

Context dependency of baseline glucocorticoids as indicators of individual quality in a capital breeder



Kim Jaatinen^{a,*}, Martin W. Seltmann^b, Tuula Hollmén^{c,d}, Shannon Atkinson^{c,e}, Kendall Mashburn^{c,e}, Markus Öst^b

^a Evolution, Ecology & Genetics, Research School of Biology, ANU College of Medicine, Biology & Environment, The Australian National University, Canberra ACT 0200, Australia

^b ARONIA Coastal Zone Research Team, Åbo Akademi University & Novia University of Applied Sciences, Finland

^c University of Alaska Fairbanks, School of Fisheries and Ocean Sciences, Institute of Marine Science, United States

^d Alaska Sealife Center, Seward, United States

^e University of Alaska Fairbanks, School of Fisheries and Ocean Sciences, Fisheries Division, Juneau, United States

ARTICLE INFO

Article history:

Received 5 March 2013

Revised 17 June 2013

Accepted 23 June 2013

Available online 10 July 2013

Keywords:

Body temperature

CORT-fitness hypothesis

Individual quality

Context-dependent CORT response

Baseline CORT

Somateria mollissima

ABSTRACT

Identifying markers of individual quality is a central goal of life-history theory and conservation biology. The 'corticosterone (CORT)-fitness hypothesis' postulates that low fitness signals impaired ability to cope with the environment, resulting in elevated baseline CORT levels. CORT can, however, be negatively, positively or neutrally related to fitness, depending on the context. In order to clarify this controversial issue, we elucidate the utility of using baseline CORT as a correlate of individual fitness in incubating female eiders across variable environments. An increase in serum CORT with decreasing body condition was evident in older, more experienced breeders, while increased clutch mass was associated with elevated serum CORT in females breeding late in the season. For faecal CORT, the expected negative association with body condition was observed only in early breeders. We found a strong increase in faecal CORT with increasing baseline body temperature, indicating the utility of body temperature as a complementary stress indicator. Females in good body condition had a lower baseline body temperature, but this effect was only observed on open islands, a harsher breeding habitat less buffered against weather variability. Females with higher reproductive investment also maintained a lower baseline body temperature. Nest success strongly decreased with increasing serum and faecal CORT concentrations, and individual stress hormone and body temperature profiles were repeatable over years. Although our data support the tenet that baseline CORT is negatively related to fitness, the complex context-dependent effects call for cautious interpretation of relationships between stress physiology and phenotypic quality.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Identifying markers that quantify individual performance in the face of environmental challenges is a central goal of life-history theory and conservation biology (e.g., Cockrem, 2005). Physiological, especially endocrine, mechanisms may serve as such markers, and these mechanisms also show promise in mediating life-history trade-offs between survival and reproduction (reviewed in Crespi et al., 2013). Glucocorticoid (GC) hormones, such as corticosterone (CORT), may play a particularly important role in this regard, as they regulate the allotment of energy between reproduction and survival (Bókonyi et al., 2009). Furthermore, as GC concentrations show high individual variation (e.g., Cockrem and Silverin, 2002) that is partly heritable (Evans et al., 2006), and affects survival

and reproductive performance (e.g., Romero and Wikelski, 2001), they are likely to be a target of selection.

The 'corticosterone-fitness hypothesis' postulates that, all other things being equal, individuals with low predicted reproductive output or survival should exhibit elevated baseline CORT levels as a result of their impaired ability to cope with their environment (Bonier et al., 2009a). Although backed up by a fair amount of empirical support in the literature (e.g., Sheriff et al., 2009), it is becoming increasingly clear that this hypothesis is overly simplistic because, in reality, all other things are seldom equal and CORT can in fact be negatively, positively or neutrally related to fitness, depending on the context (Crespi et al., 2013). For example, the relationship between GCs and fitness may change within individuals in different phases of their annual cycle, particularly during the transition from non-breeding to a reproductive state (Bonier et al., 2009b; Ouyang et al., 2011). Thus, heavy investment in reproduction may concomitantly increase both reproductive success (i.e., fitness) and CORT levels, which forms the basic tenet of the

* Corresponding author.

E-mail address: kim.jaatinen@gmail.com (K. Jaatinen).

'CORT-adaptation hypothesis' (Bonier et al., 2009a). Indeed, there is growing evidence that elevated GC levels may facilitate rather than interfere with reproductive investment (e.g., Crossin et al., 2012), spurring controversy as to the interpretation of the relationship between stress and fitness on the individual level (Bonier et al., 2010; Dingemanse et al., 2010).

Our current understanding of stress-response mechanisms is also hampered by a failure to account for other intrinsic and external factors affecting both life histories and the secretion of GCs by the hypothalamic–pituitary–adrenal (HPA) axis (Crespi et al., 2013). The relationship between CORT and fitness should preferably be measured at different times (i.e., in multiple reproductive seasons) and/or across different habitats consistently differing in their perceived harshness. So far this has mainly been attempted with across-species comparisons between distinct environmental types (e.g., Wada et al., 2006; Hau et al., 2010), whereas comparisons within a species are largely lacking (Crespi et al., 2013). This is unfortunate, because the relationship between individual quality and stress resistance is only observable at the within-species level, once potential interactions between individual quality and the environment are taken into account (D'Alba et al., 2011). For example, the ability to cope with stressors may decline with decreasing individual quality, but this relationship may only be evident in harsh environments (Charmantier and Garant, 2005; Descamps et al., 2009; Robert et al., 2012).

Recently, Crespi et al. (2013) called for more longitudinal studies measuring effects of GCs on multiple life-history traits in different environmental contexts. To heed this call we here shed light on the utility of baseline CORT as a correlate of individual fitness across variable environments. We do this in incubating eider females (*Somateria mollissima*), which are especially suited for elucidating potential connections between individual quality, CORT and fitness because they are long-lived capital breeders which rely almost entirely on bodily reserves during incubation, losing up to 40% of their pre-laying body weight (e.g., Parker and Holm, 1990). Individual differences in body condition are likely to be pronounced during incubation because there is substantial variation in both energy reserves at incubation onset and weight loss during incubation (Hanssen et al., 2002; Öst et al., 2008b), and females cannot compensate for such differences due to complete breeding anorexia. Previous studies indicate that breeding success is largely determined by body condition, breeding experience and timing of breeding (Lehikoinen et al., 2010; Öst and Steele, 2010), wherefore these traits can be regarded as correlates of individual quality in eiders (Jaatinen and Öst, 2011). The fact that females do not replenish their energy reserves during incubation facilitates direct comparison of CORT secretion and fitness between individuals nesting in vicinity of one another. Furthermore, eiders breed in two contrasting habitat types, open and forested islands, of which open islands are more exposed to weather extremes (Kilpi and Lindström, 1997) and predation risk (Ekroos et al., 2012).

We first examined links between individual quality and baseline CORT levels in blood and faeces, using multiple correlates of individual quality (breeding experience, body condition, hatch date, clutch mass). Because high CORT levels may be associated with a rise in body temperature (e.g., Cabanac and Guillemette, 2001), we also included female baseline body temperature during incubation in the analyses. We predicted that elevated baseline CORT should be found in individuals of low quality. Next, we explored whether the relationship between baseline body temperature and individual quality was modulated in the two habitats contrasting in their degree of thermal protection. We predicted that low-quality individuals would be disproportionately affected by a challenging thermal environment, reflected in concomitant changes in their baseline body temperature. Third, we tested whether baseline CORT levels were repeatable, a prerequisite for

linking baseline CORT to a fitness component (Bonier et al., 2009a; Angelier et al., 2010), and we also assessed whether baseline body temperature was repeatable within individuals over years. Finally, we determined the relationship between CORT and fitness, in terms of nest success, controlling for breeding experience, body condition and hatch date. Under the CORT-fitness hypothesis, we expected a negative correlation between baseline CORT and reproductive success, and we further expected the relationship to be consistent for both of our CORT measures, since different stress measures should positively co-vary (Crespi et al., 2013).

2. Methods

2.1. Field methods

This study was conducted at Tvärminne (59°50'N, 23°15'E), western Gulf of Finland, in 2009–2011. We also utilized data on faecal CORT concentrations from 2008 in the corresponding repeatability analysis to improve statistical power. In this study area, females nest on small open islands or on larger forested islands. Females were captured during nesting by using hand nets. Upon capture, females were weighed, measured for structural size (length of the radius-ulna) and ringed with a standard metal ring. We calculated the number of years since the bird was first ringed and used this as a minimum estimate of years of maternal experience (Öst et al., 2008a; Öst and Steele, 2010; Jaatinen and Öst, 2011). This is a reasonably accurate indicator of breeding experience because females are highly philopatric to their breeding islands (Öst et al., 2011), more than half of the successfully breeding females on our study islands are trapped annually (Jaatinen and Öst, 2011), and annual trapping effort has been similar since 1996. The incubation stage was estimated using an egg flotation test, which gives an estimate of how long a female has incubated her eggs (Kilpi and Lindström, 1997). Female eiders included in the current study incubated an average (\pm SD) of 27.21 (\pm 2.18, $n = 286$) days, and because not all females managed to hatch their clutches successfully, hatch date was therefore calculated by adding 27 days to the date of the flotation test and subtracting the number of days the female had incubated. Clutch masses were made comparable between females by measuring total clutch mass and correcting for incubation stage, as eggs become lighter as the incubation progresses. This was done by calculating the raw residuals (in grams) from a linear regression of clutch mass on incubation stage. Clutch mass is a good measurement of reproductive investment in this capital breeding species, as all eggs are produced from stored reserves (Parker and Holm, 1990), and because the frequency of conspecific brood parasitism is low in this population (ca. 6% of eggs are of non-natal origin; Waldeck et al., 2004).

Trapping took place predominantly during the end of the incubation period in late May to minimize nest desertion. The time spent on each island during a bout of female capture was held to a minimum to decrease disturbance. During each capturing session on an island, we recorded the time elapsed between the first female being either flushed or caught off her nest and the capture of each female. This variable (hereafter capture timing; average [\pm SD] = 29.88 [\pm 26.78] min, $n = 535$) was used to control for any rise in baseline CORT levels due to females potentially seeing us capture other females at a distance. Potential researcher-induced nest desertion is largely restricted to the early phases of incubation, whereas the frequency of nest visits does not influence the probability of nest desertion, provided that the first visit is timed to the later phases of incubation (Bolduc and Guillemette, 2003). A detailed account of the ethics involved in female capture is

presented in Kilpi et al. (2001), and female handling procedures were approved by the Animal Experiment Board/State Provincial Office of Southern Finland, number ESLH-2009-02969/Ym-23, and Tvärminne zoological station.

Nest success was determined upon visiting the nest to capture the ducklings for other purposes than this study. If the brood had hatched and left the nest prior to our arrival, and the nest was empty, we could tell successful hatching from predation by the condition of the eggs shells remaining. Hatched eggs have an intact leathery membrane while predated eggs are gone or leave shattered shells with the membrane, usually bloody, still attached to the shells (Öst and Steele, 2010).

Based on incubation stage, we estimated female body condition for all trapped females ($n = 482$, range: 156–167 females annually), provided that they had incubated their eggs for > 8 days (egg laying may otherwise still be in progress; Öst et al., 2008b). This was done by regressing the log-transformed projected weight at hatching against the log-transformed radius-ulna length and using the standardized residuals as a condition index (data from all years were pooled to obtain a global index). The projected weight at hatching was obtained by subtracting the expected weight loss (by calculating the expected number of days before hatching, based on incubation stage) from the body weight measured at capture. We caught females at different incubation stages, which enabled us to quantify average weight loss by regressing the logarithm of body weight against the logarithm of incubation time and projected hatching date (Öst et al., 2008b).

We measured baseline CORT concentrations from blood samples taken within 3 min of capture. The blood samples were obtained by extracting approximately 1 ml of blood from the brachial vein, and the time elapsed between capture and blood sampling (hereafter: sampling lag; average [\pm SD] = 2.74 [\pm 0.55] min, $n = 495$) monitored with a stopwatch. Faecal samples were collected in Whirl-Paks (Nasco) directly from the female or by gathering faeces from the nest. Blood and faecal samples were immediately stored on ice in a cool box and transported to the laboratory within 2–4 h. The blood samples were centrifuged at 1500g for 10 min to separate the serum. The serum was then transferred into 1.5 ml reaction tubes and stored frozen at -20°C until analysis. Immediately after blood sampling, the body temperature was measured from the rectum of the females using a digital veterinary thermometer to the nearest 0.1°C . This variable was only measured for a fraction of females in 2009 (28%, 47 out of 165 females), and from all females during 2010 and 2011, wherefore sample sizes differ slightly for this variable.

2.2. Corticosterone radioimmunoassay (RIA)

A validation of the serum corticosterone RIA kit (ImmuChem™ Double Antibody, Corticosterone, 125I RIA Kit, MP Biomedicals, Orangeburg, NY) for eiders is provided by Nilsson (2004). A double antibody RIA kit (ImmuChem™ Double Antibody, Corticosterone, 125I RIA Kit, MP Biomedicals, Orangeburg, NY) for corticosterone was validated for use with extracted faeces of European eiders. Ten randomly chosen faecal extracts of 100 μl were chosen to serve as a pool. The pool was serially diluted from neat to 1:256 in assay buffer provided by the manufacturer. Serial dilutions of faecal extract for female eiders (1:2–1:256) yielded displacement parallel to the standard curve and, as a dilution of 1:8 exhibited binding of ~59%, all subsequent individual samples were run at this dilution. Dilutions of individual samples were adjusted for concentration if sample concentrations fell above or below acceptable limits (below 20% and above 80% binding on the standard curve) and re-run. Radioactivity of the bound portion was determined using a gamma counter (Gamma C12, Diagnostic Products, Los Angeles, CA). The recovery rate of 3H-labeled corticosterone added to faecal

sample pools (\pm SD) was $78 \pm 10\%$. Intra-assay coefficients of variation were less than 10%. Average inter-assay coefficient of variation was 22.33%. The mean serum assay sensitivity was 13.1 ng/ml (Seltmann et al., 2012) and mean faeces assay sensitivity was 13.4 ng/ml. All reported concentrations were within the detection limit. Manufacturer cross-reactivity with other steroids were: desoxycorticosterone (0.34%), testosterone (0.10%), cortisol (0.05%), aldosterone (0.03%), progesterone (0.02%), and less than 0.01% for all other steroids tested. RIA values were corrected for dilution and extraction efficiency and expressed as ng/ml. The RIAs were performed according to the manufacturer's instructions with the exception that all volumes were halved (cf. Mashburn and Atkinson, 2004). Average (\pm SD) cort levels in blood and faeces were found to be 33.81 ± 28.34 ng/ml and 167.81 ± 124.16 ng/ml, respectively.

2.3. Data analysis

Because the variables breeding experience, body condition, hatch date and clutch mass may all represent interchangeable measures of individual quality, we calculated the variance inflation factors (VIF) for these variables. All VIFs were found to be below 1.11, indicating that no collinearity was present, thus allowing the variables to be present in the same models.

To test the connection between individual quality, baseline body temperature and our two baseline CORT measures, we constructed two LMMs where body condition, hatch date, breeding experience, reproductive investment (clutch mass corrected for incubation stage), and body temperature explained log-transformed baseline CORT (CORT0) and log-transformed faecal CORT (FC) concentrations, respectively. In the model explaining CORT0, we controlled for the effects of sampling lag, capture timing, incubation stage and year by including these variables as covariates in the model. In the model explaining FC, we controlled for the effects of incubation stage and year, while the variables sampling lag and capture timing were excluded. Inclusion of the two latter variables was unnecessary since their effects will not be immediately detectable in female faecal CORT levels, which represent a longer-term cumulative index of stress (Möstl et al., 2005). Both models contained all two-way interactions between body condition, hatch date, breeding experience, reproductive investment and baseline body temperature. Female identity was included as a random effect in both models to control for repeated observations of individual females and were fitted using restricted maximum likelihood (REML) estimation.

Next, we explored how breeding habitat type and individual quality jointly influenced the ability to withstand heat stress, as a preliminary analysis showed that FC levels were strongly positively correlated with baseline body temperatures. To this end, we constructed a linear mixed model (LMM) where female baseline body temperature was explained body condition, hatch date, breeding experience, reproductive investment and nesting habitat type (open or forested islands), while controlling for the effects of sampling lag, capture timing, incubation stage and year. Because baseline body temperature was measured immediately after taking CORT0 blood samples, any variation in the time between capturing the female and measuring body temperature will be due to variation in sampling lag. Therefore, the variable 'sampling lag', related to the timing of CORT0 sampling, is also applicable for controlling potential handling-induced temperature increase (cf. Cabanac and Guillemette, 2001). The model contained all two-way interactions between reproductive investment (clutch mass), body condition, hatch date, breeding experience and habitat type. The model was fitted using REML estimation and had female identity as a random effect to control for repeated observations of individual females.

Repeatabilities of CORT0, FC and baseline body temperature were analysed using the rpt.remlmm functions from the R software package rptR (Nakagawa and Schielzeth, 2010). To explore the connection between CORT0 and fitness and FC and fitness, we constructed a generalized linear mixed model (GLMM) with a binomial error distribution, where nest success (at least one egg hatched vs. no eggs hatched), was explained by CORT0 and FC, respectively. The variables hatch date, breeding experience and body condition were added to the model as well, since these variables are known to affect reproductive success in birds. Also all two-way interactions were included in the model and it was fitted using Laplace approximation. Female identity was included as a random effect to control for repeated observations of individual females.

All analyses were run using the software R 2.8.1 (R Development Core Team, 2008) and non-significant variables and interactions were removed from the final models. The residuals of all LMMs adhered to the assumption of normality.

3. Results

CORT0 levels increased with decreasing body condition, however, the effect was modulated by breeding experience so that the increase in CORT0 with decreasing body condition was more pronounced in older breeders (LMM: body condition \times breeding experience interaction: $b = -0.022$, $F_{1, 94} = 7.38$, $p < 0.01$; Fig. 1a). CORT0 levels were also affected by an interaction between residual clutch mass and hatch date (LMM: residual clutch mass \times hatch date interaction: $b = -0.00012$, $F_{1, 94} = 7.14$, $p < 0.01$; Fig. 1b), showing a pronounced increase in CORT0 with increasing clutch mass for late breeding females. CORT0 levels also decreased with increasing capture timing (i.e., the time between CORT0 sampling of the focal female and first encounter with a female on the island on that particular trapping occasion; LMM: $b = -0.33$, $F_{1, 94} = 22.43$, $p < 0.0001$), and decreased from 2009 to 2011 (LMM: $b = -0.065$, $F_{1, 94} = 11.79$, $p < 0.001$). CORT0 levels were not significantly connected to baseline body temperature (LMM: $b = 0.035$, $F_{1, 47} = 0.45$, $p = 0.50$), incubation stage (LMM: $b = -0.01$, $F_{1, 91} = 1.86$, $p = 0.18$) or sampling lag (LMM: $b = -0.013$, $F_{1, 91} = 2.18$, $p = 0.14$).

Faecal CORT concentrations were affected by an interaction between body condition and hatch date (LMM: body condition \times hatch date interaction: $b = 0.013$, $F_{1, 65} = 6.45$, $p = 0.01$; Fig. 2a), so that the FC of early breeding females decreased with increasing body condition, but the opposite was true for late breeding females, the FC of whom increased with body condition. FC levels also increased strongly with increasing baseline body temperature (LMM: $b = 0.17$, $F_{1, 65} = 16.87$, $p = 0.0001$; Fig. 2b). FC was not significantly connected to breeding experience (LMM: $b = 0.014$, $F_{1, 58} = 0.56$, $p = 0.46$), residual clutch mass (LMM: $b = 0.00007$, $F_{1, 59} = 0.93$, $p = 0.34$), year (LMM: $b = 0.037$, $F_{1, 60} = 3.078$, $p = 0.08$) or incubation stage (LMM: $b = -0.016$, $F_{1, 64} = 3.01$, $p = 0.09$).

Female baseline body temperature was affected by an interaction between body condition and habitat type (body condition \times habitat type interaction: $b = 0.21$, $F_{1, 60} = 6.10$, $p = 0.02$; Fig. 3). Thus, baseline body temperature increased with decreasing body condition on open islands, whereas it was essentially independent of body condition on forested islands. Baseline body temperature also decreased with increasing breeding experience (LMM: $b = -0.028$, $F_{1, 60} = 6.40$, $p = 0.01$; Fig. 4a), with residual clutch mass (LMM: $b = -0.00039$, $F_{1, 60} = 4.80$, $p = 0.03$), and with later hatch date (LMM: $b = -0.014$, $F_{1, 65} = 7.80$, $p = 0.007$; Fig. 4b). Capture timing affected baseline body temperature positively (LMM: $b = 0.38$, $F_{1, 65} = 25.80$, $p < 0.0001$), whereas incubation stage was negatively associated to baseline body temperature (LMM: $b = -0.060$, $F_{1, 65} = 43.80$, $p < 0.0001$). Baseline body temperature was not significantly connected to either sampling lag (LMM: $b = -0.019$, $F_{1, 53} = 0.80$, $p = 0.38$), or year (LMM: $b = -0.017$, $F_{1, 59} = 1.30$, $p = 0.27$).

Individual repeatability of CORT0, FC and baseline body temperature over years was relatively low but nonetheless significant (Table 1).

Nest fate was negatively associated with CORT0 (GLMM: $b = -0.012$, $z = -2.51$, $p = 0.01$; Fig. 5b), FC (GLMM: $b = -0.0030$, $z = -2.72$, $p < 0.01$; Fig. 5a) and hatch date (GLMM: $b = -0.19$, $z = -8.03$, $p < 0.0001$), while it was not associated with breeding experience (GLMM: $b = 0.049$, $z = 1.29$, $p = 0.20$) nor body condition (GLMM: $b = 0.21$, $z = 1.56$, $p = 0.12$).

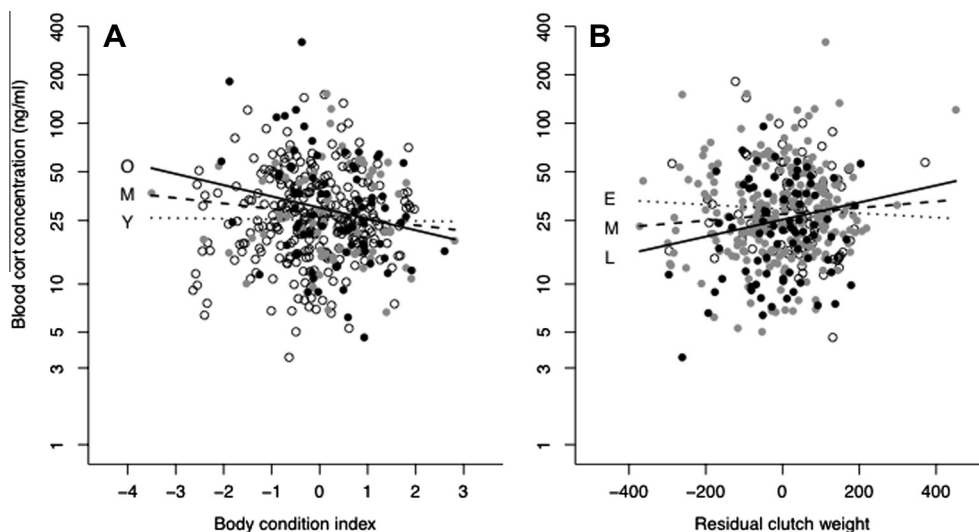


Fig. 1. Baseline serum CORT (CORT0) levels generally decreased with increasing body condition (panel A). However, this effect was modulated by breeding experience, so that CORT0 of older females (mean + 1 SD, solid line O, black filled dots) decreased more rapidly than those of intermediate-aged (mean, dashed line M, gray filled dots) and young individuals (mean - 1 SD, dotted line Y, open dots). CORT0 was also affected by reproductive investment (panel B; residual clutch mass, see Methods for details), and this effect was modulated by breeding phenology. A negative association between reproductive investment and CORT0 was evident early in the breeding season (mean - 1 SD, dotted line E, open dots), a positive association late in the season (mean + 1 SD, solid line L, filled black dots), and essentially no association during the mid-season (mean, dashed line M, filled gray dots). See Aiken and West (1991) for more details on plotting interaction terms.

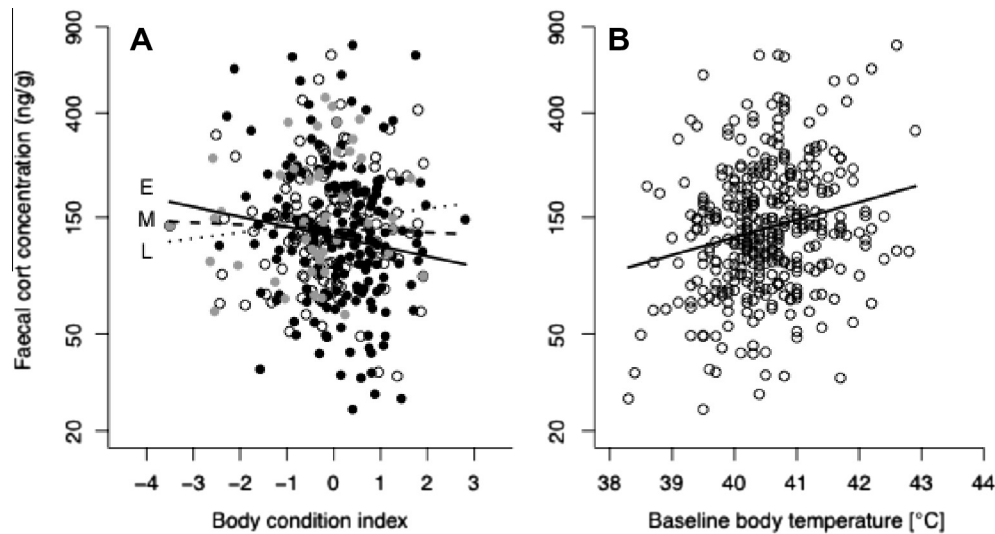


Fig. 2. Faecal CORT (FC) levels were affected by body condition (panel A) but this effect was modulated by breeding phenology. A negative association between body condition and FC was evident early in the breeding season (mean -1 SD, solid line E, filled black dots), a positive association late in the season (mean $+1$ SD, dotted line L, open dots), and essentially no association during mid-season (mean, dashed line M, filled grey dots). FC levels also increased with increasing baseline body temperature (panel B). See Aiken and West (1991) for more details on plotting interaction terms.

4. Discussion

Corticosterone mobilizes energy stores via processes such as gluconeogenesis (e.g., Wingfield et al., 1998; Warne et al., 2009). In a capital breeder like the eider, relying on its energy reserves for successfully completing incubation, it should therefore be adaptive to maintain CORT at a low level to avoid protein mobilization for energy supply (Criscuolo et al., 2006), and our *a priori* expectation was that high-quality individuals would be better at doing so. Indeed, female body condition, previously shown to be an important correlate of individual quality, was generally negatively associated with CORT levels in both serum and faeces. However, breeding experience (Fig. 1a) and breeding phenology

(Fig. 2a) modulated this relationship, and this context-dependency highlights the need for cautious interpretation of the relationship between stress physiology and phenotypic quality. We further discovered a strikingly strong association between baseline body temperature and faecal CORT levels, showing that FC increased with increasing baseline body temperature. Females in good body condition showed a lower baseline body temperature, but this effect was only observed on open islands (Fig. 3). We also found that females producing a large clutch mass, i.e., investing more into reproduction, had a lower baseline body temperature. This enhanced capacity to maintain thermal homeostasis suggests that reproductive investment may serve as an indicator of phenotypic quality, which is also corroborated by the finding that fecundity and survival are positively correlated in eiders (Yoccoz et al., 2002). Finally, there was a strong and consistent relationship between fitness and our two CORT measures, as indicated by the sharp decrease in nest success with increasing CORT concentrations in serum and faeces.

Examining how the different individual quality measures interact with baseline stress levels reveals intriguing links between stress physiology, age effects and timing of breeding. While our findings show that baseline blood CORT levels decrease with increasing body condition, thus supporting the prediction of the CORT-fitness hypothesis, older birds exhibited the steepest increase in blood CORT levels with decreasing condition, whereas the youngest breeders showed essentially no connection between CORT and body condition (Fig. 1a). This finding may indicate an age effect on stress tolerance, as aging is often accompanied by an increase in baseline CORT levels, an effect demonstrated under laboratory conditions (e.g., Purnell et al., 2004; Stein-Behrens and Sapolsky, 1992). It is conceivable that such effects of senescence on stress tolerance are exacerbated in birds in poor condition.

Interesting parallels can be drawn between, on the one hand, the interactive effect of timing of breeding and reproductive investment on serum CORT levels (Fig. 1b) and, on the other hand, the interactive effect of timing of breeding and body condition on FC levels (Fig. 2a). Thus, the general increase in serum CORT levels with increasing reproductive investment (i.e., total clutch mass) was most pronounced in the late breeding season. Correspondingly, only late breeders showed an increase in FC with increasing body condition. The common denominator linking these findings

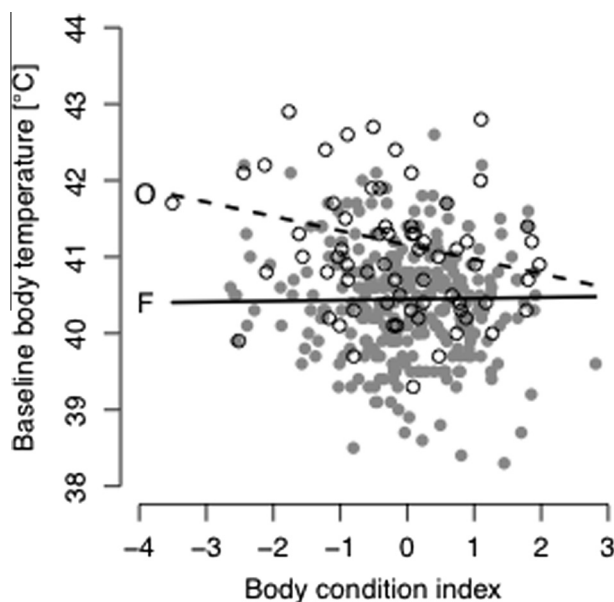


Fig. 3. Female baseline body temperature decreased with increasing body condition, but this effect was only present in the harsher breeding habitat on open islands (dashed line O, open dots), whereas no effect was observed on more protected forested islands (solid line F, filled grey dots).

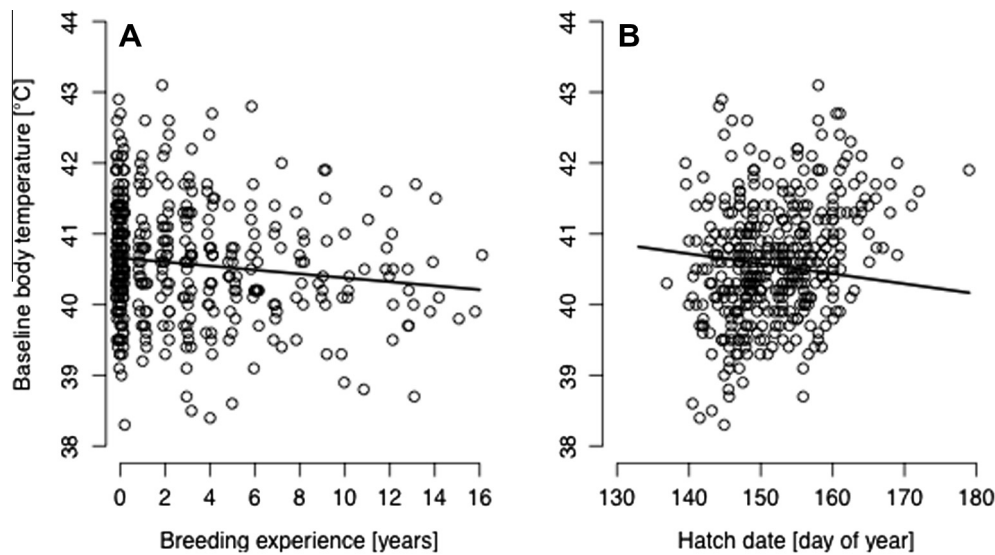


Fig. 4. Female baseline body temperature decreased with increasing breeding experience (panel A), and with advancing breeding phenology (panel B).

Table 1
Repeatabilities (*R*) of baseline serum corticosterone, faecal corticosterone and baseline body temperature between years.

Trait	Years	<i>R</i>	SE	CI	<i>p</i>	<i>N</i>
Baseline serum corticosterone	2009–2011	0.27	0.08	0.11, 0.43	<0.01	237/108
Faecal corticosterone	2008–2011	0.13	0.05	0.04, 0.26	<0.05	415/168
Baseline body temperature	2009–2011	0.25	0.06	0.16, 0.37	<0.001	371/167

Years: time frame in which data was collected; SE: standard error; CI: 95% confidence interval; *p*: significance of repeatability; *N*: observations/individuals.

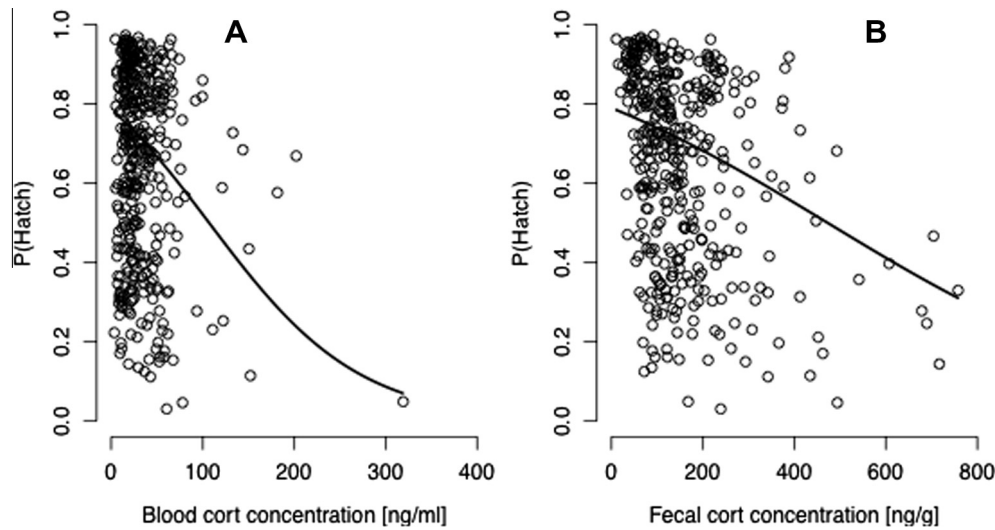


Fig. 5. Baseline CORT levels and fitness are connected in female eiders, because both serum (panel A) and faecal (panel B) CORT levels exhibited a strong negative association with the probability of hatching at least one duckling (nest success, see Methods for details).

may be that acquiring sufficient energy reserves to produce a large clutch may become increasingly difficult as the season progresses. This assumption is corroborated by the fact that clutch size in eiders declines with advancing laying date also within individuals (Öst et al., 2008b). The intensified foraging effort required to produce a large clutch late in the season may, in turn, contribute to explaining the elevated stress hormones levels, since higher CORT levels support increased parental foraging activity, a key argument underlying the CORT-adaptation hypothesis (e.g., Crossin et al., 2012). Another adaptive explanation is based on the ‘preparative

hypothesis’ (Romero, 2002). Thus, a late breeding schedule may put pressure on females to speed up the embryonic development of their eggs, which could be achieved by CORT-mediated mobilization of energy stores; only females with sufficient energy reserves (i.e., in good body condition) may afford to do so. There is indeed experimental evidence showing that increased CORT levels in incubating birds shortens the incubation period (e.g., Schmidt et al., 2009). Alternatively, the increased CORT levels of late-breeding females in good condition producing large clutches may not be adaptive, but rather represent an inevitable consequence of the

challenges involved in attaining the required resources. For example, whereas female eiders laying large clutches generally show higher survival than those laying smaller ones (Yoccoz et al., 2002), Descamps et al. (2009) showed that this pattern was actually reversed when there was heightened exposure to avian cholera. This result suggests that female eiders may only pay the fitness costs of high reproductive effort under unfavorable conditions. Finally, any differential fitness costs of increased reproductive effort between early and later breeders may be accentuated if early breeders represent higher-quality individuals. This is indeed possible, given that nest success decreases with hatch date in eiders (Öst and Steele, 2010; this study), and experienced breeders generally tend to nest earlier in the season in many bird species (e.g., Forslund and Larsson, 1992).

Our results draw attention to the utility of baseline body temperature as a complementary stress indicator because there was a strong positive connection between FC levels and body temperature (Fig 2b), and baseline body temperature was also repeatable within individuals over breeding seasons (Table 1). This association suggests that an elevated baseline body temperature represents a long-lasting rather than a momentary stress response, as faecal CORT levels represent a longer-term cumulative index of stress (Möstl et al., 2005). This notion is supported by findings showing that changes in body temperature may be detectable for days after a single stressful event (Meerlo et al., 1996), or for even longer after repeated exposure to stressful events (Hayashida et al., 2010).

We found support for our prediction that the regulation of body temperature during incubation was affected by an interaction between individual quality and environmental harshness. Thus, only poor-condition females breeding on open islands, where the environmental conditions are considerably more challenging and variable than on forested islands, showed an elevated baseline body temperature (Fig. 3). The environmental harshness of open islands is mainly due to the lack of vegetation exposing breeding eiders to wind, rain and direct sunlight. The lower limit of the thermoneutral zone of eiders is 7 °C (Gabrielsen et al., 1991) and it has been suggested that females nesting in our study population may in fact be at risk of heat stress, because of a negative relationship between average ambient temperature during the incubation period and female body weight at hatching (Öst et al., 2008b). Thus, Fast et al. (2007) suggested that increased ambient temperatures can lead to more rapid dehydration in eiders, presumably increasing the frequency of incubation recesses and lowering body hydration. However, also habitat-specific differences in perceived predation risk and predation pressure may contribute to explaining the interrelationships between breeding habitat, individual quality and baseline body temperature. Females nesting on open islands are subject to greater predation pressure by especially white-tailed sea eagles (*Haliaeetus albicilla*) than those nesting on forested islands and, consequently, their survival is significantly lower (Ekroos et al., 2012). Evidence demonstrating that exposure to predators or predator cues can induce sustained psychological stress is currently mounting (reviewed by Clinchy et al., 2013); such stress effects include significant and sustained increases in body temperature (e.g., Campeau et al., 2008). In the light of our findings, the question of whether poor-quality individuals may show a stronger body temperature response to ambient predation risk warrants further study.

Baseline serum and faecal CORT concentrations were repeatable, thus allowing inference as to the fitness consequences of variable stress hormone profiles. The results showed that females with increased CORT levels in both their blood stream and their faeces suffered from lower reproductive output, a result consistent with the CORT-fitness hypothesis. It is remarkable that both of our measures of baseline CORT (CORT0 and FC) actually outperformed body condition, the so far best predictor of eider nesting success

(Lehikoinen et al., 2010), as a predictor of nesting success. In light of the current study, the finding that poor body condition is negatively correlated with nest success (Lehikoinen et al., 2010) may in fact reflect the close association between both CORT0 and FC and body condition. It should be noted here that the elevated CORT levels of females may *per se* provide a simple mechanistic explanation for the observed decline in hatching success. This is because maternal corticosterone can be passed from the mother to the eggs (e.g., Hayward and Wingfield, 2004; Rubolini et al., 2005), and elevated CORT levels *in ovo* may, in turn, reduce both hatching success and result in weaker nestlings at hatching (e.g., Saino et al., 2005).

The fact that CORT levels were negatively correlated with a suite of individual quality metrics and with a direct measure of fitness provides strong evidence that the CORT-fitness hypothesis holds for eiders and perhaps also for other capital breeders with a precocial mode of development. In contrast, most findings of a positive association between CORT secretion and reproductive success have been done in species with extensive post-hatching parental effort, such as parental feeding (e.g., Bonier et al., 2009a; Crossin et al., 2012), where CORT may serve to boost parental care performance. We therefore suggest that the parental care strategy adopted by the species plays a decisive role in determining the sign of the relationship between CORT and fitness.

By highlighting the context dependency of the interrelationship between CORT secretion, individual quality and fitness, our study has contributed to bridging the gap between the sometimes disparate views on these important but elusive relationships (e.g., Bonier et al., 2009a). In doing so, we have showed that several correlates of individual quality are connected with measures of baseline CORT levels, as well as with a measure of fitness itself, and these relationships are further modified by the degree of environmental challenge faced by the individual. The interactive nature of many of the effects revealed by our study highlights that relationships between GCs and life-history traits are neither direct nor simple as is often hypothesized (cf. Crespi et al., 2013). Our findings particularly stress the importance of scrutinizing the degree of environmental challenge in cases where studies fail to find a difference between high- and low-quality individuals in their ability to cope with environmental stress, as differences may only become apparent in the most challenging of environments.

Acknowledgments

We thank Petteri Lehikoinen, Johan Ekroos and James Montanari for assistance in the field. Tvärminne Zoological Station provided facilities and equipment for fieldwork. The study was funded by the Academy of Finland (to KJ, MS and MÖ; grant no. 128039), the Finnish Cultural Foundation (to KJ), Onni Talas Foundation (to MS) and the Swedish Cultural Foundation (to MÖ).

References

- Aiken, L.S., West, S.G., 1991. Multiple Regression: Testing and Interpreting Interactions. Sage Publications, Newbury Park, CA.
- Angelier, F., Wingfield, J.C., Weimerskirch, H., Chastel, O., 2010. Hormonal correlates of individual quality in a long-lived bird: a test of the 'corticosterone-fitness hypothesis'. *Biol. Lett.* 6, 846–849.
- Bókonyi, V., Lendvai, A.Z., Liker, A., Angelier, F., Wingfield, J.C., Chastel, O., 2009. Stress response and the value of reproduction: are birds prudent parents? *Am. Nat.* 173, 589–598.
- Bolduc, F., Guillemette, M., 2003. Human disturbance and nesting success of common eiders: interaction between visitors and gulls. *Biol. Conserv.* 110, 77–83.
- Bonier, F., Moore, I.T., Martin, P.R., Robertson, R.J., 2009a. The relationship between fitness and baseline glucocorticoids in a passerine bird. *Gen. Comp. Endocrinol.* 163, 208–213.
- Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009b. Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* 24, 634–642.
- Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2010. Clarifying the cort-fitness hypothesis: a response to Dingemanse et al. *Trends Ecol. Evol.* 25, 262–263.

- Cabanac, A.J., Guillemette, M., 2001. Temperature and heart rate as stress indicators of handled common eider. *Physiol. Behav.* 74, 475–479.
- Campeau, S., Nyhuis, T.J., Sasse, S.K., Day, H.E.W., Masini, C.V., 2008. Acute and chronic effects of ferret odor exposure in Sprague–Dawley rats. *Neurosci. Biobehav. Rev.* 32, 1277–1286.
- Charmantier, A., Garant, D., 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proc. R. Soc. Lond. B.* 272, 1415–1425.
- Clinchy, M., Sheriff, M.J., Zanette, L., 2013. Predator induced stress and the ecology of fear. *Funct. Ecol.* 27, 56–65.
- Cockrem, J.F., 2005. Conservation and behavioral neuroendocrinology. *Horm. Behav.* 48, 492–501.
- Cockrem, J.F., Silverin, B., 2002. Variation within and between birds in corticosterone responses of great tits (*Parus major*). *Gen. Comp. Endocrinol.* 125, 197–206.
- Crespi, E.J., Williams, T.D., Jessop, T.S., Delehanty, B., 2013. Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Funct. Ecol.* 27, 93–106.
- Crisuolo, F., Bertile, F., Durant, J.M., Raclot, T., Gabrielsen, G.W., Massemin, S., Chastel, O., 2006. Body mass and clutch size may modulate prolactin and corticosterone levels in eiders. *Physiol. Biochem. Zool.* 79, 514–521.
- Crossin, G.T., Trathan, P.N., Phillips, R.A., Gorman, K.B., Dawson, A., Sakamoto, K.Q., Williams, T.D., 2012. Corticosterone predicts foraging behavior and parental care in macaroni penguins. *Am. Nat.* 180, E31–E41.
- D'Alba, L., Spencer, K.A., Nager, R.G., Monaghan, P., 2011. State dependent effects of elevated hormone: nest site quality, corticosterone levels and reproductive performance in the common eider. *Gen. Comp. Endocrinol.* 172, 218–224.
- Descamps, S., Gilchrist, H.G., Bety, J., Buttler, E.L., Forbes, M.R., 2009. Costs of reproduction in a long-lived bird: large clutch size is associated with low survival in the presence of a highly virulent disease. *Biol. Lett.* 5, 278–281.
- Dingemanse, N.J., Edelaar, P., Kempenaers, B., 2010. Why is there variation in baseline glucocorticoid levels? *Trends Ecol. Evol.* 25, 261–262.
- Ekroos, J., Öst, M., Karell, P., Jaatinen, K., Kilpi, M., 2012. Philopatric predisposition to predation-induced ecological traps: habitat-dependent mortality of breeding eiders. *Oecologia* 70, 979–986.
- Evans, M.R., Roberts, M.L., Buchanan, K.L., Goldsmith, A.R., 2006. Heritability of corticosterone response and changes in life history traits during selection in the zebra finch. *J. Evol. Biol.* 19, 343–352.
- Fast, P.L.F., Gilchrist, H.G., Clark, R.G., 2007. Experimental evaluation of nest shelter effects on weight loss in incubating common eiders *Somateria mollissima*. *J. Avian Biol.* 38, 205–213.
- Forslund, P., Larsson, K., 1992. Age-related reproductive success in the barnacle goose. *J. Anim. Ecol.* 61, 195–204.
- Gabrielsen, G.W., Mehlum, F., Karlsen, H.E., Andresen, Ø., Parker, H., 1991. Energy cost during incubation and thermoregulation in the female common eider *Somateria mollissima*. *Norsk Polarinst. Skrif.* 195, 51–62.
- Hanssen, S.A., Engebretsen, H., Erikstad, K.E., 2002. Incubation start and egg size in relation to body reserves in the common eider. *Behav. Ecol. Sociobiol.* 52, 282–288.
- Hau, M., Ricklefs, R.E., Wikelski, M., Lee, K.A., Brawn, J.D., 2010. Corticosterone, testosterone and life-history strategies of birds. *Proc. R. Soc. Lond. B.* 277, 3203–3212.
- Hayashida, S., Oka, T., Mera, T., Tsuji, S., 2010. Repeated social defeat stress induces chronic hyperthermia in rats. *Physiol. Behav.* 101, 124–131.
- Hayward, L.S., Wingfield, J.C., 2004. Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *Gen. Comp. Endocrinol.* 135, 365–371.
- Jaatinen, K., Öst, M., 2011. Experience attracts: the role of age in the formation of cooperative brood-rearing coalitions in eiders. *Anim. Behav.* 81, 1289–1294.
- Kilpi, M., Lindström, K., 1997. Habitat-specific clutch size and cost of incubation in common eiders, *Somateria mollissima*. *Oecologia* 111, 297–301.
- Kilpi, M., Öst, M., Lindström, K., Rita, H., 2001. Female characteristics and parental care mode in the crèche system of eiders, *Somateria mollissima*. *Anim. Behav.* 62, 527–534.
- Lehikoinen, A., Jaatinen, K., Öst, M., 2010. Do female ornaments indicate quality in eider ducks? *Biol. Lett.* 6, 225–228.
- Mashburn, K., Atkinson, S., 2004. Evaluation of adrenal function in serum and feces of Steller sea lions (*Eumetopias jubatus*): influences of molt, gender, sample storage, and age on glucocorticoid metabolism. *Gen. Comp. Endocrinol.* 136, 371–381.
- Meerlo, P., De Boer, S.F., Koolhaas, J.M., Daan, S., Van den Hoofdakker, R.H., 1996. Changes in daily rhythms of body temperature and activity after a single social defeat in rats. *Physiol. Behav.* 59, 735–739.
- Möstl, E., Rettenbacher, S., Palme, R., 2005. Measurement of corticosterone metabolites in birds' droppings: an analytical approach. *Ann. N. Y. Acad. Sci.* 1046, 17–34.
- Nakagawa, S., Schielzeth, H., 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol. Rev.* 85, 935–956.
- Nilsson, P.B., 2004. Characterizing glucocorticoid levels in five species of sea ducks occurring in Alaska. Master thesis, University of Alaska Fairbanks.
- Öst, M., Lehikoinen, A., Jaatinen, K., Kilpi, M., 2011. Causes and consequences of fine-scale breeding dispersal in a female-philopatric species. *Oecologia* 166, 327–336.
- Öst, M., Smith, B.D., Kilpi, M., 2008a. Social and maternal factors affecting duckling survival in eiders *Somateria mollissima*. *J. Anim. Ecol.* 77, 315–325.
- Öst, M., Steele, B.B., 2010. Age-specific nest-site preference and success in eiders. *Oecologia* 162, 59–69.
- Öst, M., Wickman, M., Matulionis, E., Steele, B.B., 2008b. Habitat-specific clutch size and cost of incubation in eiders reconsidered. *Oecologia* 158, 205–216.
- Ouyang, J.Q., Sharp, P.J., Dawson, A., Quetting, M., Hau, M., 2011. Hormone levels predict individual differences in reproductive success in a passerine bird. *Proc. R. Soc. Lond. B.* 278, 2537–2545.
- Parker, H., Holm, H., 1990. Patterns of nutrient and energy expenditure in female common eiders nesting in the high arctic. *Auk* 107, 660–668.
- Purnell, J.Q., Brandon, D.D., Isabella, L.M., Loriaux, D.L., Samuels, M.H., 2004. Association of 24-hour cortisol production rates, cortisol-binding globulin, and plasma free cortisol levels with body composition, leptin levels, and aging in adult men and women. *J. Clin. Endocrinol. Metab.* 89, 281–287.
- R Development Core Team, 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Robert, A., Paiva, V.H., Bolton, M., Jiguet, F., Bried, J., 2012. The interaction between reproductive cost and individual quality is mediated by oceanic conditions in a long-lived bird. *Ecology* 93, 1944–1952.
- Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128, 1–24.
- Romero, L.M., Wikelski, M., 2001. Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Niño events. *Proc. Natl. Acad. Sci. USA* 98, 7366–7370.
- Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R.P., Martinelli, R., Galeotti, P., Fasola, M., Saino, N., 2005. Effects of elevated egg corticosterone levels on behavior, growth and immunity of yellow-legged gull (*Larus michahellis*) chicks. *Horm. Behav.* 47, 592–605.
- Saino, N., Romano, M., Ferrari, R.P., Marinelli, R., Möller, A.P., 2005. Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *J. Exp. Zool. A Comp. Exp. Biol.* 303, 998–1006.
- Schmidt, J.B., André, R.M., Davis, K.A., Treece, S.M., Satterlee, D.G., 2009. Influence of maternal corticosterone treatment on incubation length of eggs laid by Japanese quail hens selected for divergent adrenocortical stress responsiveness. *Br. Poult. Sci.* 50, 739–747.
- Selmann, M.W., Öst, M., Jaatinen, K., Atkinson, S., Mashburn, K., Hollmén, T., 2012. Stress responsiveness, age and body condition interactively affect flight initiation distance in breeding female eiders. *Anim. Behav.* 84, 889–896.
- Sheriff, M.J., Krebs, C.J., Boonstra, R., 2009. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *J. Anim. Ecol.* 78, 1249–1258.
- Stein-Behrens, B.A., Sapolsky, R.M., 1992. Stress, glucocorticoids, and aging. *Aging Clin. Exp. Res.* 4, 197–210.
- Wada, H., Moore, I.T., Breuner, C.W., Wingfield, J.C., 2006. Stress responses in tropical sparrows: comparing tropical and temperate *Zonotrichia*. *Physiol. Biochem. Zool.* 79, 784–792.
- Waldeck, P., Kilpi, M., Öst, M., Andersson, M., 2004. Brood parasitism in a population of common eider (*Somateria mollissima*). *Behaviour* 41, 725–739.
- Warne, J.P., Akana, S.F., Ginsberg, A.B., Horneman, H.F., Pecoraro, N.C., Dallman, M.F., 2009. Disengaging insulin from corticosterone: roles of each on energy intake and disposition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1366–R1375.
- Wingfield, J.C., Breuner, C., Jacobs, J.D., Lynn, S., Maney, D., Ramenofsky, M., Richardson, R., 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am. Zool.* 38, 191–206.
- Yoccoz, N.G., Erikstad, K.E., Bustnes, J.O., Hanssen, S.A., Tveraa, T., 2002. Costs of reproduction in common eiders (*Somateria mollissima*): an assessment of relationships between reproductive effort and future survival and reproduction based on observational and experimental studies. *J. Appl. Stat.* 29, 57–64.