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Complete List of Authors:	Leivesley, Jessica; University of Stirling, Biological and Environmental Sciences Bussiere, Luc; University of Stirling, Biological and Environmental Sciences Pemberton, Josephine; University of Edinburgh, Institute of Evolutionary Biology Pilkington, Jill; University of Edinburgh, Institute of Evolutionary Biology Wilson, Kenneth; Lancaster University, Lancaster Environment Centre Hayward, Adam; Moredun Research Institute, Disease Control Division
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Survival costs of reproduction are mediated by parasite infection in wild Soay sheep

Jessica A. Leivesley[1,2], Luc F. Bussière[1], Josephine M. Pemberton[3], Jill G. Pilkington[3,4], Kenneth Wilson[5] & Adam D. Hayward[1,6]

1. Department of Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA, UK. Email: luc.bussiere@stir.ac.uk
2. Current address: Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, M5S 3B2, Canada. Email: jessica.leivesley@mail.utoronto.ca
3. Institute of Evolutionary Biology, University of Edinburgh, Ashworth Laboratories, Charlotte Auerbach Road, Edinburgh, EH9 3FL, UK. Email: j.pemberton@ed.ac.uk
4. School of Biology, University of St Andrews, Sir Harold Mitchell Building, St Andrews, KY16 9TF, UK. Email: jgp8@st-andrews.ac.uk
5. Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK. Email: ken.wilson@lancaster.ac.uk
6. Current address: Moredun Research Institute, Pentland Science Park, Penicuik, Midlothian, EH26 0PZ. Email: adam.hayward@moredun.ac.uk

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Corresponding author: Adam Hayward, Moredun Research Institute, Pentland Science Park, Penicuik, Midlothian, EH26 0PZ. Email: adam.hayward@moredun.ac.uk; Tel: +44 (0) 131 445 5111.

Abstract

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.

Introduction

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

Both observational and experimental studies have provided support for a trade-off between reproduction and parasite resistance (Festa-Bianchet 1989; Richner *et al.* 1995; Ardia *et al.* 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 demonstrate that reduced parasite resistance is associated with reduced future fitness. Experimental removal of parasites in wild populations has been shown to increase survival (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 studies can be difficult due to differences in resource acquisition between individuals (van Noordwijk & de Jong 1986). These differences may mean that some individuals have resources available to allocate to both reproduction and immunity, while others may struggle to allocate 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).

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

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

Material and methods

Study Population and Data Collection

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

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

Change in FEC across lambing season

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

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

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

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

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.

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 several occasions throughout the history of the study (Gulland 1992; Gulland *et al.* 1993; Boyd 1999; Wilson *et al.* 2003; Tempest 2005). The treatment was applied either in the August before lambing or in the spring of lambing and was considered a categorical variable: (0) no treatment before lambing; (1) treatment before lambing. We included treatment in our models to account for possible effects of treatment on FEC in our correlative study. The bolus releases anthelmintic for several weeks; to be conservative about any long-lasting effects of treatment, we considered animals given a bolus in either August or April as treated before lambing. Exclusion of treated animals from our analysis did not substantially influence our conclusions.

FEC+100 was natural log-transformed before analysis to adhere to assumptions of residual normality. Generalised additive mixed-effects models (GAMMs) were used to determine how 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 functions to FEC without being restricted to a specific polynomial form. Year and individual identity were included in all models as random effects since multiple faecal samples were taken from individuals within and across years. Analyses were performed in R 3.5.0 (R Core Team 2018) using the 'gamm4' package (Wood & Scheipl 2017).

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

Survival costs of reproduction

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

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

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

Population Density: We considered the effect of population density in the August before reproduction (year *t*-1) on reproductive status, since high density is associated with lower fecundity (Clutton-Brock *et al.* 1991). We also considered the effect of density in year *t* on 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).

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).

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

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

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

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

Results

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 (ΔAIC 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 (ΔAIC = -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.

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

Survival costs of reproduction

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

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.

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.

Reproductive allocation influences FEC

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

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

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 used. Previous work found that FEC during the lambing season varied with age and reproductive status: young animals that failed to reproduce experienced a PPR while non-reproducing adult females did not. Further, reproducing females that successfully weaned a 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 difference between individuals based on reproductive status in older animals, and a more general PPR in younger animals. Our study has, however, extended previous work by investigating not only the effects of sex and reproduction on FEC, but also the effects of different aspects of reproduction.

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

Reproduction is negatively associated with survival

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

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

A major advantage of using SEMs is that if there were other important mediating factors between reproduction and survival, Shipley's test would indicate a missing path between reproduction and survival as missing paths can not only indicate a direct association between variables, but may also reflect all direct and indirect pathways not otherwise modelled (Shipley 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 pathway was not statistically supported. In our case, the direct and indirect paths are of the same order of magnitude, which is difficult to interpret considering the direct effect was not statistically supported. Considering this, the most conservative interpretation of our SEM is that at least as much variation in survival is explained by the mediating influence of FEC as is explained by all other direct and indirect factors flowing from reproduction combined. However, as the missing direct association was not detected, we are confident that there are no important missing mediators between reproduction and survival. Therefore, the important mediating factors of survival costs of reproduction in this population appear to be FEC and body weight, or their close correlates.

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

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

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Figure Legends

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.

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

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.

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 *et al.* 1992)
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 → 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

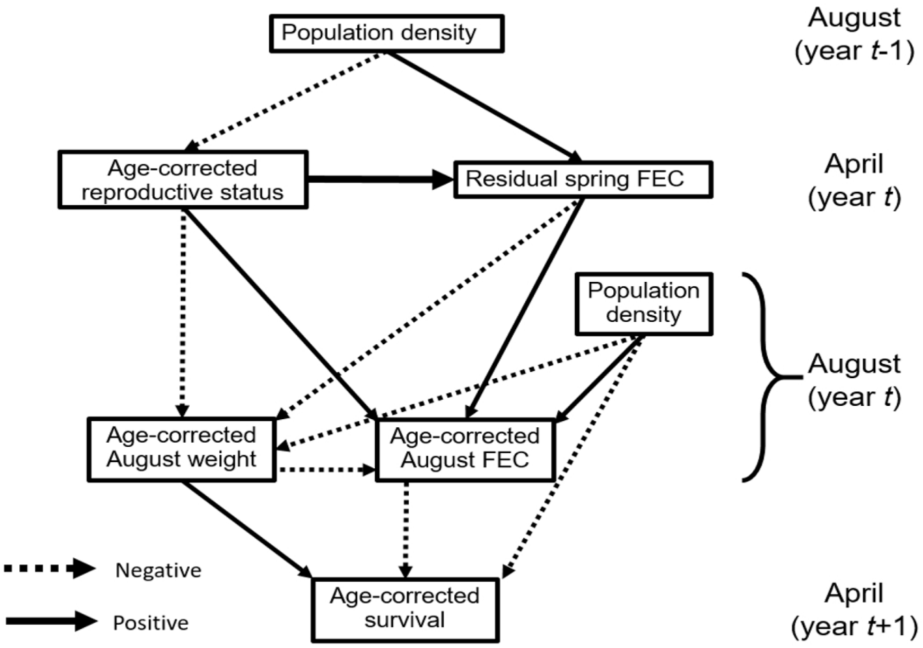

Figure 1. A priori structural equation model (SEM) based on decades of research on the Soay sheep population. Associations are depicted for female sheep only. Variables are temporally separated over two years. Reproductive status is based on whether or not the female gave birth in spring.

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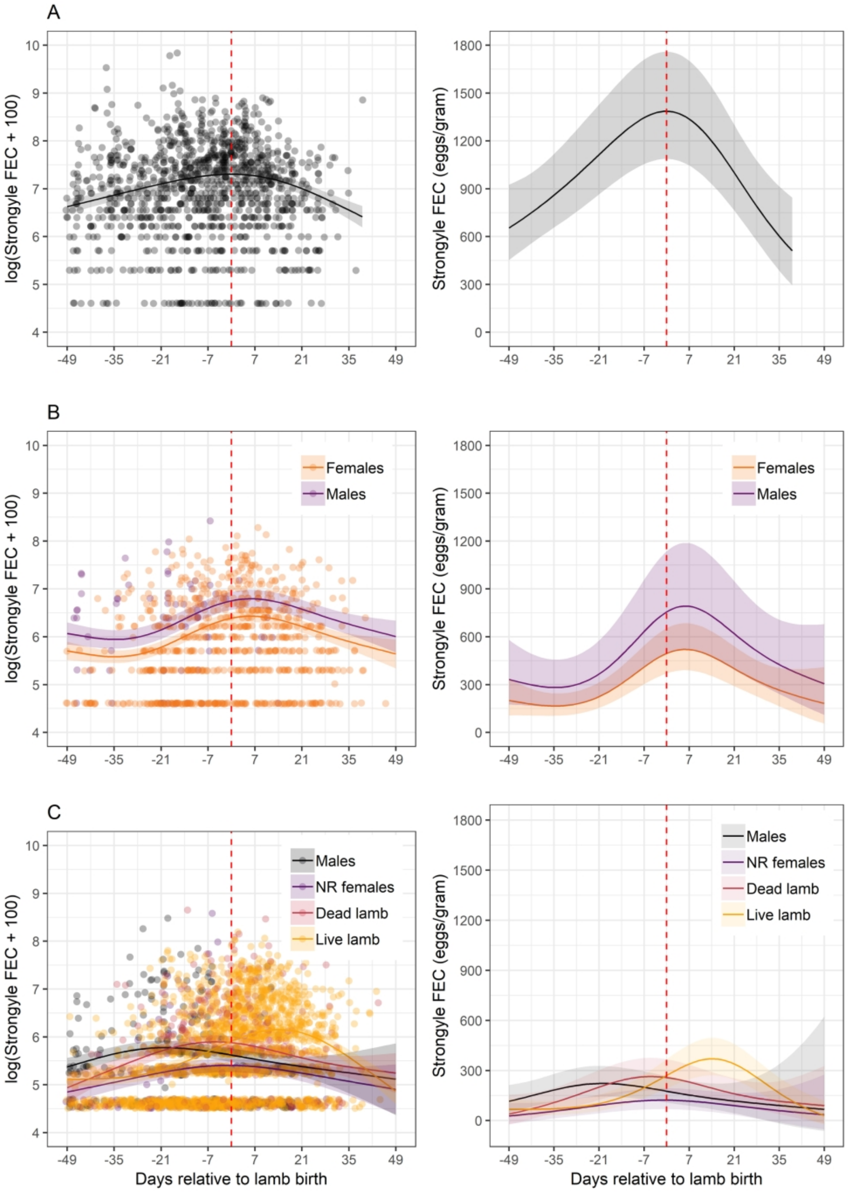

Figure 2. Comparison of change in strongyle FEC over a 99-day period during the lambing season, analysed using generalised additive mixed-effects models (GAMMs; Tables S2-S4). (A) The best-fitting model for juveniles grouped all individuals; (B) the best-fitting model for yearlings grouped individuals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

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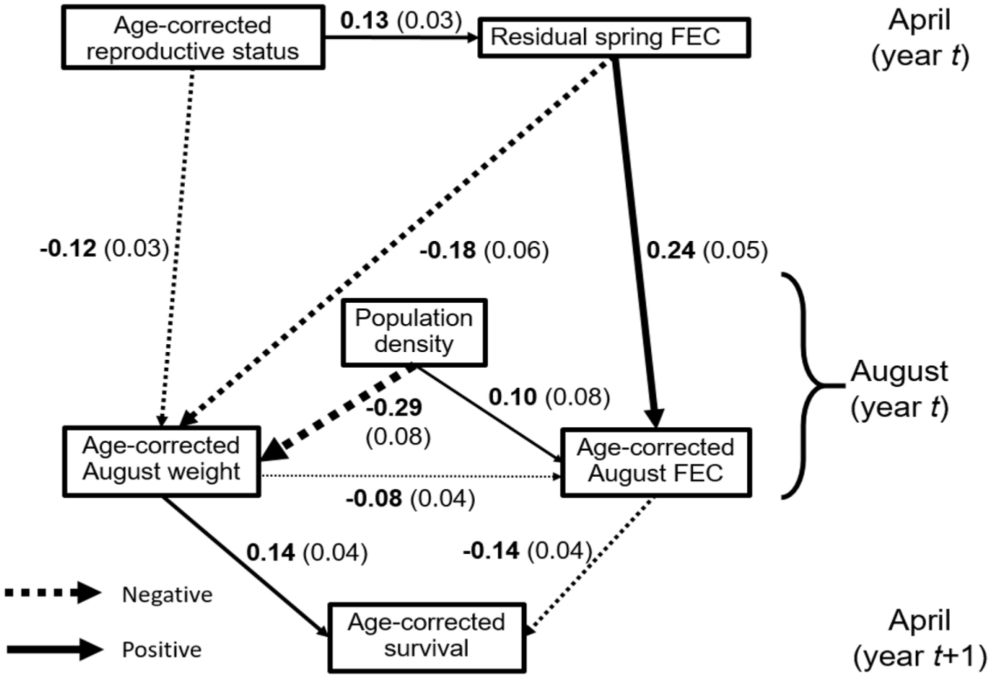

Figure 3. Our final structural equation model (SEM) showing downstream impacts of reproduction on future fitness (overwinter survival). Values on arrows and arrow widths indicate standardised path coefficients with standard errors in parentheses. All variables were standardised by dividing by one standard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

258x193mm (150 x 142 DPI)