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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.

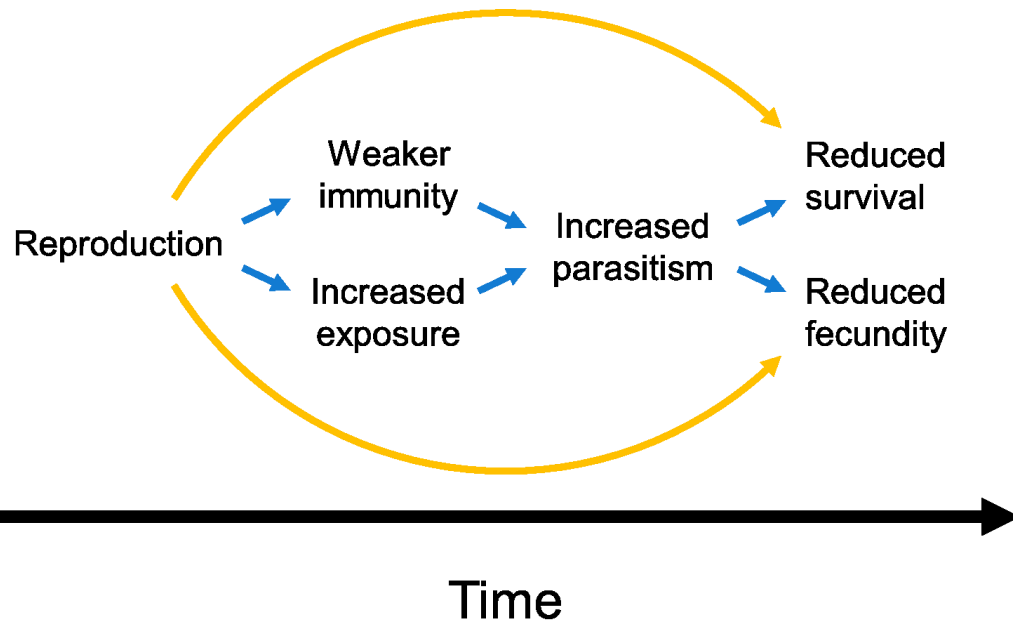
## 20 Introduction

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.

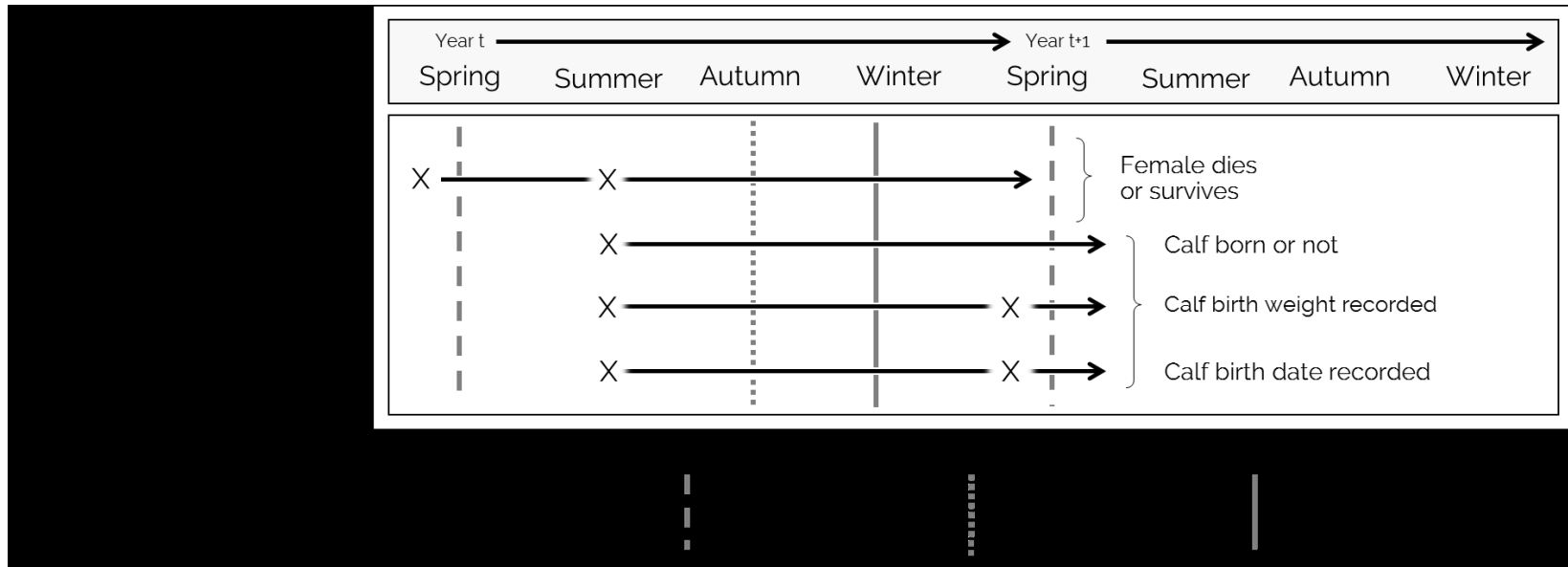
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65 Many studies examining reproduction-immunity-parasitism interrelationships have  
66 been carried out in birds, often using experiments in which reproductive effort is  
67 artificially increased by manipulating clutch sizes (Knowles *et al.*, 2009). 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 1<sup>st</sup>, 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 1<sup>st</sup> October that year) and  $Gestation_t + Lactation_t$  (gave  
206 birth to a calf which survived to 1<sup>st</sup> 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<sub>t</sub>** ~ *Gestation<sub>t</sub> + Lactation<sub>t</sub>* (+ Age + Season + Year)
- 222 2. **Anti-Tc IgA<sub>t</sub>** ~ **Total IgA<sub>t</sub>** + *Gestation<sub>t</sub> + Lactation<sub>t</sub>* (+ Age + Season + Year)

223 3. **Strongyles<sub>t</sub> ~ Anti-Tc IgA<sub>t</sub> + Gestation<sub>t</sub> + Lactation<sub>t</sub>** (+ Age + Season +  
224 Year)

225 4. Fitness-related trait<sub>t+1</sub> ~ **Strongyles<sub>t</sub> + Anti-Tc IgA<sub>t</sub> + Total IgA<sub>t</sub> +**  
226 **Gestation<sub>t</sub> + Lactation<sub>t</sub>** (+ 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.

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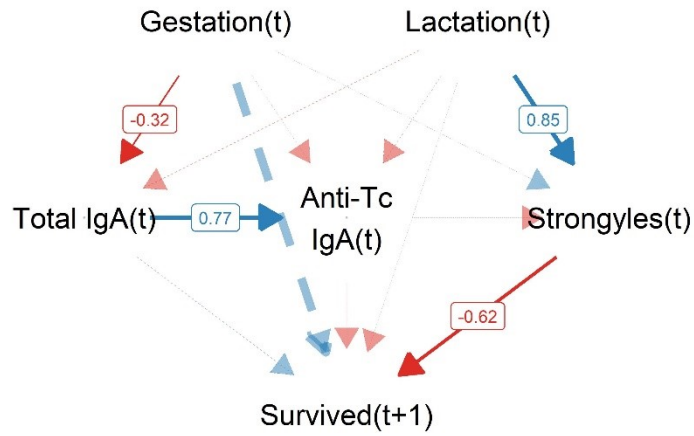
<b>Model Set</b>	<b>Fitness Measure</b>	<b>Definition</b>	<b>Dataset</b>	<b>Samples</b>	<b>Individuals</b>
<b>1</b>	Survival	Female survival to end of year t+1 (0/1)	All females (Spring and Summer year t)	485	134
<b>2</b>	Fecundity	Female reproduction in year t+1 (0/1)	All females (Summer year t)	223	107
<b>3</b>	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
<b>4</b>	Parturition date	Date of parturition year t+1 (Days from 1 <sup>st</sup> January)		336	106

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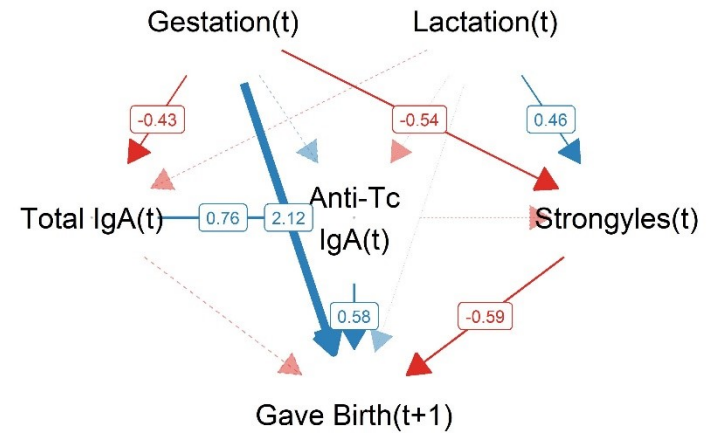
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Table 1: Descriptions of path analyses and the datasets used.

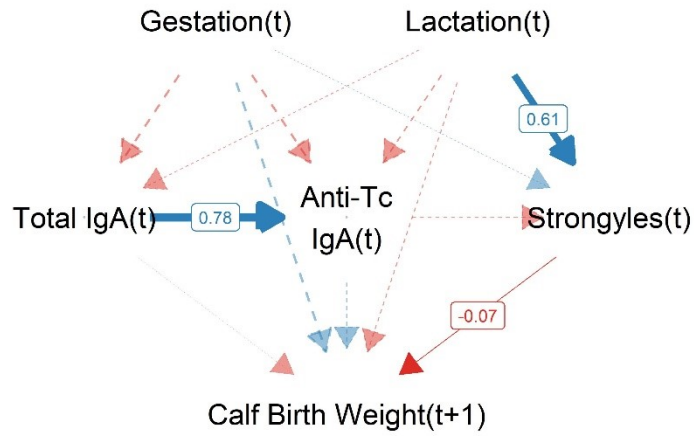
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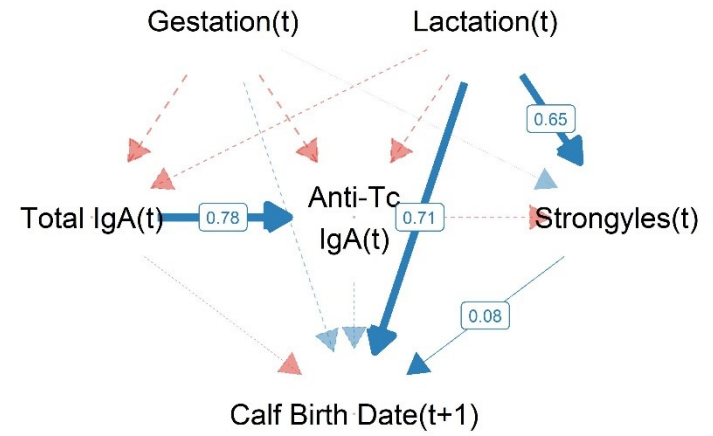
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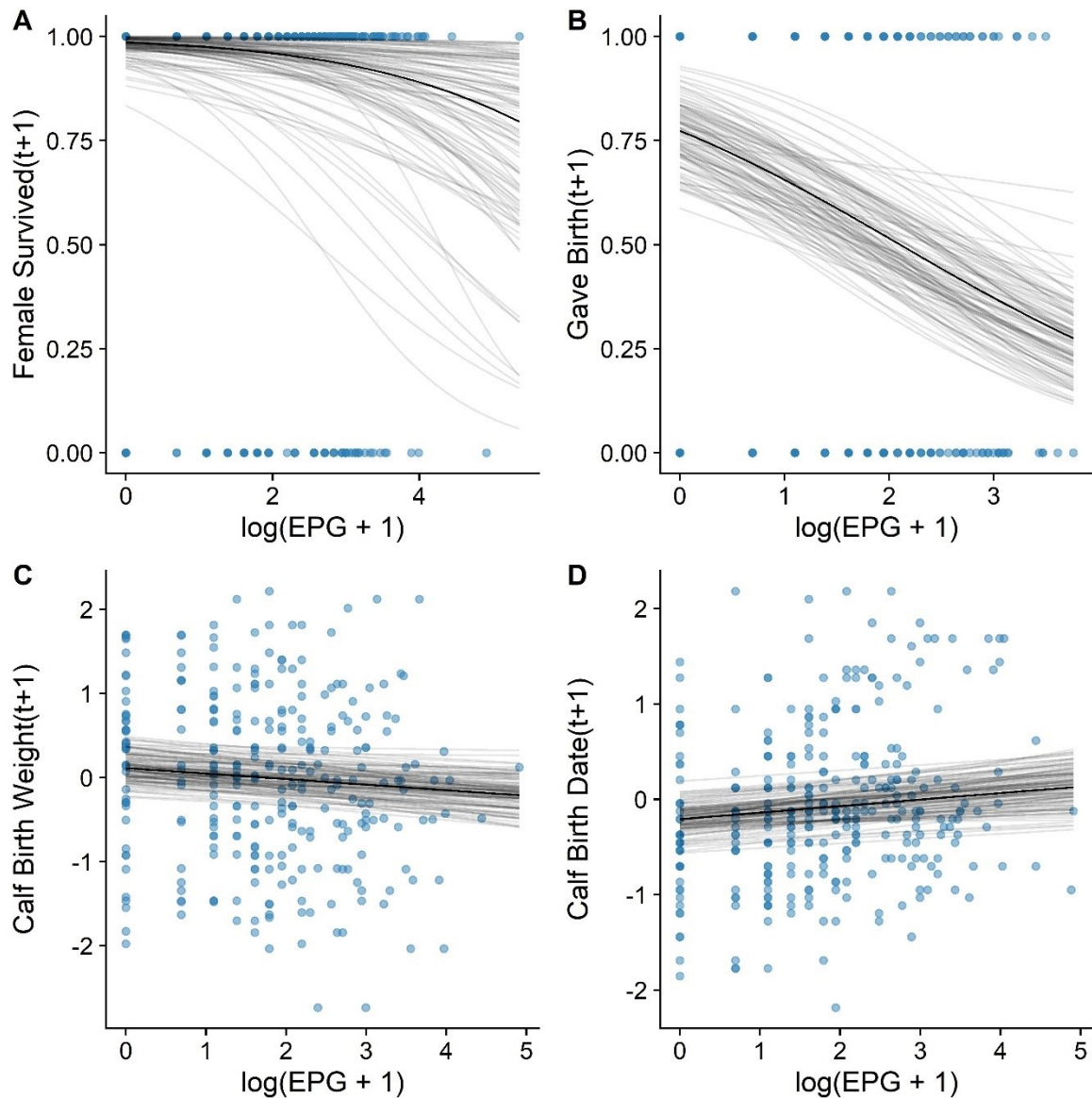
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246 Figure 3: Directed Acyclic Graphs (DAGs). Results are displayed for all four investigated female fitness-related response variables: overwinter  
247 survival (A); reproduction the following year (B); subsequent calf birth weight (C); subsequent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

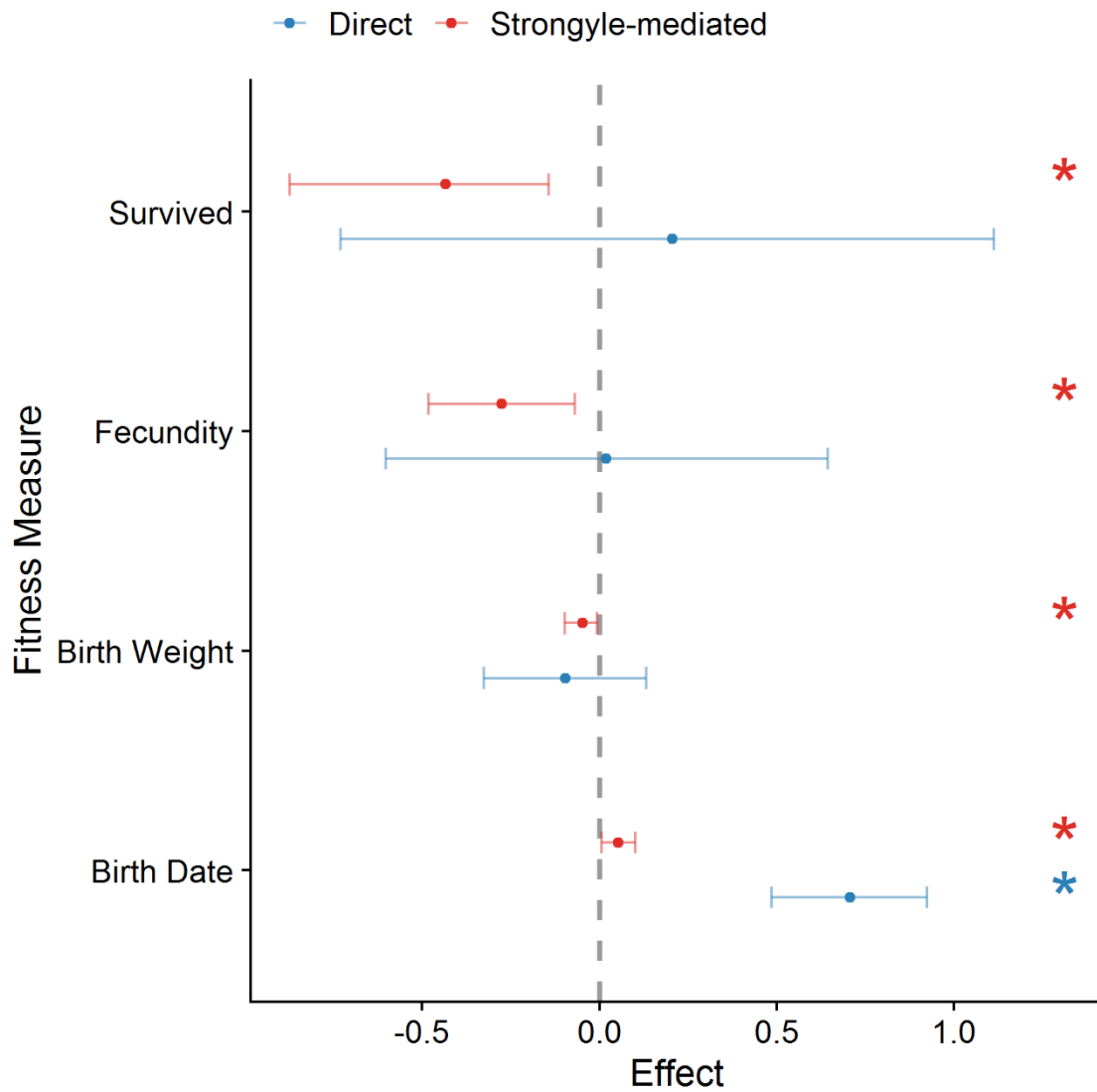


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253 Figure 4: Relationships between strongyle parasite count and subsequent survival and fecundity in  
 254 female red deer. Results are displayed for all four investigated fitness-related response variables:  
 255 overwinter survival (A); fecundity (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

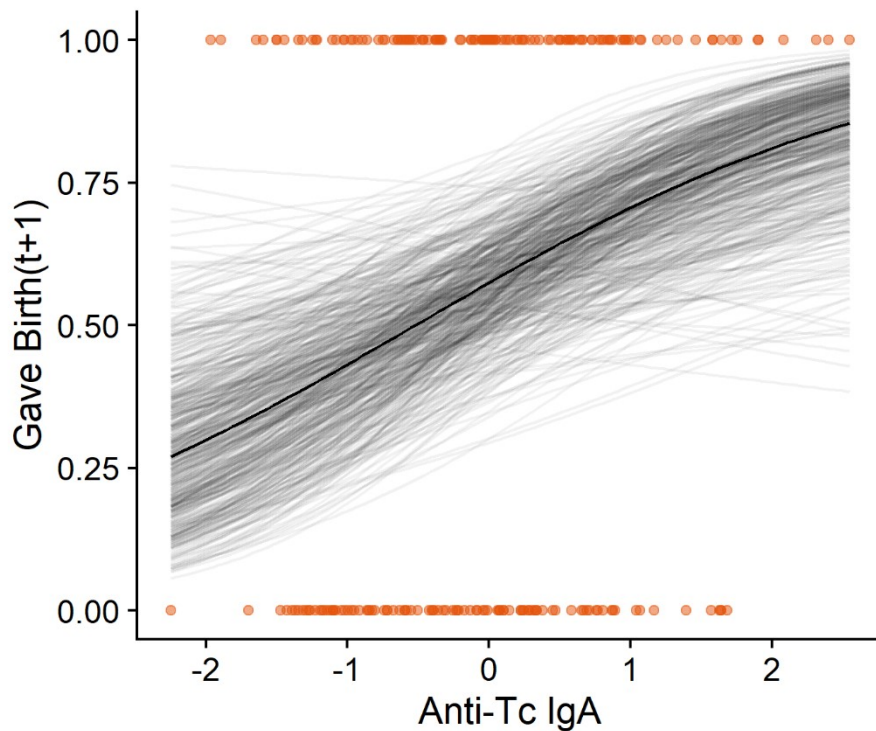
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263 Figure 5: Comparison of direct and indirect (parasite-mediated) effects of lactation on fitness-related  
 264 traits on the link scale (logistic for survival and reproduction; Gaussian for birth weight and birth date).

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).

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