View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Helsingin yliopiston digitaalinen arkisto

1 **Nest cover and faecal glucocorticoid metabolites are linked to hatching success and**
2 **telomere length in breeding eiders (*Somateria mollissima*)**
3
4 K. Noreikiene, M. Öst, M.W. Seltmann, W. Boner, P. Monaghan and K. Jaatinen
5
6 K. Noreikiene (corresponding author), Ecological Genetics Research Unit, Department of Biosciences,
7 University of Helsinki, PO Box 65, FI-00014, Helsinki, Finland; phone +358417248196;
8 kriste.noreikiene@gmail.com
9 M. Öst, Environmental and Marine Biology, Faculty of Science and Engineering, Åbo Akademi
10 University, Artillerigatan 6, FI-20520 Turku, Finland and Novia University of Applied Sciences,
11 Raseborgsvägen 9, FI-10600, Ekenäs, Finland; most@abo.fi
12 M. W. Seltmann, Department of Biology, University of Turku, FI-20014 Turku, Finland;
13 martin.seltmann@utu.fi
14 W. Boner, Institute of Biodiversity, Animal Health and Comparative Medicine, College of
15 Medical,Veterinary and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12
16 8QQ, UK; Winnie.Boner@glasgow.ac.uk
17 P. Monaghan, Institute of Biodiversity, Animal Health and Comparative Medicine, College of
18 Medical,Veterinary and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12
19 8QQ, UK; Pat.Monaghan@glasgow.ac.uk
20 K. Jaatinen, Tvärminne Zoological Station, University of Helsinki, Finland; kim.jaatinen@helsinki.fi
21

22 **ABSTRACT**

23 Habitat-associated crypsis may affect perceived predation vulnerability, selecting for different predator
24 avoidance strategies. Glucocorticoids could mediate the adjustment of escape responses to the extent of
25 crypsis, introducing an overlooked source of variation in glucocorticoid-fitness relationships. However,
26 prolonged exposure to elevated glucocorticoids may be costly leading to accelerated telomere loss and
27 consequently senescence. Here, we examined how nest cover and immunoreactive faecal glucocorticoid
28 metabolite levels (fGCM) are linked to hatching success and telomere length in breeding female eiders
29 (*Somateria mollissima* Linnaeus, 1758). We hypothesized that the degree of nest crypsis, reflecting
30 differences in perceived predation risk, would moderate the relationship between reproductive success
31 and fGCM levels. We also expected that telomere length would be shorter in birds with higher
32 glucocorticoid concentration. Results showed thatindividuals with high fGCM levels had higher
33 hatching success in nests with low cover, while low fGCM levels were more successful in well-
34 concealed nests.We found that shorter telomeres were associated with high fGCM in nesting sites
35 offering little cover and with low fGCM in well-concealed ones.This study provides the first evidence of
36 habitat-dependent moderation of the relationships between stress physiology, telomere length and
37 hatching success.

38 **Keywords:**

39 Cost of reproduction, eider, glucocorticoids, nesting habitat, *Somateria mollissima*

40

41 **INTRODUCTION**

42 Glucocorticoid (GC) stress hormones are considered to play a key role in integrating information about
43 environmental challengesand in mediating inter-individual differences in fitness (Ricklefs and Wikelski
44 2002; Boonstra 2013). So far, however, no consensus has emerged on the direction of the relationship
45 between GCs and fitness in natural populations (Wingfield and Sapolsky 2003; Bonier et al. 2009*a*, *b*,
46 Crossin et al. 2012; Jaatinen et al. 2013). This uncertainty is perhaps not surprising given that a negative
47 relationship between GCs and fitness is often assumed *a priori*, based on evidence from biomedical
48 research (Boonstra 2013). Thus, prolonged exposure to high concentrations of GCs is considered to be
49 costly and subject individuals to pathologies (Ricklefs and Wikelski 2002). In contrast to laboratory
50 models, however, wild animals are exposed to a diverse array of stressors, including predation risk. A
51 short-term increase in GC levels is a vital response immediately before, during and after a predatory
52 attack (reviewed by Wingfield et al. 1998). Less known and appreciated is the fact that circulating
53 higher baseline GC concentrations may be adaptive whenever the risk of stress exposure is high
54 ('preparative hypothesis'; Romero 2002), as GCs may prepare the organism to perform better under
55 such circumstances (Sapolsky et al. 2000). Thereby, long-term elevation of GCs may serve to prepare
56 prey for attacks by predators, for instance, by increasing vigilance (Scheuerlein et al. 2001; Cockrem
57 and Silverin 2002; Hawlena and Schmitz 2010). If the benefits of such preparative responses outweigh
58 the costs, an increase in GCs can be adaptive and continue to promote fitness (Boonstra 2013).
59 Therefore, the ambiguity in the relationship between GCs and fitness may partly reflect our incomplete
60 understanding of stress coping strategies in the wild.

61

62 The ability of the breeding habitat to provide protection has received little attention as a contextual
63 factor affecting the relationship between GCs and fitness (Crespi et al. 2013; but see D'Alba et al. 2011).
64 The degree of crypsis provided by the nest site is inexorably linked to the optimal predator avoidance

65 tactic. The optimal solution in well-concealed nest sites may be to rely on crypsis and down-regulate
66 escape behaviour (Amat and Masero 2004; Albrecht and Klvaňa 2004), whereas preparing for predatory
67 attacks by maintaining escape performance at a high level may maximize breeding success in less-
68 concealed nests (Merilaita et al. 1999). Such increased vigilance and escape performance has been
69 linked to high baseline GC levels (Sapolsky et al. 2000; Chin et al. 2009; Thaker et al. 2010).
70 Experimental evidence suggests that the nest characteristics themselves may not directly affect the stress
71 hormone levels of breeding birds, but rather that the breeders adopt different strategies of nest-site
72 selection depending on their phenotypic traits (D'Alba et al. 2011). Although a parent selecting a poorly
73 concealed nest site may have high GC levels due to high perceived predation risk, this physiological
74 response may in fact represent an appropriate predatory avoidance strategy enhancing reproductive
75 success (Boonstra 2013). While such a response may be adaptive, increased GCs may still have
76 detrimental effects on the condition and future reproductive potential of individuals in long-lived species
77 (Johnson 2007; Haussmann and Marchetto 2010).
78
79 The ability to cope with external and internal challenges varies widely between individuals (Wilson and
80 Nussey 2010) and this variation may be associated with habitat choice (e.g., D'Alba et al. 2011). Despite
81 this, only a few studies have considered the intrinsic stress tolerance quality of individuals occupying
82 different habitats (e.g., Germain and Arcese 2014). Thus, we still know very little about fitness value
83 associated with a given breeding habitat. Telomeres, nucleoprotein structures located at the ends of
84 chromosomes, hold promise as a composite indicator of physiological stress associated with internal and
85 external challenges (Mizutani et al. 2013; Young et al. 2015; LeVaillant et al. 2015). In general,
86 individuals with longer than average telomeresfor their age have longer life expectancy (Heidinger et al.
87 2012; Barrett et al. 2013; Angelier et al. 2013), a higher number of functional cells (Monaghan and
88 Haussmann 2006; Monaghan 2014), and higher stress resistance (Kotrschal et al. 2007). However,

89 chronically elevated GCs can accelerate telomere shortening (von Zglinicki 2002; Epel et al. 2004; Choi
90 et al. 2008; Haussmann and Marchetto 2010). Because the typically high reproductive investment by
91 good-quality individuals may be facilitated by elevated GC levels (Crossin et al. 2012), such investment
92 may incur costs in terms of accelerated telomere attrition (Reichert et al. 2014, Schultner et al. 2014;
93 Sudyka et al. 2014). However, breeding animals interact with their chosen breeding habitat and also
94 telomere dynamics have been linked to habitat choice (Angelier et al. 2013). This adds a previously
95 unappreciated level of complexity to the interrelationships between reproductive success, glucocorticoid
96 stress physiology and telomere dynamics. Increased GC levels may facilitate reproduction and offspring
97 care and can help individuals adjust their antipredatory behavior depending on the habitat-specific risk
98 of predation. Thus, while the immediate benefits of elevated GCs are evident, elevated GC levels have
99 also been shown to carry long-term costs in the form of telomere shortening and subsequently lowered
100 survival (Kotrschalet al. 2007).
101
102 To bring clarity to these issues, we explored potential links between the degree of visual nest
103 concealment and stress physiology, telomere length and breeding success. We hypothesized interactive
104 effects of nest cover and GCs on breeding success: low nest cover is associated with higher perceived
105 predation risk than covered nests and thus the optimal antipredatory response may differ between
106 degrees of nest concealment. We predicted that individuals with higher GC, and thereby presumably
107 enhanced anti-predator responsiveness, would have the greatest reproductive output in poorly-concealed
108 nests facilitating rapid escape, whereas individuals attaining high reproductive success in concealed
109 nests would exhibit lower GC levels and rely on crypsis instead of escape. We also hypothesized that
110 high levels of reproductive performance, either in association with elevated GC levels or independently,
111 may be linked to shorter telomeres (see Bauch et al. 2013).
112

113 As a model system, we used female eider ducks (*S. mollissima*) from a well-studied population in south-
114 western Finland. The eider is an excellent study species. Female eiders rely to a large extent on stored
115 energy resources during incubation (Parker and Holm 1990; Bolduc and Guillemette 2003; but see
116 Hobson et al. 2015; Jaatinen et al. 2016). These limited resources can be mobilized by GCs and
117 consistent individual differences in baseline GC profilesare associated with individual differences in
118 current reproductive success (Jaatinen et al. 2013), and thus potentially also long-term fitness. Also,
119 females show fidelity to nest sites (Öst et al. 2011; Ekroos et al. 2012) and the degree of nest cover is
120 repeatable between years (Öst and Steele 2010; Seltmann et al. 2014). Further, incubating females
121 encounter a spatially and temporally varying risk of attack by predators, posing a considerable threat for
122 this ground-nesting bird (Ekroos et al. 2012). The relationship between the acute (handling-induced)
123 stress response and reproductive investment has previously been shown to be modulated by predation
124 risk (Jaatinen et al. 2014). Finally, the number of years of maternal experience, a proxy for age, does not
125 explain the variability in telomere length observed among adult eider females (this study), and thus age
126 is not likely to confound or mask the associations under focus here.
127
128 A previous experimental study on female eiders showed that nest shelter did not affect baseline plasma
129 GC levels (D'Alba et al. 2011). However, this study also suggested that nest habitat was not independent
130 of individual quality and that the relationship between hatching success and CORT was affected by
131 female body condition, i.e., it was state-dependent. This study explores these possibilities further by first
132 assessing the relationships between nest cover, female hatching success and faecal glucocorticoid
133 metabolite (fGCM) level, an accumulative index of stress (Möstl et al. 2005). fGCMs provide a more
134 integrated measure of adrenocortical activity than point serum samples and thus diminish the influence
135 of temporal changes in GC secretion (Whitten et al. 1998). Thereafter, we examined the associations
136 between individual biological state, as quantified by telomere length, nest-site cover and fGCM. In all

137 analyses we also controlled for other potentially important predictors of hatching success and telomere

138 length, including female breeding experience, body condition and timing of breeding.

139

140 **MATERIALS AND METHODS**

141

142 *Field methods*

143

144 (a) Study area and population

145 The study was conducted at Tvärminne Zoological Station (59˚50´N, 23˚15´E), in the western Gulf of

146 Finland, in 2009-2011. The 23 study islands were represented by 9 forested island and 14open rocky

147 islets. Nest cover is highly variable on both island types as females readily nest under trees, bushes such

148 as junipers (which are often abundant also on open islands), rock outcrops or concealed in grassy

149 vegetation. Annually all study islands are searched through with equal thoroughness so that all nesting

150 events are recorded. The number of nests on the islands ranged between 0-94 (mean ± *SD* = 15.7 ± 2.1)

151 during the study period (Jaatinen et al. 2014). Female eiders in the study population nest at low densities

152 and previous evidence suggests that nest-site selection is not affected by female competition or nest-site

153 limitation (Öst et al. 2008; Öst and Steele 2010; Ekroos et al. 2012; Seltmann et al. 2014), which

154 contrasts with the situation described for eiders in other populations that nest in dense colonies (e.g.,

155 D'Alba et al. 2011). Thus, in our current sample of individuals, there was no association between nest

156 cover and the onset of breeding (linear mixed model: b = 0.001, SD = 0.002, t = 0.303 p > 0.05, N

157 (observations/females) = 472/346) or between nest cover and body condition (linear mixed model: b =

158 0.018, SD = 0.012, t = 1.478, p > 0.05, N (observations/females) = 472/346). Therefore, it is reasonable

159 to assume that individual quality does not create a significant confounding effect on initial nest-site

160 selection, which instead represents the outcome of an active decision-making process when females

161 come to the area to breed for the first time. In contrast, nest-site choices in subsequent breeding seasons
162 may to some degree be constrained by high fidelity to the particular breeding island (Öst et al. 2011).
163 The low breeding dispersal has been identified as a putative ecological trap for females (Ekroos et al.
164 2012). Thus, despite an increased propensity to switch nest sites after events of nest depredation (Öst et
165 al. 2011), females still exhibit high breeding philopatry regardless of the increased predation pressure on
166 adults by white-tailed sea eagles (*Haliaeetus albicilla* Linnaeus, 1758), eagle owls (*Bubo bubo*
167 Linnaeus, 1758) and several mammalian predators. On average open islands are subjected to higher
168 predation pressure than islands with a forest cover (Ekroos et al. 2012). In a short term, spatial predation
169 patterns may change due to predator movement between islands and this type of variation is more
170 pronounced that temporal variation in predation pressure (Öst et al. 2011)
171
172 **(b) Female trapping and measurements**
173 We captured nesting eider females (535 captures of 381 individuals) on their nests by using hand nets.
174 Captured females were weighed with a spring balance to the nearest 10g, measured for structural size
175 (length of the radius-ulna to the nearest 1mm), ringed, and their clutch size was recorded. Clutch size
176 varied between 2-7 eggs (mean ± *SD* = 4.69 ± 1.16). Ducklings are not ringed in this population and
177 hence female age could not be directly determined; therefore the ringing information was used to
178 calculate the number of years since the bird was first trapped, indicating minimum years of maternal
179 experience (Öst et al. 2008; Öst and Steele 2010; Jaatinen and Öst 2011; Jaatinen et al. 2012). This is a
180 reasonably good proxy for female age in the population due to the high breeding philopatry and the fact
181 that more than half of the breeding females in the population are captured annually, with a relatively
182 constant annual trapping effort since 1996 (Jaatinen and Öst 2011).
183
184 We obtained female blood samples by extracting approximately 1 ml of peripheral blood from the

185 brachial vein. Faecal samples were collected in Whirl-Paks (Nasco) directly from the female or by
186 gathering fresh faeces from the nest, and both blood and faecal samples were immediately stored on ice
187 in a cool box and transported to the laboratory within 2–4 *h*. Blood samples were centrifuged in a cold
188 centrifuge (Sigma 3K12, B. Broun, Germany) for 10 min at 1500×g to separate blood serum and cells.
189 Blood cells and faecal samples were stored frozen in –20°C until further analyses. Faecal samples
190 collected during 2009-2011 (*N*=514/369) were used for immunoreactive fGCM measurement, whereas
191 blood cells were collected only in 2011 (*N*=197) and subsequently used for telomere measurement.
192
193 We used egg floatation to determine the incubation stage at female capture (Kilpi and Lindström 1997).
194 Information on incubation stage was used to calculate a body condition index since eider females refrain
195 completely from feeding during the incubation period and lose up to 40% of their pre-laying body mass
196 (e.g., Parker and Holm 1990). Body condition indices were determined for all trapped females that had
197 been incubating eggs for at least 8 days (egg laying may otherwise not have been completed; Öst et al.
198 2008). The index was given by the standardized residuals of a regression of log-transformed projected
199 weight at hatching (response variable) on log-transformed radius–ulna length, and indices were derived
200 separately for each year (Öst and Steele 2010). A female's body mass at hatching was estimated by
201 subtracting an estimate of the expected body mass loss during the remaining incubation time from her
202 measured incubation body mass. Females were weighed once, but as females abstain from feeding
203 during incubation and females were captured at different times in their incubation, we can derive an
204 estimate of average mass loss rate during incubation as the slope of the regression of log-transformed
205 body mass (response variable) on log-transformed incubation time and projected hatching date (Öst et
206 al. 2008). The assumption of continued mass loss after female capture applies to our study population,
207 which makes this index reliable for estimating body condition (Öst and Steele 2010).
208

209 Hatching success was determined upon subsequent nest visits that were timed to coincide with the
210 expected hatching of the clutch, based on estimated incubation stage at trapping. Successful hatching
211 was determined by observing live ducklings in the nests. If the female had left the nest with her brood
212 prior to our arrival, we observed whether the egg shells remaining in the nest had intact egg membranes,
213 indicating successful hatching (Öst and Steele 2010). Such egg shell remnants can be distinguished from
214 those left after nest depredation. Thus, we were able to precisely determine the fate of the nests for the
215 majority of trapped females (*N*=449observations (333 females) out of 535 (381 females), 83.9%). Of the
216 nests with known fates in 2009-2011 (Supplementary Table 1), the majority were successful (at least one
217 egg hatched; annual mean ± *SD* = 63.1 ± 15.1%), nearly one-third were depredated (29.2 ± 13.3%),
218 while only a small fraction were abandoned (7.7 ± 6.2%). For all nests, we recorded the number of
219 successfully hatched eggs (duckling has hatched and survived to leave the nest) and unhatched eggs (the
220 number of eggs that failed to hatch due to depredation and abandonment, and to a lesser extent
221 inviability). Hatching success of undisturbed eider nests is high at ca 90 %, showing low variability
222 among clutches (Swennen 1989), and thus the small fraction of inviable eggs is unlikely to
223 systematically bias our results. All nests with known fates were included in subsequent analyses (2009-
224 2011; *N*=449/333). To quantify spatial and temporal variation in predation risk, we calculated an annual
225 island-specific predation index. This index was given by dividing the number of depredated nests with
226 the total number of censused nests on a given island in each year (Öst et al. 2011).
227 Nestcover was quantified by taking hemispherical digital photographs witha Olympus C-740 camera
228 equipped with a 42-mm Opteka fisheye lens. All nest photographs were taken right after females had
229 hatched their broods to reduce bias caused by vegetation growth. Hemispherical images were taken by
230 placing an upward facing camera on a stable surface in the nest (Öst and Steele 2010). We used the
231 program Image Tool (v. 3.00; University of Texas Health Science Center, San Antonio) to process nest
232 cover photographs. Firstly, images were converted to grey scale and pixels assigned as black or white so

233 that vegetation and other elements such as rocks providing cover were coded as black pixels whereas
234 areas of open sky were coded as white pixels. Nest cover was then calculated as the proportion of black
235 pixels in the image.
236
237 *Laboratory methods*
238 **(a) Telomere measurement**
239 Relative telomere length normalized for a non-variable copy gene (T/S), was measured in red blood
240 cells (RBC), and corresponds to the average telomere length across chromosomes (Cawthon 2002). The
241 length of telomeres in RBC reflects the telomere length of hematopoietic stem cells (Vaziri et al. 1994)
242 and has been shown to correlate with telomere length in other tissues (Reichert et al. 2013). We obtained
243 a relative telomere length measure (T/S) by using the real-time quantitative PCR (qPCR) method as
244 proposed by Cawthon (2002) and previously validated for use in birds (Criscuolo et al. 2009). Results
245 from the qPCR correlate well with the results obtained by terminal restriction fragment (TRF) analysis,
246 the conventional method of telomere measurement, and qPCR has successfully been used in a growing
247 number of studies (e.g., Criscuolo et al. 2009; Bize et al. 2009; Aviv et al. 2011; Heidinger et al. 2012).
248
249 Genomic DNA for the assay was extracted from 5µl of RBC following the protocol by Aljanabi and
250 Martinez (1997). DNA integrity was determined by agarose gel electrophoresis where 50ng of
251 undigested DNA were resolved in 1.5%agarose gel at 120V for 90min, and DNA purity and
252 concentration was measured spectrophotometrically using a NanoDrop 2000 (ThermoFisher Scientific).
253 Only intact samples, appearing as a tight crown migrating in parallel, and with a A260/A280 ratio >1.7
254 were accepted for further analyses. Because telomere length has not previously been measured in eiders,
255 we first validated the assay. We selected the *gapdh* gene (glyceraldehyde 3-phosphate dehydrogenase) to
256 test whether it could function as the non-variable copy gene in the qPCR assay. Primers which were

257 originally developed for chickens (genbank accession number: NW_001471525): *gapdhFw.*: (5′-

258 TCCTGTGACTTCAATGGTGA-3′) and *gapdh Rev.*: (5′-AAACAAGCTTGACGAAATGG-3′) also

259 successfully amplified the *gapdh* gene fragment in eiders, which resulted in a single DNA band of the

260 expected size (80bp) when visualized on an agarose gel. Negative control reactions, without template

261 DNA, showed no detectable product, suggesting that primer-dimer formation during qPCR was

262 negligible. To confirm that the *gapdh* gene fragment is non-variable in copy number we compared

263 whether the number of copies was stable at the inter-individual level (N=31) as well as at the intra-

264 individual (for repeatedly sampled individuals with at least a 10-year gap in sampling, N=4).

265 Examination by qPCR showed that the *gapdh* copy number did not differ between individuals and it also

266 did not systematically change with age in the same individuals. These results are also supported by other

267 evidence suggesting that the avian *gapdh* gene is usually a single copy gene found on autosomes and no

268 pseudogenes have been identified (Alström et al. 2011). For amplifying telomeric repeats we used the

269 universal primers developed by Cawton (2002): *tel1b* (5′-

270 CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′) and *tel2b* (5′-

271 GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′). After amplification, the products

272 were visualized on agarose gels and we observed a smear which was most intense around 78bp as

273 reported in previous studies (Criscuolo et al. 2009).

274

275 The qPCR reactions were carried out in BIO-RAD X1000 real time thermal cyclers (BIO-RAD) in 384

276 well microplates (BIO-RAD). For this purpose, we used iQ™ SYBR® Green qPCR mix (BIO-RAD)

277 which includes iTaq ™ DNA polymerase, dNTPs, $MgCl_2$ and fluorescein-SYBR. The reaction mix

278 contained iQ™ SYBR® Green qPCR mix, and 400 nM of forward and reverse primers for either

279 telomere and g*apdh* gene fragment amplification. 10 ng of sample DNA was added to each reaction and

280 each sample was measured in triplicate on the same plate. Each plate also included serial doubling

281 dilutions (from 1.25 ng/well to 40 ng/well) of a standard sample DNA plus a no template control, also
282 carried out in triplicate for both telomere and *gapdh* reactions and later used to construct standard
283 curves. The thermal cycling conditions for both amplicons, were as follows: an initial denaturation at
284 95°C for 3 min followed by 45 cycles of 95°C for 15s, 58°C for 18s and 72°C for 30s. The melt curve,
285 used to determine the specificity of the qPCR amplification, was generated by slowly increasing
286 temperature (0.1^{o}C/s) from 65 to 95 ^{o}C.

287

288 Our mean qPCR efficiencies, determined from the standard curve, were 102% (±6.31 *SD*) and 92%
289 (±4.9 *SD*) for telomere and *gapdh* reactions, respectively, and thus fell within the acceptable range (85-
290 115%) for reliable telomere measurements (Criscuolo et al. 2009). Cq values (defined as the cycle
291 number at which the fluorescence reached a fixed threshold value) were standardized for interplate
292 variation using the software GenEx6 (MultiD). Replicates of samples were scanned for outliers (*CV* >
293 5%) and resulted in the exclusion of one sample. Intraplate coefficients of variation for telomeres and
294 *gapdh* Cq values were 1.7%and 0.68%, respectively. Inter-plate *CV*'s were 2.4% for telomere and 1.15
295 % for *gapdh* Cq values. We calculated the average of Cq values for the replicates and used them to
296 calculated relative telomere length (T/S) for 172 females. It was calculated according using a formula
297 taking qPCR efficiencies into consideration (Pfaffl 2001).

298

299 **(b) Faecal glucocorticoid metabolite analysis**

300 Faecal GCM concentrations provide an integrated measure of faecal hormone profiles accumulated over
301 periods up to several days, since female alarm excreta start to accumulate in the intestinal tract
302 immediately after incubation onset, typically occurring after the second egg is laid (e.g., Andersson and
303 Waldeck 2006). Although females were trapped at different incubation stages, fGCM levels do not
304 systematically change with advancing incubation (Jaatinen et al. 2013).We measured immunoreactive

305 fGCMs by radioimmunoassay (RIA) using a double antibody kit (ImmuChemTM Double Antibody,
306 Corticosterone, 1251 RIA Kit, MP Biomedicals, Orangeburg, NY). The assay was carried successfully
307 for 423 samples from 317 individuals, which were sampled from one to three times during the study
308 period. As a biological validation of the assay, we note that immunoreactive fGCMs in eiders have
309 previously been found to be elevated up to 3 weeks after surgical interventions (Latty, 2008) and
310 repeatable within individuals (Jaatinen et al. 2013). A detailed description of the protocol for the use of
311 this RIA kit for eider fGMC can be found in Jaatinen et al. (2013). Briefly, serial dilutions (1:2 to 1:256)
312 of ten pooled faecal extracts were used for constructing a displacement curve which is parallel to the
313 standard curve. This allowed to determine a faecal dilution (1:8) where binding was close to 60% and
314 which was used for all test samples. Radioactivity of the bound portion was read in gamma counter
315 (Gamma C12, Diagnostic Products, CA). The mean recovery rate of 3H-labeled CORT added to faecal
316 samples pools was 78±10%. The cross-reactivities with other steroids were: desoxycorticosterone
317 (0.34%), testosterone (0.1%), cortisol (0.05%), aldosterone (0.03%), progesterone (0.02%) and 0.01%
318 for other steroids.The mean sensitivity of the assay for immunoreactive fGCM was 13.4 ng/g (range
319 7.70 to 21.95 ng/g) and the mean (±*SD*) fGCM level in samples was 167.81±124.16 ng/g (range 11.2 to
320 757.65 ng/g). Immunoreactive fGCM levels were always above detection limit and our intra-assay *CV*
321 was less than 10% and inter-assay *CV* was 15.28%.
322
323 *Data analysis*
324
325 To elucidate the effects of immunoreactive fGCM level and nest cover on female hatching success, we
326 constructed a generalized linear mixed effect model (GLMM) with a binomial error distribution where
327 hatched eggs of a clutch were considered a success and unhatched eggs a failure. In more detail, we
328 combined the number of hatched and unhatched eggs in each clutch using the "*cbind*" function in R (R

329 Core Team 2013). This procedure combines the number of successes and failures for each clutch and
330 thus produces a clutch-specific hatching proportion, which takes into account the total number of
331 observations (i.e., eggs) used to produce the clutch-specific hatching proportion. This response variable
332 describes the probability by which a given egg hatches (here after, P(hatch)) and we tested whether it
333 was explained by nest cover and female fGCM level (Table 1). To reduce statistical bias arising from
334 missing covariates, we included additional variables known to affect female breeding success: female
335 minimum breeding experience, body condition and hatch date. Because predation pressure varies
336 between islands and between years (Öst et al. 2011), we also included island-specific annual predation
337 risk as a covariate in the model. Year was included to account for annual differences in hatching dates
338 and hatching success, which may arise due to factors other than those explicitly considered in the model.
339 To test our hypothesis that nest cover and immunoreactive fGCM may have interacting effects on
340 hatching success, we included the interaction term between fGCM and nest cover in the model. Model
341 selection was done by removing all non-significant variables ($\alpha = 0.05$) using backward stepwise model
342 reduction, where the least significant covariates were removed one at a time until the model contained
343 only significant variables and interactions. The model was fitted using Laplace approximation and
344 female identity was included as a random effect to correct for repeated measurements on the same
345 female in different years ($N = 423$ observations of 317 females; Table 1).
346
347 To study the associations between telomere length, stress physiology and breeding microhabitat, we
348 constructed a linear model (LM) where relative telomere length was explained by immunoreactive
349 fGCM, nest cover and the interaction between these two variables (Table 2). We included minimum
350 maternal experience and body condition as covariates, to account for the potential telomere attrition with
351 advancing age and potential links between telomere length and individual body condition. Telomere
352 length was log transformed to ensure the normality of model residuals. Non-significant ($\alpha = 0.05$) were

353 removed from the model using backward stepwise model reduction as described above.

354

355 To graphically illustrate significant interaction terms, these were analysed *post hoc* using the established
356 method of simple slope analysis (Aiken and West 1991). Predictive trend lines depicted in graphs serve
357 to illustrate significant interaction between two non-discrete predictors. Grouping of females into three
358 categories depending on the concentration of immunoreactive fGCM (low– L, medium – M, high – H)
359 was done after the statistical analyses therefore significance of the interaction is not affected by the
360 grouping of females. In short, regression equations were restructured to reflect the regression of the
361 criterion on one predictor and simple slope regressions were plotted to display the interactions at the
362 mean and 1SD above and below the mean. All statistical analyses were performed in R2.13.0 (R Core
363 Team 2011).

364

365 **RESULTS**

366

367 *Hatching success*

368 We found that the relationship between immunoreactive fGCM levels and hatching success varied with
369 the degree of nest cover (fGCM × nest cover interaction: b = -0.018; SE = 0.004; Z = -4.187; p <0.001;
370 N (observations/individuals)=423/317; $R^2_{marg.}$ = 0.18; Table 1, Fig.1). For eider females with low fGCM,
371 hatching success was positively associated with nest site cover. However, the opposite was observed for
372 females with high fGCM levels; high proportional hatching success was associated with low nest cover.
373 Hatching success decreased with advancing hatching date, as it did with increasing island-specific
374 annual predation risk (Table 1). However, minimum maternal experience and body condition were not
375 significantly associated with proportional hatching success, and there was no significant year effect

376 (Table 1).

377

378 *Telomere length*

379 Variation in telomere length was explained by an interaction between female immunoreactive fGCM

380 level and nest cover (fGCM × nest cover interaction: $b = 0.002$; $SE= 0.001$; $t =2.014$; $p <0.05$; $df = 155$;

381 $R^2_{adj.} = 0.03$; Table 2, Fig. 2). Longer telomeres were associated with high nest concealment for females

382 with high fGCM while the opposite trend was observed for low fGCM females. Importantly, we did not

383 detect a significant association between telomere length and female minimum years of maternal

384 experience, and female body condition was likewise not significantly associated with telomere length

385 (Table 2).

386

387 **DISCUSSION**

388 Consistent with the hypothesized role of GCs in adaptively regulating escape responses to the habitat-

389 specific risk of detection by predators, we found that individuals with high immunoreactive fGCM

390 levels had the highest hatching success in nests offering little cover, whereas females with low fGCM

391 profiles had the highest hatching success in well-covered nests (Fig. 1). Thereby, variation in nest-site

392 preferences may facilitate the coexistence of different baseline GC levels in populations subjected to

393 habitat-specific risks of attack by predators (Rivers et al. 2014), cautioning against uncritically assuming

394 a uniformly negative association between baseline GC levels and fitness (Bonier et al. 2009*b*). While we

395 observed no link between telomere length and a proxy of age in female eiders, we found that shorter

396 telomeres were associated with high fGCM in nest sites with little shelter and with low fGCM in well-

397 concealed ones (Fig. 2). Interestingly, this habitat-associated pattern of telomere dynamics may imply a

398 potential cost of reproduction, since the females with shorter telomeres also had higher reproductive

399 success. This result agrees with that of a recent study on common terns (*Sterna hirundo* Linnaeus,

400 1758), showing that individuals with short telomeres had higher reproductive performance (Bauch et al.
401 2013).
402
403 The interactive effects of immunoreactive faecal GCs and nest cover on hatching success are consistent
404 with the presence of habitat-specific antipredator strategies. Cross-species comparisons have shown that
405 ecologically similar co-inhabiting species may show contrasting escape tactics when at risk from
406 predation (Lima 1990; Wirsing et al. 2010). Thus, some species always select dense vegetation because
407 of the protection it provides against predators ('cover-dependent escape tactic'), whereas others prefer a
408 clear path of escape to the air ('aerial escape tactic'; Lima 1990). These different antipredatory tactics
409 may also be present within species (Cuadrado et al. 2001; Thaker et al. 2010; Brink et al. 2011). In the
410 case of eiders, evidence suggests that well-concealed nest-sites sites may be associated with a reduced
411 risk of detection by predators but also potentially higher costs of escape, favouring cover-dependent
412 escape tactics at such nest-sites. First, predation pressure (number of killed females/nesting attempt) is
413 lower and female survival is higher on forested islands than on open ones (Ekroos et al. 2012). Second,
414 it has been experimentally shown that the risk of egg predation decreases with increasing nest cover (Öst
415 et al. 2008), suggesting that concealed nests may attract less attention from visually hunting predators.
416 Third, the presumed benefit of immobility in the presence of predators ('freezing') in densely vegetated
417 habitat is enhanced by the fact that once detected by a predator, dense vegetation may prevent successful
418 escape (Öst and Steele 2010). In contrast, the optimal strategy in poorly concealed nests may be to rely
419 on early escape from predators in anticipation of the higher risk of predator detection (Amat and Masero
420 2004; Albrecht and Klvaňa 2004).
421
422 D'Alba et al. (2011) argued that exposed nest sites are occupied by female eiders of lower phenotypic
423 quality and that the effects of GCs on hatching success appear to vary independent of nest shelter. In line

424 with these conclusions, our own previous work indicated a consistently negative association between
425 immunoreactive fGCMs and hatching success in eiders (Jaatinen et al. 2013). However, our present
426 study showed that a detailed examination of nest-site preferences profoundly changes these conclusions,
427 providing a more nuanced view of the interrelationships between baseline fGCM, reproductive success
428 and breeding habitat. This discrepancy may relate to the orchestrating role of fGCM in simultaneously
429 affecting both reproductive physiology and antipredator behaviours; both of which are intimately linked
430 to reproductive success (Crossin et al. 2016). GCs are associated with the anticipation or awareness of
431 danger when confronted with the threat of predation (e.g., Korte 2001; Cockrem and Silverin 2002).
432 Thus, GCs enhance vigilance behavior (e.g., Romero and Butler 2007) and causally affect flight
433 initiation distance (Thaker et al. 2010). In incubating female eiders, flight initiation distance increases
434 with the magnitude of the acute handling-induced corticosterone (a major GC in birds) response
435 (Seltmann et al. 2012), while handling-induced corticosterone responsiveness decreases with increasing
436 nest cover (Schmidt et al. 2009; Jaatinen et al. 2014). This earlier work also suggests a positive link
437 between enhanced GC responsiveness and reproductive success under high risk of predation (Jaatinen et
438 al. 2014). Our result showing a positive correlation between high fGCM levels and hatching success in
439 poorly concealed nests corroborates this notion, while also suggesting a nest-cover dependent nature of
440 the association. Although the mechanisms underlying a positive association between corticosterone
441 secretion and fitness under high risk of predation remain obscure, it is perhaps pertinent that
442 minimization of incubation time may be particularly beneficial in microhabitats offering limited
443 protection from predators. Thus, experimental evidence suggests that corticosterone shortens incubation
444 time in birds (Schmidt et al. 2009) and female eiders having a long flight initiation distance,
445 characterized by higher stress-induced corticosterone secretion, have a shorter incubation period
446 (Seltmann et al. 2012).
447

448 We were unable to detect any effect of our proxy for female age on telomere length in adult female
449 eiders. This result could be an artefact of selective disappearance, i.e. individuals with shorter telomeres
450 disappearing earlier from the population (van de Pol and Verhulst 2006). However, body condition, a
451 correlate of life expectancy (Ekroos et al. 2012) showing individual repeatability between years
452 (Jaatinen and Öst 2011), was also not significantly associated with telomere length (Table 2). This lack
453 of a relationship between telomere length and body condition adds credence to the possibility that
454 telomere length may not be associated with age *per se* in adult eiders. Likewise, a lack of an association
455 between age and telomere length in adulthood has been found in some other long-lived birds (e.g.,
456 Mizutani et al. 2009; Pauliny et al. 2012; Rattiste et al. 2015), although there are exceptions (e.g., Bize
457 et al. 2009). In this study, we quantified relative rather than absolute telomere length which could
458 potentially mask some between-individual differences in telomere length (see Young et al. 2013).
459 Nonetheless, some studies on long-lived birds where absolute telomere length was quantified also failed
460 to observe telomere shortening with age (e.g., Hall et al. 2004). Potentially, this lack of correlation may
461 be attributed to lifelong persistence of active telomerase (Haussmann et al. 2007), a possibility
462 warranting further investigation.
463
464 How can we reconcile the finding that individuals with short telomeres had higher breeding performance
465 (Fig. 2) with the widely-held notion that individuals with longer telomeres, after controlling for any age
466 effects, are of higher phenotypic quality (Pauliny et al. 2006; Le Vaillant et al. 2015)? However, as
467 argued by Bauch et al. (2013), increased investment in reproduction may induce telomere loss, and this
468 effect may become particularly pronounced if some individuals consistently perform better than others
469 throughout their lives. Viewed in this light, individual variation in telomere length may implicate long-
470 term cumulative reproductive costs, rather than merely reflecting the current reproductive burden
471 (Bauch et al. 2013). This argument may also be valid in the case of eiders. For example, female identity

472 explains more than half of the variation in nest fate (i.e., at least one egg hatched vs. all eggs unhatched)
473 (Öst and Steele 2010). Nevertheless, some open questions remain, the solution of which will require
474 further, preferably experimental evaluation. One particular challenge relates to the observation that
475 females with low fGCM levels in covered nests had shorter telomeres, given the alleged role of
476 glucocorticoids in accelerating telomere loss (Haussmann and Marchetto2010). Although this effect may
477 seem small (Fig. 2), it deserves further longitudinal study, because our current, cross-sectional analysis
478 inevitably only provides a snapshot of telomere length, and thus it cannot unveil the underlying
479 complexity of the telomere shortening and restoration process (Monaghan and Haussmann 2006).
480
481 Because behavioural reactivity and physiological stress coping mechanisms are tightly linked (Koolhaas
482 et al. 1999), the same forces are likely to maintain variation in both set of traits in the population.
483 Theory predicts that individuals with higher GCs should perform better under unpredictable
484 environmental conditions, whereas low GC levels are favoured under stable conditions (Cockrem 2005).
485 These fundamental context-dependent differences in optima could serve to maintain phenotypic
486 variability in the population under temporally or spatially fluctuating selection pressures. In line with
487 this general expectation, our study demonstrates that short-term reproductive output tends to become
488 equalized for individuals with different stress profiles if individually repeatable habitat choices are taken
489 into account. Nest cover is an important habitat feature especially for ground-nesting birds, as it can
490 influence adult and egg predation risk (Martin 1993), offer variable thermal conditions (e.g., Kilpi and
491 Lindström 1997) and thereby influence habitat predictability. Our current results showed that females
492 with high fGCM have higher reproductive success but shorter telomeres in open compared to concealed
493 nest sites. Since concealed nest sites are less exposed to weather extremes (Kilpi and Lindström 1997;
494 Fast et al. 2007) and predator attacks (Ekroos et al. 2012) and thereby likely to offer a more stable
495 environment, our findings agree with the hypothesis that variation in environmental predictability can

496 promote the co-existence of different behavioural and physiological phenotypes within the same
497 population (Cockrem 2005).
498
499 In summary, we have demonstrated that the relationships between breeding microhabitat, telomere
500 length and reproductive success may differ depending on individual stress coping strategies in a wild
501 population, subject to temporally and spatially varying predation pressure. Our results are consistent
502 withadaptive adjustment of GC levels to match local environmental conditions, thereby tending to
503 equalize fitness across nests of different concealment. Accordingly, our results may help to explain the
504 considerable variation in nest concealment at the intraspecific level (Öst and Steele 2010). Here we have
505 argued that this adjustment may be driven by threat-sensitive predation avoidance, where different
506 behavioural tactics are favoured in contrasting nest microhabitats. However, since our study is
507 necessarily correlational, causality remains to be demonstrated (but see D'Alba et al. 2011). Equally
508 unclear at this point is whetherfemale eiders with high reproductive success, incurring an apparent cost
509 in terms of telomere shortening, also have shorter lifespan, i.e., whether they actually pay a cost of
510 reproduction. In fact, circumstantial evidence suggests a positive relationship between fecundity and
511 survival in this species (Yoccoz et al. 2002). To address these open questions, we encourage future
512 longitudinal studies investigating within-individual relationships between stress physiology, fitness and
513 telomere dynamics, preferably involving experimental manipulations of predation risk.
514
515 **ACKNOWLEDGEMENTS**
516 We are ever so grateful for the years of field endeavours Ben Steele has put down on the project. We
517 also thank numerous field assistants, particularly Petteri and Aleksi Lehikoinen, Heikki Eriksson and
518 Sara Neggazi for all their hard work. We thank Kirsi Kähkönen for her advice concerning technical
519 issues. Tvärminne Zoological Station provided excellent facilities. This work was supported by the

520 Academy of Finland (grant no. 266208 to KJ, grant no. 128039 to MÖ and grant no. 1265211 to KN via
521 Juha Merilä), the Finnish Cultural Foundation (to KN and KJ), the Swedish Cultural Foundation in
522 Finalnd (to KJ via Alf Norkko), the Doctoral Programme in Wildlife Biology Research at the University
523 of Helsinki (to KN), Societas pro Fauna et Flora Fennica (to KN and MWS), Otto A. Malm foundation
524 (to KN), Oskar Öflund foundation (to KN), the Finnish Society for Sciences and Letters (to MWS),
525 Foundation for Nature Conservation(to KN) and the Swedish Cultural Foundation in Finland (to MÖ).
526

527 REFERENCES

528 Albrecht, T., and Klvaňa, P. 2004. Nest crypsis, reproductive value of a clutch and escape decisions in
529 incubating female mallards *Anasplatyrhynchos*. Ethology, 110: 603–613.

530 Aljanabi, S. M., and Martinez, I. 1997. Universal and rapid salt-extraction of high quality genomic DNA
531 for PCR-based techniques. Nucleic Acids Res. **25**: 4692–4693.

532 Alström, P., Fregin, S., Norman, J. A., Ericson, P. G. P., Christidis, L., and Olsson, U.
533 2011.Multilocusanalysis of a taxonomically densely sampled dataset reveal extensive non-
534 monophyly in the avian family Locustellidae. Mol. Phylogenet. Evol. **58**: 513–526.

535 Amat, J. A., and Masero, J. A. 2004. Predation risk on incubating adults constrains the choice of
536 thermally favourable nest sites in a plover. Anim. Behav. **67**: 293–300.

537 Andersson, M., and Waldeck, P. 2006. Reproductive tactics under severe egg predation: an eiders'
538 dilemma. Oecologia, **148**: 350–355.

539 Angelier, F., Vleck, C. M., Holberton, R. L., and Marra, P. P. 2013. Telomere length, non-breeding
540 habitat and return rate in male American redstarts. Funct. Ecol. **27**: 342–350.

541 Aviv, A., Hunt, S. C., Lin, J., Cao, X., Kimura, M., and Blackburn, E. 2011. Impartial comparative
542 analysis of measurement of leukocyte telomere length/DNA content by Southern blots and
543 qPCR. Nucleic Acids Res. **39**: e134.

544 Barrett, E. L. B., Burke, T. A., Hammers, M., Komdeur, J., and Richardson, D. S. 2013. Telomere length
545 and dynamics predict mortality in a wild longitudinal study. Mol. Ecol. **22**: 249–259.

546 Bauch, C., Becker, P. H., and Verhulst, S. 2013. Telomere length reflects phenotypic quality and costs of
547 reproduction in a long-lived seabird. Proc. Roy. Soc. B Biol. Sci. **280**: 20122540.

548 Bize, P., Criscuolo, F., Metcalfe, N. B., Nasir, L., and Monaghan, P. 2009. Telomere dynamics rather
549 than age predict life expectancy in the wild. Proc. Roy. Soc. B Biol. Sci. **276**: 1679–1683.

550 Bolduc, F., and Guillemette, M. 2003.Incubation constancy and mass loss in the common eider
551 *Somateria mollissima*. Ibis, **145**: 329–332.

552 Bonier, F., Moore, I. T., Martin, P. R., and Robertson, R. J. 2009a.The relationship between fitness and
553 baseline glucocorticoids in a passerine bird. Gen. Comp. Endocrinol. **163**: 208–213.

554 Bonier, F., Martin, P. R., Moore, I. T., and Wingfield, J. C. 2009b. Do baseline glucocorticoids predict
555 fitness? Trends Ecol. Evol. **24**: 634–642.

556 Boonstra, R. 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in
557 nature. Funct. Ecol. **27**: 11–23.

558 Brink, V. van den, Dolivo, V., Falourd, X., Dreiss, A. N., and Roulin, A. 2011.Melanic color-dependent
559 antipredator behavior strategies in barn owl nestlings. Behav. Ecol. **23**: 473–480.

560 Cawthon, R. M. 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res. 30: e47.

561 Chin, E. H., Love, O. P., Verspoor, J. J., Williams, T. D., Rowley, K., and Burness, G. 2009. Juveniles
562 exposed to embryonic corticosterone have enhanced flight performance. Proc. Roy. Soc. B Biol.
563 Sci. **276**: 499–505.

564 Choi, J., Fauce, S. R., and Effros, R. B. 2008. Reduced telomerase activity in human T lymphocytes
565 exposed to cortisol. Brain. Behav. Immun. **2**: 600–605.

566 Cockrem, J. F. 2005. Conservation and behavioral neuroendocrinology. Horm. Behav. **48**: 492–501.

567 Cockrem, J. F., and Silverin, B. 2002. Sight of a predator can stimulate a corticosterone response in the
568 great tit (*Parus major*). Gen. Comp. Endocrinol. **125**: 248–255.

569 Crespi, E. J., Williams, T. D., Jessop, T. S., and Delehanty, B. 2013. Life history and the ecology of
570 stress: how do glucocorticoid hormones influence life-history variation in animals? Funct. Ecol.
571 **27**: 93–106.

572 Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N. B., Foote, C. G., Griffiths, K., Gault, E. A., and
573 Monaghan, P. 2009. Real-time quantitative PCR assay for measurement of avian telomeres. J.
574 Avian Biol. **40**: 342–347.

575 Crossin, G. T., Trathan, P. N., Phillips, R. A., Gorman, K. B., Dawson, A., Sakamoto, K. Q., and
576 Williams, T. D. 2012. Corticosterone predicts foraging behavior and parental care in macaroni
577 penguins. Am. Nat. **180**: E31–41.

578 Crossin, G. T., Love, O. P., Cooke, S. J., and Williams, T. D. 2016. Glucocorticoid manipulations in
579 free-living animals: considerations of dose delivery, life-history context and reproductive state.
580 Funct. Ecol. **30**: 116–125.

581 Cuadrado, M., Martín, J., and López, P. 2001. Camouflage and escape decisions in the common
582 chameleon *Chamaeleo chamaeleon*. Biol. J. Linn. Soc. **72**: 547–554.

583 D'Alba, L., Spencer, K. A., Nager, R. G., and Monaghan, P. 2011. State dependent effects of elevated
584 hormone: nest site quality, corticosterone levels and reproductive performance in the common
585 eider. Gen. Comp. Endocrinol. **172**: 218–224.

586 Ekroos, J., Öst, M., Karell, P., Jaatinen, K., and Kilpi, M. 2012. Philopatric predisposition to predation-
587 induced ecological traps: habitat-dependent mortality of breeding eiders. Oecologia, **170**: 979–
588 986.

589 Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E.,Morrow, J. D., and Cawthon, R. M.
590 2004. Accelerated telomere shortening in response to life stress. Proc. Natl. Acad. Sci. U. S. A.
591 **101**: 17312–17315.

592 Fast, P. L. F., Grant Gilchrist, H., and Clark, R. G. 2007.Experimental evaluation of nest shelter effects
593 on weight loss in incubating common eiders *Somateria mollissima*. J. Avian Biol. **38**: 205–213.

594 Germain, R. R., and Arcese, P. 2014. Distinguishing individual quality from habitat preference and
595 quality in a territorial passerine. Ecology, **95**: 436–445.

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

599 Haussmann, M. F., Winkler, D. W., Huntington, C. E., Nisbet, I. C. T., Vleck C.M. 2007.Telomerase
600 activity is maintained throughout the lifespan of long-lived birds. Exp. Gerontol. 42:610–618.

601 Haussmann, M. F., and Mauck, R. A. 2008. Telomeres and longevity: testing an evolutionary hypothesis.
602 Mol. Biol. Evol. **25**: 220–228.

603 Haussmann, M. F., and Marchetto, N. M. 2010. Telomeres: linking stress and survival, ecology and
604 evolution. Curr. Zool. **56**: 714-727.

605 Hawlena, D., and Schmitz, O. J. 2010. Physiological stress as a fundamental mechanism linking
606 predation to ecosystem functioning. Am. Nat. **176**: 537–556.

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

609 Hobson, K. A., Jaatinen, K., and Öst, M. 2015. Differential contributions of endogenous and exogenous
610 nutrients to egg components in wild Baltic common eiders (*Somateria mollissima*): a test of
611 alternative stable isotope approaches. The Auk, **132**: 624–633.

612 Jaatinen, K., and Öst, M. 2011. Experience attracts: the role of age in the formation of cooperative
613 brood-rearing coalitions in eiders. Anim. Behav. **81**: 1289–1294.

614 Jaatinen, K., Seltmann, M. W., Hollmén, T., Atkinson, S., Mashburn, K., and Öst, M. 2013. Context
615 dependency of baseline glucocorticoids as indicators of individual quality in a capital breeder.
616 Gen. Comp. Endocrinol. **191**: 231–238.

617 Jaatinen, K., Seltmann, M. W., and Öst, M. 2014. Context-dependent stress responses and their
618 connections to fitness in a landscape of fear. J. Zool. **294**: 147–153.

619 Jaatinen, K., Öst, M., and Hobson, K.A. 2016. State-dependent capital and income breeding: a novel
620 approach to evaluating individual strategies with stable isotopes. Front. Zool. **13**: 24.
621
622 Johnson, M.D. 2007.Measuring habitat quality: a review. Condor, **109**: 489–504.

623 Kilpi, M., and Lindström, K. 1997. Habitat-specific clutch size and cost of incubation in common eiders,
624 *Somateria mollissima*. Oecologia, **111**: 297–301.

625 Kim, S.-Y.and Monaghan, P. 2005. Interacting effects of nest shelter and breeder quality on behaviour
626 and breeding performance of herring gulls. Anim. Behav. **69**: 301–306.

627 Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De
628 Jong, I. C., Ruis, M. A., and Blokhuis, H. J. 1999. Coping styles in animals: current status in
629 behavior and stress-physiology. Neurosci. Biobehav. Rev. **23**: 925–935.

630 Korte, S. M. 2001. Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci. Biobehav.
631 Rev. **25**: 117–142.

632 Kotrschal, A., Ilmonen, P., and Penn, D. J. 2007. Stress impacts telomere dynamics. Biol. Lett. **3**: 128–
633 130.

634 Latty, C. J. 2008.Responses of captive common eiders to implanted satellite transmitters with
635 percutaneous antennas. M.Sc. thesis, University of Alaska Fairbanks, USA

636 Le Vaillant, M., Viblanc, V. A., Saraux, C., Le Bohec, C., Le Maho, Y., Kato, A., Criscuolo, F., and
637 Ropert-Coudert, Y. 2015. Telomere length reflects individual quality in free-living adult king
638 penguins. Polar Biol. **38**: 2059–2067.

639 Lima, S. L. 1990. Protective cover and the use of space: different strategies in finches. Oikos, **58**: 151–
640 158.

641 Martin, T. E. 1993. Nest predation and nest sites. Bioscience, **43**: 523.

642 Merilaita, S., Tuomi, J., and Jormalainen, V. 1999. Optimization of cryptic coloration in heterogeneous

643 habitats. Biol. J. Linn. Soc. **67**: 151–161.

644 Mizutani, Y., Tomita, N., Kazama, K., Takahashi, H., Hasegawa, O., and Niizuma, Y. 2009. Relationship
645 between telomere length and age in Black-tailed Gull. Jpn. J. Ornithol. **58**: 192–195.

646 Mizutani, Y., Tomita, N., Niizuma, Y., and Yoda, K. 2013. Environmental perturbations influence
647 telomere dynamics in long-lived birds in their natural habitat. Biol. Lett. **9**: 20130511.

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

649 Monaghan, P., and Haussmann, M. F. 2006. Do telomere dynamics link lifestyle and lifespan? Trends
650 Ecol. Evol. **21**: 47–53.

651 Möstl, E., Rettenbacher, S., and Palme, R. 2005. Measurement of corticosterone metabolites in birds'
652 droppings: an analytical approach. Ann. N. Y. Acad. Sci. **1046**: 17–34.

653 Öst, M., and Steele, B. B. 2010.Age-specific nest-site preference and success in eiders. Oecologia*,* **162**:
654 59–69.

655 Öst, M., Wickman, M., Matulionis, E., and Steele, B. 2008. Habitat-specific clutch size and cost of
656 incubation in eiders reconsidered. Oecologia*,* **158**: 205–216.

657 Öst, M., Lehikoinen, A., Jaatinen, K., and Kilpi, M. 2011. Causes and consequences of fine-scale
658 breeding dispersal in a female-philopatric species. Oecologia*,* **166**: 327–336.

659 Parker, H., and Holm, H. 1990.Patterns of nutrient and energy expenditure in female common eiders
660 nesting in the high arctic. The Auk, **107**: 660–668.

661 Pauliny, A., Wagner, R. H., Augustin, J., Szép, T., and Blomqvist, D. 2006. Age-independent telomere

662 length predicts fitness in two bird species. Mol. Ecol. **15**: 1681–1687.

663 Pauliny, A., Larsson, K., and Blomqvist, D. 2012. Telomere dynamics in a long-lived bird, the barnacle
664 goose. BMC Evol. Biol. **12**: 257.

665 Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic
666 Acids Res. **29**: e45.

667 R Development Core Team 2011. R: A language and environment for statistical computing. R
668 Foundation for Statistical Computing, Vienna, Austria.ISBN 3-900051-07-0

669 Rattiste, K., Klandorf, H., Urvik, J., Sepp, T., Asghar, M., Hasselquist, D., Cooey, C., Hõrak, P. 2015.
670 Skin pentosidine and telomere length do not covary with age in a long-lived seabird.
671 Biogerontology*,* **16**:435–441.

672 Reichert, S., Criscuolo, F., Verinaud, E., Zahn, S., and Massemin, S. 2013.Telomere length correlations
673 among somatic tissues in adult zebra finches. PLoS ONE, **8**: e81496.

674 Reichert, S., Stier, A., Zahn, S., Arrivé, M., Bize, P., Massemin, S., and Criscuolo, F. 2014. Increased
675 brood size leads to persistent eroded telomeres. Behav. Evol. Ecol. **2**: 9.

676 Ricklefs, R. E., and Wikelski, M. 2002. The physiology/life-history nexus. Trends Ecol. Evol. **17**: 462–
677 468.

678 Rivers, J. W., Liebl, A. L., Martin, L. B., and Betts, M. G. **2014.** Corticosterone in territorial male
679 Swainson's thrushes varies in relation to forest age but not vegetation cover. J. Ornithol. **155**:
680 539–548.

681 Romero, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living

682 vertebrates. Gen. Comp. Endocrinol. **128**:1–24.

683 Romero, M. L., and Butler, L. K. 2007.Endocrinology of stress. Int. J. Comp. Psychol. **20: 89–95.**

684 Sapolsky, R. M., Romero, L. M., and Munck, A. U. 2000. How do glucocorticoids influence stress
685 responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr.
686 Rev. **21**: 55–89.

687 Scheuerlein, A., Van't Hof, T. J., and Gwinner, E. 2001.Predators as stressors? Physiological and
688 reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*).
689 Proc. Roy. Soc. B Biol. Sci. **268**: 1575–1582.

690 Schmidt, J. B., Andree', R. M., Davis, K. A., Treese, S. M., and Satterlee, D. G. 2009. Influence of
691 maternal corticosterone treatment on incubation length of eggs laid by Japanese quail hens
692 selected for divergent adrenocortical stress responsiveness. Br. Poult. Sci. **50**: 739–747.

693 Schultner, J., Moe, B., Chastel, O., Bech, C., Kitaysky, A. S. 2014. Migration and stress during
694 reproduction govern telomere dynamics in a seabird. Biol. Lett. **10**:20130889.

695 Seltmann, M. W., Öst, M., Jaatinen, K., Atkinson, S., Mashburn, K., and Hollmén, T. 2012. Stress
696 responsiveness, age and body condition interactively affect flight initiation distance in breeding
697 female eiders. Anim. Behav. **84**: 889–896.

698 Seltmann, M. W., Jaatinen, K., Steele, B. B., and Öst, M. 2014. Boldness and stress responsiveness as
699 drivers of nest-site selection in a ground-nesting bird. Ethology*,* **120**: 77–89.

700 Sudyka, J., Arct, A., Drobniak, S., Dubiec, A., Gustafsson, L., and Cichoń, M. 2014. Experimentally
701 increased reproductive effort alters telomere length in the blue tit (*Cyanistes caeruleus*). J. Evol.

702 Biol. **27**: 2258–2264.

703 Swennen, C. 1989. Gull predation upon eider (*Somateria mollissima*) ducklings: destruction or
704 elimination of the unfit? Ardea*,* **77**: 21–45.

705 Thaker, M., Vanak, A. T., Lima, S. L., and Hews, D. K. 2010. Stress and aversive learning in a wild
706 vertebrate: the role of corticosterone in mediating escape from a novel stressor. Am. Nat. **175**:
707 50–60.

708 van de Pol, M., and Verhulst, S. 2006. Age-dependent traits: a new statistical model to separate within-
709 and between-individual effects. Am. Nat*.* **167**: 766–773.

710 Vaziri, H., Dragowska, W., Allsopp, R. C., Thomas, T. E., Harley, C. B., and Lansdorp, P. M. 1994.
711 Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age.
712 Proc. Natl. Acad. Sci. U. S. A. **91**: 9857–9860.

713 vonZglinicki, T. 2002. Oxidative stress shortens telomeres. Trends Biochem. Sci. **27**: 339–344.

714 Whitten, P.L., Brockman, D.K., Stavisky, R.C. 1998. Recent advances in noninvasive techniques to
715 monitor hormone-behavior interactions. Yearb. Phys. Anthropol. **41**: 1–23.

716 Wilson, A. J., and Nussey, D. H. 2010. What is individual quality? An evolutionary perspective. Trends
717 Ecol. Evol*.* **25**: 207–214.

718 Wingfield, J. C., and Sapolsky, R. M. 2003. Reproduction and resistance to stress: when and how. J.
719 Neuroendocrinol. **15**: 711–724.

720 Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., and
721 Richardson, R. D. 1998. Ecological bases of hormone-behavior interactions: the “emergency life

722 history stage". Am. Zool. **38**: 191–206.

723 Wirsing, A. J., Cameron, K. E., and Heithaus, M. R. 2010. Spatial responses to predators vary with prey
724 escape mode. Anim. Behav. **79**: 531–537.

725 Yoccoz, N., Erikstad, K., Bustnes, J., Hanssen, S., and Tveraa, T. 2002. Costs of reproduction in
726 common eiders (*Somateria mollissima*): An assessment of relationships between reproductive
727 effort and future survival and reproduction based on observational and experimental studies. J.
728 Appl. Stat. 29: 57–64.

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

732 Young, R. C., Kitaysky, A. S., Barger, C. P., Dorresteijn, I., Ito, M., and Watanuki, Y. 2015. Telomere
733 length is a strong predictor of foraging behavior in a long-lived seabird. Ecosphere, **6**: 1–26.

734
735

736

737 **Table1**

738 Model selection and GLMM (binomial error distribution, log link function and female identity as a

739 random factor) testing the effects of a set of independent variables on proportional hatching success

740 (P(hatch)).

741

Independent variable	**Parameter estimate (*b*)**	***SE***	***Z* value**	***p***	***N***
Minimum maternal experience (years)	0.036	0.065	0.555	0.58	421/316
Body condition	-0.118	0.174	-0.675	0.50	408/309
Year	0.359	0.215	1.669	0.09	423/317
Hatching date	**-0.226**	**0.025**	**-8.981**	**< 0.001**	**423/317**
Island-specific predation	**-4.812**	**1.433**	**-3.358**	**< 0.001**	**423/317**
Nestcover	**2.485**	**0.933**	**2.662**	**< 0.001**	**423/317**
fGCM (ng/g)	**0.007**	**0.003**	**2.434**	**0.01**	**423/317**
fGCM ×nestcover	**-0.018**	**0.004**	**-4.187**	**< 0.001**	**423/317**

742 The final model (in **bold**) was selected by removing all non-significant variables ($\alpha = 0.05$). Variables

743 included in the initial model included the *a priori* defined two-way interaction between fGCM and nest

744 cover. Abbreviations: fGCM –faecal glucocorticoid metabolites; df-degrees of freedom; N:

745 observations/unique individuals; SE-standard error. Sample sizes differ because data were not available

746 for all independent variables.

747

748

749 **Table 2**

750 Model selection and linear model (LM) testing the effects of the set of independent variables on

751 telomere length.

Independent variable	**Parameter estimate (*b*)**	***SE***	***t* value**	***df***	***p***
Minimum maternal experience (years)	0.008	0.011	0.697	154	0.49
Body condition	0.017	0.043	0.404	136	0.69
fGCM (ng/g)	**-0.001**	**0.001**	**-1.274**	**155**	**0.20**
Nest cover	**-0.365**	**0.310**	**-1.179**	**155**	**0.24**
fGCM × nestcover	**0.002**	**0.001**	**2.014**	**155**	**<0.05**

752 The final model (in **bold**) was selected by removing all non-significant variables ($\alpha = 0.05$). Variables

753 included in the initial model included the *a priori* defined two-way interaction between fGCM and nest

754 cover. Abbreviations: fGCM: faecal glucocorticoid metabolites; *df*: degrees of freedom; *SE*: standard

755 error.

756

757

758

759

760 Figure legends

761

762 Fig. 1. Proportional hatching success is affected by an interaction between proportional nest
763 cover and immunoreactive faecal glucocorticoid metabolite (fGCM, ng/g) level, so that the hatching
764 success of females with low fGCM (mean – 1*SD*, solid line, L, black dots) positively correlates with
765 increasing proportional nest cover, whereas females with high fGCM (mean + 1*SD*, dotted line, H, open
766 circles) tend to have lower hatching success in concealed nests. Females with intermediate fGCM
767 concentrations (mean, dashed line, M, grey dots) exhibit an intermediate response.

768

769

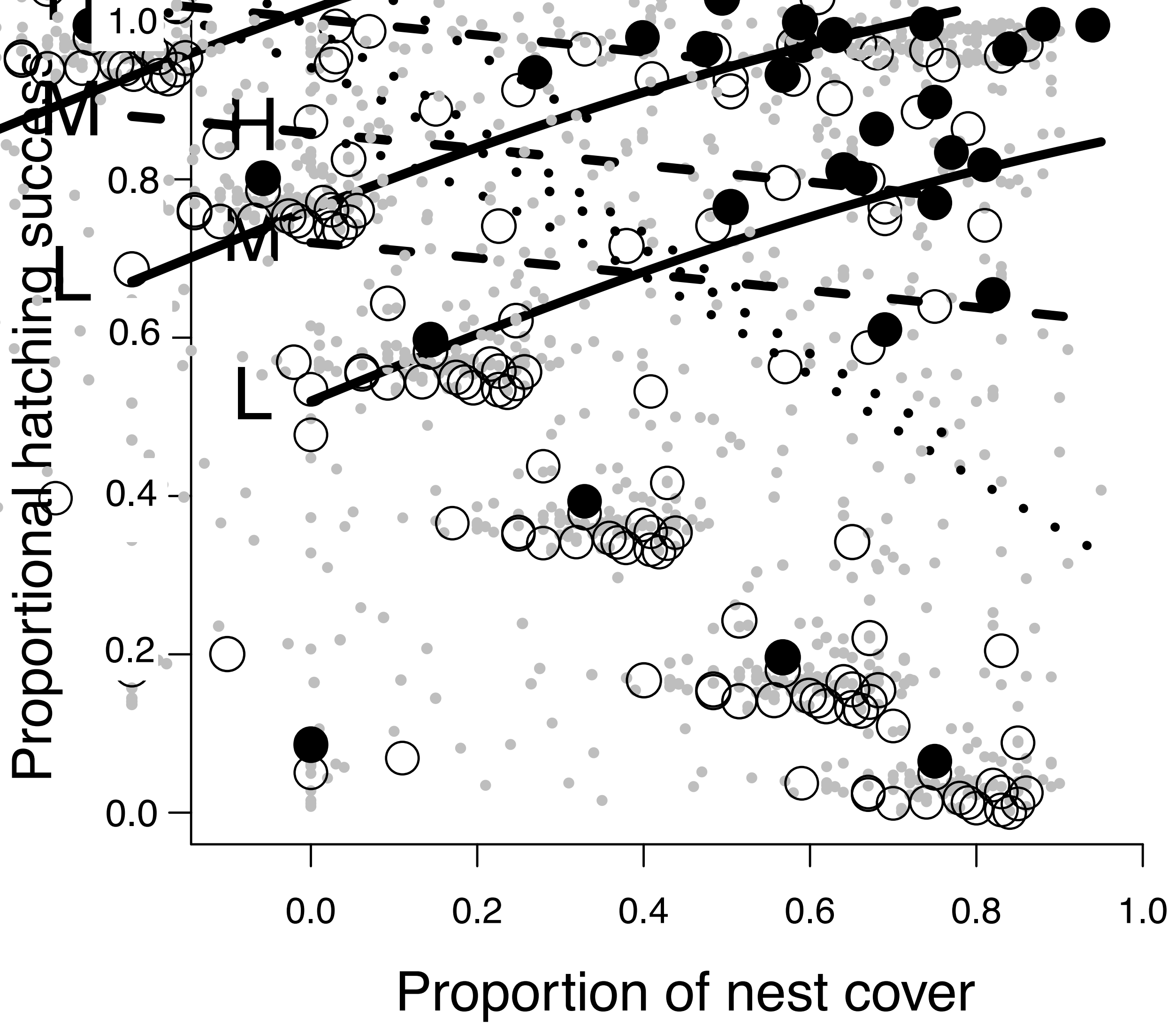
770

771 Fig. 2. Female telomere length is connected to nest cover, but this relationship is modulated by the
772 immunoreactive faecal glucocorticoid metabolite (fGCM, ng/g) level. Telomere length is positively
773 associated with proportional nest cover for females with high fGCMs (mean+1*SD*, dotted line, H, open
774 circles), whereas for nesting females with low fGCM (mean-1*SD*, solid line, L, black dots) this
775 association is negative. Females exhibiting intermediate fGCM levels (mean dashed line, M, grey dots)
776 show an intermediate response.

777

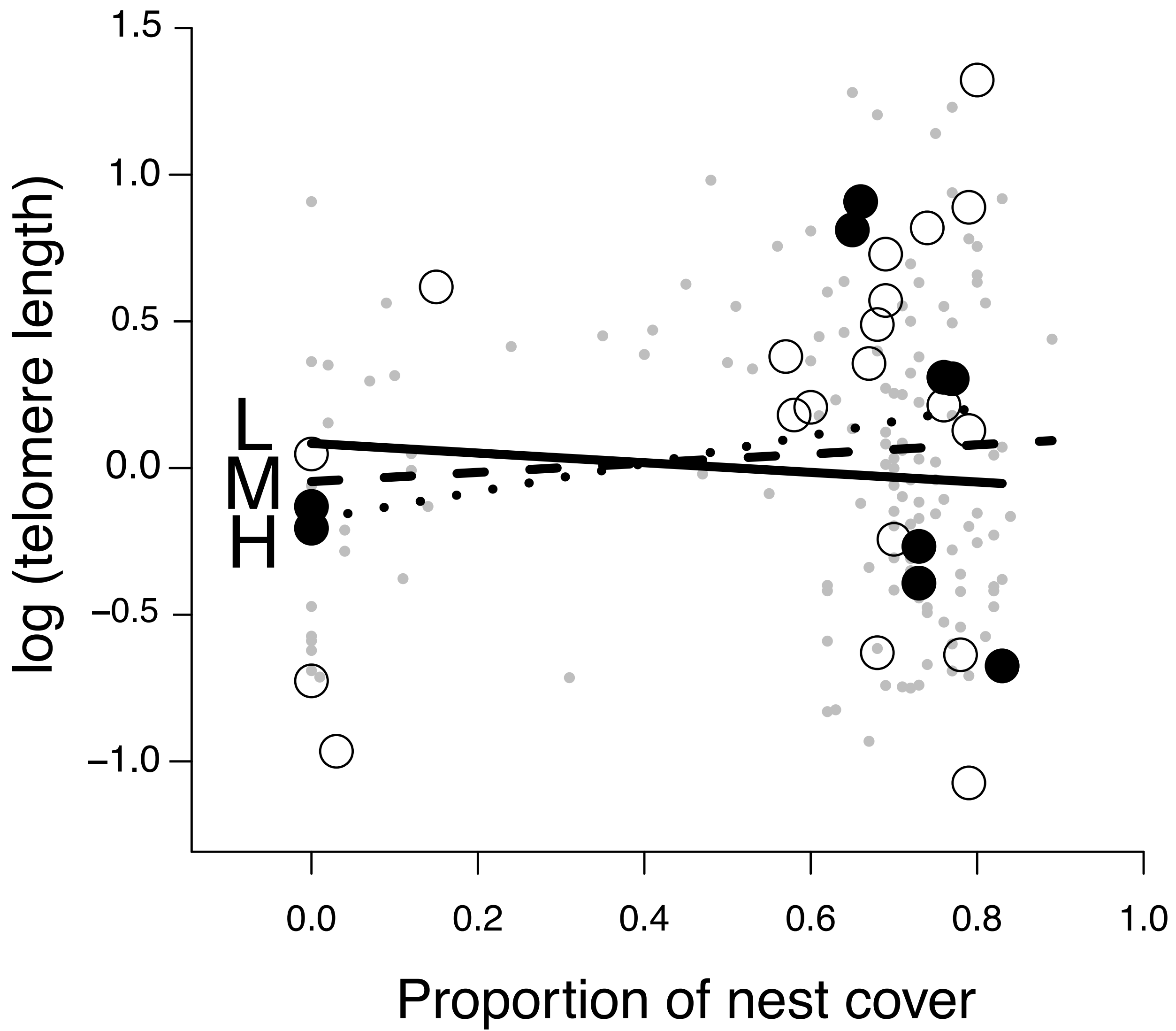
778

779 Fig. 1



780

781



782

783

784 **Supplementary Table1**

785 Nest fate during 2009-2011. Nest fate was categorized as either depredated, abandoned or successful (at

786 least one successfully hatched offspring) (see Methods).

Year	Depredatednests	Abandoned nests	Successful nests	Total number of known-fate nests	Total number of nests
2009	22 (14.76%)	7 (4.70%)	120 (80.54%)	149	165
2010	43 (31.85%)	20 (14.81%)	72 (53.33%)	135	173
2011	67 (40.60%)	6 (3.64%)	92 (55.75%)	165	197

787

788

789