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

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ABSTRACT

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.

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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 Schrempf, 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 telomere

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dynamics better predict longevity than chronological age (Haussmann et al., 2005; Pauliny 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 (Jessop 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 partners, obtain sub-optimal breeding sites or are otherwise inexperienced breeders (de Forest and Gaston, 1996; Newton, 1998; Lewis et al., 2006; Angelier et al., 2007; Coulson, 2011; Hypothesis 2). Alternatively, older individuals may have habituated to stress (Hypothesis 3) or senescence may cause deterioration in adrenal tissue (Hypothesis 4), both of which may dampen stress responses. Finally, reduced levels of glucocorticoid hormones among older age classes may reflect the selective disappearance of phenotypes with high stress responses (Forslund & Pärt 1995; Hypothesis 5). It is important to emphasize that these five hypotheses are not mutually exclusive and multiple mechanisms may operate simultaneously.

We examined these five hypotheses in two long-lived, cliff-nesting seabirds. We predicted that if Hypothesis 1 was true, then diminished response to a stressful event should be proportional to future reproductive potential and the stress response should exhibit the greatest suppression late in life, when future reproductive prospects decline rapidly due to the accelerating probability of mortality. If Hypothesis 2 was true, then the stress response should be inversely proportional to average reproductive success at any age, and the rate of diminishment of the stress response should be highest early in life, when reproductive success increases rapidly. We predicted that if Hypotheses 3 or 4 were true, then pre-breeding birds should show similar age-related trends in their stress responses as breeding birds. 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 (Astheimer 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 habituation 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
Hypotheses tested in the current study.

Hypothesis	Prediction
1. Restraint for future opportunities	Stress response is proportional to residual reproductive success at a given age. Change only occurs in reproductive individuals.
2. Restraint when probability of current success is low	Stress response is inversely proportional to average reproductive success at a given age. Change only occurs in reproductive individuals.
3. Constraint via habituation	Stress response declines with age and is similar among reproductive and non-reproductive (pre-breeding) individuals.
4. Constraint via decay of adrenal tissue	Stress response declines with age and is similar among reproductive and non-reproductive (pre-breeding) individuals. Stress response declines with age following ACTH injection.
5. Selection against high-HPA individuals	Individuals with high stress responses have lower survival.

(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 murres) 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 (Coulson and Fairweather, 2001; Steiner et al., 2010; Coulson, 2011) and, with >100,000 individuals banded, the longevity record stands at 28 years (http://www.euring.org/data_and_codes/longevity-voous.htm). We sampled murres that were 3–29 years of age. Survival and repro-duction begins to decline after age 20 in murres (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 murres (reviewed in Elliott et al., 2014b). We therefore sampled over a suffi-cient 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 cor-ticosterone 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 murres 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 murres (44 controls) with 0.2 mL dexamethasone (Sigma D1756: 0.5 mg = kittiwakes; 1 mg = murres) immediately following the baseline blood sample and with 0.2 mL adrenocorticotropic hormone (Sigma A6303: 45 IU = kittiwakes; 100 IU = murres) 60 min later. We based ACTH dosages on a similar study in another charadriiform species (Heidinger et al., 2010). Five murres injected with 200 IU did not have a higher corticosterone response; increased ACTH dosage is more likely to increase the duration of the corticosterone response than its magnitude. We randomly selected every other bird (kittiwakes) or every third bird (murres) as ACTH-challenged birds. Control birds were injected with phosphate-buffered saline at each step. As described earlier, we obtained blood samples 60 min after dexamethasone and 30 min after ACTH injections. We used dexamethasone as a glucocorticoid agonist that binds to glucocorticoid receptors more readily than natural glucocorti-coids, inducing inhibitory feedback that reduces the production of ACTH and, therefore, corticosterone (Astheimer et al., 1994; Boonstra et al., 2001; Schmidt et al., 2012). Dexamethasone is used to minimize the confounding effect of endogenous ACTH release during ACTH challenge. Including sampling time (time since capture or last injection) and hours since sunrise within our models did not influence the statistical significance of any of our results.

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; Schultner 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 gluco-corticoid 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 (LS6500, 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: $F_{1,22}$ = 1.24, P = 0.32; murre: $F_{1,22}$ = 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 ^{125}I 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 murres, 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 corticoste-rone. A high responsiveness would be associated with a higher level of glucose for a given concentration of corticosterone.

Corticosterone-binding globulin and free corticosterone

Corticosterone-binding globulin (CBG) is a protein that binds corti-costerone 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 nM Tris buffer with 0.3% PEI. 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 (H_{free}) as:

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

where $K_d = 1.20 \pm 0.25$ nM for kittiwakes and 2.18 ± 0.32 nM for murres, B_{max} is the concentration of CBG molecules and H_{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 alone is key to maximizing reproductive success at our study sites.

We attached temperature loggers (Lotek 1100, 5 g or 0.5% of body mass) that recorded time flying and time at the colony (Elliott et al., 2008). The loggers were attached to the band using duct tape and total restraint was <5 min. Using the data from the logger, we recorded time to return to the colony following capture without blood-sampling (see Wilson and Gaston, 2001). To examine the relationship between corticosterone and investment in offspring, we recorded time to return to the offspring following restraint and blood-sampling for corticosterone analyses.

Metabolic rate

Alongside behavioural and endocrine responses, the stress response also involves a cardiovascular response that increases energy consumption by initiating a 'fight or flight' response that primes the body for active movement away from the stressor (Nephew et al., 2003; Lecomte, 2010; Wascher et al., 2011; Viblanc et al., 2012). Higher metabolic rate, associated with increase heart rate, is primarily associated with the sympathetic nervous system and may be regulated independently of behavioural or endocrine stress responses (Nephew et al., 2003). We examined metabolic rate during the first 10 min of capture to measure the cardiovascular or metabolic response to stress.

We placed post-absorptive, resting, thermoneutral birds in a small oxygen chamber and used a Fox Box II to measure oxygen consumption and carbon dioxide production over 4 h after removing water vapour

and, for the oxygen analyzer, carbon dioxide (same dataset as reported by Elliott et al., 2013, 2014b). We used Withers (2001) to convert measured percent oxygen into instantaneous oxygen consumption rate, and averaged values over five minute intervals. We then subtracted the lowest 30-minute average instantaneous oxygen consumption rate ("un-stressed resting metabolic rate") from the average instantaneous oxygen consumption rate over the first 10 min (two intervals). We expressed that value as a residual on body mass.

Reproductive success and survival

We measured reproductive success (number of young fledged per pair) by monitoring sites daily. We assumed that all chicks that left after day 40 (kittiwakes) or day 14 (murres) fledged 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 $\sum_{i=j+1}^{40} R_i(1 - M_i)$ 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), age² (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 age². 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 Akaike'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: $t_{79} = 6.23$, 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
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 (dexamethasone 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, age^2, sex, year (for those variables measured in more than one year) and recapture history.

	Baseline (free)		Max (free)		Max (total)		ACTH (free)		DEX (free)		Δ glucose		Return time		CBG		Calmness	
	t	P	t	P	t	P	t	P	t	P	t	P	t	P	t	P	t	P
Pre-breeding kittiwakes																		
Body mass	**−3.34**	**0.001**	1.40	0.18	1.62	0.11	0.94	0.45	1.84	0.05					−0.14	0.89		
Relative lay date	**2.99**	**0.004**	−1.75	0.10	−1.49	0.14	−0.88	0.51	−0.14	0.82					−0.6	0.55		
Age	0.44	0.66	−0.43	0.67	−0.81	0.42	0.45	0.66	−0.69	0.49					0.71	0.48		
Age^2	0.32	0.84	0.05	0.95	0.22	0.83	0.65	0.54	0.14	0.88					−0.24	0.81		
Sex	0.78	0.44	0.32	0.76	−0.14	0.89	0.15	0.87	1.02	0.31					0.28	0.78		
Recapture history	0.88	0.38	0.66	0.51	−0.28	0.78	0.55	0.71	0.14	0.88					0.38	0.71		
Kittiwakes (chicks)																		
Body mass	**−2.02**	**0.04**	1.28	0.21	1.43	0.16	0.86	0.40	1.03	0.30	0.03	0.98	−1.43	0.16	0.61	0.55	1.23	0.23
Relative hatch date	1.45	0.09	0.25	0.84	−0.81	0.42	1.01	0.31	0.14	0.88	0.35	0.73	1.34	0.19	−0.47	0.64	0.03	0.98
Age	−1.33	0.11	**−3.35**	**0.002**	−1.61	0.11	0.49	0.63	**−3.22**	**0.003**	**−2.04**	**0.04**	**−2.89**	**0.006**	2.01	0.05	1.94	0.05
Age^2	1.62	0.06	**2.45**	**0.02**	1.73	0.09	0.38	0.72	−0.03	0.98	**3.46**	**0.001**	**2.56**	**0.01**	−2.34	0.01	0.23	0.82
Sex	0.54	0.60	0.29	0.79	1.03	0.31	0.85	0.40	0.18	0.85	0.01	0.99	2.01	0.05	0.19	0.85	**2.45**	**0.02**
Year	0.17	0.90	0.25	0.85	0.53	0.6									0.35	0.73		
Recapture history	0.03	0.98	0.41	0.60	0.34	0.73	0.81	0.42	0.22	0.82	0.23	0.82	0.12	0.90	−0.66	0.51	1.62	0.11
Murres (chicks)																		
Body mass	−1.55	0.08	0.95	0.35	−1.56	0.12	−0.02	0.98	1.41	0.17	0.45	0.65	−1.57	0.12	0.96	0.34	0.29	0.80
Relative hatch date	0.55	0.61	−0.34	0.74	0.78	0.44	0.19	0.85	0.67	0.51	0.02	0.98	1.01	0.32	0.44	0.66	−0.68	0.50
Age	−1.57	0.07	**−2.31**	**0.03**	**−2.45**	**0.02**	1.01	0.32	**−2.42**	**0.02**	**−2.81**	**0.007**	**−3.88**	**0.001**	**2.05**	**0.04**	**4.21**	**0.001**
Age^2	1.58	0.07	**3.14**	**0.006**	**2.74**	**0.008**	0.71	0.50	**2.06**	**0.04**	**2.64**	**0.008**	**2.14**	**0.03**	−1.87	0.07	1.22	0.23
Sex	0.22	0.81	−0.04	0.95	0.39	0.7	0.55	0.59	0.45	0.66	0.67	0.26	−0.45	0.65	−0.48	0.63	1.46	0.15
Year	0.02	0.99	0.08	0.97	−0.37	0.71							0.14	0.90	−0.49	0.63	1.23	0.23

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

$t_{60} = 5.42$, P < 0.0001; kittiwakes: 1.24 ± 0.25 times higher; $t_{79} = 4.91$, 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 ($t_{70} = 1.26$, P = 0.21). Average reproductive success was more parsimonious at explaining variation in free CORT with age than residual reproductive success (kittiwake: ΔAIC = 18.43; murre: ΔAIC = 8.93; Fig. 1 resembles the inverse shape of Fig. 2a more closely than the shape of Fig. 2b).

Behaviour

Birds with lower stress-induced corticosterone were calmer during handling (chick-rearing kittiwakes: $t_{75} = 3.15$, P = 0.002; murres: $t_{41} = 2.04$, P = 0.04) and took less time to return to their chicks (chick-rearing kittiwakes: $t_{75} = 3.15$, P = 0.002; murres: $t_{36} = 5.06$, P < 0.0001). Return time declined with age before increasing again for the oldest age classes (Table 2, Fig. 1). Older birds tended to be calmer during handling (Table 2) and had a lower oxygen consumption rate (residual on body mass) during the first 10 min of handling relative to their long-term baseline (kittiwakes: $t_{32} = 2.24$, P = 0.03; murres: $t_{38} = 2.51$, P = 0.02). Reproductive success was highest at intermediate ages whereas residual reproductive success declined with age (Fig. 2).

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 dexamethasone, 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 because the birds in that study were not first injected with dexamethasone 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
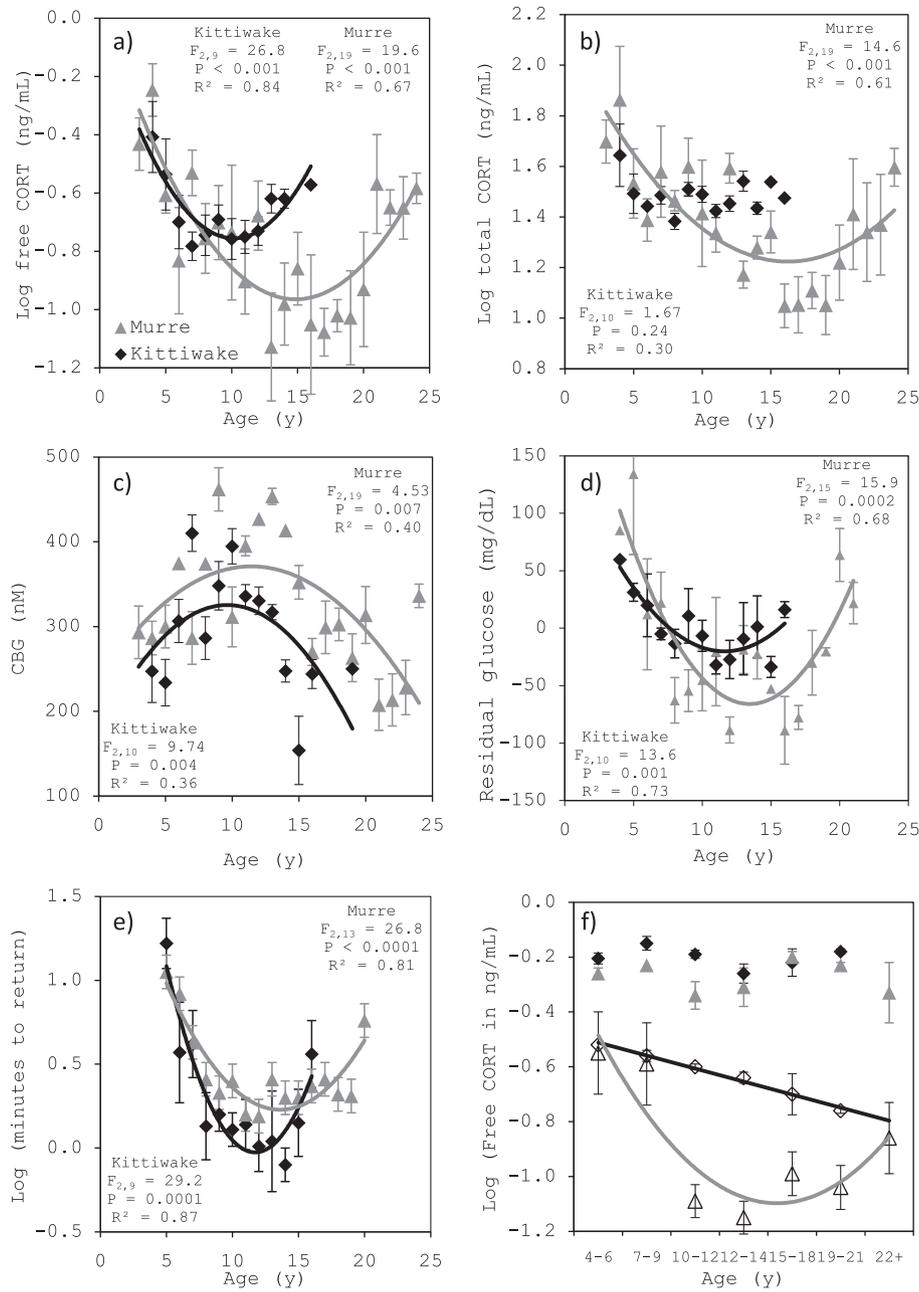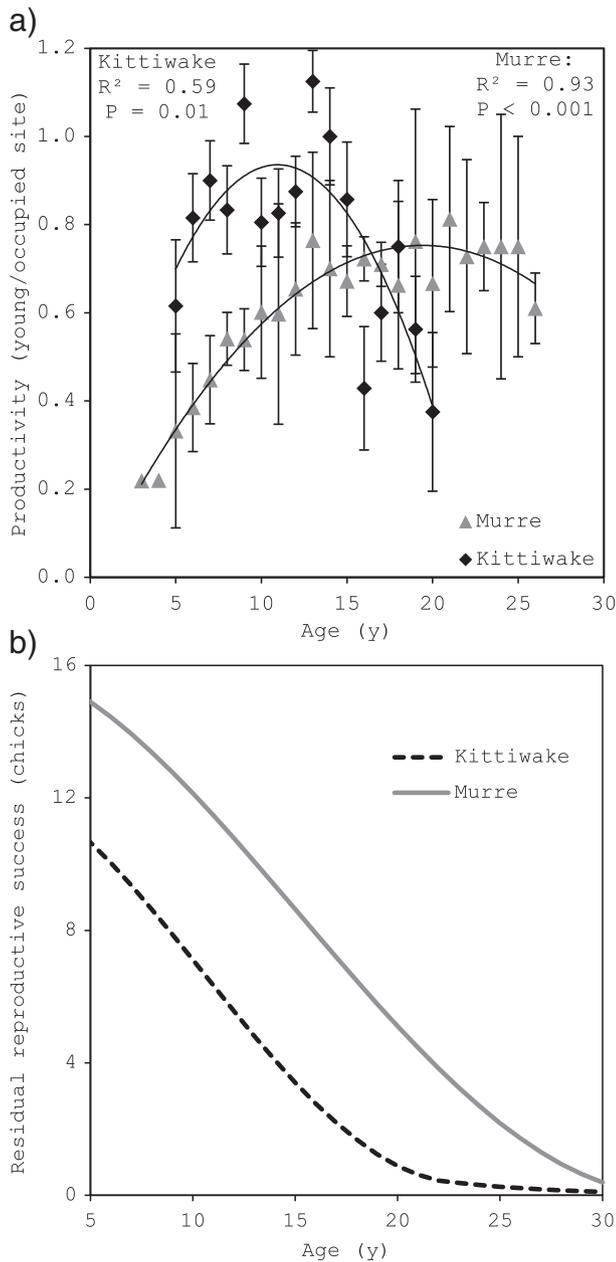
Fig. 1. (a) Free corticosterone, (b) total corticosterone and (c) corticosterone-binding globulin levels after 60 min ("maximal") of restraint, (d) residual of the change in circulating glucose (mg/dL) between capture and 60 minute restraint on free corticosterone levels after 60 min of restraint, (e) average return time to offspring after capture and restraint for <5 min, and (f) corticosterone levels 60 min after injection with dexamethasone (open symbols) and 30 min after injection with ACTH (closed symbols) relative to age in black-legged kittiwakes (diamonds) and thick-billed murres (triangles). Values are averages ± SE at each age class; the last age class includes all individuals in that age class or older due to the small sample sizes for old birds ("16" = 16+; age for pooling defined as the youngest age when only one individual is in an age category). All logarithms are base 10.

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

Fig. 2. (a) Average (±SE) reproductive success and (b) estimated likely residual reproductive success for kittiwakes and murres at our study sites, based on age-specific trends in reproductive success and survival, and assuming no reproductive success after age 30.

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.

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 (Otte et al., 2005; Lendvai et al., 2007; Heidinger et al., 2008; Angelier 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

Table 3
A comparison of age-related variation in glucocorticoid response and reproductive success across breeding vertebrates.

Species	Glucocorticoid response	Reproductive success	Reference
Green turtle *Chelonia mydas*	Higher in juvenile	Lower in juvenile	Jessop and Hamann (2005)
Human *Homo sapiens*	Increases with age	Declines with age	Reviewed by Otte et al. (2005)
Dog *Canus lupus*	Increases with age	Not reported	Reul et al. (1991)
Horse *Equus ferus*	Declines with age	Not reported	Liburt et al. (2013)
Rat *Rattus norvegicus*	Declines with age	Not reported	Hess and Riegle (1972); Brett et al. (1983)
Black-legged kittiwake	Lowest in middle age	Highest in middle age	Our study
Common tern *Sterna hirundo*	Declines with age	Increases with age	Heidinger et al. (2006)
Florida scrub-jay *Aphelocoma coerulescens*	Lowest in middle age	Highest in middle age	Wilcoxen et al. (2011)
Snow petrel *Pagodroma nivea*	Declines with age	Increases with age	Goutte et al. (2011) but see Angelier et al. (2007)
Thick-billed murre	Lowest in middle age	Highest in middle age	Our study
Wandering albatross *Diomedea exulans*	Lowest in middle age	Highest in middle age	Lecomte (2010)

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 glucrticoids 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 nestling 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.

References

Angelier, F., Moe, B., Weimerskirch, H., Chastel, O., 2007. Age-specific reproductive success in a long-lived bird: do older parents resist stress better? J. Anim. Ecol. 76, 1181–1191.
Angelier, F., Clément-Chastel, C., Welcker, J., Gabrielsen, G.W., Chastel, O., 2009. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. Funct. Ecol. 23, 784–793.
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*). Oecologia 147, 12–23.
Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1994. Gender and seasonal differences in the adrenocortical response to ACTH challenge in an Arctic passerine, *Zonotrichia leucophrys gambelii*. Gen. Comp. Endocrinol. 94, 33–43.
Barsano, 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.
Bateson, P., Bradshaw, E.L., 1997. Physiological effects of hunting red deer (*Cervus elaphus*). Proc. R. Soc. Lond. 264B, 1707–1714.
Bize, P., Criscuolo, F., Metcalfe, N.B., Nasir, L., Monaghan, P., 2009. Telomere dynamics rather than age predict life expectancy in the wild. Proc. R. Soc. Lond. B 276, 1–5.
Boonstra, R., 2004. Coping with changing northern environments: the role of the stress axis in birds and mammals. Integr. Comp. Biol. 44, 95–108.
Boonstra, R., McColl, C.J., Karels, T.J., 2001. Reproduction at all costs: the adaptive stress response of male arctic ground squirrels. Ecology 82, 1930–1946.
Brett, L.P., Chong, G.S., Coyle, S., Levine, S., 1983. The pituitary–adrenal response to novel stimulation and ether stress in young adult and aged rats. Neurobiol. Aging 4, 133–138.
Breuner, C.W., Orchinik, M., 2002. Beyond carrier proteins: plasma binding proteins as mediators of corticosteroid action in vertebrates. J. Endocrinol. 175, 99–112.

Breuner, C.W., Lynn, S.E., Julian, G.E., Cornelius, J.M., Heidinger, B.J., Love, O.P., Sprague, R.S., Wada, H., Whitman, B.A., 2006. Plasma-binding globulins and acute stress response. Horm. Metab. Res. 38, 260–268.

Breuner, C.W., Delehanty, B., Boonstra, R., 2013. Evaluating stress in natural populations of vertebrates: total CORT is not good enough. Funct. Ecol. 27, 24–36.

Carere, C., van Oers, K., 2004. Shy and bold great tits (Parus major): body temperature and breath rate in response to handling stress. Phys. Behav. 82, 905–912.

Carere, C., Caramaschi, D., Fawcett, T.W., 2010. Covariation between personalities and individual differences in coping with stress: converging evidence and hypotheses. Curr. Zool. 56, 728–740.

Chastel, O., Lacroix, A., Weimerskirch, H., Gabrielsen, G.W., 2005. Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird. Horm. Behav. 47, 459–466.

Cichon, M., Sendecka, J., Gustafsson, L., 2003. Age-related decline in humoral immune function in collared flycatchers. J. Evol. Biol. 16, 1205–1210.

Clary, D., Skyner, L.J., Ryan, C.P., Gardiner, L.E., Anderson, W.G., Hare, J.F., 2014. Shyness–boldness, but not exploration, predicts glucocorticoid stress response in Richardson's ground squirrels (Urocitellus richardsonii). Ethology 120, 1–9.

Cockrem, J.F., 2007. Stress, corticosterone responses and avian personalities. J. Ornithol. 148, 169–178.

Costantini, D., Ferrari, C., Pasquaretta, C., Cavallone, E., Carere, C., Hardenberg, A.V., Reale, D., 2012. Interplay between plasma oxidative status, cortisol and coping styles in wild alpine marmots, Marmota marmot. J. Exp. Biol. 215, 374–383.

Coulson, J., 2011. The Kittiwake. A&C Black, London UK.

Coulson, J.C., Fairweather, J.A., 2001. Reduced reproductive performance prior to death in the black-legged kittiwake: senescence or terminal illness? J. Avian Biol. 32, 146–152.

Crespin, L., Harris, M.P., Lebreton, J.D., Wanless, S., 2006. Increased adult mortality and reduced breeding success with age in a population of common guillemot Uria aalge using marked birds of unknown age. J. Avian Biol. 37, 273–282.

de Forest, L.N., Gaston, A.J., 1996. The effect of age on timing of breeding and reproductive success in the thick-billed murre. Ecology 77, 1501–1511.

Desprez, M., Pradel, R., Cam, E., Monnat, J.-Y., Gimenez, O., 2011. Now you see him, now you don't: experience, not age, is related to reproduction in kittiwakes. Proc. R. Soc. Lond. B 278, 3060–3066.

Elliott, K.H., Woo, K., Gaston, A.J., Benvenuti, S., Dall Antonia, L., Davoren, G.K., 2008. Seabird foraging behaviour indicates prey type. Mar. Ecol. Prog. Ser 354, 289–304.

Elliott, K.H., Shoji, A., Campbell, K.L., Gaston, A.J., 2010. Oxygen stores and foraging behavior of two sympatric. planktivorous auks Aquat. Biol 8, 221–235.

Elliott, K.H., Welcker, J., Gaston, A.J., Hatch, S., Palace, V., Hare, J.F., Speakman, J.R., Anderson, W.G., 2013. Thyroid hormones correlate with resting metabolic rate, not daily energy expenditure, in two charadriiform seabirds. Biol. Open http://dx.doi.org/10.1242/bio.20134358.

Elliott, K.H., Le Vaillant, M., Kato, A., Gaston, A.J., Ropert-Coudert, Y., Hare, J.F., Speakman, J.R., Croll, D.A., 2014a. Age-related variation in energy expenditure in a long-lived bird within the envelope of an energy ceiling. J. Anim. Ecol. 83, 136–146.

Elliott, K.H., Hare, J.F., Le Vaillant, M., Gaston, A.J., Ropert-Coudert, Y., Anderson, W.G., 2014b. Ageing gracefully: physiology but not behaviour declines with age in a diving seabird, Funct. Ecol. (in press) http://dx.doi.org/10.1111/1365-2435.12316.

Fernandez-Real, J.M., Pugeat, M., Grasa, M., Broch, M., Vendrell, J., Brun, J., Ricart, W., 2002. Serum corticosteroid-binding globulin concentration and insulin resistance syndrome: a population study. J. Clin. Endocrinol. Metab. 87, 4686–4690.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Forslund, P., Pärt, T., 1995. Age and reproduction in birds:hypotheses and tests. Trends Ecol. Evol 10, 374–378.

Froy, H., Phillips, R.A., Wood, A.G., Nussey, D.H., Lewis, S., 2013. Age-related variation in reproductive traits in the wandering albatross: evidence for terminal improvement following senescence. Ecol. Lett. 16, 642–649.

Gaston, A.J., Elliott, K.H., 2013. The effects of climate-influenced parasitism, predation and predator–predator interactions on the reproduction and survival of an Arctic marine bird. Arctic 66, 43–51.

Gaston, A.J., Gilchrist, H.G., Hipfner, J., 2005. Climate change, ice conditions and reproduction in an Arctic nesting marine bird: Brünnich's guillemot (Uria lomvia L.). J. Anim. Ecol. 74, 832–841.

Gilchrist, H.G., Gaston, A.J., Smith, J.N.M., 1998. Wind and prey nest sites as foraging constraints on an avian predator, the glaucous gull. Ecology 79, 2403–2414.

Gill, V.A., Hatch, S.A., 2002. Components of productivity in black-legged kittiwakes Rissa tridactyla: response to supplemental feeding. J. Avian Biol. 33, 113–126.

Goutte, A., Antoine, É., Chastel, O., 2011. Experimentally delayed hatching triggers a magnified stress response in a long-lived bird. Horm. Behav. 59, 167–173.

Hall, M.E., Nasir, L., Daunt, F., Gault, E.A., Croxall, J.P., Wanless, S., Monaghan, P., 2004. Telomere loss in relation to age and early environment in long-lived birds. Proc. R. Soc. Lond. 271B, 1571–1576.

Harris, B.N., Saltzman, W., 2013. Effects of aging on hypothalamic–pituitary–adrenal (HPA) axis activity and reactivity in virgin male and female California mice (Peromyscus californicus). Gen. Comp. Endocrinol. 186, 41–49.

Hatch, S.A., 2013. Kittiwake diets and chick production signal a 2008 regime shift in the Northeast Pacific. Mar. Ecol. Prog. Ser. 477, 271–284.

Haussmann, M.F., Winkler, D.W., O'Reilly, K.M., Huntington, C.E., Nisbet, I.C., Vleck, C.M., 2003. Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. Proc. R. Soc. Lond. 270B, 1387–1392.

Haussmann, M.F., Winkler, D.W., Vleck, C.M., 2005. Longer telomeres associated with higher survival in birds. Biol. Lett. 1, 212–214.

Heidinger, B.J., Nisbet, I.C.T., Ketterson, E.D., 2006. Older parents are less responsive to a stressor in a long-lived seabird: a mechanism for increased reproductive performance with age? Proc. Roy. Soc. B 273, 2227–2231.

Heidinger, B.J., Nisbet, I.C.T., Ketterson, E.D., 2008. Changes in adrenal capacity contribute to a decline in the stress response with age in a long-lived seabird. Gen. Comp. Endocrinol. 156, 564–568.

Heidinger, B.J., Chastel, O., Nisbet, I.C.T., Ketterson, E.D., 2010. Mellowing with age: older birds are less responsive to a stressor in a long-lived seabird. Funct. Ecol. 24, 1037–1044.

Heidinger, B.J., Blount, J.D., Boner, W., Griffiths, K., Metcalfe, N.B., Monaghan, P., 2012. Telomere length in early life predicts lifespan. Proc. Natl. Acad. Sci. U. S. A. 109, 1743–1748.

Heinze, J., Schrempf, A., 2012. Terminal investment: individual reproduction of ant queens increases with age. PLoS One 7, e5201.

Hess, G.D., Riegle, G.D., 1972. Effects of chronic ACTH stimulation on the adrenocortical function in young and aged rats. Am. J. Physiol. 222, 1458–1461.

Holmes, D., Martin, K., 2009. A bird's-eye view of aging: what's in it for ornithologists? Auk 126, 1–23.

Holmes, D.J., Ottinger, M.A., 2003. Birds as long-lived animal models for the study of aging. Exp. Gerontol. 38, 1365–1375.

Jacobs, S.R., Elliott, K.H., Gaston, A.J., Guigueno, M.F., Redman, P., Speakman, J.R., Weber, J.M., 2012. Determining seabird body condition using non-lethal measures. Physiol. Biochem. Zool. 85, 85–95.

Jessop, T.S., Hamann, M., 2005. Interplay between age class, sex and stress response in green turtles (Chelonia mydas). Aust. J. Zool. 53, 131–136.

Jodice, P.G.R., Lanctot, R.B., Gill, V.A., Roby, D.D., Hatch, S.A., 2000. Sexing adult black-legged kittiwakes by DNA, behavior, and morphology. Waterbirds 23, 405–415.

Kitaysky, A.S., Piatt, J.F., Hatch, S.A., Kitaiskaia, E.V., Benowitz-Fredericks, M., Shultz, M.T., Wingfield, J.C., 2010. Food availability and population processes: severity of nutritional stress during reproduction predicts survival of long-lived seabirds. Funct. Ecol. 24, 625–637.

Le Vaillant, M., Wilson, R.P., Kato, A., Saraux, C., Hanuise, N., Prud'Homme, O., et al., 2012. King penguin adjust their diving behaviour with age. J. Exp. Biol. 215, 3685–3692.

Lecomte, V., 2010. La sénescence en milieu naturel: une etude pluridisciplinaire chez deux oiseaux marins longévifs, le grand albatross et le pétrel des neiges. University of Poitiers, University of Poitiers Ph.D. thesis.

Lecomte, V.J., Sorci, G., Cornet, S., Jaeger, A., Faivre, B., Arnoux, E., et al., 2010. Patterns of aging in the long-lived wandering albatross. Proc. Natl. Acad. Sci. U. S. A. 107, 6370–6375.

Lendvai, Á.Z., Giraudeau, M., Chastel, O., 2007. Reproduction and modulation of the stress response: an experimental test in the house sparrow. Proc. Roy. Soc. B 274, 391–397.

Lewis, S., Wanless, S., Elston, D.A., Schultz, M.D., Mackley, E., du Toit, M., Underhill, J.G., Harris, M.P., 2006. Determinants of quality in a long-lived colonial species. J. Anim. Ecol. 75, 1304–1312.

Liburt, N.R., McKeever, K.H., Malinowski, K., Smarsh, D.N., Geor, R.J., 2013. Responses of the hypothalamic–pituitary–adrenal axis to stimulation tests before and after exercise training in old and young Standardbred mares. J. Anim. Sci. 91, 5208–5219.

Love, O.P., Breuner, C.W., Vezina, F., Williams, T.D., 2004. Mediation of a corticosterone-induced reproductive conflict. Horm. Behav. 46, 59–65.

McNamara, J.M., Houston, A.I., Barta, Z., Scheuerlein, A., Fromhage, L., 2009. Deterioration, death and the evolution of reproductive restraint in late life. Proc. R. Soc. B 276, 4061–4066.

Mendel, C.M., 1989. The free hormone hypothesis: a physiologically based mathematical model. Endocr. Rev. 10, 232–274.

Mizutani, Y., Tomita, N., Kazama, K., Takahashi, H., Hasegawa, O., Niizuma, Y., 2009. Relationship between telomere length and age in black-tailed gull. Jpn. J. Ornithol. 58, 192–195.

Monaghan, P., 2014. Organismal stress, telomeres and life histories. J. Exp. Biol. 217, 57–66.

Nephew, B.C., Kahn, N., Romero, L.M., 2003. Heart rate and behaviour are regulated independently of corticosterone following diverse acute stressors. Gen. Comp. Endocrinol. 133, 173–180.

Newton, I., 1998. Population Limitation in Birds. Academic Press, New York.

Nisbet, I.C.T., 2001. Detecting and measuring senescence in wild birds: experience with long-lived seabirds. Exp. Gerontol. 36, 833–843.

Nisbet, I.C.T., Apanius, V., Friar, M.S., 2002. Breeding performance of very old common terns. J. Field Ornithol. 73, 117–124.

Otte, C., Hart, S., Neylan, T.C., Marmar, C.R., Yaffe, K., Mohr, D.C., 2005. A meta-analysis of cortisol response to challenge in human aging: importance of gender. Psychoneuroendocrinology 30, 80–91.

Ouyang, J.Q., Quetting, M., Hau, M., 2012. Corticosterone and brood abandonment in a passerine bird. Anim. Behav 84, 261–268.

Palacios, M.G., Cunnick, J.E., Winkler, D.W., Vleck, C.M., 2007. Immunosenescence in some but not all immune components in a free-living vertebrate, the tree swallow. Proc. R. Soc. B Biol. Sci 274, 951–957.

Pauliny, A., Wagner, R.H., Augustin, J., Szép, T., Blomqvist, D., 2006. Age-independent telomere length predicts fitness in two bird species. Mol. Ecol. 15, 1681–1687.

Perogamvros, I., Kayahara, M., Trainer, P.J., Ray, D.W., 2011. Serum regulates cortisol bioactivity by corticosteroid-binding globulin-dependent and independent mechanisms, as revealed by combined bioassay and physicochemical assay approaches. Clin. Endocrinol. 75, 31–38.

Rattiste, K., 2004. Reproductive success in presenescent common gulls (Larus canus): the importance of the last year of life. Proc. R. Soc. Lond. 271B, 2059–2065.

Reed, T.E., Kruuk, L.E., Wanless, S., Frederiksen, M., Cunningham, E.J., Harris, M.P., 2008. Reproductive senescence in a long-lived seabird: rates of decline in late-life performance are associated with varying costs of early reproduction. Am. Nat. 171, E89–E101.

Reul, J.M., Rothuizen, J., de Kloet, E.R., 1991. Age-related changes in the dog hypothalamic–pituitary–adrenocortical system: neuroendocrine activity and corticosteroid receptors. J. Steroid Biochem. Mol. Biol. 40, 63–69.

Ricklefs, R.E., 2010. Insights from comparative analysis of aging in birds and mammals. Aging Cell 9, 273–284.

Riechert, J., Chastel, O., Becker, P.H., 2012. Why do experienced birds reproduce better? Possible endocrine mechanisms in a long-lived seabird, the common tern. Gen. Comp. Endocrinol 178, 391–399.

Saino, N., Ferrari, R.P., Romano, M., Rubolini, D., Möller, A.P., 2003. Humoral immune response in relation to senescence, sex and sexual ornamentation in the barn swallow (*Hirundo rustica*). J. Evol. Biol. 16, 1127–1134.

Salomons, H.M., Mulder, G.A., van de Zande, L., Haussmann, M.F., Linskens, M.H., Verhulst, S., 2009. Telomere shortening and survival in free-living corvids. Proc. R. Soc. Lond. 276B, 3157–3165.

Sapolsky, R.M., Altmann, J., 1991. Incidence of hypercortisolism and dexamethasone resistance increases with age among wild baboons. Biol. Psychiatry 30, 1008–1016.

Satterthwaite, W.H., Kitaysky, A.S., Hatch, S.A., Piatt, J.F., Mangel, M., 2010. Unifying quantitative life-history theory and field endocrinology to assess prudent parenthood in a long-lived seabird. Evol. Ecol. Res. 12 (6), 779–792.

Schmidt, K.L., Furlonger, A.A., Lapierre, J.M., MacDougall-Shackleton, E.A., MacDougall-Shackleton, S.A., 2012. Regulation of the HPA axis is related to song complexity and measures of phenotypic quality in song sparrows. Horm. Behav. 61, 652–659.

Schultner, J., Kitaysky, A.S., Welcker, J., Hatch, S., 2013a. Fat or lean: adjustments of endogenous energy stores to predictable and unpredictable changes in allostatic load. Funct. Ecol. 27, 45–55.

Schultner, J., Kitaysky, A.S., Gabrielsen, G.W., Hatch, S.A., Bech, C., 2013b. Differential reproductive responses to stress reveal the role of life-history strategies within a species. Proc. R. Soc. B 280, 20132090.

Seltmann, 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.

Smith, P.A., Gaston, A.J., 2012. Environmental variation and the demography and diet of thick-billed murres. Mar. Ecol. Prog. Ser. 454, 237–249.

Stearns, S., 1992. The Evolution of Life-Histories. Oxford University Press, New York.

Steiner, U.K., Tuljapurkar, S., Orzack, S.H., 2010. Dynamic heterogeneity and life history variability in the kittiwake. J. Anim. Ecol. 79, 436–444.

Suomi, S.J., Novak, M.A., Well, A., 1996. Aging in rhesus monkeys: different windows on behavioral continuity and change. Dev. Psychol. 32, 1116–1128.

Thierry, A.-M., Massemin, S., Handrich, Y., Raclot, T., 2013. Elevated corticosterone levels and severe weather conditions decrease parental investment of incubating Adélie penguins. Horm. Behav. 63, 475–483.

Turbill, C., Ruf, T., 2010. Senescence is more important in the natural lives of long- than short-lived mammals. PLoS One 5, e12019.

Velando, A., Drummond, H., Torres, R., 2006. Senescent birds redouble reproductive effort when ill: confirmation of the terminal investment hypothesis. Proc. R. Soc. Lond. 273, 1443–1448.

Viblanc, V.A., Smith, A.D., Gineste, B., Groscolas, R., 2012. Coping with continuous human disturbance in the wild: insights from penguin heart rate response to various stressors. BMC Ecol. 12, 10.

Vincenzi, S., Hatch, S., Mangel, M., Kitaysky, A., 2013. Food availability affects onset of reproduction in a long-lived seabird. Proc. R. Soc. B 280, 20130554.

Wada, H., Hahn, T.P., Breuner, C.W., 2007. Development of stress reactivity in white-crowned sparrow nestlings: total corticosterone response increases with age, while free corticosterone response remains low. Gen. Comp. Endocrinol. 150, 405–413.

Wascher, C.A.F., Scheiber, I.B.R., Weiss, B.M., Kotrschal, K., 2011. Heart rate responses to induced challenge situations in greylag geese (*Anser anser*). J. Comp. Psychol. 125, 116–119.

Withers, P.C., 2001. Design, calibration and calculation for flow-through respirometry systems. Aust. J. Zool 49, 445–461. http://dx.doi.org/10.1071/ZO00057.

Wilcoxen, T.E., Boughton, R.K., Bridge, E.S., Rensel, M.A., Schoech, S.J., 2011. Age-related differences in baseline and stress-induced corticosterone in Florida scrub-jays. Gen. Comp. Endocrinol. 173, 461–466.

Williams, G.C., 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11, 398–411.

Wilson, L., Gaston, A.J., 2001. Effects of handling stress on Brunnich's guillemots *Uria lomvia*. Ringing Migr. 20, 320–327.

Wingfield, J.C., Kitaysky, A.S., 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr. Comp. Biol. 42, 600–609.

Young, R.C., Kitaysky, A.S., Haussmann, M.F., Descamps, S., Orben, R.A., Elliott, K.H., Gaston, A.J., 2013. Age, sex, and telomere dynamics in a long-lived seabird with male-biased parental care. PLoS One 8, e74931.