



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

Journal:	<i>Ecology Letters</i>
Manuscript ID	ELE-00215-2019.R1
Manuscript Type:	Letters
Date Submitted by the Author:	08-Apr-2019
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
Key Words:	Life history, Infection, Immunity, Trade-offs, Fitness, Peri-parturient rise, Costs of reproduction
<p>Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.</p> <p>2019-04-12 Leivesley et al final documents.zip</p>	

Survival costs of reproduction are mediated by parasite infection in wild

Soay sheep

Jessica A. Leivesley^{1,2}, Luc F. Bussière¹, Josephine M. Pemberton³, Jill G. Pilkington^{3,4},
Kenneth Wilson⁵ & 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

Statement of authorship: JAL and ADH designed the study; JAL performed all statistical analyses after discussion with ADH and LFB; JGP and KW made significant contributions to collection of parasitology data across the history of the Soay sheep project, which is directed by JMP; JAL wrote the first draft of the manuscript and LFB, JMP, KW and ADH contributed substantially to revisions.

Data accessibility statement: Should the manuscript be accepted for publication, the data supporting the results will be archived in the Dryad data repository under a DOI, which will be included at the end of the article.

Running title: Parasite-mediated reproductive costs

1
2
3
4
5 **Keywords:** Life history; infection; immunity; trade-offs; fitness; peri-parturient rise; costs of
6 reproduction
7

8
9 **Type of article:** Letters
10

11 **Word Count:** Abstract = 150 Main text = 4999
12

13
14 **References:** 82

15 **Figures:** 3

16 **Tables:** 2

17 **Text boxes:** 0
18
19
20

21
22
23 **Corresponding author:** Adam Hayward, Moredun Research Institute, Pentland Science
24

25 Park, Penicuik, Midlothian, EH26 0PZ. Email: adam.hayward@moredun.ac.uk; Tel: +44 (0)
26

27 131 445 5111.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 1 Abstract
4

5 2 A trade-off between current and future fitness potentially explains variation in life-history
6 strategies. A proposed mechanism behind this is parasite-mediated reproductive costs:
7 individuals that allocate more resources to reproduction have fewer to allocate to defence
8 against parasites, reducing future fitness. We examined how reproduction influenced faecal
9 egg counts (FEC) of strongyle nematodes using data collected between 1989-2008 from a wild
10 population of Soay sheep in the St. Kilda archipelago, Scotland (741 individuals). Increased
11 reproduction was associated with increased FEC during the lambing season: females that gave
12 birth, and particularly those that weaned a lamb, had higher FEC than females that failed to
13 reproduce. Structural equation modelling revealed future reproductive costs: a positive effect
14 of reproduction on spring FEC and a negative effect on summer body weight were negatively
15 associated with overwinter survival. Overall, we provide evidence that parasite resistance and
16 body weight are important mediators of survival costs of reproduction.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3 51 exist. The costs of reproduction are also likely dependent on age and sex (e.g. Descamps *et al.*
4
5 52 2009). In mammals, while there are costs of bearing offspring, the greatest costs are often
6
7 53 attributed to lactation (Clutton-Brock *et al.* 1989; Froy *et al.* 2016).
8
9

10 54
11
12 55 Long-term, individual-based studies in natural systems are critical to testing life-history theory,
13
14 56 but are rare as they are difficult to maintain (Lindenmayer *et al.* 2012). Here, we quantified the
15
16 57 pathways linking reproduction, parasite egg counts, body weight, and survival using 19 years
17
18 58 of data collected from a wild population of Soay sheep (*Ovis aries*). Many parasite species are
19
20 59 present, but gastrointestinal strongyle nematodes have the greatest effect on health and
21
22 60 mortality of any parasite in the population and are highly prevalent (Gulland 1992; Gulland &
23
24 61 Fox 1992). Strongyles have a direct life cycle: adults live up to 60 days in the host (Armour *et*
25
26 62 *al.* 1966), producing eggs that are shed in faeces; eggs hatch and moult to L3 larvae that are
27
28 63 ingested by sheep during grazing. Infection intensity of strongyles is quantified with faecal egg
29
30 64 count (FEC), which is strongly positively correlated with worm burden (Wilson *et al.* 2004).
31
32
33
34
35
36

37 65
38 66 Survival of female Soay sheep reproducing in spring (mean survival = 90.8%) is lower than
39
40 67 non-reproducing females (95.5%) over the subsequent winter (Clutton-Brock *et al.* 1996;
41
42 68 Tavecchia *et al.* 2005). This difference is more pronounced in years of harsh winters (Clutton-
43
44 69 Brock *et al.* 1996). Two observations suggest that parasites could mediate this reproduction-
45
46 70 survival association. First, a rise in FEC occurs during late pregnancy and early lactation in this
47
48 71 population (Tempest 2005), a phenomenon known as the peri-parturient rise (Lloyd 1983;
49
50 72 Fthenakis *et al.* 2015). Second, higher FEC and lower body weight are associated with lower
51
52 73 overwinter survival (Clutton-Brock *et al.* 1992; Gulland 1992; Hayward *et al.* 2011, 2018). We
53
54 74 therefore evaluated whether allocation to reproduction incurred survival costs mediated by its
55
56
57
58
59
60

1
2
3 75 effect on FEC and body weight. Our results support the notion that costs of reproduction,
4
5 76 manifested as reduced survival, are mediated by both strongyle FEC and body weight.
6
7
8 77

9
10 78 Material and methods

11
12 79 **Study Population and Data Collection**

13
14 80 The St. Kilda archipelago (54°49'08"34"W) lies 65km west of the Outer Hebrides, Scotland,
15
16 81 and consists of four islands: Hirta, Soay, Boreray, and Dun. A population of unmanaged Soay
17
18 82 sheep, descendants of primitive European domestic sheep that were introduced to the island of
19
20 83 Soay several thousand years ago (Clutton-Brock & Pemberton 2004), inhabit the island of
21
22 84 Hirta. Population dynamics on Hirta are characterised by periods of growth followed by large
23
24 85 declines in population size due to adverse winter weather, poor food availability, and parasite
25
26 86 infections, which combine to reduce body weight and increase mortality (Gulland 1992;
27
28 87 Coulson *et al.* 2001; Craig *et al.* 2006).
29
30
31
32
33 88

34
35 89 A longitudinal individual-based study on the sheep in the Village Bay area of Hirta began in
36
37 90 1985 and continues to the present (Clutton-Brock & Pemberton 2004). The population is
38
39 91 monitored daily during the lambing season in March-May. >95% of lambs are caught within a
40
41 92 week of birth and are given an identification tag, weighed, and have blood and tissue samples
42
43 93 taken. Throughout the history of the study, faecal samples have been collected from adults
44
45 94 during lambing; often, the same individuals are repeatedly sampled within a year. Lambs suckle
46
47 95 throughout the spring and are weaned by August. In August, around 50% of the population are
48
49 96 captured to collect a variety of measures including body weight and faecal samples.
50
51
52
53
54 97

55
56 98 **Change in FEC across lambing season**
57
58
59
60

1
2
3 99 We first determined how differences in reproductive allocation were associated with the PPR
4
5 100 in strongyle faecal egg count (FEC). Faecal samples were collected from both sexes across the
6
7 101 springs of 1989-2008 (1st March – 29th May) and FEC was quantified using a modified version
8
9 102 of the McMaster technique (Craig *et al.* 2006). We constructed models assessing changes in
10
11 103 FEC during the lambing season including the following predictors.
12
13
14
15 104

16
17 105 *Relative date:* The date on which FEC samples were taken was standardised relative to the date
18
19 106 of lamb birth (lamb birth = day 0). Relative dates for non-reproducing females and males were
20
21 107 based on the average lambing date for a given year. We analysed FEC data restricted to 49 days
22
23 108 either side of lamb birth (relative date = -49 to +49). Our results and conclusions were
24
25 109 unchanged if we also used year-specific mean lambing dates to calculate relative date for
26
27 110 reproducing females, and if we used absolute date of sample collection for all individuals.
28
29
30
31 111

32
33 112 *Reproductive status:* Each year, females were divided into two categories: (0) did not
34
35 113 reproduce; (1) produced at least one lamb.
36
37
38 114

39
40 115 *Litter size:* A categorical variable in reproducing females each year: (1) a single lamb; (2) twin
41
42 116 lambs.
43
44
45 117

46
47 118 *Litter survival:* A categorical variable measured at the end of each August in reproducing
48
49 119 females: (1) both twins alive; (2) one twin alive; (3) both twins dead; (4) a singleton alive; (5)
50
51 120 a singleton dead.
52
53
54 121

55
56 122 *Anthelmintic treatment:* This distinguishes individuals that received an anthelmintic bolus
57
58 123 or drench as part of experimental treatments to remove parasites, which have been applied on
59
60

1
2
3 124 several occasions throughout the history of the study (Gulland 1992; Gulland *et al.* 1993; Boyd
4
5 125 1999; Wilson *et al.* 2003; Tempest 2005). The treatment was applied either in the August before
6
7 126 lambing or in the spring of lambing and was considered a categorical variable: (0) no treatment
8
9 127 before lambing; (1) treatment before lambing. We included treatment in our models to account
10
11 128 for possible effects of treatment on FEC in our correlative study. The bolus releases
12
13 129 anthelmintic for several weeks; to be conservative about any long-lasting effects of treatment,
14
15 130 we considered animals given a bolus in either August or April as treated before lambing.
16
17 131 Exclusion of treated animals from our analysis did not substantially influence our conclusions.
18
19 132
20
21
22
23 133 FEC+100 was natural log-transformed before analysis to adhere to assumptions of residual
24
25 134 normality. Generalised additive mixed-effects models (GAMMs) were used to determine how
26
27 135 FEC changed during the lambing season and to determine how reproductive allocation was
28
29 136 associated with the change in FEC. GAMMs allowed us to fit non-parametric smoothing
30
31 137 functions to FEC without being restricted to a specific polynomial form. Year and individual
32
33 138 identity were included in all models as random effects since multiple faecal samples were taken
34
35 139 from individuals within and across years. Analyses were performed in R 3.5.0 (R Core Team
36
37 140 2018) using the ‘*gam4*’ package (Wood & Scheipl 2017).
38
39 141
40
41
42
43 142 We performed separate analyses to model changes in FEC for different age groups: juveniles
44
45 143 (one year old in the spring of faecal sampling), yearlings (two years old), and adults (three or
46
47 144 more years), because these groups are known to differ in FEC (Wilson *et al.* 2004). We
48
49 145 analysed 1129 FECs collected from 381 juveniles, 761 from 208 yearlings, and 2536 from 446
50
51 146 adults (Table S1). We tested associations between reproductive allocation and FEC in each age
52
53 147 group during the lambing season by grouping reproductive allocation in different ways based
54
55 148 on sex, reproductive status, litter size (adults only), and litter survival; the full list of models
56
57
58
59
60

1
2
3 149 and groupings for each age class is shown in Tables S2-S4. Three models were compared for
4
5 150 each grouping where: (1) the intercept of FEC varied between the groups, but the change in
6
7 151 FEC across the season was consistent across groups; (2) the intercept of FEC was the same in
8
9 152 all groups, but the change in FEC across the season varied between groups; (3) both the
10
11 153 intercept of FEC and the change in FEC across the season varied between groups. In each
12
13 154 model, the anthelmintic treatment status of individuals was included as a fixed effect. All
14
15 155 models were compared using AIC values with the best-fitting model having the lowest AIC
16
17 156 value (Burnham & Anderson 2002).
18
19
20
21
22 157

23 24 158 **Survival costs of reproduction**

25
26 159 We found that increased reproductive allocation in females was associated with higher FEC
27
28 160 during the spring (see Results). We next investigated how reproduction influenced female
29
30 161 survival, incorporating extensive prior knowledge about this system. Structural equation
31
32 162 models (SEMs) enabled us to test our *a priori* expectation that reproductive allocation has
33
34 163 effects on survival that are at least partly mediated by effects of reproduction on spring and
35
36 164 summer FEC and summer body weight. SEMs are well suited to testing the parasite-mediated
37
38 165 costs of reproduction because they specifically quantify the degree to which the relationship
39
40 166 between two variables is mediated by a third. SEMs were constructed using 601 records
41
42 167 collected from 325 females of all ages (many females are represented in more than one year),
43
44 168 including the following set of variables. All variables in the SEM were corrected for
45
46 169 anthelmintic treatment and age (see below). As recommended when evaluating SEMs (Grace
47
48 170 *et al.* 2014), our *a priori* path diagram (Fig. 1) is based on evidence for causal relationships
49
50 171 that might link reproduction and survival and known associations between traits in the Soay
51
52 172 sheep system (Table 1).
53
54
55
56
57
58
59
60

1
2
3 174 *Residual Spring FEC:* Our GAMM analysis (see Results) was made possible by having
4
5 175 longitudinal FEC measures from the same individuals within years. However, for our SEMs,
6
7 176 we needed a single value of FEC per individual per year to pass to the model. Thus, we
8
9 177 extracted random effect estimates from a GAMM of $\text{Ln}(\text{FEC}+100)$ and included age (as a
10
11 178 three-level categorical variable: juvenile, yearling, adult), treatment, and relative date (as a
12
13 179 smoothed term) as explanatory variables. We summed the overall intercept, individual ID, and
14
15 180 year effect estimates to obtain a year-specific FEC value for every individual (year t),
16
17 181 accounting for variation in age, date, and treatment (i.e. the value represents the expected FEC
18
19 182 at day 0). This value was predicted to be positively associated with August FEC and negatively
20
21 183 associated with August body weight in year t (Gulland 1992).
22
23
24
25
26
27

28 185 *Reproductive status:* Following our characterisation of the PPR in Soay sheep, females were
29
30 186 grouped into two categories based on reproductive allocation: individuals either reproduced in
31
32 187 year t ($n=490$) or did not ($n=111$). Of the reproducing female records, there were 89 records
33
34 188 where no lambs survived. We chose to group females in this way, rather than based on lamb
35
36 189 survival, since there was clear evidence for a different PPR in reproducing versus non-
37
38 190 reproducing females (see Results). We predicted that reproductive status would be positively
39
40 191 associated with residual spring FEC and negatively associated with August weight, since body
41
42 192 weight is expected to reflect the allocation trade-off between reproduction and somatic
43
44 193 maintenance (Clutton-Brock *et al.* 1996).
45
46
47
48

49 194
50
51 195 *Population Density:* We considered the effect of population density in the August before
52
53 196 reproduction (year $t-1$) on reproductive status, since high density is associated with lower
54
55 197 fecundity (Clutton-Brock *et al.* 1991). We also considered the effect of density in year t on
56
57 198 summer weight and FEC, and survival, since high density is associated with higher FEC
58
59
60

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

1
2
3 224 model with a binomial distribution and age and treatment fitted as explanatory variables.
4
5 225 Residuals were extracted to obtain age-corrected measures of reproductive status and survival.
6
7 226 Variables were then standardised by dividing by one standard deviation. Our results and
8
9 conclusions were unaffected by the exclusion of treated animals from the analysis.
10
11
12 228
13
14 229 We first explored each pathway in our SEM using separate (generalised) linear mixed-effects
15
16 230 models using the R package ‘lme4’ (Bates *et al.* 2015). Year and individual identity were
17
18 231 included in each model as random effects except for spring FEC, where only year was included
19
20 232 as a random effect since between-individual effects were negligible. We then conducted formal
21
22 233 ‘piecewise’ structural equation modelling to join the multiple models into a single SEM
23
24 234 (Shipley 2009), using the R package ‘piecewiseSEM’ (Lefcheck 2015). Shipley’s test of d-
25
26 235 separation was used to assess the overall fit of the model and to determine whether any paths
27
28 236 were missing (Shipley 2009); missing paths were added into the model (Grace *et al.* 2015).
29
30 237 Non-supported paths were removed from the SEM (based on AIC comparison) to improve
31
32 238 parameter estimation of the remaining paths. After removal of non-supported paths, the model
33
34 239 could not be improved by adding or removing any path.
35
36
37
38
39
40
41

42 241 Results

43 242 **Characterisation of the peri-parturient rise (PPR)**

44
45 243 All age-sex groups showed a PPR in spring faecal egg count (FEC) (Fig. 2). Generally, this
46
47 244 was characterised by a peak in FEC close to parturition (day 0). For juveniles, the best-fitting
48
49 245 model grouped all individuals together (Δ AIC relative to next best model = -2; Table S2; Table
50
51 246 S6). Juveniles had consistently higher FEC than the other age groups, with a peak of 1386epg
52
53 247 (eggs per gram; 95% CI = 1088–1759epg) on day 0 (Fig. 2A). There was marginal evidence
54
55 248 suggesting that the best-fitting model for yearlings grouped individuals by sex (Δ AIC = -1;
56
57
58
59
60

1
2
3 249 evidence ratio = 0.63; Table S3; Table S7). Males had a higher peak spring FEC, 792epg (518–
4
5 250 1189epg) than females, 521epg (91–685epg; Fig. 2B). Both sexes' peak occurred on day 6.
6
7

8 251
9
10 252 For adults, the best-fitting model grouped individuals based on litter survival in August: (1)
11
12 253 males; (2) non-reproducing females; (3) reproducing females with no surviving lambs; (4)
13
14 254 reproducing females with at least one surviving lamb ($\Delta\text{AIC} = -4$; Table S4; Table S8). Females
15
16 255 with at least one surviving lamb had a higher and later peak FEC than those that reproduced
17
18 256 but whose lambs died before weaning, but both had much higher peak than non-reproducing
19
20 257 females. The peak FEC of females with at least one surviving lamb was 370epg (270–495epg)
21
22 258 on day 15, whereas the peak FEC of females whose lambs died was 264epg (178–376epg) on
23
24 259 day -5 (Fig. 2C). Non-reproducing females had the lowest spring FEC, peaking at 123epg (69–
25
26 260 194epg) on day -1 (Fig. 2C). Males had an intermediate peak FEC, 222epg (143–327epg)
27
28 261 which occurred on day -20 (Fig. 2C).
29
30
31
32

33 262

35 263 **Survival costs of reproduction**

37 264 During the model specification stage, population density in the previous August was dropped
38
39 265 from the SEM as its inclusion did not improve the models. The SEM of the minimal adequate
40
41 266 models did not support two paths included in our *a priori* model: reproductive status \rightarrow August
42
43 267 FEC and population density \rightarrow survival ($\Delta\text{AIC} = -8.5$). The final SEM adequately fitted the
44
45 268 data (i.e., there were no missing paths; *Fisher's C* = 8.59; *P* = 0.57; Fig. 3). Reproductive status
46
47 269 had effects on survival mediated by residual spring FEC, August weight, and August FEC.
48
49 270 Pathways linking residual spring FEC and overwinter survival were mediated by effects of
50
51 271 residual spring FEC on August weight and August FEC. Weight and FEC in August were also
52
53 272 linked to survival. We also found effects of August density (year *t*) on August weight and
54
55
56
57
58
59
60

1
2
3 273 August FEC, both of which influenced age-corrected survival. Table 2 shows the total influence
4
5 274 of each variable (the products of standardised predictors along each path) on survival.
6
7
8 275

9
10 276 Discussion
11

12 277 Using data collected from a wild population of Soay sheep, we found increased reproductive
13
14 278 allocation was associated with greater gastrointestinal nematode faecal egg count (FEC) during
15
16
17 279 the lambing season. Further, the association between reproduction and overwinter survival was
18
19 280 mediated by effects of reproduction on spring and summer FEC and summer body weight.
20
21 281 Overall, our work demonstrates how parasites can mediate associations between reproduction
22
23
24 282 and future survival.
25
26 283

27
28 284 **Reproductive allocation influences FEC**
29

30 285 We found the effects of sex and/or reproductive allocation on spring FEC became more
31
32 286 apparent in adults compared to juveniles and yearlings: juvenile FEC was not influenced by
33
34 287 sex or reproductive status; there was marginal evidence that yearling FEC was influenced by
35
36 288 sex; and adult FEC was influenced by both. Juveniles had generally high FEC, as predicted
37
38 289 from previous research on other wild vertebrates (Wilson *et al.* 1996; Isomursu *et al.* 2006;
39
40 290 Hayward 2013; Watson *et al.* 2016) and humans (Simon *et al.* 2015), which suggests that
41
42 291 juveniles have not developed fully effective immune defences. Yearling males tended to have
43
44 292 higher spring FEC than females (marginal statistical support; estimate = 0.36 ± 0.17 SE, Δ AIC
45
46 293 = -1; evidence ratio = 0.63); this result is consistent with studies from diverse species that have
47
48 294 found males to have less effective immune responses (Tschirren *et al.* 2003; Hayward 2013;
49
50 295 Klein & Flanagan 2016; Watson *et al.* 2016).
51
52
53
54
55

56 296
57
58
59
60

1
2
3 297 The best-supported model for adults grouped animals into four categories: males, non-
4
5 298 reproducing females, reproducing females with no surviving lambs, and reproducing females
6
7 299 with surviving lambs. The different patterns of FEC between females with surviving or no
8
9 300 surviving lambs suggest that lactation may play a role in these differences (Fig. 2C). Lactation
10
11 301 uses resources that could otherwise be allocated to immunity (Coop & Kyriazakis 1999) and
12
13 302 studies on red deer (*Cervus elaphus*) suggest that lactation is more expensive than gestation
14
15 303 (Clutton-Brock *et al.* 1989; Froy *et al.* 2016). Moreover, experimental studies on domestic
16
17 304 sheep have shown that provisioning protein during lactation reduces FEC (Houdijk *et al.* 2003).
18
19 305 The number of surviving offspring that a female had did not influence the trajectory of FEC
20
21 306 changes, which could be because peak lactation is fixed (Johnson *et al.* 2001), or because only
22
23 307 females with the greatest resource pool produce twins (Cassinello & Gomendio 1996; Hewison
24
25 308 & Gaillard 2001). Another possibility is that high FEC just before lamb birth may cause ewes
26
27 309 to lose their lamb soon after birth and thus experience a decrease in FEC thereafter, while ewes
28
29 310 that keep their lamb have their FEC continue to rise (Fig. 2C). There is evidence that dairy
30
31 311 sheep treated with anthelmintic produce less milk (Fthenakis *et al.* 2005), but a link between
32
33 312 worm infections and lamb survival has not been demonstrated explicitly (Fthenakis *et al.* 2015).
34
35 313 A rise in FEC across the season was also seen in males. This is likely due to males being in
36
37 314 poor condition following the winter (Gulland & Fox 1992), the weaker antibody responses
38
39 315 males exhibit (Hayward *et al.* 2014; Watson *et al.* 2016), and the re-emergence of larvae from
40
41 316 arrested development in spring (Langrová *et al.* 2008).
42
43
44
45
46
47
48

317

49
50
51
52 318 Our results agree with brood manipulation studies on birds showing that increased allocation
53
54 319 to reproduction is associated with greater parasite burdens and less effective immune responses
55
56 320 (Nordling *et al.* 1998; Hanssen *et al.* 2005; Knowles *et al.* 2009). Our work also largely agrees
57
58 321 with previous work on the Soay sheep population, despite differences in the data and analyses
59
60

1
2
3 322 used. Previous work found that FEC during the lambing season varied with age and
4
5 323 reproductive status: young animals that failed to reproduce experienced a PPR while non-
6
7 324 reproducing adult females did not. Further, reproducing females that successfully weaned a
8
9 325 lamb experienced a peak in FEC after lamb birth, while those that lost their lamb had an earlier
10
11 326 peak (Tempest 2005). Along with previous work, our findings suggest a more pronounced
12
13 327 difference between individuals based on reproductive status in older animals, and a more
14
15 328 general PPR in younger animals. Our study has, however, extended previous work by
16
17 329 investigating not only the effects of sex and reproduction on FEC, but also the effects of
18
19 330 different aspects of reproduction.
20
21
22
23
24
25 331

26
27
28 332 It is possible that our results could reflect variation in exposure between groups rather than
29
30 333 reflecting a trade-off with reproduction. There is known heterogeneity in larval distribution
31
32 334 around Village Bay (Wilson *et al.* 2004), and there may be differences in feeding rates between
33
34 335 the reproductive groups of females, resulting in differences in exposure to larval parasites and
35
36 336 influencing the patterns of FEC seen here. However, it has been shown that Soays actively
37
38 337 avoid grazing more contaminated areas in spring and that males, barren females, and females
39
40 338 with lambs all avoid parasite-rich vegetation to a similar degree (Hutchings *et al.* 2002).
41
42 339 Moreover, experimental work in domestic sheep shows that greater infective doses (i.e.
43
44 340 increased exposure) do not result in a greater PPR (Kidane *et al.* 2009). Finally, new work on
45
46 341 the Soay sheep has shown that increased reproductive effort is associated with reduced
47
48 342 strongyle-specific antibody responses (Hayward *et al.* 2019). Therefore, it appears unlikely that
49
50 343 variation in exposure is the main driver of the variation seen in the PPR in this study.
51
52
53
54
55
56 344

57
58
59 345 **Reproduction is negatively associated with survival**
60

1
2
3 346 As predicted by our hypothesis of parasite-mediated reproductive costs, structural equation
4
5 347 models (SEMs) revealed that reproducing females had reduced survival, mediated by spring
6
7 348 and summer FEC and summer weight (Fig. 3). These pathways are consistent with a trade-off
8
9
10 349 between reproductive allocation during spring and overwinter survival, mediated by parasite
11
12 350 resistance and body weight. While previous studies in wild populations suggest that
13
14 351 reproduction incurs survival (Stearns 1992; Clutton-Brock *et al.* 1996; Hodges *et al.* 2015) and
15
16 352 immunity costs (Festa-Bianchet 1989; Richner *et al.* 1995; Nordling *et al.* 1998; Knowles *et*
17
18 353 *al.* 2009; Graham *et al.* 2010; Hayward *et al.* 2014), and that parasites influence survival
19
20 354 (Hanssen *et al.* 2005; la Puente *et al.* 2010; Hayward *et al.* 2011), we have explicitly quantified
21
22 355 the pathways through which reproduction influences survival, via associations between
23
24 356 reproduction, FEC and body weight (Fig. 3).
25
26
27
28
29
30
31
32

33 358 Spring FEC was positively associated with August FEC (Fig. 3). This could arise via three non-
34
35 359 mutually exclusive mechanisms: effects of reproduction on FEC persisted across several
36
37 360 months; reproduction and infection in spring lead to reduced parasite resistance in summer;
38
39 361 and/or FEC is repeatable across seasons (Coltman *et al.* 2001). Increased August FEC was
40
41 362 linked to reduced overwinter survival, as expected from previous work in this population
42
43 363 (Gulland & Fox 1992; Hayward *et al.* 2011). Reproductive allocation was also linked to
44
45 364 reduced survival through the association between spring FEC and August body weight.
46
47 365 Previous studies showing such explicit support for any mediators of a reproduction-survival
48
49 366 trade-off are rare (Hamel *et al.* 2010). Work on tree swallows (*Tachycineta bicolor*) showed
50
51 367 that experimentally-increased brood size was associated with reduced antibody responses to
52
53 368 sheep red blood cell (SRBC) antigen, and that individuals that survived to the next season had
54
55
56
57 369 higher SRBC responses (Ardia *et al.* 2003). However, SRBC responses may not reflect
58
59
60

1
2
3 370 resistance to prevalent parasites, and this analysis did not quantify how resistance mediates the
4
5 371 association between reproduction and survival.
6
7
8

9 372

10
11
12 373 A major advantage of using SEMs is that if there were other important mediating factors
13
14 374 between reproduction and survival, Shipley's test would indicate a missing path between
15
16 375 reproduction and survival as missing paths can not only indicate a direct association between
17
18 376 variables, but may also reflect all direct and indirect pathways not otherwise modelled (Shipley
19
20 377 2009; Lefcheck 2015). Shipley's test estimates that in our model (Fig. 3), the direct pathway
21
22 378 between reproduction and survival would have an estimate of 0.0053 ± 0.04 , but that this
23
24 379 pathway was not statistically supported. In our case, the direct and indirect paths are of the
25
26 380 same order of magnitude, which is difficult to interpret considering the direct effect was not
27
28 381 statistically supported. Considering this, the most conservative interpretation of our SEM is
29
30 382 that at least as much variation in survival is explained by the mediating influence of FEC as is
31
32 383 explained by all other direct and indirect factors flowing from reproduction combined.
33
34 384 However, as the missing direct association was not detected, we are confident that there are no
35
36 385 important missing mediators between reproduction and survival. Therefore, the important
37
38 386 mediating factors of survival costs of reproduction in this population appear to be FEC and
39
40 387 body weight, or their close correlates.
41
42
43
44
45
46
47
48
49
50

51 388

52 389 In our *a priori* path diagram, weight is conceived as a potentially mediating link between
53
54 390 reproduction and survival because we expect that costs of reproduction involve reduced storage
55
56 391 of nutritional reserves. However, we also acknowledge that innate variation between
57
58 392 individuals that are not directly caused by reproduction ('condition' in the broad sense) might
59
60

1
2
3 393 cause differences in weight that affect survival. In that sense, variation in weight is not merely
4
5 394 a consequence of prior reproduction, but probably reflects unmeasured variation between
6
7 395 individuals that we cannot disentangle from weight in our analysis. One direction for future
8
9
10 396 research could involve modelling the latent causes of weight variation, including reproduction
11
12 397 and infection. A recent study on the Soay sheep showed that variation in plasma proteins,
13
14 398 independently of body weight and potentially reflecting variation in acquisition of resources,
15
16 399 predicted over winter survival (Garnier *et al.* 2017). Assessing how reproductive effort is
17
18 400 associated with such nutritional markers could therefore provide more insight into the link
19
20 401 between reproduction, weight, and survival. The nature of the association between August
21
22 402 weight and August FEC also represented a challenge, since these are measured simultaneously
23
24 403 and the association is likely mutually antagonistic (Koski & Scott 2001; Beldomenico *et al.*
25
26 404 2008). In our SEM, we suggested that weight influences FEC, which we believed to be the
27
28 405 most plausible direction for the association: weight should be more stable than FEC, which can
29
30 406 fluctuate greatly within a short time-period. Body weight also has higher repeatability and
31
32 407 heritability than FEC (Coltman *et al.* 2001) and although body weight may fluctuate, due to
33
34 408 bladder fullness or a wet fleece, these fluctuations are minor compared to the larger fluctuations
35
36 409 in FEC (Pollott *et al.* 2004). A final compromise made in our analyses was the decision to
37
38 410 perform our SEMs with parameters derived from GAMMs. The caveats of performing
39
40 411 statistical analyses on model estimates (e.g. best linear unbiased predictors, BLUPs) are well-
41
42 412 known (Hadfield *et al.* 2010); for example, the error with which model estimates are generated
43
44 413 are not carried forward. Faced with the challenge of condensing multiple FEC values collected
45
46 414 during spring from one female collected in one year into a single value, while accounting for
47
48 415 age and other factors, our approach was the only viable option. The fact that our results reliably
49
50 416 reflect prior knowledge of the system gives us confidence that our SEM is effective at
51
52 417 representing the pathways linking reproduction, parasites, and survival.
53
54
55
56
57
58
59
60

1
2
3 418
4
5
6
7 419 In this study, we found that increased allocation to reproduction was associated with increased
8
9 420 FEC during late pregnancy and early lactation. We demonstrated that the negative relationship
10
11 421 between reproductive allocation and survival is mediated through effects of reproduction on
12
13 422 FEC and body weight. The results of our study have several far-reaching implications. First,
14
15 423 like previous studies (Festa-Bianchet 1989; Richner *et al.* 1995; Ardia *et al.* 2003; Hanssen *et*
16
17 424 *al.* 2005; Graham *et al.* 2010; la Puente *et al.* 2010; Mills *et al.* 2010; East *et al.* 2015), we have
18
19 425 shown that reproduction is associated with increased FEC/reduced immune responses, and that
20
21 426 these are associated with reduced survival. However, we have also shown explicit links
22
23 427 between reproduction, parasites, and survival in a wild system, providing support for a key
24
25 428 theory of maintenance of variation in resistance (Sheldon & Verhulst 1996; Rolff & Siva-Jothy
26
27 429 2003; Graham *et al.* 2011). Second, the trade-off between reproduction and survival is
28
29 430 fundamental, explaining variation in ageing, mating strategies, and sexual traits (Rowe &
30
31 431 Houle 1996; Blomquist 2009; Lemaître *et al.* 2015). Our results therefore have broad
32
33 432 implications for how trade-offs can shape variation within populations. Finally, as the role of
34
35 433 parasites in regulating populations is well-known (Anderson & May 1978; Hudson *et al.* 1992),
36
37 434 we demonstrate that reproductive costs could play a role in regulating populations through the
38
39 435 effects that they have on parasites. Overall, our work has explicitly quantified the survival costs
40
41 436 of reproduction mediated through FEC and the effects of FEC on body weight, providing
42
43 437 support for the theory of parasite-mediated reproductive costs.
44
45
46
47
48
49
50
51 438
52
53 439
54
55 440
56
57 441
58
59
60

1
2
3 442 Acknowledgements
4

5 443 We thank the National Trust for Scotland for permission to work on St Kilda and QinetiQ and
6
7 444 Elicor for logistics and other support on the island. We are especially grateful to Dan Nussey
8
9 445 and Andrea Graham for insightful comments on an earlier draft of the manuscript and to
10
11 446 Jonathan Chase, one other Editor and five reviewers for highly constructive comments which
12
13 447 greatly improved the manuscript. We are indebted to the many people who have collected
14
15 448 spring faecal egg count data over the years, particularly Frances Gulland and Louisa Tempest.
16
17 449 We are grateful to all project members and many volunteers who have helped with field work
18
19 450 on the island and all those who have contributed to keeping the project going over many years,
20
21 451 including T. Clutton-Brock, M. Crawley, S. Albon, L. Kruuk, T. Coulson and D. Nussey. The
22
23 452 long-term project on St Kilda, including field assistant JGP, has been largely funded by the UK
24
25 453 Natural Environment Research Council. JAL and LFB were both supported by the University
26
27 454 of Stirling. ADH has received support from a University of Stirling Impact Research
28
29 455 Fellowship and a Moredun Foundation Research Fellowship.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 467 References
4

5 468 1. Anderson, R.M. & May, R.M. (1978). Regulation and Stability of Host-Parasite Population
6
7 469 Interactions: I. Regulatory Processes. *J. Anim. Ecol.*, 47, 219–247
8
9

10 470

11
12 471 2. Ardia, D.R., Schat, K.A. & Winkler, D.W. (2003). Reproductive effort reduces long-term
13
14 472 immune function in breeding tree swallows (*Tachycineta bicolor*). *Proc. R. Soc. B*, 270,
15
16 473 1679–1683
17
18

19 474

20
21 475 3. Armour, J., Jarrett, W.F.H., Jennings, F.W. (1996) Experimental *Ostertagia circumcincta*
22
23 476 infections in sheep: development and pathogenesis of a single infection. *American Journal of*
24
25 477 *Veterinary Research* 27, 1267-1278
26
27

28 478

29
30 479 4. Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects
31
32 480 models using lme4. *J. Stat. Softw.*, 67, 1–48
33
34

35 481

36
37 482 5. Beldomenico, P.M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M. & Begon, M. (2008).
38
39 483 Poor condition and infection: a vicious circle in natural populations. *Proc. R. Soc. B*, 275,
40
41 484 1753–1759
42
43

44 485

45
46 486 6. Blomquist, G.E. (2009). Trade-off between age of first reproduction and survival in a
47
48 487 female primate. *Biol. Lett.*, 5, 339–42
49
50

51 488

52
53 489 7. Boyd, H.E.G. (1999). The Early Development of Parasitism in Soay Sheep on St Kilda,
54
55 490 PhD Thesis, University of Cambridge
56
57

58 491
59
60

- 1
2
3 492 8. Burnham, K. & Anderson, D.R. (2002). *Model Selection and Multimodel Inference*.
4
5 493 Springer-Verlag, New York
6
7
8 494
9
10 495 9. Cassinello, J. & Gomendio, M. (1996). Adaptive variation in litter size and sex ratio at birth
11
12 496 in a sexually dimorphic ungulate. *Proc. R. Soc. B*, 263, 1461–1466
13
14
15 497
16
17 498 10. Clutton-Brock, T.H., Albon, S.D. & Guinness, F.E. (1989). Fitness costs of gestation and
18
19 499 lactation in wild mammals. *Nature*, 337, 260–262
20
21 500
22
23 501 11. Clutton-Brock, T.H. & Pemberton, J.M. (Eds.). (2004). *Soay Sheep: Dynamics and*
24
25 502 *Selection in an Island Population*. 1st edn. Cambridge University Press, Cambridge
26
27 503
28
29 504 12. Clutton-Brock, T.H., Price, O.F., Albon, S.D. & Jewell, P.A. (1991). Persistent instability
30
31 505 and population regulation in Soay sheep. *J. Anim. Ecol.*, 60, 593–608
32
33 506
34
35 507 13. Clutton-Brock, T.H., Price, O.F., Albon, S.D. & Jewell, P.A. (1992). Early development
36
37 508 and population fluctuations in Soay sheep. *J. Anim. Ecol.*, 61, 381–396
38
39 509
40
41 510 14. Clutton-Brock, T.H., Stevenson, I.R., Marrow, P., MacColl, A.D., Houston, A.I. &
42
43 511 McNamara, J.M. (1996). Population fluctuations, reproductive costs and life-history tactics in
44
45 512 female Soay sheep. *J. Anim. Ecol.*, 65, 675–689
46
47 513
48
49 514 15. Coltman, D.W., Pilkington, J.G., Kruuk, L.E.B., Wilson, K. & Pemberton, J.M. (2001).
50
51 515 Positive Genetic Correlation between Parasite Resistance and Body Size in a Free-Living
52
53 516 Ungulate Population. *Evolution*, 55, 2116–2125
54
55
56
57
58
59
60

- 1
2
3 517
4
5 518 16.Coop, R.L. & Kyriazakis, I. (1999). Nutrition – parasite interaction. *Vet. Parasitol.*, 84,
6
7 519 187–204
8
9 520
10
11 521 17.Coulson, T., Catchpole, E.A., Albon, S.D., Morgan, B.J.T., Pemberton, J.M., Clutton-
12
13 522 Brock, T.H., *et al.* (2001). Age, Sex, Density, Winter Weather, and Population Crashes in
14
15 523 Soay Sheep. *Science*, 292, 1528–1531
16
17 524
18
19 525 18.Craig, B.H., Pilkington, J.G. & Pemberton, J.M. (2006). Gastrointestinal nematode species
20
21 526 burdens and host mortality in a feral sheep population. *Parasitology*, 133, 485–96
22
23 527
24
25 528 19.Descamps, S., Boutin, S., McAdam, A.G., Berteaux, D. & Gaillard, J.-M. (2009). Survival
26
27 529 costs of reproduction vary with age in North American red squirrels. *Proc. R. Soc. B*, 276,
28
29 530 1129–35
30
31 531
32
33 532 20.East, M., Otto, E., Helms, J., Theirer, D., Cable, J. & Hofer, H. (2015). Does lactation lead
34
35 533 to resource allocation trade-offs in the spotted hyaena? *Behav. Ecol. Sociobiol.*, 69, 805–814
36
37 534
38
39 535 21.Festa-Bianchet, M. (1989). Individual differences, parasites, and the costs of reproduction
40
41 536 for bighorn ewes (*Ovis canadensis*). *J. Anim. Ecol.*, 58, 785–795
42
43 537
44
45 538 22.Froy, H., Walling, C.A., Pemberton, J.M., Clutton-Brock, T.H. & Kruuk, L.E.B. (2016).
46
47 539 Relative costs of offspring sex and offspring survival in a polygynous mammal. *Biol. Lett.*,
48
49 540 12, 20160417
50
51 541
52
53
54
55
56
57
58
59
60

- 1
2
3 542 23.Fthenakis, G.C., Mavrogianni, V.S., Gallidis, E. & Papadopoulos, E. (2015). Interactions
4
5 543 between parasitic infections and reproductive efficiency in sheep. *Vet. Parasitol.*, 208, 56–66
6
7 544
8
9
10 545 24. Fthenakis, G.C., Papadopoulos, E., Himonas, C. (2005) Effects of Three Anthelmintic
11
12 546 Regimes on Milk Yield of Ewes and Growth of Lambs. *J. Vet. Med.* 52, 78–82
13
14
15 547
16
17 548 25.Garnier, R., Cheung, C.K., Watt, K.A., Pilkington, J.G., Pemberton, J.M. & Graham, A.L.
18
19 549 (2017). Joint associations of blood plasma proteins with overwinter survival of a large
20
21 550 mammal. *Ecol. Lett.*, 20, 175–183
22
23
24 551
25
26 552 26.Grace, J.B., Adler, P.B., Harpole, S.W., Borer, E.T. & Seabloom, E.W. (2014). Causal
27
28 553 networks clarify productivity - richness interrelations, bivariate plots do not. *Funct. Ecol.*, 28,
29
30 554 787–798
31
32
33 555
34
35 556 27.Grace, J.B., Scheiner, S.M. & Schoolmaster, D.R. (2015). Structural equation modeling:
36
37 557 building and evaluating causal models. In: *Ecological Statistics: Contemporary Theory and*
38
39 558 *Application* (eds. Fox, G.A., Negrete-Yankelovich, S. & Sosa, V.J.). Oxford University Press,
40
41 559 Oxford, pp. 168–199
42
43 560
44
45 561 28.Graham, A.L., Hayward, A.D., Watt, K.A., Pilkington, J.G., Pemberton, J.M. & Nussey,
46
47 562 D.H. (2010). Fitness correlates of heritable variation in antibody responsiveness in a wild
48
49 563 mammal. *Science*, 330, 662–664
50
51
52 564
53
54 565 29.Graham, S.P., Earley, R.L., Guyer, C. & Mendonça, M.T. (2011). Innate immune
55
56 566 performance and steroid hormone profiles of pregnant versus nonpregnant cottonmouth
57
58
59
60

- 1
2
3 567 snakes (*Agkistrodon piscivorus*). *Gen. Comp. Endocrinol.*, 174, 348–353
4
5 568
6
7
8 569 30. Gulland, F.M. (1992). The role of nematode parasites in Soay sheep (*Ovis aries* L.)
9
10 570 mortality during a population crash. *Parasitology*, 105, 493–503
11
12 571
13
14 572 31. Gulland, F.M., Albon, S.D., Pemberton, J.M., Moorcroft, P.R. & Clutton-Brock, T.H.
15
16 573 (1993). Parasite-associated polymorphism in a cyclic ungulate population. *Proc. R. Soc. B*,
17
18 574 254, 7–13
19
20 575
21
22 576 32. Gulland, F.M. & Fox, M. (1992). Epidemiology of nematode infections of Soay sheep.
23
24 577 *Parasitology*, 105, 481–492
25
26 578
27
28 579 33. Hadfield, J.D., Wilson, A.J., Garant, D., Sheldon, B.C. & Kruuk, L.E.B. (2010). The
29
30 580 misuse of BLUP in ecology and evolution. *Am. Nat.*, 175, 116–125
31
32 581
33
34 582 34. Hamel, S., Gaillard, J.M., Yoccoz, N.G., Loison, A., Bonenfant, C. & Descamps, S.
35
36 583 (2010). Fitness costs of reproduction depend on life speed: Empirical evidence from
37
38 584 mammalian populations. *Ecol. Lett.*, 13, 915–935
39
40 585
41
42 586 35. Hanssen, S.A., Hasselquist, D., Folstad, I. & Erikstad, K.E. (2005). Cost of reproduction
43
44 587 in a long-lived bird: incubation effort reduces immune function and future reproduction.
45
46 588 *Proc. R. Soc. B*, 272, 979–1082
47
48 589
49
50 590 36. Hayward, A.D. (2013). Causes and consequences of intra- and inter-host heterogeneity in
51
52 591 defence against nematodes. *Parasite Immunol.*, 35, 362–373
53
54
55
56
57
58
59
60

- 1
2
3 592
4
5
6 593 37.Hayward, A.D., Garnier, R., Watt, K.A., Pilkington, J.G., Grenfell, B.T., Matthews, J.B.,
7
8 594 *et al.* (2014). Heritable, heterogeneous, and costly resistance of sheep against nematodes and
9
10 595 potential feedbacks to epidemiological dynamics. *Am. Nat.*, 184, S58-76
11
12 596
13
14 597 38.Hayward, A.D., Pemberton, J.M., Berenos, C., Wilson, A.J., Pilkington, J.G. & Kruuk,
15
16 L.E.B. (2018). Evidence for selection-by-environment but not genotype-by-environment
17 598 interactions for fitness-related traits in a wild mammal population. *Genetics*, 208, 349–364
18
19 599
20
21 600
22
23 601 39.Hayward, A.D., Pilkington, J.G., Wilson, K., McNeilly, T.N. & Watt, K.A. (2019).
24
25 602 Reproductive effort influences intra-seasonal variation in parasite-specific antibody responses
26
27 603 in wild Soay sheep. *Funct. Ecol.*, In Press (accepted)
28
29 604
30
31 605 40.Hayward, A.D., Wilson, A.J., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. &
32
33 606 Kruuk, L.E.B. (2011). Natural selection on a measure of parasite resistance varies across ages
34
35 607 and environmental conditions in a wild mammal. *J. Evol. Biol.*, 24, 1664–1676
36
37 608
38
39 609 41.Hewison, A.J.M. & Gaillard, J.M. (2001). Phenotypic quality and senescence affect
40
41 610 different components of reproductive output in roe deer. *J. Anim. Ecol.*, 70, 600–608
42
43 611
44
45 612 42.Hodges, C.J., Bowers, E.K., Thompson, C.F. & Sakaluk, S.K. (2015). Cascading costs of
46
47 613 reproduction in female house wrens induced to lay larger clutches. *J. Evol. Biol.*, 28, 1383–
48
49 614 1393
50
51 615
52
53
54
55
56
57
58 616 43.Houdijk, J.G., Kyriazakis, I., Jackson, F., Huntley, J.F. & Coop, R.L. (2003). Is the
59
60

- 1
2
3 617 allocation of metabolisable protein prioritised to milk production rather than to immune
4
5 618 functions in *Teladorsagia circumcincta*-infected lactating ewes? *Int. J. Parasitol.*, 33, 327–
6
7 619 338
8
9 620
10
11
12 621 44.Hudson, P.J., Newborn, D. & Dobson, A.P. (1992). Regulation and stability of a free-
13
14 622 living host-parasite system: *Trichostrongylus tenuis* in red grouse. I. Monitoring and parasite
15
16 623 reduction experiments. *J. Anim. Ecol.*, 61, 477–486
17
18
19 624
20
21 625 45.Hutchings, M.R., Milner, J.M., Gordon, I.J., Kyriazakis, I. & Jackson, F. (2002). Grazing
22
23 626 decisions of Soay sheep, *Ovis aries*, on St Kilda: a consequence of parasite distribution?
24
25 627 *Oikos*, 96, 235–244
26
27
28 628
29
30 629 46.Isomursu, M., Rätti, O., Helle, P. & Hollmén, T. (2006). Sex and age influence intestinal
31
32 630 parasite burden in three boreal grouse species. *J. Avian Biol.*, 37, 516–522
33
34
35 631
36
37 632 47.Johnson, M.S., Thomson, S.C. & Speakman, J.R. (2001). Limits to sustained energy
38
39 633 intake, lactation in the laboratory mouse *Mus musculus*. *J. Exp. Biol.*, 204, 1925–1935
40
41
42 634
43
44 635 48.Katoch, R., Yadav, A., Godara, R., Khajuria, J.K., Borkataki, S. & Sodhi, S.S. (2012).
45
46 636 Prevalence and impact of gastrointestinal helminths on body weight gain in backyard
47
48 637 chickens in subtropical and humid zone of Jammu, India. *J. Parasit. Dis.*, 36, 49–52
49
50
51 638
52
53 639 49.Kidane, A., Houdijk, J.G., Tolkamp, B.J., Athanasiadou, S. & Kyriazakis, I. (2009).
54
55 640 Consequences of infection pressure and protein nutrition on periparturient resistance to
56
57 641 *Teladorsagia circumcincta* and performance in ewes. *Vet. Parasitol.*, 165, 78–87
58
59
60

- 1
2
3 642
4
5 643 50.Klein, S.L. & Flanagan, K.L. (2016). Sex differences in immune responses. *Nat. Rev.*
6
7 644 *Immunol.*, 16, 626–638
8
9 645
10
11 646 51.Knowles, S., Nakagawa, S. & Sheldon, B.C. (2009). Elevated reproductive effort
12
13 647 increases blood parasitaemia and decreases immune function in birds: A meta-regression
14
15 648 approach. *Funct. Ecol.*, 23, 405–415
16
17 649
18
19 650 52.Koski, K.G. & Scott, M.E. (2001). Gastrointestinal nematodes, nutrition and immunity:
20
21 651 Breaking the Negative Spiral. *Annu. Rev. Nutr.*, 21, 297–321
22
23 652
24
25 653 53.Langrová, I., Makovcová, K., Vadlejš, J., Jankovská, I., Petrtýl, M., Fechtner, J., *et al.*
26
27 654 (2008). Arrested development of sheep strongyles: onset and resumption under field
28
29 655 conditions of Central Europe. *Parasitol. Res.*, 103, 387–392
30
31 656
32
33 657 54.Lefcheck, J.S. (2015). piecewiseSEM: Piecewise structural equation modelling in r for
34
35 658 ecology, evolution, and systematics. *Methods Ecol. Evol.*, 7, 573–579
36
37 659
38
39 660 55.Lemaître, J.-F., Berger, V., Bonenfant, C., Douhard, M., Gamelon, M., Plard, F., *et al.*
40
41 661 (2015). Early-late life trade-offs and the evolution of ageing in the wild. *Proc. R. Soc. B*, 282,
42
43 662 20150209
44
45 663
46
47 664 56.Lindenmayer, D.B., Likens, G.E., Andersen, A., Bowman, D., Bull, C.M., Burns, E., *et al.*
48
49 665 (2012). Value of long-term ecological studies. *Austral Ecol.*, 37, 745–757
50
51 666
52
53
54
55
56
57
58
59
60

- 1
2
3 667 57.Lloyd, S. (1983). Effect of pregnancy and lactation upon infection. *Vet. Immunol.*
4
5 668 *Immunopathol.*, 4, 153–176
6
7 669
8
9
10 670 58.Mills, S.C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T. & Poikonen, T. (2010).
11
12 671 Fitness trade-offs mediated by immunosuppression costs in a small mammal. *Evolution*, 64,
13
14 672 166–179
15
16 673
17
18
19 674 59.Milner, J.M., Elston, D.A. & Albon, S.D. (1999). Estimating the contribution of
20
21 675 population density and climatic fluctuations to interannual variation in survival of Soay
22
23 676 sheep. *J. Anim. Ecol.*, 68, 1235–1247
24
25 677
26
27
28 678 60.van Noordwijk, A. & de Jong, G. (1986). Acquisition and allocation of resources - their
29
30 679 influence on variation in life-history tactics. *Am. Nat.*, 128, 137–142
31
32 680
33
34
35 681 61.Nordling, D., Andersson, M.S., Zohari, S. & Lars, G. (1998). Reproductive effort reduces
36
37 682 specific immune response and parasite resistance. *Proc. R. Soc. B*, 265, 1291–1298
38
39 683
40
41
42 684 62.Pelletier, F., Page, K.A., Ostiguy, T., Festa-Bianchet, M. & Lundberg, P. (2005). Fecal
43
44 685 counts of lungworm larvae and reproductive effort in bighorn sheep, *Ovis canadensis*. *Oikos*,
45
46 686 110, 473–480
47
48 687
49
50
51 688 63.Penn, D.J. & Smith, K.R. (2007). Differential fitness costs of reproduction between the
52
53 689 sexes. *Proc. Natl. Acad. Sci. USA.*, 104, 553–8
54
55 690
56
57
58 691 64.Pollott, G.E., Karlsson, L.J.E., Eady, S. & Greeff, J.C. (2004). Genetic parameters for
59
60

- 1
2
3 692 indicators of host resistance to parasites from weaning to hogget age in Merino sheep. *J.*
4
5 693 *Anim. Sci.*, 82, 2852–2864
6
7
8 694
9
10 695 65.la Puente, J.M.J., Merino, S., Tomas, G., Moreno, J., Morales, J., Lobato, E., *et al.* (2010).
11
12 696 The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment.
13
14 697 *Biol. Lett.*, 6, 663–665
15
16
17 698
18
19 699 66.R Core Team. (2018). R: A language and environment for statistical computing.
20
21 700
22
23 701 67.Richner, H., Christe, P. & Oppliger, A. (1995). Paternal investment affects prevalence of
24
25 702 malaria. *Proc. Natl. Acad. Sci. USA*, 92, 1192–1194
26
27
28 703
29
30 704 68.Rolff, J. & Siva-Jothy, M.T. (2003). Invertebrate Ecological Immunology. *Science*, 301,
31
32 705 472–475
33
34
35 706
36
37 707 69.Rowe, L. & Houle, D. (1996). The Lek Paradox and the Capture of Genetic Variance by
38
39 708 Condition Dependent Traits. *Proc. R. Soc. B*, 263, 1415–1421
40
41
42 709
43
44 710 70.Sheldon, B.C. & Verhulst, S. (1996). Ecological immunology: Costly parasite defences
45
46 711 and trade-offs in evolutionary ecology. *Trends Ecol. Evol.*, 11, 317–321
47
48
49 712
50
51 713 71.Shipley, B. (2009). Confirmatory path analysis in a generalized multilevel context.
52
53 714 *Ecology*, 90, 363–368
54
55
56 715
57
58 716 72.Simon, A.K., Hollander, G.A. & McMichael, A. (2015). Evolution of the immune system
59
60

- 1
2
3 717 in humans from infancy to old age. *Proc. R. Soc. B*, 282, 20143085
4
5 718
6
7
8 719 73.Stearns, S.C. (1992). Trade-offs. In: *The Evolution of Life Histories*. 1st edn. Oxford
9
10 720 University Press, Oxford, pp. 72-90
11
12 721
13
14 722 74.Tavecchia, G., Coulson, T., Morgan, B.J.T., Pemberton, J.M., Pilkington, J.G., Gulland,
15
16 F.M.D., *et al.* (2005). Predictors of reproductive cost in female Soay sheep. *J. Anim. Ecol.*,
17 723
18 74, 201–213
19 724
20
21 725
22
23 726 75.Tempest, L.J. (2005). Parasites and the cost of reproduction in Soay sheep, PhD Thesis.
24
25 University of Stirling
26 727
27
28 728
29
30 729 76.Tschirren, B., Fitze, P.S. & Richner, H. (2003). Sexual dimorphism in susceptibility to
31
32 parasites and cell-mediated immunity in great tit nestlings. *J. Anim. Ecol.*, 72, 839–845
33 730
34
35 731
36
37 732 77.Watson, M.J. (2013) What drives population-level effects of parasites? Meta-analysis
38
39 meets life-history. *Int. J. Parasitol. Parasites Wildl.* 2, 190–196
40 733
41
42 734
43
44 735 78.Watson, R.L., McNeilly, T.N., Watt, K.A., Pemberton, J.M., Pilkington, J.G., Waterfall,
45
46 M., *et al.* (2016). Cellular and humoral immunity in a wild mammal: Variation with age &
47 736
48 sex and association with overwinter survival. *Ecol. Evol.*, 6, 8695–8705
49 737
50
51 738
52
53 739 79.Williams, G.C. (1966). Natural selection, the cost of reproduction and a refinement of
54
55 Lack's principle. *Am. Nat.*, 100, 687–690
56 740
57
58 741
59
60

- 1
2
3 742 80. Wilson, K., Grenfell, B.T., Pilkington, J.G., Boyd, G. & Gulland, F.M.D. (2004). Parasites
4
5 743 and their impact. In: *Soay Sheep: Dynamics and Selection in an Island Population* (eds.
6
7 744 Clutton-Brock, T.H. & Pemberton, J.M.). Cambridge University Press, Cambridge, pp. 113–
8
9 745 165
10
11
12 746
13
14 747 81. Wilson, K., Grenfell, B.T. & Shaw, D.J. (1996). Analysis of aggregated parasite
15
16 748 distributions: a comparison of methods. *Funct. Ecol.*, 10, 592–601
17
18
19 749
20
21 750 82. Wood, S. & Scheipl, F. (2017). *gam4*: Generalized Additive Mixed Models using
22
23 751 “mgcv” and “lme4”. R package version 0.2-5
24
25
26 752
27
28 753
29
30 754
31
32 755
33
34 756
35
36 757
37
38 758
39
40 759
41
42 760
43
44 761
45
46 762
47
48 763
49
50 764
51
52 765
53
54 766
55
56
57
58
59
60

1
2
3 767 Figure Legends
4

5 768
6

7
8 769 **Figure 1.** *A priori* structural equation model (SEM) based on decades of research on the Soay
9
10 770 sheep population. Associations are depicted for female sheep only. Variables are temporally
11
12 771 separated over two years. Reproductive status is based on whether or not the female gave birth
13
14 772 in spring.
15

16 773

17
18
19 774 **Figure 2.** Comparison of change in strongyle FEC over a 99-day period during the lambing
20
21 775 season, analysed using generalised additive mixed-effects models (GAMMs; Tables S2-S4).
22

23
24 776 (A) The best-fitting model for juveniles grouped all individuals; (B) the best-fitting model for
25
26 777 yearlings grouped individuals by sex; (C) the best-fitting model for adults grouped individuals
27
28 778 by lamb survival. Panels on the left show predictions on the log-transformed scale (the scale at
29
30 779 which data were analysed), with lines showing model estimates, shaded areas indicating ± 1 SE
31
32 780 and points showing raw data. On the right, predictions are back-transformed onto the original
33
34 781 scale. Raw data are not shown on the back-transformed plots for clarity of viewing and ease of
35
36 782 interpretation.
37

38 783

39
40
41
42 784 **Figure 3.** Our final structural equation model (SEM) showing downstream impacts of
43
44 785 reproduction on future fitness (overwinter survival). Values on arrows and arrow widths
45
46 786 indicate standardised path coefficients with standard errors in parentheses. All variables were
47
48 787 standardised by dividing by one standard deviation.
49

50 788
51
52
53
54
55
56
57
58
59
60

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)

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

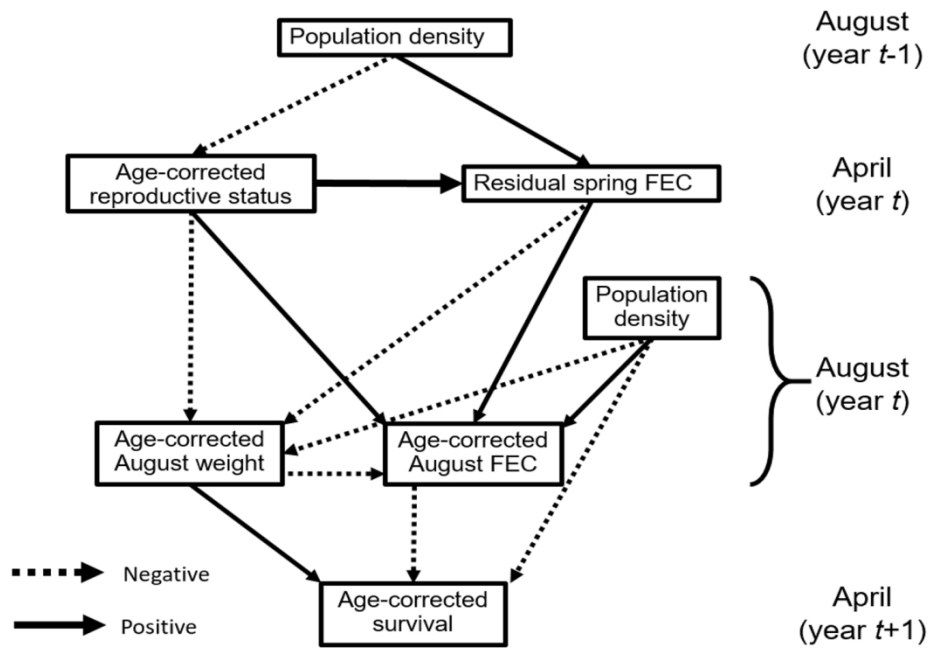


Figure 1. A priori structural equation model (SEM) based on decades of research on the Soay sheep population. Associations are depicted for female sheep only. Variables are temporally separated over two years. Reproductive status is based on whether or not the female gave birth in spring.

272x198mm (150 x 138 DPI)

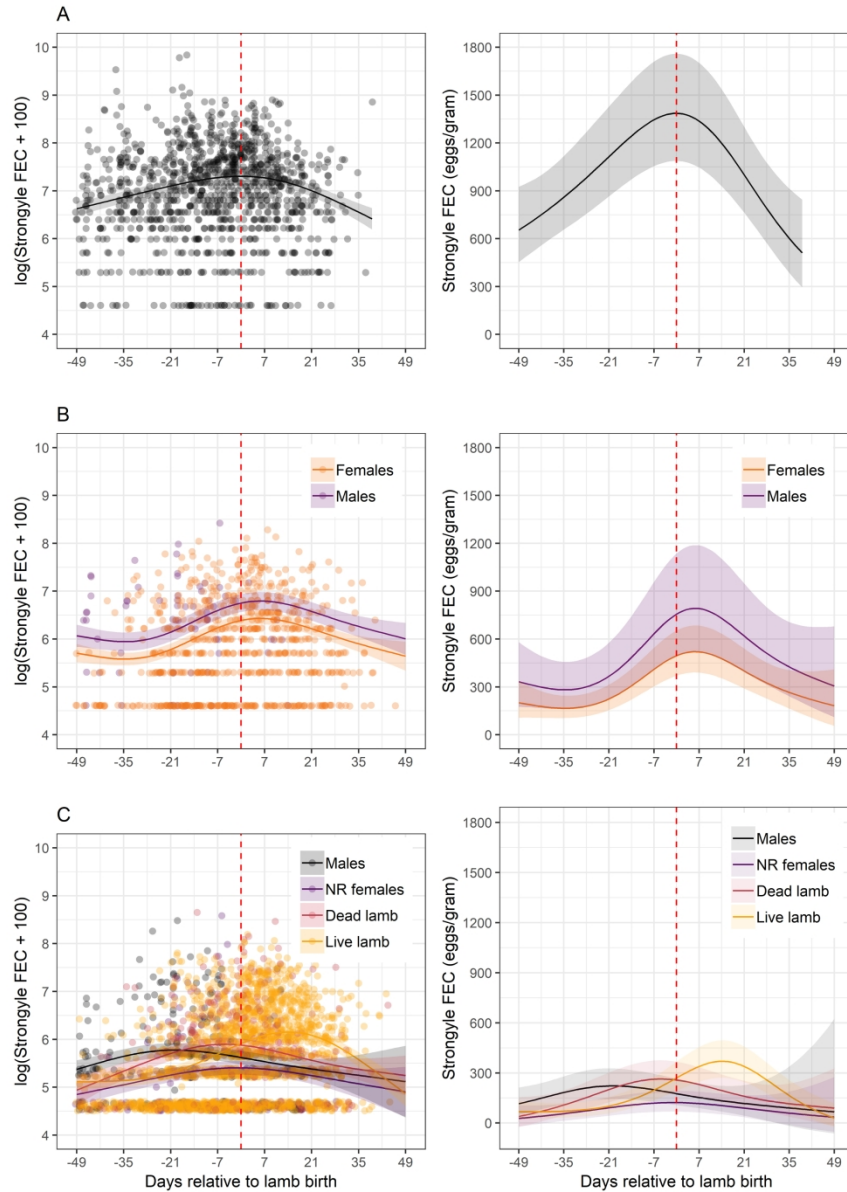


Figure 2. Comparison of change in strongyle FEC over a 99-day period during the lambing season, analysed using generalised additive mixed-effects models (GAMMs; Tables S2-S4). (A) The best-fitting model for juveniles grouped all individuals; (B) the best-fitting model for yearlings grouped individuals 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 $\pm 1SE$ 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.

199x279mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

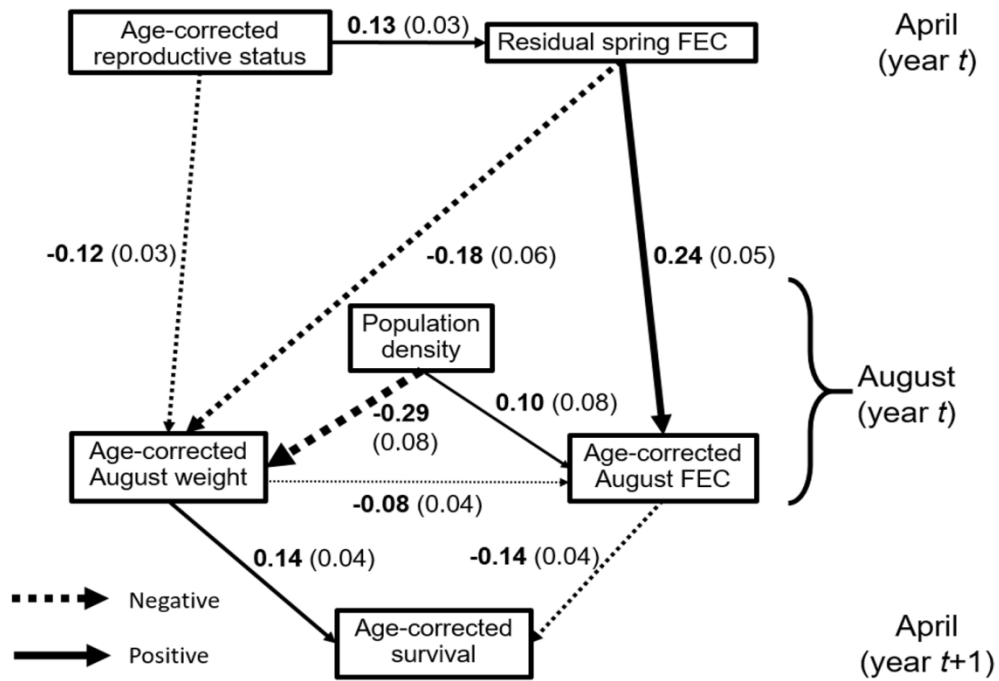


Figure 3. Our final structural equation model (SEM) showing downstream impacts of reproduction on future fitness (overwinter survival). Values on arrows and arrow widths indicate standardised path coefficients with standard errors in parentheses. All variables were standardised by dividing by one standard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

258x193mm (150 x 142 DPI)