

Survival costs of reproduction are mediated by parasite infection in wild

Soay sheep

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1 Abstract

2 A trade-off between current and future fitness potentially explains variation in life-history
3 strategies. A proposed mechanism behind this is parasite-mediated reproductive costs:
4 individuals that allocate more resources to reproduction have fewer to allocate to defence
5 against parasites, reducing future fitness. We examined how reproduction influenced faecal
6 egg counts (FEC) of strongyle nematodes using data collected between 1989-2008 from a wild
7 population of Soay sheep in the St. Kilda archipelago, Scotland (741 individuals). Increased
8 reproduction was associated with increased FEC during the lambing season: females that gave
9 birth, and particularly those that weaned a lamb, had higher FEC than females that failed to
10 reproduce. Structural equation modelling revealed future reproductive costs: a positive effect
11 of reproduction on spring FEC and a negative effect on summer body weight were negatively
12 associated with overwinter survival. Overall, we provide evidence that parasite resistance and
13 body weight are important mediators of survival costs of reproduction.

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26 Introduction

27 Life-history theory is dominated by the principle that reproduction is associated with large
28 resource costs, meaning individuals constantly face trade-offs, such as that between current
29 and future reproduction (Williams 1966). Theory predicts that current reproduction is expected
30 to reduce future survival because individuals command finite resources, which they must
31 apportion between life-history characteristics (Stearns 1992). The term ‘investment’ implies
32 direct future fitness costs of provisioning resources to a characteristic, whereas the term
33 ‘allocation’ does not imply any direct fitness cost (Hamel *et al.* 2010). The theory of parasite-
34 mediated reproductive costs specifies that resource allocation trade-offs between reproduction
35 and immunity are central to regulating the reproduction-survival trade-off, because increased
36 allocation of resources to reproduction should suppress allocation of resources to immunity
37 and reduce future survival (Sheldon & Verhulst 1996).

38

39 Both observational and experimental studies have provided support for a trade-off between
40 reproduction and parasite resistance (Festa-Bianchet 1989; Richner *et al.* 1995; Ardia *et al.*
41 2003; Pelletier *et al.* 2005; Graham *et al.* 2010; Mills *et al.* 2010). Despite this, to support the
42 hypothesis of parasite-mediated reproductive costs in an observational study we must also
43 demonstrate that reduced parasite resistance is associated with reduced future fitness.
44 Experimental removal of parasites in wild populations has been shown to increase survival
45 (Gulland 1992; la Puente *et al.* 2010; Watson 2013), suggesting increased parasite burdens may
46 mediate effects of reproduction on survival. However, detecting trade-offs in observational
47 studies can be difficult due to differences in resource acquisition between individuals (van
48 Noordwijk & de Jong 1986). These differences may mean that some individuals have resources
49 available to allocate to both reproduction and immunity, while others may struggle to allocate
50 resources to both, resulting in observed positive correlations between traits where trade-offs

51 exist. The costs of reproduction are also likely dependent on age and sex (e.g. Descamps *et al.*
52 2009). In mammals, while there are costs of bearing offspring, the greatest costs are often
53 attributed to lactation (Clutton-Brock *et al.* 1989; Froy *et al.* 2016).

54

55 Long-term, individual-based studies in natural systems are critical to testing life-history theory,
56 but are rare as they are difficult to maintain (Lindenmayer *et al.* 2012). Here, we quantified the
57 pathways linking reproduction, parasite egg counts, body weight, and survival using 19 years
58 of data collected from a wild population of Soay sheep (*Ovis aries*). Many parasite species are
59 present, but gastrointestinal strongyle nematodes have the greatest effect on health and
60 mortality of any parasite in the population and are highly prevalent (Gulland 1992; Gulland &
61 Fox 1992). Strongyles have a direct life cycle: adults live up to 60 days in the host (Armour *et*
62 *al.* 1966), producing eggs that are shed in faeces; eggs hatch and moult to L3 larvae that are
63 ingested by sheep during grazing. Infection intensity of strongyles is quantified with faecal egg
64 count (FEC), which is strongly positively correlated with worm burden (Wilson *et al.* 2004).

65

66 Survival of female Soay sheep reproducing in spring (mean survival = 90.8%) is lower than
67 non-reproducing females (95.5%) over the subsequent winter (Clutton-Brock *et al.* 1996;
68 Tavecchia *et al.* 2005). This difference is more pronounced in years of harsh winters (Clutton-
69 Brock *et al.* 1996). Two observations suggest that parasites could mediate this reproduction-
70 survival association. First, a rise in FEC occurs during late pregnancy and early lactation in this
71 population (Tempest 2005), a phenomenon known as the peri-parturient rise (Lloyd 1983;
72 Fthenakis *et al.* 2015). Second, higher FEC and lower body weight are associated with lower
73 overwinter survival (Clutton-Brock *et al.* 1992; Gulland 1992; Hayward *et al.* 2011, 2018). We
74 therefore evaluated whether allocation to reproduction incurred survival costs mediated by its

75 effect on FEC and body weight. Our results support the notion that costs of reproduction,
76 manifested as reduced survival, are mediated by both strongyle FEC and body weight.

77

78 Material and methods

79 **Study Population and Data Collection**

80 The St. Kilda archipelago (54°49'08"34'W) lies 65km west of the Outer Hebrides, Scotland,
81 and consists of four islands: Hirta, Soay, Boreray, and Dun. A population of unmanaged Soay
82 sheep, descendants of primitive European domestic sheep that were introduced to the island of
83 Soay several thousand years ago (Clutton-Brock & Pemberton 2004), inhabit the island of
84 Hirta. Population dynamics on Hirta are characterised by periods of growth followed by large
85 declines in population size due to adverse winter weather, poor food availability, and parasite
86 infections, which combine to reduce body weight and increase mortality (Gulland 1992;
87 Coulson *et al.* 2001; Craig *et al.* 2006).

88

89 A longitudinal individual-based study on the sheep in the Village Bay area of Hirta began in
90 1985 and continues to the present (Clutton-Brock & Pemberton 2004). The population is
91 monitored daily during the lambing season in March-May. >95% of lambs are caught within a
92 week of birth and are given an identification tag, weighed, and have blood and tissue samples
93 taken. Throughout the history of the study, faecal samples have been collected from adults
94 during lambing; often, the same individuals are repeatedly sampled within a year. Lambs suckle
95 throughout the spring and are weaned by August. In August, around 50% of the population are
96 captured to collect a variety of measures including body weight and faecal samples.

97

98 **Change in FEC across lambing season**

99 We first determined how differences in reproductive allocation were associated with the PPR
100 in strongyle faecal egg count (FEC). Faecal samples were collected from both sexes across the
101 springs of 1989-2008 (1st March – 29th May) and FEC was quantified using a modified version
102 of the McMaster technique (Craig *et al.* 2006). We constructed models assessing changes in
103 FEC during the lambing season including the following predictors.

104

105 *Relative date:* The date on which FEC samples were taken was standardised relative to the date
106 of lamb birth (lamb birth = day 0). Relative dates for non-reproducing females and males were
107 based on the average lambing date for a given year. We analysed FEC data restricted to 49 days
108 either side of lamb birth (relative date = -49 to +49). Our results and conclusions were
109 unchanged if we also used year-specific mean lambing dates to calculate relative date for
110 reproducing females, and if we used absolute date of sample collection for all individuals.

111

112 *Reproductive status:* Each year, females were divided into two categories: (0) did not
113 reproduce; (1) produced at least one lamb.

114

115 *Litter size:* A categorical variable in reproducing females each year: (1) a single lamb; (2) twin
116 lambs.

117

118 *Litter survival:* A categorical variable measured at the end of each August in reproducing
119 females: (1) both twins alive; (2) one twin alive; (3) both twins dead; (4) a singleton alive; (5)
120 a singleton dead.

121

122 *Anthelmintic treatment:* This distinguishes individuals that received an anthelmintic bolus
123 or drench as part of experimental treatments to remove parasites, which have been applied on

124 several occasions throughout the history of the study (Gulland 1992; Gulland *et al.* 1993; Boyd
125 1999; Wilson *et al.* 2003; Tempest 2005). The treatment was applied either in the August before
126 lambing or in the spring of lambing and was considered a categorical variable: (0) no treatment
127 before lambing; (1) treatment before lambing. We included treatment in our models to account
128 for possible effects of treatment on FEC in our correlative study. The bolus releases
129 anthelmintic for several weeks; to be conservative about any long-lasting effects of treatment,
130 we considered animals given a bolus in either August or April as treated before lambing.
131 Exclusion of treated animals from our analysis did not substantially influence our conclusions.
132
133 FEC+100 was natural log-transformed before analysis to adhere to assumptions of residual
134 normality. Generalised additive mixed-effects models (GAMMs) were used to determine how
135 FEC changed during the lambing season and to determine how reproductive allocation was
136 associated with the change in FEC. GAMMs allowed us to fit non-parametric smoothing
137 functions to FEC without being restricted to a specific polynomial form. Year and individual
138 identity were included in all models as random effects since multiple faecal samples were taken
139 from individuals within and across years. Analyses were performed in R 3.5.0 (R Core Team
140 2018) using the ‘*gam4*’ package (Wood & Scheipl 2017).

141
142 We performed separate analyses to model changes in FEC for different age groups: juveniles
143 (one year old in the spring of faecal sampling), yearlings (two years old), and adults (three or
144 more years), because these groups are known to differ in FEC (Wilson *et al.* 2004). We
145 analysed 1129 FECs collected from 381 juveniles, 761 from 208 yearlings, and 2536 from 446
146 adults (Table S1). We tested associations between reproductive allocation and FEC in each age
147 group during the lambing season by grouping reproductive allocation in different ways based
148 on sex, reproductive status, litter size (adults only), and litter survival; the full list of models

149 and groupings for each age class is shown in Tables S2-S4. Three models were compared for
150 each grouping where: (1) the intercept of FEC varied between the groups, but the change in
151 FEC across the season was consistent across groups; (2) the intercept of FEC was the same in
152 all groups, but the change in FEC across the season varied between groups; (3) both the
153 intercept of FEC and the change in FEC across the season varied between groups. In each
154 model, the anthelmintic treatment status of individuals was included as a fixed effect. All
155 models were compared using AIC values with the best-fitting model having the lowest AIC
156 value (Burnham & Anderson 2002).

157

158 **Survival costs of reproduction**

159 We found that increased reproductive allocation in females was associated with higher FEC
160 during the spring (see Results). We next investigated how reproduction influenced female
161 survival, incorporating extensive prior knowledge about this system. Structural equation
162 models (SEMs) enabled us to test our *a priori* expectation that reproductive allocation has
163 effects on survival that are at least partly mediated by effects of reproduction on spring and
164 summer FEC and summer body weight. SEMs are well suited to testing the parasite-mediated
165 costs of reproduction because they specifically quantify the degree to which the relationship
166 between two variables is mediated by a third. SEMs were constructed using 601 records
167 collected from 325 females of all ages (many females are represented in more than one year),
168 including the following set of variables. All variables in the SEM were corrected for
169 anthelmintic treatment and age (see below). As recommended when evaluating SEMs (Grace
170 *et al.* 2014), our *a priori* path diagram (Fig. 1) is based on evidence for causal relationships
171 that might link reproduction and survival and known associations between traits in the Soay
172 sheep system (Table 1).

173

174 *Residual Spring FEC:* Our GAMM analysis (see Results) was made possible by having
175 longitudinal FEC measures from the same individuals within years. However, for our SEMs,
176 we needed a single value of FEC per individual per year to pass to the model. Thus, we
177 extracted random effect estimates from a GAMM of $\text{Ln}(\text{FEC}+100)$ and included age (as a
178 three-level categorical variable: juvenile, yearling, adult), treatment, and relative date (as a
179 smoothed term) as explanatory variables. We summed the overall intercept, individual ID, and
180 year effect estimates to obtain a year-specific FEC value for every individual (year t),
181 accounting for variation in age, date, and treatment (i.e. the value represents the expected FEC
182 at day 0). This value was predicted to be positively associated with August FEC and negatively
183 associated with August body weight in year t (Gulland 1992).

184

185 *Reproductive status:* Following our characterisation of the PPR in Soay sheep, females were
186 grouped into two categories based on reproductive allocation: individuals either reproduced in
187 year t ($n=490$) or did not ($n=111$). Of the reproducing female records, there were 89 records
188 where no lambs survived. We chose to group females in this way, rather than based on lamb
189 survival, since there was clear evidence for a different PPR in reproducing versus non-
190 reproducing females (see Results). We predicted that reproductive status would be positively
191 associated with residual spring FEC and negatively associated with August weight, since body
192 weight is expected to reflect the allocation trade-off between reproduction and somatic
193 maintenance (Clutton-Brock *et al.* 1996).

194

195 *Population Density:* We considered the effect of population density in the August before
196 reproduction (year $t-1$) on reproductive status, since high density is associated with lower
197 fecundity (Clutton-Brock *et al.* 1991). We also considered the effect of density in year t on
198 summer weight and FEC, and survival, since high density is associated with higher FEC

199 (Gulland & Fox 1992), lower summer body weight (Milner *et al.* 1999), and lower survival
200 (Clutton-Brock *et al.* 1991).

201

202 *August FEC:* We estimated summer strongyle FEC from samples taken during August captures
203 in year t . As above, to improve model fit and adhere to assumptions, FEC+100 estimates were
204 natural log-transformed. We expected this to be positively associated with spring FEC and
205 negatively with August body weight (Coltman *et al.* 2001).

206

207 *August Weight:* Body weight was measured during August captures in year t and was expected
208 to be positively associated with survival (Clutton-Brock *et al.* 1996).

209

210 *Overwinter survival:* If the individual was observed during censuses conducted during May in
211 the following year ($t+1$) the individual was considered to have survived the winter; resighting
212 probability of live individuals is close to 100%. This was considered a categorical variable: (1)
213 survived to May 1st; (0) died before May 1st.

214

215 We used structural equation models (SEMs) to assess how reproduction was associated with
216 future survival in females and how this was mediated by FEC and body weight. We wanted to
217 estimate the partial effect of recent reproduction having controlled for age, and therefore
218 derived age-corrected measures of August body weight, August FEC, reproductive status, and
219 survival. Age-corrected weight and FEC measures were generated by fitting them as response
220 variables in linear models with age (as a categorical variable, with ages ≥ 9 grouped together;
221 Table S5) and anthelmintic treatment as explanatory variables. Model residuals were then
222 extracted as measures of age-corrected summer weight and FEC. Reproductive status and
223 survival were also age-corrected: each was fitted as a response variable in a generalised linear

224 model with a binomial distribution and age and treatment fitted as explanatory variables.
225 Residuals were extracted to obtain age-corrected measures of reproductive status and survival.
226 Variables were then standardised by dividing by one standard deviation. Our results and
227 conclusions were unaffected by the exclusion of treated animals from the analysis.

228

229 We first explored each pathway in our SEM using separate (generalised) linear mixed-effects
230 models using the R package ‘lme4’ (Bates *et al.* 2015). Year and individual identity were
231 included in each model as random effects except for spring FEC, where only year was included
232 as a random effect since between-individual effects were negligible. We then conducted formal
233 ‘piecewise’ structural equation modelling to join the multiple models into a single SEM
234 (Shipley 2009), using the R package ‘piecewiseSEM’ (Lefcheck 2015). Shipley’s test of d-
235 separation was used to assess the overall fit of the model and to determine whether any paths
236 were missing (Shipley 2009); missing paths were added into the model (Grace *et al.* 2015).
237 Non-supported paths were removed from the SEM (based on AIC comparison) to improve
238 parameter estimation of the remaining paths. After removal of non-supported paths, the model
239 could not be improved by adding or removing any path.

240

241 Results

242 **Characterisation of the peri-parturient rise (PPR)**

243 All age-sex groups showed a PPR in spring faecal egg count (FEC) (Fig. 2). Generally, this
244 was characterised by a peak in FEC close to parturition (day 0). For juveniles, the best-fitting
245 model grouped all individuals together (Δ AIC relative to next best model = -2; Table S2; Table
246 S6). Juveniles had consistently higher FEC than the other age groups, with a peak of 1386epg
247 (eggs per gram; 95% CI = 1088–1759epg) on day 0 (Fig. 2A). There was marginal evidence
248 suggesting that the best-fitting model for yearlings grouped individuals by sex (Δ AIC = -1;

249 evidence ratio = 0.63; Table S3; Table S7). Males had a higher peak spring FEC, 792epg (518–
250 1189epg) than females, 521epg (91–685epg; Fig. 2B). Both sexes' peak occurred on day 6.

251

252 For adults, the best-fitting model grouped individuals based on litter survival in August: (1)
253 males; (2) non-reproducing females; (3) reproducing females with no surviving lambs; (4)
254 reproducing females with at least one surviving lamb ($\Delta\text{AIC} = -4$; Table S4; Table S8). Females
255 with at least one surviving lamb had a higher and later peak FEC than those that reproduced
256 but whose lambs died before weaning, but both had much higher peak than non-reproducing
257 females. The peak FEC of females with at least one surviving lamb was 370epg (270–495epg)
258 on day 15, whereas the peak FEC of females whose lambs died was 264epg (178–376epg) on
259 day -5 (Fig. 2C). Non-reproducing females had the lowest spring FEC, peaking at 123epg (69–
260 194epg) on day -1 (Fig. 2C). Males had an intermediate peak FEC, 222epg (143–327epg)
261 which occurred on day -20 (Fig. 2C).

262

263 **Survival costs of reproduction**

264 During the model specification stage, population density in the previous August was dropped
265 from the SEM as its inclusion did not improve the models. The SEM of the minimal adequate
266 models did not support two paths included in our *a priori* model: reproductive status \rightarrow August
267 FEC and population density \rightarrow survival ($\Delta\text{AIC} = -8.5$). The final SEM adequately fitted the
268 data (i.e., there were no missing paths; *Fisher's C* = 8.59; *P* = 0.57; Fig. 3). Reproductive status
269 had effects on survival mediated by residual spring FEC, August weight, and August FEC.
270 Pathways linking residual spring FEC and overwinter survival were mediated by effects of
271 residual spring FEC on August weight and August FEC. Weight and FEC in August were also
272 linked to survival. We also found effects of August density (year *t*) on August weight and

273 August FEC, both of which influenced age-corrected survival. Table 2 shows the total influence
274 of each variable (the products of standardised predictors along each path) on survival.

275

276 Discussion

277 Using data collected from a wild population of Soay sheep, we found increased reproductive
278 allocation was associated with greater gastrointestinal nematode faecal egg count (FEC) during
279 the lambing season. Further, the association between reproduction and overwinter survival was
280 mediated by effects of reproduction on spring and summer FEC and summer body weight.
281 Overall, our work demonstrates how parasites can mediate associations between reproduction
282 and future survival.

283

284 **Reproductive allocation influences FEC**

285 We found the effects of sex and/or reproductive allocation on spring FEC became more
286 apparent in adults compared to juveniles and yearlings: juvenile FEC was not influenced by
287 sex or reproductive status; there was marginal evidence that yearling FEC was influenced by
288 sex; and adult FEC was influenced by both. Juveniles had generally high FEC, as predicted
289 from previous research on other wild vertebrates (Wilson *et al.* 1996; Isomursu *et al.* 2006;
290 Hayward 2013; Watson *et al.* 2016) and humans (Simon *et al.* 2015), which suggests that
291 juveniles have not developed fully effective immune defences. Yearling males tended to have
292 higher spring FEC than females (marginal statistical support; estimate = 0.36 ± 0.17 SE, Δ AIC
293 = -1; evidence ratio = 0.63); this result is consistent with studies from diverse species that have
294 found males to have less effective immune responses (Tschirren *et al.* 2003; Hayward 2013;
295 Klein & Flanagan 2016; Watson *et al.* 2016).

296

297 The best-supported model for adults grouped animals into four categories: males, non-
298 reproducing females, reproducing females with no surviving lambs, and reproducing females
299 with surviving lambs. The different patterns of FEC between females with surviving or no
300 surviving lambs suggest that lactation may play a role in these differences (Fig. 2C). Lactation
301 uses resources that could otherwise be allocated to immunity (Coop & Kyriazakis 1999) and
302 studies on red deer (*Cervus elaphus*) suggest that lactation is more expensive than gestation
303 (Clutton-Brock *et al.* 1989; Froy *et al.* 2016). Moreover, experimental studies on domestic
304 sheep have shown that provisioning protein during lactation reduces FEC (Houdijk *et al.* 2003).
305 The number of surviving offspring that a female had did not influence the trajectory of FEC
306 changes, which could be because peak lactation is fixed (Johnson *et al.* 2001), or because only
307 females with the greatest resource pool produce twins (Cassinello & Gomendio 1996; Hewison
308 & Gaillard 2001). Another possibility is that high FEC just before lamb birth may cause ewes
309 to lose their lamb soon after birth and thus experience a decrease in FEC thereafter, while ewes
310 that keep their lamb have their FEC continue to rise (Fig. 2C). There is evidence that dairy
311 sheep treated with anthelmintic produce less milk (Fthenakis *et al.* 2005), but a link between
312 worm infections and lamb survival has not been demonstrated explicitly (Fthenakis *et al.* 2015).
313 A rise in FEC across the season was also seen in males. This is likely due to males being in
314 poor condition following the winter (Gulland & Fox 1992), the weaker antibody responses
315 males exhibit (Hayward *et al.* 2014; Watson *et al.* 2016), and the re-emergence of larvae from
316 arrested development in spring (Langrová *et al.* 2008).

317

318 Our results agree with brood manipulation studies on birds showing that increased allocation
319 to reproduction is associated with greater parasite burdens and less effective immune responses
320 (Nordling *et al.* 1998; Hanssen *et al.* 2005; Knowles *et al.* 2009). Our work also largely agrees
321 with previous work on the Soay sheep population, despite differences in the data and analyses

322 used. Previous work found that FEC during the lambing season varied with age and
323 reproductive status: young animals that failed to reproduce experienced a PPR while non-
324 reproducing adult females did not. Further, reproducing females that successfully weaned a
325 lamb experienced a peak in FEC after lamb birth, while those that lost their lamb had an earlier
326 peak (Tempest 2005). Along with previous work, our findings suggest a more pronounced
327 difference between individuals based on reproductive status in older animals, and a more
328 general PPR in younger animals. Our study has, however, extended previous work by
329 investigating not only the effects of sex and reproduction on FEC, but also the effects of
330 different aspects of reproduction.

331

332 It is possible that our results could reflect variation in exposure between groups rather than
333 reflecting a trade-off with reproduction. There is known heterogeneity in larval distribution
334 around Village Bay (Wilson et al. 2004), and there may be differences in feeding rates between
335 the reproductive groups of females, resulting in differences in exposure to larval parasites and
336 influencing the patterns of FEC seen here. However, it has been shown that Soays actively
337 avoid grazing more contaminated areas in spring and that males, barren females, and females
338 with lambs all avoid parasite-rich vegetation to a similar degree (Hutchings *et al.* 2002).
339 Moreover, experimental work in domestic sheep shows that greater infective doses (i.e.
340 increased exposure) do not result in a greater PPR (Kidane *et al.* 2009). Finally, new work on
341 the Soay sheep has shown that increased reproductive effort is associated with reduced
342 strongyle-specific antibody responses (Hayward *et al.* 2019). Therefore, it appears unlikely that
343 variation in exposure is the main driver of the variation seen in the PPR in this study.

344

345 **Reproduction is negatively associated with survival**

346 As predicted by our hypothesis of parasite-mediated reproductive costs, structural equation
347 models (SEMs) revealed that reproducing females had reduced survival, mediated by spring
348 and summer FEC and summer weight (Fig. 3). These pathways are consistent with a trade-off
349 between reproductive allocation during spring and overwinter survival, mediated by parasite
350 resistance and body weight. While previous studies in wild populations suggest that
351 reproduction incurs survival (Stearns 1992; Clutton-Brock *et al.* 1996; Hodges *et al.* 2015) and
352 immunity costs (Festa-Bianchet 1989; Richner *et al.* 1995; Nordling *et al.* 1998; Knowles *et*
353 *al.* 2009; Graham *et al.* 2010; Hayward *et al.* 2014), and that parasites influence survival
354 (Hanssen *et al.* 2005; la Puente *et al.* 2010; Hayward *et al.* 2011), we have explicitly quantified
355 the pathways through which reproduction influences survival, via associations between
356 reproduction, FEC and body weight (Fig. 3).

357

358 Spring FEC was positively associated with August FEC (Fig. 3). This could arise via three non-
359 mutually exclusive mechanisms: effects of reproduction on FEC persisted across several
360 months; reproduction and infection in spring lead to reduced parasite resistance in summer;
361 and/or FEC is repeatable across seasons (Coltman *et al.* 2001). Increased August FEC was
362 linked to reduced overwinter survival, as expected from previous work in this population
363 (Gulland & Fox 1992; Hayward *et al.* 2011). Reproductive allocation was also linked to
364 reduced survival through the association between spring FEC and August body weight.
365 Previous studies showing such explicit support for any mediators of a reproduction-survival
366 trade-off are rare (Hamel *et al.* 2010). Work on tree swallows (*Tachycineta bicolor*) showed
367 that experimentally-increased brood size was associated with reduced antibody responses to
368 sheep red blood cell (SRBC) antigen, and that individuals that survived to the next season had
369 higher SRBC responses (Ardia *et al.* 2003). However, SRBC responses may not reflect

370 resistance to prevalent parasites, and this analysis did not quantify how resistance mediates the
371 association between reproduction and survival.

372

373 A major advantage of using SEMs is that if there were other important mediating factors
374 between reproduction and survival, Shipley's test would indicate a missing path between
375 reproduction and survival as missing paths can not only indicate a direct association between
376 variables, but may also reflect all direct and indirect pathways not otherwise modelled (Shipley
377 2009; Lefcheck 2015). Shipley's test estimates that in our model (Fig. 3), the direct pathway
378 between reproduction and survival would have an estimate of 0.0053 ± 0.04 , but that this
379 pathway was not statistically supported. In our case, the direct and indirect paths are of the
380 same order of magnitude, which is difficult to interpret considering the direct effect was not
381 statistically supported. Considering this, the most conservative interpretation of our SEM is
382 that at least as much variation in survival is explained by the mediating influence of FEC as is
383 explained by all other direct and indirect factors flowing from reproduction combined.
384 However, as the missing direct association was not detected, we are confident that there are no
385 important missing mediators between reproduction and survival. Therefore, the important
386 mediating factors of survival costs of reproduction in this population appear to be FEC and
387 body weight, or their close correlates.

388

389 In our *a priori* path diagram, weight is conceived as a potentially mediating link between
390 reproduction and survival because we expect that costs of reproduction involve reduced storage
391 of nutritional reserves. However, we also acknowledge that innate variation between
392 individuals that are not directly caused by reproduction ('condition' in the broad sense) might

393 cause differences in weight that affect survival. In that sense, variation in weight is not merely
394 a consequence of prior reproduction, but probably reflects unmeasured variation between
395 individuals that we cannot disentangle from weight in our analysis. One direction for future
396 research could involve modelling the latent causes of weight variation, including reproduction
397 and infection. A recent study on the Soay sheep showed that variation in plasma proteins,
398 independently of body weight and potentially reflecting variation in acquisition of resources,
399 predicted over winter survival (Garnier *et al.* 2017). Assessing how reproductive effort is
400 associated with such nutritional markers could therefore provide more insight into the link
401 between reproduction, weight, and survival. The nature of the association between August
402 weight and August FEC also represented a challenge, since these are measured simultaneously
403 and the association is likely mutually antagonistic (Koski & Scott 2001; Beldomenico *et al.*
404 2008). In our SEM, we suggested that weight influences FEC, which we believed to be the
405 most plausible direction for the association: weight should be more stable than FEC, which can
406 fluctuate greatly within a short time-period. Body weight also has higher repeatability and
407 heritability than FEC (Coltman *et al.* 2001) and although body weight may fluctuate, due to
408 bladder fullness or a wet fleece, these fluctuations are minor compared to the larger fluctuations
409 in FEC (Pollott *et al.* 2004). A final compromise made in our analyses was the decision to
410 perform our SEMs with parameters derived from GAMMs. The caveats of performing
411 statistical analyses on model estimates (e.g. best linear unbiased predictors, BLUPs) are well-
412 known (Hadfield *et al.* 2010); for example, the error with which model estimates are generated
413 are not carried forward. Faced with the challenge of condensing multiple FEC values collected
414 during spring from one female collected in one year into a single value, while accounting for
415 age and other factors, our approach was the only viable option. The fact that our results reliably
416 reflect prior knowledge of the system gives us confidence that our SEM is effective at
417 representing the pathways linking reproduction, parasites, and survival.

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419 In this study, we found that increased allocation to reproduction was associated with increased
420 FEC during late pregnancy and early lactation. We demonstrated that the negative relationship
421 between reproductive allocation and survival is mediated through effects of reproduction on
422 FEC and body weight. The results of our study have several far-reaching implications. First,
423 like previous studies (Festa-Bianchet 1989; Richner *et al.* 1995; Ardia *et al.* 2003; Hanssen *et*
424 *al.* 2005; Graham *et al.* 2010; la Puente *et al.* 2010; Mills *et al.* 2010; East *et al.* 2015), we have
425 shown that reproduction is associated with increased FEC/reduced immune responses, and that
426 these are associated with reduced survival. However, we have also shown explicit links
427 between reproduction, parasites, and survival in a wild system, providing support for a key
428 theory of maintenance of variation in resistance (Sheldon & Verhulst 1996; Rolff & Siva-Jothy
429 2003; Graham *et al.* 2011). Second, the trade-off between reproduction and survival is
430 fundamental, explaining variation in ageing, mating strategies, and sexual traits (Rowe &
431 Houle 1996; Blomquist 2009; Lemaître *et al.* 2015). Our results therefore have broad
432 implications for how trade-offs can shape variation within populations. Finally, as the role of
433 parasites in regulating populations is well-known (Anderson & May 1978; Hudson *et al.* 1992),
434 we demonstrate that reproductive costs could play a role in regulating populations through the
435 effects that they have on parasites. Overall, our work has explicitly quantified the survival costs
436 of reproduction mediated through FEC and the effects of FEC on body weight, providing
437 support for the theory of parasite-mediated reproductive costs.

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Table 1. Response and explanatory variables included in structural equation model (SEM) analysis, justified with published evidence supporting each association.

Response Variable	Explanatory Variables	Sign of Relationship	Reference
Reproductive Status	Previous Density	Negative	(Clutton-Brock <i>et al.</i> 1992)
Spring FEC	Previous Density	Positive	(Gulland & Fox 1992)
	Reproductive Status	Positive	(Wilson <i>et al.</i> 2004)
August Weight	Reproductive Status	Negative	(Clutton-Brock <i>et al.</i> 1996)
	Spring FEC	Negative	(Gulland 1992)
	August Density	Negative	(Milner <i>et al.</i> 1999b)
August FEC	Reproductive Status	Positive	(Wilson <i>et al.</i> 2004)
	Spring FEC	Positive	(Coltman <i>et al.</i> 2001)
	August Weight	Negative	(Coltman <i>et al.</i> 2001)
	August Density	Positive	(Gulland & Fox 1992)
Overwinter survival	August Weight	Positive	(Clutton-Brock <i>et al.</i> 1996)
	August FEC	Negative	(Coltman <i>et al.</i> 1999)
	August Density	Negative	(Clutton-Brock <i>et al.</i> 1991)

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Table 2. The total influence of each variable (the products of standardised coefficients along each path) shown in Figure 3, on the probability of overwinter survival.

Path	Standardised Path Estimate
Reproductive Status → August Weight → Survival	-0.0168
Reproductive Status → August Weight → August FEC → Survival	-0.0012
Reproductive Status → Spring FEC → August FEC → Survival	-0.0044
Reproductive Status → Spring FEC → August Weight → Survival	-0.0033
Reproductive Status → Spring FEC → August Weight → August FEC → Survival	-0.0002
Spring FEC → August Weight → Survival	-0.0252
Spring FEC → August Weight → August FEC → Survival	-0.0018
August Weight → Survival	0.1400
August Weight → August FEC → Survival	0.0100
August FEC → Survival	-0.1400
August Density → August FEC → Survival	-0.0140
August Density → August Weight → Survival	-0.0406
August Density → August Weight → August FEC → Survival	-0.0028

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789 Figure Legends

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791 **Figure 1.** *A priori* structural equation model (SEM) based on decades of research on the Soay
792 sheep population. Associations are depicted for female sheep only. Variables are temporally
793 separated over two years. Reproductive status is based on whether or not the female gave birth
794 in spring.

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796 **Figure 2.** Comparison of change in strongyle FEC over a 99-day period during the lambing
797 season, analysed using generalised additive mixed-effects models (GAMMs; Tables S2-S4).
798 (A) The best-fitting model for juveniles grouped all individuals; (B) the best-fitting model for
799 yearlings grouped individuals by sex; (C) the best-fitting model for adults grouped individuals
800 by lamb survival. Panels on the left show predictions on the log-transformed scale (the scale at
801 which data were analysed), with lines showing model estimates, shaded areas indicating ± 1 SE
802 and points showing raw data. On the right, predictions are back-transformed onto the original
803 scale. Raw data are not shown on the back-transformed plots for clarity of viewing and ease of
804 interpretation.

805

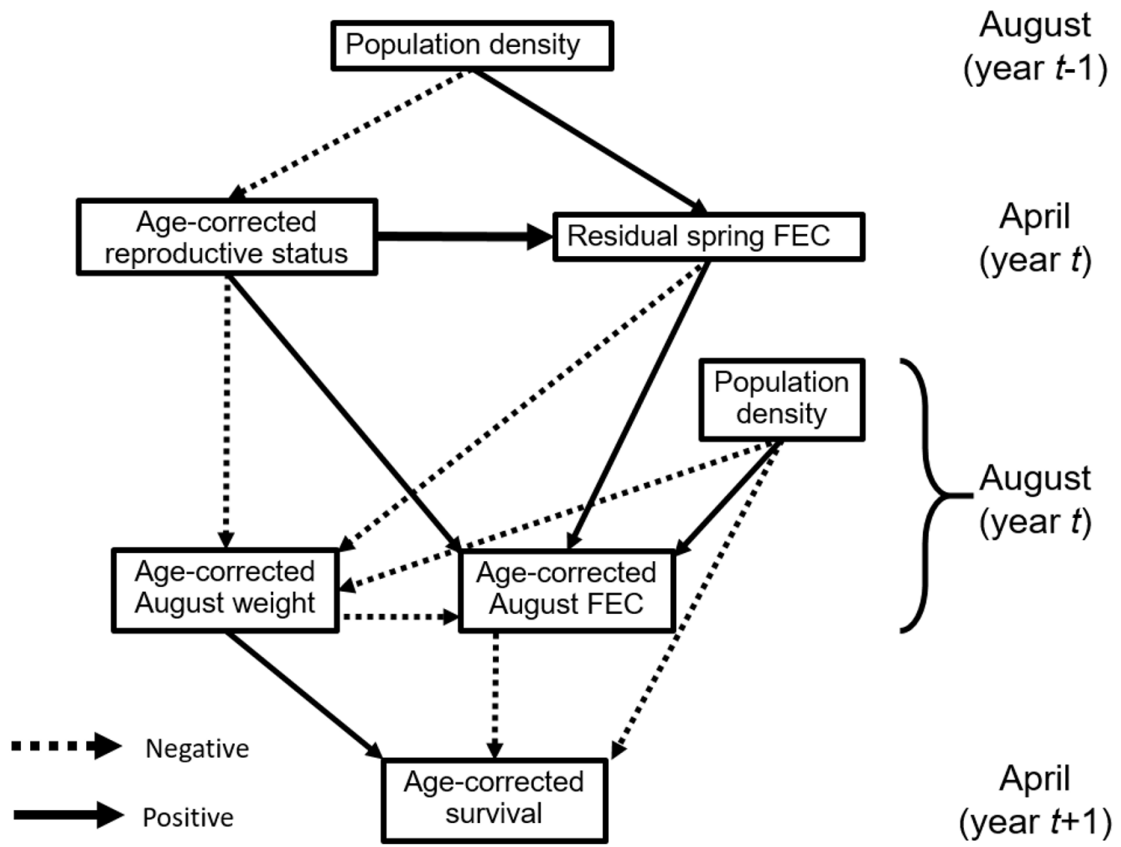
806 **Figure 3.** Our final structural equation model (SEM) showing downstream impacts of
807 reproduction on future fitness (overwinter survival). Values on arrows and arrow widths
808 indicate standardised path coefficients with standard errors in parentheses. All variables were
809 standardised by dividing by one standard deviation.

810

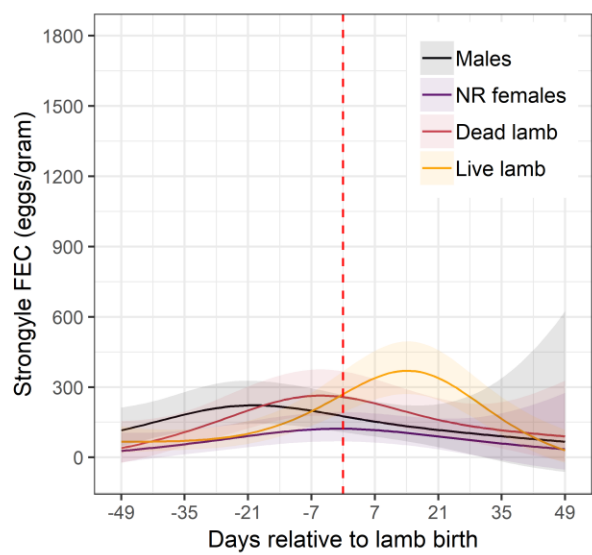
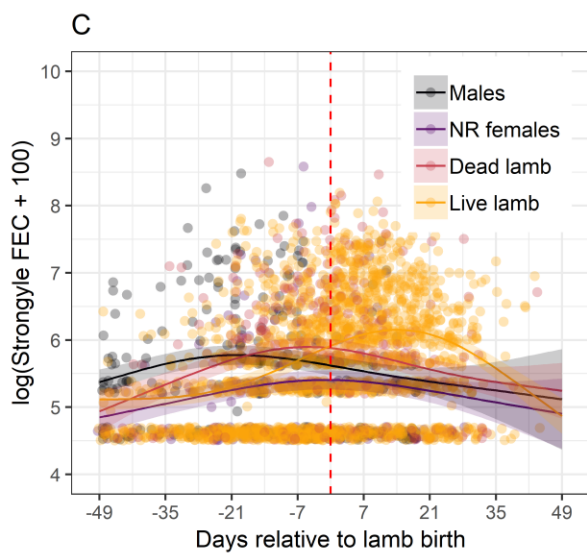
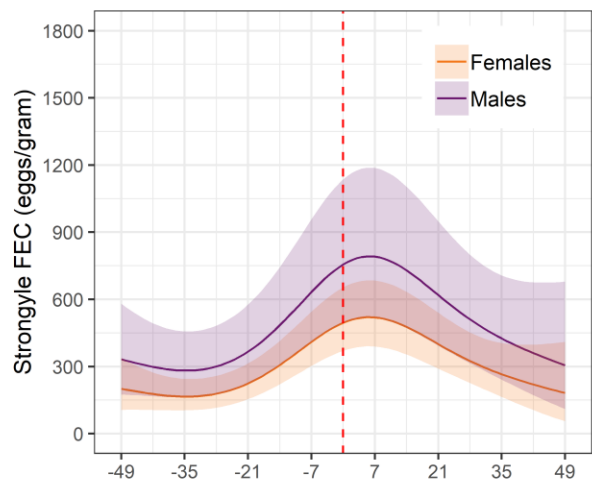
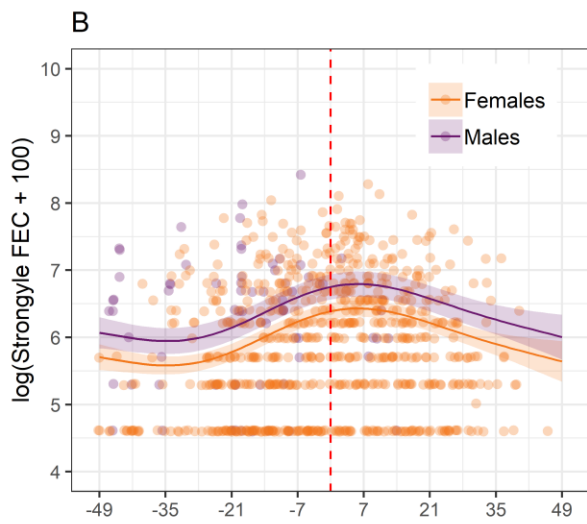
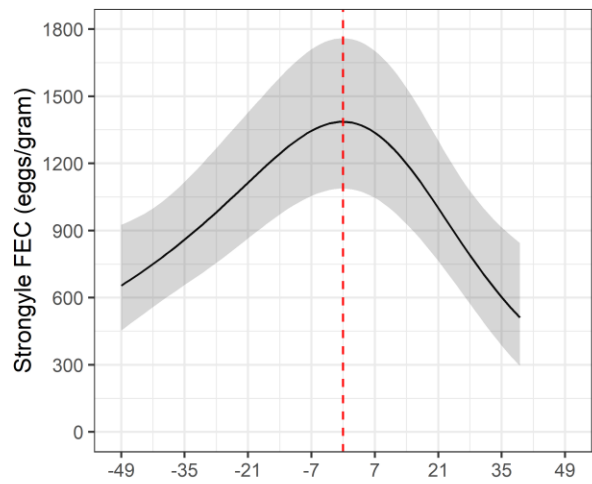
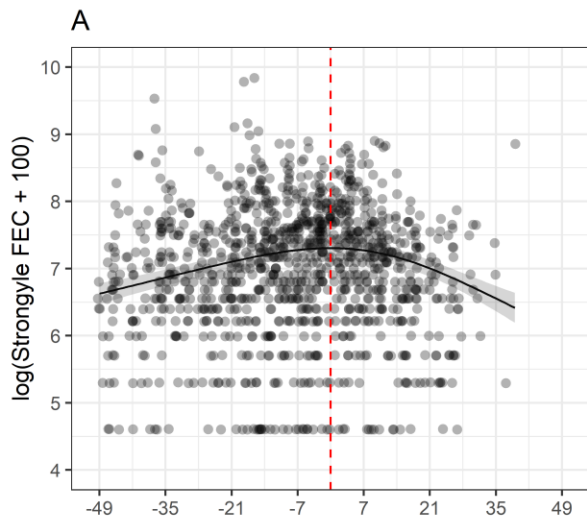
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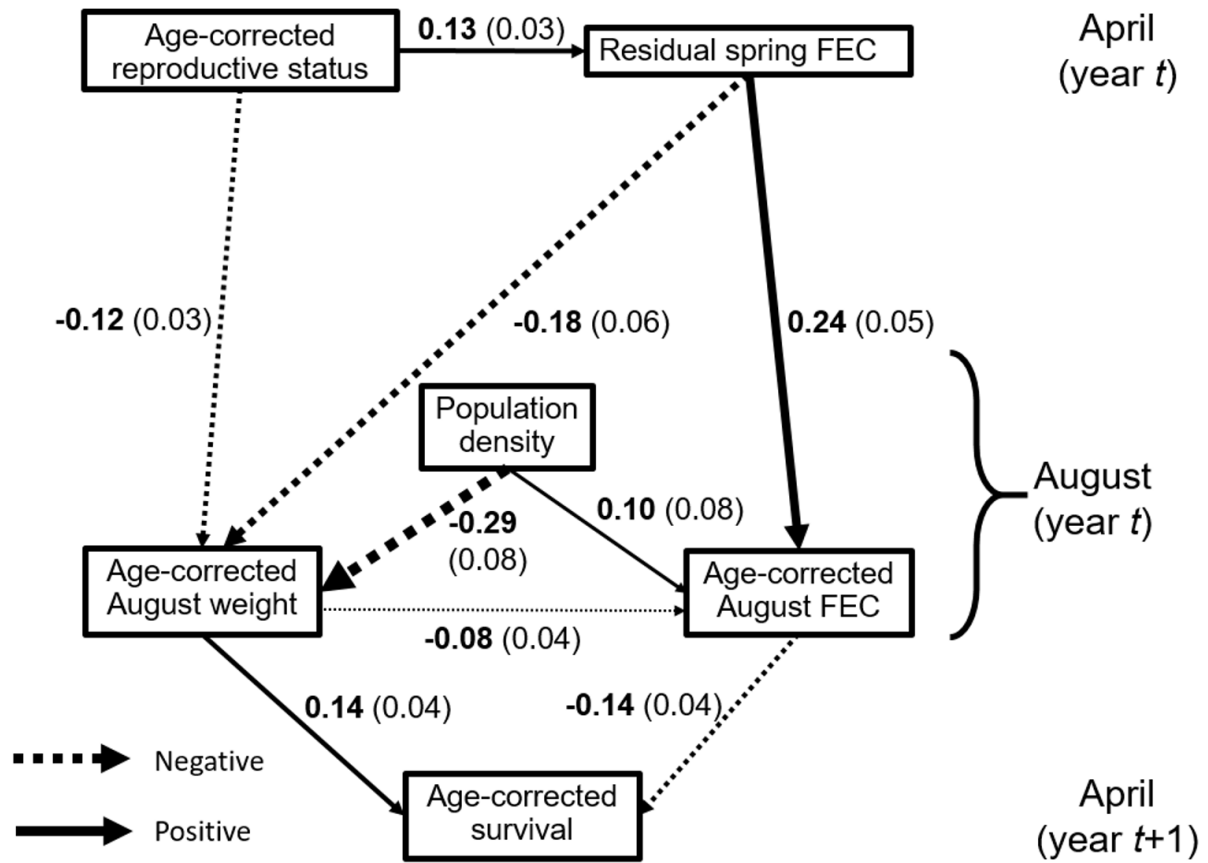
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