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5 **Environmental conditions shape the temporal pattern of investment in reproduction and**
6 **survival**

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21 **Abstract**

22 The relationship between environmental stress exposure and ageing is likely to vary with
23 stressor severity, life history stage, and the time scale over which effects are measured. Such
24 factors could influence whether stress exposure accelerates or slows the ageing process, but
25 their interactions have not previously been experimentally investigated. We found that
26 experimental exposure of zebra finches to mildly challenging environmental circumstances
27 from young to old adulthood, which increased exposure to stress hormones, reduced breeding
28 performance during early adulthood, but had positive effects when individuals were bred in
29 old adulthood. This difference was not due to selective mortality, since the effects were
30 evident within individuals, and no evidence of habituation in the response to the stressor was
31 found. The more stressful environment had no effects on survival during young or old
32 adulthood, but substantially improved survival during middle age. Changes in the effects at
33 different ages could be due to the duration and nature of the challenging exposure, or to
34 variation in coping capacity or strategy with age. These results show that living under
35 challenging environmental circumstances can influence ageing trajectories in terms of both
36 reproductive performance and longevity. Our results provide experimental support for the
37 emerging idea that stress exposure needs to be optimised rather than minimised to obtain the
38 best health outcomes.

39

40 **Keywords:** environmental stress, glucocorticoids, reproduction, survival, hormesis.

41

42 **Introduction**

43 Ageing, broadly defined as the decline in performance with advancing age, has been well
44 documented among different animal taxa both in the wild and under laboratory conditions [1,
45 2]. The pattern of ageing, that is the timing of onset and the rate at which deterioration occurs,
46 is highly variable both among and within species. One of the major foci of ageing research is

47 the endeavour to understand the causes of such heterogeneity [3-5]. This involves identifying
48 selection pressures driving the evolution of species-specific patterns of ageing [1], the
49 underlying cellular mechanisms [6], and the genetic and environmental factors that generate
50 variation among individuals of the same species [7]. Evolutionary explanations of ageing are
51 largely based on cost-benefit trade-offs. Two main theories currently predominate - a genetic
52 approach centred on the antagonistically pleiotropic effects of genes that confer beneficial
53 effects early in life but deleterious effects later in life [8, 9], and a resource allocation
54 approach, embodied in the disposable soma theory, which is concerned with the fitness effects
55 of differential investment in self-maintenance and reproduction [3, 10, 11]. These two
56 approaches are complementary, make similar predictions and have both been applied largely
57 in the context of variation in lifespan and reproductive performance among different species
58 [1, 3, 12, 13].

59 Variation among individuals of the same species in the pattern of ageing can also be
60 viewed using the same framework. Allocation of resources to self maintenance will vary due
61 to differing capacities, constraints, priorities and resource availability. It is well recognised
62 that intra-specific variation in the pattern of ageing is strongly influenced by environmental
63 conditions. Shifts in “priority rules” underlying optimal allocation of limiting resources
64 between self-maintenance and reproduction are expected to become more evident when
65 animals are exposed to challenging environments, such as when facing unpredictable, adverse
66 environmental circumstances influencing factors such as weather, food availability, disease,
67 parasite and predation risk [14, 15]. The resultant increase in energy expenditure and stress
68 exposure might directly damage the soma and result in faster age-related deterioration [16-

69 19]. Alternatively, harsher environmental conditions could influence the optimal balance of
70 resource allocation between self-maintenance and reproduction with consequences for age-
71 related reproductive effort and survival patterns [7, 20-21]. Strategic rescheduling of
72 investment may occur, with individuals delaying reproduction if conditions are likely to
73 improve or bringing it forward if life expectancy is likely to be reduced, with consequences
74 for age-specific reproductive success and the pattern of senescence [22, 23].

75 Effects of stressful environments on ageing patterns could also vary at different life
76 stages, for example in early life and in adulthood, or early adulthood and old age, because
77 vulnerability to damage, and the resulting fitness consequences, may differ. An additional
78 layer of complexity is added by the fact that the ageing process itself can alter both
79 vulnerability and resilience to stress exposure, and stress exposure can diminish or exacerbate
80 ageing [24]. These interactions are influenced by the severity of the stress experienced, with
81 severe stress generally accelerating ageing, while milder stress exposure can induce resilience
82 and extend lifespan [25]. Furthermore, the consequences of exposure to even mild stressors
83 are likely to change with age due at least in part to impaired functioning of the stress-response
84 systems with age [24]. Much attention has been devoted to the long lasting effects of stress
85 exposure in early life, with much less attention being given to effects in adulthood, and less
86 still to how these effects might change across the life course [24, 26]. In the majority of
87 studies conducted to date, manipulations of environmental conditions have been conducted
88 over a relatively short period, and at a single life stage. This is in part due to the time
89 investment required, the logistics of following individuals over time, and to some extent also
90 to the largely untested assumption that what holds at one life history stage also holds at others.

91 Here we report the results of an experiment in which female zebra finches
92 (*Taeniopygia guttata*) were repeatedly exposed to a relatively mild environmental stressor, to
93 which they did not habituate, from early in their adult lives. We have previously shown that
94 this has no effect on survival in young adulthood, but increased survival during middle age in
95 comparison with a control group not exposed to the environmental stressor [27]. The survival
96 advantage could have occurred due to a re-scheduling of resource allocation to reproduction,
97 and/or stress-induced resilience. In order to examine whether this response to the mildly
98 stressful environment involved any differences in reproductive investment over controlled
99 age-specific breeding events, we examined reproductive performance of these birds from
100 young adulthood into old age. We also examined whether the previously observed survival
101 advantage of stress-exposed birds in middle age persisted into old age, or whether there was
102 evidence that resilience then declined. Lastly, we examined whether there were any change in
103 baseline levels of the stress hormone corticosterone with age, and whether there was any
104 evidence of habituation to the stressor when the birds were older.

105

106 **Materials and Methods**

107 *(a) Study subjects*

108 The study was performed in female zebra finches that were produced in two replicates
109 from parents of the same stock population at the University of Glasgow (replicate 1 birds
110 were produced in April-June 2011; replicate 2 birds were produced in August-September
111 2011). To minimise potential mate familiarity [28], the stock females were paired with

112 different mates in the two breeding events, and the resulting offspring used to form the two
113 experimental replicates. For each replicate, the environmental manipulations started when the
114 study females were young, fully grown, sexually mature adults (5 months old on average:
115 mean \pm SE: 152 ± 1 day) [27]. Females were housed in treatment-specific cages ($n = 7$ - 10 per
116 120 x 50 x 50 cm cage) and randomly allocated to one of two experimental groups: (1)
117 challenging environment (replicate 1: $n = 45$ females; replicate 2: $n = 62$ females), or (2)
118 control environment (replicate 1: $n = 46$ females; replicate 2: $n = 61$ females). When possible,
119 females that hatched in the same nest (part of the same brood) were counterbalanced between
120 the two treatment groups and family of origin was taken into account in all analyses. All birds
121 were maintained throughout the experiment at a photoperiod of 14h:10h light:dark cycle and
122 the temperature was maintained between 20-24°C. All procedures were carried out under UK
123 Home Office Project Licence 60/4109.

124

125 (b) *Environmental conditions*

126 Upon 5 months of age, females were randomly assigned to either a challenging or
127 control environmental condition. In the challenging environmental condition, food was made
128 unavailable for a continuous period of 4.9 hours (~ one third of the daylight hours), 4 days per
129 week, on a random time schedule. For the remaining two thirds of the day and on the
130 remaining 3 days per week, they were provided with *ad libitum* food. Thus the manipulation
131 changed the temporal availability of food, but, when available, food was abundant.
132 Challenged females were always kept on this food regime, except when they were breeding
133 and received *ad libitum* access to food continuously from the time they were paired with a

134 male or shortly afterwards until after they completed breeding (~ two months for each
135 breeding event). Females in the control group were always provided with *ad libitum* food and
136 experienced exactly the same breeding regime as the challenged birds (see paragraph below).
137 During the third breeding event only, at 1.8 years old (see also paragraph below), the birds in
138 the challenging environment were given a single, daily, exposure to the glucocorticoid stress
139 hormone corticosterone to determine whether a more protracted environmental challenge
140 during pre-breeding/pair formation influenced reproductive investment. Specifically, two
141 weeks prior to this breeding event, challenged birds were given oral doses of corticosterone
142 (Sigma-Aldrich, Poole, UK) following each period of episodic food withdrawal. The hormone
143 was administered by providing the birds with seed soaked in corticosterone suspended in
144 peanut oil at a concentration of 0.0825mg/ml (corticosterone dose/bird was ~ 4.075µg; 1 g of
145 seed soaked/bird) for 10 min immediately after the end of each episodic food withdrawal.
146 Corticosterone dosing was based on previous work in zebra finches (29). Control birds
147 received 1g of seed soaked in peanut oil only for the same amount of time as the challenged
148 females. The unpredictable food regime and corticosterone seed manipulation were continued
149 until individual clutches were completed (mean ± SE: 25.8 ± 0.3 days; range: 20-33 days). A
150 small number of females did not attempt to breed and in these birds the oral corticosterone
151 treatment was suspended 14 days after pairing (total duration 28 days). Following this
152 breeding event, all experimental females were placed back on the unpredictable food regime
153 only (i.e., no exposure to corticosterone soaked seeds) until the next breeding event at 3.5
154 years of age (~1.5 years later). There were no effects of the duration of corticosterone
155 supplementation on measures of reproductive performance (clutch size or number of chicks

156 reared) at both 1.8 years of age and 3.5 years of age (Pearson's r: $-0.02 < r < 0.2$, $p \geq 0.2$ for
157 all), suggesting that this short-term additional corticosterone treatment did not influence
158 breeding investment.

159 We have previously shown that there is no overall significant effect of the
160 experimental treatment on body mass up to three years of age [27]. Consistent with other
161 studies, we have also found that the experimental food manipulation resulted in increases in
162 overall exposure to glucocorticoids [30, 31]. More specifically, at the end of the episodes of
163 food withdrawal, the challenged birds showed higher baseline corticosterone (the predominant
164 avian glucocorticoid hormone) levels than those birds living in the control environment and
165 this physiological response was consistent over prolonged exposure periods (up to 6 weeks)
166 indicating no habituation of the birds to the unpredictable food shortages (on average 1.4 fold
167 increase; full details in [27]). These data were collected during young adulthood (< 1 year of
168 age). We also measured corticosterone in a randomly chosen subset of study females (34
169 control and 32 challenging environment) when they were 3.5 years old. Average baseline
170 corticosterone levels decreased by around 50% in both treatment groups in old adulthood
171 compared to young adulthood, but despite this the birds in the challenging environment
172 continued to show a similar magnitude of increase in baseline corticosterone at the end of the
173 episodic food withdrawals also into old adulthood (on average 1.8 fold increase; Table S0;
174 full details in Supplementary). Thus, our environmental protocol mimicked the physiological
175 effects of an environmental stressor naturally experienced by animals living under protracted
176 exposure to unpredictable environmental conditions [32].

177

178 (c) Breeding schedule and breeding performance

179 Study females from both treatment groups were allowed to produce clutches of eggs
180 four times during the study. For breeding, females were paired with a randomly assigned,
181 relatively young male ranging in age from 6 months to 1.8 years; experimental females were
182 paired with the same male partner in their first and second breeding event whereas in the
183 following two breeding events they were always paired with a different male. Control and
184 challenged females were paired with males for the first time when they were on average 6
185 months old (188 ± 0.89 days of age; all females survived to this first breeding event),
186 approximately 1 month after the start of the environmental manipulation. Each pair was
187 housed in their own cage (60 X 50 X 50 cm) and provided with a nest box and nest material
188 (coconut fibre and jute, Haiths Ltd). The females were paired again at the following ages: 1.1
189 years (408 ± 0.82 days of age), 1.8 years (653 ± 0.78 days of age), and finally when they were
190 3.5 years old (1270 ± 0.92 days of age) – mean \pm SE for all. For the breeding event at 1.1
191 years the pairs were not allowed to rear any chicks since the eggs were required for assays of
192 egg composition, and were collected shortly after laying and replaced with dummy eggs.
193 Dummy eggs were removed once individual clutches were complete and the pairs then
194 separated. During the breeding at 1.8 years, most of the clutches (157 out of 187; logistic
195 reasons) were cross-fostered at the end of the incubation period in order to examine egg
196 effects on chick survival as part of a separate study to disentangle maternal from rearing
197 environmental treatment effects; a small subset of cross-fostered clutches (43) was also
198 subjected to brood size manipulation experiments (data to be reported in full elsewhere).

199 When lifetime breeding performance and survival are examined below, we only
200 considered those birds whose clutch size was not manipulated (43 out of 214 birds excluded
201 from these analyses; total sample size 171 birds). We quantified breeding performance of the
202 study females by recording the following: (1) likelihood of breeding (laying a clutch), (2)
203 latency to lay (i.e. time from pairing to the laying of the first egg); (3) clutch size; (4)
204 fledgling success (proportional data: number of chicks fledged/clutch size), and (5) the
205 number of chicks fledged (assessed when the offspring were ~ 30 days old, including also
206 those females that did not lay a clutch in order to assess the overall breeding performance).

207

208 *(d) Survival*

209 We monitored the survival of the birds for 4 years (i.e. till 1456 days of age).
210 Experimental birds were inspected daily and all the birds considered here died of intrinsic
211 causes, not of accidental injury or aggression. Where birds showed clear signs that death was
212 imminent and their welfare was very severely compromised (the birds were not able to fly
213 and/or feed independently and our veterinarian confirmed that death was imminent), they
214 were culled under the advice of our veterinarian in line with UK Home Office legislation (n =
215 24 out of 85 females that died – total sample size, n = 171). Generally, deaths were
216 unpredictable with the majority of the birds being found dead on the cage floor without
217 having shown prior symptoms.

218

219 **Data analysis**

Analyses were performed in R (version 3.2.5; R core team, 2014). Unless otherwise specified, all final models included the effects of experimental design factors expected to influence the response variables either as parameters of interest integral to the question being investigated or for the purpose of adjustment. These relevant factors were always retained in the main models rather than tested using backwards or forwards selection to avoid overfitting. We used Generalised Linear Mixed Models (GLMMs, R package “lme4” and “lmerTest” - [33, 34]) to examine whether the challenging treatment influenced reproductive performance and whether any potential effect of the treatment varied across the age-specific breeding events at 6 months, 1.1 years, 1.8 years, and 3.5 years old as appropriate. Unless otherwise specified, final models included the following factors: treatment, age, replicate, and the interaction treatment x age. In initial models, we tested the potential interaction effect of the treatment with replicate to check consistencies of treatment effects between the two replicates. Age was modelled as categorical rather than continuous variable due to the relatively reduced number of data points per individual bird (up to 2, or 4 as appropriate); female individual identity was always added as random factor to control for correlations between reproductive performance traits within individuals due to the presence of repeated-measurements in the data. As appropriate, we also entered family of origin and male partner identity as additional random factors to control for potential pseudo-replication due to the presence of sisters in the experiment and because some males were used more than once across the breeding events. In preliminary analyses, we also tested if previous reproductive investment decision level (investment in egg laying up until the event under consideration) influenced current reproductive investment (clutch size) at 1.1 years, 1.8 years, or 3.5 years in

242 an interaction with the treatment. Chick mortality is low in this captive situation, and clutch
243 size correlates well with the number of chicks reared in our population (Pearson's $r = 0.6$, $p <$
244 0.0001); thus investment in egg laying is a good proxy for overall reproductive investment
245 level at each breeding event. Across all breeding events, we found no interaction effect of the
246 treatment with previous reproductive decisions on clutch size ($p \geq 0.5$), excluding the
247 possibility of conditionality between previous and current reproductive decisions in relation to
248 life time environmental conditions. We first examined if there were any treatment differences
249 in whether or not the females attempted to breed (i.e. laid eggs) using GLMM with a binomial
250 error distribution and logit link function. The interactions treatment x age and treatment x
251 replicate could not be assessed in the latter model due to reduced statistical power because
252 relatively few birds did not attempt to breed during the first three breeding events. For those
253 females that bred (i.e. laid a clutch), we then analysed the latency to lay the first egg using
254 GLMMs with a Gaussian distribution error— data were log10 transformed to improve
255 normality of model residuals. Clutch size (mean: 4.3, range: 1-8 eggs) was analysed using a
256 GLMM with a Gaussian distribution error rather than with a Poisson distribution because the
257 data were strongly under-dispersed (dispersion parameter < 0.39) and model residuals were
258 normally distributed. Fledging success was analysed with GLMM using a binomial error
259 distribution and logit link function [35], and the number of chicks fledged (range 0-6 chicks)
260 was analysed using a GLMM with a Poisson distribution (dispersion parameters: 0.8-1.3). In
261 the fledging success and number of chicks fledged statistics we did not include the data at 1.8
262 years of age as these response variables could have been influenced by the cross-fostering
263 experiment conducted as part of a separate study to disentangle maternal from rearing

264 environmental treatment effects (data to be fully reported elsewhere). In order to assess
265 within-female treatment effects and to exclude potential survival bias in the results caused by
266 loss of specific phenotypes from the population (e.g. poor quality breeders dying in early
267 adulthood), we also performed the analyses using only those females that survived to the
268 breeding event up to 3.5 years of age (103 out of 171 birds). We used the R package
269 “lsmeans” (36) to perform pairwise post-hoc contrasts for significant outcomes in the main
270 models (Tukey *p* values adjustment).

271 We have previously shown in the birds from the same study population used here that
272 the challenging environmental conditions improved life expectancy up to three years of age
273 (Mixed Effects Cox Models, *p* = 0.02; full details in [27]). We have also shown that there was
274 no link between body mass at 1 year of age and subsequent survival up to three years of age
275 [27]. Importantly, the positive effect of the challenging treatment on survival was evident
276 prior to the start of the additional short-term corticosterone manipulation at ~1.8 years of age
277 (data right-censored at 600 days of age: Mixed Effects Cox Models, *p* = 0.04) excluding the
278 possibility that the short-term change in the severity of the stress treatment at 1.8 years of age
279 per se was the main factor triggering the change in the survival trajectories of our study birds.
280 Here, we further examined survival in old age (between three and four years) and tested the
281 extent to which survival probability was dependent on individuals’ lifetime reproductive
282 effort. We excluded the females that were subjected to the brood size manipulation
283 experiments at 1.8 years of age (43 birds) from all breeding performance and survival
284 analyses performed here to exclude any possibility that those manipulations altered
285 subsequent survival independently of the environmental conditions. However, the results do

286 not differ qualitatively when these birds are included (data not shown). Data were right
287 censored to allow inclusions of birds still alive at the end of the survival monitoring period
288 (49.7% out of 171 birds). We first checked if our annual measurements of body mass (i.e. 1
289 year, 2 years, and 3 years) predicted survival up to 4 years of life using time-dependent
290 covariate Cox model analyses (R package “survival” [37]) and found no effect of this
291 covariate on survival (body mass, body mass x treatment, body mass x replicate, $p \geq 0.2$).
292 Therefore body mass was dropped from the following analyses. In the following Cox Model
293 analyses (R packages: “survival” and “coxme” [37, 38] we entered treatment, replicate, and
294 their interaction as fixed factors, and family identity as a random factor as appropriate. Model
295 diagnostics using Schoenfeld’s residuals plotting suggested that the proportional hazards
296 hypothesis was not met due to a non-linear effect of the treatment with time emerging after 3
297 years of age, whereas it was met in our previous analysis up to 3 years. As mortality rates
298 were clearly very low from 5 months to 1 year (4 control and 2 challenged birds dead out of
299 171 females), and because of the change in the effect of the treatment over time after 3 years
300 of age, we consequentially introduced in the analyses treatment time-dependent coefficients
301 by breaking the data into three time intervals: (1) young adulthood, from the start of the
302 experiment (5 months old) up to 1 year of age; (2) middle adulthood, from 1 to 3 years of age,
303 and (3) old adulthood, from 3 to 4 years of age. In the model we also checked the potential
304 interaction effect of treatment with replicate. The proportional hazard assumption was met in
305 these models. To test if survival was influenced by the individual’s lifetime reproductive
306 effort, we performed separate GLMs (binomial family distribution error with logit link
307 function) entering replicate, along with lifetime egg laying effort (calculated as lifetime

308 number of eggs laid divided by total number of breeding events, ranging from 1 to 4 events
309 depending on the individual's lifespan), or chick rearing effort (calculated as lifetime number
310 of chicks reared by each female divided by total number of breeding events in which chicks
311 were reared, including the event at 1.8 years of age, ranging from 1 to 3 events depending on
312 the individual's lifespan) as continuous covariate – this standardisation allowed us to
313 overcome collinearity between longevity and lifetime number of eggs laid/chicks reared
314 (Pearson's $r = 0.1$, $0.07 < p < 0.2$) as females that survived longer ended up with larger
315 number of eggs and chicks reared over the lifespan (Pearson's $r = 0.5-0.7$, $p < 0.0001$ for both
316 covariates). We performed the latter GLMs separately by treatment in order to simplify model
317 interpretation and avoid issues of collinearity between the treatment and the lifetime
318 reproductive effort. Unless otherwise specified, values are presented as means \pm SE.

319

320 **Results**

321 **Breeding failure**

322 Irrespective of environmental conditions, the probability of breeding failure was influenced
323 by female age. More females failed to produce a clutch in the later breeding events at 1.8
324 years and 3.5 years of age than the earlier events (1.8 years *vs* 6 months and 1.8 years *vs* 1.1
325 years, $p \leq 0.048$; 3.5 years months *vs* 6 months and 3.5 years *vs* 1.1 years, $p \leq 0.0007$; full
326 results in Table S1a, Supplementary; descriptive statistics in Table S2, Supplementary). There
327 was no difference in the probability of breeding failure between the breeding events at 6
328 months and 1.1 years old ($p = 1.0$), or between 1.8 years and 3.5 years of age ($p = 0.2$; Table

329 S1a, S2). We found no significant effect of the treatment or replicate on the likelihood of
330 breeding failure (Table S1a). Similar results were obtained when we carried out this analysis
331 using only those females that survived to breed in old age at 3.5 years old (Table S3a and S4,
332 Supplementary).

333

334 ***Latency to lay the first egg within the clutch***

335 Irrespective of their environmental conditions, females at 1.1 years and 1.8 years laid their
336 first egg sooner following pairing than they did at at 6 months of age (1.1 years *vs* 6 months,
337 and 1.8 years *vs* 6 months, $p < 0.0001$ for both; full results in Table S1b, Supplementary;
338 Figure 1a); there were no differences in latency between 1.8 and 1.1 years ($p = 0.4$; Figure
339 1a). Latency to lay increased again when the birds were old at 3.5 years to a level similar to
340 that at 6 months of age ($p = 0.8$; Table S1b; Figure 1a). Replicate 2 birds laid their first
341 clutches slightly sooner compared to replicate 1 birds (replicate 1: 8.5 ± 0.4 days; replicate 2:
342 7.2 ± 0.3 days), and there were no treatment effects on latency to lay either as a main factor or
343 in its interaction with age (Table S1b; Figure 1a). Again, similar estimate parameters were
344 obtained when carrying out the analysis only on those females that opted to breed and
345 survived to the breeding event at 3.5 years of age (Table S3b and Figure S1a, Supplementary).

346

347 ***Clutch size***

348 Irrespective of environmental conditions, clutch size (range 1-8 eggs) was influenced by
349 female age: it increased at 1.1 years relative to 6 months of age ($p = 0.007$), did not differ

350 between 1.1 years and 1.8 years of age ($p = 0.15$), and then decreased in old adulthood
351 relative to the earlier life breeding events ($p \leq 0.0001$ for all contrasts, full results in Table
352 S1c, Supplementary; Figure 1b). We found no effect of treatment on clutch size (Table S1c;
353 Figure 1b). Replicate 2 females produced overall slightly larger clutches than replicate
354 1females (replicate 1: 4.0 ± 0.1 eggs; replicate 2: 4.5 ± 0.1 eggs - Table S1c). Similar
355 parameter estimates were obtained when carrying out the analyses only using those females
356 that opted to breed and survived up the breeding event at 3.5 years of age (Table S3c and
357 Figure S1b, Supplementary).

358

359 ***Fledgling success***

360 We examined fledgling success at the first and last breeding event (no chicks were reared at
361 the breeding event at 1.1 years, and at 1.8 years of age a separate egg cross-fostering
362 experiment was performed so these data have not been included). Fledgling success was
363 reduced when the birds were 3.5 years relative to 6 months ($p < 0.0001$, full results in Table
364 S1d, Supplementary; Figure 1c). There was no effect of replicate either as a main factor or in
365 its interaction with the treatment (Table S1d). The effect of the treatment on fledgling success
366 was age-dependent (Table S1d, Figure 1c). At 6 months of age there was no detectable
367 reduction in fledgling success in the challenged females relative to controls ($p = 0.2$; Figure
368 1c), while at 3.5 years, challenged females had higher fledgling success than the age-matched
369 controls ($p = 0.01$; Figure 1c). The same results were observed when the analysis was carried
370 out using only those females that opted to breed and survived to the breeding event at 3.5
371 years of age (Table S3d and Figure S1c, Supplementary).

372

373 ***Number of chicks fledged***

374 As with the fledging success, we examined overall breeding performance at the first and last
375 breeding event. As expected from the clutch size results, the number of chicks fledged (0-6)
376 was much reduced in old adulthood compared to 6 months of age in both control and
377 challenged females ($p < 0.0001$, full results in Table S1e, Supplementary; Figure 1d).
378 Replicate 2 birds reared more fledglings than replicate 1 birds (replicate 1: 1.6 ± 0.1 chicks;
379 replicate 2: 2.1 ± 0.1 chicks, Table S1e), however this effect was consistent between control
380 and challenged females (Table S1e). The effect of the treatment on the number of chicks
381 fledged was influenced by female age. Challenged females fledged fewer chicks (on average
382 20%) compared to controls at 6 months of age ($p = 0.04$; Figure 1c), whereas at 3.5 years,
383 challenged females reared more offspring compared to age-matched controls ($p = 0.008$,
384 Figure 1d). Similar parameter estimates were obtained when performing analyses only using
385 those females that survived to 3.5 years of age ($p = 0.1$, Table S3e and Figure S1d,
386 Supplementary).

387

388 ***Survival***

389 Mortality was very low between 5 months and 1 year of age and there were no differences in
390 survival between the two treatment groups ($p = 0.5$, full results in Table S5, Supplementary;
391 Figure 2a). Survival curves started diverging after 1 year of age (Figure 2b), and from 1 to 3
392 years old, the challenged females had on average a 48% reduction in relative risk of death

393 compared to controls ($p = 0.03$, Table S5), as previously shown [27]. However, when we
394 examined survival during old age, between ages 3 and 4, this effect disappeared; survival of
395 challenged birds was no longer better than controls ($p = 0.8$, Table S5; Figure 2c). There was
396 no effect of replicate as main factor or in its interaction with the treatment (Table S5). When
397 examining survival up to 4 years of age in relation to lifetime breeding effort, we found no
398 relationships between either laying effort, nor chicks rearing effort within both treatment
399 groups (Table S6, Supplementary).

400

401 **Discussion**

402 This is the first experimental longitudinal study in a vertebrate species to directly compare the
403 effects of living in a challenging environment at different adult life stages, from early to old
404 adulthood. Our key findings are that (i) regardless of environmental conditions, female
405 reproductive performance changed across adult life (6 months, 1.1 years, 1.8 years and 3.5
406 years) with peak performance generally occurring during middle adulthood (1.1 and 1.8
407 years) followed by a marked decline in old adulthood (3.5 years) – importantly this later life
408 decline occurred within individuals consistent with previous literature on ageing across
409 diverse vertebrate taxa [2], (ii) females exposed to the challenging environmental
410 circumstances produced relatively fewer chicks than those living in the control environmental
411 conditions when they were young (6 months of age), but, in contrast, were able to rear more
412 chicks when they were old (3.5 years of age), again this effect occurred within individuals,
413 (iii) females living in the more challenging conditions showed no difference relative to
414 controls in more benign conditions in the probability of survival when they were young adults

415 (5 months to 1 year of age), had a higher probability of survival in middle age (1 to 3 years of
416 age), with this benefit then disappearing at older ages (from 3 to 4 years).

417 Our stressful environmental protocol did not influence either the likelihood of
418 breeding or the latency to lay the first egg when the females were given the opportunity to
419 breed across the four breeding events, from early to old adulthood. During young adulthood,
420 the challenged females showed an overall reduction in the number of fledglings produced
421 compared to the controls. This effect on overall breeding performance was due to additive
422 treatment-dependent reduction in performance observed at the clutch (primarily) and fledging
423 success level. Interestingly, in old adulthood (3.5 years of age), challenged females, despite
424 laying similar clutch sizes to the controls, fledged proportionally more of their chicks than
425 females living in the more predictable environment, possibly due to treatment differences in
426 parental behaviour and/or in egg quality. Altogether, our results thus show that the mild stress
427 exposure induced by the challenging environmental conditions resulted in females showing a
428 relatively reduced breeding performance when they were young, but increased performance in
429 old age. This effect occurred within individuals, and thus was not due to any differential
430 survival effects. It could be due to challenged females having either an impaired breeding
431 capacity in young adulthood as a result of their exposure to increased levels of glucocorticoid
432 hormones, or to their showing a strategic restraint in breeding effort during early adulthood.
433 We have shown that our challenging environmental protocol did increase overall exposure to
434 stress hormones without causing habituation (measured to old adulthood, 3.5 years). A
435 reduction in reproductive performance in response to stress exposure has been reported in
436 other studies that examined responses to stressful environments, including food shortages or

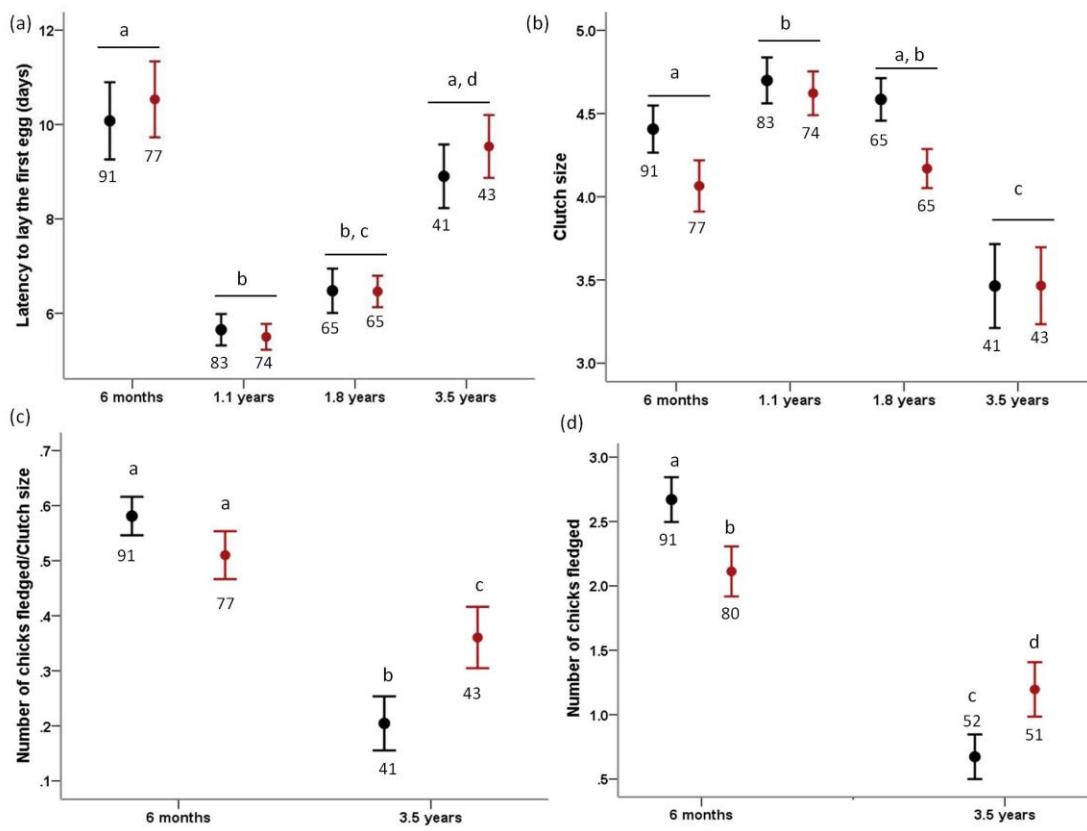
437 increased predation pressure [32, 39-41]. However, because the birds in our study then
438 showed increased breeding performance in old adulthood, despite still being exposed to
439 higher levels of stress hormones, suggests that their breeding capacity was not impaired and
440 supports a strategic restraint interpretation. It has been suggested that stress exposure induces
441 shifts in energy allocation in order to promote self-maintenance strategies at the expense of
442 reproductive behaviours and parenting [42]. It has also been suggested that environmental
443 stressors could trigger protective and compensatory effects on reproductive physiology (see
444 [15] for a review on the potential mechanisms). Therefore, increases in stress exposure levels
445 experienced by the challenged birds might have activated adaptive changes that allowed
446 individuals to better cope with the protracted exposure to the somewhat harsher
447 environmental conditions, at the expense of earlier reproductive investment perhaps in favour
448 of long-term maintenance processes, including survival [43]. We found no relationships
449 between lifetime breeding effort (egg laying/chicks rearing) and survival within both
450 treatment groups. The slight treatment-dependent reduction in clutch size during the early-
451 middle adulthood breeding events within the pool of birds that survived up to the final
452 breeding event in old adulthood provides only very limited support to this possibility. It
453 would be interesting in future studies to see whether similar treatment effects would be
454 observed in animals free to reproduce. Such a design was not possible in our experiment since
455 we were interested in determining the varying effects of the treatment with maternal age on
456 breeding performance, while controlling for the age of the male partner. Our experimental
457 design does not allow us to separate the effects of age and duration of the challenging
458 exposure, since the two are interlinked as would be the case in nature. Our comparison

459 between exposure to repeated stress or not simulates responses of animals living in
460 environments in which the occurrence of key stressors such as low food availability, high
461 population density or high predation risk differ, as has been recorded in the wild in diverse
462 species, such as black-legged kittiwakes *Rissa tridactyla* [44], Belding's ground squirrels
463 *Spermophilus beldingi* [45], snowshoe hares *Lepus americanus* (see 46 for further discussion
464 of this). The facts that breeding performance increased at old age in the birds living in the
465 more stressful environment, and that the stress response of the birds to the random food
466 withdrawals was not diminished with age, suggests that the observed effects on reproductive
467 performance are not due to any accumulated negative effects of stress exposure. Our data on
468 survival show that exposure to the challenging environmental conditions had little effect on
469 survival probability when the birds were young, as mortality was very low during this period
470 in our study population as in previous work in captive zebra finches [17,47]. Survival of the
471 birds in the challenging environments was better than the controls during middle age, with this
472 effect disappearing into old adulthood. Our environmental exposure protocol only affected the
473 temporal availability of food, which was otherwise abundant and thus the effects on survival
474 that we found are not likely to be attributable to caloric restriction. Indeed, body mass was not
475 predictive of survival in our study. The challenge induced by our environmental manipulation
476 was mild, giving rise to repeated and prolonged increases in baseline glucocorticoid secretion
477 (this study, 27). The effect of the treatment on survival was substantial, with the challenged
478 birds having on average 48% decrease in the relative risk of mortality compared to control
479 females during middle age. It is possible that the challenging environment may have induced
480 effects that reduced the rate of ageing through hormetic processes [48, 49]. This possibility

481 fits also with our reproductive data in old adulthood as the challenged females showed less
482 pronounced age-specific declines in reproductive performance relative to those females
483 exposed to the more benign environmental conditions. These long-term beneficial effects of
484 mild challenging exposure resemble those induced by various low-levels/mild repeated
485 stressors that have been shown to delay or slow the onset of senescence across a large variety
486 of animals, including humans [49-53]. Our data are therefore compatible with the treatment
487 exposure having induced stimulatory hormetic responses that slowed at least in part the rate of
488 ageing. The majority of the work focussing on hormetic effects have used single or repeated
489 exposure to mild stressors over relatively brief periods [54, 55]. There is good experimental
490 evidence that exposure to mild stressors can ‘prime’ responses such that individuals are better
491 able to cope with challenges experienced in later life [52, 56, 57]. However, the survival
492 benefits seem to be contingent on the environmental conditions to which the physiology of the
493 animal has been conditioned being encountered again later in life [47]. In our study, the birds
494 exposed to the challenging environment were continuously exposed to it from when they first
495 experienced it at five months old, which may have enabled them to reap the best survival
496 benefit from the resilience induced by the challenging exposure. We do not know the
497 mechanism underlying the disappearance of the positive effect of the challenging
498 environmental conditions on survival in old age. Overall our data highlights the need of more
499 longitudinal/long-term studies to further our understanding of interacting effects among
500 duration of exposure to stress, stressor severity, and aging patterns – disentangling such
501 factors would require exposing animals of different ages to different stressors duration and
502 severity.

503 In conclusion, the results of this study suggest that the apparent organismal effects of
504 living in a mildly challenging environment might vary at different life stages, something
505 which has previously received very little consideration. We found evidence of negative effects
506 of living under challenging environmental conditions on breeding performance across young
507 adulthood, but positive effects in old age. Survival was not affected in young adulthood,
508 improved in middle age, but then not affected in old age. These results, in addition to showing
509 that exposure to challenging environments can modulate life histories with consequences for
510 patterns of senescence, also emphasise that the duration of studies, the life history stage at
511 which they take place, and the point at which the effects are examined can influence the
512 interpretation. That repeated exposure to stress might slow the ageing process is an extremely
513 interesting prospect and fits with the emerging idea that, rather than being minimised,
514 exposure to stress levels across the life course needs to be optimised in order to obtain the best
515 health benefits [25, 48].

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Figure 1

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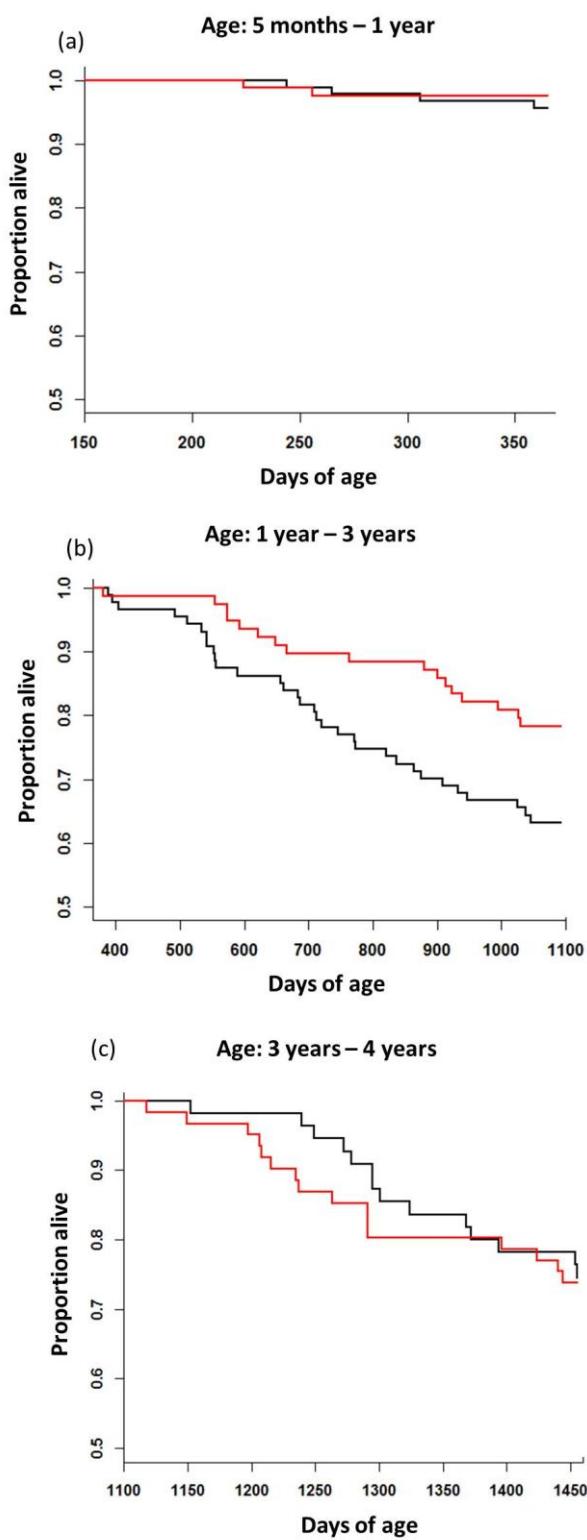


Figure 2

Figure legends

545 **Figure 1.** (a) Latency to lay the first egg, (b) clutch size, (c) fledging success (number of
 546 chicks fledged/clutch size; proportional data) and (d) number of chicks fledged by the
 547 females exposed to the challenging environmental conditions (in red) and control
 548 environmental conditions (in black) across the age-specific breeding events. Note that eggs
 549 were allowed to hatch only during the breeding event at six months, 1.8 years and 3.5 years of
 550 age; at 1.8 years, cross-fostering was used and these data were omitted from these analyses
 551 (full details in ‘Data Analysis’). Different letters indicate significant post hoc pairwise
 552 contrasts ($p < 0.05$ after Tukey’s multiple comparison adjustment—full statistics in electronic
 553 supplementary material, table S1); numbers indicate sample sizes separately by treatment and
 554 age.

555

556 **Figure 2.** (a) Survival trajectories from the start of the experiment up to 1 year of age (i.e.
 557 150-365 days); (b) from 1 to 3 years of age (i.e. 365-1096 days) and (c) from 3 to 4 years of
 558 age (i.e. 1096-1456 days) of zebra finch females exposed to challenging (in red) or control (in
 559 black) environmental conditions. Birds exposed to the challenging environment showed
 560 improved survival from 1 to 3 years of age ($p = 0.03$), whereas no treatment effects were
 561 found either from five months to 1 year of age, or from 3 to 4 years of age (full statistics in
 562 electronic supplementary material, table S5).

563

564

565 **Ethics statement.** All procedures were carried out under Home Office Project Licence
 566 (60/4109).

567 **Data accessibility.** Data are available from Dryad Digital Repository:
 568 <https://doi.org/10.5061/dryad.6r273>.

569 **Authors’ contributions.** VM, WB, BH, and PM designed the study; all authors conducted
 570 the experiment, VM and PM analysed the data and wrote the manuscript, all authors
 571 contributed to manuscript revisions of earlier drafts of the manuscript.

572 **Competing interests.** We declare we have no competing interests.

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582

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723

Supplementary Material

724

725 **Environmental conditions shape the temporal pattern of investment in reproduction and**
726 **survival**

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737 **Baseline corticosterone monitoring**

738 *Sampling and laboratory analyses*

739 To monitor the effects of the unpredictable food withdrawals on baseline corticosterone levels
740 we sampled a subset of randomly selected birds from both replicates when the birds were ca
741 3.5 years old (1266.5 ± 1.5 days of age, mean \pm SE; control: 34 females; challenged: 32
742 females), and after approximately 1.5 years of non-interrupted exposure to the unpredictable
743 food withdrawals (since the termination of the breeding round at ca 1.8 years of age). At the
744 end of a period of food withdrawal in the challenged birds, birds from both treatment groups
745 were blood sampled (~ 75 μ l) within 3 min of entering the room to obtain a baseline blood
746 sample (1). We recorded bleed time from each individual bird. Blood samples were stored on
747 ice, centrifuged to separate plasma from red blood cells, and frozen at -80 °C until laboratory
748 analyses. Blood samples were always collected between 13.15 and 15.50 h. Corticosterone
749 levels were measured using an enzyme-immunoassay (EIA - Assay Designs Corticosterone
750 Kit 901-097, Enzo Life Sciences, Exeter UK) following the same method as described
751 previously (2). Briefly, corticosterone was extracted two times in 1 ml of diethyl ether
752 (Rathburn Chemicals, Walkerburn, UK) from plasma aliquots (~17 μ l). Tracer amounts
753 (~1500 v.p.) of corticosterone label ([1, 2, 6, 7-3M] NET 399, PerkinElmer, Waltham, MA,
754 USA) were added to each sample to estimate extraction efficiencies. After extraction,
755 corticosterone concentrations (ng/ml) were measured following the manufacturer's
756 instructions. Samples from both treatment groups were standardised across assay plates and
757 the average extraction efficiency was 85%, the average intra-assay coefficient of variation
758 (CV) was 10%, and the inter-assay CV calculated using the same quality control sample run
759 in each plate was 11%. Eight samples fell below the detection limit of the assay and were
760 assigned the minimum detectable value (0.37 ng/ml). The same quality control sample used in
761 the current batch of assays was also used when we measured baseline corticosterone levels
762 from samples collected in early adulthood (~ 6 months of age) from randomly selected birds
763 from the same study population (26 controls and 29 challenged birds - full data published
764 elsewhere, REF 2), and corticosterone concentrations in the quality control were also
765 comparable with the earlier assays (inter-assay CV was 12%).

766

767 *Data analysis*

768 By including our previous corticosterone data collected from birds in the same study
769 population when the birds were ~ 6 months of age (full results published elsewhere, 2), we
770 used Generalised Linear Mixed Models (GLMMs) with Gaussian error distribution to monitor
771 the effects of age and/or the unpredictable food withdrawals on baseline corticosterone levels

772 (“lme4” package in R, [3]). In the final model fixed factors were treatment, age (6 months vs
773 3.5 years), replicate, and the interaction treatment and age; family identity and individual
774 identity were entered as random factors as there were sisters in the experiment and a few
775 individuals ($n = 15$) were sampled at both ages. We checked the potential co-variation
776 between the response variable and bleed time, as well as the interaction of the treatment with
777 replicate to assess consistency of treatment effects on baseline corticosterone between the two
778 replicates. CORT levels were ln-transformed to improve normality of model residuals.

779

780 **Results**

781 There was a main effect of age due a decrease in baseline corticosterone in the birds sampled
782 at 3.5 years of age relative to those sampled at 6 months in both treatment groups (age: $p <$
783 0.0001, interaction: $p = 0.3$, full model output in Table S0). However, at both age periods the
784 challenged birds responded with similar baseline corticosterone increases to the random
785 episodes of food withdrawals relative to the age-matched controls sampled at the same time of
786 the day (6 months, control: 2.32 ± 0.21 , challenged: 3.93 ± 0.52 ; 3.5 years, control: $1.11 \pm$
787 0.19 ng/ml; challenged: 2.03 ± 0.36 ng/ml, un-transformed mean \pm SE for all; treatment: $p =$
788 0.02, Table S0). There was no effect of replicate on baseline corticosterone levels (Table S0).

789

790 **References**

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795 Gerontology* 69, 170-175.
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800

801 **Table S0.** GLMM modelling (Gaussian error distribution) to assess the effects of the random
802 episodes of food withdrawals on baseline corticosterone levels. Fixed factors estimates are
803 indicated in parenthesis, r indicates random factor and its associated variance. Significant
804 factors are highlighted in bold. The non-significant interaction treatment x replicate
805 (likelihood ratio test, $p > 0.05$) was removed from the final models.

806

Factor	Estimate	SE	t-value	p-value
Family identity (r)	0			
Individual identity (r)	0			
Residual	0.396			
Intercept	0.789	0.138	5.718	<0.0001
Treatment	0.405	0.170	2.381	0.019
(challenging environment)				
Age (3.5 years)	-0.936	0.164	-5.705	<0.0001
Replicate (2)	-0.098	0.115	-0.856	0.394
Treatment x Age	0.321	0.230	1.394	0.166
Bleed time				0.4
Treatment x Replicate				0.6

807

808

809 **Table S1.** GLMM modelling to test the effects of treatment, age at breeding, and selected
 810 fixed parameters (see “Data Analysis”, Material and Methods) on (a) whether or not the
 811 females attempted to breed (i.e. laid eggs); (b) latency to lay the first egg; (c) clutch size, (d)
 812 fledging success, and (e) number of chicks fledged. Fixed factors estimates are indicated in
 813 parenthesis, r indicates random factor with its estimated variance. Significant factors are
 814 highlighted in bold and post-hoc pairwise comparisons for significant outcomes are shown in
 815 Table S2 and Figure 1. The non-significant interaction treatment x replicate was removed
 816 from the final models (likelihood ratio test, $p > 0.05$). In (a) the additional random factors
 817 family identity and male partner identity were dropped from final analysis because the models
 818 did not converge.

819

(a) Breeding failure

Parameter	Estimate	SE	Z	p
Female ring identity (r)	1.429			
Intercept	4.254	0.798	5.328	<0.0001
Treatment (challenging environment)	0.383	0.430	0.891	0.373
Replicate (2)	0.465	0.439	1.059	0.289
Age (1.1 years)	-0.376	0.782	-0.481	0.630
Age (1.8 years)	-1.943	0.677	-2.872	0.004
Age (3.5 years)	-2.817	0.704	-4.001	<0.0001
Treatment x Age	–	–	–	–
Treatment x Replicate	–	–	–	–

(b) Latency to lay the first egg

Parameter	Estimate	SE	t	p
Female ring identity (r)	0.008			
Partner identity (r)	0.009			
Family identity (r)	0.005			
Intercept	0.947	0.032	30.044	<0.0001
Treatment (challenging environment)	0.026	0.039	0.674	0.500
Replicate (2)	-0.058	0.029	-1.991	0.049
Age (1.1 years)	-0.221	0.032	-6.994	<0.0001
Age (1.8 years)	-0.198	0.038	-5.190	<0.0001
Age (3.5 years)	-0.023	0.045	-0.514	0.608
Treatment x Age (1.1 years)	-0.026	0.046	0.553	0.581
Treatment x Age (1.8 years)	0.014	0.055	0.257	0.798
Treatment x Age (3.5 years)	-0.012	0.064	-0.189	0.850
Treatment x Replicate				0.2

(c) Clutch size

Parameter	Estimate	SE	t	p
Female ring identity (r)	0.545			
Partner identity (r)	0.037			
Family identity (r)	0.015			
Intercept	4.124	0.149	27.768	<0.0001
Treatment (challenging environment)	-0.340	0.192	-1.775	0.077
Replicate (2)	0.525	0.132	3.975	0.0002
Age (1.1 years)	0.253	0.167	1.520	0.130
Age (1.8 years)	0.132	0.183	0.720	0.472
Age (3.5 years)	-0.992	0.215	-4.606	<0.0001
Treatment x Age (1.1 years)	0.295	0.244	1.209	0.228
Treatment x Age (1.8 years)	-0.030	0.263	-0.115	0.910
Treatment x Age (3.5 years)	0.462	0.306	1.511	0.132
Treatment x Replicate				0.9

(d) Fledging success

Parameter	Estimate	SE	z	p
Female ring identity (r)	1.044			
Family identity (r)	<0.0001			
Intercept	0.316	0.197	1.599	0.110
Treatment (challenging environment)	-0.305	0.235	-1.296	0.195
Replicate (2)	0.298	0.219	1.357	0.175
Age (3.5 years)	-2.068	0.274	-7.561	<0.0001
Treatment x Age (3.5 years)	1.196	0.358	3.336	0.0009
Treatment x Replicate				0.7

(e) Number of chicks fledged

Parameter	Estimate	SE	z	p
Female ring identity (r)	0.128			
Family identity (r)	<0.0001			
Intercept	0.797	0.100	8.005	<0.0001
Treatment (challenging environment)	-0.235	0.115	-2.043	0.041
Replicate (2)	0.221	0.106	2.076	0.038

Age (3.5 years)	-1.415	0.183	-7.728	<0.0001
Treatment x Age (3.5 years)	0.831	0.238	3.495	0.0005
Treatment x Replicate				0.6

820

821

822 **Table S2.** Percentage values of zebra finch females subjected to the control or challenging
823 environmental conditions that did not opt to breed (i.e. did not attempt to lay a clutch) during
824 the four age-specific breeding events; sample sizes refers to the total number of birds within
825 each treatment group, the gradual decrease in sample size with age was due to mortality of
826 experimental females across the experiment. Different letters indicate significant differences
827 ($p < 0.05$ after Tukey multiple comparison adjustment).

Age at breeding	Control	Challenging
6 months	0%, n = 91 ¹	3.9%, n = 80 ¹
1.1 years	3.6%, n = 86 ¹	1.4%, n = 75 ¹
1.8 years	13.8%, n = 74 ²	7.7%, n = 70 ²
3.5 years	26.8%, n = 41 ³	18.6%, n = 51 ³

828
829
830

831 **Table S3.** GLMM modelling to test the effects of treatment, age at breeding, and selected
 832 fixed parameters (see “Data Analysis”, Material and Methods) on (a) whether or not the
 833 females attempted to breed (i.e. laid eggs); (b) latency to lay the first egg; (c) clutch size, (d)
 834 fledging success, and (e) number of chicks fledged. These analyses are performed only using
 835 the females that were alive up to the final breeding event at 3.5 years of age. Fixed factors
 836 estimates are indicated in parenthesis, r indicates random factor and its associated variance.
 837 Significant factors are highlighted in bold and post-hoc pairwise comparisons for significant
 838 outcomes are shown in Table S4 and Figure S1. The non-significant interaction treatment x
 839 replicate was removed from the final models (likelihood ratio test, $p > 0.05$). In (a) the
 840 additional random factors family identity and male partner identity were dropped from final
 841 analysis because the models did not converge.

842

(a) Breeding failure

Parameter	Estimate	SE	Z	p
Female ring identity (r)	0.979			
Intercept	4.949	1.153	4.292	<0.0001
Treatment (challenging environment)	0.088	0.473	0.185	0.853
Replicate (2)	0.146	0.475	0.308	0.758
Age (1.1 years)	-0.71	1.239	-0.573	0.566
Age (1.8 years)	-2.05	1.086	-1.887	0.059
Age (3.5 years)	-3.275	1.055	-3.105	0.002
Treatment x Age	—	—	—	—
Treatment x Replicate	—	—	—	—

(b) Latency to lay the first egg

Parameter	Estimate	SE	t	p
Female ring identity (r)	0.006			
Partner identity (r)	0.014			
Family identity (r)	0.004			
Intercept	0.975	0.04	24.367	<0.0001
Treatment (challenging environment)	0.012	0.05	0.233	0.816
Replicate (2)	-0.095	0.033	-2.866	0.005
Age (1.1 years)	-0.237	0.04	-5.948	<0.0001
Age (1.8 years)	-0.205	0.047	-4.332	<0.0001
Age (3.5 years)	-0.028	0.05	-0.548	0.587
Treatment x Age (1.1 years)	0.03	0.057	0.53	0.597
Treatment x Age (1.8 years)	0.02	0.067	0.0301	0.763
Treatment x Age (3.5 years)	0.004	0.071	0.05	0.957

Treatment x Replicate 0.1

(c) Clutch size

Parameter	Estimate	SE	t	p
Female ring identity (r)	0.249			
Partner identity (r)	0.14			
Family identity (r)	0.062			
Intercept	4.263	0.195	21.899	<0.0001
Treatment (challenging environment)	-0.468	0.244	-1.920	0.056
Replicate (2)	0.568	0.164	3.464	0.001
Age (1.1 years)	0.103	0.204	0.505	0.615
Age (1.8 years)	0.119	0.220	0.540	0.590
Age (3.5 years)	-1.110	0.234	-4.747	<0.0001
Treatment x Age (1.1 years)	0.256	0.291	0.878	0.382
Treatment x Age (1.8 years)	0.006	0.315	0.020	0.984
Treatment x Age (3.5 years)	0.535	0.330	1.618	0.107
Treatment x Replicate				0.3

(d) Fledging success

Parameter	Estimate	SE	Z	p
Female ring identity (r)	0.691			
Family identity (r)	<0.0001			
Intercept	0.510	0.233	2.185	0.029
Treatment (challenging environment)	-0.234	0.274	-0.851	0.395
Replicate (2)	0.395	0.241	1.638	0.101
Age (3.5 years)	-2.125	0.277	-7.668	<0.0001
Treatment x Age (3.5 years)	1.128	0.365	3.095	0.002
Treatment x Replicate				1.0

(e) Number of chicks fledged

Parameter	Estimate	SE	Z	p
Female ring identity (r)	0.077			
Family identity (r)	0			
Intercept	0.907	0.117	7.729	<0.0001
Treatment (challenging environment)	-0.214	0.134	-1.601	0.109
Replicate (2)	0.273	0.119	2.3	0.021

Age (3.5 years)	-1.501	0.186	-8.056	<0.0001
Treatment x Age (3.5 years)	0.816	0.243	3.353	0.0008
Treatment x Replicate				0.7

843

844 **Table S4.** Percentage values of zebra finch females subjected to the control or challenging
845 environmental conditions that did not opt to breed (i.e. did not attempt to lay a clutch) during
846 the four age-specific breeding events within the pool of females that survived up to the final
847 breeding event at 3.5 years of age; sample sizes refers to the total number of birds within each
848 treatment group. Different letters indicate significant differences ($p < 0.05$ after Tukey
849 multiple comparison adjustment).

850

851

Age at breeding	Control	Challenging
6 months	0%, n = 52 ¹	3.9%, n = 51 ¹
1.1 years	3.6%, n = 52 ¹	1.4%, n = 51 ¹
1.8 years	13.8%, n = 52 ^{1, 2}	7.7%, n = 51 ^{1, 2}
3.5 years	26.8%, n = 52 ²	18.6%, n = 51 ²

852 **Table S5.** Time-dependent Cox Regression modelling to test the effects of the treatment on
853 survival. Coefficient estimates are referred to treatment = challenging environment, replicate
854 = 2; Coef indicates the hazard rate; Exp (Coef) indicates the hazard ratios, and SE (Coef)
855 indicates the standard error of the hazard rate. The non-significant interaction term of
856 replicate with treatment was consequentially removed from the final model.

857

Parameter	Coef	Exp (Coef)	SE (Coef)	Z	p
Treatment:Age interval 150-365 days	-0.553	0.575	0.866	-0.64	0.523
Treatment:Age interval 365-1096 days	-0.656	0.519	0.300	-2.18	0.029
Treatment:Age interval 1096-1456 days	0.111	1.118	0.367	0.30	0.762
Replicate	0.272	1.313	0.221	1.23	0.218
Treatment:age interval 150-365 days:Replicate					0.8
Treatment:Age interval 365-1096 days:Replicate					0.4
Treatment:Age interval 1096- 1456:Replicate					0.2

858

859 **Table S6.** GLMs modelling to assess whether the probability of survival up to 4 years of age
 860 was influenced by lifetime breeding effort (a) lifetime egg laying effort, or (b) lifetime chick
 861 rearing effort; see “Statistical analysis” paragraph for full details) within the females exposed
 862 to the control environmental conditions or challenging environmental conditions. Fixed factor
 863 estimates are indicated in parenthesis. Significant effects are highlighted in bold. The non-
 864 significant factor replicate in interaction with the treatment was subsequently removed from
 865 the final model (likelihood ratio test, $p > 0.05$).

866

(a) Lifetime egg laying effort

Control environment

Parameter	Estimate	SE	Z	p
Intercept	-0.069	0.801	-0.086	0.932
Lifetime egg laying effort	0.044	0.200	0.222	0.824
Replicate (2)	0.166	0.439	0.379	0.704
Lifetime egg laying effort x Replicate				0.7

Challenging environment

Parameter	Estimate	SE	Z	p
Intercept	-1.549	0.897	-1.727	0.084
Lifetime egg laying effort	0.239	0.228	1.047	0.295
Replicate (2)	0.696	0.482	1.444	0.149
Lifetime egg laying effort x Replicate				0.3

(b) Lifetime chick rearing effort

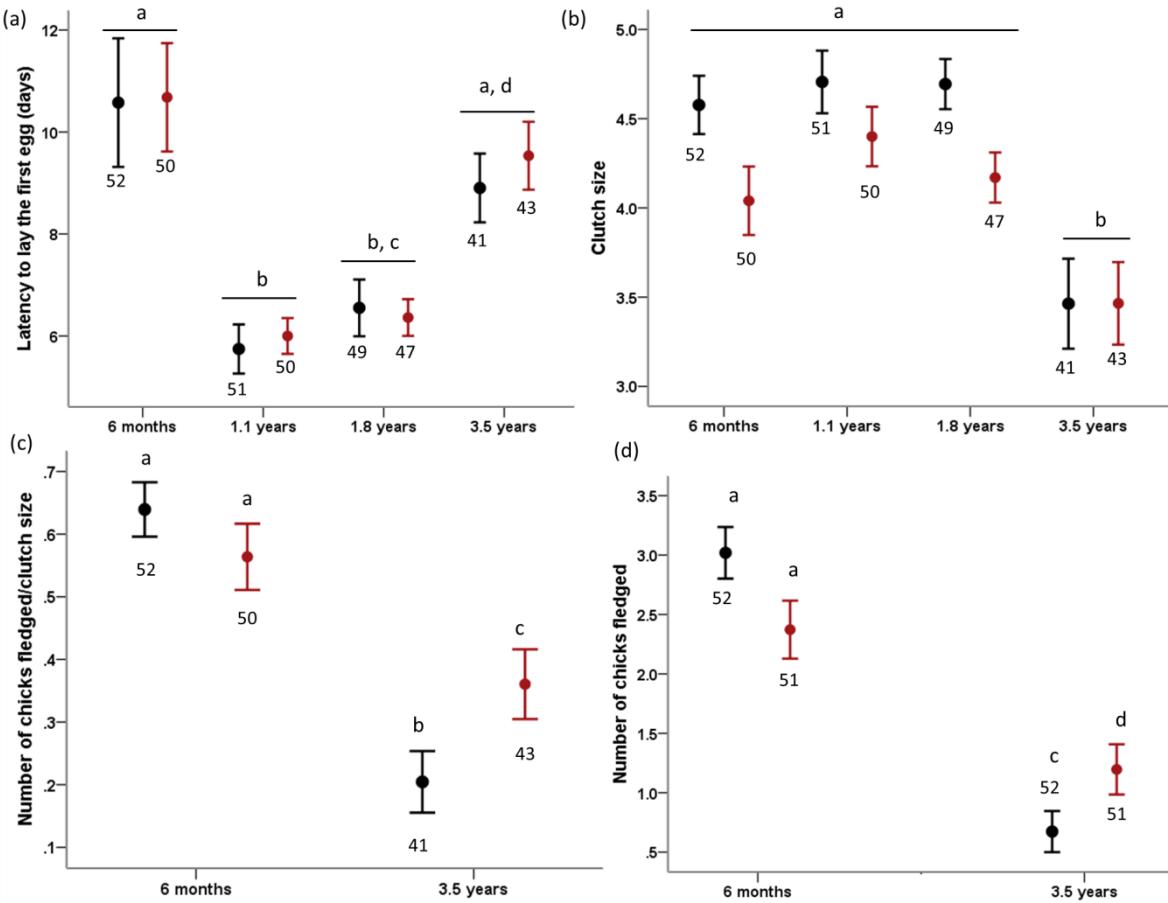
Control environment

Parameter	Estimate	SE	Z	p
Intercept	-0.083	0.486	-0.170	0.865
Lifetime chick rearing effort	0.094	0.198	0.475	0.635
Replicate (2)	0.165	0.427	0.388	0.698
Lifetime chick rearing effort x Replicate				0.5

Challenging environment

Parameter	Estimate	SE	Z	p
Intercept	-0.339	0.474	-0.715	0.474
Lifetime chick rearing effort	-0.222	0.211	-1.051	0.293

	Replicate (2)	0.967	0.484	2.000	0.046
	Lifetime chick rearing effort x Replicate				0.1
867					
868					



869

870 **Figure S1.** (a) Latency to lay the first egg, (b) clutch size, (c) fledging success (number of
 871 chicks fledged/clutch size; proportional data), and (d) number of chicks fledged in the females
 872 exposed to the challenging environmental conditions (in red) and control environmental
 873 conditions (in black) across the age-specific breeding events in the experimental birds that
 874 were alive up to 3.5 years of age. Data are shown as means \pm SE. Note that eggs were allowed
 875 to hatch only during the breeding event at 6 months, 1.8 years and 3.5 years of age; cross-
 876 fostering experiments were conducted at 1.8 years of age and these data were dropped from
 877 analyses of fledging success and number of chicks fledged (full details in “Data Analysis”).
 878 Different letters indicate significant differences (post-hoc tests, $p < 0.05$ after Tukey multiple
 879 comparison adjustment – full statistics in Table S3); numbers indicate sample sizes separately
 880 by treatment and age.

881