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Fitness costs of parasites explain multiple life history tradeoffs in a wild mammal

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1 Fitness costs of parasites explain 2 multiple life history tradeoffs in a 3 wild mammal

4 Summary

5 Reproduction in wild animals can divert limited resources away from immune
6 defence, resulting in increased parasite burdens. A longstanding prediction of life
7 history theory states that these parasites can harm the reproductive individual,
8 reducing its subsequent survival and fecundity, producing reproduction-fitness
9 tradeoffs. Here, we examined associations among reproductive allocation,
10 immunity, parasitism, and subsequent survival and fecundity in a wild population of
11 individually identified red deer (*Cervus elaphus*). Using path analysis, we
12 investigated whether costs of lactation in terms of downstream survival and
13 fecundity were mediated by changes in strongyle nematode count and mucosal
14 antibody levels. Lactating females exhibited increased parasite counts, which were
15 in turn associated with substantially decreased fitness in the following year in terms
16 of overwinter survival, fecundity, subsequent calf weight, and parturition date. This
17 study offers observational evidence for parasite regulation of multiple life history
18 tradeoffs, supporting the role of parasites as an important mediating factor in wild
19 mammal populations.

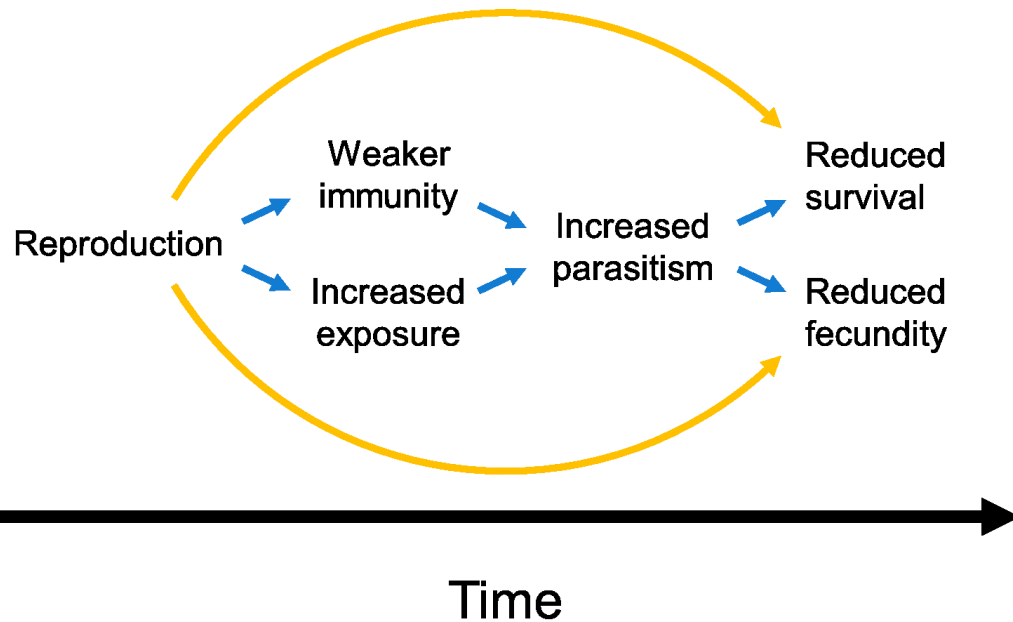
20 Introduction

21 A fundamental tenet of life history theory states that reproduction should reduce
22 subsequent survival and fecundity (Williams, 1966; Stearns, 1989). While evidence
23 for such trade-offs is widespread, the mechanisms behind them remain poorly
24 understood. One hypothesised mechanism is that reproductive costs act through
25 parasites, where increased reproductive allocation diverts limited resources away
26 from immune defence, resulting in increased parasite burdens, which reduce
27 subsequent survival and fecundity (Sheldon and Verhulst, 1996; Harshman and
28 Zera, 2007). Parasite mediation of life history tradeoffs involves two necessary
29 components: that reproduction increases parasitism, and that these parasites cause
30 harm or require resources to combat them, reducing subsequent fitness. There is
31 abundant evidence for each component of this theory across a range of taxa: firstly,
32 reproduction is often associated with weaker or altered immune allocation (Neggazi
33 *et al.*, 2016; Rödel *et al.*, 2016; Krams *et al.*, 2017) or with increased parasitism
34 (Festa-Bianchet, 1989; Cizauskas *et al.*, 2015; Debeffe *et al.*, 2016). Secondly,
35 increased parasitism is often associated with decreased subsequent probability of
36 survival (Coltman *et al.*, 1999; Leivesley *et al.*, 2019) or reproduction (Albon *et al.*,
37 2002; Vandegrift *et al.*, 2008; Hughes *et al.*, 2009). Despite evidence for one or other
38 of these processes in isolation, reproduction-associated increases in parasitism
39 have rarely been linked to downstream survival and fecundity consequences within
40 the same study system to provide full support for parasite mediation of life history
41 tradeoffs.

42 While it is true that reproduction, immunity, and parasites all compete for host
43 resources, mechanisms governing life history tradeoffs are hypothesised to occur in
44 a temporal sequence rather than occurring simultaneously (Figure 1). First,
45 reproduction diverts resources away from immunity, reducing immune allocation
46 (Sheldon and Verhulst, 1996). Resultant weaker immunity, plus potentially
47 increased exposure associated with altered behaviour of reproductive individuals,
48 can then result in higher parasite burden (Knowles *et al.*, 2009; Albery *et al.*, 2020).
49 Finally, subsequent survival and fecundity is reduced by damage from parasites
50 (Harshman and Zera, 2007; Graham *et al.*, 2011). This combination of mechanisms
51 comprises an indirect cost of reproduction acting through parasites. Additional
52 (direct) costs of reproduction can simultaneously act through other mechanisms
53 such as reduced condition, hormonal or phenological regulation, or damage caused
54 by oxidative stress (Stjernman *et al.*, 2004; Harshman and Zera, 2007; Speakman,
55 2008; Figure 1). This causal sequence is important, because parasites' observed

56 relationship with life history traits can depend on whether a preceding,
57 contemporary, or subsequent trait is chosen for examination.

58



59

60 Figure 1: The hypothesised mechanism for parasite-dependent mediation of life history
61 tradeoffs. Blue (interior) arrows denote indirect, parasite-mediated costs of reproduction for
62 downstream survival and fecundity, while the orange (exterior) arrows denote direct costs
63 through resource allocation, hormonal regulation, or similar mechanisms.

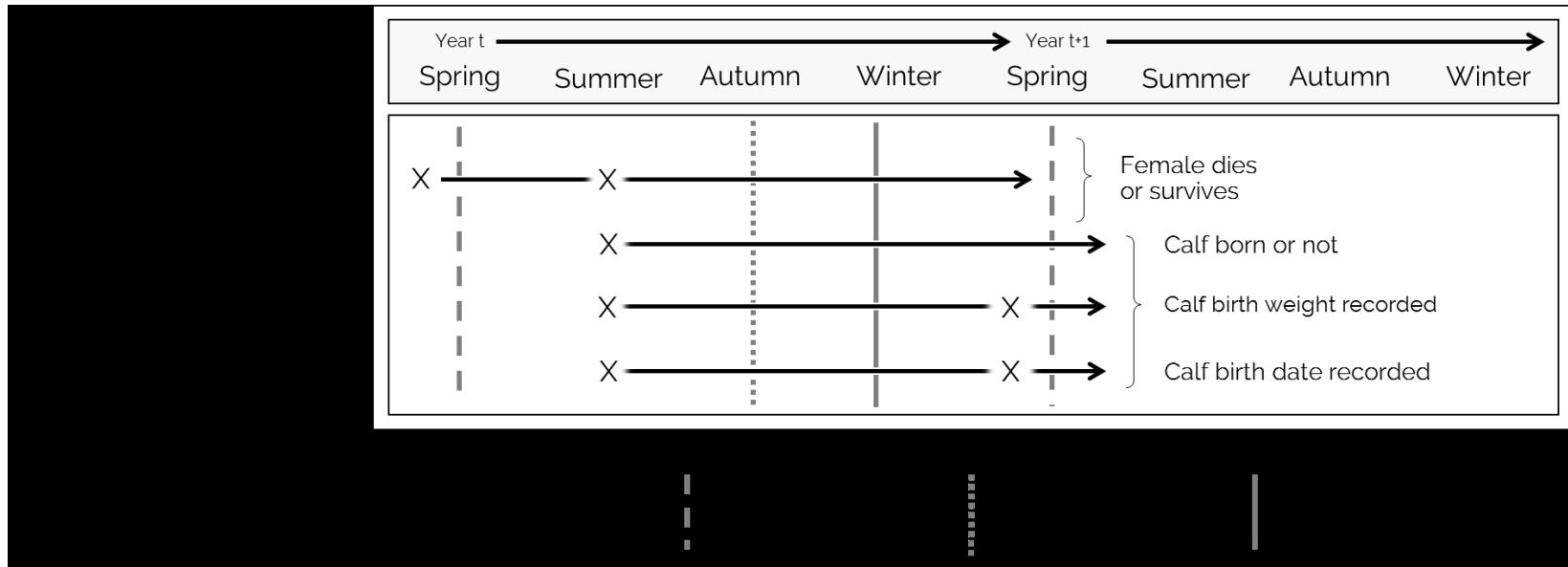
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65 Many studies examining reproduction-immunity-parasitism interrelationships have
66 been carried out in birds, often using experiments in which reproductive effort is
67 artificially increased by manipulating clutch sizes (Knowles *et al.*, 2009). Such
68 manipulations are not possible in many mammal species, and thus most of our
69 knowledge of these trade-offs is based on observational studies. In observational
70 contexts, or in concert with experiments, path analysis can be used to infer links
71 between parasites and their fitness consequences (Pacejka *et al.*, 1998; Stjernman
72 *et al.*, 2004; Brambilla *et al.*, 2015; Leivesley *et al.*, 2019). Notably, a recent analysis
73 in a wild population of Soay sheep used path analysis to demonstrate
74 observationally that reproduction reduced survival through increased parasite count
75 and reduced body weight (Leivesley *et al.*, 2019), but without examining impacts on
76 subsequent reproductive traits.

77 The wild red deer (*Cervus elaphus*) on the Isle of Rum provide a classic example of
78 a life history tradeoff under natural conditions: female deer that invest in lactation
79 have reduced survival probability and fecundity in the subsequent year compared
80 to non-lactating females (Clutton-Brock *et al.*, 1989; Froy *et al.*, 2016). Females that
81 give birth to a calf that dies within the first few months of its life have similar survival
82 and fecundity the following year to those that do not give birth, implying that
83 gestation has a minimal cost relative to lactation (Clutton-Brock *et al.*, 1989). The
84 deer are infected with several helminth parasites; egg counts and mucosal antibody
85 (IgA) levels are measured via non-invasive collection of faecal samples (Albery *et*
86 *al.*, 2018, 2020). A previous study demonstrated decreased mucosal IgA among
87 females that gestate a calf (Albery *et al.*, 2020). Lactation (but not gestation) is
88 associated with increased parasite counts, partially mirroring the previously
89 observed costs of lactation for subsequent survival and fecundity (Clutton-Brock *et*
90 *al.*, 1989; Froy *et al.*, 2016). Together, these studies demonstrate a cost of
91 reproduction for subsequent survival, fecundity, parasitism, and immunity
92 respectively in this population. However, we have yet to establish the degree to
93 which subsequent fitness costs of reproduction can be explained by changes in
94 parasitism.

95 Here, we use path analysis to link reproduction-immunity-parasitism tradeoffs in the
96 Isle of Rum red deer with survival and reproductive traits in the following year,
97 investigating whether immunity and parasitism are capable of mediating life history
98 tradeoffs, and attempting to separate immune and parasite mediation from direct
99 effects of reproduction acting through alternative mechanisms. We expected that
100 the substantially increased parasite counts associated with lactation would be
101 associated with decreased subsequent survival, fecundity, parturition date, and calf
102 birth weight, such that parasites provide a mechanistic explanation for the costs of
103 lactation seen in this system.

104



105

106 Figure 2: The four models fitted in this study in the context of the red deer reproductive cycle and sampling regime, over an example two-year
 107 period. Reproduction begins in spring and summer, at the start of each deer year, and one sampling trip was undertaken each summer (August),
 108 after the calving season had finished. Mating occurs in the autumn, and the mortality season begins in winter and lasts until early spring. A second
 109 sampling trip occurred each spring (April), after mating and mortality, but before the beginning of the subsequent calving season. The fitness
 110 variables investigated were quantified at the start of the subsequent deer year: if a female survived to May 1 the following year she was counted as
 111 1 in the survival analysis, 0 if not, and the presence, weight, and birth date of her calf in the following spring were used as response variables in
 112 the remaining three models. The sampling trips included in each model were selected according to feasibility of causal links. For example, females
 113 become pregnant in the autumn, so we did not include the spring sampling season in the reproduction model as they would already be pregnant at
 114 this point, making it unlikely that parasite counts in April have a direct effect on their probability of having a calf 1-2 months later. NB the
 115 parasitological and immunological data were derived through specific faecal sampling trips (represented by X marks), while survival and calving
 116 data were collected through routine censusing operations, separate from the faecal sampling trips, providing data that we then linked with the
 117 immunoparasitology to infer fitness consequences.

118 Methods

119 Study system, sampling, and labwork

120 The study population lives in the north block of the Isle of Rum National Nature
121 Reserve (57°N 6°20'W). The deer are entirely wild and unmanaged, and have been
122 monitored continuously since the 1970s (see Clutton-Brock *et al.*, 1982 for an
123 overview of the project). The life history data collected on the population provide
124 high-resolution estimates of individuals' dates of birth and death, reproduction, and
125 familial relationships. The "deer year" begins on May 1st, and the deer give birth
126 ("calving") in May-June, having conceived in the previous autumn (Figure 2). Deer
127 on Rum give birth to a single calf, and do not reproduce every year. During the
128 calving season, we aim to capture and mark as many of the calves born as possible
129 soon after birth, so that they can be monitored for the rest of their lives. Sex and
130 capture weight (to the nearest 100g) are recorded. ~20% of calves die within the
131 first few weeks of life, and giving birth to a calf that dies within this period has little
132 cost to the mother in terms of her survival and reproduction probability the following
133 year (Clutton-Brock *et al.*, 1989). In contrast, if a calf survives into the winter, the
134 mother has spent ~6 months lactating to it, expending considerable resources in
135 doing so, and this cost is associated with substantially decreased fecundity and
136 survival probability the following year (Clutton-Brock *et al.*, 1989; Froy *et al.*, 2016).

137 During early spring (April) and late summer (August), either side of the calving
138 season, we conducted two-week field trips to collect faecal samples from the deer
139 noninvasively. These trips were conducted separately from the censuses and
140 calving and mortality searches that facilitated the life history data collection.
141 Sampling was undertaken across five trips 2016-2018, with 701 faecal samples
142 collected in total; see Table 1 for details of datasets. We watched known individuals
143 for defaecation, marked the spot where the droppings landed, and then collected
144 them while minimising disturbance to the deer, generally within an hour. In the
145 evening, samples were aliquotted and processed. A subsample was extracted by
146 centrifugation and frozen at -20C for faecal antibody analysis (Watt *et al.*, 2016;
147 Albery *et al.*, 2020). Another subsample was kept as anaerobic as possible in a
148 Ziploc bag at 4°C to avoid egg hatching.

149 Within three weeks of collection, the faecal samples stored at 4°C were counted for
150 the eggs of gastrointestinal strongyle nematodes using a salt flotation-centrifugation
151 faecal egg count (FEC), accurate to 1 egg per gram (EPG) (Kenyon *et al.*, 2013;
152 Taylor, M. A.; Coop, R. L.; Wall, 2015), and within 3 weeks of collection. This

153 followed previously established methodology for the study population (Albery *et al.*,
154 2018, 2020). Strongyles are ubiquitous ruminant parasites that are present at high
155 prevalence in the study population, exhibiting greater-intensity infections in lactating
156 individuals (Albery *et al.*, 2020). Previous studies in this population have also
157 examined the nematode parasite *Elaphostrongylus cervi* and trematode parasite
158 *Fasciola hepatica* (Albery *et al.*, 2018, 2020). We chose to analyse strongyles but
159 not *E. cervi* or *F. hepatica* for several reasons: we did not want to add too many links
160 to the analysis for reasons of interpretability; strongyles are expected to inflict the
161 greatest costs (Hoberg *et al.*, 2001) and exhibited the most profound changes
162 associated with reproduction in terms of significance and effect size (Albery *et al.*,
163 2020); we did not expect *E. cervi* to have strong fitness effects (Irvine *et al.*, 2006);
164 and *F. hepatica* is present at relatively low prevalence in adult females, preventing
165 it from being fitted easily as an explanatory variable (Albery *et al.*, 2018).

166 We carried out antibody detection ELISAs designed to quantify mucosal IgA in
167 sheep using the faecal extractions stored at -20C (Watt *et al.*, 2016; Albery *et al.*,
168 2020). This protocol quantifies both total IgA levels as a measure of general immune
169 investment, and anti-*Teladorsagia circumcincta* IgA levels (anti-Tc IgA) as a specific
170 anti-strongyle measure. *T. circumcincta* is primarily a sheep strongyle, but the anti-
171 Tc IgA assay shows high cross-reactivity with a range of strongyle nematodes
172 including the mouse helminth *Heligmosomoides polygyrus* (Froy *et al.*, 2019). The
173 deer are infected with a selection of strongyle nematodes (Irvine *et al.*, 2006),
174 including *Teladorsagia circumcincta* (unpublished data); thus, anti-Tc IgA is used to
175 approximate anti-strongyle immune responses in the deer (Albery *et al.*, 2020). To
176 control for collection factors which introduce confounding variation in antibody levels
177 we used the residuals from a model including extraction session, time to freezing,
178 and collection day, as in previous studies (Albery *et al.*, 2019, 2020).

179 We also assayed faecal samples collected in November (Albery *et al.*, 2018).
180 However, females exhibited very low strongyle prevalence in the autumn compared
181 with spring and summer, preventing our FEC data from approximating normality and
182 providing little variation to test when fitted as an explanatory variable. Hence,
183 autumn data were excluded from our analyses.

184 Statistical analysis

185 All code and data are available at <https://github.com/gfalbery/DeerPaths>. To
186 investigate links among our variables we used path analysis using the D-sep
187 method, in which a set of linear models are fitted to the data, with some variables

188 appearing as both response and explanatory variables (Shibley, 2009). Combining
189 the linear models in this way allows identification of potential causal links and
190 mediating variables.

191 We created four Directed Acyclic Graphs (DAGs), each examining a different
192 fitness-related trait measured in the year following measurement of parasite burden
193 (see Figure 2). These measures included two fitness components: the female's
194 overwinter survival (0/1, where 1=survived to May 1 in year t+1) and fecundity in
195 year t+1 (0/1, where 1=gave birth in the following calving season). We also
196 examined two fitness-associated calving traits: the birth weight of a female's calf the
197 year t+1 (continuous, Gaussian distributed, based on a regression of capture weight
198 on capture age in days) and parturition date year t+1 (continuous, Gaussian
199 distributed, based on Julian date that year).

200 Our analyses used three immune and parasite measures: Total IgA level; Anti-Tc
201 IgA level; Strongyle count per gram of faeces (continuous, log(count+1)-transformed
202 to approximate normality). All were examined using a Gaussian error distribution.
203 We included two mutually exclusive binary reproductive categories representing the
204 reproductive effort made in year t (Clutton-Brock *et al.*, 1989): $Gestation_t$ (gave birth
205 to a calf which died before 1st October that year) and $Gestation_t + Lactation_t$ (gave
206 birth to a calf which survived to 1st October; hereafter referred to as simply
207 "Lactation", as all individuals that lactated must have also undergone the cost of
208 gestation). We also included variables to control for annual, seasonal, and age-
209 related variation: Year (categorical, with three levels: 2015, 2016, 2017); Season
210 (two levels: Summer, Spring); and Age (continuous, in years).

211 Each of the four DAGs was composed of four similar models, fitted using the INLA
212 package (Rue and Martino, 2009) in R version 3.5 (R Core Team, 2020). All
213 measures included female identity as a random effect to control for
214 pseudoreplication. First, we ran a set of three "input models", where the response
215 variable was an antibody or parasite measure. The aim of these models was to
216 quantify the association between reproduction in year t and the immune/parasite
217 measures, and to quantify links between these measures.

218 The models were specified as follows for each of our analyses, with
219 immune/parasite measures in bold and reproductive traits in italics. Variables in
220 brackets were included in the models, but are not displayed in the DAGs for clarity.

- 221 1. **Total IgA_t** ~ *Gestation_t + Lactation_t* (+ Age + Season + Year)
- 222 2. **Anti-Tc IgA_t** ~ **Total IgA_t** + *Gestation_t + Lactation_t* (+ Age + Season + Year)

223 3. **Strongyles_t ~ Anti-Tc IgA_t + Gestation_t + Lactation_t** (+ Age + Season +
224 Year)

225 4. Fitness-related trait_{t+1} ~ **Strongyles_t + Anti-Tc IgA_t + Total IgA_t +**
226 **Gestation_t + Lactation_t** (+ Age + Year)

227 Combining the estimates from models 1-3 with the estimates from model 4 allows
228 calculation of the direct and indirect (parasite- or immune-mediated) effects of
229 lactation and gestation on survival and reproduction in year t+1 (Figure 1). As an
230 example, we compared the magnitude and credibility intervals of direct lactation
231 effects (effect of lactation in the fitness-related trait model [model 4]) with indirect
232 effects (lactation effects on strongyle count [model 3] multiplied by the effects of
233 strongyle count on the fitness-related trait [model 4]). We took 1000 posterior draws
234 from each of the lactation-strongyle link and the strongyle-fitness trait link and
235 multiplied them together, and then derived the 95% credibility intervals for this link.
236 We compared these estimates with those for the direct lactation-fitness trait link to
237 investigate whether effects of lactation were likely to act independently and/or
238 through strongyle count. The models, fitness measures, and datasets used in each
239 analysis are described in Table 1.

240

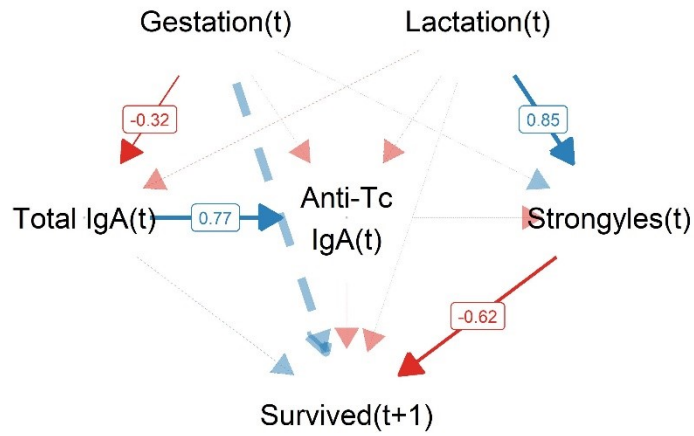
Model Set	Fitness Measure	Definition	Dataset	Samples	Individuals
1	Survival	Female survival to end of year t+1 (0/1)	All females (Spring and Summer year t)	485	134
2	Fecundity	Female reproduction in year t+1 (0/1)	All females (Summer year t)	223	107
3	Calf birth weight	Calf weight the year t+1 (Kg)	Females that reproduced the following May-June (Summer year t and Spring year t)	300	94
4	Parturition date	Date of parturition year t+1 (Days from 1 st January)		336	106

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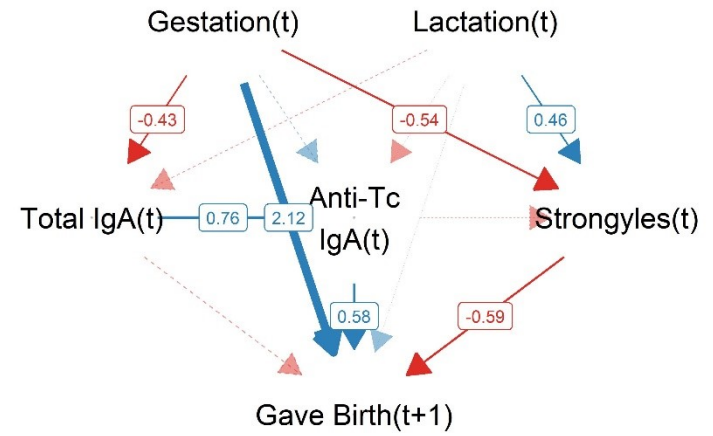
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Table 1: Descriptions of path analyses and the datasets used.

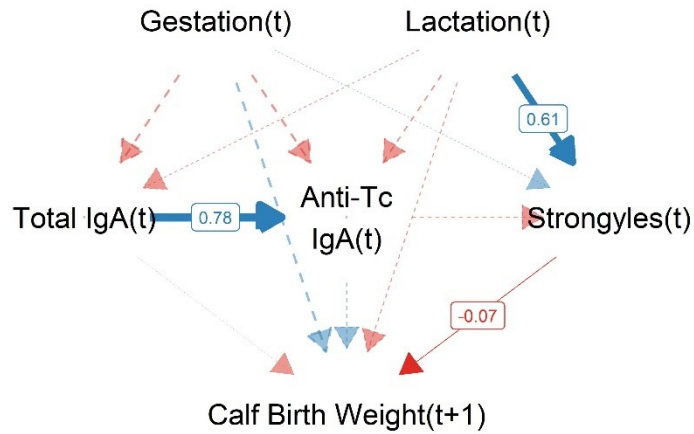
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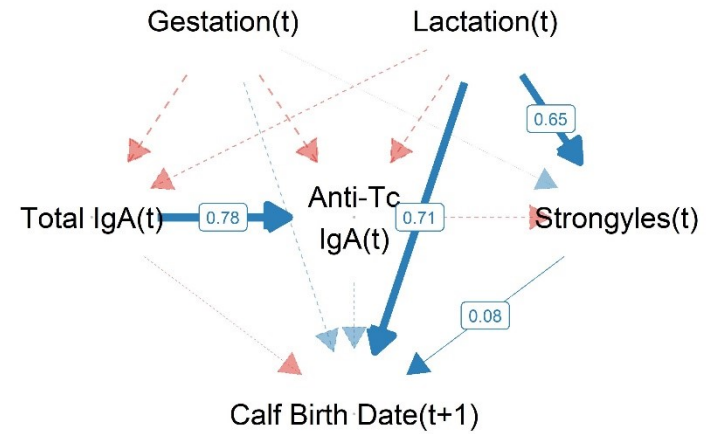
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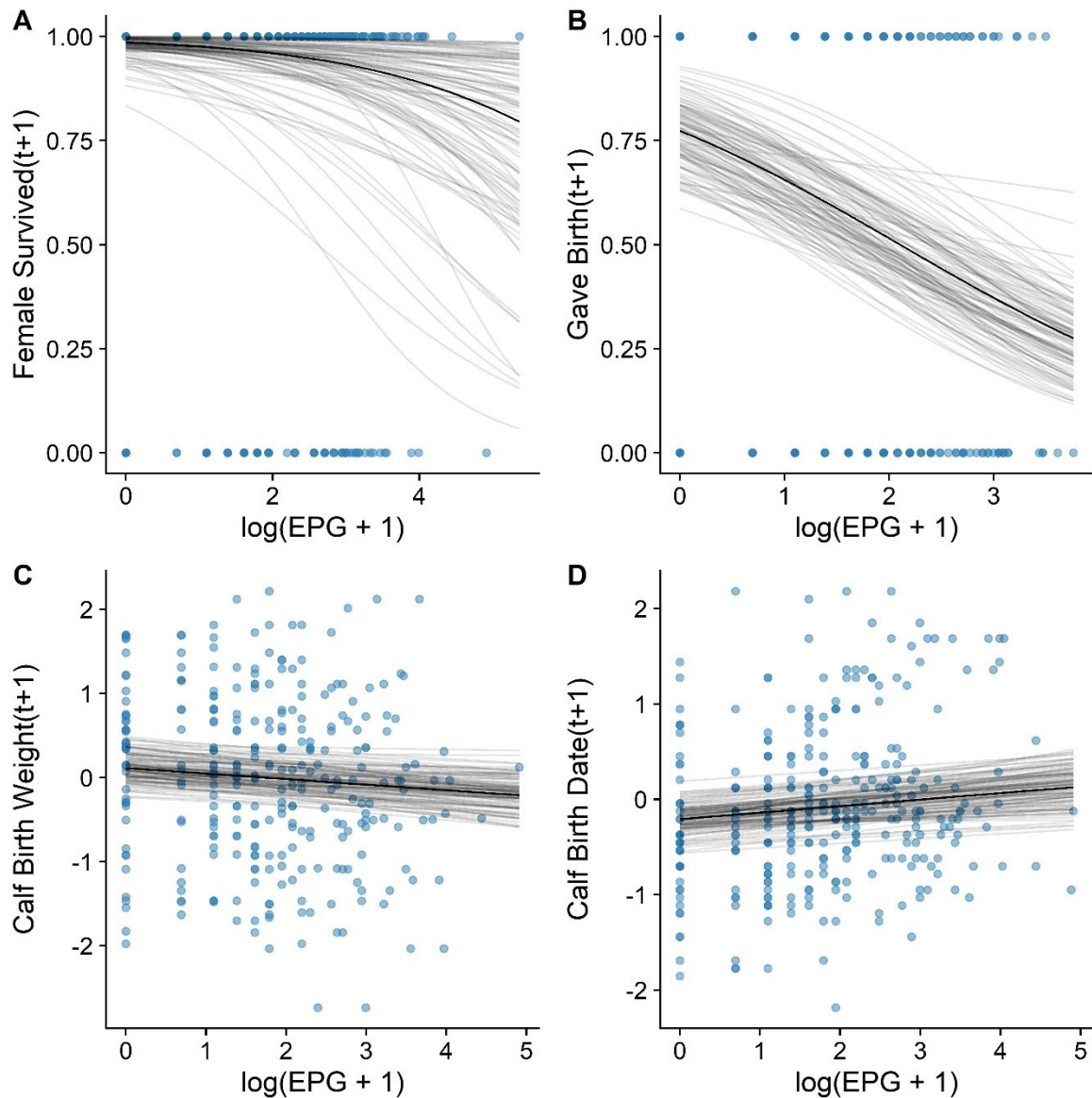
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246 Figure 3: Directed Acyclic Graphs (DAGs). Results are displayed for all four investigated female fitness-related response variables: overwinter
247 survival (A); reproduction the following year (B); subsequent calf birth weight (C); subsequent parturition date (D). Link colour depends on the

248 direction of the effect (blue=positive, red=negative); link width indicates the magnitude of the effect; and only solid, opaque links are significant
249 (estimates did not overlap with zero). Labels denote the link-scale effect sizes (slopes) for the significant effects, derived from GLMMs (full model
250 effects displayed in SI Figure 1).

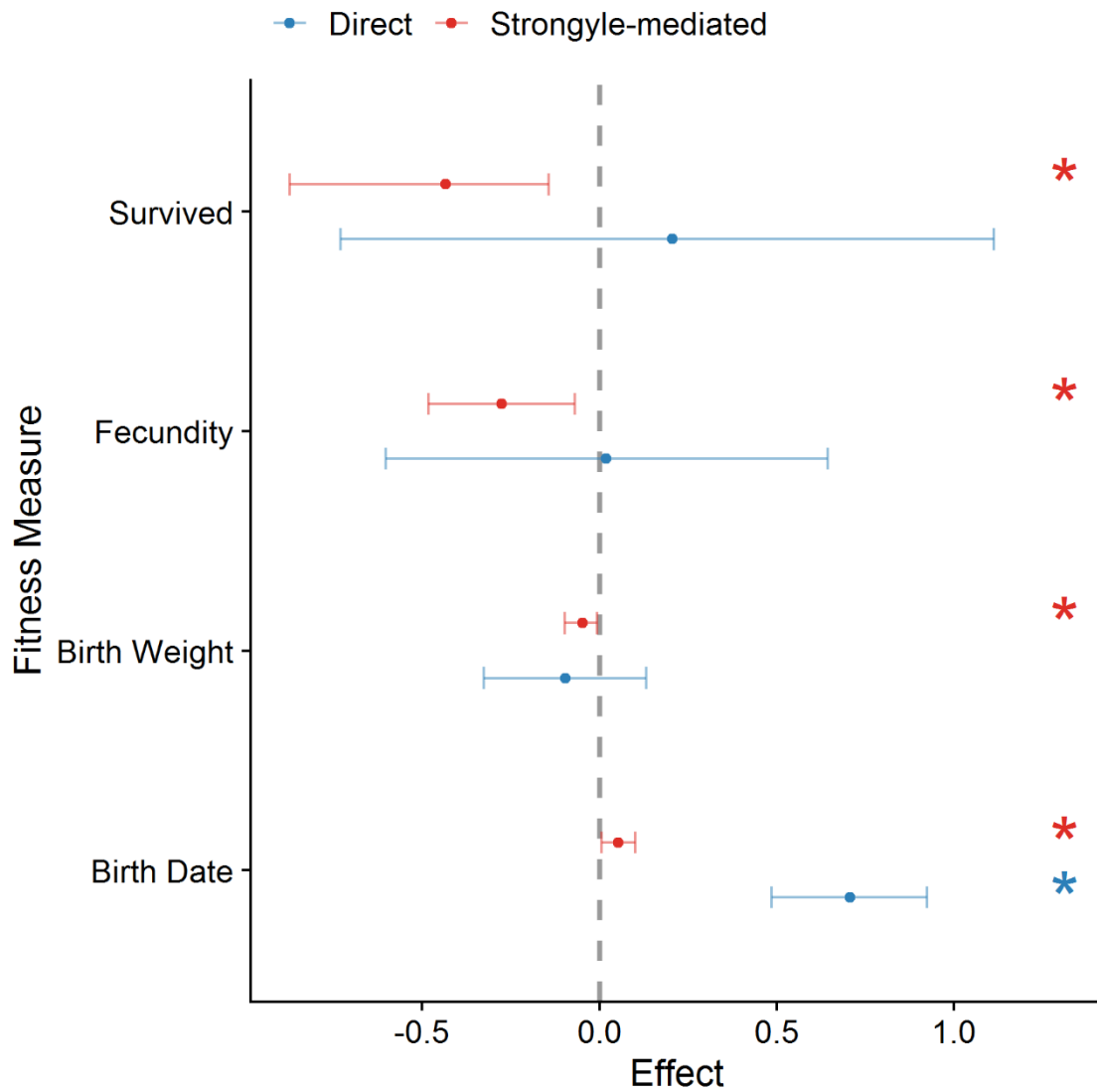


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253 Figure 4: Relationships between strongyle parasite count and subsequent survival and fecundity in
 254 female red deer. Results are displayed for all four investigated fitness-related response variables:
 255 overwinter survival (A); fecundity (B); subsequent calf birth weight (C); subsequent parturition date
 256 (D). The solid black lines denote the fitted slope of parasitism on the response variable. Transparent
 257 grey lines represent 100 draws from the posterior distribution to display variation in the estimated
 258 slope. Credibility intervals did not overlap with zero for any of the four relationships. Strongyle count
 259 was $\log(x+1)$ -transformed for analysis and plotting.

260

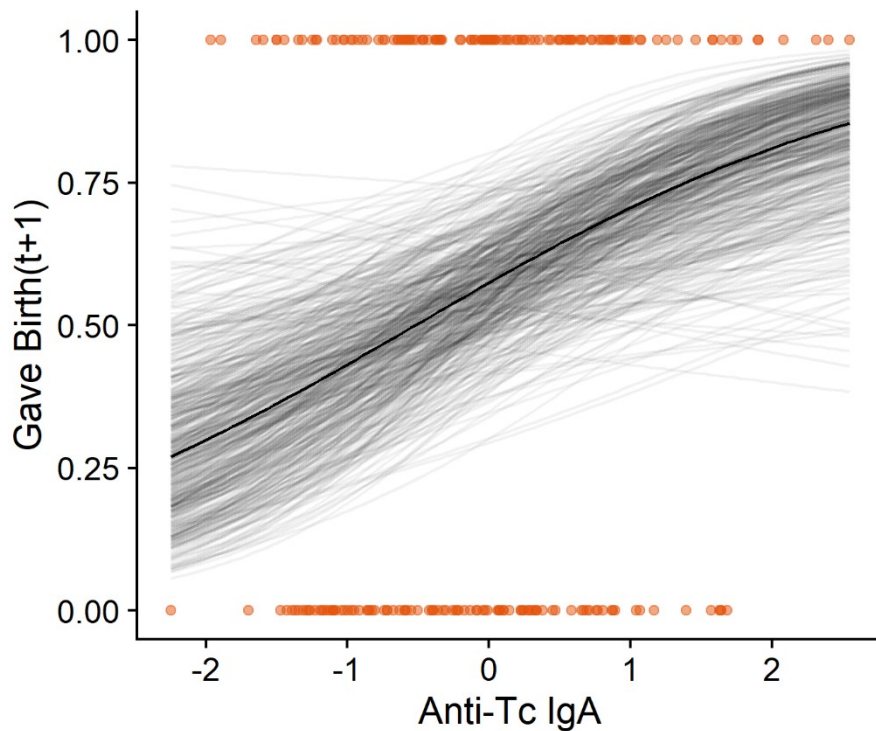
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263 Figure 5: Comparison of direct and indirect (parasite-mediated) effects of lactation on fitness-related
 264 traits on the link scale (logistic for survival and reproduction; Gaussian for birth weight and birth date).

265 Points represent mean effect estimates derived from the model posterior distributions; blue
 266 corresponds to direct effects, and red corresponds to indirect effects. Parameters with asterisks were
 267 significant: i.e., their credibility intervals did not overlap with zero.



268

269

270 Figure 6: Higher anti-*Teladorsagia circumcincta* IgA was associated with an increased probability of
 271 reproducing the following year in female red deer. Anti-Tc IgA was cube root-transformed and
 272 calculated from the residuals of a linear model including collection variables, and was then scaled to
 273 have a mean of 0 and a standard deviation of 1. The line represents the output of the reproduction
 274 probability model which includes lactation and strongyles as explanatory factors. The solid black line
 275 represents the mean of the anti-Tc IgA effect in the reproduction probability model, which includes
 276 lactation and strongyles as explanatory factors. Transparent grey lines represent 500 draws from the
 277 posterior distribution to display variation in the estimated slope.

278 Results

279 Path analyses consistently revealed strong positive associations between lactation and
 280 parasite count, and negative associations between parasite counts and subsequent survival
 281 and fecundity (Figures 3-5). In contrast, estimates for lactation's direct association with
 282 subsequent survival and fecundity overlapped with zero for all response variables except
 283 parturition date, supporting parasite-mediated costs of reproduction (Figures 3-5). Below, for
 284 each of the four fitness-related response variables, we describe the magnitude of the direct
 285 association with parasitism and with lactation, and lactation's association with parasitism
 286 multiplied by parasitism's association with the subsequent fitness-related trait (i.e., survival or
 287 fecundity). The latter gives an estimate of the indirect effect of lactation on the fitness-related
 288 trait acting through strongyle count. For effect sizes we give the mean and 95% credibility

289 intervals (CI). 1 log(EPG+1) increase corresponds to a ~3x increase in strongyle count. Full
290 model effect sizes are displayed in the supplementary information (Figure S11; Table S11).

291 Parasite count had a strong association with subsequent survival probability despite high
292 survival rates in the population (Figure 3A,4A). Females with the lowest counts (0 EPG, 10%
293 of samples) had a survival probability of ~100%, while those with the highest (>25EPG, 7% of
294 samples) had a survival probability of <90% (Figure 4A). Lactation was associated with
295 increased strongyle count (+0.85, CI 0.64, 0.99), so that a substantial cost of lactation for
296 survival acted through parasitism (Figure 5). Although this effect was highly significant on the
297 link (logistic) scale (Figure S11, Table S11), given the high survival rates in the population, at
298 the mean EPG value this lactation-associated increased strongyle parasitism would
299 correspond to only a ~2% decrease in survival probability. In contrast, estimates for the direct
300 effect of lactation on survival overlapped widely with zero, and the point estimate was greater
301 than zero, implying that individuals that lactate were slightly more likely to survive when the
302 effects of parasitism were accounted for (Figures 3A, 5).

303 Strongyles' association with subsequent reproduction had a similar effect size to its
304 association with overwinter survival (Figures 3B, 4B, 5; Table S11; Figure S11). An increase of
305 1 log(EPG+1) was associated with a decrease of ~15% probability of reproducing. 0 EPG
306 (17% of samples) corresponded to a ~77% chance of reproducing the following year, and
307 those with >20 EPG (6% of samples) had a reproduction probability of <36% (Figure 4B). The
308 direct effect of lactation on subsequent fecundity was negligible and had very large credibility
309 intervals, as with survival (Figure 5). In addition to the association with parasite count,
310 individuals with higher levels of anti-Tc IgA were more likely to reproduce the following year
311 (Figure 3B; Figure 6). An increase of 1 standard deviation of anti-Tc IgA levels corresponded
312 to an increase of ~10% in the probability of reproducing. Individuals with the lowest anti-Tc
313 IgA levels (less than -1 SD units) had a reproduction probability of <50%, compared to >75%
314 for those with the highest levels (>1 SD units; Figure 6). Finally, individuals that paid the cost
315 of gestation were much more likely to reproduce the following year, independently of the
316 effects of antibodies and parasites (Figure 3B).

317 Calving traits exhibited weaker associations with parasitism than did survival and
318 reproduction, although the results still implied an indirect cost of lactation acting through
319 strongyle count (Figures 3-5). The DAG for calf birth weight was similar to that for survival
320 (Figure 3C). An increase of 1 log(EPG+1) corresponded to a slight decrease in calf birth weight
321 the following year (0.07 SD units, or about 86g; Figure 4C). Females with the highest strongyle
322 intensities (>25 EPG) gave birth to calves which were ~400g lighter than those with the lowest
323 intensities (0 EPG), or around 6.24 kg compared to 6.65 kg. As with survival, there was poor

324 support for a direct association between lactation and birth weight (Figure 5). The estimates
325 for this direct effect were close to zero, and credibility intervals overlapped substantially with
326 zero (Figure 5). Lactation's positive effect on strongyle count once again resulted in a
327 significant negative indirect effect of lactation on subsequent calf birth weight acting through
328 strongyles, but the estimates were very small and nearly overlapped with zero (-0.0438kg, CI
329 -0.1111.6, -0.005.6).

330 In contrast to the other fitness-related traits investigated, there was support for a positive and
331 direct effect of lactation on parturition date the following year: that is, females whose calf
332 survived until the winter were likely to calve later in the following year (~8.5 days later, CI: 5.9,
333 11.2; Figure 3D,5), regardless of parasite count. There was a much weaker association
334 between strongyle count and parturition date: an increase of 1 log(EPG+1) produced a delay
335 in calving of ~0.93 days (CI: 0.12, 1.75; Figure 3D,4D,5). Lactation resulted in an increase of
336 0.7 log(EPG+1). Combining this estimate with the effect of parasitism on birth date gives an
337 estimate for an indirect effect of lactation acting on birth date totalling 0.58 days' delay (CI
338 0.06, 1.31; Figure 5). Parturition date was thus the only metric examined here for which
339 lactation's direct effect was larger than its indirect effect acting through strongyle count
340 (Figures 3-5).

341 There was a strong positive association between total IgA and anti-Tc IgA, as expected given
342 our previous findings (Albery *et al.*, 2020; Table SI1, Figure SI1). However, lactation had no
343 significant effect on anti-Tc IgA in our DAGs (Figure 3, Table SI1, Figure SI1).

344 Discussion

345 This study provides observational evidence for strong parasite-dependent mediation of
346 multiple life history tradeoffs in a large wild mammal. Lactation was associated with higher
347 parasite intensities which translated to reduced fecundity and survival probability in the
348 subsequent year. Among individuals that did reproduce the following year, those with high
349 strongyle counts gave birth slightly later in the year and to slightly smaller calves. These
350 findings represent the second evidence for such mediation of reproduction-survival tradeoffs
351 in a wild mammal (Leivesley *et al.*, 2019), and new evidence of parasites mediating
352 reproductive tradeoffs with subsequent reproductive traits. It is likely that much of the
353 subsequent reduction in survival and reproductive fitness associated with lactation in the Rum
354 red deer population (Clutton-Brock *et al.*, 1989; Froy *et al.*, 2016) is caused by strongyle
355 parasites, or that strongyle count closely corresponds to latent condition variables that are
356 responsible for mediating fitness. This finding supports parasites' role as an important
357 mediating factor in this system.

358 Lactation's negative association with subsequent fitness-related traits acted largely through
359 strongyle count for all response variables except parturition date. This may represent a
360 parasite-mediated cost, where pathology and resource allocation associated with increased
361 parasitism are the primary cause of increased overwinter mortality and reduced subsequent
362 fecundity in lactating individuals (Clutton-Brock *et al.*, 1989). Allocation of resources to
363 lactation and associated physiological changes likely reduces resources available for
364 resistance and damage repair mechanisms, rendering lactating females more susceptible to
365 strongyles (Sheldon and Verhulst, 1996; Speakman, 2008). Notably, while most literature on
366 reproduction-parasitism-fitness relationships revolves around energetic/resource tradeoffs, it
367 is also possible that non-energetic, mechanistic relationships between reproduction,
368 physiology, and survival are partially responsible (Speakman, 2008; Stahlschmidt *et al.*, 2013);
369 for example, the greater parasite counts in lactating individuals may be the product of greater
370 exposure through heightened forage intake (necessitated by greater resource demand), rather
371 than originating from increased susceptibility incurred by greater reproductive allocation
372 (Albery *et al.*, 2020). Because strongyle intensity exhibits strong seasonality, strongyle
373 abundance on the pasture could likewise influence the shape of these tradeoffs; however,
374 because intensity peaks in spring (Albery *et al.*, 2018), it is unlikely that variation in
375 environmental abundance is responsible for driving the differential costs of gestation and
376 lactation that we see.

377 High parasite counts in lactating females may cause gut pathology, interfering with nutrient
378 absorption and thereby exacerbating the nutritional scarcity of the winter period, leading to
379 overwinter mortality (Gulland, 1992; Pedersen and Greives, 2008; Maublanc *et al.*, 2009), as
380 well as reducing females' ability to achieve the body condition necessary to conceive and carry
381 a calf to term (Albon *et al.*, 1986). This reduction in body condition could likewise cause
382 females to give birth later in the year and to a calf that is smaller. There are two time points at
383 which strongyles may reduce fecundity: first, parasites may impact females in the resource-
384 abundant summer and early autumn, preventing them from conceiving in the autumn mating
385 season. In this case, strongyle-associated pathology may occur somewhat independently of
386 overwinter nutritional scarcity. Alternatively, strongyles may cause females to lose their
387 pregnancies over winter. This possibility may be tested in the future by investigating whether
388 more highly parasitised females are less likely to be observed mating (demonstrating reduced
389 conception rates), or only less likely to give birth.

390 Lactation exerts a substantial resource cost that results in reduced condition; therefore, it is
391 also possible that we observed a negative relationship between parasitism and fitness
392 because both were determined by condition, rather than because parasites were causally
393 responsible for reducing fitness. Strongyle counts are associated with decreased body

394 condition in shot individuals in this population, supporting this possibility (Irvine *et al.*, 2006).
395 Similarly, an important role for condition is supported by our observation that higher anti-Tc
396 IgA levels were associated with increased fecundity the following year, independently of any
397 associations between 1) strongyles and fecundity and 2) anti-Tc IgA and strongyles
398 themselves (Figure 6). It is highly likely that anti-Tc IgA is well-correlated with an unmeasured
399 component of individual quality such as fat content (Demas *et al.*, 2003) which is linked to
400 fitness both in the deer (Albon *et al.*, 1986) and in other systems (Milenkaya *et al.*, 2015). This
401 possibility reflects the confounding effects of individual quality in observational studies of
402 tradeoffs (van Noordwijk and de Jong, 1986). We were unfortunately unable to replicate
403 previous findings of lactation costs for mucosal antibodies (Albery *et al.*, 2020), likely due to
404 extremely reduced sample sizes (485 samples and fewer here compared to 837 samples
405 previously), and so we were unable to link the anti-Tc IgA-fitness association as part of a
406 reproduction-fitness tradeoff. Another potential fitness-mediating factor is body weight, which
407 is often used to control for condition-driven versus parasite-driven fitness effects; however,
408 analyses in Soay sheep often show that strongyle-fitness associations occur independently
409 of, or in addition to body weight (e.g. Sparks *et al.*, 2018; Froy *et al.*, 2019; Leivesley *et al.*,
410 2019). In addition, although condition-parasitism relationships are well-documented, negative
411 effects are far from ubiquitous and their slopes are relatively shallow on average (Sánchez *et al.*,
412 2018). This difficulty untangling fitness consequences of parasitism from their condition-
413 dependent correlates is indicative of a wider problem in observational disease ecology studies;
414 specifically, confirming fitness consequences of infection often requires treatment with
415 antiparasitics to compare the fitness of treated *versus* untreated individuals (Graham *et al.*,
416 2011; Fenton *et al.*, 2014; Pedersen and Fenton, 2015). We emphasize that while path
417 analysis is useful for inference of likely causal links (Shipley, 2009), the approach does not
418 confirm causality any more than other types of statistical analysis. For example, links among
419 variables may belie more complex associations and/or may be confounded with hidden (latent)
420 variables. Path analysis must therefore be used carefully, and in conjunction with specific *a*
421 *priori* hypotheses and feasible causal pathways (e.g. see Figure 1).

422 Whether or not strongyles are the effectors, our findings nevertheless support the use of these
423 parasites as a proxy for an individual's health and as predictors of its subsequent fitness. If
424 our results are indicative of underlying fitness costs, strongyles will have a strong mediating
425 effect on population dynamics, for two principal reasons: first, by reducing both survival and
426 fecundity simultaneously, and second, by exhibiting different relationships with past and
427 subsequent reproduction. As such, it stands to reason that their impact will prevent females
428 from reproducing in a single year, potentially stabilising population fluctuations. Further years
429 of data will reveal how parasite abundances relate to the population dynamics of the deer, and

430 particularly whether inter-annual variation in strongyle numbers can explain population size
431 (Wilson *et al.*, 2004). At higher population densities the deer exhibit delayed maturity and
432 reduced fecundity (Albon *et al.*, 1983); the lactation-strongyle-fecundity tradeoff offers a
433 potential mechanism behind this fecundity reduction, particularly as parasitism should
434 increase at higher densities (Altizer *et al.*, 2003; Wilson *et al.*, 2004). Local population density
435 also influences fitness-related traits in this population (Coulson *et al.*, 1997) and parasitism
436 demonstrates fine-scale spatial variation (Albery *et al.*, 2019), so this life history mediation
437 could likewise occur at relatively fine spatial scales. A similar study in Soay sheep on St Kilda
438 demonstrated that strongyles mediate a reproduction-survival tradeoff, but without examining
439 similar reproduction-fecundity tradeoffs, partly because most sheep do not take years off
440 between reproduction events (Leivesley *et al.*, 2019). The fecundity reduction seen in the deer
441 and the strength of these parasite-mediated tradeoffs potentially contribute to the population's
442 relatively weak population fluctuations, particularly compared to the large fluctuations seen in
443 the Soay sheep (Clutton-Brock and Pemberton, 2004). As such, parasite-dependent life
444 history mediation may be an important contributing factor determining the strength of
445 oscillatory population dynamics.

446 Finally, having uncovered costs of parasitism in adult females, it would be interesting to
447 investigate whether other age and sex categories experience similar fitness effects: e.g., do
448 more highly parasitised males sire fewer calves, and are more highly parasitised calves less
449 likely to survive to maturity? Do maternal costs transfer to their calves, providing another
450 potentially important mediating mechanism (Martin and Festa-Bianchet, 2010)? Future studies
451 in this population could elaborate on these findings by investigating how maternal and calf
452 parasitism correlate and correspond to maternal and calf fitness, quantifying transgenerational
453 immunity-parasitism-fitness correlations: a topic that is largely understudied and likely
454 influences ecological and epidemiological dynamics considerably (Roth *et al.*, 2018).

455 [References](#)

- 456 **Albery, G. F., Kenyon, F., Morris, A., Morris, S., Nussey, D. H. D. H. and Pemberton, J.**
457 **M. J. M.** (2018). Seasonality of helminth infection in wild red deer varies between
458 individuals and between parasite taxa. *Parasitology* **145**, 1–11. doi:
459 10.1017/S0031182018000185.
- 460 **Albery, G. F., Becker, D. J., Kenyon, F., Nussey, D. H. and Pemberton, J. M.** (2019). The
461 fine-scale landscape of immunity and parasitism in a wild ungulate population.
462 *Integrative and Comparative Biology* **icz016**, 1–11. doi: 10.1093/icb/icz016.
- 463 **Albery, G. F., Watt, K. A., Keith, R., Morris, S., Morris, A., Kenyon, F., Nussey, D. H. and**
464 **Pemberton, J. M.** (2020). Reproduction has different costs for immunity and parasitism

- 465 in a wild mammal. *Functional Ecology* **34**, 229–239. doi: 10.1111/1365-2435.13475.
- 466 **Albon, S. D., Mitchell, B. and Staines, B. W.** (1983). Fertility and Body Weight in Female
467 Red Deer: A Density-Dependent Relationship. *Journal of Animal Ecology* **52**, 969–980.
- 468 **Albon, S. D., Mitchell, B., Huby, B. J. and Brown, D.** (1986). Fertility in female red deer
469 (*Cervus elaphus*): the effects of body composition, age and reproductive status. *Journal*
470 *of Zoology (London)* **209**, 447–460. doi: 10.1111/j.1469-7998.1986.tb03603.x.
- 471 **Albon, S. D., Stien, A., Irvine, R. J., Langvatn, R., Ropstad, E. and Halvorsen, O.** (2002).
472 The role of parasites in the dynamics of a reindeer population. *Proceedings of the*
473 *Royal Society B: Biological Sciences* **269**, 1625–1632. doi: 10.1098/rspb.2002.2064.
- 474 **Altizer, S., Nunn, C. L., Thrall, P. H., Gittleman, J. L., Antonovics, J., Cunningham, A.**
475 **A., Dobson, A. P., Ezenwa, V., Jones, K. E., Pedersen, A. B., Poss, M. and Pulliam,**
476 **J. R. C.** (2003). Social Organization and Parasite Risk in Mammals: Integrating Theory
477 and Empirical Studies. *Annual Review of Ecology, Evolution, and Systematics* **34**, 517–
478 547. doi: 10.1146/annurev.ecolsys.34.030102.151725.
- 479 **Brambilla, A., Biebach, I., Bassano, B., Bogliani, G., von Hardenberg, A., Hardenberg,**
480 **A. Von, Brambilla, A. and von Hardenberg, A.** (2015). Direct and indirect causal
481 effects of heterozygosity on fitness-related traits in Alpine ibex. *Proceedings of the*
482 *Royal Society B: Biological Sciences* **282**, 20141873. doi: 10.1098/rspb.2014.1873.
- 483 **Cizauskas, C. A., Turner, W. C., Pitts, N. and Getz, W. M.** (2015). Seasonal patterns of
484 hormones, macroparasites, and microparasites in wild african ungulates: The interplay
485 among stress, reproduction, and disease. *PLoS ONE* **10**, 1–29. doi:
486 10.1371/journal.pone.0120800.
- 487 **Clutton-Brock, T. H. and Pemberton, J. M. (Josephine M. .** (2004). *Soay sheep :*
488 *population dynamics and selection on St. Kilda*. Cambridge University Press.
- 489 **Clutton-Brock, T. H., Guinness, F. E. and Albon, S. D.** (1982). *Red Deer: Behavior and*
490 *Ecology of Two Sexes*. University of Chicago Press, Chicago, IL.
- 491 **Clutton-Brock, T. H., Albon, S. D. and Guinness, F. E.** (1989). Fitness costs of gestation
492 and lactation in wild mammals. *Nature* **337**, 260–262. doi: 10.1038/337260a0.
- 493 **Coltman, D. W., Pilkington, J. G., Smith, J. a, Pemberton, J. M. and Josephine, M.**
494 (1999). Parasite-Mediated Selection against Inbred Soay Sheep in a Free-Living, Island
495 Population. *Evolution* **53**, 1259–1267. doi: 10.2307/2640828.
- 496 **Coulson, T., Albon, S., Guinness, F., Pemberton, J. and Clutton-Brock, T.** (1997).

- 497 Population Substructure, Local Density, and Calf Winter Survival in Red Deer (*Cervus*
498 *elaphus*). *Ecology* **78**, 852. doi: 10.2307/2266064.
- 499 **Debeffe, L., McLoughlin, P. D., Medill, S. A., Stewart, K., Andres, D., Shury, T., Wagner,**
500 **B., Jenkins, E., Gilleard, J. S. and Poissant, J.** (2016). Negative covariance between
501 parasite load and body condition in a population of feral horses. *Parasitology* **143**, 983–
502 997. doi: 10.1017/S0031182016000408.
- 503 **Demas, G. E., Drazen, D. L. and Nelson, R. J.** (2003). Reductions in total body fat
504 decrease humoral immunity. *Proceedings of the Royal Society B: Biological Sciences*
505 **270**, 905–911. doi: 10.1016/j.microc.2018.08.056.
- 506 **Fenton, A., Knowles, S. C. L., Petchey, O. L. and Pedersen, A. B.** (2014). The reliability
507 of observational approaches for detecting interspecific parasite interactions :
508 comparison with experimental results. *International Journal for Parasitology* **44**, 437–
509 445. doi: 10.1016/j.ijpara.2014.03.001.
- 510 **Festa-Bianchet, M.** (1989). Individual Differences, Parasites, and the Costs of Reproduction
511 for Bighorn Ewes (*Ovis canadensis*). *Journal of Animal Ecology* **58**, 785–795. doi:
512 10.2307/5124.
- 513 **Froy, H., Walling, C. A., Pemberton, J. M., Clutton-brock, T. H. and Kruuk, L. E. B.**
514 (2016). Relative costs of offspring sex and offspring survival in a polygynous mammal.
515 *Biology Letters* **12**, 20160417. doi: 10.1098/rsbl.2016.0417.
- 516 **Froy, H., Sparks, A. M., Watt, K., Sinclair, R., Bach, F., Pilkington, J. G., Pemberton, J.**
517 **M., Mcneilly, T. N. and Nussey, D. H.** (2019). Senescence in immunity against
518 helminth parasites predicts adult mortality in a wild mammal. *Science* **365**, 1296–1298.
- 519 **Graham, A. L., Shuker, D. M., Pollitt, L. C., Auld, S. K. J. R., Wilson, A. J., Little, T. J.,**
520 **Wilson, J. and Little, T. J.** (2011). Fitness consequences of immune responses:
521 Strengthening the empirical framework for ecoimmunology. *Functional Ecology* **25**, 5–
522 17. doi: 10.1111/j.1365-2435.2010.01777.x.
- 523 **Gulland, F. M. D.** (1992). The role of nematode parasites in Soay sheep (*Ovis aries* L.)
524 mortality during a population crash. *Parasitology* **105 (Pt 3)**, 493–503. doi:
525 10.1017/S0031182000074679.
- 526 **Harshman, L. G. and Zera, A. J.** (2007). The cost of reproduction: the devil in the details.
527 *Trends in Ecology and Evolution* **22**, 80–86. doi: 10.1016/j.tree.2006.10.008.
- 528 **Hoberg, E. P., Kocan, A. A. and Rickard, L. G.** (2001). Gastrointestinal strongyles in wild

529 ruminants. *Diseases of Wild Mammals* 193–227. doi: 10.1002/9780470377000.ch8.

530 **Hughes, J., Albon, S. D., Irvine, R. J. and Woodin, S.** (2009). Is there a cost of parasites
531 to caribou? *Parasitology* **136**, 253–65. doi: 10.1017/S0031182008005246.

532 **Irvine, R. J., Corbishley, H., Pilkington, J. G. and Albon, S. D.** (2006). Low-level parasitic
533 worm burdens may reduce body condition in free-ranging red deer (*Cervus elaphus*).
534 *Parasitology* **133**, 465–475. doi: 10.1017/S0031182006000606.

535 **Kenyon, F., McBean, D., Greer, A. W., Burgess, C. G. S., Morrison, A. A., Bartley, D. J.,**
536 **Bartley, Y., Devin, L., Nath, M. and Jackson, F.** (2013). A comparative study of the
537 effects of four treatment regimes on ivermectin efficacy, body weight and pasture
538 contamination in lambs naturally infected with gastrointestinal nematodes in Scotland.
539 *International Journal for Parasitology: Drugs and Drug Resistance* **3**, 77–84. doi:
540 10.1016/j.ijpddr.2013.02.001.

541 **Knowles, S. C. L., Nakagawa, S. and Sheldon, B. C.** (2009). Elevated reproductive effort
542 increases blood parasitaemia and decreases immune function in birds: A meta-
543 regression approach. *Functional Ecology* **23**, 405–415. doi: 10.1111/j.1365-
544 2435.2008.01507.x.

545 **Krams, I. A., Rumvolt, K., Saks, L., Krams, R., Elferts, D., Vrublevska, J., Rantala, M. J.,**
546 **Kecko, S., Cīrule, D., Luoto, S. and Krama, T.** (2017). Reproduction compromises
547 adaptive immunity in a cyprinid fish. *Ecological Research* **32**, 559–566. doi:
548 10.1007/s11284-017-1467-y.

549 **Leivesley, J. A., Bussi re, L. F., Pemberton, J. M., Pilkington, J. G., Wilson, K. and**
550 **Hayward, A. D.** (2019). Survival costs of reproduction are mediated by parasite
551 infection in wild Soay sheep. *Ecology Letters* ele.13275. doi: 10.1111/ele.13275.

552 **Martin, J. G. A. and Festa-Bianchet, M.** (2010). Bighorn Ewes Transfer the Costs of
553 Reproduction to Their Lambs. *The American Naturalist* **176**, 414–423. doi:
554 10.1086/656267.

555 **Maublanc, M. L., Bideau, E., Picot, D., Rame, J. L., Dubois, M., Fert , H., Gerard, J. F.,**
556 **Rames, J., Dubois, M., Fert , H. and Gerard, J. F.** (2009). Demographic crash
557 associated with high parasite load in an experimental roe deer (*Capreolus capreolus*)
558 population. *European Journal of Wildlife Research* **55**, 621–625. doi: 10.1007/s10344-
559 009-0298-8.

560 **Milenkaya, O., Catlin, D. H., Legge, S. and Walters, J. R.** (2015). Success but Not Survival
561 in a Sedentary , Tropical Bird. *PLoS Neglected Tropical Diseases* **10**, 1–18. doi:

562 10.5061/dryad.3n2j5.

563 **Neggazi, S. A., Noreikiene, K., Öst, M. and Jaatinen, K.** (2016). Reproductive investment
564 is connected to innate immunity in a long-lived animal. *Oecologia* **182**, 347–356. doi:
565 10.1007/s00442-016-3657-7.

566 **Pacejka, A. J., Gratton, C. M. and Thompson, C. F.** (1998). Do potentially virulent mites
567 affect house wren (*Troglodytes aedon*) reproductive success? *Ecology* **79**, 1797–1806.
568 doi: 10.1890/0012-9658(1998)079[1797:DPVMAH]2.0.CO;2.

569 **Pedersen, A. B. and Fenton, A.** (2015). The role of antiparasite treatment experiments in
570 assessing the impact of parasites on wildlife. *Trends in Parasitology* **31**, 200–211. doi:
571 10.1016/j.pt.2015.02.004.

572 **Pedersen, A. B. and Greives, T. J.** (2008). The interaction of parasites and resources
573 cause crashes in a wild mouse population. *Journal of Animal Ecology* **77**, 370–377. doi:
574 10.1111/j.1365-2656.2007.01321.x.

575 **R Core Team** (2020). R: A language and environment for statistical computing. R
576 Foundation for Statistical Computing, Vienna, Austria.

577 **Rödel, H. G., Zapka, M., Stefanski, V. and von Holst, D.** (2016). Reproductive effort alters
578 immune parameters measured post-partum in European rabbits under semi-natural
579 conditions. *Functional Ecology* **30**, 1800–1809. doi: 10.1111/1365-2435.12663.

580 **Roth, O., Beemelmans, A., Barribeau, S. M. and Sadd, B. M.** (2018). Recent advances
581 in vertebrate and invertebrate transgenerational immunity in the light of ecology and
582 evolution. *Heredity* **121**, 225–238. doi: 10.1038/s41437-018-0101-2.

583 **Rue, H. and Martino, S.** (2009). Approximate Bayesian inference for latent Gaussian
584 models by using integrated nested Laplace approximations. *Statistical Methodology* **71**,
585 319–392.

586 **Sánchez, C. A., Becker, D. J., Teitelbaum, C. S., Barriga, P., Brown, L. M., Majewska, A.**
587 **A., Hall, R. J. and Altizer, S.** (2018). On the relationship between body condition and
588 parasite infection in wildlife: a review and meta-analysis. *Ecology Letters* **21**, 1869–
589 1884. doi: 10.1111/ELE.13160.

590 **Sheldon, B. C. and Verhulst, S.** (1996). Ecological immunology - costly parasite defenses
591 and trade- offs in evolutionary ecology. *Trends in Ecology & Evolution* **11**, 317–321. doi:
592 10.1016/0169-5347(96)10039-2.

593 **Shipley, B.** (2009). Confirmatory path analysis in a generalized multilevel context. *Ecology*

594 **90**, 363–368. doi: 10.1890/08-1034.1.

595 **Sparks, A. M., Watt, K., Sinclair, R., Pilkington, J. G., Pemberton, J. M., Johnston, S. E.,**
596 **McNeilly, T. N. and Nussey, D. H.** (2018). Natural selection on antihelminth antibodies
597 in a wild mammal population. *American Naturalist* **192**, 745–760. doi: 10.1086/700115.

598 **Speakman, J. R.** (2008). The physiological costs of reproduction in small mammals.
599 *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**, 375–398.
600 doi: 10.1098/rstb.2007.2145.

601 **Stahlschmidt, Z. R., Rollinson, N., Acker, M. and Adamo, S. A.** (2013). Are all eggs
602 created equal? Food availability and the fitness trade-off between reproduction and
603 immunity. *Functional Ecology* **27**, 800–806. doi: 10.1111/1365-2435.12071.

604 **Stearns, S. C.** (1989). Trade-Offs in Life-History Evolution. *Functional Ecology* **3**, 259–268.
605 doi: 10.2307/2389364.

606 **Stjernman, M., Raberg, L. and Nilsson, J.** (2004). Survival costs of reproduction in the
607 blue tit (*Parus caeruleus*): a role for blood parasites? *Proceedings of the Royal Society*
608 *B: Biological Sciences* **271**, 2387–2394. doi: 10.1098/rspb.2004.2883.

609 **Taylor, M. A.; Coop, R. L.; Wall, R. L.** (2015). Veterinary Parasitology. 1–1006. doi:
610 10.1002/9781119073680.

611 **van Noordwijk, A. J. and de Jong, G.** (1986). Acquisition and Allocation of Resources:
612 Their Influence on Variation in Life History Tactics. *The American Naturalist* **128**, 137–
613 142. doi: 10.1086/284547.

614 **Vandegrift, K. J., Raffel, T. R. and Hudson, P. J.** (2008). Parasites prevent summer
615 breeding in white-footed mice, *Peromyscus leucopus*. *Ecology* **89**, 2251–2258. doi:
616 10.1890/07-1935.1.

617 **Watt, K. A., Nussey, D. H., Maclellan, R., Pilkington, J. G. and McNeilly, T. N.** (2016).
618 Fecal antibody levels as a noninvasive method for measuring immunity to
619 gastrointestinal nematodes in ecological studies. *Ecology and Evolution* **6**, 56–67. doi:
620 10.1002/ece3.1858.

621 **Williams, G. C.** (1966). Natural Selection, the Costs of Reproduction, and a Refinement of
622 Lack's Principle. *The American naturalist* **100**, 687–690.

623 **Wilson, K., Grenfell, B. T., Pilkington, J. G., Boyd, H. E. G. and Gulland, F. M. D.** (2004).
624 Parasites and their impact. In *Soay Sheep: Dynamics and Selection in an Island*
625 *Population* (ed. T. Clutton-Brock and J. Pemberton), pp. 113–165. Cambridge

626 University Press.

627