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1 **Fear of predators reduces body and physiological condition affecting offspring**  
2 **survival and the 'quality' of the survivors**

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8

9 **Abstract**

10 1. Predators affect the survival of developing young by eating them. More recently, the fear of  
11 being eaten has been uncovered as an important driver of offspring survival significant enough to  
12 reduce the population growth rate, but the mechanisms driving these effects are not well  
13 understood.

14 2. We exposed song sparrows to predator or non-predator playbacks throughout three breeding  
15 seasons. We followed offspring as eggs through development and into maturity. We quantified  
16 the repercussions of being reared in a fearful environment on the body (fat, mass, structural size)  
17 and physiological condition of offspring using a combination of 12 different measures  
18 categorized into six systems (oxygen carrying capacity, energy reserves, immune function,  
19 antioxidant protection, oxidative stress/damage, glucocorticoids). We further tracked offspring  
20 survival in the nest to post-independence and determined which measures of condition  
21 significantly predicted survival.

22 3. We report that fear of predators affected offspring condition at all stages of life, with survival  
23 consequences. Predator-playback offspring hatched from heavier eggs but quickly lost this  
24 advantage and never regained it. Nestlings reared by 'frightened' parents were as heavy and  
25 much leaner than non-predator playback offspring. This pattern extended throughout

26 development and into independence when offspring previously reared by frightened parents were  
27 leaner and significantly lighter. Predator-playback offspring also were evidently in poorer  
28 physiological condition, having shorter telomeres, while late dependency stage fledglings carried  
29 relatively less uric acid and more haptoglobin perhaps indicative of chronic susceptibility to  
30 infection. Mass and fat both predicted survival during peak mortality periods (nestling and early  
31 fledging), with the leanest lightest offspring from the predator-playback treatment being least  
32 likely to survive. Thereafter, only mass predicted survival regardless of playback treatment.  
33 Similarly, at this later stage, offspring with less telomere loss and high uric acid levels survived  
34 best regardless of playback group.

35 4. Our findings reveal that fear of predators is a potent form of early life adversity which  
36 persistently alters the condition of offspring affecting survival and the ‘quality’ of the survivors.  
37 Development is fueled by food and fear-induced reductions in parental provisioning at the  
38 earliest stages likely instigated these effects, with population level consequences.

39

## 40 **1 INTRODUCTION**

41 The very earliest stages of life are most precarious for developing young. Adversity can reduce  
42 the growth trajectory, body condition, and physiology of young thereby reducing their prospects  
43 of surviving to developmental maturity (e.g. Zippel et al., 2019). The earlier development is  
44 disturbed, the stronger are the effects (Lindström, 1999). Amongst the survivors, early life  
45 adversity can permanently alter the morphological and physiological state of offspring, leaving a  
46 signature of early-life hardship with long-term consequences on their reproduction and survival  
47 as adults, regardless of the environment those adults later face (Burton & Metcalfe, 2014;  
48 Callaghan & Tottenham, 2016; Monaghan & Haussmann, 2015). If early life adversity affects

49 entire populations then the outcome could be a reduction in the population growth rate because  
50 the number of offspring that survive sets the ceiling on the number of offspring available to  
51 recruit (reviewed in Cox et al., 2014; Dudeck et al., 2018). The long-term legacy of early life  
52 adversity imprinted for life on offspring could further interfere with the population growth rate  
53 when those survivors recruit (i.e. silver-spoon or cohort effects, e.g. Payo-Payo et al., 2016;  
54 Pigeon & Pelletier, 2018). In two classic examples, one in a bird (Reid et al., 2003, red-billed  
55 chough) one in a mammal (Albon et al., 1987, red deer), cohorts born in more favourable years  
56 (high temperatures and/or rainfall) with presumably more food available, survived longer and  
57 produced more of their own offspring, whose offspring also produced more offspring, compared  
58 to those born in years when the weather was poor and the food supply presumably low. Such  
59 effects introduce a time lag that can negatively affect the population growth rate (Sibly & Hone,  
60 2002).

61         Relatively recently, the perceived presence of predators (cf. actual predation) has been  
62 uncovered as a significant cause of early life adversity for individuals that has population-level  
63 impacts on wildlife (reviewed in Zanette & Clinchy, 2020). Manipulations of perceived  
64 predation risk in both birds and mammals frequently alter the birth rate (i.e. propagules  
65 produced) in addition to the survival rates of those propagules leading to fewer offspring  
66 produced per capita (reviewed in Zanette & Clinchy, 2020), now known to be substantial enough  
67 to significantly reduce the population growth rate (Allen et al., 2022). Of offspring that survive,  
68 Allen et al. (2022) further revealed that a long-term imprint of their early rearing condition  
69 remains evident into adulthood such that cohorts reared by ‘frightened’ parents were expected to  
70 reduce the population growth rate even further because those adult offspring sang fewer songs

71 (Allen et al., 2022), an indicator of reduced survival and lifetime reproductive success (Reid et  
72 al., 2005).

73         While predator risk can clearly affect the survival of offspring and leave a lasting legacy  
74 on the survivors, the mechanistic drivers of these ‘fear’ effects are less clear. Development is  
75 largely fueled by food and a lack of food has been proposed as a key mechanism that creates  
76 early life adversity reducing both survival and the phenotype of the survivors (reviews in Burton  
77 & Metcalfe, 2014; Lindström, 1999). In both birds and mammals, reductions in the body mass of  
78 offspring, due to a lack of food, can be predictive of later survival (e.g. Magrath, 1991; reviewed  
79 in Ronget et al., 2018) presumably because body mass is often positively correlated with body  
80 fat which are the reserves necessary to tap into at times that food becomes relatively scarce  
81 (Ronget et al., 2018). The perceived presence of predators can reduce the amount of food that  
82 dependent offspring receive leading to changes in body mass and survival. For example, using  
83 audio playbacks of predators or a non-predator control, Zanette et al. (2011) reported that song  
84 sparrow nestlings reared by frightened parents were provisioned less, lighter in mass, and also  
85 more likely to die. However, no predator risk manipulation in wildlife has examined the body  
86 condition and survival of offspring throughout each stage of their development and then  
87 determine whether body condition is directly predictive of survival at each stage.

88         Aside from body condition, early life adversity could also affect the physiological  
89 condition of offspring which could additionally affect survival probabilities and/or the long  
90 lasting “quality” of the survivors (Gicquel et al., 2022; Marasco et al., 2022; Slos & Stoks,  
91 2008), but few manipulations have examined how the physiological condition of offspring is  
92 altered by perceived predation risk. Of those that have, predation risk effects have been  
93 demonstrated on oxidative damage (e.g.s Janssens & Stoks, 2013; Slos & Stoks, 2008), telomere

94 lengths (Kärkkäinen et al., 2019; Noguera & Velando, 2019), immunity (Roncalli et al., 2020),  
95 corticosterone and androgens (Ibáñez-Álamo et al., 2011), and thermoregulatory stress of  
96 unfeathered hatchlings (Zanette et al., 2011). All manipulations thus far have focussed on single  
97 traits or physiological systems but perceived predation risk may be expected to have pleiotropic  
98 effects on multiple traits in multiple physiological systems (e.g. Boonstra et al., 1998; Fowler &  
99 Williams, 2017; Travers et al., 2010). Moreover, components of the same physiological system  
100 do not necessarily covary suggesting that animals may make adjustments independently (e.g.  
101 Fowler & Williams, 2017) requiring measurement of multiple components for each system when  
102 possible (also see Boonekamp et al., 2018).

103         We worked within the same manipulation as Allen et al. (2022) in which we reported the  
104 population-level consequences of fear. Here, our interest was to examine the extent to which the  
105 fear of predators might alter the condition of offspring at all stages of development and into  
106 maturity. We focussed on aspects of body condition (e.g. fat and mass) and physiological  
107 condition which we assessed using 12 different measures categorized into six different  
108 physiological systems including oxygen carrying capacity, energy reserves, immune function,  
109 antioxidant protection, oxidative stress/damage, and glucocorticoids. We next determined which  
110 condition measures (if any) were significantly predictive of survival at each stage. Finally, we  
111 examined whether measures significantly predictive of survival were more or less affected by  
112 playback treatment. We had previously found that fear of predators led to a 53 % reduction in the  
113 number of recruited offspring produced largely due to significant reductions in offspring survival  
114 (Allen et al. 2022) in the nest and in the first week out of the nest. Thereafter, mortality rates  
115 were similar between the playback treatments even when offspring were still fully dependent on

116 their parents for food. Consequently, we anticipated that condition measures would negatively  
117 affect predator playback offspring survival most at these earliest stages.

118

## 119 **2 MATERIALS AND METHODS**

### 120 Study area and species.

121 We conducted our manipulation on wild, free-living, song sparrows distributed across five ( $\leq$   
122 220 ha) Gulf Islands, in B.C. (details in Allen et al., 2022). The sparrows are resident and occupy  
123 exclusive territories. They build open-cup nests typically in low shrubs and build a new nest  
124 upon each attempt. Egg-laying occurs April-July, the typical clutch size is 3 - 4 eggs, incubation  
125 lasts 13 days, mothers alone incubate the eggs, both parents feed the nestlings, nestlings  
126 normally fledge 12 days after hatching, all pairs can successfully rear two broods. After fledging,  
127 offspring cannot engage in sustained flight for approximately 7 days (Dybala et al., 2013; L.  
128 Zanette, unpub. data), and they remain completely dependent on their parents for food  
129 provisioning for the first 3 weeks post-fledging (i.e. 0 – 21 d post-fledge), and most are  
130 independent thereafter (Dybala et al. 2013).

131

### 132 Experimental design

133 Details of the manipulation and radio-tagging are in Allen et al. (2022; also see Zanette et al.  
134 2011). Briefly, we monitored the nests of 51 and 53 adults from the predator and non-predator  
135 playback manipulations, respectively, at 11 – 15 study sites over the three breeding seasons  
136 (2010, 2013, 2014). We located nests using behavioural cues from parents. We manipulated  
137 perceived predation risk by broadcasting either predator or non-predator sounds over the course  
138 of nesting. We eliminated direct killing by mammalian and large bird predators by encircling all

139 nests with portable electric fences and covering them with seine netting. The smaller predatory  
140 (Swan et al., 2015) brown-headed cowbird was removed from each site with live traps.  
141 Playbacks included 8 species of predator and 8 species of non-predator sounds, with multiple  
142 exemplars per species, broadcast at random times. Predators included the Common Raven  
143 (*Corvus corax*), Northwestern Crow (*Corvus caurinus*), Cooper's Hawk (*Accipiter cooperii*),  
144 Brown-headed Cowbird (*Molothrus ater*), raccoon (*Procyon lotor*), Barred Owl (*Strix*  
145 *varia*), Western Screech-Owl (*Otus kennicotti*), and Northern Saw-whet Owl (*Aegolius arcadius*).  
146 Nonpredator species, here listed in the order matching the corresponding predator, comprised the  
147 Canada Goose (*Branta canadensis*), Mallard Duck (*Anas platyrhynchos*), Northern Flicker  
148 (*Colaptes auratus*), Rufous Hummingbird (*Selasphorus rufus*), harbor seal (*Phoca vitulina*),  
149 wood frog (*Rana sylvatica*), Common Loon (*Gavia immer*), and Pacific chorus frog (*Pseudacris*  
150 *regilla*). All of the predators are known to kill adult song sparrows or their offspring. Sounds  
151 were interspersed with periods of silence including a 4-d on 4-d off cycle at which time speakers  
152 were moved to new locations within each site. This combination of techniques has been  
153 successful in manipulating fear in free-living wildlife, while avoiding habituation, in not only  
154 this long-term experiment (Allen et al., 2022; Zanette et al., 2011) but in others done in different  
155 systems on animals as diverse as cougars (Suraci et al., 2019) raccoons (Suraci et al., 2016), and  
156 opossums, bobcats, skunks (Suraci et al., 2019). In 2010 and 2013 playbacks were begun before  
157 first nests were built, and at hatch in 2014. In all years, playbacks ended when the last nestling  
158 fledged.

159           The survival of fledged offspring was established by tracking 151 randomly selected  
160 radio-tagged young from fledging to 49 days post-fledging corresponding to the end of the



161 breeding season (31 August) in 2013 and 2014. All radio-tags weighed  $< 3\%$  of the bird's body  
162 mass and were fitted with a leg-loop harness and tracked with hand-held antennas and receivers.

163

## 164 Fear effects on offspring condition

### 165 **Assessment of body condition**

166 We measured egg mass as an estimation of condition given that heavier eggs would hatch out  
167 heavier nestlings who would thus have an initial mass advantage. A variety of manipulations  
168 have repeatedly reported that when food intake is restricted either by the bottom-up food supply,  
169 or via predator-induced reductions in food-intake (Zanette et al., 2006, 2009; Zanette et al.,  
170 2011), song sparrows will lay fewer eggs that are relatively heavy. Alleviating this restriction  
171 leads to more, relatively light eggs. Consequently, we expected eggs from the predator playback  
172 treatment to be relatively heavy. Eggs were weighed to 0.01 g using an electronic balance and  
173 initial egg mass estimated following the standard procedure validated for song sparrows (see  
174 Zanette et al. 2011).

175 Six-day-old nestlings were measured for mass, fat, and structural size (tarsus and wing  
176 chord). Fat levels consisted of an index summed over the furculum (scored 0 to 7) and abdomen  
177 (scored 0 to 3) (following Travers et al., 2010). We expected that our index of total fat was an  
178 accurate reflection of nestling condition because it was significantly and positively correlated  
179 with nestling mass (Spearman rank correlation:  $r = 0.35$ ,  $P < 0.001$ ). We estimated hunger levels  
180 by hand-feeding nestlings after processing them but before placing them back into the nest. The  
181 food consisted of moistened egg biscuit (Living World Egg Biscuit) rolled into a standard sized 1  
182 cm diameter ball, and our index was scaled from 0 (no food accepted) to three (three  
183 standardized balls accepted). Mass was measured as per eggs. The structural size of offspring

184 was evaluated by measuring the right tarsus (to 0.01 mm, using calipers) and right wing chord (to  
185 0.5 mm, using a wing ruler).

186 Fat, mass, tarsus and wing chord lengths were again measured for fledged, radio-tagged  
187 offspring at three stages. Once when newly fledged (1 d post-fledge, median), once again in the  
188 late dependency stage (14 d post-fledge, median), and once when the offspring were independent  
189 (40 d post-fledge, median).

190

### 191 **Assessment of physiological condition**

192 We assessed the physiological condition of 64 radio-tagged nestlings (35 non-predator, 29  
193 predator treatment) with three variables including haematocrit (% pack cell volume), glucose  
194 (mmol L), and red blood cells' relative telomere length (T/S ratio). The physiological condition  
195 of 33 fledged offspring (17 non-predator, 16 predator treatment) was assessed using 12  
196 physiology variables over six physiological systems including (1) oxygen carrying capacity:  
197 haematocrit (% pack cell volume); (2) energy reserves: nonesterified fatty acids (NEFAs, mmol  
198 L), glucose (mmol L); (3) immune function: haptoglobin (mg mL), lysis agglutination (natural  
199 antibody titres); (4) antioxidant protection: uric acid (mg dL), plasma antioxidant capacity  
200 (OXY; total antioxidant titres, mmol HClO L); (5) oxidative stress/damage: reactive oxygen  
201 metabolites (dROMs; mg H<sub>2</sub>O<sub>2</sub> dL), telomere length; the rate of telomere shortening (6)  
202 glucocorticoids: baseline plasma corticosterone (ng mL), free plasma corticosterone (ng mL).  
203 The rate of telomere shortening was estimated as telomere length at late dependency (d 14 post-  
204 fledge) minus the nestling stage (d 6 post-hatch) such that high values in the negative represent  
205 the greatest rates of loss.

206           In all cases, blood was collected from the brachial vein within 3 min of capture and  
207 glucose was immediately measured using a glucose meter. All remaining blood was stored on ice  
208 for transport. Red blood cells (for telomeres) and plasma were extracted following centrifugation  
209 after measurement for haematocrit (packed cell volume/total volume) and were frozen at -20°C  
210 within 10 h. Nestlings were sampled in the course of banding (d 6 post-hatch). Fledglings were  
211 sampled in the late dependency stage (d 14 post-fledge). All procedures and assays followed  
212 those published previously (glucocorticoids: Clinchy et al., 2004, 2011; telomeres: Criscuolo et  
213 al., 2009; all other assays: Fowler et al., 2018; Fowler & Williams, 2017) with further details in  
214 the Supplementary Information, Appendix 1. The physiological traits used allowed us to capture  
215 multiple physiological systems including those that reportedly vary with perceived predation risk  
216 (see Introduction). Within this context, our choice of traits was a compromise between the  
217 amount of plasma available and assay availability.

218

219 **Replication statement**

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale	Stage	Measure
Treatment	Average per Nest	37,42	egg	mass
Treatment	Average per Nest	68,71	nestling	body condition variables
Treatment	Individual	65,54	newly fledge	body condition variables
Treatment	Individual	56,44	late dependency	body condition variables
Treatment	Individual	22,17	independent	body condition variables
Treatment	Individual	247,197	nestling	survival and body condition variables
Treatment	Individual	64,54	newly fledge	survival and body condition variables
Treatment	Individual	56,44	late dependency	survival and body condition variables
Treatment	Individual	35,30	nestling	physiology variables
Treatment	Individual	17,16	late dependency	physiology variables
Treatment	Individual	29,26	nestling	survival and physiology variables
Treatment	Individual	17,16	late dependency	survival and physiology variables

220

## 221 Statistical analyses

222 Our previous results revealed that the fear of predation had effects on the survival of offspring  
223 during the nestling and early post-fledging stage, with no discernable fear effects on survival at  
224 later stages of life (Allen et al., 2022). We now examine whether fear also affected (i) the body  
225 and physiological condition of offspring across stages. Then, we analyzed (ii) the body followed  
226 by physiology of offspring to determine which combination of variables for each were  
227 significantly predictive of survival across stages. Fear might lead to differences in offspring  
228 condition variables perhaps reflecting the consequences of being reared in a poor environment

229 (i.e. the ‘quality’ of offspring) independent of condition effects on survival (i.e. the ‘quantity of  
230 offspring). Consequently, we included all indices of body and then physiological condition in  
231 models (i) and (ii). Finally, we (iii) examined whether condition traits significantly predictive of  
232 survival led to differential effects on survival according to playback treatment.

233         We analyzed average egg mass per nest from 2010 and 2013, excluding 2014 when  
234 playbacks began at hatch. Data for the body condition of nestlings included the average fat levels  
235 and mass of each brood in 2010, 2013, 2014. Experimental food intake of nestlings was  
236 measured in 2013 and 2014 and averaged per nest. Egg mass, nestling body condition and  
237 experimental food intake were analyzed with 3-factor linear mixed-model ANOVAs (GLMMs),  
238 including playback treatment, nest number (1 or 2), and year, with parent identity as a random  
239 effect. Body condition variables of radio-tagged post-fledged young were collected in 2013 and  
240 2014, and analyzed with 3-factor GLMMs including playback treatment, year, and stage (newly  
241 fledged, late dependency, independence) entered as a repeated measures term. Individual  
242 offspring identity and nest identity were the random effects. Because neither nest number nor  
243 year interacted with playback treatment, we only present results for our main variable of interest,  
244 playback treatment.

245         We collected physiology data in 2013 on radio-tagged birds. We examined the  
246 physiology of nestling and late dependency stage offspring by entering all of our physiology  
247 variables into one multivariate model. Because our aim was to find the combination of variables  
248 that significantly differentiated offspring from the two playback treatments, we used  
249 Discriminant Function Analysis (DFA, Travers et al., 2010) after testing for assumptions. We  
250 chose backward elimination as our data reduction technique, removing the least non-significant  
251 variable at each step until only significant variables remained. We cross -validated our models

252 using a jack-knife procedure in which the data set was split into a training and testing set. The  
253 training set used all observations but one to build a model and that model is then used to predict  
254 the group membership of the one observation left out. Nestling physiology included 3 variables  
255 (telomere length, haematocrit, glucose). For late dependency stage offspring, we excluded free  
256 corticosterone because it was highly correlated with baseline corticosterone ( $R^2 = 0.993$ ) thereby  
257 exceeding the minimum acceptable tolerance level of 0.01, leaving us with 11 physiology  
258 variables to analyze.

259 We next tested whether body condition variables were predictive of: 1) nestling survival  
260 to fledgling; 2) survival of newly fledged radio-tagged young to 7 d post-fledge, which is the  
261 most perilous time for fledged offspring in this species and many others; 3) survival of late  
262 dependency stage (14 d) radio-tagged offspring to the end of the study (49 d post-fledge), well  
263 past independence using Cox-Proportional Hazards models. We included the variables fat + mass  
264 + fat \* mass, removing the interaction term if not significant and re-running the models. We  
265 tested the interaction because we reasoned that offspring of a given mass might be more prone to  
266 mortality if they also were less fat. Next, we crossed fat and mass with playback treatment to  
267 assess whether playback treatment rendered offspring more or less vulnerable to mortality, again  
268 removing higher order interactions if not significant. We also conducted Principal Component  
269 Analysis on fat and mass and determined whether the PC1 scores for individuals were predictive  
270 of survival in Cox-Proportional Hazards models. At all developmental stages PC1 explained at  
271 least 66% of the variation whereby heavy offspring were also fat.

272 To assess how physiology might impact survival, we combined all physiology variables  
273 (3 for nestlings, 11 for late dependency stage fledglings) into two Cox-Proportional Hazards  
274 models and again conducted backward elimination (using the same criteria as above) to find the

275 combination of variables that significantly predicted survival. Backward elimination has the  
276 benefit of considering correlations amongst variables at the outset thereby assessing their joint  
277 predictive power. For nestlings, we examined survival to the first 7 d out of the nest. Examining  
278 survival in the nest was not possible because all nestlings sampled fledged, which was  
279 intentional because one objective was to continue to monitor and further sample offspring once  
280 fledged. For late dependency stage fledglings, we examined survival into post-independence (49  
281 d post-fledge). All of our Cox-Proportional Hazards models included parent identity as a random  
282 effect. We first ran our models stratifying by playback treatment to gain insight into the  
283 combination of variables that significantly predicted survival in general. Statistically significant  
284 variables remaining from backward elimination were then examined individually and crossed  
285 with playback treatment to determine whether there were any differential effects on survival as a  
286 result of fear. Combining all 11 physiology measures into a Principal Component analysis  
287 provided the scores for each offspring which we also tested against survival using Cox-  
288 Proportional Hazards.

289         To gain further insight into the signal of physiology that our measures might be reflecting  
290 we conducted Spearman Rank Correlations of all 11 analyzed variables in addition to fat and  
291 mass. We also conducted Principal Component Analysis on the entirety of our condition  
292 measures (body and physiology, 13 variables) to confirm these associations. We present only  
293 eigenvectors  $> 0.32$  (explaining more than 10 % of the variation). We then tested whether the  
294 scores calculated from the Principal Component Analysis was predictive of survival with Cox-  
295 Proportional Hazards.

296         For body condition, no measure of body size (tarsus length or wing chord) either differed  
297 between the playback treatments or was predictive of the survival probability of offspring at any

298 stage (all  $p > 0.18$ ). Consequently, we report only results for fat and mass. SAS Studio was used  
299 for our survival models and SPSS v 28 for everything else.

300

### 301 **3 RESULTS**

#### 302 Fear effects on offspring condition

303 Fear effects on condition were evident at all stages in the life of offspring (Table S1). To begin,  
304 predator playback females laid fewer eggs (Allen et al., 2022), but those eggs were 8 % heavier  
305 on average (predator vs. non-predator:  $3.01 \pm 0.043$  g vs.  $2.79 \pm 0.047$  g; GLMM:  $F_{1,79} = 11.7$ ,  $P$   
306  $= 0.001$ ). Although offspring from the predator playback treatment hatched from heavier eggs,  
307 nestlings ended up less fat (Fig. 1; GLMM:  $F_{1,76.4} = 7.8$ ,  $P = 0.007$ ), and were evidently hungrier  
308 than non-predator playback nestlings given that they ate more food provided by us (predator vs.  
309 non-predator:  $2.91 \pm 0.075$  vs  $2.79 \pm 0.089$ ; GLMM:  $F_{1,68.3} = 4.2$ ,  $P = 0.043$ ). Nestlings from the  
310 predator playback treatment were as heavy as those from the non-predator playback group,  
311 despite their initial mass advantage at the egg stage (Fig. 1;  $F_{1,80.9} = 0.2$ ,  $P = 0.62$ ).

312 Of the three physiology measures we took at the nestling stage, only telomere length  
313 significantly differentiated between the playback treatments (DFA,  $F_{1,63} = 4.9$ ,  $P = 0.03$ ; all other  
314  $P > 0.46$ ; SI Table 2), whereby nestlings had significantly shorter telomere lengths when reared  
315 in the predator versus the non-predator playback treatment (means  $\pm$  sem:  $1.12 \pm 0.148$  versus  
316  $1.57 \pm 0.166$ , respectively).

317 The reduced fat levels of nestlings reared in the predator playback treatment carried  
318 through to fledge day, extended well into the late dependency stage, and on into independence  
319 (Fig 1; Repeated Measures GLMM, main effect of playback treatment:  $F_{1,77.9} = 15.4$ ,  $P < 0.000$ ;  
320 playback treatment x stage interaction:  $F_{2,101.1} = 0.2$ ,  $P = 0.84$ ). Post-fledged offspring from the



321 predator playback treatment also tended to be lighter in mass across stages (Fig 1; Repeated  
322 Measures GLMM, main effect of playback treatment:  $F_{1,120.3} = 3.3$ ,  $P = 0.072$ ), though the  
323 differences in mass between the playback treatments were only pronounced at independence (Fig  
324 1; playback treatment x stage interaction:  $F_{2,61.4} = 2.9$ ,  $P = 0.065$ ).

325 The physiology of late dependency stage fledglings also differed according to playback  
326 treatment (DFA, *Wilk's*  $\lambda_2 = 0.7$ ,  $P = 0.006$ ). Of the 11 physiology measures analyzed, offspring  
327 from the predator playback treatment were characterized as having a combination of lower uric  
328 acid levels (Factor Structure Coefficient, -0.44;  $F_{1,30} = 9.1$ ,  $P = 0.005$ ) and more haptoglobin  
329 (0.56;  $F_{1,30} = 7.0$ ,  $P = 0.013$ ) compared to non-predator playback birds. None of our other  
330 physiology measures remained in the model (SI Table 2).

331

### 332 Fear effects on offspring condition and survival

333 Fat and mass were both important predictors of survival at all stages in the life of offspring, and  
334 also varied according to playback treatment (Table S1). At the nestling stage, the average fat  
335 levels (Spearman Rank Correlation,  $R = -0.3$ ,  $P = 0.0006$ ) and mass of the brood ( $R = -0.3$ ,  $P =$   
336  $0.0003$ ) were each negatively correlated with the proportion of the brood that expired. Individual  
337 nestlings within a brood that were both lean and light were less likely to survive to fledge (Fig. 2;  
338 fat \* mass: *Wald*  $\chi^2 = 4.1$ ,  $P = 0.034$ ), but it was the much leaner nestlings from the predator  
339 playback treatment that were most susceptible to mortality in contrast to those fatter nestlings  
340 that heard non-predator sounds (Fig. 2; playback treatment \* fat \* mass: *Wald*  $\chi^2 = 13.9$ ,  $P =$   
341  $0.0002$ ). For example, as Fig 2 indicates, for a given mass on the x-axis, nestlings from the  
342 predator playback treatment that died were less fat (y-axis) than survivors, and also were less fat  
343 than any nestling from the non-predator playback treatment. Principal Component scores

344 incorporating fat and mass also were significantly predictive of nestling survival whereby  
345 heavier and fatter nestlings survived best ( $Wald \chi^2 = 42.8, P < 0.0001$ ). None of our three  
346 physiology measures were predictive of survival, nor were the Principal Component scores based  
347 on these three measures (all  $P > 0.20$ )

348 Newly fledged young were less likely to survive the critical first seven days out of the  
349 nest if they were both lean (Fig 2;  $Wald \chi^2 = 4.0, P = 0.037$ ) and light ( $Wald \chi^2 = 5.7, P = 0.013$ ;  
350 mass \* fat:  $Wald \chi^2 = 3.2, P = 0.059$ ; Principal Component Scores on fat and mass:  $Wald \chi^2 =$   
351  $13.6, P = 0.0002$ ), and again, it was the leanest fledglings from the predator playback treatment  
352 that were least likely to make it through those first seven days (Fig 2; playback treatment \* mass  
353 \* fat:  $Wald \chi^2 = 4.4, P = 0.03$ ).

354 Mass remained a significant predictor of survival for offspring in the late dependency  
355 stage whereby lighter offspring were less likely to survive than heavier ones by the end of the  
356 study when all offspring were independent juveniles (i.e. 49 d post-fledge: CPH,  $Wald \chi^2 = 8.1,$   
357  $P = 0.003$ ). Moreover, relatively light late dependency stage young were somewhat more prone  
358 to mortality if from the predator than the non-predator playback treatment, though the effects  
359 were not statistically significant (CPH, survival to independent juvenile stage:  $Wald \chi^2 = 2.7, P$   
360  $= 0.080$ ). Fat no longer carried an effect on survival at this stage either as a main effect or an  
361 interaction with mass (CPH, all  $P > 0.66$ ). Principal Component scores incorporating fat and  
362 mass were significantly predictive of survival at this late stage ( $Wald \chi^2 = 8.6, P = 0.003$ ).

363 In the late dependency stage, physiology was predictive of survival to post-independence  
364 regardless of playback treatment (all  $P$ -values  $> 0.24$ ; SI Table S2). Specifically, of our 11  
365 physiology measures analyzed, it was the combination of telomere shortening rate (Fig. 3; CPH:  
366  $Wald \chi^2 = 7.5, P = 0.004$ ) and uric acid level (Fig. 3; CPH:  $Wald \chi^2 = 3.9, P = 0.034$ ) that

367 remained after backward elimination (see Table 1 for the order of elimination). Here, the hazard  
368 ratios indicated that there was a 5.3 fold ( $\pm 1.84$ ) and a 1.1 fold increase ( $\pm 1.03$ ) in the  
369 likelihood of mortality with every one unit drop in each of these variables, respectively, for all  
370 offspring (interactions with playback treatment, all  $P > 0.26$ ). Principal Component scores  
371 incorporating all 11 physiological measures were not significantly predictive of survival in the  
372 late dependency stage (*Wald*  $\chi^2 = 1.2$ ,  $P = 0.20$ )

373 That 9 of 11 physiology measures were removed from our survival model does not  
374 suggest that those 9 variables were unimportant. Indeed, examining the physiology variables  
375 more closely revealed that 5 of 9 excluded variables were correlated with the two that remained  
376 (Table 1). That is, telomere shortening rate was most closely correlated with measures associated  
377 with energy supply (nonesterified fatty acids), and fat and mass to some extent. Uric acid was  
378 most closely correlated with our two measures of immune function (lysis and haptoglobin), in  
379 addition to corticosterone, and antioxidant protection (OXY) to some extent. Principal  
380 component analysis on all body and physiology variables confirmed that less telomere shortening  
381 (eigenvector = 0.35) positively loaded with energy supply (NEFA, 0.47), fat (0.45), and mass  
382 (0.53; all other eigenvectors  $< 0.32$ ; PC1, eigenvalue 2.6, explaining 20 % of the variation).  
383 Indeed, the Principal Component scores for this axis incorporating both body and physiological  
384 condition was significantly predictive of whether or not late stage offspring would survive  
385 beyond independence (*Wald*  $\chi^2 = 4.3$ ,  $P = 0.029$ ). Uric acid levels (eigenvector = 0.56)  
386 negatively loaded with immune function (lysis, -0.47; PC2, eigenvalue 2.1, explaining 16 % of  
387 the variation). The Principal Component scores for this axis were not significantly predictive of  
388 offspring survival (*Wald*  $\chi^2 = 0.2$ ,  $P = 0.54$ ).

389

## 390 **4 DISCUSSION**

391 We previously reported that fear of predators led to a 53 % reduction in the number of recruited  
392 offspring produced largely due to significant reductions in offspring survival (Allen et al. 2022)  
393 that occurred primarily in the nest and in the first week out of the nest with no playback  
394 treatment differences in survival thereafter. Here, we demonstrate that fear of predators affected  
395 the body and physiological condition variables of offspring at these earliest stages, such that  
396 predator playback offspring put on less fat and had shorter telomeres lengths than non-predator  
397 playback offspring. We further report that, being especially lean and light, predator playback  
398 offspring were the least likely to survive those early stages. The condition of offspring continued  
399 to diverge between playback treatments even at later stages of life, as expected if early life  
400 adversity sets an irreversible trajectory on condition. Predator playback offspring remained  
401 leaner and lighter and had higher haptoglobin and lower uric acid levels. None of these condition  
402 variables, however, differentially affected the survival of predator playback birds. Instead, lighter  
403 offspring, and those with both high telomere shortening rates and low levels of uric acid were  
404 less likely to survive irrespective of treatment.

405 Predator-playback offspring hatched from heavier eggs but quickly lost this body  
406 condition advantage and never regained it. Instead, their early poor body condition stayed with  
407 them throughout the entire developmental period, into independence, and beyond (Table S1).  
408 The importance of body condition is that it was linked to the survival prospects of offspring.  
409 Offspring survival was, in turn, a key driver of the population growth rate in a fear manipulation  
410 conducted on these same individuals (Allen et al., 2022). Measures of early offspring body  
411 condition are often reported as strong predictors of later offspring survival in both birds (Cox et  
412 al., 2014; Dybala et al., 2013; Magrath, 1991; Naef-Daenzer & Gruebler, 2016; Ronget et al.,

413 2018), and mammals (e.g. Plard et al., 2015; Ronget et al., 2018), as we too found. Moreover,  
414 that early offspring survival drives variation in the population growth rate for many birds and  
415 mammals also is known (Bjorkvoll et al., 2016; Cox et al., 2014; Dybala et al., 2013; Gaillard et  
416 al., 1989; Gaillard & Yoccoz, 2003; Reid et al., 2011). The insight from our manipulation is that  
417 the perceived presence of predators is a key mechanism involved in compromising body  
418 condition, thereby reducing survival, and the population growth rate of prey.

419       Most studies examining the relationship between early body condition and later survival  
420 have focussed on mass. Body mass is often positively correlated with fat such that lighter  
421 animals have fewer body reserves to rely on when challenged with restrictions in food intake  
422 (reviewed in Ronget et al., 2018), as we also found. The effects of reduced fat on survival would  
423 be expected to be particularly severe during early growth when energy demands are highest. The  
424 results from our manipulation would appear consistent with these ideas. That predator playback  
425 offspring were fed less (Zanette et al., 2011) and were evidently hungrier likely led to lower  
426 offspring fat levels, and both fat and mass were predictive of survival during the nestling and  
427 early fledging stages, the times of highest growth. The effects of fat and mass on survival were  
428 particularly harsh on predator-playback chicks and newly fledged young, who had a much  
429 reduced chance of surviving those early stages of life compared to non-predator playback birds  
430 (Allen et al., 2022). The next time we measured fat and mass was when growth was largely  
431 complete (late dependency stage). Here, even though predator playback offspring were still  
432 leaner and somewhat lighter than were offspring from the non-predator playback treatment,  
433 mortality rates were similar between treatments. At this time, it was mass, but not fat, that was a  
434 significant predictor of survival for offspring regardless of playback treatment. Taken together,  
435 the implications here are that (i) the mechanism driving the often reported relationship between

436 early mass and later survival may be primarily due to fat levels during early development; (ii)  
437 because the fat levels of predator playback offspring were lower at all stages measured, but  
438 dropped out as a predictor of survival near independence, suggests that fat levels in later life may  
439 be a useful signal of early life adversity.

440         Physiology may contain another possible signal of early life adversity because predator  
441 playback young at the late dependency stage were characterized as having relatively low uric  
442 acid and high haptoglobin levels. Uric acid is a by-product of protein metabolism and reduced  
443 parental food provisioning (Zanette et al., 2011) would mean less protein to metabolise for  
444 predator playback young. Haptoglobin is a first-line of defense in fighting the inflammation  
445 associated with infection (Roast et al., 2020) and can be a reliable indicator of infection status in  
446 birds (Sebastiano et al., 2017). One other manipulation examining predator effects on immune  
447 responses demonstrated that predator exposure increased the susceptibility of adult house  
448 sparrows to infection and did so at least 6-weeks post exposure (Navarro, 2004), thereby  
449 persistently triggering the immune system long-term. It is possible then that predator playback  
450 offspring had been, and perhaps continued to be, more susceptible to infection and so chronically  
451 engaged in immune responses beginning at least as early as the nestling stage.

452         The combination of low uric acid and high haptoglobin levels also may interact in their  
453 effects on offspring condition. Immune responses can result in the production of reactive oxygen  
454 species which are beneficial in damaging pathogens, but harmful because they can also damage  
455 host cells (Armour et al., 2020). Uric acid is a by-product of protein metabolism that also has  
456 antioxidant functionality, which can neutralize oxidative damage (Cohen et al., 2007) and having  
457 less uric acid, would reduce this capacity. Similarly, if predator-playback offspring were more  
458 susceptible to infection in early life, then this might have exacerbated the negative effects on

459 their body condition evident as a result of being fed less by their parents (Roncalli et al., 2020).  
460 Poor feeding generally results in poor body condition and animals in poor body condition are  
461 least able to fend off infection which can then worsen condition thereby increasing the incidence  
462 of infection (the “vicious circle”, reviewed in Strandin et al., 2018). Infection itself can degrade  
463 body condition because immune defences and challenges require the mobilization of energy and  
464 nutrients which the body has to tap into, diverting those resources from other processes including  
465 growth (Armour et al., 2020; Strandin et al., 2018; but see Hasselquist & Nilsson, 2012). Food  
466 manipulation experiments, mostly on young birds (Hasselquist & Nilsson, 2012), indicate that  
467 food does affect responses to immune challenges (meta-analysis in Strandin et al., 2018).

468         Merrill et al. (2017) suggested that haptoglobin levels measured during development is an  
469 indicator of early life adversity and an excellent predictor of the quality of individuals once they  
470 become breeders themselves as measured by their sexual signals. Merrill et al. (2017)  
471 demonstrated that when given an immune challenge, developing male zebra finches in the lab  
472 had higher haptoglobin levels which were negatively correlated with song complexity later in life  
473 as adults (uric acid levels were not measured). Like most cells in the body, brain nuclei  
474 associated with song learning grow rapidly during development in early life and are sensitive to  
475 perturbations in development the most common being reductions in parental food provisioning  
476 (MacDonald et al., 2006; Nowicki et al., 1998, 2000), which can be predator-induced (Allen et  
477 al., 2022; Zanette et al., 2011). Consequently, the songs adults sing frequently reflects their past  
478 levels of development stress (Buchanan et al., 2003; MacDougall-Shackleton & Spencer, 2012).  
479 Because song is a sexually selected trait, song complexity can be predictive of an individual’s  
480 longevity and lifetime reproductive success, as is the case in song sparrows (Reid et al., 2005).  
481 Indeed, song sparrows in Allen et al. (2022) did sing fewer songs as adults when raised by

482 fearful parents. It is possible that the haptoglobin levels (and perhaps uric acid levels) we found  
483 were forecasting the effect that predator-induced early life stress would have on this important  
484 sexual signal.

485 In addition to reflecting the effect of early life developmental stresses, and possibly  
486 signifying future quality as an adult, uric acid was also an important predictor of the probability  
487 that late dependency stage offspring would survive to the end of the breeding season, but in this  
488 case it affected all offspring regardless of playback treatment. Another significant and even  
489 stronger predictor here was the rate of telomere shortening (also see Criscuolo et al., 2019;  
490 Sheldon et al., 2022). Indeed, the overall physiological profile of late stage offspring  
491 (incorporating all 11 physiology measures) did not predict survival while telomere shortening  
492 rates and uric acid levels together did.

493 Telomere shortening rates (reviewed in Monaghan & Ozanne, 2018) are often associated  
494 with oxidative stress which can occur when reactive oxygen species are produced in excess of  
495 the circulating antioxidants available to counteract their effects, potentially leading to DNA  
496 damage (Gormally et al., 2019) including the DNA caps formed by telomeres. We did not find,  
497 however, that telomere shortening rates were correlated to our measures of oxidative  
498 stress/damage. Instead, high telomere shortening rates were associated with low levels of fat and  
499 mass (also see Boonekamp et al., 2014) and energetics (non-esterified fatty acid levels, NEFA).  
500 NEFA is produced mainly by hydrolysis of triglycerides during body fat reserve mobilization  
501 indicating that individuals that did not mobilize much fat lost the most telomere sequences.  
502 Unravelling the complexity of both the direct and indirect effects of all condition indices on  
503 survival warrant further study. Furthermore, that our Principal Component analyses were  
504 significantly predictive of survival at all stages but only when body condition was part of the



505 model may indicate that body condition best signals the survival prospects of offspring which  
506 might be expected if body condition drives physiology for example.

507         Although telomere shortening rates predicted offspring survival, it did not vary according  
508 to the playback treatment, perhaps indicating that telomere shortening is not a good indicator of  
509 past rearing stresses and/or predictive of the future quality of the survivors. On the other hand,  
510 measuring telomere shortening between two points of time requires that the individual be alive at  
511 each of those two points. As mentioned, differential mortality between the playback treatments  
512 occurred only in the early dependency stages, from nestlings to the first few days out of the nest  
513 and was similar thereafter. Repeated measures of individuals during the peak mortality phase  
514 might have revealed differences in telomere shortening according to playback treatment. We  
515 know of only one other experiment on this topic. Kärkkäinen et al. (2019) placed nest boxes for  
516 Pied Flycatchers near nesting Eurasian Pygmy Owls or at control sites. Telomere shortening  
517 occurred amongst the flycatchers between 5 and 12 days post-hatch but shortening rate was the  
518 same between predator treatments. Thus, telomere shortening rate may not be a function of  
519 perceived predation risk, and is perhaps more of a genetic trait than one that reflects early life  
520 hardships (e.g. Boonekamp et al., 2014). Alternatively, the manipulation also did not  
521 differentially affect parental care as measured by chick growth, such that there would be little *a*  
522 *priori* reason to expect telomere shortening rates to differ between treatments (effects on survival  
523 were not measured).

524         Kärkkäinen et al. (2019) did find that nestlings reared near owls had longer telomere  
525 lengths than did control chicks. We found the opposite; offspring reared with predator playbacks  
526 had shorter telomere lengths as nestlings. Few other studies have examined telomere length in  
527 developing young but Noguera & Velando (2019) reported shorter telomeres due to predator

528 exposure as did Tablado (2022) for great tits, while Monteforte et al. (2017) found no predator-  
529 induced effects in guppies. A relatively short telomere length is expected to reflect, amongst  
530 other things, poor biological state and increased disease susceptibility, and potentially decreased  
531 survival (McLennan et al., 2016; Watson et al., 2015). Telomere length was not predictive of  
532 survival at any stage in our study. Nonetheless, nestlings reared in the predator playback  
533 treatment were evidently in poorer body condition and perhaps more susceptible to infection. As  
534 mentioned, we do not have the telomere lengths of nestlings that died to compare with the  
535 survivors. Of those nestlings destined to fledge, however (i.e. the survivors), predator-induced  
536 reductions in telomere lengths may be a reflection of their relatively poor upbringing.

537

## 538 **5 CONCLUSIONS**

539 Manipulating the fear of predators in free-living wildlife is logistically challenging but results to  
540 date reveal that fear has effects on the number of offspring produced in addition to the survival  
541 of those offspring which have a significant impact on the population growth rate. Reductions in  
542 parental care and feeding behaviour appear to be the most likely driver of these responses.  
543 Consistent with this, we demonstrate that offspring reared by frightened parents suffer a  
544 reduction in body and physiological condition in the very earliest stages which offspring carry  
545 with them throughout much, if not all, of their life. Also, predator-induced reductions  
546 specifically in fat levels again in the earliest stages, were the best explanation for the mechanism  
547 leading to the differential mortality that occurs at this time between the playback treatment  
548 groups. Once the period of predator-induced differential mortality is over, aspects of body and  
549 physiological condition continue to predict survival but for all offspring in both treatments. Our  
550 findings point to fear of predators as a potent form of early life adversity which has a two-fold

551 effect on offspring in altering both survival and the ‘quality’ of the survivors. That fear can  
552 generate this dual effect might explain why fear of predators can have such large impact on  
553 populations.

## 554 555 **AUTHORS’ CONTRIBUTIONS**

556 LYZ and MC conceived the study. All authors contributed to the design of at least one aspect of  
557 the methodology. LYZ, MC, MCA collected the data. TDW, MAF, FC, SZ conducted the bulk  
558 of the physiological assays. LYZ and MCA analyzed the data. LYZ wrote the manuscript with  
559 feedback from authors.  
560

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570

## 571 **CONFLICT OF INTEREST STATEMENT**

572 The authors declare that they have no conflict of interest.  
573

## 574 **DATA AVAILABILITY STATEMENT**

575 Data will be made available at [https://owl.uwo.ca/portal/site/d42edc38-490d-4455-9b0b-](https://owl.uwo.ca/portal/site/d42edc38-490d-4455-9b0b-e9355dbc693e/tool/d26f8475-8ac1-4bfa-a0bd-c3efb69c337a?panel=Main)  
576 [e9355dbc693e/tool/d26f8475-8ac1-4bfa-a0bd-c3efb69c337a?panel=Main](https://owl.uwo.ca/portal/site/d42edc38-490d-4455-9b0b-e9355dbc693e/tool/d26f8475-8ac1-4bfa-a0bd-c3efb69c337a?panel=Main) should this manuscript  
577 be accepted for publication.  
578

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796

## 797 **SUPPORTING INFORMATION**

798 Additional supporting information may be found in the online version of this article.

799 TABLE S1 Overview of condition results

800 TABLE S2 DFA and CPH backward elimination procedure

801 APPENDIX S1 Assays for physiology variables

802

## 803 **FIGURE CAPTIONS**

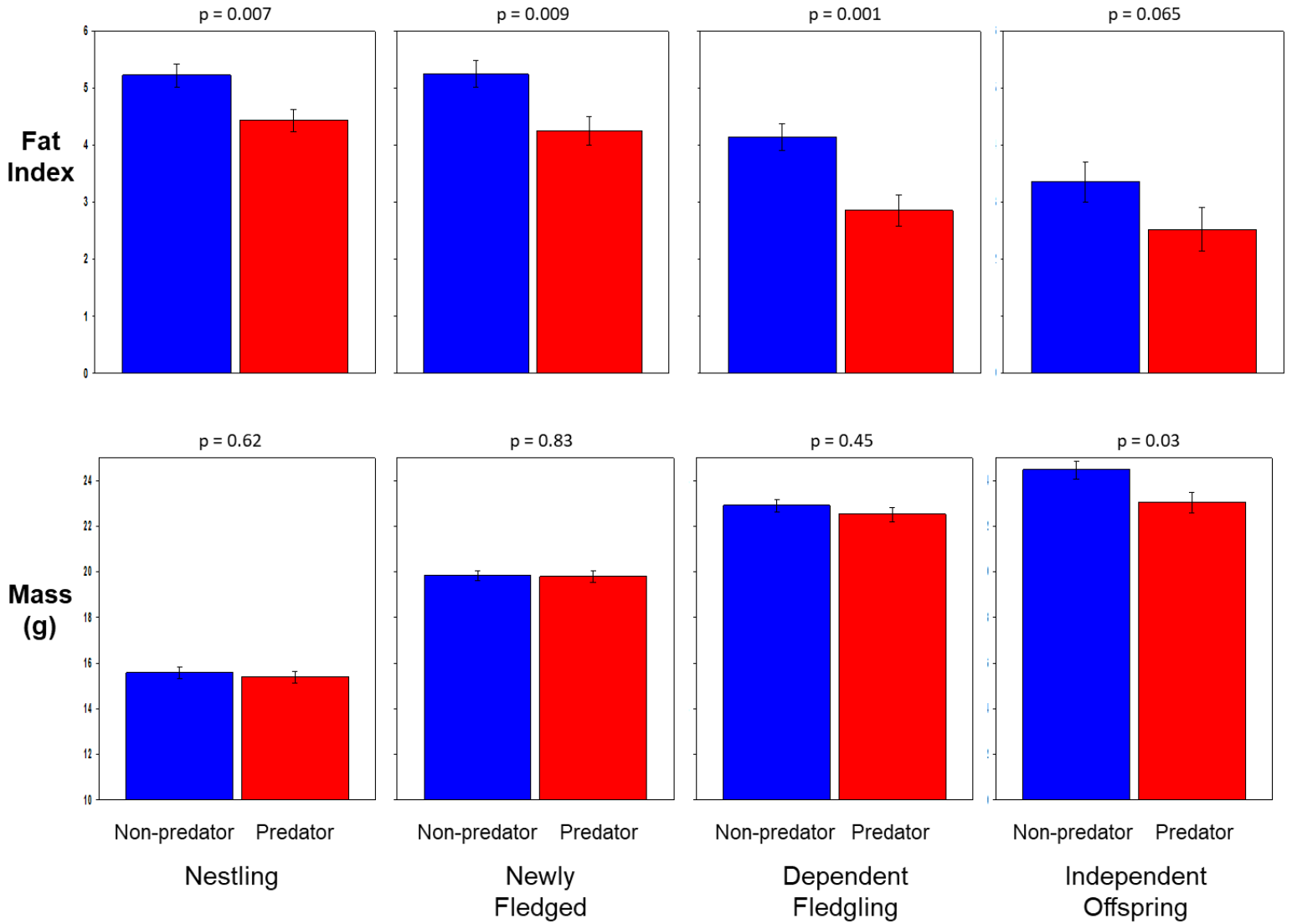
804 Fig. 1. The average  $\pm$  sem of song sparrow offspring in terms of their fat score and mass from  
805 the predator (red bars) and non-predator (blue bars) playback treatment at four stages of  
806 development including the nestling stage; newly fledged (1 d post-fledge); late dependency stage  
807 (14 d); post-independent (40 d). *P*-values were calculated from our GLMM for nestlings (main  
808 effect of playback treatment, nestlings) and sequential Bonferroni post-hoc tests for post-fledge  
809 offspring.

810

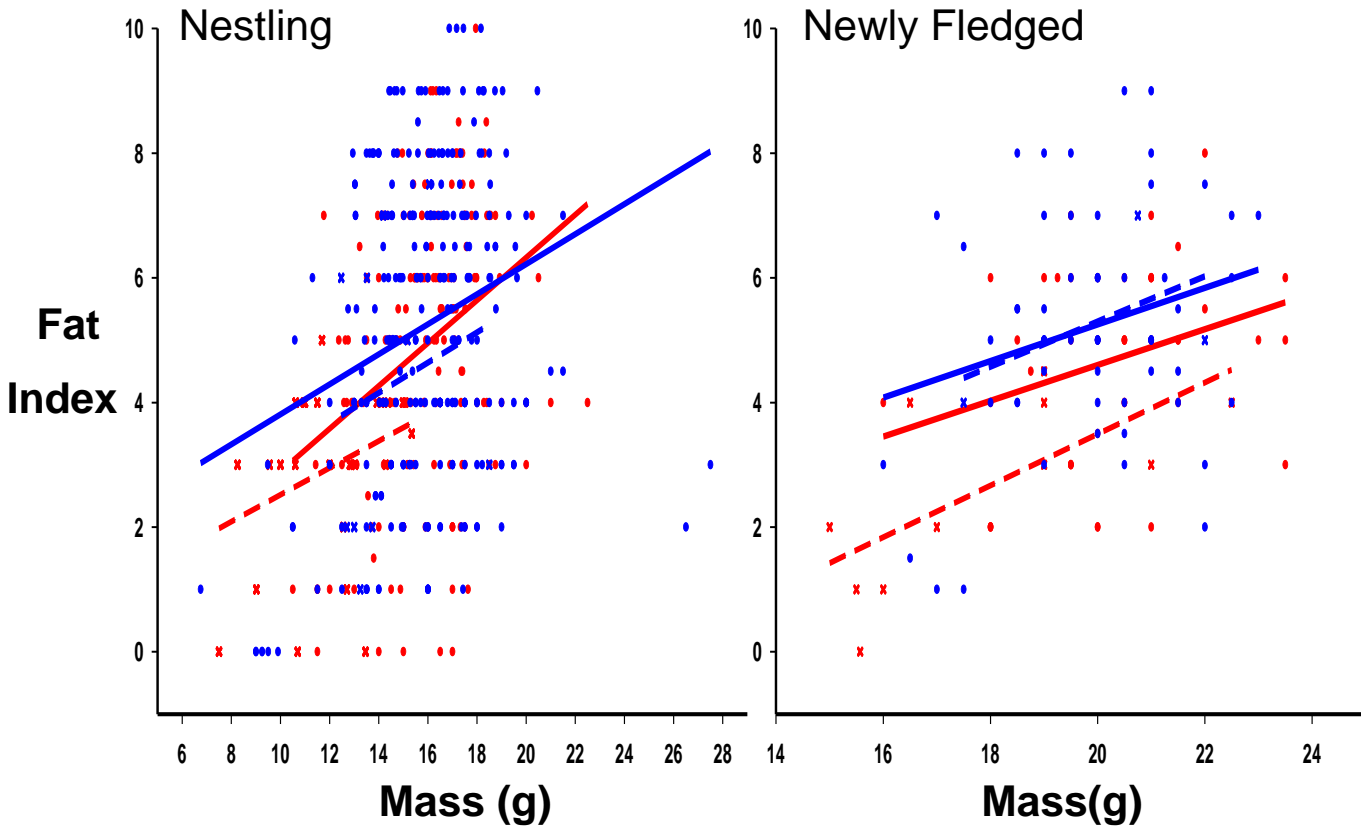
811 Fig. 2. Our Cox-Proportional Hazards Models indicated that both the fat and mass of offspring  
812 predicted the survival of nestlings and newly fledged young. We visualized the results by  
813 presenting the relationship between fat reserves and mass for song sparrow offspring for in the  
814 predator (red) and non-predator (blue) playback treatments. Solid lines are correlations for  
815 offspring that survived from the nestling stage to fledging (solid dots are individuals); and when  
816 newly fledged to the first week out of the nest. Dashed lines are correlations for those offspring  
817 that died (x's are individuals).

818

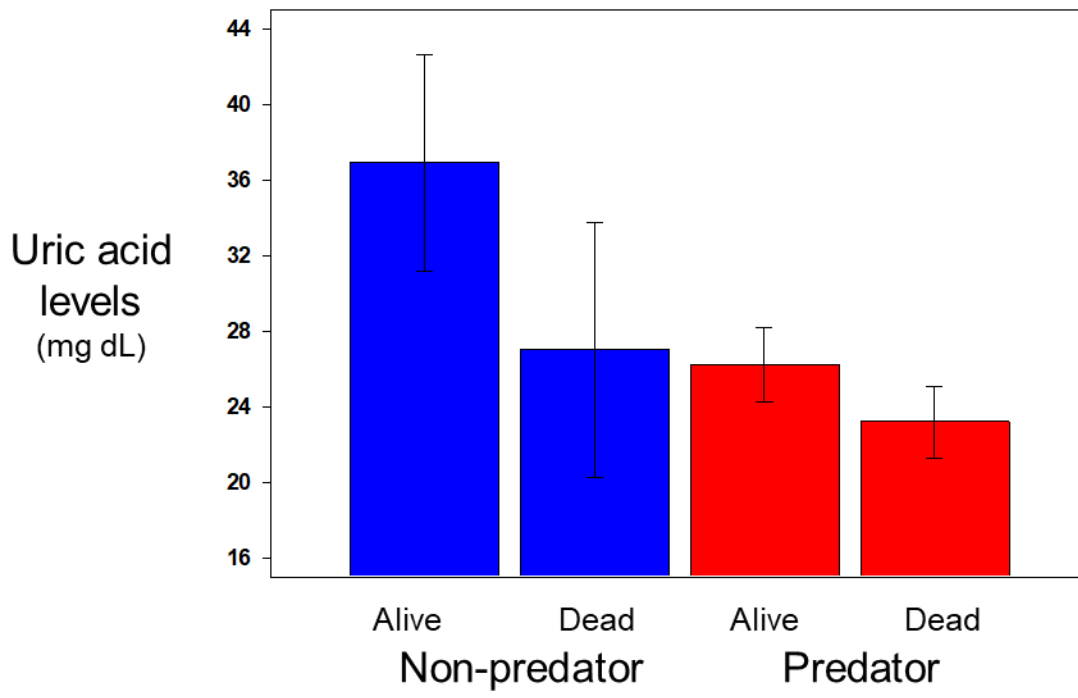
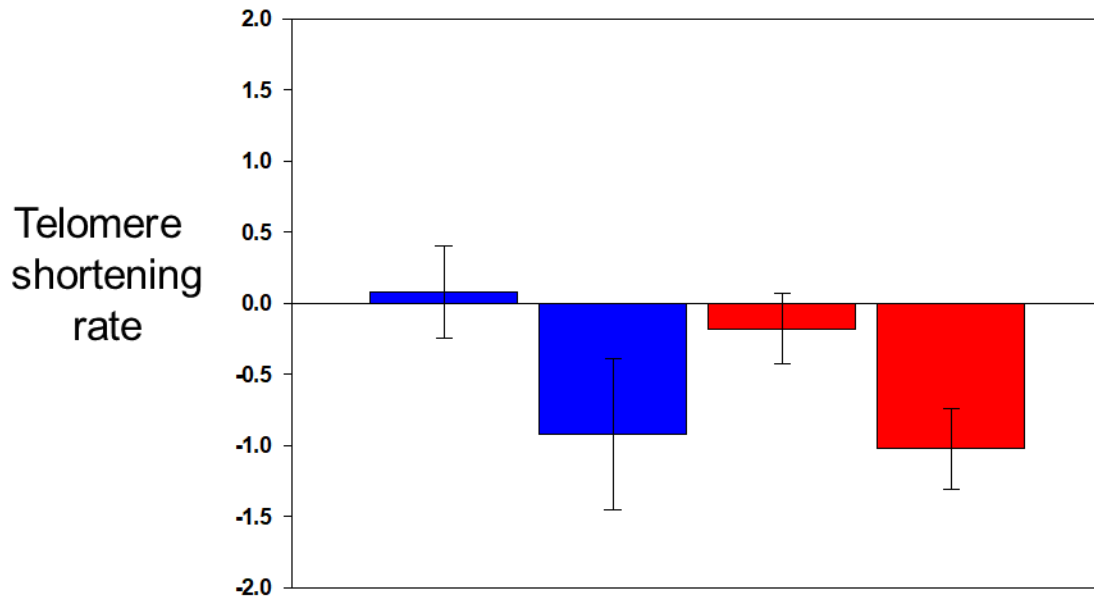
819 Fig. 3. Our Cox-proportional Hazards Model on our 11 physiology measures analyzed, identified  
820 telomere shortening rates and uric acid levels as significant predictors of the survival of offspring  
821 from the late dependency stage to post-independence. Here, we visualize the results for the  
822 predator (red bars) and non-predator (blue bars) playback treatments, by calculating the median  
823 values of telomere shortening and uric acid for offspring that survived to independence versus  
824 those that died. SEMs were estimated by bootstrapping the data.



- Non-predator, Alive
- Predator, Alive
- - -x- - Non-predator, Dead
- - -x- - Predator, Dead







**TABLE 1** Spearman Rank Correlations (*R*) and *P*-values of all 11 variables used in the Cox Proportional Hazards Model conducted to assess which combination of variables best predicts survival of late dependency stage fledged offspring (14 d) to post-independence (49 d after fledging). Backward Elimination sequence 1 indicates that that variable was removed first. Also included are correlations with fat and mass. Cox Proportional Hazards revealed that telomere shortening rates and uric acid best predicted survival. Correlations  $\geq 0.30$  are highlighted with *P*-values  $\leq 0.10$  in red and *P*-values  $\leq 0.05$  in red and bold.

Backward Elimination Process	Physiological System	Physiological Variable	Telomere Shortening Rate*		Uric Acid		Corticosterone		Lysis		dROMS		Haptoglobin		Telomere Length		Anti-oxidant capacity		Glucose		Haematocrit		NEFA		Fat	
			<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Remains	Oxidative Stress/Damage	Telomere Shortening	x	x																						
Remains	Antioxidant Protection	Uric Acid	-0.05	0.80	x	x																				
1	Glucocorticoids	Corticosterone	-0.16	0.37	<b>-0.39</b>	<b>0.03</b>	x	x																		
2	Immune Function	Lysis	0.07	0.70	<b>-0.45</b>	<b>0.01</b>	0.11	0.54	x	x																
3	Oxidative Stress/Damage	dROMS	0.04	0.83	0.02	0.90	0.01	0.94	0.04	0.83	x	x														
4	Immune Function	Haptoglobin	-0.18	0.34	<b>0.41</b>	<b>0.02</b>	-0.20	0.26	-0.21	0.23	0.06	0.73	x	x												
5	Oxidative Stress/Damage	Telomere Length	0.12	0.51	0.05	0.78	-0.01	0.96	-0.22	0.22	0.07	0.70	-0.15	0.41	x	x										
6	Antioxidant Protection	Antioxidant Capacity	0.02	0.89	<b>0.31</b>	<b>0.07</b>	0.12	0.49	0.07	0.71	0.12	0.50	0.24	0.17	-0.16	0.39	x	x								
7	Energy Reserves	Glucose	-0.23	0.21	0.05	0.79	0.14	0.42	<b>0.36</b>	<b>0.04</b>	-0.06	0.72	-0.15	0.40	-0.03	0.89	0.26	0.14	x	x						
8	Oxygen Carrying Capacity	Haematocrit	0.18	0.32	-0.17	0.36	0.01	0.96	-0.17	0.35	<b>0.33</b>	<b>0.06</b>	0.01	0.97	-0.03	0.85	0.13	0.49	-0.16	0.36	x	x				
9	Energy Reserves	NEFA	<b>0.41</b>	<b>0.02</b>	0.09	0.62	-0.23	0.19	-0.01	0.97	-0.07	0.69	0.00	0.98	<b>0.30</b>	<b>0.08</b>	-0.12	0.50	-0.29	0.10	-0.01	0.94	x	x		
	Body Condition	Fat	<b>0.30</b>	<b>0.09</b>	0.01	0.96	-0.24	0.18	0.22	0.21	0.17	0.36	0.09	0.61	-0.03	0.85	0.00	0.99	0.07	0.70	0.14	0.44	0.22	0.21	x	x
	Body Condition	Mass	<b>0.33</b>	<b>0.06</b>	0.10	0.58	<b>-0.37</b>	<b>0.04</b>	0.24	0.18	-0.07	0.68	0.00	0.99	0.15	0.40	-0.10	0.58	-0.08	0.65	<b>-0.33</b>	<b>0.06</b>	<b>0.61</b>	<b>&lt;0.001</b>	<b>0.43</b>	<b>0.01</b>

Sample sizes for physiology = 33

Sample sizes for fat and mass = 40

\*Telomere shortening rate: positive correlations = high telomere loss associated with low quantities of the corresponding variable, whereas low telomere loss associated with high quantities; negative correlations = high telomere loss and high quantities of the corresponding variable, whereas low telomere loss associated with low quantities

## **Supplementary Information for**

### **Fear of predators reduces body and physiological condition affecting offspring survival and the 'quality' of the survivors**

#### **This Supplementary Information includes:**

TABLE S1 Overview of condition results

TABLE S2 DFA and CPH backward elimination procedure

APPENDIX S1 Assays for physiology variables



**TABLE S2 DFA and CPH backward elimination procedure**

<b>Nestling Stage</b>						
Physiology Variable	DFA: Backward Elimination Step	P-value	Variable Status			
Tel Length	1	0.025	In			
Haematocrit	1	0.299	In			
Glucose	1	0.405	Removed			
Tel Length	2	0.031	In			
Haematocrit	2	0.461	Removed			
Tel Length	3	0.031	In			
<b>Late Dependency Stage</b>						
Physiology Variable	DFA: Backward Elimination Step	P-value	Variable Status	CPH: Backward Elimination Step	P-value	Variable Status
Uric acid	1	0.028	In	1	0.197	In
Haptoglobin	1	0.026	In	1	0.647	In
Tel Shortening	1	0.203	In	1	0.121	In
Glucose	1	0.567	In	1	0.311	In
Haematocrit	1	0.151	In	1	0.454	In
OXY	1	0.935	In	1	0.434	In
dROMS	1	0.786	In	1	0.708	In
Lysis	1	0.778	In	1	0.842	In
NEFA	1	0.885	In	1	0.101	In
Cort	1	0.534	In	1	0.909	Removed
Tel Length	1	0.944	Removed	1	0.330	In
Uric acid	2	0.020	In	2	0.186	In
Haptoglobin	2	0.014	In	2	0.652	In
Tel Shortening	2	0.190	In	2	0.120	In
Glucose	2	0.553	In	2	0.308	In
Haematocrit	2	0.138	In	2	0.430	In
OXY	2	0.945	Removed	2	0.419	In
dROMS	2	0.788	In	2	0.716	In
Lysis	2	0.779	In	2	0.859	Removed
NEFA	2	0.896	In	2	0.100	In
Cort	2	0.518	In	2		OUT
Tel Length	2		OUT	2	0.328	In
Uric acid	3	0.012	In	3	0.092	In
Haptoglobin	3	0.011	In	3	0.616	In
Tel Shortening	3	0.179	In	3	0.119	In
Glucose	3	0.525	In	3	0.307	In
Haematocrit	3	0.124	In	3	0.216	In
OXY	3		OUT	3	0.383	In
dROMS	3	0.774	In	3	0.745	Removed
Lysis	3	0.756	In	3		OUT
NEFA	3	0.888	Removed	3	0.086	In
Cort	3	0.506	In	3		OUT
Tel Length	3		OUT	3	0.327	In

Physiology Variable	DFA: Backward Elimination Step	P-value	Variable Status	CPH: Backward Elimination Step	P-value	Variable Status
Uric acid	4	0.010	In	4	0.098	In
Haptoglobin	4	0.008	In	4	0.653	Removed
Tel Shortening	4	0.139	In	4	0.128	In
Glucose	4	0.506	In	4	0.285	In
Haematocrit	4	0.113	In	4	0.147	In
OXY	4		OUT	4	0.381	In
dROMS	4	0.768	Removed	4		OUT
Lysis	4	0.766	In	4		OUT
NEFA	4		OUT	4	0.089	In
Cort	4	0.506	In	4		OUT
Tel Length	4		OUT	4	0.340	In
Uric acid	5	0.009	In	5	0.045	In
Haptoglobin	5	0.007	In	5		OUT
Tel Shortening	5	0.137	In	5	0.039	In
Glucose	5	0.494	In	5	0.215	In
Haematocrit	5	0.103	In	5	0.164	In
OXY	5		OUT	5	0.381	In
dROMS	5		OUT	5		OUT
Lysis	5	0.801	Removed	5		OUT
NEFA	5		OUT	5	0.097	In
Cort	5	0.474	In	5		OUT
Tel Length	5		OUT	5	0.406	Removed
Uric acid	6	0.004	In	6	0.064	In
Haptoglobin	6	0.006	In	6		OUT
Tel Shortening	6	0.135	In	6	0.027	In
Glucose	6	0.390	In	6	0.242	In
Haematocrit	6	0.099	In	6	0.127	In
OXY	6		OUT	6	0.546	Removed
dROMS	6		OUT	6		OUT
Lysis	6		OUT	6		OUT
NEFA	6		OUT	6	0.107	In
Cort	6	0.452	Removed	6		OUT
Tel Length	6		OUT	6		OUT
Uric acid	7	0.002	In	7	0.075	In
Haptoglobin	7	0.007	In	7		OUT
Tel Shortening	7	0.139	In	7	0.032	In
Glucose	7	0.409	Removed	7	0.238	Removed
Haematocrit	7	0.088	In	7	0.144	In
OXY	7		OUT	7		OUT
dROMS	7		OUT	7		OUT
Lysis	7		OUT	7		OUT
NEFA	7		OUT	7	0.079	In
Cort	7		OUT	7		OUT
Tel Length	7		OUT	7		OUT
Uric acid	8	0.001	In	8	0.081	In
Haptoglobin	8	0.003	In	8		OUT
Tel Shortening	8	0.079	In	8	0.038	In
Glucose	8		OUT	8		OUT
Haematocrit	8	0.094	Removed	8	0.268	Removed

Physiology Variable	DFA: Backward Elimination Step	P-value	Variable Status	CPH: Backward Elimination Step	P-value	Variable Status
OXY	8		OUT	8		OUT
dROMS	8		OUT	8		OUT
Lysis	8		OUT	8		OUT
NEFA	8		OUT	8	0.105	In
Cort	8		OUT	8		OUT
Tel Length	8		OUT	8		OUT
Uric acid	9	0.004	In	9	0.081	In
Haptoglobin	9	0.007	In	9		OUT
Tel Shortening	9	0.147	Removed	9	0.015	In
Glucose	9		OUT	9		OUT
Haematocrit	9		OUT	9		OUT
OXY	9		OUT	9		OUT
dROMS	9		OUT	9		OUT
Lysis	9		OUT	9		OUT
NEFA	9		OUT	9	0.176	Removed
Cort	9		OUT	9		OUT
Tel Length	9		OUT	9		OUT
Uric acid	10	0.005	In	10	0.034	In
Haptoglobin	10	0.014	In	10		OUT
Tel Shortening	10		OUT	10	0.004	In
Glucose	10		OUT	10		OUT
Haematocrit	10		OUT	10		OUT
OXY	10		OUT	10		OUT
dROMS	10		OUT	10		OUT
Lysis	10		OUT	10		OUT
NEFA	10		OUT	10		OUT
Cort	10		OUT	10		OUT
Tel Length	10		OUT	10		OUT

## **Appendix I Assays for physiology variables**

**Plasma antioxidant capacity** ( $\mu\text{mol HClO ml}^{-1}$ ; OXY) were determined using the OXY kit from Diacron International, Italy (catalog number MC435), modified after Guindre-Parker et al. (Guindre-Parker et al., 2013). Briefly, samples were diluted 1:100 with distilled water and then 5  $\mu\text{l}$  per well used in the assay, 200  $\mu\text{l}$  R1 added to each well and then incubated at 37°C for 1 min before 2  $\mu\text{l}$  of R2 added to each well. Plates were read at 490 nm. Mean intra-assay coefficient of variation was 5.4% and inter-assay variation was 9.7%

**Reactive oxygen metabolites** ( $\text{mg H}_2\text{O}_2 \text{ dl}^{-1}$ ; dROMs) were measured using the dROM kit from Diacron International, Italy (catalog number MC002), modified after Guindre-Parker et al. (Guindre-Parker et al., 2013). Briefly, 15  $\mu\text{l}$  plasma were added to 300  $\mu\text{l}$  working reagent, incubated for 75 minutes at 37°C and then centrifuged at 10,000 rpms for 30 seconds. The supernatant was removed (leaving precipitate on the bottom) and 130  $\mu\text{l}$  used per well. Samples were assayed in duplicate and the plate read at 490 nm and a cubic curve fit used. Mean intra-assay coefficient of variation was 6.6% and inter-assay variation was 13.5%

**Non-esterified fatty acids** ( $\text{mmol.l}^{-1}$ ; NEFA) were measured with the HR-2 kit (Wako Diagnostics, Mountain View, CA) following the manufacturers guidelines. Samples were assayed in duplicate using 5  $\mu\text{l}$  sample volume following 1:2 dilution with dd  $\text{H}_2\text{O}$ . Mean intra- and inter-assay coefficient of variation was 5.2% and 6.0% respectively.

**Uric acid** ( $\text{mg.dL}^{-1}$ ) levels were measured with the Quantichrom assay (DIUA-250) following the manufacturer's guidelines. Samples were assayed in duplicate using 5  $\mu\text{l}$  sample volume following 1:2 dilution with dd  $\text{H}_2\text{O}$ . Mean intra- and inter-assay coefficient of variation was 3.0% and 2.5% respectively.

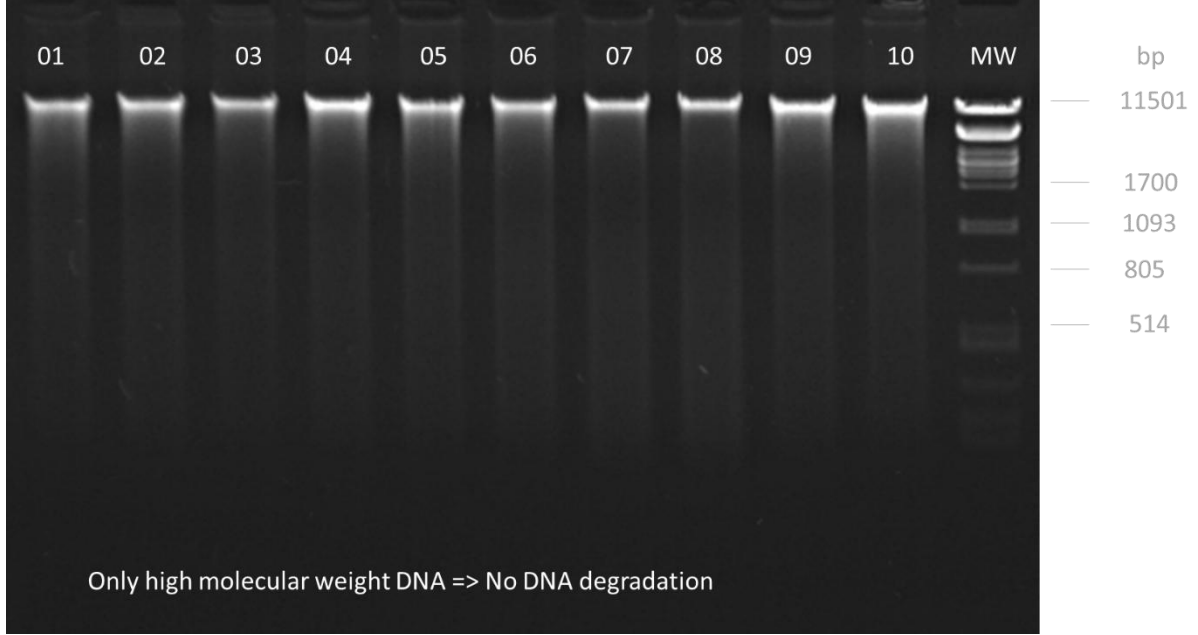


**Haptoglobin** ( $\text{mg}\cdot\text{ml}^{-1}$ ) was assayed in duplicate using the TP801 kit from TriDelta Development, Ltd. We decreased sample volumes to 5  $\mu\text{l}$ , after (Zylberberg et al., 2012) and decreased the other reagents (R1 & R2) by 2/3. Plates were read at 630 nm. Mean intra- and inter-assay coefficient of variation was 10.2% and 5.7% respectively for assays from both years.

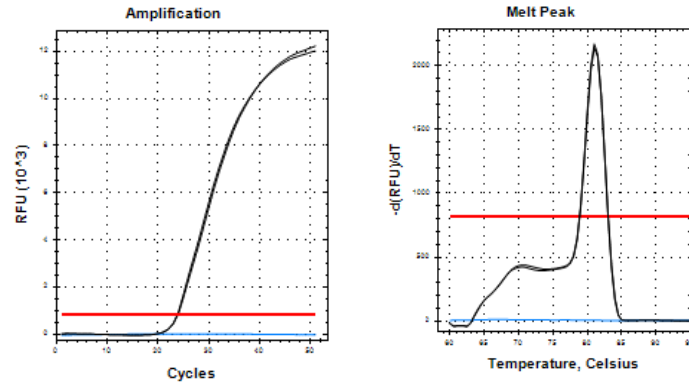
**Lysis agglutination** (Natural antibody; NAb) titers were assayed using the hemolysis-hemoagglutination assay developed by (Matson et al., 2005) and modified by (Zylberberg et al., 2012) for small volumes (10  $\mu\text{l}$ ). Briefly, a round bottomed 96-well plate is used. A row is dedicated to an individual bird. 10  $\mu\text{l}$  is placed in well 1, and then 10  $\mu\text{l}$  of 0.01M PBS added to each of wells 2-12 in the row. 10  $\mu\text{l}$  of plasma is placed in well 2 and mixed with the PBS. Then 10  $\mu\text{l}$  of well 2 is removed and added to well 3, and so on until well 11 is reached, performing a serial dilution. Well 12 is only PBS, it is the negative control. Then, 10  $\mu\text{l}$  of 1% rabbit blood (washed and diluted from 50%, HemoStat Labs) is added to all wells. Lysis and agglutination scores were photographed and counted at 90 min post removal from 37°C incubation. Agglutination was quantified 20 minutes (room temperature) post removal from incubation and lysis quantified 70 minutes (room temperature removal) from incubation. Scores were counted twice by the same observer (MAF); if scores different more than 1.5 points, they were counted a third time and the mean of scores are reported e.g. (Matson et al., 2012).

**Telomere lengths** were measured on 146 samples, in which DNA was extracted from red blood cells as previously described (Criscuolo et al. 2020). DNA quantity and quality were assessed based on spectrophotometer absorbance (Nano-Drop 1000, Thermo Fisher Scientific, [Waltham, MA, USA](#), ratios A260/280 and A260/230) and DNA integrity by gel migration (see below an example : one gel migration conducted on 10 extracted DNA of song sparrow, together with a DNA size marker ( $\lambda\text{DNA}/\text{PstI}$  Digest = MW, Jena Bioscience GmbH)).

Song Sparrow



Relative telomere length (RTL) was obtained by amplification of DNA sequences of telomere ends (T) and of a control gene (S, mainly controlling for the quantity of DNA actually present in the wells) using the qPCR methodology. All samples were analyzed in one run using a 384 wells thermocycler (CFX-384,



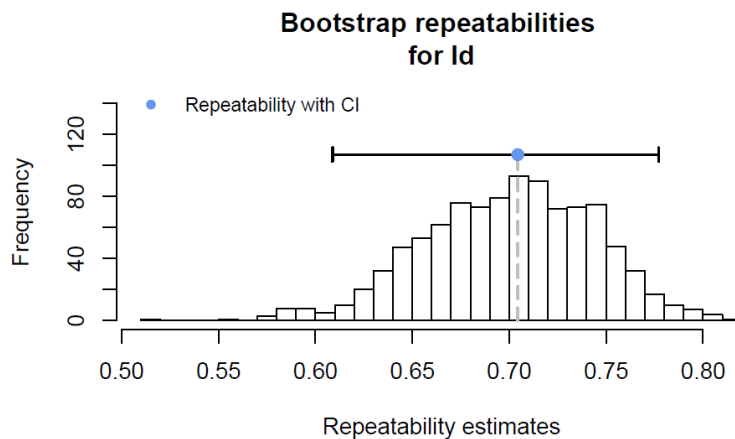
Biorad Hercules, CA, USA), which combines a first plate for telomere sequence's amplification and a second plate for the control gene sequence amplification. Amplifications were done in a well-final volume of 10  $\mu$ L, using 2.5  $\mu$ L of DNA pre-diluted at a concentration of 2  $\text{ng}\cdot\mu\text{L}^{-1}$ , completed with 200 nM (in 0,4  $\mu$ L) of forward and reverse primers, 5  $\mu$ L of SYBR Green PCR mix (GoTaq® qPCR master mix, Promega, Madison, WI, USA) and sterilized distilled water. The telomere primers were Tel1b 5' - CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT - 3' and Tel2b 5' - GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT - 3'. The control gene used in our species was RAG-1 gene (recombination activating protein 1 gene, NCBI number KC789832). . Amplification conditions were set as 95°C for 2 min, followed by 40 cycles of data collection at 95°C for 1 min and 60°C for 1min for RAG1 , and at 95°C for 2 min followed by 30 cycles of 15 s at 95°C, 30 s at 56°C, and 1 min at 72°C for the telomere sequences. All runs ended by a melting curve to check for absence of non-specific amplifications (see figure below, right panel, amplification profiles of the control gene of one DNA sample of song sparrow (black line) and of the negative control (blue line); left panel the associated melting curves).

Each plate contained one negative control (sterilized distilled water, to control for non-specific amplification) and a two-fold serial-dilution of a golden sample (20 ng, 10 ng, 5 g, 2.5 ng, 1,25

ng, 0.625 ng, 0.3125 ng) used to assess the amplification efficiencies of each run. Those were of 99,9 % (RAG1) and 100 % (telomere). All samples were run in duplicates to assess repeatability of the qPCR amplification (DNA and qPCR mix were filled into plates by a calibrated automated liquid handling workstation, Epmotion, Eppendorf, Montesson, France). The final RTL value was calculated following the equation of Pfaffl (2001) and expressed as T/S ratio. The intra-assay repeatability of the T/S ratio was calculated using the ICC and the rptR packages of the R program. Intra-assay ICC was of 0,7 and the repeatability test using a boot-strapping (nboot = 1000) method is given below.

Repeatability for Identity using RTL values:

$R = 0.704$ ;  $SE = 0.044$ ;  $CI = [0.609, 0.777]$ ;  $P = 2.26e-24$  [LRT]



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