Exposure to food insecurity increases energy storage and reduces somatic maintenance in European starlings

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Abstract

Birds exposed to food insecurity—defined as temporally variable access to food—respond adaptively by storing more energy. In order to do this, they may reduce energy allocation to other functions such as somatic maintenance and repair. To investigate this trade-off, we exposed juvenile European starlings (*Sturnus vulgaris*, n = 69) to 19 weeks of either uninterrupted food availability, or a regime where food was unpredictably unavailable for five hours on five days each week. Our measures of energy storage were repeated measurements of mass, and fat score at the end of the treatment. Our measures of somatic maintenance were growth rate of a repeatedly plucked tail feather, and erythrocyte telomere length, which we measured five times by analysis of the terminal restriction fragment. The insecure birds were heavier at all measurement points, but by an amount that varied across time points. They also had higher fat scores. We found no evidence that they consumed any more food overall, though our food consumption data was incomplete. Plucked tail feathers regrew more slowly in the insecure birds. Telomere length was reduced in the insecure birds, specifically, in the longer percentiles of the within-individual telomere length distribution. We conclude that increased energy storage in response to food insecurity is achieved at the expense of investment in somatic maintenance and repair.

Key words: food insecurity, insurance hypothesis, somatic maintenance, telomeres, birds, starlings

35 **Introduction**

36 When birds such as starlings are exposed to food insecurity—defined as temporally variable access
37 to food—they respond by storing fat and gaining body mass [1–6]. This is an adaptive response: the
38 greater the risk of a period of shortfall, the larger the energy buffer it is optimal to store [7–10].
39 Something very similar may occur in humans, at least in females: experience of food insecurity,
40 measured by questionnaire, is associated with higher body mass index [8,11]. It has been widely
41 assumed that the mechanism underlying food-insecurity driven mass gain is increased food
42 consumption during the times when food is available [12–14]. However, the empirical evidence does
43 not currently support this assumption. In food insecurity experiments, birds can gain weight whilst
44 not increasing their food consumption, or even whilst decreasing it [3,5,6,15]. Likewise, food-
45 insecure women have higher body mass indices without apparently consuming any more calories
46 [16–20]. Another possibility is that food-insecure individuals sequester more energy for fat storage
47 by reducing their energy expenditure rather than increasing their intake. In related and relevant
48 work, Wiersma and Verhulst [21] showed that when foraging was made more costly by mixing food
49 with chaff, zebra finches decreased their daily energy expenditure, despite the greater time spent
50 foraging.

51 There are several ways that an animal might reduce energy expenditure. Zebra finches have been
52 shown to reduce energy expenditure in response to food insecurity [22], though recent evidence
53 from European starlings was inconclusive [5]. Beyond physical activity, animals may down-regulate
54 investments in somatic maintenance and repair. In zebra finches, Marasco et al. [23] found that
55 long-term exposure to food insecurity increased the rate of accumulation of DNA damage (as
56 measured by 8-hydroxy-2′-deoxyguanosine). Wiersma and Verhulst [21] found that zebra finches
57 whose foraging costs were increased regrew a plucked tail feather more slowly than control birds.
58 Another possible marker of somatic maintenance is telomere length (TL). Telomeres are repetitive
59 DNA sequences at the ends of chromosomes that serve to maintain chromosome integrity [24]. They
60 gradually shorten with age due to end-replication problems and other processes [25], shortening
61 that is accelerated by oxidative stress [review; 26]. Across non-human vertebrates, shorter TL or
62 accelerated telomere shortening is associated with ecological challenges such as infection, high
63 competition, poor food or harsh abiotic conditions [meta-analysis; 27]. In nestling starlings,
64 nutritional shortfall and increased begging effort accelerate telomere shortening [28]. Individuals
65 can invest in maintaining TL through antioxidant defences [29]. Thus, change in TL in a proliferating
66 tissue such as erythrocytes can be used as an index of investment in somatic maintenance and
67 repair.

68 In the present study, we exposed groups of captive wild-caught juvenile European starlings to an
69 extended period (19 weeks) of either food insecurity, or constant food availability. Our method of
70 imposing food insecurity was similar to that of several earlier studies [2,23,30]: the removal of
71 access to any food for a five hour period in the fifteen hour day, whose timing during the day varied
72 randomly. In the present case, this was done five days out of seven, with uninterrupted access to
73 food on the remaining two days. Note that this manipulation introduces both restriction of food
74 access, and temporal unpredictability, to the insecure birds compared to the controls. It was not the
75 aim of this study to distinguish the effect of unpredictability from that of restriction, as some other
76 studies have done [1,3]. Food insecurity in the wild may typically involve both, and we were simply
77 seeking a food insecurity regime sufficient to affect the birds in naturalistic manner. We measured
78 body mass repeatedly, as well as fat scores at the end of the treatment period. In addition, we
79 measure two potential markers of somatic maintenance and repair, induced feather regrowth and
80 erythrocyte telomere length (TL). We measured TL by terminal telomere restriction fragment

81 analysis. This has the advantages, compared to the popular qPCR relative telomere length assay [31],
82 of higher precision, and providing, for each sample, a distribution of the lengths of the telomeres
83 present, not just a single estimate of central tendency [32]. We also gathered some information on
84 food consumption, though for logistical reasons the consumption data did not cover every day of the
85 study period. Our general hypothesis was that food insecurity would produce an increase in energy
86 storage and decreased expenditure on somatic maintenance and repair. Hence, we predicted body
87 fat and mass would increase, whilst the rate of feather regrowth and TL would decrease, under food
88 insecurity compared to the control treatment.

89

90 **Methods**

91 *Ethics and permissions*

92 This study was completed under UK Home Office licence 70/8089 (licence holder Melissa Bateson)
93 and with approval of the Animal Welfare Ethical Review Board at Newcastle University. Capture of
94 birds from the wild was done with landowner permission under Natural England permit number
95 2016-57171-SCI-SCI. A copy of the ARRIVE guidelines 2.0 essential items check list [33] is included as
96 Supporting Information.

97 *Birds and aviaries*

98 We captured 70 European starlings in Northumberland over four days in October 2016 using a
99 whoosh net at a site we had been baiting. The number of birds was limited by aviary capacity
100 constraints, but was several-fold larger than the numbers of animals used in comparable previous
101 experiments (typically 6 – 24; [3–6,21,34]). Juvenile status (having hatched in Spring 2016) was still
102 recognisable from plumage, and only juveniles were retained. Birds were transported in cloth bags
103 to the laboratory (approximately 30 minutes), where they were weighed and inspected. Sex was
104 established from visual appearance (and subsequently confirmed genetically, although only after
105 treatment allocation). An initial blood sample was taken (see blood sampling, below); one tail
106 feather pulled (see feather regrowth, below); and a numbered plastic leg ring fitted. Birds were also
107 treated with topical Ivermectin to kill common parasites. Birds were then released into one of four
108 indoor aviaries, where they remained for the duration of the experiment. The aviaries varied slightly
109 in size, with width 239-246 cm, depth 209-219 cm, and height 240cm. The light cycle of the aviaries
110 was 15L:8D with dim lighting simulating dawn/dusk during the first/last 30 mins of the light period.
111 Drinking water was available at all times and environmental enrichment was provided in the form of
112 rope perches, water baths and wood shaving substrate. Diet throughout the study was a mixture of
113 commercially available dry cat food (Royal Canin Ltd.), turkey crumb (Special Diets Services 'Poultry
114 Starter (HPS)'), and insect mix for birds (Orlux insect patée). Birds were left to settle in their aviaries
115 with *ad* libitum food for 11-19 days prior to the beginning of the experimental treatment.

116 Catching for weighing or blood sampling, as outlined below, was done in the dark one hour prior to
117 the birds' dawn, and birds were placed into cloth bags until they were processed and re-released
118 into their aviaries.

119 One bird was euthanised prior prior to the beginning of the treatment, owing to lethargy and very
120 low body weight. This left a final sample of 69 birds, assessed as 29 females and 40 males. On
121 conclusion of the experiment, birds were given a period of *ad libitum* food, inspected by a
122 veterinarian, transported to the site of capture in cloth bags, and released.

123 *Experimental treatments*

124 Two aviaries each were assigned to the two experimental treatments ('insecure' and 'control').
125 Assignment was by alternation within sex, on removal from the bags, and so was effectively random
126 apart from sex balancing. This produced 35 birds (20 male) in the insecure treatment and 34 (20
127 male) in the control treatment. For five days a week (Monday to Friday), food was provided in
128 automated pet feeders (Andrew James Ltd; three per aviary). These worked by sequentially
129 revealing four compartments at pre-programmed times. For the control treatment, all
130 compartments were full of food. Thus, although the feeders moved from compartment to
131 compartment at the same times as for the insecure treatment, food was always available. For the
132 insecure treatment, one compartment was empty, and thus no food was available for five hours out
133 of the day. The timing of the period without food was varied pseudo-randomly from day to day, but
134 was the same for the two aviaries in the insecure treatment. Food deprivation could begin at any
135 hour within the period of full light, and could last until dusk (i.e. the earliest onset of the 5h period
136 was after the 30 minutes of dawn, and the latest end of the 5 h period was at the beginning of the
137 30 minutes of dusk). Food in each non-empty compartment was sufficient that it never ran out. On
138 the remaining two days of the week, uninterrupted food access was provided to both aviaries all day
139 in open bowls. During week 9, uninterrupted food access was provided to both groups every day, as
140 the facility was closed for a public holiday. The experimental treatment was continued for a total of
141 19 weeks.

142 *Body mass and fat scoring*

143 Birds were weighed before dawn. Body masses and fat scores by the main scorer were not made
144 blind to treatment. At all weighing points, body mass was measured by placing the bird in a plastic
145 cone on a digital scale measuring to a resolution of 0.1 g. Body mass was measured on arrival,
146 immediately prior to the beginning of the experimental treatment (henceforth baseline), then after
147 2, 5, 8, 11, 14, 17 and 19 weeks of treatment. In addition, all birds were manually fat scored at week
148 19 (0-8, Biometrics Working Group system [35]) by CA, who was not blind to treatment. Fat score
149 was positively correlated with mass ($r = 0.58$, $p < 0.001$). A subset of 14 birds was also fat scored
150 independently by a different, experienced avian fat scorer blind to treatment. The intra-class
151 correlation coefficient (ICC1) for the two raters was 0.75 (95% CI 0.72 – 079).

152 *Food consumption*

153 Food consumption was estimated for four days out of every seven by weighing the food remaining in
154 the automated feeders. Due to logistical constraints, it was not possible to weigh the food on
155 Fridays, Saturdays or Sundays. Thus, the food consumption data are incomplete and do not cover
156 the two days per week when the insecure birds had ad libitum food. Food consumption was only
157 measured at the aviary level. Food weighings were not blind to treatment. We averaged across the
158 four days of each week to produce one consumption number for each aviary in each week, and
159 converted this to g per bird per day to correct for the different numbers of birds in each aviary.

160 *Feather regrowth*

161 On capture, we removed the left outer retrix (tail feather) by grasping the rachis with blunt-ended
162 forceps and gently pulling until the feather released. This was repeated after 5 and 17 weeks of
163 treatment, by which times the pulled feather had largely grown back. The length of the regrowing
164 feather was measured in mm using digital callipers, from the base of the pin to the most distal point
165 of the feather tip, after 2, 5, 8, 11, 14, 17 and 19 weeks of treatment. These measurements were
166 blind to treatment. At week 17, three birds had feather lengths substantially shorter than they had

167 been at week 14. These were assumed to represent breakage or accidental loss and excluded from
168 analysis.

169 *Telomere length (TL)*

170 TL was measured in erythrocytes by telomere restriction fragment analysis under non-denaturing
171 conditions. Blood samples (around 140 µl) were taken by puncture of an alar vein with a 25-gauge
172 needle and collection into capillary tubes. Samples were transferred to EDTA-treated plastic tubes
173 on ice. They were then centrifuged to separate cells from plasma (10 minutes at RCF 1400 g), and
174 pellets of cells frozen to -80° C. Blood samples were taken on arrival (henceforth baseline; note that
175 this is two weeks earlier than the baseline date for mass), and after 2, 8, 14 and 19 weeks of
176 treatment.

177 TL analysis followed the methods of Bauch et al. [36]. In brief, we washed the cells and isolated DNA
178 from 5 µl of erythrocytes using CHEF Genomic DNA Plug kit (Bio- Rad, Hercules, CA, USA). Cells in the
179 agarose plugs were digested overnight with Proteinase K at 50˚C. Half of a plug per sample was
180 restricted simultaneously with HindIII (60 U), HinfI (30 U) and MspI (60 U) for ~18 h in NEB2 buffer
181 (New England Biolabs Inc., Beverly, MA, USA). The restricted DNA was then separated by pulsed-field
182 gel electrophoresis in a 0.8% agarose gel (Pulsed Field Certified Agarose, Bio-Rad) at 14˚C for 24h,
183 3.5V/cm, initial switch time 0.5 s, final switch time 7.0 s. For size calibration, we added 32P-labelled
184 size ladders (DNA Molecular Weight Marker XV, Roche Diagnostics, Basel, Switzerland; NEB
185 MidRange PFG Marker I, New England Biolabs, range 15–242.5 kb). Gels were dried (gel dryer, Bio-
186 Rad, model 538) at room temperature and hybridized overnight at 37˚C with a 32P-endlabelled
187 oligonucleotide (5’-CCCTAA-3’)4 that binds to the single-strand overhang of telomeres of non-
188 denatured DNA. Subsequently, unbound oligonucleotides were removed by washing the gel for 30
189 min at 37˚C with 0.25x saline-sodium citrate buffer. The radioactive signal of the sample specific TL
190 distribution was detected by a phosphor screen (MS, Perkin-Elmer Inc., Waltham, MA, USA),
191 exposed overnight, and visualized using a phosphor imager (Cyclone Storage Phosphor System,
192 Perkin-Elmer Inc.).

193 TL distributions were quantified using IMAGEJ (v. 1.38x). The TL parameters potentially relevant to
194 aging and somatic state are not just average TL, but aspects of an individual’s TL distribution (for
195 example, the length of the shortest or longest telomeres). We therefore calculated the mean of the
196 TL distribution (i.e. henceforth aTL), and additionally the percentiles, in 5% intervals, from 10% to
197 90%. For each sample the limit at the side of the short telomeres of the distribution was lane-
198 specifically set at the point of the lowest signal (i.e. background intensity). The limit on the side of
199 the long telomeres of the distribution was set lane-specifically where the signal dropped below Y,
200 where Y is the sum of the background intensity plus 10% of the difference between peak intensity
201 and background intensity. The coefficient of variation of a control sample run on 15 gels was 6%. The
202 intra-class correlation coefficient (ICC) across individuals was 0.77, including treatment week as a
203 fixed factor. This represents a minimum estimate of the technical repeatability of the TL
204 measurements. All telomere measurements were blind to treatment.

205 *Statistical analysis*

206 Data were analysed in R, version 3.6.0 [37], using linear mixed models with R packages ‘lme4’ and
207 ‘lmerTest’. Model estimation used restricted maximum likelihood. Significance testing used
208 Satterthwaite’s method with $\alpha = 0.05$. Models of experimental effects used insecurity status as the
209 fixed predictor, where this status was control for all birds at baseline, and insecure for the insecurity
210 groups subsequent to the onset of the experimental treatment. Taking the data from the onset of

211 the experimental treatments onwards and using treatment group as the fixed predictor produces
212 very similar results. Preliminary inspection revealed week-to-week changes with no linear trend,
213 especially for mass (perhaps due to temperature and seasonal variation). We therefore included
214 treatment week as a fixed factor rather than a continuous covariate. The interaction between
215 treatment week and insecurity was also included. The distributions of residuals were checked and
216 found satisfactory for the assumptions of the models. For binary comparisons of means, we report
217 Cohen's d as measures of effect size.

218 Models for mass, TL and feather regrowth included random effects of bird to account for repeated
219 measures. Adding aviary as an additional level of random effect did not improve AIC or change
220 results, and hence was not included in the analyses presented below. For TL, in a first model, the
221 outcome variable was aTL. In a follow up model, we used all available percentiles of the TL
222 distribution. For this model, the fixed predictors were insecurity, week, percentile, and all possible
223 interactions, with random effects of sample identity and bird.

224 In addition to analyses of individual outcomes, we present meta-analyses, in which we combine the
225 evidence for a treatment effect from the two measures of energy storage (mass and fat score), and
226 the two principal measures of somatic investment (average telomere length and feather regrowth).
227 For these models, we standardized the dependent variables for comparability of parameter
228 estimates, and excluded the interaction between week and insecurity. Meta-analyses were
229 conducted using R package 'metafor'.

230

231 **Results**

232 *Mass and fat scores*

233 Mass at baseline did not differ significantly between the treatment groups (control: mean 75.20g, se
234 0.72; insecure: 74.80g, se 0.76; $t = -0.16$, $p = 0.87$). Insecure birds were heavier than control birds at
235 all time points after the onset of the treatment, by varying amounts (figure 1A). The main effect of
236 insecurity was marginally non-significant ($F(1, 505.38) = 3.24$, $p = 0.07$), but there was a significant
237 interaction between insecurity and week ($F(6, 469.37) = 2.22$, $p = 0.04$). The mass difference by
238 insecurity status was substantial at weeks 14 (1.76 g; d = 0.40, 95% CI -0.08-0.89) and 19 (2.08 g; d =
239 0.48, 95% CI -0.01-0.97) and negligible at, weeks 5 (0.13 g; d = 0.03, 95% CI -0.45-0.51) and 8 (0.10 g;
240 d = 0.02, 95% CI -0.46 – 0.50).

241 Fat scores at week 19 were significantly higher for the insecure group (mean 3.59, se 0.13) than the
242 control group (mean 3.06, se 0.14; $t = 2.76$, $p = 0.01$; d = 0.66, 95% CI 0.17 – 1.15; figure 1B).

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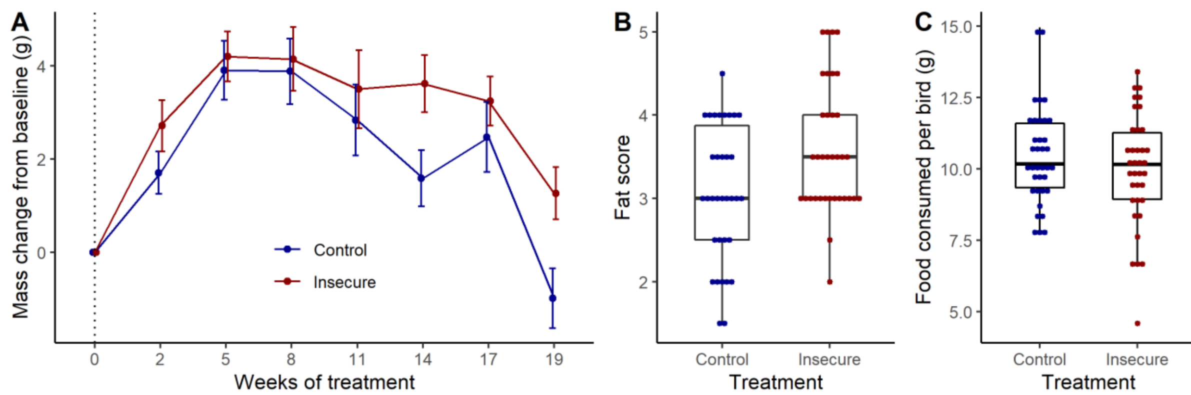

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245 Figure 1. Effects of experimental treatment on mass, fat and food consumption. A. Mass change
246 from baseline ± 1 se, by treatment across the experimental period. B. Fat scores after 19 weeks of
247 treatment, by treatment. Points represent birds. C. Food consumption (g per bird per day), by
248 treatment. Points represent aviary weeks.

249

250 *Food consumption*

251 We calculated food consumed per bird at the aviary level, as described in Methods (i.e. there was
252 one data point per aviary per week). We fitted a model with food consumed per bird as the
253 outcome, and insecurity, week and their interactions as fixed predictors. The main effect of
254 insecurity was not significant ($F(1, 2) = 0.22$, $p = 0.68$), and the interaction between week and
255 insecurity was marginally non-significant ($F(17, 34) = 1.91$, $p = 0.05$). The insecure birds consumed
256 slightly less per bird overall (control: 10.48 g, se 0.17; insecure: 10.01 g, se 0.33; d = -0.25, 95% -0.73
257 – 0.22; figure 1C).

258

259 *Telomere length*

260 Mean aTL at baseline was 17351 bp (sd 1032, range 15110 – 20179). Individual TL showed high
261 degrees of consistency over time; for example, the correlation matrix of aTL across individuals at the
262 various time points is shown in table 1. Correlations over time for percentiles of the TL distribution
263 were similar. On average, individuals' TL shortened by 142 bp (sd 522) between baseline and the
264 final TL measurement point 21 weeks later ($t = -2.26$, $p = 0.03$).

265 Table 1. Correlations across individuals for average TL at different time points.

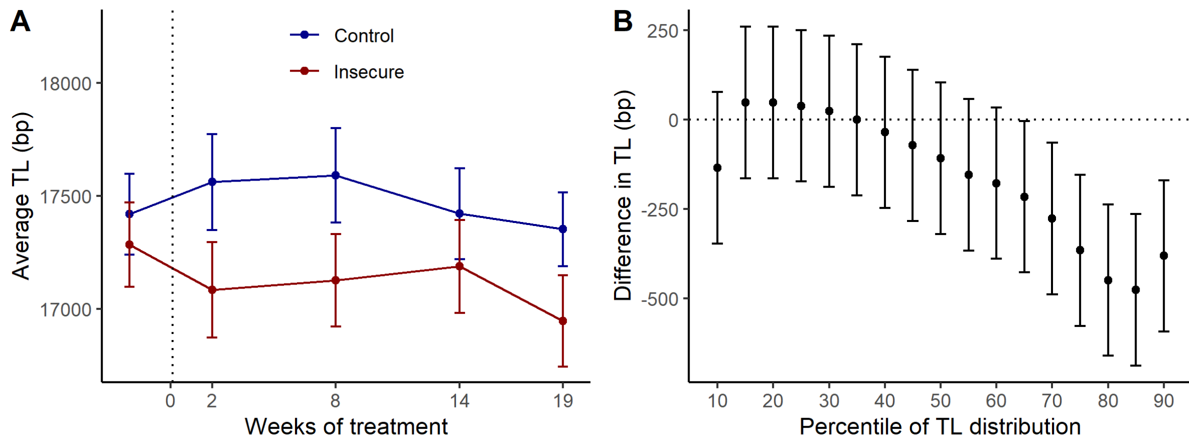
	Week 2	Week 8	Week 14	Week 19
Baseline	0.76	0.75	0.77	0.87
Week 2		0.76	0.77	0.75
Week 8			0.80	0.80
Week 14				0.85

266

267 At baseline, aTL did not differ significantly between treatment groups (control: mean 17420, se 179;
268 insecure: mean 17285, se 187; $t = 0.52$, $p = 0.61$). In the model using aTL as the outcome variable,
269 the main effect of insecurity was marginally non-significant ($F(1, 326.78) = 3.03$, $p = 0.08$). The
270 interaction between insecurity and week was not significant ($F(3, 262.43) = 1.30$, $p = 0.27$). Figure 2A
271 shows aTL by treatment group at each measurement point; insecure birds had shorter aTL than

272 control birds at all time points. The difference between the two groups was largest at week 2 (-516
273 bp; d = -0.45, 95% CI -0.96 – 0.06) and smallest at week 14 (-204 bp; d = -0.17, 95% CI -0.65 – 0.30).

274 We followed up this analysis with a model using the full range of percentiles of the TL distribution.
275 The main effect of insecurity was not significant in this model (F(1, 298.3) = 2.57, p = 0.11), and
276 neither was the main effect of week (F(4, 244.2) = 1.53, p = 0.19). There was however a significant
277 interaction between insecurity and percentile (F(16, 5257.6) = 11.04, p < 0.001). No other
278 interactions were significant. As figure 2B shows, the insecure birds had shorter TL at the longer
279 percentiles of the TL distribution.



280

281 Figure 2. Effects of insecurity on telomere length. A. Average TL by insecurity status though the
282 treatment. Error bars represent one standard error. The dotted vertical line represents the onset of
283 the treatments. B. Difference between insecure and control birds by percentile of the TL distribution,
284 collapsed across the weeks after the onset of the treatment. Data represent difference in marginal
285 means (± 1 se), estimated from the statistical model. A negative number indicates shorter TL in the
286 insecure birds.

287

288 *Feather regrowth*

289 For feather regrowth, as well as an expected large effect of week (F(6, 363.84) = 147.96, p < 0.001),
290 there was a significant effect of treatment (F(1, 66.37) = 5.40, p = 0.02). Birds from the insecure
291 groups had slightly but consistently shorter feathers at all time points other than the final one (figure
292 3). The interaction between treatment and week was not significant (F(6, 363.84 = 0.35, p = 0.91).
293 Averaging across the measurement points, insecure birds had an average feather length of 53.90
294 mm (se 1.01) compared to 56.20 mm (se 0.42) for the control birds, corresponding to an effect size
295 (Cohen's d) of -0.50 (95% CI 0.01 – 0.99).

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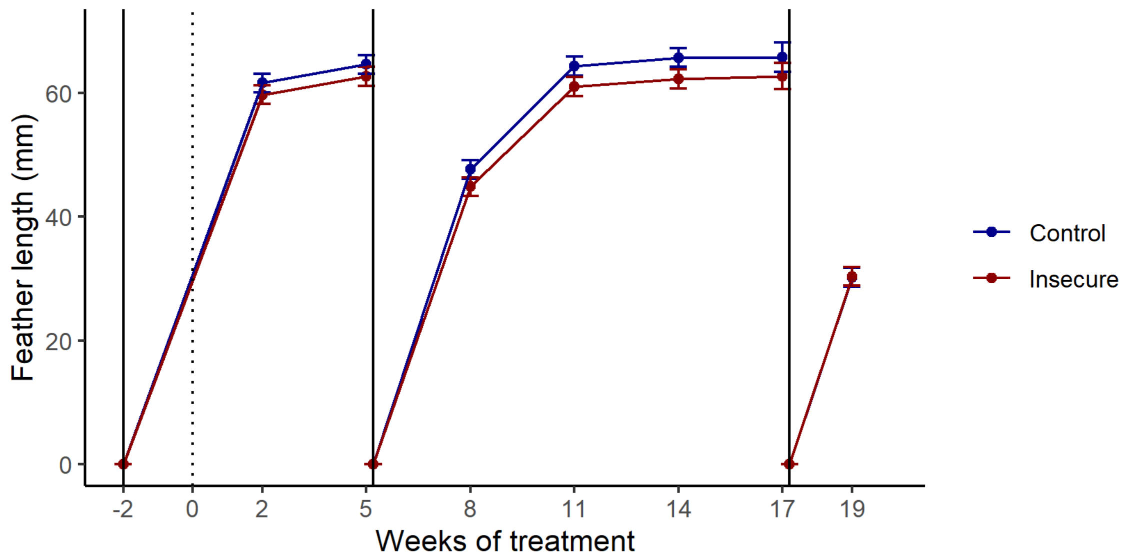

297

298 Figure 3. Length of regrowing tail feathers (mm) by insecurity and time point. The pulling of the
299 feather is indicated by the vertical solid lines. The beginning of the treatment phase is shown with a
300 vertical dotted line. Shown are estimated marginal means plus or minus one standard error. At
301 weeks 17 and 19, the data are overlapping.

302 *Meta-analysis*

303 In a fixed effects meta-analysis of the two measures of energy storage, mass and fat score, there was
304 a significant positive effect of insecurity (figure 5; B = 0.27, se 0.09, 95% z = 2.79, $p < 0.001$). For the
305 measures of somatic investment, average TL and feather regrowth, there was a significant negative
306 effect of insecurity (figure 5; B = -0.16, se 0.06, 95% z = -2.86, $p < 0.001$).

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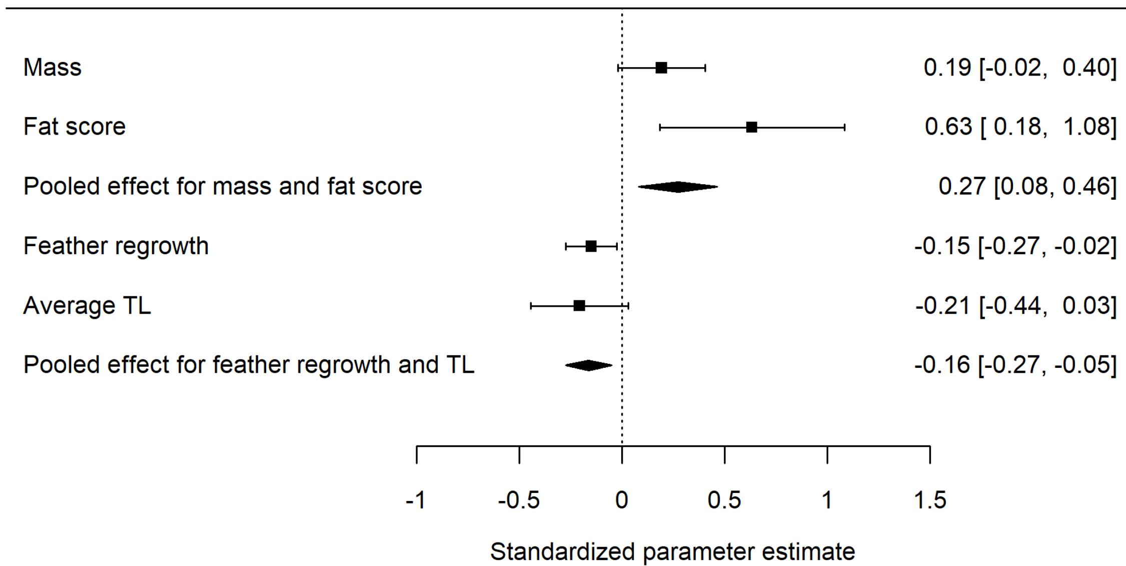

309

310 Figure 5. Meta-analysis of study measures. Squares represent standardized effect sizes, and whiskers
311 represent 95% confidence intervals. Diamonds represent pooled effect sizes and their 95%
312 confidence interval from a fixed-effects meta-analysis model.

313 **Discussion**

314 We experimentally exposed groups of young starlings to food insecurity or uninterrupted food
315 access over a period of more than four months. Overall, our results support the hypothesis that the
316 birds experiencing food insecurity increased energy storage, and reduced somatic investment and
317 repair. When the evidence from fat scores and masses was combined meta-analytically there was a
318 clear pattern of increased energy storage, though the effect on mass considered separately was
319 significant only in interaction with time point. On the somatic maintenance side, again the pattern of
320 reduced investment was clearer when TL and feather regrowth were combined meta-analytically.
321 Considered separately, the effect of insecurity on TL was significant only in interaction with
322 percentile of the TL distribution: the longer telomeres were those affected.

323 Our findings that food insecurity increased energy storage conceptually replicate earlier findings in
324 starlings and other passerine birds [3–6,38]. The insecurity effect on mass varied from week to week;
325 when averaged over all the weeks, the effect size was small. This is consistent with our recent
326 findings from a series of experiments using a different method of inducing food insecurity in
327 starlings. There, we found evidence for mass gain under food insecurity overall, but with effects that
328 varied in magnitude from experiment to experiment and were null in some experiments [5]. How
329 successful the laboratory protocols are at simulating natural food insecurity is not clear; it may be
330 that they underestimate the magnitude or reliability of the shifts in the wild, given, for example, that

331 in the current experiment, birds would have been able to learn that the absence of food is always
332 short-lived.

333 There are several non-mutually exclusive mechanisms that could explain how food insecurity
334 induces fat storage. The first is that food insecure birds consume more food in the periods when
335 food is available [12–14]. We found no evidence for increased food consumption. This result is not
336 definitive: in the present experiment, we only measured food consumption on four days out of every
337 seven, and only at the coarse level of the whole aviary. Thus, we cannot exclude that the food
338 insecure aviaries consumed more food than the control aviaries on the two ad libitum days per week
339 where food consumption was not monitored. Nonetheless, the non-significant trend we observed
340 was in the direction of insecure birds eating less rather than more. This finding is consistent with a
341 number of other avian studies where food consumption was measured more completely, in which
342 food insecure birds gained weight despite eating no more food, or less food [3,5,6,15]. It is also
343 consistent with the human evidence that food insecure women gain weight without apparently
344 consuming any more calories, [16–20], though those studies suffer from the limitation that food
345 consumption is self-reported.

346 A second possible mechanism is that food insecure birds assimilate more of the potential caloric
347 content of the food they do consume. In our previous study in the same species [5], we used bomb
348 calorimetry to measure the energy density of guano. We found lower energy density of guano in
349 food insecure birds, suggesting greater assimilation of the caloric content. We did not collect guano
350 in the present experiment, and hence have no information on whether assimilation was increased,
351 though this is plausible given previous findings [5,39].

352 Third, food insecure birds may reduce energy expenditure on other functions. Our findings on
353 feather regrowth and TL suggest in particular that energy allocation to somatic maintenance and
354 repair was reduced. These findings are consistent with Wiersma and Verhulst's [21] demonstration
355 of reduced feather regrowth in zebra finches for whom foraging had been made less profitable, and
356 the evidence from Marasco et al. [23], also in zebra finches, of a faster accumulation of DNA damage
357 over time in birds exposed to a food insecurity regime very similar to the present one. Whereas our
358 choice of energy storage measures was straightforward, in that mass and fat score are the directly
359 relevant quantities, our choice of feather regrowth and TL was opportunistic. There were other
360 potential measures we could have chosen but did not, such as DNA damage or immune function.
361 Previous studies suggest food insecurity may have similar negative effects on those measures
362 [15,23]. The choice of measures in the present case was dictated by convenience and our prior
363 expertise in telomere dynamics [40–42]. The fact that both our chosen measures showed some
364 evidence of a reduction under food insecurity was either fortunate, or suggests that reduction of
365 investment under food insecurity is detectable across a range of possible markers of somatic
366 investment. Such reduced investment would provide a general pathway to explain the reliable
367 associations between food insecurity and subsequent poor health in humans [43,44]. It is, however,
368 difficult to reconcile with findings that long-term exposure to a food insecurity regime increased life
369 expectancy in zebra finches [45]. We note also that we did not measure other components of energy
370 expenditure, such as movement, thermoregulation [46], preparation for reproduction, or song and
371 song learning [47], that could have also been reduced under food insecurity.

372 Our investigation of TL under food insecurity was notable for its high precision, compared to many
373 other avian TL studies. This precision arose from measuring TL five times on the same individuals,
374 and using the terminal restriction fragment approach rather than the more widespread qPCR assay
375 [see 32 for discussion of alternative TL measurement methods]. This method has several advantages.
376 First, as used here it excludes interstitial telomere sequences, which can be numerous and variable

377 between individuals in birds. Thus, it provides a clean measure of terminal TL, which is the
378 parameter of interest. Second, using this method we were able to characterise the absolute lengths,
379 in base pairs, of telomeres in the European starling, whereas our previous work [40,41] reported
380 only relative abundance of the telomeric sequence. The average TL for the whole sample, 17351
381 base pairs, falls squarely within the range observed in birds, fairly similar to the values seen in blue
382 tits and zebra finches measured by the same method [48]. Third, measuring the terminal restriction
383 fragment provides a distribution of TL for each sample. This revealed that, though the effect of food
384 insecurity on average TL was non-significant, there was an interaction between insecurity and
385 percentile of the TL distribution, with insecurity appearing to shorten the longest telomeres within
386 individuals. In common terns, Bauch et al. [49] found that the length of the longest telomeres was a
387 better predictor than average telomere length of survival and reproductive success. Bauch et al.
388 suggest that this is due to the effects of environmental stressors being most visible in the longest
389 percentiles of the TL distribution, where telomeres shorten fastest in absolute terms. Our findings
390 represent a direct corroboration of this claim.

391 Our repeated measurement of TL also allowed us to characterise TL dynamics, albeit that the
392 timescale was short for examining age-related shortening given the rate at which TL changes after
393 early life. As in other studies using high-precision methods, TL was individually highly consistent over
394 time, with those individuals with long average TL at the beginning of the study generally having long
395 TL at the end [50]. Despite the restricted study period, we were able to observe TL shortening. For
396 average TL over the study period, the mean loss was 142 bp (se 66.9; by treatment it was 209 bp for
397 the insecure birds (se 66.3), and 77 bp for the control birds (se 115)). Averaging across the
398 treatments suggests an annual shortening rate of around 350 bp/year. This is in the range estimated
399 for other passerine birds [51], albeit that our birds were young and likely to be losing TL faster than
400 the whole-life rate.

401 Our results confirm that when faced with food insecurity, starlings can respond adaptively by
402 increasing energy allocated to fat storage, even without taking in any more food overall. At the same
403 time, they reduce allocation to somatic maintenance and repair. The costs of doing this are real and
404 measurable, in terms of slowed feather regrowth and erosion of the longest telomeres. Over time,
405 such reduced investments would presumably have measurable impacts on health. Classical
406 theoretical work on optimal energy reserves treated increased predation risk as the fitness cost of
407 fat storage [9,52,53]. The present work suggests that increased predation risk does not adequately
408 capture all of the costs, since energy intake is limited, and the energy to fund storage must be
409 diverted from other fitness-relevant functions.

410

411 **Data availability**

412 Code and raw data relating to this study are freely available in the Zenodo repository at
413 https://zenodo.org/record/5036419 (doi: 10.5281/zenodo.5036419).

414 **Competing interests**

415 The authors have no competing interests to declare.

416

417 **References**

418 1. Bauer CM, Glassman LW, Cyr NE, Romero LM. 2011 Effects of predictable and unpredictable
419 food restriction on the stress response in molting and non-molting European starlings
420 (Sturnus vulgaris). *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **160**, 390–399.
421 (doi:10.1016/j.cbpa.2011.07.009)

422 2. Witter MS, Swaddle JP, Cuthill IC. 1995 Periodic food availability and strategic regulation of
423 body mass in the European Starling , Sturnus vulgaris. *Funct. Ecol.* **9**, 568–574.

424 3. Cuthill IC, Maddocks SA, Weall CV, Jones EKM. 2000 Body mass regulation in response to
425 changes in feeding predictability and overnight energy expenditure. *Behav. Ecol.* **11**, 189–195.
426 (doi:10.1093/beheco/11.2.189)

427 4. Witter MS, Swaddle JP. 1997 Mass regulation in juvenile Starlings: response to change in food
428 availability depends on initial body mass. *Funct. Ecol.* **11**, 11–15. (doi:10.1046/j.1365-
429 2435.1997.00041.x)

430 5. Bateson M, Andrews C, Dunn J, Egger C, Gray F, Mchugh M, Nettle D. 2021 Food insecurity
431 increases energetic efficiency, not food consumption: an exploratory study in European
432 starlings. *PeerJ* **9**, e11541.

433 6. Bednekoff PA, Krebs JR. 1995 Great tit fat reserves: effects of changing and unpredictable
434 feeding day length. *Funct. Ecol.* **9**, 457–462. (doi:10.2307/2390009)

435 7. Higginson AD, McNamara JM, Houston AI. 2016 Fatness and fitness: Exposing the logic of
436 evolutionary explanations for obesity. *Proc. R. Soc. B Biol. Sci.* **283**, 20152443.

437 8. Nettle D, Andrews C, Bateson M. 2017 Food insecurity as a driver of obesity in humans: The
438 insurance hypothesis. *Behav. Brain Sci.* **40**, e105. (doi:10.1017/S0140525X16000947)

439 9. Lima SL. 1986 Predation risk and unpredictable feeding conditions : determinants of body
440 mass in birds. *Ecology* **67**, 377–385.

441 10. Bednekoff PA, Houston AI. 1994 Optimising fat reserves over winter - a dynamic model. *Oikos*
442 **71**, 408–415.

443 11. Townsend MS, Peerson J, Love B, Achterberg C, Murphy SP. 2001 Food insecurity is positively
444 related to overweight in women. *J. Nutr.* **131**, 1738–1745.

445 12. Anselme P, Güntürkün O. 2018 How foraging works: uncertainty magnifies food-seeking
446 motivation. *Behav. Brain Sci.* , 1–106. (doi:10.1017/s0140525x18000948)

447 13. Brunstrom JM, Cheon BK. 2018 Do humans still forage in an obesogenic environment?
448 Mechanisms and implications for weight maintenance. *Physiol. Behav.*
449 (doi:10.1016/j.physbeh.2018.02.038)

450 14. Anselme P, Otto T, Güntürkün O. 2017 How unpredictable access to food increases the body
451 fat of small passerines: A mechanistic approach. *Behav. Processes* **144**, 33–45.
452 (doi:10.1016/j.beproc.2017.08.013)

453 15. Cornelius EA, Vezina F, Regimbald L, Hallot F, Petit M, Love OP, Karasov WH. 2017 Chickadees
454 faced with unpredictable food increase fat reserves but certain components of their immune
455 function decline. *Physiol. Biochem. Zool.* **90**, 190–200. (doi:10.1086/689913)

456 16. Kowaleski-Jones L, Wen M, Fan JX. 2018 Unpacking the paradox: testing for mechanisms in
457 the food insecurity and BMI association. *J. Hunger Environ. Nutr.* **0248**, 1–15.
458 (doi:10.1080/19320248.2018.1464997)

459 17. Nettle D, Bateson M. 2019 Food-Insecure Women Eat a Less Diverse Diet in a More
460 Temporally Variable Way: Evidence from the US National Health and Nutrition Examination
461 Survey, 2013-4. *J. Obes.* **2019**. (doi:10.1155/2019/7174058)

462 18. Zizza CA, Duffy PA, Gerrior SA. 2008 Food insecurity is not associated with lower energy
463 intakes. *Obesity* **16**, 1908–1913. (doi:10.1038/oby.2008.288)

464 19. Tarasuk VS, Beaton GH. 1999 Women's dietary intakes in the context of household food
465 insecurity. *J. Nutr.* **129**, 672–679.

466 20. Jansen EC, Kasper N, Lumeng JC, Brophy Herb HE, Horodynski MA, Miller AL, Contreras D,
467 Peterson KE. 2017 Changes in household food insecurity are related to changes in BMI and
468 diet quality among Michigan Head Start preschoolers in a sex-specific manner. *Soc. Sci. Med.*
469 **181**, 168–176. (doi:10.1016/j.socscimed.2017.04.003)

470 21. Wiersma P, Verhulst S. 2005 Effects of intake rate on energy expenditure, somatic repair and
471 reproduction of zebra finches. *J. Exp. Biol.* **208**, 4091–4098. (doi:10.1242/jeb.01854)

472 22. Dall SRX, Witter MS. 1998 Feeding interruptions, diurnal mass changes and daily routines of
473 behaviour in the zebra finch. *Anim. Behav.* **55**, 715–725. (doi:10.1006/anbe.1997.0749)

474 23. Marasco V, Stier A, Boner W, Griffiths K, Heidinger B, Monaghan P. 2017 Environmental
475 conditions can modulate the links among oxidative stress, age, and longevity. *Mech. Ageing*
476 *Dev.* **164**, 100–107. (doi:10.1016/j.mad.2017.04.012)

477 24. Blackburn EH. 1991 Structure and function of telomeres. *Nature* **350**, 569–573.
478 (doi:10.1038/350569a0)

479 25. Shay JW, Wright WE. 2019 Telomeres and telomerase: three decades of progress. *Nat. Rev.*
480 *Genet.* **20**, 299–309. (doi:10.1038/s41576-019-0099-1)

481 26. Reichert S, Stier A. 2017 Does oxidative stress shorten telomeres in vivo? A review. *Biol. Lett.*
482 **13**. (doi:10.1098/rsbl.2017.0463)

483 27. Chatelain M, Drobniak SM, Szulkin M. 2020 The association between stressors and telomeres
484 in non-human vertebrates: a meta-analysis. *Ecol. Lett.* **23**, 381–398. (doi:10.1111/ele.13426)

485 28. Nettle D, Andrews C, Reichert S, Bedford T, Kolenda C, Parker C, Martin-Ruiz C, Monaghan P,
486 Bateson M. 2017 Early-life adversity accelerates cellular ageing and affects adult
487 inflammation: Experimental evidence from the European starling. *Sci. Rep.* **7**, 40794.
488 (doi:10.1038/srep40794)

489 29. Pineda-Pampliega J, Herrera-Dueñas A, Mulder E, Aguirre JI, Höfle U, Verhulst S. 2020
490 Antioxidant supplementation slows telomere shortening in free-living white stork chicks.
491 *Proc. R. Soc. B Biol. Sci.* **287**, 1–7. (doi:10.1098/rspb.2019.1917)

492 30. Marasco V, Boner W, Griffiths K, Heidinger B, Monaghan P. 2018 Environmental conditions
493 shape the temporal pattern of investment in reproduction and survival. *Proc. R. Soc. B Biol.*
494 *Sci. Sci.* **285**, 20172442. (doi:10.1016/B978-0-323-60984-5.00062-7)

495 31. Cawthon RM. 2002 Telomere measurement by quantitative PCR. *Nucleic Acids Res.* **30**, 1–6.

496 32. Nussey DH *et al.* 2014 Measuring telomere length and telomere dynamics in evolutionary
497 biology and ecology. *Methods Ecol. Evol.* **5**, 299–310. (doi:10.1111/2041-210X.12161)

498 33. du Sert NP *et al.* 2020 The ARRIVE guidelines 2.0: Updated guidelines for reporting animal
499 research. *PLoS Biol.* **18**, 1–12. (doi:10.1371/journal.pbio.3000410)

500 34. Witter M, Cuthill I, Bonser R. 1994 Experimental investigations of mass-dependent predation
501 risk in the European starling, Sturnus vulgaris. *Anim. Behav.* **48**, 201–222.

502 35. Redfern CP, Clark JA. 2001 *Ringer's Manual*. Thetford: British Trust for Ornithology.

503 36. Bauch C, Boonekamp JJ, Korsten P, Mulder E, Verhulst S. 2019 Epigenetic inheritance of
504 telomere length in wild birds. *PLoS Genet.* **15**, e1007827. (doi:10.1101/284208)

505 37. R Core Development Team. 2020 R: A Language and Environment for Statistical Computing.

506 38. Witter M, Swaddle JP. 1995 Dominance, competition, and energetic reserves in the European
507 starling, Sturnus vulgaris. *Behav. Ecol.* **6**, 343–348. (doi:10.1093/beheco/6.3.343)

508 39. Bautista LM, Tinbergen J, Wiersma P, Kacelnik. A. 1998 Optimal foraging and beyond: How
509 starlings cope with changes in food availability. *Am. Nat.* **152**, 543–61. (doi:10.2307/2463356)

510 40. Nettle D, Monaghan P, Boner W, Gillespie R, Bateson M. 2013 Bottom of the heap: Having
511 heavier competitors accelerates early-life telomere loss in the European starling, Sturnus
512 vulgaris. *PLoS One* **8**, e83617. (doi:10.1371/journal.pone.0083617)

513 41. Nettle D, Monaghan P, Gillespie R, Brilot B, Bedford T, Bateson M. 2015 An experimental
514 demonstration that early-life competitive disadvantage accelerates telomere loss. *Proc. R.*
515 *Soc. B Biol. Sci.* **282**, 20141610. (doi:10.1098/rspb.2014.1610)

516 42. Boonekamp JJ, Mulder GA, Salomons HM, Dijkstra C, Verhulst S. 2014 Nestling telomere
517 shortening, but not telomere length, reflects developmental stress and predicts survival in
518 wild birds. *Proc. R. Soc. B Biol. Sci.* **281**, 20133287. (doi:10.1098/rspb.2013.3287)

519 43. Men F, Gundersen C, Urquia ML, Tarasuk V. 2020 Association between household food
520 insecurity and mortality in Canada: A population-based retrospective cohort study. *Can. Med.*
521 *Assoc. J.* **192**, E53–E60. (doi:10.1503/cmaj.190385)

522 44. Walker RJ, Chawla A, Garacci E, Williams JS, Mendez C, Ozieh MN, Egede LE. 2019 Assessing
523 the relationship between food insecurity and mortality among U.S. adults. *Ann. Epidemiol.*
524 **32**, 43–48. (doi:10.1016/j.annepidem.2019.01.014)

525 45. Marasco V, Boner W, Heidinger B, Grif K, Monaghan P. 2015 Repeated exposure to stressful
526 conditions can have beneficial effects on survival. **69**, 170–175.
527 (doi:10.1016/j.exger.2015.06.011)

528 46. Briga M, Verhulst S. 2017 Individual variation in metabolic reaction norms over ambient
529 temperature causes low correlation between basal and standard metabolic rate. *J. Exp. Biol.*
530 **220**, 3280–3289. (doi:10.1242/jeb.160069)

531 47. Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK. 2004 Developmental stress, social
532 rank and song complexity in the European starling (Sturnus vulgaris). *Proc. R. Soc. B Biol. Sci.*
533 **271 Suppl**, S121-3. (doi:10.1098/rsbl.2003.0122)

534 48. Atema E, Mulder E, van Noordwijk AJ, Verhulst S. 2019 Ultralong telomeres shorten with age
535 in nestling great tits but are static in adults and mask attrition of short telomeres. *Mol. Ecol.*
536 *Resour.* **19**, 648–658. (doi:10.1111/1755-0998.12996)

537 49. Bauch C, Becker PH, Verhulst S. 2014 Within the genome, long telomeres are more
538 informative than short telomeres with respect to fitness components in a long-lived seabird.
539 *Mol. Ecol.* **23**, 300–310. (doi:10.1111/mec.12602)

540 50. Benetos A *et al.* 2013 Tracking and fixed ranking of leukocyte telomere length across the
541 adult life course. *Aging Cell* **12**, 615–621. (doi:10.1111/acel.12086)

542 51. Tricola GM *et al.* 2018 The rate of telomere loss is related to maximum lifespan in birds.
543 *Philos. Trans. R. Soc. B Biol. Sci.* **373**. (doi:10.1098/rstb.2016.0445)

544 52. McNamara JM, Houston AI. 1990 The Value of Fat Reserves and the Tradeoff Between
545 Starvation and Predation. *Acta Biotheor.* **38**, 37–61. (doi:10.1007/BF00047272)

546 53. Houston AI, McNamara JM, Hutchinson JMC. 1993 General results concerning the trade-off
547 between gaining energy and avoiding predation. *Philos. Trans. R. Soc. B Biol. Sci.* **341**, 375–
548 397. (doi:10.1098/rstb.1993.0123)

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The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	