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# Survival costs of reproduction are mediated by parasite infection in wild Soay sheep

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#### 1 <u>Abstract</u>

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

#### 26 <u>Introduction</u>

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 hypothesis of parasite-mediated reproductive costs in an observational study we must also 42 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 mediate effects of reproduction on survival. However, detecting trade-offs in observational 46 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 exist. The costs of reproduction are also likely dependent on age and sex (e.g. Descamps *et al.*2009). In mammals, while there are costs of bearing offspring, the greatest costs are often
attributed to lactation (Clutton-Brock *et al.* 1989; Froy *et al.* 2016).

54

Long-term, individual-based studies in natural systems are critical to testing life-history theory, 55 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

effect on FEC and body weight. Our results support the notion that costs of reproduction,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 springs of 1989-2008 (1<sup>st</sup> March – 29<sup>th</sup> May) and FEC was quantified using a modified version 101 102 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

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

117

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

121

*Anthelminthic treatment:* This distinguishes individuals that received an anthelminthic bolus
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 associated with the change in FEC. GAMMs allowed us to fit non-parametric smoothing 136 137 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 'gamm4' package (Wood & Scheipl 2017).

141

We performed separate analyses to model changes in FEC for different age groups: juveniles (one year old in the spring of faecal sampling), yearlings (two years old), and adults (three or more years), because these groups are known to differ in FEC (Wilson *et al.* 2004). We analysed 1129 FECs collected from 381 juveniles, 761 from 208 yearlings, and 2536 from 446 adults (Table S1). We tested associations between reproductive allocation and FEC in each age group during the lambing season by grouping reproductive allocation in different ways based 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 anthelminthic 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 sheep system (Table 1). 172

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 Ln(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

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

201

*August FEC:* We estimated summer strongyle FEC from samples taken during August captures
in year *t*. As above, to improve model fit and adhere to assumptions, FEC+100 estimates were
natural log-transformed. We expected this to be positively associated with spring FEC and
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 1<sup>st</sup>; (0) died before May 1<sup>st</sup>.

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  $\geq 9$  grouped together; 221 Table S5) and anthelminthic 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 model with a binomial distribution and age and treatment fitted as explanatory variables.
Residuals were extracted to obtain age-corrected measures of reproductive status and survival.
Variables were then standardised by dividing by one standard deviation. Our results and
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 models using the R package 'lme4' (Bates et al. 2015). Year and individual identity were 230 231 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 <u>Results</u>

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

All age-sex groups showed a PPR in spring faecal egg count (FEC) (Fig. 2). Generally, this was characterised by a peak in FEC close to parturition (day 0). For juveniles, the best-fitting model grouped all individuals together ( $\Delta$ AIC relative to next best model = -2; Table S2; Table S6). Juveniles had consistently higher FEC than the other age groups, with a peak of 1386epg (eggs per gram; 95% CI = 1088–1759epg) on day 0 (Fig. 2A). There was marginal evidence suggesting that the best-fitting model for yearlings grouped individuals by sex ( $\Delta$ AIC = -1; evidence ratio = 0.63; Table S3; Table S7). Males had a higher peak spring FEC, 792epg (518–
1189epg) than females, 521epg (91–685epg; Fig. 2B). Both sexes' peak occurred on day 6.

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 ( $\Delta AIC = -4$ ; 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 ( $\triangle AIC = -8.5$ ). 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

of each variable (the products of standardised predictors along each path) on survival.

275

#### 276 Discussion

Using data collected from a wild population of Soay sheep, we found increased reproductive allocation was associated with greater gastrointestinal nematode faecal egg count (FEC) during the lambing season. Further, the association between reproduction and overwinter survival was mediated by effects of reproduction on spring and summer FEC and summer body weight. Overall, our work demonstrates how parasites can mediate associations between reproduction 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 =  $0.36\pm0.17$  SE,  $\Delta$ AIC = -1; evidence ratio = 0.63); this result is consistent with studies from diverse species that have 293 294 found males to have less effective immune responses (Tschirren et al. 2003; Hayward 2013; 295 Klein & Flanagan 2016; Watson et al. 2016).

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

Our results agree with brood manipulation studies on birds showing that increased allocation to reproduction is associated with greater parasite burdens and less effective immune responses (Nordling *et al.* 1998; Hanssen *et al.* 2005; Knowles *et al.* 2009). Our work also largely agrees 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 peak (Tempest 2005). Along with previous work, our findings suggest a more pronounced 326 327 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 the371 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 between reproduction and survival would have an estimate of 0.0053±0.04, but that this 378 379 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.

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In our *a priori* path diagram, weight is conceived as a potentially mediating link between reproduction and survival because we expect that costs of reproduction involve reduced storage of nutritional reserves. However, we also acknowledge that innate variation between 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.

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 fundamental, explaining variation in ageing, mating strategies, and sexual traits (Rowe & 430 431 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 (Clutton-Brock <i>et al.</i> 1992)	
Reproductive Status	Previous Density	Negative		
Spring FEC	Previous Density	Positive	(Gulland & Fox 1992)	
	Reproductive Status	Positive	(Wilson et al. 2004)	
August Weight	Reproductive Status	Negative	(Clutton-Brock et al. 1996)	
	Spring FEC	Negative	(Gulland 1992)	
	August Density	Negative	(Milner et al. 1999b)	
August FEC	Reproductive Status	Positive	(Wilson et al. 2004)	
	Spring FEC	Positive	(Coltman et al. 2001)	
	August Weight	Negative	(Coltman et al. 2001)	
	August Density	Positive	(Gulland & Fox 1992)	
Overwinter survival	August Weight	Positive	(Clutton-Brock et al. 1996)	
	August FEC	Negative	(Coltman et al. 1999)	
	August Density	Negative	(Clutton-Brock et al. 1991)	

**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 $\rightarrow$ August Weight $\rightarrow$ Survival	-0.0168
Reproductive Status $\rightarrow$ August Weight $\rightarrow$ August FEC $\rightarrow$ Survival	-0.0012
Reproductive Status $\rightarrow$ Spring FEC $\rightarrow$ August FEC $\rightarrow$ Survival	-0.0044
Reproductive Status $\rightarrow$ Spring FEC $\rightarrow$ August Weight $\rightarrow$ Survival	-0.0033
Reproductive Status $\rightarrow$ Spring FEC $\rightarrow$ August Weight $\rightarrow$ August FEC $\rightarrow$ Survival	-0.0002
Spring FEC $\rightarrow$ August Weight $\rightarrow$ Survival	-0.0252
Spring FEC $\rightarrow$ August Weight $\rightarrow$ August FEC $\rightarrow$ Survival	-0.0018
August Weight $\rightarrow$ Survival	0.1400
August Weight $\rightarrow$ August FEC $\rightarrow$ Survival	0.0100
August FEC $\rightarrow$ Survival	-0.1400
August Density $\rightarrow$ August FEC $\rightarrow$ Survival	-0.0140
August Density $\rightarrow$ August Weight $\rightarrow$ Survival	-0.0406
August Density $\rightarrow$ August Weight $\rightarrow$ August FEC $\rightarrow$ Survival	-0.0028

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

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

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