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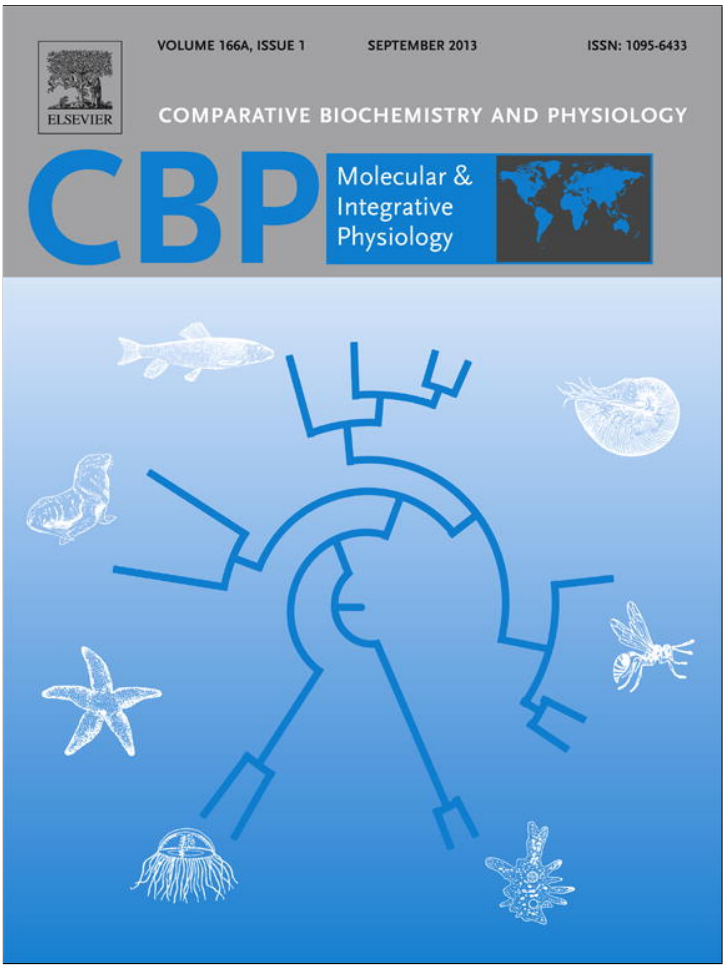

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Linking sex differences in corticosterone with individual reproductive behaviour and hatch success in two species of uniparental shorebirds

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ABSTRACT

In birds, corticosterone (CORT) appears to facilitate reproductive activity because baseline and stress-induced CORT levels are elevated in breeding individuals compared with other times of the year. In particular, CORT is lower in the sex providing most of the parental care (i.e., incubation), which could be an important adaptation to tolerate stressors that result in abandoning reproduction. Therefore, one explanation for sex differences in CORT is that lower levels are favoured during the incubation/parental phase of reproduction. Using two species of uniparental shorebird – polyandrous red phalaropes (*Phalaropus fulicarius*) and polygynous white-rumped sandpipers (*Calidris fuscicollis*) – we predicted that the incubating sex would have lower baseline and stress-induced CORT, and incubating individuals with lower CORT would more effectively defend nests against a simulated intrusion, would return more quickly afterwards, and would ultimately have higher hatch success. We found that phalaropes followed the predicted pattern: incubating individuals (males) had lower baseline and stress-induced CORT than females but for baseline CORT these differences existed prior to males commencing incubation. Incubating male phalaropes with lower baseline and stress-induced CORT returned to incubate more quickly after a disturbance and there was non-significant tendency for baseline CORT to be lower in successful nests. In sandpipers, we observed no sex differences and no significant relationships between individual CORT levels and nest defence behaviours or hatch success. Our results demonstrate that in phalaropes at least, selection favours lower baseline and stress-induced CORT during the nesting period. These results can explain sex differences in stress-induced levels of CORT, however sex differences in baseline CORT were present prior to incubation.

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1. Introduction

Glucocorticoids, such as corticosterone (CORT) in birds, mediate the behavioural and physiological responses to both unpredictable short-term (i.e., acute stressors) and predictable long-term (i.e., life history stages) challenges (Landys et al., 2006). As such, individual differences in baseline hormone expression during normal physiological conditions and acute response to stress are thought to be subject to strong selection (Breuner et al., 2008; Bonier et al., 2009).

At seasonal baseline levels, increases in CORT reflect energetic demands (Bonier et al., 2009). Elevated CORT liberates energy stores via gluconeogenesis (Remage-Healey and Romero, 2001) and modifies behaviour such as feeding rate (Wingfield et al., 1990), aggression (Kitaysky et al., 2003) and/or locomotory activity (Wingfield et al., 1998). Following exposure to an acute stressor, CORT increases rapidly from baseline levels to initiate behavioural and physiological changes that are thought to promote current survival (Sapolsky et al., 2000; Breuner et al., 2008); one such effect is to abandon parental duties (e.g., Silverin, 1986). Consequently, both baseline (Bonier et al., 2009) and acute (Breuner et al., 2008) CORT levels are expected to be linked to fitness.

CORT levels typically vary seasonally (Breuner and Orchinik, 2001) suggesting that it is adaptively regulated in response to different life history stages, such as breeding. Adrenal activity is upregulated during the breeding season (Romero and Wingfield, 1998) and as a consequence, breeding individuals tend to have a higher baseline and acute CORT response to stress (hereafter termed 'stress-induced CORT') than at other times of the year or when compared with non-breeding individuals (Astheimer et al., 1994; Romero and Wingfield, 1998; Cornelius et al., 2012).

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Sex differences in CORT are thought to reflect sex-role differences and facilitate sex-specific reproductive behaviours. Among breeding birds, males often have higher baseline and stress-induced CORT than females in species that display sexually dimorphic reproductive behaviours (Astheimer et al., 1994; Holberton and Wingfield, 2003; Meddle et al., 2003). In tree (*Spizella arborea*) and white-crowned sparrows (*Zonotrichia leucophrys gambelii*), males have higher stress-induced CORT during the preparental stage when behaviours differ between the sexes, but males were similar to females during the parental stage when both sexes fed the young (Holberton and Wingfield, 2003). These observations have led to the notion that lower stress-induced CORT in incubating birds functions to prevent nest abandonment by the incubating sex during stressful conditions (O'Reilly and Wingfield, 2001). In support of this, the sex providing more parental care has lower stress-induced CORT (males in polyandrous species and females in polygynous species) and there is no apparent sex difference when both sexes contribute evenly (O'Reilly and Wingfield, 2001; but see O'Reilly and Wingfield, 2003). Furthermore, females have weaker CORT responses in species where they provide more parental care possibly due to the value of the brood as a function of renesting opportunity during shorter breeding seasons (Bókony et al., 2009).

Because of these observations, sex differences in CORT are thought to be the result of selection for reduced CORT levels in parental birds. In support of this, breeding adult birds with naturally high levels of baseline CORT have higher abandonment rates (Groscolas et al., 2008; Spée et al., 2010), as do birds with artificially elevated CORT (Silverin, 1986; Angelier et al., 2009a; Spée et al., 2011). Yet, a natural, acute stress response may be an important part of mounting a strong and successful nest defence. Nest defence is positively related to reproductive success (Garcia, 2003; Goławski and Mitrus, 2008), and a strong nest defence is especially important for ground nesting birds where predation accounts for a significant proportion of failed nest attempts and abandonment is otherwise infrequent (Smith and Wilson, 2010). Acute glucocorticoid exposure increases aggression in a variety of animals (Wingfield and Silverin, 1986; Hayden-Hixon and Ferris, 1991; DeNardo and Licht, 1993; Haller et al., 1997; Kitaysky et al., 2003) and so the ability to mount a defence of the nest could be positively related to the magnitude of the adrenocortical response (i.e., stress-induced CORT). However, much of the work to date has been focussed on implants that cause 'short-term' effects that last hours to days, rather than an actual measure of the acute stress response.

As a means of explaining sex differences in CORT levels, we sought to link individual behaviour and hatch success with baseline and stress-induced CORT levels in two species of uniparental shorebird: polygynous white-rumped sandpipers (*Calidris fuscicollis*) and polyandrous red phalaropes (*Phalaropus fulicarius*). In polygynous sandpipers, females are the sole incubators but in phalaropes males incubate and females do not. We outline three predictions addressed by this study. Prediction 1) If baseline and stress-induced CORT levels are attenuated to facilitate incubation and prevent nest abandonment, we predict that the incubating sex (female sandpipers and male phalaropes) should have lower levels than the non-incubating sex. Prediction 2) Because CORT is expected to be related to defensive and nest guarding behaviour, we predict that individual incubators with low baseline and stress-induced CORT levels should flush less readily from nests, perform stronger nest defence displays and return more quickly to the nest following a disturbance. Prediction 3) Individuals with lower CORT levels will have higher hatch success.

2. Materials and methods

2.1. Field site and species

We examined white-rumped sandpipers (*C. fuscicollis*) and red phalaropes (*P. fulicarius*) (hereafter referred to as sandpipers and phalaropes) at East Bay Migratory Bird Sanctuary, Nunavut, Canada (N63 59 13.5 W81 41 48.3) during June–July 2008–2010. These two species are small-bodied ground nesting shorebirds. At our site, mean male and female body mass of sandpipers is 43.7 g and 42.6 g respectively, and for phalaropes 49.4 g and 57.1 g. During incubation, both species rely on crypsis to avoid nest predation until a predator approaches too closely. Once the predator has approached too closely, the two species employ slightly different tactics: sandpipers undertake rather convincing distraction displays (e.g., 'broken-wing display' or 'rodent run') with relatively few individuals leaving the nest site without producing any display. By contrast, phalaropes frequently flush without producing a display, or less frequently, perform a milder form of a broken-wing display (pers. obs.). The nesting habitats of the two species overlap completely at our field site with most individuals of both species nesting in upland *Dryas* hummocks and sedge meadows; the landscape if predominantly flat with vegetation less than 15 cm in height.

2.2. Trapping and blood collection

We trapped male sandpipers, and both male (prior to incubation) and female phalaropes during the courtship period; we were unable to capture adequate numbers of female sandpipers at this time, and most of those we did capture were carrying eggs and so considered in a physiologically distinct stage. We termed pre-incubation birds 'courtship'. We used the presence of a brood patch to determine whether male phalaropes were incubating. We caught courtship birds by stalking them while they were feeding or displaying/defending territories using a Super Talon net gun (Fly Dragon Technology Co., Ltd) (Edwards and Gilchrist, 2011). We trapped incubating female sandpipers and incubating male phalaropes using either bownets or Fundy Jerk Traps placed on the nest. These traps allow birds to come and go from the nest and are manually triggered so can be placed for longer periods of time without interrupting feeding and incubation intervals.

We caught a total of 62 white-rumped sandpipers (45 incubating females and 17 courtship males) and 112 phalaropes (38 courtship females, 37 courtship males and 36 incubating males). Both species are polygamous and courtship activities of male sandpipers and female phalaropes extend into the incubation period. Nevertheless, courtship activity declines making courtship birds more scarce and incubating birds more abundant as the season progresses. Additionally, we could not confirm that birds trapped later in the season were not in an ambiguous or confounding physiological state such as could be expected by including failed or post-breeding individuals in the study. For these reasons, trapping periods did not perfectly overlap for some stages. We trapped male sandpipers from 156 to 173 Julian Day (JD) (mean 164) and incubating females from 172 to 204 JD (mean 186). In phalaropes, females were trapped from 161 to 188 JD (mean 171), courtship males 160 to 188 JD (mean 172) and incubating males from 171 to 201 JD (mean 189).

A stopwatch was started immediately when the trap was deployed (either the net gun was shot or the nest trap triggered) and a 'baseline' blood sample was taken within 4 min, but usually in less than 3 min (90% of samples; mean baseline bleed time: 2.3 min ± 0.8 min). The bird was measured, banded and subjected to a standard stress protocol (Wingfield et al., 1992). Blood was collected in heparinised capillary tubes, and volumes drawn were typically between 50 and 100 μL per bleed as blood samples were used as part of a larger study. After 30 min (mean stress-induced bleed time after first bleed: 30.4 min ± 1.7 min) a second blood sample was taken from the other wing to reflect the 'stress-induced' value for CORT (hereafter, stress-induced CORT). There was no difference between trapping methods in the length of time it took to collect a blood sample. Blood samples were kept cool for up to 6 h at which point they were centrifuged and the plasma frozen at −20 °C until

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further analysis. Courtship males and incubating males represented different individuals. All capture and collection protocols were approved by Laurentian University Animal Care Committee and Environment Canada.

Incubating and non-incubating birds were trapped using different methods, which could influence CORT levels. For example, stalking birds could elicit a premature increase in CORT levels, even though birds did not show obvious signs of distress when doing so. To compare the effects of trapping technique, we trapped a subsample ($n = 9$) of incubating male phalaropes in 2010 while they were away from their nests using the net gun (in the same manner as courtship birds). There was no difference in CORT levels between incubating birds caught with the net gun and those caught using traps placed at the nest site ($t_{37} = 1.75$, $P = 0.2$).

2.3. Nest searching and assessing hatch success

We found nests throughout the breeding season during extensive searches of our 3×4 km study area, either by observing birds returning to the nest or by flushing incubating birds off the nest. We estimated hatch date using one of two methods in order to confirm the fate of the nest. We could confirm lay date (commencement of incubation) in nests located during the laying sequence and we used the average incubation duration for the species to predict the expected hatch date. When completed clutches were found, we floated the eggs in water to estimate the stage of incubation (Liebezeit et al., 2007). We visited nests daily 4 days prior to the estimated hatch date to account for error in our hatch estimates but increased the frequency of our visits as hatch date drew near. We used hatch success as a measure of reproductive success because chicks typically leave the nest after 24 h of hatch (Colwell, 2010) and are difficult to track once they do.

2.4. Nest defence behaviours

We measured four aspects of nest defence behaviours in response to a human approaching the nest while the birds were incubating. These trials were conducted during the second visit to the nest for all nests whose clutches were completed when found, and either the second or third visit for those nests that were initially found during the laying sequence. As a means to correct for variation in nest defence behaviours, nests were approached from upwind following a clear line of sight to the nest to ensure that the observer was in full view of the incubating bird.

First, we measured 'primary flush distance', which was the distance between the observer and the bird when it first flushed from the nest. In the 2 min after being flushed, we estimated the closest distance between the bird and the observer, termed 'secondary distance'. When the trial was complete, primary flush and secondary distances were measured by either pacing over short distances (<10 m) or by handheld GPS unit (>10 m) to points marked during the trial. Upon flushing the bird off the nest, we categorically defined their subsequent behaviour as: 1) the bird left the area without performing nest defence behaviours, 2) performed either a 'rodent-run' or 'broken-wing display', 3) performed an escalated version of the previous behaviour that included calling, 4) the bird performed short hovering flights near the observer to attract attention, in conjunction with calls and other behavioural displays. Finally, we recorded 'return time' as the length of time the bird took to return to incubate after the disturbance. To estimate return time, the observer would withdraw to about 75–100 m from the nest and observe the parent with binoculars for up to 15 min. The distance from which we observed the nest varied due to topography, and for a small number of nests we could not get accurate measurements of return time because we lost sight of the bird. We limited the observation to 15 min in case our presence prevented the bird from returning. However, all but one bird had returned before 15 min, and birds typically were no longer displaying nest defence behaviours once we were more than 30 m from the nest upon leaving the nest area.

We collected wind speed using a handheld wind metre (Dwyer Instruments, Michigan City, IN, USA). Cloud cover was categorised as either overcast including broken skies or clear, including scattered clouds. Nests were not disturbed during high winds (>30 km/h at ground level) or during precipitation. A centrally located weather station (within 3.5 km of nests) recorded the daytime low temperature.

In order to obtain blood samples that were closely linked to nest defence behaviours, we deployed traps on the nests during the nest visit where we collected the behavioural measures. We returned to trigger the traps 4–6 h after their placement to allow birds to calm after the previous visit. While traps were deployed, all birds returned to incubate and no nests were lost to predators.

2.5. Laboratory analyses

Plasma corticosterone levels were measured in duplicate with a double antibody radioimmunoassay (MP Biomedicals, Orangeburg, NY, USA) as per previously published protocols (Washburn et al., 2002). The CORT antibody has low cross-reactivity with cortisol (0.05%), deoxycorticosterone (0.34%), aldosterone (0.03%) and 17ß-E_2 (<0.01%). Briefly, the assay was conducted following the manufacturer's directions, except that the volumes of the reagents were halved and the plasma was diluted (5 μL of plasma plus 245 μL of assay buffer) for baseline samples, and (2.5 μL plasma plus 247.5 μL of assay buffer) for stress-induced samples. Intra-assay variation was 7.2%. The inter-assay variation was 15.6% ($n = 15$ assays) and the lowest point on the standard curve was 3.125 pg CORT/tube.

Male sandpipers were targeted based on their territorial behaviour or appearance (i.e., enlarged vocal sacs or small body size), or sexed in the hand by the presence of a cloacal protuberance. However, we could not be certain of the correct sex in all cases and so sex was corroborated genetically in non-incubating sandpipers following standard procedures using P2/P8 (Griffiths et al., 1998) and 2550F/2718R primers (Fridolfsson and Ellegren, 1999). DNA was extracted from blood by heating samples at 95 °C in a 5% Chelex 100 suspension and results were visualised on 3% agarose gels.

2.6. Statistical analyses

We tested for sex differences in baseline and stress-induced CORT, as well as their effect on nest defence behaviours and hatch success, using linear models. We aim to determine whether sex differences exist between males and females, and ultimately whether these can be explained by selection through incubation activities as has been previously hypothesized (O'Reilly and Wingfield, 2001). Because female phalaropes and male sandpipers do not incubate, we could make comparisons between the sexes at this stage. We tested for sex differences in CORT separately for each species, because for phalaropes we had three groups to compare between, while in sandpipers we had two. We used Tukey post hoc tests to determine differences among groups in phalaropes.

Baseline CORT was related to the time to first bleed in phalaropes ($t_{1,22} = 3.62$, $P = 0.002$) but not sandpipers ($t_{1,40} = 0.02$, $P = 0.98$) despite there being no difference in the average time of first bleed between species. The effect was still significant when using only those phalaropes sampled within 3 min ($n = 101$). The time to the second bleed was not related to stress-induced hormone values for either species. Excluding individuals with first bleed times over 3 min did not change the interpretation of the results in sex analyses, or relations between CORT and behaviours or hatch success.

We identified nine variables that could influence both baseline and stress-induced CORT levels: date of trapping, time of trapping, time to first and second bleeds (above), nest age, wind speed, cloud

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cover, daily low temperature and year. To reduce the potential number of variables included in models, we used hierarchical partitioning to highlight potential covariates that explain corticosterone levels using the hier.part package in the programme R (R Development Core Team, 2008). We also initially included assay run as a fixed effect in linear models, however, because including assay run in analyses did not change our conclusions, results are presented without assay run included. Analyses of CORT values on nest defence behaviours and hatch success were done independently for each species because hierarchical partitioning identified a different group of variables for each species.

We explored flush variables using Principal Components Analysis (PCA) to collapse the four flush variables. We square root transformed sandpiper CORT values because they were not normality distributed. In some cases outliers were still present but their exclusion did not alter the results and so they were retained for the analysis. All analyses were completed in R 2.13.1 (R Core Team, 2008).

3. Results

3.1. Hierarchical partitioning

Hierarchical partitioning revealed that time of day had substantial explanatory power for both baseline and stress-induced corticosterone levels in sandpipers and so was included in the analysis. In phalaropes, timing to the first bleed (time between capture and when the first blood sample was taken) had the greatest explanatory power of baseline corticosterone, and along with time of day and year, was included in the model with baseline values. All variables had poor explanatory power for stress-induced corticosterone in phalaropes.

3.2. Prediction 1: sex differences in CORT

We began by determining whether CORT levels differed by sex or breeding stage for our two species. There were no sex differences in baseline CORT in sandpipers (Fig. 1A, $F_{1,63} = 0.09$, $P = 0.76$) but incubating females had higher stress-induced CORT levels than courtship males (Fig. 1B, $F_{1,61} = 10.52$, $P = 0.002$). In phalaropes, males had lower baseline CORT levels in a model including elapsed time of first bleed, year and time of day (Fig. 2A, sex/breeding stage: $F_{2,107} = 9.17$, $P < 0.001$) with the most extreme difference between courtship females and incubating males. Pre-incubating males were higher but not statistically distinguishable from incubating males (Tukey: $P = 0.31$). In a reduced dataset including only the birds caught within the period of overlapping trap dates, the effect was still present but marginally significant ($F_{2,49} = 3.05$, $P = 0.056$); females had the highest levels of baseline CORT, whereas there was no distinction between courtship and incubating males. A similar pattern was present across females, courtship males and incubating males for stress-induced CORT levels (Fig. 2B, $F_{2,109} = 20.14$, $P < 0.001$) although the difference between courtship males and females was marginal (Tukey: $P = 0.06$). When we once again restricted the analysis to only those birds caught within the period of overlapping dates the pattern was similar as before ($F_{2,54} = 11.72$, $P < 0.001$), however courtship males changed to being statistically different from females but indistinguishable from incubating males (Tukey: $P = 0.20$).

3.3. Prediction 2: nest defence traits and parental CORT levels

The results from the PCA were similar for both species (Table 1). The first two components were retained for both species and PC1 explained 54.1% and 41.8% of the variation for sandpipers and phalaropes respectively and PC2 explained 25.7 and 30.7. Flush distance, secondary distance and behaviour loaded heavily on PC1 such that birds with high scores were those with a close flush distance, a close secondary distance and a strong behavioural display. For both species, PC2 was composed primarily of return time but for REPH flush distance also contributed. Thus, a bird's tendency to return quickly to the nest following a disturbance was independent of the intensity to which it defended its nest.

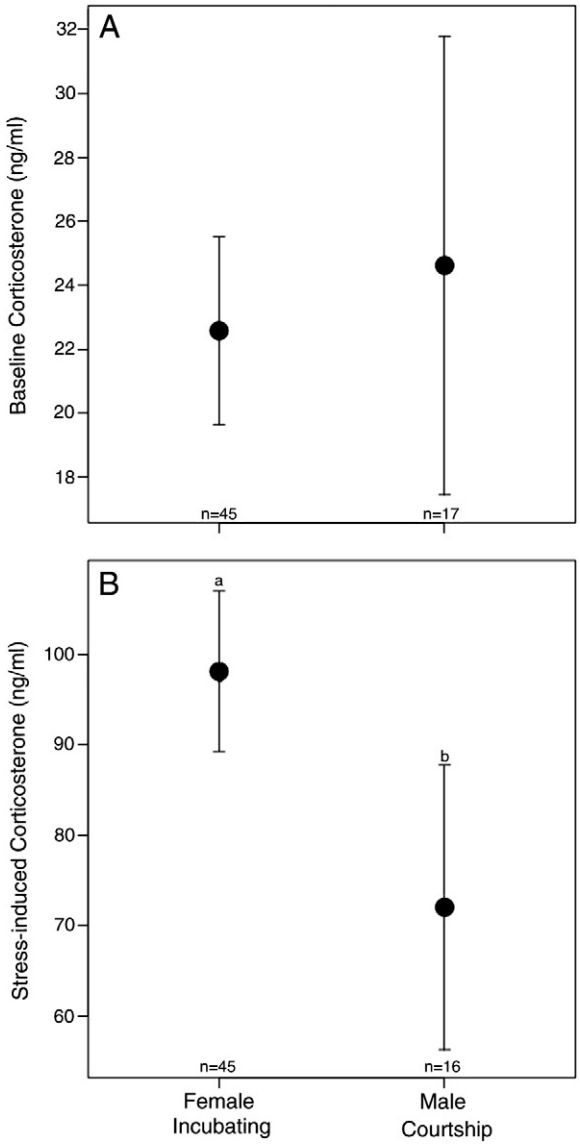

Fig. 1. Sex differences in baseline (A) and stress-induced (B) corticosterone levels among breeding male and incubating female white-rumped sandpipers. Males were trapped during courtship and females were trapped while incubating. Lower case letters denote significant differences ($\alpha = 0.05$) using ANOVA. Bars represent 95% confidence limits.

PC1, composed of close flush distance, close secondary distance and elevated defensive behaviours, was not related to baseline or stress-induced CORT in either species (Table 2). PC2, composed primarily of return time, was positively related, albeit not significantly so ($P = 0.09$), to baseline CORT in female sandpipers (Table 2). In male phalaropes, PC2 was positively related to both baseline and stress-induced CORT (Table 2).

Because CORT can be related to defence behaviours through body condition, we repeated these models including body mass and tarsus length as factors. The significant factors in the previous models remained significant with the inclusion of these terms suggesting that individual differences in condition contributed little to the effect. In sandpipers, the relationship between CORT and body condition, which we interpreted as the partial r of body mass from the previous models, was negligible for baseline ($r_{partial} = 0.09$) and stress-induced

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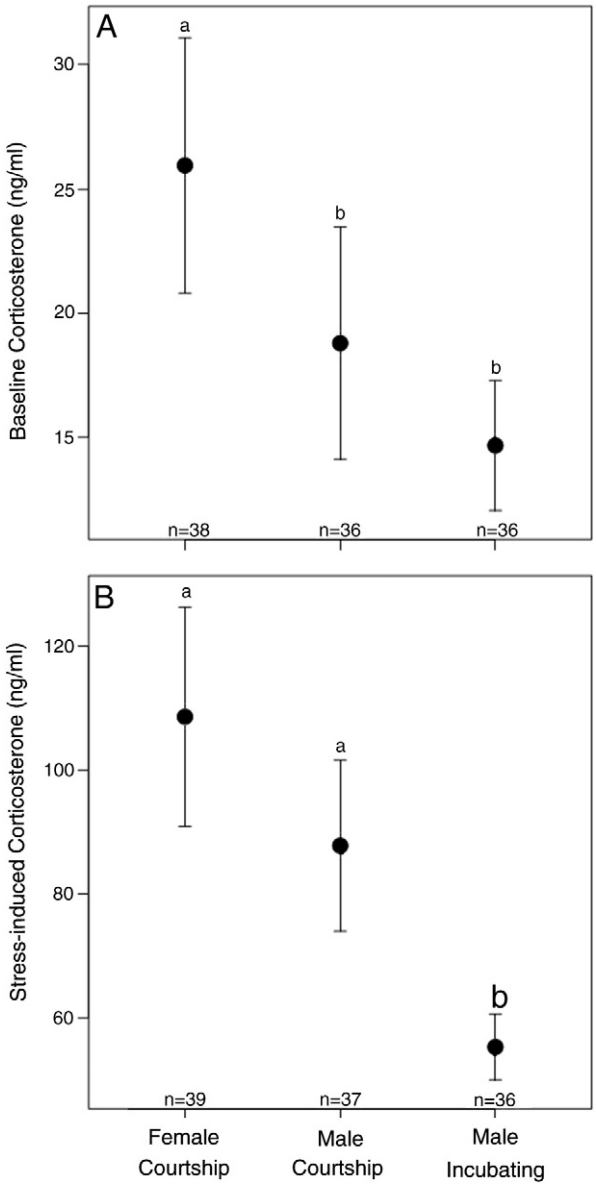

Fig. 2. Sex differences in baseline (A) and stress-induced (B) corticosterone levels among female red phalaropes and males caught during courtship and incubation stages. Lower case letters denote significant differences ($\alpha = 0.05$) using ANOVA. Bars represent 95% confidence limits.

CORT ($r_{partial} = -0.01$). In phalaropes, the relationships were stronger for both baseline ($r_{partial} = -0.22$) and stress-induced CORT ($r_{partial} = 0.25$).

3.4. Prediction 3: hatch success and parental CORT levels

Hatching success was not related to either baseline or stress-induced CORT levels in sandpipers (Table 2, Fig. 3). In phalaropes, there was a non-significant tendency for both baseline and stress-induced CORT to be lower in successful phalaropes (Table 2, Fig. 3). To determine whether our inability to detect an effect in phalaropes was the result of low statistical power, we conducted a post hoc power analysis on the main effect (hatch success) using parameters specified from the previous linear models. Statistical power was low to detect an effect in baseline CORT (power = 0.45; recommended level = 0.80) and poor for stress-induced CORT (power = 0.16).

Table 1
Loadings for nest defence behaviours from principal component analysis.

	WRSA		REPH	
	PC1	PC2	PC1	PC2
Flush distance	0.57	−0.09	0.49	−0.48
Secondary distance	0.60	0.11	0.61	0.09
Behaviour	−0.55	0.26	−0.62	−0.31
Return time	0.13	0.96	−0.01	0.82

4. Discussion

We noted marked species differences in the relationships among CORT and sex and nesting behaviours despite both species being sampled under the same environmental conditions and over the same period of time. Two predictions were supported in phalaropes (where males incubate; Table 3) with weak support for the third, while none were supported in sandpipers (in which females incubate).

4.1. Prediction 1: sex differences in CORT

Because selection should favour tolerance to stressors during incubation, we sought to establish whether the incubating sex had lower CORT levels in our two species. We expected baseline and stress-induced CORT levels to be lower in female sandpipers and male phalaropes. In sandpipers, we could not compare directly a change within females from the courtship period to incubation because we were not able to catch an adequate number of females during the courtship period. However, if CORT levels are attenuated during incubation, we should see the most distinct differences between courtship males and incubating females. However, this was not the case for baseline CORT, and incubating females actually had higher stress-induced CORT. This result is similar to another species in the same genus, western sandpipers (*Calidris mauri*), where males and females share incubation duties (O'Reilly and Wingfield, 2001; O'Reilly and Wingfield, 2003). In fact, the actual baseline and stress-induced CORT values for male and female white-rumped sandpipers we report in our study are very similar to the monogamous western sandpiper (O'Reilly and Wingfield, 2001). Interestingly, female western sandpipers are known to abandon incubation duties frequently compared with males (referenced in Myers et al., 1982), but it is not known whether this abandonment is related to stressors during incubation, or possibly to seek additional mating opportunities as reported in other sandpiper species (Thomas et al., 2007). Regardless, the high acute stress response of female white-rumped sandpipers in our study does not appear to result in increased abandonment since at our site sandpipers and phalaropes are similar in hatch success.

In phalaropes, females had higher baseline and stress-induced CORT than males. Baseline CORT was higher in female phalaropes possibly due to high energetic demands placed on polyandrous females, such as producing multiple clutches in a short period of time (Ross, 1979; Liker et al., 2001). Baseline levels were similar in courtship and incubating male phalaropes suggesting that the two stages are similar in their energetic requirements, otherwise we might expect the more energy intensive stage to have higher CORT (Landys et al., 2006). Using the complete dataset, incubating male phalaropes had the lowest stress-induced CORT levels and the difference between courtship males and females approached significance. However, when we used a subset of the individuals from each stage trapped over a similar time period, females had higher levels than males at either stage. In the smaller dataset, incubating males had lower levels than courtship males but the difference was no longer significant. Therefore, we demonstrate that sex differences in both baseline and stress-induced CORT are present prior to incubation beginning.

We found that in both species, females had higher stress-induced CORT than males, which is contrary to our predictions for sandpipers

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Table 2
Results of linear models used to test for relationships between baseline and stress-induced corticosterone levels and reproductive variables. Nest defence behaviours were included in models as principal component scores (PC; see Table 1).

Species	n		Model		SE	t	P
White-rumped sandpiper	39	Baseline	*PC1 + PC2 + Capturetime*	PC1	0.12	0.82	0.42
				PC2	0.18	2.00	**0.05**
	32		*Hatchsuccess + Capturetime*	Hatch	0.54	−0.15	0.88
	39	Stress-induced	*PC1 + PC2 + Capturetime*	PC1	0.18	0.27	0.79
				PC2	0.25	−1.03	0.31
	32		*Hatchsuccess + Capturetime*	Hatch	0.68	−0.42	0.68
Red phalarope	17	Baseline	*PC1 + PC2 + Bleedtime + Capturetime + Yr*	PC1	0.96	−0.15	0.88
				PC2	0.92	3.29	**0.007**
	17		*Hatchsuccess + Bleedtime + Capturetime + Yr*	Hatch	2.48	−1.99	0.07
	18	Stress-induced	*PC1 + PC2*	PC1	2.73	0.52	0.61
				PC2	2.60	2.16	**0.05**
	18		*Hatchsuccess*	Hatch	5.68	−1.28	0.22

Bold data indicates two-sided tests are significant at $\alpha = 0.05$.

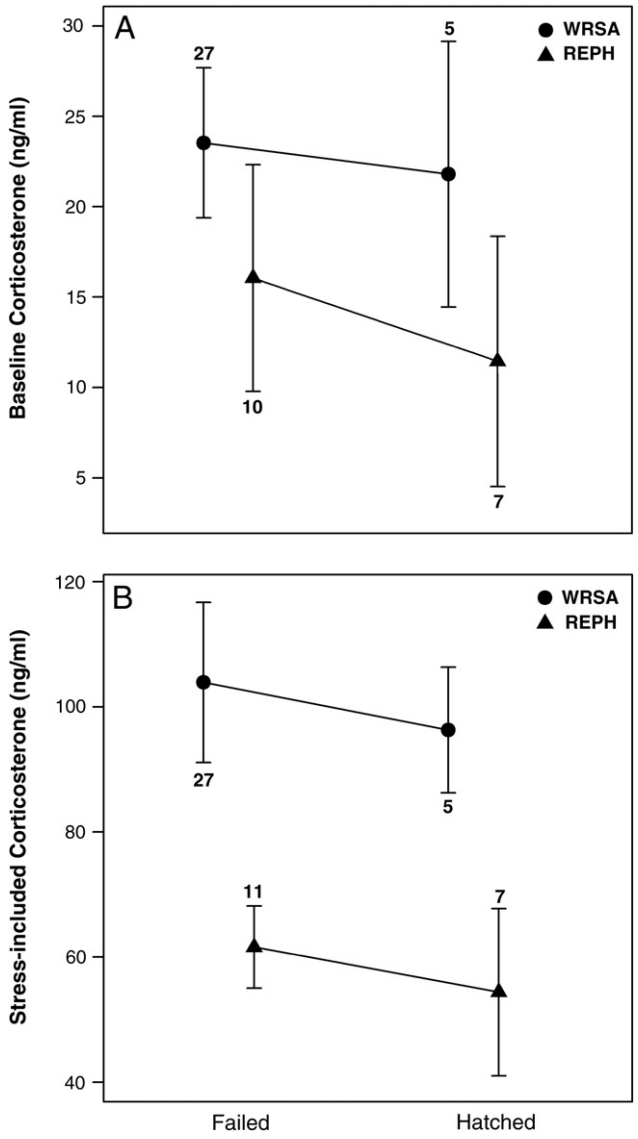

Fig. 3. Baseline (A) and stress-induced (B) CORT levels in incubating white-rumped sandpipers (WRSA, circles) and red phalaropes (REPH, triangles) with regard to nest fate. The relationship was not significant (ANOVA) for baseline CORT although there was a non-significant tendency for CORT to be lower phalaropes that incubated nests that hatched successfully. Stress-induced levels were higher in failed nesters for both species. Bars represent 95% confidence limits and sample sizes are included for each group.

and contrary to studies of species where sex roles differ (passerines: Astheimer et al., 1994; Holberton and Wingfield, 2003; Meddle et al., 2003). In pectoral sandpipers (*Calidris melanotus*), a polygynous shorebird where males do not incubate, males had higher stress-induced CORT than females (O'Reilly and Wingfield, 2001). Given the close similarities in breeding behaviour between pectoral and white-rumped sandpipers, it is not clear what aspect of white-rumped sandpiper breeding biology could account for lower stress-induced CORT levels in males.

There have been two important observations suggesting that a dampened stress response may be important for tolerating stressors that could ultimately reduce reproductive success. The most obvious is the observation that the incubating sex has a lower CORT response (O'Reilly and Wingfield, 2001). Another is that birds breeding at high latitudes have lower acute CORT, possibly resulting from the need to deal with more extreme weather at northern latitudes (Silverin et al., 1997; Silverin and Wingfield, 1998; O'Reilly and Wingfield, 2001). However, an alternative explanation for these sex differences, which is not mutually exclusive, is that CORT levels may be higher in both sexes in the pre-incubating period or solely in the sex competing for mates as a result of higher reproductive-related stress stemming from shortened breeding seasons at higher latitudes. Indeed, northern species also tend to have higher testosterone levels (Goymann et al., 2004; Garamszegi et al., 2008). Increased reproductive effort can be accompanied by severe stress-related physiological changes in a variety of animals (e.g., mammals: Boonstra et al., 2001; birds: Greives et al., 2007), and territorial defence and courtship in birds are themselves energetically costly (Barnett and Briskie, 2007; Lynn et al., 2010). Because CORT levels are typically higher during the breeding season compared with other stages during the year (Astheimer et al., 1994; Romero et al., 1997; Romero and Wingfield, 1998), higher levels typically found in the competing sex may reflect an adaptive mechanism to deal with stressful and demanding breeding requirements. In phalaropes, the differences in mean values of baseline and stress-induced CORT between females and courtship males, and between courtship males and incubating males suggest that sex differences are due to both conditions facing courtship birds (i.e., polyandrous females), as well as moderate reductions in incubating males. However, the results from sandpipers contradict either mechanism and suggest that these are not general mechanisms.

4.2. Predictions 2 and 3: nest defence traits, CORT and hatch success

The patterns detected in male phalaropes demonstrate that stress-induced CORT levels are lower among incubating birds. We expect these sex differences to come about via selection for individual phenotypes that facilitate nest defence and prevent nest abandonment.

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Table 3
Summary of the results highlighting whether the predictions were supported for baseline/stress-induced CORT. With similar predictions for both baseline and stress-induced CORT, we predicted that [1] the incubating sex has lower CORT, [2] individuals with lower CORT have stronger nest defence, and [3] individuals with lower CORT have higher hatch success. For the purposes of this table, we use only time to return to the nest (PC2) as our measure of nest defence, since PC1 did not support any of our predictions (see text).

Species	Incubating sex	Prediction 1		Prediction 2		Prediction 3	
		Baseline	Stress-induced	Baseline	Stress-induced	Baseline	Stress-induced
White-rumped sandpiper	Female	No	No	No	No	No	No
Red phalarope	Male	Partial	Yes	Yes	Yes	Weak	No

In phalaropes, the strength of nest defence (PC1) was not related to CORT levels (Table 2). However, male phalaropes with lower baseline and stress-induced CORT levels returned more quickly to the nest following a disturbance (PC2). No such relationships were found in sandpipers.

We failed to detect any significant effects of CORT on hatch success. In phalaropes, there were clear tendencies for successful phalaropes to have both lower baseline and stress-induced CORT but our ability to detect a statistically significant effect was hindered by a lack of statistical power. Not only did we have a relatively small sample size, but the sample was also biased towards successful nests. Hatch success for phalaropes was 16% at our site over the years of this study (unpublished data) yet successful nests comprised about 40% of our sample because nests failed faster than they could be trapped. Thus, our sample under-represents failed nests, nests of individuals expected to have the highest CORT levels.

Interestingly, the nest defence variables segregated into two principal components that were similar for both species. The first principal component was comprised of flush distance, secondary distance, and behavioural nest defence while the second component was comprised solely of the time taken to return to the nest following a disturbance. In other words, we found that an individual's tendency to perform a strong nest defence display was not related to nest attendance.

Nest defence behaviours are good candidates to be related to CORT levels because they result in increased energetic expenditure, and involve elements of aggressive confrontation and boldness, all of which are related to CORT and the adrenocortical stress response (Wingfield and Silverin, 1986; Hayden-Hixon and Ferris, 1991; DeNardo and Licht, 1993; Haller et al., 1997; Landys et al., 2006; Atwell et al., 2012). While nest defence displays (as in PC1 in the present study) improve reproductive success (e.g., Garcia, 2003; Goławski and Mitrus, 2008), our results suggest that tolerance of the approach of a threat, the intensity of the observed nest defence display and the distance the bird remained from the threat are not related to the adrenocortical axis (Table 2). Consequently, these behaviours could be mediated through hormones other than CORT. For example, prolactin is responsible for eliciting parental behaviours (Buntin, 1996) and in particular has been linked to nest attendance and rates of nest desertion (Groscolas et al., 2008; Angelier et al., 2009a). That CORT and prolactin levels can vary independently of each other (Angelier et al., 2009b) suggests that they could have independent effects on breeding behaviour in some species.

A number of studies have shown that artificially elevated levels of CORT or naturally high baseline CORT results in nest abandonment and reduced reproductive success in birds (Silverin, 1986; Love et al., 2004; Groscolas et al., 2008; Angelier et al., 2009a; Spée et al., 2010, 2011). In a number of studies, birds implanted with CORT had hormonal and behavioural effects lasting days, yet in some cases these changes have been interpreted as demonstrating acute changes in CORT (Breuner et al., 2008). Acute increases in CORT elicit physiological changes in the span of min (Landys et al., 2006) and acute changes can have different or even the opposite effects to chronic increases (aggression: see above; energy mobilisation/fat deposition: McEwen, 2004; immunity: Dhabhar and McEwen, 1997; Martin, 2009). To our knowledge, there are few studies of the adaptive significance of the natural acute stress response with respect to breeding activity measured at the level of the individual. With that said, our results agree with the studies mentioned above: phalaropes with lower CORT returned to the nest more quickly following a disturbance and lower stress-induced CORT was related to higher hatch success in both species.

5. Conclusion

Based on our results, CORT appears to play a role in regulating reproductive-based traits in phalaropes, but not sandpipers. However, we provided only partial support for the idea that sex differences in CORT are related to parental role. Despite both species being exposed to the same environmental conditions (i.e., weather and predation risk), female sandpipers did not conform to our predictions (Table 3). In phalaropes, females generally had higher CORT than males at either the courtship or incubating stage, suggesting that sex differences are not solely due to selection placed upon incubators. We suggest that elevated CORT in the sex competing for mates could play a permissive role in reproductive behaviours while facilitating physiological changes required during this time. However, we found evidence for a decline in CORT from the courtship to incubation stage in male phalaropes, which can be explained by individuals with lower CORT returning to incubate more quickly after a disturbance. In phalaropes, we found a general concordance in how baseline and stress-induced CORT related to our three predictions. More study is needed to understand what environmental or physiological factors account for species differences under similar conditions, because our results suggest that CORT is not regulating the reproductive behaviours of sandpipers.

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