship was similar for males and females during the incubation and the chick rearing in 2015 (Table 2) but not during the chick rearing in 2011 (Table 2), when females exhibited a stronger relationship than males (Fig. 3).

3.1.2. Prolactin

Baseline PRL was considerably higher during the incubation than

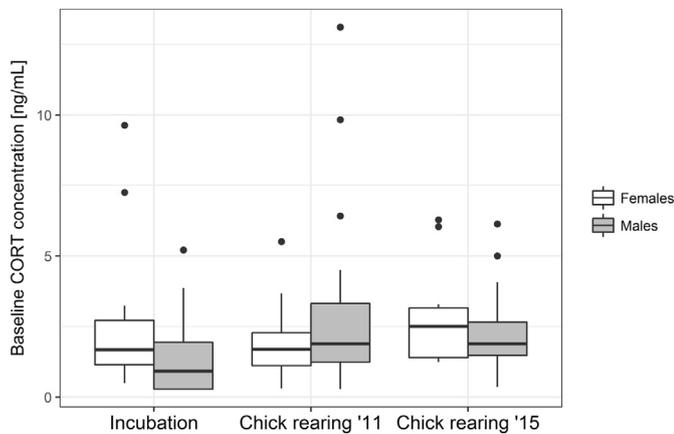


Fig. 1. Corticosterone (CORT) baseline level during the incubation and chick rearing phases (two different seasons) in little auk males and females. The bottom and top of the box are the first (Q1) and third quartiles (Q3; IQR), and the line inside the box is the median. The end of upper whisker represents $Q3 + 1.5 \text{ IQR}$, whereas the lower whisker represents $Q1 - 1.5 \text{ IQR}$, with the points above and below being outliers.

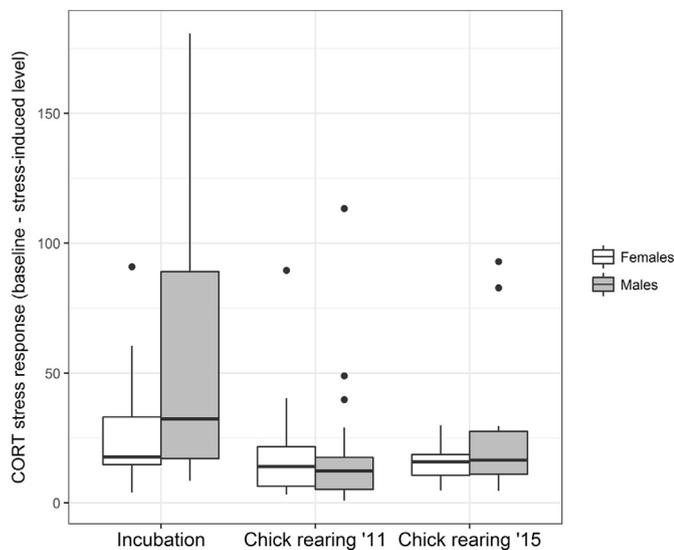


Fig. 2. Corticosterone (CORT) stress response (relative change in respect to baseline level) during the incubation and chick rearing phases (two different seasons) in little auk males and females. The bottom and top of the box are the first (Q1) and third quartiles (Q3; IQR), and the line inside the box is the median. The end of upper whisker represents $Q3 + 1.5 \text{ IQR}$, whereas the lower whisker represents $Q1 - 1.5 \text{ IQR}$, with the points above and below being outliers.

the chick-rearing stage (Table 3). Overall, the sexes did not differ in the baseline PRL level but females exhibited higher concentration than males during the incubation phase (the phase \times sex interaction, Table 3; Fig. 4). The PRL stress response differed significantly between the breeding phases, being primarily negative during incubation (i.e. decrease in hormone concentration in response to stress) and positive during the chick rearing phase (i.e. increase in the hormone concentration in response to stress) (Table 3; Fig. 5). The magnitude of the stress response (i.e. absolute values) differed significantly between the phases, being lower during the incubation than the chick rearing phase (Table 3). There were significant differences between the sexes, with females exhibiting stronger response than males (both raw and absolute values, Table 3). The significance of this difference, however, was due primarily to the difference between the sexes during the chick rearing phase (the phase \times sex interaction in both models, with raw and

absolute values, Table 3; Fig. 5). The PRL stress response was significantly and positively related to the baseline PRL level during both breeding phases (Table 3; Fig. 6). This relationship was basically similar in both sexes. The significant sex difference that was noted during the chick rearing phase was driven by the sex difference in the magnitude of the PRL stress response (Table 3; Fig. 6).

4. Discussion

We examined the baseline levels of CORT and PRL, the changes in the concentrations of these hormones after acute stress (stress response), and the relationship between their baseline levels and their changes in response to stress in the little auk, with respect to the breeding phase (incubation and chick rearing) and the sex of the birds.

We did not find any significant differences in baseline CORT levels between the incubation and chick rearing periods; this was consistent with our predictions. Although the parental care provided in the two breeding phases takes fundamentally different forms, the basic physiological stress appears to be similar. The exact endocrine mechanisms underlying this pattern remain poorly understood, but it is likely that changes in parental activities (egg incubation \rightarrow chick feeding) are not the only trigger of metabolic adjustment associated with reproduction. A similar pattern of baseline CORT levels over the breeding season has been also found in some other seabirds, e.g. adult grey-faced petrels (Adams et al., 2005) and the tufted puffin *Fratecula cirrhata* (Williams et al., 2008), although baseline CORT changes during breeding have also been reported to be season-specific (e.g. Wingfield et al., 1999; Williams et al., 2008). Taken together, all these results suggest that overall physiological stress may be the same for the two breeding phases. Similarly, we did not find any significant effect of the season-phase on the CORT stress response, although stress response did tend to be higher during the incubation compared to the chick rearing phase.

In the line with our predictions, the baseline PRL level was higher (five-fold) during incubation than chick rearing period. However, contrary to our predictions, the PRL stress response differed between the incubation and chick rearing, with a contrasting trend in the two phases – a decrease during the former and increase during the latter phase. These contrasting patterns are likely to be due to considerable differences in the baseline level of this hormone (see below), which could affect the direction of its changes. The magnitude of the stress response, however, also differed between the breeding phases: it was larger during incubation. This result, together with the tendency towards a higher CORT stress response during incubation than chick rearing, suggests that the brood value for the little auk parents may change over the season, so we may simply have overestimated it for the incubation phase. However, an alternative interpretation of the observed inter-phase difference in hormonal stress response in the little auk could be inter-phase difference related to predation pressure, which is likely to be stronger during incubation, especially for burrow-nesting seabirds. Adult little auks are more likely to be predated on by polar foxes *Vulpes lagopus* or glaucous gulls *Larus hyperboreus* while incubating in the nest chamber and having a smaller capacity to escape. During the chick rearing phase only glaucous gulls represent a real danger, and little auks can easily fly away (Wojczulanis-Jakubas et al., 2005).

Given the equal contributions of little auk males and females to parental duties during incubation and chick rearing (Harding et al., 2004; Wojczulanis-Jakubas et al., 2009), we did not expect any sex differences in the hormones levels. Similar baseline and stress-induced CORT and PRL levels have been reported in other species sharing comparable levels of parental care and energy expenditure by the two sexes (Wingfield and Sapolsky, 2003; Adams et al., 2005; Bókony et al., 2009; Riou et al., 2010). With regard to the CORT stress response, we found that males were more stress-sensitive than females during the incubation period. The inter-phase difference, may well be associated with the different stress-exposure of the sexes. Although both parents

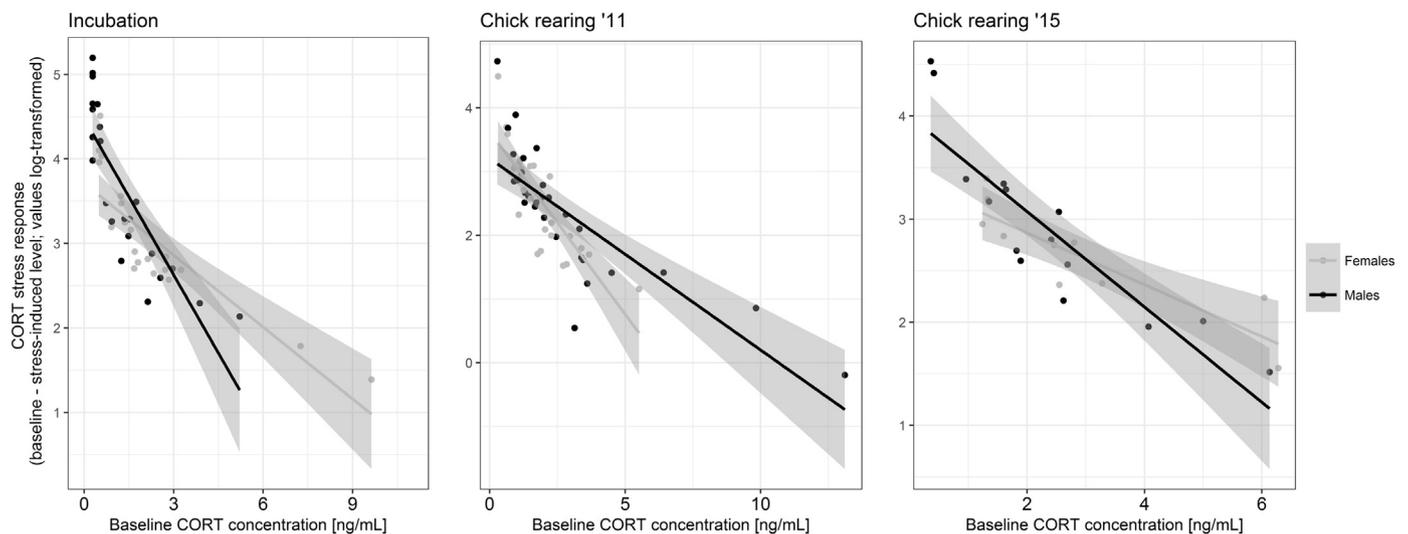


Fig. 3. Relationship between corticosterone (CORT) baseline level and CORT stress response during the incubation (left-hand panel) and chick rearing phase (two seasons: 2011 and 2015, middle and right-hand panels) in little auk males and females.

Table 3

Modelling output of the prolactin (PRL) analyses (general linear models).

Model formula	Parameter	Estimate	SE	t	P
PRL baseline ~ phase * sex	Intercept	47.1	6.11	7.7	< 0.001
	Phase (incubation)	125.48	8.65	14.51	< 0.001
	Sex (male)	-3.22	8.77	-0.37	0.71
	Phase (incubation) × sex (male)	-35.05	11.99	-2.92	0.004
PRL stress response – raw values ~ phase * sex	Intercept	0.9	0.16	5.45	< 0.001
	Phase (incubation)	-1.3	0.23	-5.67	< 0.001
	Sex (male)	-0.73	0.23	-3.11	0.003
	Phase (incubation) × sex (male)	0.75	0.32	2.36	0.02
PRL stress response – absolute value ~ phase * sex	Intercept	1.17	0.12	9.51	< 0.001
	Phase (incubation)	-0.77	0.17	-4.42	< 0.001
	Sex (male)	-0.62	0.18	-3.49	< 0.001
	Phase (incubation) × sex (male)	0.63	0.24	2.63	0.01
Incubation:					
PRL stress response ~ PRL baseline + PRL baseline: sex	Intercept	0.08	0.12	0.68	0.5
	PRL baseline	-0.003	0.001	-3.83	< 0.001
	PRL baseline × sex (male)	-0.001	0.0004	-1.76	0.09
Chick rearing:					
PRL stress response ~ PRL baseline + PRL baseline: sex	Intercept	1.84	0.38	4.78	< 0.001
	PRL baseline	-0.02	0.01	-2.86	0.007
	PRL baseline × sex (male)	-0.02	0.01	-2.35	0.02

share the incubation duties equally, females depart to the sea after their shift, while males spend more time in the colony, which is probably associated with their role in territory guidance/defence (Wojczulanis-Jakubas et al., 2009). When in the colony, males are more likely to become a prey of glaucous gulls than being at the sea, which can be more stressful and result in higher CORT response during the incubation phase. Males also engage more frequently in antagonistic interactions when in the colony (Wojczulanis-Jakubas et al., 2014). All in all, this may make them more stress-responsive than females.

We also found unexpected sex differences in both the PRL baseline level and the PRL stress response. First, females had a higher PRL baseline level than males during incubation. Since parental activities during the incubation period are shared equally or even male-biased, if one considers the presence in the territory (Wojczulanis-Jakubas et al., 2009), this sex difference is intriguing and difficult to explain. We can only speculate that it may be a question of some sex-specific physiological changes influencing the hormonal balance, perhaps related to egg production. Second, females also exhibited a higher PRL stress response than males during the chick rearing phase. This is likewise to explain in the light of arguments supporting the prediction of no sex difference in the PRL stress response. Nevertheless, this difference may be related to earlier female brood desertion. It has been suggested that

females desert the brood as they are more stress-sensitive than males, and therefore unable to perform the final parental duty, escorting the chick during its departure from the colony a dangerous activity owing to intensified predation pressure in that phase (Wojczulanis-Jakubas et al., 2005). A previous study (Wojczulanis-Jakubas et al., 2013), designed to examine this hypothesis, did not find any significant sex-differences in the PRL level, although it investigated only the stress-induced PRL level. Here, our analyses took baseline PRL concentrations into account, and we did not find significant sex differences. Importantly, the level of PRL stress response during the chick rearing increased, instead of the decrease expected in the context of the hypothesis tested in Wojczulanis-Jakubas et al. (2013). Thus, given the link between PRL concentration and parental care (Buntin, 1996; Van Roo et al., 2003), females experiencing an increase of PRL in response to a stressful situation would be more likely to continue parental care. Assuming that females cease chick provisioning mostly because and when uni-parental care is sufficient (as suggested in Wojczulanis-Jakubas and Jakubas, 2012), the increase in PRL in response to a stressful situation may induce females to stay longer, when this is necessary for successful breeding. Indeed, the timing of brood abandonment by females seems to be flexible and is likely to depend on environmental conditions (Harding et al., 2004; Wojczulanis-Jakubas and

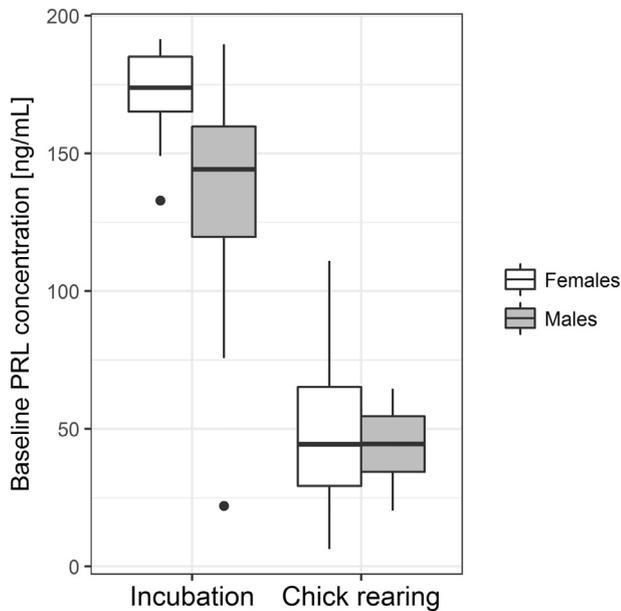


Fig. 4. Prolactin (PRL) baseline level during the incubation and chick rearing phase in little auk males and females. The bottom and top of the box are the first (Q1) and third quartiles (Q3; IQR), and the line inside the box is the median. The end of upper whisker represents $Q3 + 1.5 \text{ IQR}$, whereas the lower whisker represents $Q1 - 1.5 \text{ IQR}$, with the points above and below being outliers.

Jakubas, 2012).

The positive relationship between baseline levels of CORT and PRL and their stress responses highlights the need to interpret stress-induced levels of these hormones with respect to their baseline concentration. This may be particularly important for PRL, the baseline level of which changes considerably over the breeding season. Our results show that high baseline concentrations of both CORT and PRL are related to attenuated stress response. The increase in CORT puts the organism into a “life emergency state” (Wingfield et al., 1994). However, when the baseline CORT concentration is already high, even a small change can

trigger the desired escape behaviour. It may also be a question of the availability of a receptor binding the hormones (Lattin and Romero, 2013); hormones produced in amounts greater than the number of available receptors are ineffective.

The situation regarding PRL is even more complicated, as we found that the PRL baseline concentration affects not only the magnitude but also the direction of the stress response, i.e. an increase and/or decrease in response to the stressor. During incubation, when the baseline PRL level was five times higher than during chick rearing, its concentration dropped in response to acute stress, and increased during chick rearing, when the circulating level was relatively low. Such a differential PRL response had been reported earlier, but without any consensus being reached on its causality (reviewed in Angelier et al., 2016). The observed pattern in the little auk may be adaptive, especially during the chick rearing phase. Since the baseline level is relatively low at that time, an increase of PRL level in response to stress could have evolved to ensure that parental care continues to be provided, despite various breeding-associated stressors (the same interpretation as proposed for the sex differences, see above). The observed decrease in PRL during the incubation phase in response to stress may be a ceiling effect: the level of circulating hormone may be too high, so it decreases, since further increases do not trigger any behavioural changes. The baseline level during the incubation period could be more than enough to ensure the parental care. Thus, even if it fell, the birds would not abandon their offspring. Moreover, some studies on vertebrates have demonstrated a link between high PRL concentration and high oxidative stress [e.g. (Olavarria et al., 2010, 2012; Romero et al., 2012)]. Thus, the mechanism preventing a further increase in PRL, when it is already at a high level, could have evolved to protect parents from oxidative damage.

To summarize: we found similar CORT baseline levels in the two breeding phases, and significantly higher PRL baseline levels during the incubation than during the chick rearing phase; this is consistent with our predictions. Contrary to our expectations, however, we found differences in the stress response between the phases (PRL) and the sexes (CORT and PRL). Overall, our results suggest a similar level of physiological stress during the two breeding phases and a greater stress sensitivity to stress during the incubation period, which may be related

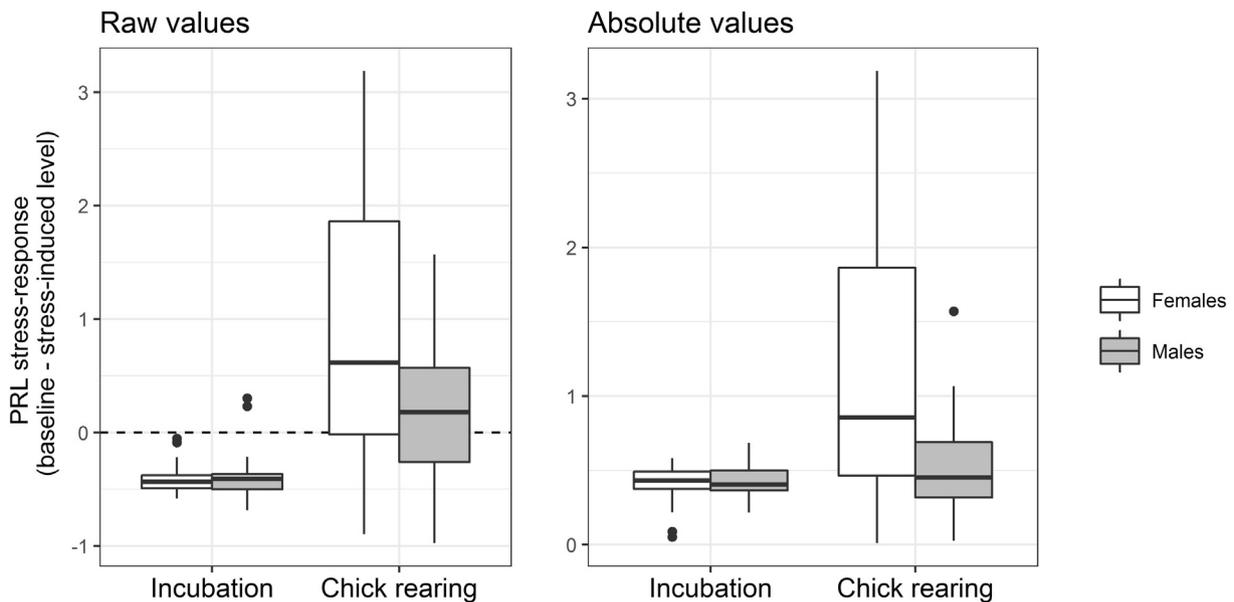


Fig. 5. Prolactin (PRL) stress response (relative change in respect to baseline level) with raw (left-hand panel) and absolute values (right-hand panel) during the incubation and chick rearing phase in little auk males and females. Note the dashed line in the left-hand panel, dividing the stress response into positive and negative values (a respective increase and decrease in the prolactin level with respect to the baseline concentration). The bottom and top of the box are the first (Q1) and third quartiles (Q3; IQR), and the line inside the box is the median. The end of upper whisker represents $Q3 + 1.5 \text{ IQR}$, whereas the lower whisker represents $Q1 - 1.5 \text{ IQR}$, with the points above and below being outliers.

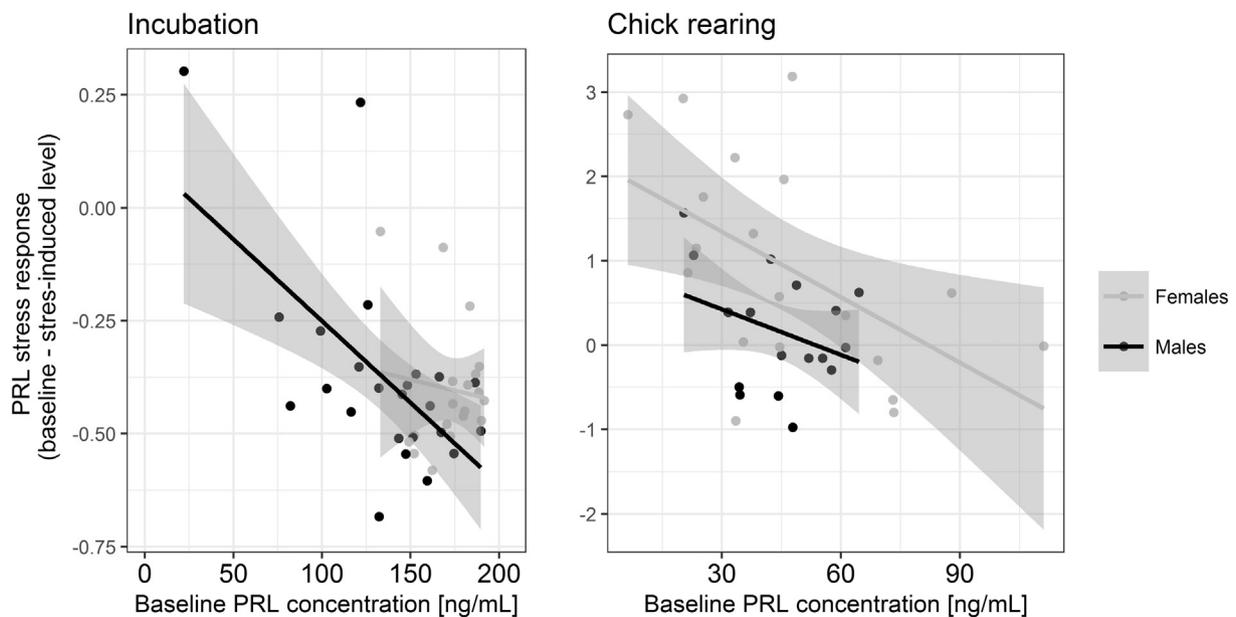


Fig. 6. Relationship between prolactin (PRL) baseline level and PRL stress response during the incubation (left-hand panel) and chick rearing phases (right-hand panel) in little auk males and females.

to the perception of the risk associated with predation pressure. The sex differences revealed in our study suggest different levels of exposure of males and females to stressors. All this, which to some extent differs from what could be expected on the basis of the literature, shows that hormonal profiles may be species-specific attributes and as such are likely to be adapted to local conditions.

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Appendix A

Table A.1
Relationship between the baseline level of corticosterone and prolactin in particular phase of breeding and sex (Pearson correlation).

Phase/sex group	r	N	P
Chick rearing 2011/female	0.09	23	0.67
Chick rearing 2011/male	0.17	21	0.43
Incubation/female	0.21	19	0.36
Incubation/male	0.24	21	0.26

Table A.2
Rank of general linear models explaining baseline corticosterone concentration.

Model rank	Intercept	Season-phase	Sex	Season-phase × sex	df	logLik	AICc	Delta AICc	Weight
1	2.211				2	−259.003	522.1	0.00	0.420
2	2.358	+			4	−257.542	523.4	1.32	0.217
3	2.227		+		3	−258.999	524.2	2.09	0.147
8	1.900	+	+	+	7	−254.659	524.3	2.19	0.141
4	2.383	+	+		5	−257.534	525.6	3.47	0.074

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