Regular article

The prudent parent meets old age: A high stress response in very old seabirds supports the terminal restraint hypothesis

Kyle H. Elliott a,⁎, Kathleen M. O’Reilly b, Scott A. Hatch c, Anthony J. Gaston d, James F. Hare a, W. Gary Anderson a

a Department of Biological Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
b Department of Biology, University of Portland, Portland, OR 97203, USA
c U.S. Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, AK 99508, USA
d National Wildlife Research Centre, Environment Canada, Carleton University, Ottawa, ON K1A 0H3, Canada

A R T I C L E   I N F O

Article history:
Received 18 June 2014
Revised 26 September 2014
Accepted 5 November 2014
Available online 13 November 2014

Keywords:
Black-legged kittiwake
Free hormone hypothesis
Glucocorticoids
Rösa tridactyla
Senescence
Shy/reactive and bold/proactive personalities
Thick-billed murre
Uria lomvia

A B S T R A C T

The reproductive success of wild animals usually increases with age before declining at the end of life, but the proximate mechanisms underlying those patterns remain elusive. Young animals are expected to invest less in current reproduction due to high prospects for future reproduction (the “restraint” hypothesis). The oldest animals may also show restraint when conditions are sub-optimal where even a small increase in reproductive investment may lead to death (“terminal restraint”). Alternatively, reproduction may be constrained by lack of experience and senescence (the “constraint” hypothesis). In two species of breeding seabirds, behavioural (time to return the offspring, calmness during restraint) and physiological (metabolism, glucose and corticosterone) parameters responded similarly to stress with advancing age, implying a generalized stress response. Across those parameters, birds were “shy” (high stress response) when young or old, and “bold” (low stress response) when middle-aged. Specifically, free corticosterone, the principal avian glucocorticoid responsible for directing energy away from reproduction and towards immediate survival following stress, was highest in both young and very old stressed birds. All age groups had a similar adrenal capacity to produce corticosterone, implying that middle-aged birds were showing restraint. Because the stress response, was highest at ages when the probability of current reproduction was lowest rather than at ages when the probability of future reproduction was highest we concluded that birds restrained reproductive investment based on current conditions rather than potential future opportunities. In particular, old birds showed terminal restraint when stressed. Hormonal cues promoted investment in adult survival over reproductive output at both the start and end of life consistent with the restraint hypothesis.

© 2014 Elsevier Inc. All rights reserved.

Introduction

Life-history theory predicts that iteroparous organisms will reduce investment in current reproduction when costs to future reproductive output outweigh the benefits of current reproduction (Williams, 1957; Stearns, 1992). Young animals, in particular, are expected to invest less energy in reproduction as they have more subsequent chances to reproduce (the “restraint” hypothesis, e.g. Heidinger et al., 2006; Desprez et al., 2011). Increased investment in current reproduction as the probability of substantial future reproductive output decreases may in part explain why reproductive success usually increases with age (de Forest and Gaston, 1996; Newton, 1998; Lewis et al., 2006; Angelier et al., 2007; Coulson, 2011; Hypothesis 1). One potential mechanism to account for such patterns is reluctance by older birds to abandon reproduction during stressful situations such as reduced food availability or risk of predation (Heidinger et al., 2006, 2010; Wilcoxen et al., 2011; Goutte et al., 2011; Riechert et al., 2012; Elliott et al., 2014a).

The oldest animals may also show restraint in reproduction during stressful years because even a small increase in energy expended during reproduction may lead to death (‘terminal restraint hypothesis’, McNamara et al., 2009; Heinze and Schrepff, 2012; Hypothesis 2). For instance, longevity may be limited by low physiological condition (high ‘biological age’) rather than high chronological age if death occurs after physiological condition is reduced beyond a minimum threshold (McNamara et al., 2009). If each reproductive event increases biological age, then lifetime reproductive success may be higher if old individuals, near their maximum biological age, forgo reproduction during stressful years, when biological age is increased by a greater degree than during non-stressful years, and only invest heavily in reproduction during non-stressful years (McNamara et al., 2009). The assumption that individuals base life-history decisions on biological age rather than chronological age is supported by several studies that have shown that telomeres

http://dx.doi.org/10.1016/j.yhbeh.2014.11.001
0018-506X/© 2014 Elsevier Inc. All rights reserved.
dynamics better predict longevity than chronological age (Haussmann et al., 2005; Paulin et al., 2006; Bize et al., 2009; Salomons et al., 2009; Heidinger et al., 2012).

Alternatively, both very young and very old animals may lack the experience or physical capacity necessary to maintain high levels of energy intake (the “constraint” hypothesis; Hypotheses 3 and 4), leading to reduced reproductive success (Finkel and Holbrook, 2000; Costantini et al., 2012). Finally, higher reproductive success among older age classes may reflect the selective mortality of low-quality phenotypes that produce few offspring (Forslund & Pärt 1995; Hypothesis 5).

Many studies have observed an increase in reproductive success in long-lived animals with age followed by a reduction at the end of life (de Forest and Gaston, 1996; Newton, 1998; Lewis et al., 2006; Angelier et al., 2007; Coulson, 2011), but fewer studies have examined the proximate mechanisms, which can provide clear, testable predictions for each of the hypotheses explaining the ultimate causes of these patterns (Table 1). The endocrine stress response provides a mechanism through which the competing demands of life-history trade-offs can be regulated, including those between current versus future reproduction. Glucocorticoid hormones released in response to a stressful event shift energy investment away from reproduction and towards immediate survival (Wingfield and Kitaysky, 2002; Satterthwaite et al., 2010; Kitaysky et al., 2010; Schultner et al., 2013a,b). For instance, as their name suggests, glucocorticoids mobilize fuel stores and increase circulating glucose necessary to fuel the muscles during rapid movement, such as flight from a predator (Bateson and Bradshaw, 1997; Wingfield and Kitaysky, 2002). Older breeding individuals may secrete lower levels of glucocorticoids in response to a stress during breeding (Jesop and Hamann, 2005; Heidinger et al., 2006; Goutte et al., 2011). As one manner by which glucocorticoid hormones shift energy investment away from reproduction and towards adult survival is by increasing the propensity of an adult to desert its offspring and to reduce the rate at which the adult provisions its offspring, the net result of a lower glucocorticoid response can be to maintain investment in offspring during a stressful period (Lendvai et al., 2007; Goutte et al., 2011; Angelier et al., 2009; Kitaysky et al., 2010; Seltmann et al., 2012).

Although reduced levels of glucocorticoid hormones with age could represent restraint via increased investment in offspring during a stressful event when the probability for future reproductive success is low (Heidinger et al., 2006; Heidinger et al., 2010; Hypothesis 1, Table 1), alternative explanations exist. For instance, both young and very old individuals may show restraint and be more likely to abandon offspring in the face of a stressor because they are unlikely to successfully reproduce, as they are unable to forage adequately, have inadequate energy intake (the “constraint” hypothesis; Hypotheses 3 and 4), leading to reduced reproductive success (Finkel and Holbrook, 2000; Costantini et al., 2012). Finally, higher reproductive success among older age classes may reflect the selective disappearance of phenotypes with high stress responses. Glucocorticoids are secreted by the adrenal tissue in response to adrenocorticotropic hormone (ACTH), which is secreted by the pituitary gland. By first using the glucocorticoid receptor agonist dexamethasone to suppress natural production of ACTH (and therefore glucocorticoids), minimizing the confounding effect of endogenous ACTH release, and then exogenously injecting ACTH, we assessed the responsiveness of adrenal tissue (Asheimer et al., 1994; Boonstra et al., 2001; Schmidt et al., 2012). If Hypothesis 4, but not 3, was true, we predicted declines in glucocorticoid response to the ACTH challenges. Finally, if Hypothesis 5 was true, we predicted that individuals with high stress responses would have lower survival. To provide a stronger context for examining these relationships, we also examined behavioural and physiological adjustments occurring in tandem with the glucocorticoid response.

### Methods

We studied pre-breeding (5 to 20 May 2012; N = 43) and chick-rearing (10 to 15 July 2010 and 12 to 20 July 2012; N = 89) black-legged kittiwakes Rissa tridactyla at Middleton Island, Alaska, and chick-rearing (20 to 31 July 2008 and 24 to 30 July 2011; N = 72) thick-billed murres Uria lomvia at Coats Island, Nunavut. We did not study pre-laying murres due to the difficulty of capturing those individuals. Kittiwakes had chicks 10–20 days old (fledging age averages –40 days) and murres had chicks 3–10 days old (departure age averages –20 days). Murres nested on a natural cliff (Gaston et al., 2005) while kittiwakes nested on an abandoned radar tower fitted with one-way glass that facilitated capture (Gill and Hatch, 2002; Hatch, 2013). We used primarily kittiwakes and murres banded as chicks and therefore of known age. However, as there were few very old birds, we included 14 kittiwakes banded as adults at least 10 years previously and three murres banded as adults at least 20 years previously. We added 5 years to the number of years since first capture to estimate their age (de Forest and Gaston, 1996; Vincenzi et al., 2013; Young et al., 2013). We captured murres by the neck using a noose pole and kittiwakes by the leg using a hook. To avoid reductions in stress response due to handling, we used murres that had likely not been handled since banded as a chick. For kittiwakes, we included years of handling as a covariate. We restrained birds by placing them in cloth bags that snugly fit the body. Only one adult per nest was captured. We did not correct body mass for linear size as such corrections do not improve estimation of lean or lipid mass in either murres or kittiwakes (Jacobs et al., 2012). For instance, in our kittiwake data set, body mass alone explains 92% of the variation in the residual of body mass on the first principal component of head-bill, tarsus, culmen and wing length, and consequently size-corrected mass explains little of the variation beyond mass alone. Murres were sexed using a combination of genetic markers and behavioural observations.

### Table 1

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Restraint for future opportunities</td>
<td>Stress response is proportional to residual reproductive success at a given age. Change only occurs in reproductive individuals.</td>
</tr>
<tr>
<td>2. Restraint when probability of current success is low</td>
<td>Stress response is inversely proportional to average reproductive success at a given age. Change only occurs in reproductive individuals.</td>
</tr>
<tr>
<td>3. Constraint via habitatting</td>
<td>Stress response declines with age and is similar among reproductive and non-reproductive (pre-breeding) individuals.</td>
</tr>
<tr>
<td>4. Constraint via decay of adrenal tissue</td>
<td>Stress response declines with age and is similar among reproductive and non-reproductive (pre-breeding) individuals. Stress response declines with age following ACTH injection.</td>
</tr>
<tr>
<td>5. Selection against high-HPA individuals</td>
<td>Individuals with high stress responses have lower survival.</td>
</tr>
</tbody>
</table>
(Elliott et al., 2010) whereas kittiwakes were sexed by position during copulation or head–bill length (Jodice et al., 2000). To examine the responsiveness of the adrenal tissue, we challenged birds with ACTH (N = 44 pre-breeding kittiwakes, N = 45 chick-rearing kittiwakes and N = 27 chick-rearing murre) after suppressing the stress response using dexamethasone. Research was approved by the Protocol Management Review Committee of the University of Manitoba under protocol F11-020.

We sampled kittiwakes that were 3–22 years of age. Survival and/or reproduction begins to decline after age 12 in kittiwakes (Coulsdon and Fairweather, 2001; Steiner et al., 2010; Coulsdon, 2011) and, with >100,000 individuals banded, the longevity record stands at 28 years (http://www.euring.org/data_and_codes/longevity-vou.htm). We sampled murrels that were 3–29 years of age. Survival and reproduction begins to decline after age 20 in murrels (Crespin et al., 2006; Lewis et al., 2006; Reed et al., 2008) and, with >100,000 individuals banded, the longevity record stands at 32 years for thick-billed murrels (reviewed in Elliott et al., 2014b). We therefore sampled over a sufficient age range to detect age-related trends, including senescence, for both species.

### Blood sampling

We obtained blood samples after 0, 60 and 90 min and recorded corticosterone by radioimmunoassay, corticosterone-binding globulin by a competitive binding assay and glucose levels enzymatically. We obtained 0.5 mL of blood from kittiwakes and 1 mL of blood from murrels within 3 min, and after 60 min and 90 min of restraint. Blood samples were stored on ice for ~4 h and centrifuged at 2000 g for 10 min. The plasma was then removed, stored at −20 °C for the remainder of the field season (<X1 month) and then shipped to the University of Manitoba on dry ice and stored at −80 °C until analysis. We also recorded behaviour during the first 10 min of restraint by counting the number of times the bird attempted to escape. We classified birds that never attempted to escape as “calm”, those that attempted to escape for 0–2 min (out of the 10 min) as “normal” and those that attempted to escape >2 min as “not calm”.

### Injections

During the chick-rearing experiments, we injected intramuscularly (pectoralis muscle) 45 chick-rearing kittiwakes (42 controls), 40 pre-breeding kittiwakes (44 controls) and 27 chick-rearing murrels (44 controls) with 0.2 mL dexamethasone (Sigma D1756: 0.5 mg = kittiwakes; 1 mg = murre) immediately following the baseline blood sample and with 0.2 mL adrenocorticotropic hormone (Sigma A6303: 45 IU = kittiwakes; 100 IU = murre) 60 min later. We based ACTH dosages on a similar study in another charadriiform species (Heidinger and Kitaysky, 2002). We therefore examined a variety of effects that direct energy away from reproduction and towards immediate survival (Wingfield and Kitaysky, 2002; Satterthwaite et al., 2010; Kitaysky et al., 2010; Schultzner et al., 2013a,b). As such, we measured circulating levels of corticosterone as our primary index of the stress response in wild birds. One of the primary effects of glucocorticoid hormones is to increase blood glucose levels to mobilize fuel to escape from a stressful event, such as a predator (Bateson and Bradshaw, 1997; Wingfield and Kitaysky, 2002). We therefore examined the relative increase in glucose levels for a given increase in corticosterone levels as a measure of the responsiveness of tissues to corticosterone.

We used a radioimmunoassay (RIA) to measure corticosterone levels in duplicate using 25 μL of plasma extracted in 1.2 mL of ethanol (2009–2012) or 25 μL of unextracted plasma (2008). For a subset of samples, there was no difference between ethanol-extracted plasma corticosterone levels, unextracted plasma corticosterone levels and dichloromethane-extracted plasma corticosterone levels after accounting for extraction efficiency. In 2009–2012, extracted samples were reconstituted in 100 μL of RIA buffer (0.1 M phosphate buffer, 0.9% NaCl [w/v], and 0.5% bovine serum albumin [w/v]). A standard curve was created by adding known concentrations (0.01 to 50 ng mL−1) of corticosterone standard (Steraloids) into RIA buffer. To each of the test tubes, we then added 100 μL of tritiated (5000 disintegrations per minute) corticosterone (GE Healthcare, 78.1 MBq mol−1, Code TRK406) and 100 μL of sheep serum anti-corticosterone (diluted 1:50,000; antibodies-online, Code ABIN343319). After 1 h incubation at room temperature, samples were incubated at 4 °C for a minimum of 18 h. The reaction was stopped through the addition of 100 μL of dextran (0.5% w/v)-coated charcoal (5% w/v) to each assay tube. After 15 min on ice, assay tubes were centrifuged for 30 min at 4 °C (2500 g), and the supernatant was decanted into 7 mL scintillation vials. Finally, 4 mL of scintillation fluid (Ultima Gold, Perkin Elmer) was added to each assay tube, and tubes were counted on a liquid scintillation counter (157500, Beckman Coulter) for 5 min. All samples were counted in duplicate, and corticosterone concentration of each sample was interpolated from the standard curve that was counted in triplicate. Inter-assay and intra-assay coefficients of variation for corticosterone were 7.3 and 5.2%, respectively. Serial dilution of plasma demonstrated good parallelism with the corticosterone standard curve (ANCOVA; kittiwake: F1,22 = 1.24, P = 0.32; murre: F1,22 = 0.62, P = 0.44; no significant interactions). The minimum detection level was 0.025 ng mL−1. In 2008, all samples were measured via double antibody radioimmunoassay (MP Biomedical kit 07-120103) directly on unextracted plasma following the kit instructions. The single assay had intra-assay variability of 5.4%.

For kittiwakes, we recorded glucose levels by placing a drop of blood on a test strip and reading the value using a Bayer contour meter. For murrels, we recorded glucose levels by adding 5 μL of plasma (5 μL gave a coefficient of variation of 3.1% whereas adding the recommended 2 μL gave a coefficient of variation of 7.2%) to 96-well plates with the reagents from the Wako LabAssay kit (product code: 298-65701). As glucose levels are expected to increase in response to corticosterone, glucose and corticosterone levels are expected to be correlated among stressed individuals. To examine the responsiveness of tissues to corticosterone we examined the residual of glucose levels on corticosterone. A high responsiveness would be associated with a higher level of glucose for a given concentration of corticosterone.

### Corticosterone and glucose assays

Corticosterone is the principal avian glucocorticoid hormone that increases in circulation in response to a stressful event and causes a variety of effects that direct energy away from reproduction and towards immediate survival (Wingfield and Kitaysky, 2002; Satterthwaite et al., 2010; Kitaysky et al., 2010; Schultzner et al., 2013a,b). As such, we measured circulating levels of corticosterone as our primary index of the stress response in wild birds. One of the primary effects of glucocorticoid hormones is to increase blood glucose levels to mobilize fuel to escape from a stressful event, such as a predator (Bateson and Bradshaw, 1997; Wingfield and Kitaysky, 2002). We therefore examined the relative increase in glucose levels for a given increase in corticosterone levels as a measure of the responsiveness of tissues to corticosterone.

Corticosterone-binding globulin and free corticosterone

Corticosterone-binding globulin (CBG) is a protein that binds corticosterone in circulation. The free hormone hypothesis posits that it is only the free hormone that is biologically active (Breuner et al., 2013).
Thus, variation in CBG levels with age may influence variation in the stress response with age by altering the amount of free, biologically-active hormone available to the tissues. For instance, CBG levels decline with age in humans (e.g. Fernandez-Real et al., 2002), meaning that more free hormone is available for the tissues for a given total hormone level among older humans than young humans. We therefore quantified corticosterone-binding globulin (CBG) levels (Breuner and Orchinik, 2002; Love et al., 2004).

We stripped endogenous corticosterone from plasma by incubating 5 μL of plasma in 495 μL of dextran-coated Norit charcoal for 30 min at room temperature prior to centrifuging for 10 min at 3000 g. We determined total binding capacity (in triplicate; 50 μL of stripped plasma, 50 μL buffer, 50 μL of tritiated corticosterone diluted 1:300) and non-specific binding (in duplicate; 50 μL of stripped plasma, 50 μL 1 μg/mL corticosterone, 50 μL of tritiated corticosterone) for each individual. Following 2 h incubation, we used a 24-sample Brandel harvester to separate the free and bound fractions using glass fibre filters (Whatman GF/B) pre-soaked for 1 h in 25 mM Tris buffer with 0.3% FEL. We suspended filters in scintillation fluid for 24 h before measuring radioactivity. Intra-assay variability was 3.2% and inter-assay variability was 9.6%. We estimated concentration of free corticosterone (Hfree) as:

\[
H_{\text{free}} = 0.5 \left[ H_{\text{total}} - B_{\text{max}} - K_d \left( \sqrt{(B_{\text{max}} - H_{\text{total}} + K_d)^2 + 4H_{\text{total}}K_d} \right) \right]
\]

where \( K_d = 1.20 \pm 0.25 \text{ nM} \) for kittiwakes and 2.18 ± 0.32 nM for murres, \( B_{\text{max}} \) is the concentration of CBG molecules and \( H_{\text{total}} \) is the concentration of total corticosterone (Barsano and Bauman, 1989).

Time to return to the colony (temperature loggers)

Glucocorticoid hormones can direct energy away from reproduction and towards immediate survival by increasing desertion rates or otherwise reducing investment in offspring (Lendvai et al., 2007; Goutte et al., 2011; Angelier et al., 2009; Kitaysky et al., 2010; Seltmann et al., 2012). To investigate behavioural responses to stressful events, we examined how much time it took for adult birds to return to their offspring following capture. As predation by gulls is a major cause of chick failure for both species at our study sites, and defence by the adult is key to preventing gull predation, minimizing time the offspring is unattended successfully (de Forest and Gaston, 1996; Gill and Hatch, 2002; Hatch, 2013). We measured survival by recording the number of individuals that were resighted in subsequent years from 2009 to 13. We used programme MARK to calculate resighting probabilities (always >85%) for each year and survival rates for each age class. For individual birds, we assume that resighting rates are representative of survival due to the high resighting probabilities. We calculated residual reproductive success at each age \( j \) as \( \sum_{i=0}^{40} R_i (1 - M_i) \) where \( R_i \), reproductive success at age \( i \), was based on the quadratic function presented in Fig. 2, and \( M_i \), mortality at age \( i \), was based on age-specific mortality rates at our study sites (KHE, unpubl. data). We assumed that any age when the function gave a negative value for reproductive success had a reproductive success of zero. We ended the summation at 40 because the function was negative beyond that age.

Statistical analyses

To obtain normality, we log-transformed corticosterone levels and return time. We used simple linear regressions to examine the relationships between corticosterone and glucose, return time and metabolic rate. We used general linear models (Gaussian link) to examine the effect of age (always considered to be continuous), age\(^2\) (to account for nonlinear relationships with age), year, time since laying (relative date, which is negative for pre-laying birds), sex, time of day (hours since sunrise), body mass, date and recapture history (kittiwakes only; we avoided murres that had been handled previously as adults) on corticosterone levels, the residual of glucose on corticosterone levels, return time and metabolic rates. We used nonlinear regressions to examine the relationship between reproductive success and both age and age\(^2\). We used t-tests to compare log-transformed maximum free corticosterone levels among individuals that were and were not resighted in the following season. We used Aikake’s information criterion to compare the parsimony of models for free CORT as a function of residual reproductive success and average reproductive success for each age.

Results

Corticosterone and glucose

Baseline free corticosterone levels declined with body mass in kittiwakes and with relative date in pre-breeding but not chick-rearing kittiwakes (Table 2). Total corticosterone at 60 min declined with age in chick-rearing murres, but not kittiwakes, before increasing again for the oldest murres (Table 2, Fig. 1). Maximum free corticosterone declined with age in chick-rearing murres and kittiwakes but not pre-breeding kittiwakes before increasing again for the oldest age classes (Table 2, Fig. 1). Free corticosterone in kittiwakes and murres challenged via ACTH was not related to any of the examined parameters (Table 2, Fig. 1). CBG capacity at baseline was correlated with CBG capacity at 60 min among individuals (murres: \( t_{61} = 4.39, P < 0.0001, R^2 = 0.47 \); kittiwakes: \( t_{61} = 6.23, P < 0.0001, R^2 = 0.64 \), and was higher at baseline than at 60 min (paired t-test: murres: 1.39 ± 0.26 times higher;
Table 2 shows results from general linear models including various independent variables for explaining log-transformed baseline and maximum free corticosterone (60 min after capture) for control (non-injected) and ACTH-injected (dexmethasone at 0 min, ACTH at 60 min—relationships reported for samples collected both at 60 min (“DEX”) and 90 min (“ACTH”)) thick-billed murres and black-legged kittiwakes. Also shown are maximum total corticosterone (60 min after capture), corticosterone-binding globulin (CBG, 60 min after capture), change in glucose between baseline and 60 min after capture and time to return to offspring following short restraint. Independent variables included body mass at capture, relative lay or hatch date, age, sex, year (for those variables measured in more than one year) and recapture history.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (free)</th>
<th>Max (free)</th>
<th>Max (total)</th>
<th>ACTH (free)</th>
<th>DEX (free)</th>
<th>Δ glucose</th>
<th>Return time</th>
<th>CBG</th>
<th>Calmness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>P</td>
<td>t</td>
<td>P</td>
<td>t</td>
<td>P</td>
<td>t</td>
<td>P</td>
<td>t</td>
</tr>
<tr>
<td><strong>Pre-breeding kittiwakes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>− 3.34</td>
<td>0.001</td>
<td>1.40</td>
<td>0.18</td>
<td>1.62</td>
<td>0.11</td>
<td>0.94</td>
<td>0.45</td>
<td>1.84</td>
</tr>
<tr>
<td>Relative lay date</td>
<td>2.99</td>
<td>0.004</td>
<td>−1.75</td>
<td>0.10</td>
<td>−1.49</td>
<td>0.14</td>
<td>−0.88</td>
<td>0.51</td>
<td>−0.14</td>
</tr>
<tr>
<td>Age</td>
<td>0.44</td>
<td>0.66</td>
<td>−0.43</td>
<td>0.67</td>
<td>−0.81</td>
<td>0.42</td>
<td>0.45</td>
<td>0.66</td>
<td>−0.09</td>
</tr>
<tr>
<td>Age²</td>
<td>0.32</td>
<td>0.84</td>
<td>0.05</td>
<td>0.95</td>
<td>0.22</td>
<td>0.83</td>
<td>0.65</td>
<td>0.54</td>
<td>0.14</td>
</tr>
<tr>
<td>Sex</td>
<td>0.78</td>
<td>0.44</td>
<td>0.32</td>
<td>0.76</td>
<td>−0.14</td>
<td>0.89</td>
<td>0.15</td>
<td>0.87</td>
<td>1.02</td>
</tr>
<tr>
<td>Recapture history</td>
<td>0.88</td>
<td>0.38</td>
<td>0.51</td>
<td>0.36</td>
<td>0.51</td>
<td>0.28</td>
<td>0.55</td>
<td>0.71</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Kittiwakes (chicks)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>− 2.02</td>
<td>0.04</td>
<td>1.28</td>
<td>0.21</td>
<td>1.43</td>
<td>0.16</td>
<td>0.86</td>
<td>0.40</td>
<td>1.03</td>
</tr>
<tr>
<td>Relative hatch date</td>
<td>1.45</td>
<td>0.09</td>
<td>0.25</td>
<td>0.84</td>
<td>−0.61</td>
<td>0.42</td>
<td>1.01</td>
<td>0.31</td>
<td>1.34</td>
</tr>
<tr>
<td>Age</td>
<td>− 1.33</td>
<td>0.11</td>
<td>−3.35</td>
<td>0.002</td>
<td>−1.61</td>
<td>0.11</td>
<td>0.49</td>
<td>0.63</td>
<td>−2.22</td>
</tr>
<tr>
<td>Age²</td>
<td>1.62</td>
<td>0.06</td>
<td>2.45</td>
<td>0.02</td>
<td>1.73</td>
<td>0.09</td>
<td>0.38</td>
<td>0.72</td>
<td>−0.03</td>
</tr>
<tr>
<td>Sex</td>
<td>0.54</td>
<td>0.60</td>
<td>0.29</td>
<td>0.79</td>
<td>1.03</td>
<td>0.31</td>
<td>0.85</td>
<td>0.40</td>
<td>0.18</td>
</tr>
<tr>
<td>Year</td>
<td>0.17</td>
<td>0.90</td>
<td>0.25</td>
<td>0.85</td>
<td>0.53</td>
<td>0.6</td>
<td>0.35</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Recapture history</td>
<td>0.03</td>
<td>0.98</td>
<td>0.41</td>
<td>0.60</td>
<td>0.34</td>
<td>0.73</td>
<td>0.81</td>
<td>0.42</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Murres (chicks)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass</td>
<td>− 1.55</td>
<td>0.08</td>
<td>0.95</td>
<td>0.35</td>
<td>−1.56</td>
<td>0.12</td>
<td>−0.02</td>
<td>0.98</td>
<td>1.41</td>
</tr>
<tr>
<td>Relative hatch date</td>
<td>0.53</td>
<td>0.61</td>
<td>−0.34</td>
<td>0.74</td>
<td>0.78</td>
<td>0.44</td>
<td>0.19</td>
<td>0.85</td>
<td>0.67</td>
</tr>
<tr>
<td>Age</td>
<td>− 1.57</td>
<td>0.07</td>
<td>−2.31</td>
<td>0.03</td>
<td>−2.45</td>
<td>0.02</td>
<td>1.01</td>
<td>0.32</td>
<td>2.81</td>
</tr>
<tr>
<td>Age²</td>
<td>1.58</td>
<td>0.07</td>
<td>3.14</td>
<td>0.006</td>
<td>2.74</td>
<td>0.008</td>
<td>0.71</td>
<td>0.50</td>
<td>2.06</td>
</tr>
<tr>
<td>Sex</td>
<td>0.22</td>
<td>0.81</td>
<td>−0.04</td>
<td>0.95</td>
<td>0.39</td>
<td>0.7</td>
<td>0.55</td>
<td>0.59</td>
<td>0.45</td>
</tr>
<tr>
<td>Year</td>
<td>0.02</td>
<td>0.99</td>
<td>0.08</td>
<td>0.97</td>
<td>−0.37</td>
<td>0.71</td>
<td>0.14</td>
<td>0.90</td>
<td>−0.49</td>
</tr>
</tbody>
</table>

Values in bold were significant at α = 0.05 significance level.

$t_{0.05} = 5.42, P < 0.0001$; kittiwakes: $1.24 \pm 0.25$ times higher than $t_{0.05} = 4.91, P = 0.0001$. CBG capacity at 60 min increased, and then decreased, with age (Table 2, Fig. 1). The change in glucose levels from baseline to 60 min tended to increase with corticosterone levels at 60 min (chick-rearing kittiwakes: $t_{76} = 2.32, P = 0.02$; chick-rearing murres: $t_{56} = 1.85, P = 0.07$). After accounting for corticosterone levels, glucose declined with age, but was also higher in very old birds (Table 1, Fig. 1). Maximum free corticosterone levels were no higher in resighted compared with non-resighted kittiwakes ($t_{87} = 0.62, P = 0.54$) and murres ($t_{50} = 1.26, P = 0.21$). Average reproductive success was more parsimonious in explaining variation in free CORT with age than residual reproductive success (kittiwake: $Δ\text{AIC} = 18.43$; murre: $Δ\text{AIC} = 8.93$; Fig. 1 resembles the inverse shape of Fig. 2a more closely than the shape of Fig. 2b).

**Discussion**

**Support for Hypothesis 2: tradeoff between immediate survival and current reproduction**

As would be expected based on a life-history trade-off between current reproduction and adult immediate survival, several measures of the stress response were attenuated at ages when reproductive success was usually high (Fig. 1a, 1d, 1e, Fig. 2). The stress response during reproduction matched the parabolic trajectory of average reproductive success with age (Hypothesis 2), rather than mirroring the decelerating trajectory of residual reproductive success (Hypothesis 1). We carefully controlled for habituation or chose naïve birds to avoid habituation. In contrast to Hypotheses 3 and 4, such parabolic trends were not apparent during pre-breeding and did not result from variation in adrenal capacity as assessed by ACTH challenges. Control of the stress response apparently does not occur via reductions and senescence in adrenal tissue with advancing age, as occurs in some mammals (Sapolsky and Altmann, 1991; Otte et al., 2005; Liburt et al., 2013). Rather, control of the stress response may occur at higher neural centres; corticosterone levels were lower in old birds after injection with dexmethasone, suggesting that the anterior pituitary of old individuals is more sensitive to inhibitory feedback, as is the case in old male mice (Harris and Saltzman, 2013). In contrast, the glucocorticoid response declined with age following ACTH challenge in another charadriiform bird (Heidinger et al., 2008). One possibility is that the birds in that study were not first injected with dexmethasone to suppress the stress response prior to ACTH challenge, the response to the ACTH challenge may have been additive to the existing stress response (Heidinger et al., 2008). Finally, “low stress” birds were no more likely to survive than “high stress” birds and so the selective disappearance of “high stress” birds did not account for trends in the stress response with age (Hypothesis 5).

We concluded that individuals suppress the stress response when they have a high probability of current successful reproduction. As has been the case in many other studies (Table 3), the stress response closely tracked the inverse of age-specific reproductive success, not residual reproductive success. Selection based on residual reproductive success is also unlikely on a theoretical basis. Most birds in even the oldest age classes within our study still have residual reproductive success higher than current reproductive success (Fig. 2), and selection at old ages is likely weak. Natural selection may be more likely to produce a mechanism that reduces responsiveness to a stressor when current...
reproductive prospects are favourable regardless of age as opposed to a mechanism reducing responsiveness to stressors when future reproductive prospects are limited years into the future. Presumably, birds are able to assess their own condition, their partner’s quality, their site quality and local conditions to determine whether current reproduction is probable, and convey that integrated message to the hypothalamus, modulating the stress response.

Our study provides evidence that the stress response serves as a mechanistic link modulating life-history trade-offs between successfully reproducing and avoiding capture by a predator. A major source of reproductive failure for cliff-nesting kittiwakes and murres is the loss of offspring to avian predators or to dislodgement (de Forest and Gaston, 1996; Coulson, 2011). Even a momentary neglect dramatically increases the chance of predation by gulls or dislodgement of precariously placed offspring. Thus, low-CORT-response birds that remain with their offspring or return quickly following disturbance are more likely to reproduce successfully (Gilchrist et al., 1998). However, birds that do not abandon in the face of risk may experience increased mortality. Murres aged 18–23—ages with low stress responses in our dataset—were disproportionately represented among those killed by polar bears (Ursus maritimus) and heat/mosquito stress on Coats Island (Gaston and Elliott, 2013). In both cases casualties occurred because those breeding birds presumably with low CORT responses refused to abandon their breeding effort when confronted with lethal threats.

Although we have interpreted our cross-sectional dataset as supporting Hypothesis 2, the five hypotheses in Table 1 are not mutually exclusive and it is possible that one or more of the other hypotheses, independently or in concert, play a role in determining age-related...
functional senescence adds to the growing consensus that long-lived birds show lower rates of physiological senescence than short-lived animals (Nisbet, 2001; Ricklefs, 2010; Holmes and Martin, 2009). Specifically, studies of long-lived wild birds detected few signs of declining immunity (Apanius and Nisbet, 2006; Lecomte et al., 2010), behaviour (Le Vaillant et al., 2012; Elliott et al., 2014b), adult telomere length (Haußmann et al., 2003; Hall et al., 2004; Mizutani et al., 2009; Young et al., 2013) or reproductive physiology (Nisbet et al., 2002; Coulson and Fairweather, 2001) with age that routinely accompany physiological ageing in mammals and short-lived birds (Cichon et al., 2003; Holmes and Ottinger, 2003; Haußmann et al., 2003; Heidinger et al., 2012; Saino et al., 2003; Palacios et al., 2007; Salomons et al., 2009). One possibility is that because a higher proportion of mortality is attributable to senescence in long-lived than short-lived animals (Ricklefs, 2010; Turbill and Ruf, 2010), long-lived animals experience stronger selection for postponed physiological senescence.

The terminal restraint hypothesis states that old individuals will reduce reproductive investment during poor years to maintain physiological condition and increase their odds of surviving until better years (McNamara et al., 2009). This may be especially true given that they have a low probability of current success. In support of the terminal restraint hypothesis, some long-lived birds have lower reproductive success during the last year of life, when they are presumably in poor condition, regardless of their age (Coulson and Fairweather, 2001; Rattiste, 2004). In contrast, other long-lived birds invest more, rather than less, during their final breeding attempt (Velando et al., 2006; Froy et al., 2013). Based on our results, we suggest that such differential investment depends on whether individuals assess a particular year as ‘good’ or ‘poor’, and urge the accumulation of longitudinal physiological data, such as telomere length, alongside environmental and demographic data to further explore the terminal restraint hypothesis.

![Graph a)](attachment:reproductive_success.png)

A generalized stress response: evidence for ‘bold’ and ‘shy’ personalities that change with age?

Multiple measures of the stress response changed in concert with age, including the glucocorticoid response, calmness during restraint, time to return to the offspring, metabolic rate and glucose production. Furthermore, these parameters were often intercorrelated at the level of the individual. Thus, although individuals can differentially upregulate different components of their stress response in response to different kinds of stress (Nephew et al., 2003; Viblanc et al., 2012), we found that some, primarily young and very old, individuals upregulated all components simultaneously in response to a particular stressor (restraint). For instance, individuals with low glucocorticoid stress responses had reduced ability to mount a glucose stress response at the tissue level, suggesting that tissue responsiveness to glucocorticoids via glucocorticoid receptors is regulated in tandem with the total adrenal response such that when the stress response is reduced, there is also less glucose production and release for a given amount of hormone. As the physiological and behavioural stress responses were intercorrelated across individuals within our study and many studies have shown a link between high stress responses and shy or reactive personalities (Carere and van Oers, 2004; Cockrem, 2007; Carere et al., 2010; Seltmann et al., 2012; Clary et al., 2014), we propose that the personalities of individual birds moved from shy/reactive to bold/proactive and back to shy/reactive throughout their lifespan. Personality changes with age in many animals (e.g. Suomi et al., 1996). There was also considerable individual variation in stress responses within each age class, reflecting the range of personalities that may be manifested by individuals at any given age.

Our study adds to the growing literature demonstrating a connection between the glucocorticoid stress response and reduced parental behaviour (Otto et al., 2005; Lendvai et al., 2007; Heidinger et al., 2008; Angellier et al., 2009; Ouyang et al., 2012; Thierry et al., 2013). For example, older ancient murrelets (Synthliboramphus antiquus) were less likely to abandon than younger murrelets following restraint, presumably reflecting variation in trends. Furthermore, environmental or cohort effects may have caused temporal variation that confounded our age-related trends. However, we found similar trends in two species in different oceans with opposing trends—reproductive success for kittiwakes at Middleton Island increased dramatically during the past decade (Hatch, 2013) while chick growth rates for murres at Coats Island declined over the past two decades (Gaston et al., 2005; Smith and Gaston, 2012). Thus, if poor nutrition during the chick stage primes the individual to have a high stress response throughout its life (Monaghan, 2014), we would expect the stress response to decline with age in kittiwakes and increase with age in murres, but we see similar patterns across both species.

Evidence for reduced physiological senescence and terminal restraint

That age-related variation in stress responses and, implicitly, reproductive success is associated with strategic restraint rather than...
an attenuated stress response with age (Elliott et al., 2010). Similarly, older murres expended more energy and were less likely to abandon than young murres after capture and attachment of an electronic recorder (Elliott et al., 2014a). Prolactin, a hormone associated with parental behaviour and secreted directly by the pituitary gland, also declines during stressful events as part of the generalized stress response organized at the level of the brain or via the downstream effect of glucocorticoids (Chastel et al., 2005; Angelier et al., 2007; Heidinger et al., 2010). The link between the stress response and parental behaviours may be due to a direct effect of glucocorticoids on behaviour, a prolactin stress response parallel but independent of glucocorticoids, or an effect of glucocorticoids on prolactin.

Age-related variation in both CBG and total CORT was important

CBG levels declined during stress, increasing the amount of free CORT reaching the tissues and magnifying the acute stress response (Breuner et al., 2006, 2013). Thus, variation in CBG could be expected to play an important role in age-related variation of the stress response, as is the case in humans (Fernandez-Real et al., 2002). Although both murres and kittiwakes showed similar trends in free CORT with age, the age-related variation in kittiwakes was primarily associated with variation in CBG while the age-related variation in murres was primarily associated with variation in total CORT (Table 2, Fig. 1). In contrast, both total CORT and CBG increase with age in the first few days of life for acutely-stressed nesting zebra finches, and consequently free CORT does not change with age until later in life (Wada et al., 2007). Given that free CORT (i) showed similar age-related patterns for both species, (ii) showed parallel age-related patterns to several other measures of the stress response; and (iii) correlated at the individual level with other physiological responses, we suggest that variation in free, rather than total CORT, is the most biologically-relevant parameter. Our results are therefore consistent with the free hormone hypothesis, which states that the concentration of free hormone is the main determinant of biological activity (Mendel, 1989; Love et al., 2004; Perogamvros et al., 2011; Breuner et al., 2013).

Conclusions

We provide evidence that the general stress response mediates a trade-off between current and future reproduction (Wingfield and Kitaysky, 2002; Boonstra, 2004; Satterthwaite et al., 2010). When the probability of current reproduction is high, individuals suppress their stress response, favouring reproduction over immediate survival in the presence of stressors, such as famine and predation. Similarly, Atlantic kittiwakes with high reproductive success have a reduced stress response, and invest more in offspring when stressed, compared to kittiwakes at our study site where reproductive success is low (Schultner et al., 2013b). Future research should focus on the role of prolactin and ACTH in the stress response via direct measurements. We predict that the magnitude of both prolactin decrease and ACTH increase will show a similar curvilinear relationship, peaking at middle ages.

Acknowledgments

I. Dorresteijn, K. Elner, Z. Fitzner, E. Grosbøll, M. Guigueno, E. Kennedy, M. LeVaililant, J. Provencher, E. Rogers, J. Schultner, P. Smith, T. Van Nus, K. Woo, and especially M. Guigueno, A. Westphal and T. Wood helped in the field. J. Nakoolak kept us safe from bears. L. Chivers, B. Delehanty, C. Ryan, J. Welcker and T. Williams provided very useful comments on an earlier draft of the manuscript. The manuscript was a chapter in KHE’s PhD dissertation and benefited from comments by committee members J. Anderson, K. Campbell and F. Schweizer. R. Armstrong at the Nunavut Research Institute, M. Mallory at the Canadian Wildlife Service Northern Research Division and C. Eberli at National Wildlife Research Centre in Ottawa provided logistical support. KHE received financial support through a NSERC Vanier Canada Graduate Scholarship, ACUNS Garfield Weston Northern Studies scholarship and AINA Jennifer Robinson Scholarship. Research support came from the NSERC (JFH, WGA), the Sigma Xi grant-in-aid of research (KHE), the Society of Canadian Ornithologists/Bird Studies Canada James Bailie Award (KHE), the American Ornithologists’ Union (KHE), the Northern Scientific Training Program (KHE, JFH), the M. J. Murdock Charitable Trust (KMO), the Environment Canada (AJG) and the Polar Continental Shelf Project (AJG). Any use of trade names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Apanius, V., Nisbet, I.C., 2006. Serum immunoglobulin G levels are positively related to reproductive performance in a long-lived seabird, the common tern (Sterna hirundo). Gen. Comp. Endocrinol. 147, 12–23.
Baranso, C.P., Bauman, G., 1989. Simple algebraic and graphic methods for the apportionment of hormone (and receptor) into bound and free fractions in binding equilibria; or how to calculate bound and free hormone? Endocrinology 124, 1101–1106.


