



RESEARCH ARTICLE

Functional Ecology



Reproduction has different costs for immunity and parasitism in a wild mammal

Gregory F. Albery¹ | Kathryn A. Watt¹ | Rosie Keith¹ | Sean Morris¹ |
Alison Morris¹ | Fiona Kenyon² | Daniel H. Nussey¹ | Josephine M. Pemberton¹

¹Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK

²Moredun Research Institute, Pentlands Science Park, Midlothian, UK

Correspondence

Gregory F. Albery
Email: gfalbery@gmail.com

Funding information

Rural and Environment Science and Analytical Services Division; Natural Environment Research Council, Grant/Award Number: NE/L002558/1 and NE/L00688X/1

Handling Editor: David Gremillet

Abstract

1. Life history theory predicts that reproductive allocation draws resources away from immunity, resulting in increased parasitism. However, studies of reproductive trade-offs rarely examine multiple measures of reproduction, immunity and parasitism. It is therefore unclear whether the immune costs of reproductive traits correlate with their resource costs, and whether increased parasitism emerges from weaker immunity.
2. We examined these relationships in wild female red deer (*Cervus elaphus*) with variable reproductive allocation and longitudinal data on mucosal antibody levels and helminth parasitism. We noninvasively collected faecal samples, counting propagules of strongyle nematodes (order: Strongylida), the common liver fluke *Fasciola hepatica* and the red deer tissue nematode *Elaphostrongylus cervi*. We also quantified both total and anti-strongyle mucosal IgA to measure general and specific immune allocation.
3. Contrary to our predictions, we found that gestation was associated with decreased total IgA but with no increase in parasitism. Meanwhile, the considerable resource demand of lactation had no further immune cost but was associated with higher counts of strongyle nematodes and *E. cervi*. These contrasting costs arose despite a negative correlation between antibodies and strongyle count, which implied that IgA was indicative of protective immunity.
4. Our findings suggest that processes other than classical resource allocation trade-offs are involved in mediating observed relationships between reproduction, immunity and parasitism in wild mammals. In particular, reproduction-immunity trade-offs may result from hormonal regulation or maternal antibody transfer, with parasitism increasing as a result of increased exposure arising from resource acquisition constraints. We advocate careful consideration of resource-independent mechanistic links and measurement of both immunity and parasitism when investigating reproductive costs.

KEYWORDS

disease ecology, ecoimmunology, helminths, life history, parasites, reproduction, trade-off, wild mammal

1 | INTRODUCTION

Costly traits are central to the fields of life history theory and eco-immunology. Trade-offs arising between reproductive allocation and other aspects of life history are a fundamental prediction of the former (Harshman & Zera, 2007; Stearns, 1989; Williams, 1966), while the latter examines the ecology of costly immune responses (Graham et al., 2011; Sheldon & Verhulst, 1996). Because reproduction and immunity compete for host resources, in resource-limited environments, animals that reproduce should have fewer resources to allocate to immune defences (Deerenberg, Arpanius, Daan, & Bos, 1997; French, Denardo, & Moore, 2007; Sheldon & Verhulst, 1996). If immunity is protective, these individuals will experience higher parasitism as a result. Intuitively, traits with higher resource demands should result in the diversion of more resources away from immunity, leading to higher parasite burdens. However, recent advances have demonstrated that the interrelationships between host resources, immunity and parasitism can be unexpectedly complex (Cressler, Nelson, Day, & Mccauley, 2014). In addition, reproduction may alter allocation to different immune components (Becker et al., 2018; Rödel, Zapka, Stefanski, & von Holst, 2016), yet the reasons for this differential allocation are poorly understood. Few studies in wild mammals have examined trade-offs with multiple reproductive traits, so it is unclear whether different components of reproduction have different costs for immune defence, and whether their costs are proportional to their resource demand. Furthermore, studies of reproductive trade-offs rarely quantify both immunity and parasitism to examine the importance of susceptibility versus exposure in driving higher parasite intensities in reproductive females (Bradley & Jackson, 2008; Knowles, Nakagawa, & Sheldon, 2009). Here, we examine the partitioning of reproductive costs for multiple measures of immunity and parasitism to investigate the possible mechanisms governing a reproduction–immunity–parasitism trade-off in a wild mammal.

Mammalian reproduction is a complex, multistage process, featuring extensive maternal allocation which varies in intensity through the reproductive period (Langer, 2008; Maestripieri & Mateo, 2009). As such, different components of reproduction vary substantially in their resource and fitness costs. In particular, lactation is a highly energetically demanding process which carries costs for immunity, parasitism or fitness in a range of mammals (Beasley, Kahn, & Windon, 2010; Christe, Arlettaz, & Vogel, 2000; Clutton-Brock, Albon, & Guinness, 1989; Froy, Walling, Pemberton, Clutton-brock, & Kruuk, 2016; Jones, Sakkas, Houdijk, Knox, & Kyriazakis, 2012; Rödel et al., 2016; Woodroffe & Macdonald, 1995). Meanwhile, only one of these studies uncovered an immunological cost of gestation (Christe et al., 2000), which requires fewer resources than does lactation (Clutton-Brock et al., 1989). However, although experimentally modifying resource availability can affect the severity of reproduction–immunity trade-offs (French et al., 2007; Jones et al., 2012), this is not always the case (Stahlschmidt, Rollinson, Acker, & Adamo, 2013). Similarly, studies in birds have questioned whether the energetic costs of immunity are sufficient to drive trade-offs (Eraud, Duriez, Chastel, &

Faivre, 2005; Svensson, Råberg, Koch, & Hasselquist, 1998). Such findings imply that reproduction–immunity trade-offs can be linked mechanistically as well as through resource reallocation. Potential such links include production of reactive oxygen species, reduction in immunologically active fat stores, or resource-independent hormonal regulation (Speakman, 2008; Svensson et al., 1998).

Different components of mammalian reproduction can have qualitatively different effects on host immunity as well as varying quantitatively in terms of their resource demand. For example, pregnancy necessitates modulation of the immune system to avoid mounting an immune response to the developing foetus, which will directly affect anti-parasite defence (Weinberg, 1984, 1987). Similarly, lactation draws immune molecules away from the mother for transfer to offspring, reducing their availability for the mother's own defence (Grindstaff, Brodie, & Ketterson, 2003; Hasselquist & Nilsson, 2009). Reproduction also induces a suite of physiological and behavioural changes which will affect susceptibility and exposure to parasites indirectly: for example, it has been suggested that bats compensate for the energetic demand of lactation by reducing costly grooming behaviour, with ectoparasite burden increasing as a result (Speakman, 2008). It is unclear how such mechanistic links between components of reproduction and immunity interact with resource allocation to influence immune defence and parasite intensity in wild mammals.

The wild red deer (*Cervus elaphus*) in the North block of the Isle of Rum exhibit a well-studied life history trade-off, in which reproduction substantially decreases the mother's probability of overwinter survival and reproduction the following year (Clutton-Brock et al., 1989; Froy et al., 2016). However, not all components of reproduction are equally costly: gestation has a negligible detectable fitness cost compared to that of lactation (Clutton-Brock et al., 1989). Moreover, while giving birth late and caring for a male calf compared to a female calf are associated with decreased maternal fitness, their effects are small compared to the cost of lactation itself (Froy et al., 2016). The study population has a high prevalence of gastrointestinal helminths, and parasite burdens can be quantified noninvasively through faecal propagule counts (Albery et al., 2018). A previous investigation into the parasite community of Rum deer living outside the study area identified multiple genera of strongyle nematodes, including *Trichostrongylus*, *Oesophagostomum*, *Cooperia*, a group of ostertagiids and the red deer-specific nematode *Elaphostrongylus cervi* (Irvine, Corbishley, Pilkington, & Albon, 2006). Mucosal antibodies, and especially the IgA isotype, are important effectors of ruminant adaptive immunity to gut helminths (Butler, 1969; McRae, Stear, Good, & Keane, 2015). Mucosal IgA can be quantified in wild ruminant faeces, correlating positively with the same isotype measured in plasma or serum and negatively with helminth faecal egg counts (Watt, Nussey, Maclellan, Pilkington, & McNeilly, 2016).

In this study, we measured both total and helminth-specific mucosal IgA and propagule counts of multiple helminth species, using faecal samples collected from the Isle of Rum study population. We quantified the associations between several reproductive traits of known fitness cost and subsequent measures of immunity and

parasitism. We also examined covariance between IgA and parasites to discern whether increased IgA was associated with decreased parasite intensity independently of shared reproductive and seasonal effects, implicating IgA as an indicator of protective immunity. We predicted that reproductive allocation would be associated with reduced antibody levels and increased parasite counts, and that aspects of reproduction previously found to be more costly for fitness, especially lactation, should likewise be more costly in terms of both immunity and parasitism. Furthermore, providing parasitism is mediated by immune susceptibility, aspects of reproduction that are costly for immunity should have similar costs in terms of parasitism.

2 | MATERIALS AND METHODS

2.1 | Study system, sampling and parasitology

The study population is located in the North block of the Isle of Rum National Nature Reserve in the Inner Hebrides, Scotland (57°N 6°20'W). The resident population comprises ~350 animals at any one time and is regularly censused to keep track of each individual and its life history. See Clutton-Brock, Guinness, and Albon (1982) for a full summary of the project and the deer reproductive cycle. Briefly, the deer mate in September and October and give birth in May–June, after an approximately 235 day gestation. Females do not reproduce every year and produce a maximum of one calf per year. During the calving season, daily monitoring of pregnant females enables the recording of precise birth dates. Neonates are caught, sexed, weighed and individually marked, enabling lifelong individual identification. Calves are dependent on their mothers for much of their first year. Regular population censusing throughout the year and winter mortality searches allow dates of death to be reliably assigned to the nearest month for the vast majority of individuals. Most calf deaths occur either within the first few weeks of life, or

in the following winter ~6–9 months later. Females that successfully raise a calf to the age of one, or that lose the calf in its first winter, have lower rates of overwinter survival and reproduction the following year compared to those that do not reproduce that year or that lose their calf in the summer (Clutton-Brock et al., 1989; Froy et al., 2016). Many calves die over the winter, but the mothers of these calves have paid the cost of lactation associated with feeding them until the winter, whether or not the calf survive. Therefore, these females are treated as a single category here (Clutton-Brock et al., 1989; Froy et al., 2016).

We collected faecal samples from female deer across the annual reproductive cycle. As a 'deer year' runs from May to April, this study examines the effects of reproduction over a year, beginning in May, on egg counts and antibody levels until the following April. A description of the sample collection procedure can be found in Albery et al. (2018). Sampling occurred over seven two-week sampling trips spanning April 2016–April 2018, in August ('summer'), November ('autumn') and April ('spring'). Note that our dataset included an April sampling trip from the deer reproductive cycle starting May 2015, without an accompanying August and November trip from this reproductive cycle. Figure 1 illustrates how sampling relates to different aspects of reproductive allocation by female deer across the annual cycle. We classify a female's reproductive status for a given year as 'No Calf', 'Calf Died' and 'Calf Survived' (Figure 1). 'No Calf' samples were collected from females that did not reproduce in the calving season preceding the sampling trip; 'Calf Died' samples were collected from females that gave birth to a calf in the preceding calving season which died before October 1st of that year; and 'Calf Survived' samples were collected from females that gave birth to a calf in the preceding calving season which survived past October 1st of that year. We excluded females that were reproducing for the first time from our analyses, as their reproductive success is heavily confounded with their young age (mean age 4.21 years). In addition, females may or

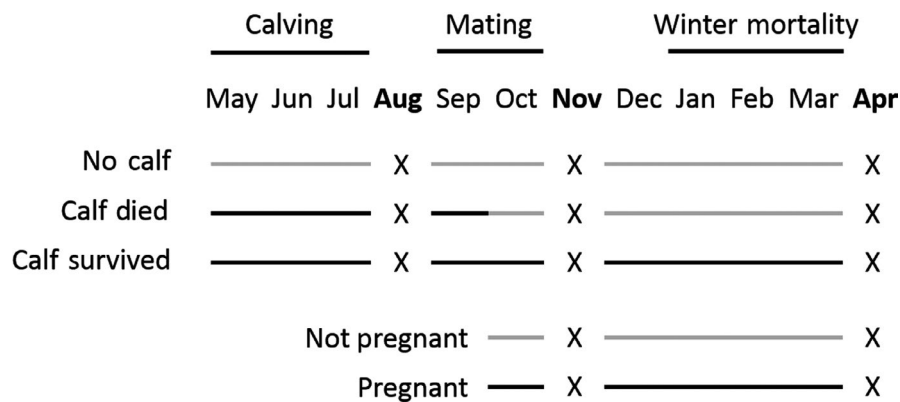


FIGURE 1 The faecal sampling regime in the context of a deer reproductive cycle ('deer year'). A cross (X) represents a 2-week sampling trip. The deer year begins on May 1st when calving begins; individuals are assigned to one of three reproductive status categories (top three lines) according to the birth and survival of their calf over the course of the following year. Individuals are also assigned a pregnancy status in November and April based on reproduction in the following calving season (bottom two lines). Black lines represent periods in which the calf is living or the female is pregnant; grey lines represent periods in which the calf is dead or non-existent or the female is not pregnant. All but two individuals in the 'Calf Died' category lost their calves before August; the remaining two lost theirs between August and October

may not become pregnant during the autumn rut. Samples were therefore assigned a pregnancy status, beginning in November, based on whether or not the female gave birth to a calf in the following spring (Figure 1). It is possible that some females that did not produce a calf conceived but lost the pregnancy. This is most likely to occur very early in gestation, in which case the female has not borne much of the cost of pregnancy. Pregnancy becomes obvious in spring from body shape, and udder size and such females always produce a calf; we therefore do not believe that cryptic pregnancies would introduce substantial variation into our analysis.

In total, 837 faecal samples were collected noninvasively from 140 mature females. All samples were collected by observing known females from a distance, marking the spot in which defecation happened and promptly collecting the pellets. In the evening after collection, samples were homogenized by hand and subsampled, with 1–15 g frozen at -20°C for antibody quantification and the remainder refrigerated at 4°C for parasitological analysis. Subsamples were transferred to Edinburgh at these temperatures. Parasite propagule counts were carried out as previously described, without correcting for dry weight, and included counts of strongyle nematodes (order: Strongylida; counted using a sedimentation–salt flotation method), the common liver fluke *Fasciola hepatica* (counted using a sedimentation method) and the red deer-specific tissue nematode *E. cervi* (isolated and counted using a baermannization method; see Albery et al., 2018 for detailed methods). Because of the difficulty identifying strongyle nematodes from egg morphology, we group them together here at the order level. Final sample sizes for each variable are displayed in Table S1. All samples were counted as a subsample and divided by the weight of the subsample, providing an eggs per gram (EPG) or larvae per gram (LPG) value.

2.2 | Antibody extraction and quantification

Faecal antibodies were quantified using a protocol modified from Watt et al. (2016). Faecal matter was stored at -20°C until extraction. Extractions occurred either in January–March 2017 (session 'A', samples collected April–November 2016; $N = 132$), January 2018 ('B', samples collected April–November 2016; $N = 212$) or within the sampling trip ('C', samples collected April 2017–April 2018, $N = 460$). 0.6 g (± 0.005 g) of the homogenate was weighed out into an Eppendorf tube and mixed thoroughly with 0.9 ml of protease inhibitor solution (cOmplete™ Mini Protease Inhibitor Cocktail tablets; Roche; 1 tablet mixed with 7 ml Phosphate Buffered Saline). The mixture was left to stand for a minimum of 5 min to allow the protease to act and then centrifuged at 10,000 g for 5 min. The supernatant was removed using a micropipette and stored in a separate Eppendorf tube at -20°C until ELISA.

We measured two antibodies by faecal ELISA: total IgA and anti-*Teladorsagia circumcincta* third larval stage IgA (anti-Tc IgA), using a method developed in sheep (Watt et al., 2016). *T. circumcincta* is an abundant and important sheep strongyle and is also

present in the Rum deer (Sam Ebdon and GF Albery, unpublished data). This method for detecting anti-*T. circumcincta* antibodies shows high cross-reactivity with other strongyle species (Froy et al., 2019). Anti-Tc IgA correlates therefore negatively with order-level strongyle faecal egg count and with species-level counts of other strongyle species in wild Soay sheep (Watt et al., 2016; Froy et al., 2019). We therefore interpret this assay as representing a general anti-strongyle response rather than a response to *T. circumcincta* specifically. ELISA plates were coated the night before using sheep-derived capture antibodies (Bethyl Laboratories) for total IgA and with third larval stage antigen for anti-Tc IgA (Moredun Research Institute). For total IgA, the samples were diluted in the ratio 1:64; due to lower antibody concentrations undiluted supernatant was used for the anti-Tc IgA assay. After this stage, the ELISA protocol was carried out as described in Watt et al. (2016). The total IgA dilution was selected by carrying out serial dilutions on a set of samples and selecting the dilution at which different concentrations of antibodies were deemed to have the widest spread of optical densities. ELISA readings diluted linearly as expected. Samples were corrected using controls according to the calculation: Final OD = (sample OD – mean plate negative OD)/(mean plate positive OD – mean plate negative OD). All samples were run on duplicate plates, which were highly correlated ($R = .98$ across all duplicates). The mean value for the two duplicates was taken for each sample and used for analysis.

2.3 | Statistical analysis

We used four sets of Generalized Linear Mixed Models (GLMMs) to test how reproductive traits were associated with antibody levels and parasite intensity. Analyses were carried out in R version 3.5.0 (R Core Team 2018) with the package MCMCglmm (Hadfield, 2010). All models were run for 2.6×10^6 iterations with a 2,000 iteration thinning interval and a 6×10^5 iteration burn in period. Models were run on 5 chains, and convergence of the chains was assessed using the Gelman–Rubin criterion. Posterior prediction was used to confirm that the model estimates recapitulated the data distribution and between-group differences. p_{MCMC} values for differences between factor categories were calculated using the proportional overlap of estimates' posterior distributions, divided by half the number of iterations.

2.4 | Full models

We first constructed five univariate GLMMs using the full dataset (837 samples from 140 individuals). Three models used an overdispersed Poisson distribution in MCMCglmm, which accounts for overdispersion in the data in order to approximate a negative binomial distribution, with strongyle, *F. hepatica* and *E. cervi* intensity as response variables (Albery et al., 2018). Models initially included

the following fixed effects, without interactions: Year (factor with three levels representative of the deer reproductive cycle beginning in 2015, 2016 and 2017); Season (factor with three levels: Summer, Autumn and Spring); Age in years (continuous) and Reproductive Status (factor with three levels: No Calf, Calf Died and Calf Survived). Individual identity was fitted as a random effect. All continuous variables except parasite counts were scaled to have a mean of 0 and a standard deviation of 1 before analysis.

The two remaining models examined antibodies as response variables. As mucosal antibodies are vulnerable to degradation by temperature-dependent faecal proteases, we had to account for the extraction session and time to freezing and extraction (Figures S5 and S6). There was an uneven distribution of year, season and status categories across different extraction sessions, so that these variables could not all be fitted in the same model. Therefore, to control for collection factors and quantify reproductive status effects conservatively (risking losing some information), we first transformed antibody levels to approximate normality (square-root transform for total IgA and cube-root transform for anti-Tc IgA) and fitted a linear model with fixed effects including extraction sessions performed at different times (factor with three levels); day of collection within a sampling trip (continuous integers, range 0–11); time elapsed from sample collection until freezing (continuous, in hours). The scaled residual values from these models (mean = 0, $SD = 1$) were used as the response variables in two Gaussian GLMMs with the same fixed and random effects as the parasite GLMMs.

Previous work on the Rum deer revealed extensive seasonal fluctuations in parasite count (Albery et al., 2018). We therefore followed up the above five models by fitting a season by reproductive status interaction in order to investigate whether the effects of reproductive status varied by season. Each model was compared with and without this interaction to investigate whether it affected Deviation Information Criterion (DIC) values as a measure of model fit (threshold values for distinguishing between models $\Delta DIC = 2$) or changed model estimates.

2.5 | Pregnancy models

Pregnancy may directly affect immunity, and effects attributed to reproductive status could be due to correlated variation in pregnancy status over the sampling year. To check this, we ran a second set of models investigating the role of pregnancy status. This used a subset of samples collected in November and April (518 samples from 122 individuals), as mating occurs in the early autumn and females could not be pregnant in August. These five models featured the same explanatory variables as the full status models, with only two levels in the season category (Autumn and Spring), and with Pregnancy included as a binary variable. We compared these models with and without the pregnancy term as a fixed effect to investigate whether its inclusion changed reproductive status effect sizes or affected model fit.

2.6 | Calving trait models

We next used a restricted dataset consisting of individuals that had given birth in the year of sampling (571 samples from 116 individuals) to investigate whether specific traits associated with a calving event influenced antibody levels and parasitism. We fitted the same fixed and random effects as the full model set, but with only two factor levels in the reproductive status category (Calf Died and Calf Survived), and including several variables relating to each birth: Parturition Date (continuous, centred around median birth date that year); Birth Weight (continuous, in kilograms, calculated from a projection using capture weight and age in hours, slope 0.01696 kg/hr); Calf Sex (Female or Male).

2.7 | Multivariate model

Multivariate mixed-effects models can be used to investigate covariance between measures of immunity and parasitism, while accounting for fixed effects. To test whether antibodies and parasites were correlated, we fitted a model with strongyles, *E. cervi*, total IgA and anti-Tc IgA as response variables, with the same fixed effects as the full univariate models. We specified Poisson and Gaussian distributions for the parasites and antibodies, respectively, as in the univariate models. Unstructured variance/covariance matrices were fitted for random and error terms, allowing estimation of the phenotypic correlations between the response variables. Phenotypic covariance between two response variables A and B ($Cov_{\text{phenotypicA,B}}$) is calculated using the random (G) and residual (R) variance structure of the model, with the formula $Cov_{\text{phenotypicA,B}} = Cov_{\text{IndividualA,B}} + Cov_{\text{ResidualA,B}}$. Phenotypic correlation between two response variables ($r_{\text{phenotypicA,B}}$) was calculated by dividing the phenotypic covariance by the square root of the sum of the variance in both response variables: $r_{\text{phenotypicA,B}} = Cov_{\text{phenotypicA,B}} / (V_{\text{phenotypeA}} + V_{\text{phenotypeB}})^{0.5}$.

3 | RESULTS

Reproduction was associated with both lower antibody levels and increased parasite counts, but patterns differed considerably between different response variables (Figure 2, Figure S1). Compared to 'No Calf' individuals, 'Calf Survived' status was associated with higher intensity strongyle ($p_{\text{MCMC}} < .001$) and *E. cervi* infection ($p_{\text{MCMC}} = .01$), and with lower total IgA ($p_{\text{MCMC}} = .016$) and anti-Tc IgA levels ($p_{\text{MCMC}} < .001$). 'Calf Survived' females also had higher parasite counts than 'Calf Died' individuals ($p_{\text{MCMC}} < .001$ for strongyles and *E. cervi*), but these reproductive status categories did not differ in total IgA ($p_{\text{MCMC}} = .502$) or anti-Tc IgA ($p_{\text{MCMC}} = .336$; Figures 2 and 3). 'Calf Died' individuals did not differ from 'No Calf' females in strongyle, *E. cervi* or anti-Tc IgA levels (Figure 2) but had lower total IgA levels ($p_{\text{MCMC}} = .018$). That is, 'Calf Died' individuals had slightly lower total IgA than 'No Calf' females, but with similar parasite intensities, while 'Calf Survived' individuals had the same

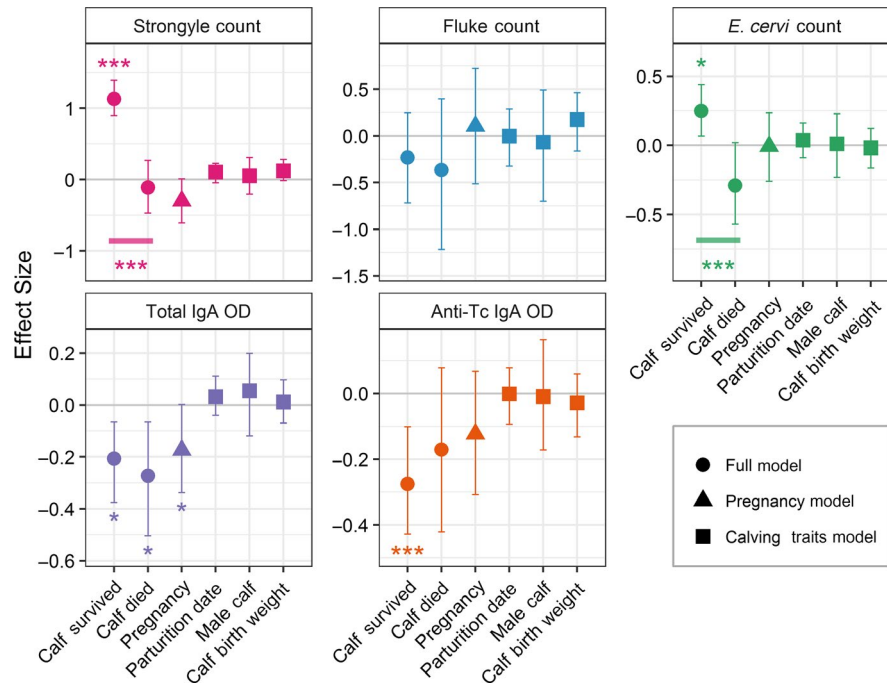


FIGURE 2 Model outputs depicting the effects of reproductive traits derived from all three univariate model sets. Points and error bars represent model estimates and 95% credibility estimates. Effect sizes for categorical variables (status, pregnancy and calf sex) denote differences from the first (absent) category of each, contained in the intercept ('No Calf', 'Not Pregnant' and 'Female Calf', respectively). Effect sizes for continuous variables (parturition date and calf birth weight) represent the change in the response variable associated with a change of one standard deviation of the explanatory variable. Asterisks represent significant differences derived from MCMCgIimm posterior distribution overlaps: ***, ** and * denote $p < .001$, $p < .01$ and $p < .05$, respectively. Bars denote differences between status categories

antibody levels as 'Calf Died' individuals, but with increased parasite intensities. *F. hepatica* was not associated with reproductive status, but decreased with age ($p_{\text{MCMC}} = .004$) as did *E. cervi* ($p_{\text{MCMC}} < .001$; Figures S1 and S7).

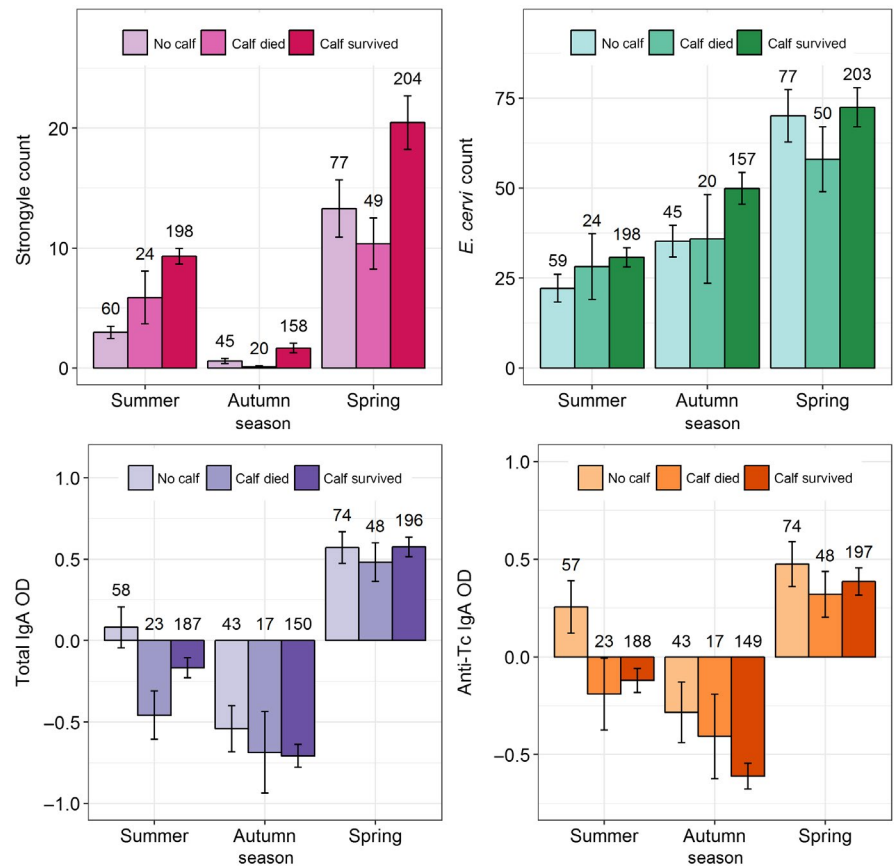
Strongyles and both antibodies all exhibited the same seasonality, peaking in the spring and being lowest in the autumn, with the summer intermediate (Figure 3, all differences $p_{\text{MCMC}} < .001$). *Fasciola hepatica* was higher in the spring than in the summer or autumn ($p_{\text{MCMC}} < .034$), and *E. cervi* was lowest in the summer, with the autumn intermediate ($p_{\text{MCMC}} < .001$). There was also some between-year variation: strongyle levels increased between 2015 and 2016, and again in 2017 (all $p_{\text{MCMC}} < .001$), while total IgA levels decreased in 2017 compared to 2015 and 2016 ($p_{\text{MCMC}} < .024$). Anti-Tc IgA was also lower in 2017 than 2016 ($p_{\text{MCMC}} < .001$). Inclusion of season-by-status interactions improved strongyle model fit ($\Delta\text{DIC} = -3.79$), but did not improve the fit of any other models ($\Delta\text{DIC} < 2$). Fixed status effects remained largely unchanged in magnitude or significance, suggesting that the observed associations with reproductive status were consistent across seasons (Figure 3). All interaction terms implied an attenuation of reproductive status effects from summer through winter to spring, rather than any major qualitative change in this association (Figure 3). Both 'Calf Died' and 'Calf Survived' females had increased antibody levels and decreased parasite intensities relative to 'No Calf' females over this period. See Figure S2 for a comparison of the full model estimates and DIC changes when a season-by-status interaction was included.

Pregnancy models examining April and November samples revealed marginally lower total IgA in pregnant females ($p_{\text{MCMC}} = .034$, Figure 2, Figures S1 and S3). Including pregnancy status in our models did not alter the direction or significance of reproductive status effects; in fact, in the case of total IgA and anti-Tc IgA it increased the significance of the 'Calf Survived' category's effect (Figure S3). It also slightly improved the fit of the total IgA model ($\Delta\text{DIC} = -3.00$). No other models were impacted notably by the inclusion of the pregnancy term, although it slightly reduced the effect size of the 'Calf Survived' category in influencing strongyle count (Figure S3). Although the 'Calf Died' category was not statistically significant in the total IgA pregnancy model as it was in the full model, the fact that adding and removing pregnancy as a variable did not change the model estimate (Figure S3) implies that this did not arise from confounding effects of pregnancy.

None of the calving traits modelled (parturition date, calf birth weight or calf sex) were associated with maternal parasite or antibody levels (Figure 2, Figure S1).

The fixed effects of the multivariate model were very similar to those of the full models (Figure S4). Phenotypic correlations (R_p) derived from the variance structure of the multivariate model are as follows. There were positive correlations between strongyles and *E. cervi* ($R_p = .26$, $p_{\text{MCMC}} < .001$) and between total and anti-Tc IgA ($R_p = .424$, $p_{\text{MCMC}} < .001$). Strongyle count was also weakly negatively correlated with total IgA ($R_p = -.074$, $p_{\text{MCMC}} = .016$) and slightly more strongly with anti-Tc IgA ($R_p = -.142$, $p_{\text{MCMC}} < .001$).

FIGURE 3 Bar charts displaying raw mean ($\pm SE$) parasite counts and antibody levels of each reproductive status category in each season. Antibody measures were taken from the residuals of a model with square-root-transformed (total IgA) or cube-root-transformed (anti-Tc IgA) antibody OD as the response variable and including collection variables as fixed effects. Numbers above bars denote sample sizes



4 | DISCUSSION

Lactation is associated with weaker immunity or increased parasitism in a range of mammals (Festa-Bianchet, 1989; Jones et al., 2012; Rödel et al., 2016; Woodroffe & Macdonald, 1995). In accordance with these studies, we found that lactating females had both decreased antibody levels and increased parasite counts relative to non-reproductive females. In contrast, gestation is rarely found to be costly for immunity or parasitism in mammals (Irvine et al., 2006; Rödel et al., 2016; Woodroffe & Macdonald, 1995) and carries no detectable fitness cost in the Rum red deer (Clutton-Brock et al., 1989). Here, deer that gave birth to a calf that died within 6 months, thereby incurring a limited lactation cost, had lower total IgA levels than non-reproducing females. Gestation therefore carried an immune cost in this study. We predicted that resource depletion incurred through allocation to a given reproductive trait would lead to reduced antibody levels and that this would lead to increased parasite intensity (Knowles et al., 2009; Sheldon & Verhulst, 1996). Our results deviated from our expectations in two ways: first, gestation's long-lasting immune cost was not accompanied by increased parasite intensities. Second, the considerable additional resource allocation of prolonged lactation was not associated with additional immune costs relative to gestation, but was instead associated with an increase in parasite intensity. These results have two implications: reproduction-immunity trade-offs were unlikely to be mediated by simple resource reallocation, and reproduction-parasitism trade-offs were unlikely to be mediated solely by immunity—despite

our observation that higher immune allocation was associated with lower parasite counts between individuals.

If gestation's lack of detectable fitness cost in our study population (Clutton-Brock et al., 1989) demonstrates a small resource cost, why was gestation associated with reduced total IgA levels, and why did the additional resource cost of lactation not decrease antibody levels further? First, it is possible that reproductive hormones suppress the immune system without being sensitive to resource availability (Foo, Nakagawa, Rhodes, & Simmons, 2017; Svensson et al., 1998). Similarly, gestation may lead to alterations in immune allocation and antibody production, so that lower IgA resulted from selective allocation to alternative immune cells or functions rather than from lower absolute resource allocation to immunity. Reproductive mammals are commonly found to exhibit different (rather than weaker) immunity, but specific patterns of immune prioritization are unpredictable. For example, reproductive vampire bats (*Desmodus rotundus*) prioritize innate over adaptive immunity (Becker et al., 2018), while reproductive rabbits (*Oryctolagus cuniculus*) exhibit reduced white blood cell counts but stronger humoral immunity (Rödel et al., 2016). Assessing whether reproductive deer allocate resources preferentially to aspects of immunity other than mucosal antibodies would therefore necessitate examining numerous additional immune measures—however, in this study we were restricted to quantifying mucosal antibodies using noninvasive faecal samples as the deer are rarely handled as adults (Clutton-Brock et al., 1982).

Alternatively, gestation and early lactation may necessitate export of IgA from the gut to the blood for transfer to offspring

(Jeffcoate et al., 1992; Sheldrake, Husband, Watson, & Cripps, 1984). In ungulates, a substantial proportion of maternal antibody transfer occurs via the colostrum in the first few days of life (Hurley & Theil, 2011). It is feasible that this diversion of IgA from the gut occurs around parturition and is detectable for an extended period of time without an underlying resource allocation trade-off, creating lower IgA levels in all reproductive females regardless of their calf's survival. The necessity of transferring immune effectors to offspring may therefore be an important obligate mechanism contributing to reduced antibody levels in reproductive wild mammals (Rödel et al., 2016). In a proposed mechanism for the periparturient rise in helminth egg count in domestic sheep, exportation of IgA from the gut around parturition releases helminths from immune control (Jeffcoate et al., 1992). However, in this study, the lower total IgA and intermediate anti-Tc IgA levels in female deer that only paid the cost of gestation were not accompanied by any change in parasitism. This is surprising, given that the results of our multivariate model implied that both IgA measures are representative of increased resistance to strongyles. However, the phenotypic correlations of strongyles with total IgA and with anti-Tc IgA are relatively weak ($R = .07$ and $.14$, respectively); this is unsurprising, given the messy nature of ecoimmunological data, yet it also implies the need for other mechanisms of resistance and exposure contributing to parasitism. As such, additional immune measures would be desirable. Our application of a veterinary reagent to a non-model species may have introduced some variation; however, ecoimmunological tools for non-model systems are thin on the ground (Garnier & Graham, 2014), and we believe that our findings of reproduction–immunity trade-offs and a significant negative correlation with parasitism implies that this measure is immunologically relevant.

If antibody levels were indicative of allocation to protective immunity, how were the deer that paid the immune cost of gestation able to maintain low strongyle and *E. cervi* intensities? Or, what produced the higher parasite counts in lactating individuals? Lactating females' anti-Tc IgA levels were lower than non-reproductive females', which could explain their increased parasitism in the absence of a contrast with any other reproductive categories. However, levels of total and anti-Tc IgA in lactating females were not detectably lower than those exhibited by females that paid the cost of gestation (Figure 2). This disparity suggests that additional processes such as exposure were important in driving the high parasite intensities in lactating females (Knowles et al., 2009; Sheldon & Verhulst, 1996). The energetic and resource demand of milk production necessitates substantially increased forage intake and grazing time (Hamel & Côté, 2008, 2009) and may reduce feeding selectivity or the ability to exhibit parasite avoidance behaviours (Hutchings, Judge, Gordon, Athanasiadou, & Kyriazakis, 2006; Speakman, 2008). In addition, hinds inevitably share space with their calves and those of other females, which exhibit very high parasite intensities (Albery et al., 2018). Thus, lactating females may suffer increased exposure to infective larvae, resulting in higher parasite burdens. This mechanism offers an explanation for our observation that lactation was associated with increased parasite counts, while gestation was not, as individuals that lost their calf as

a neonate were not then saddled with a necessity for such high resource acquisition. Given that we observed some inter-annual variation in parasitism, it is possible that annual variation in density and resource availability may alter the severity of this reproduction–parasitism trade-off. Based on our results, we suggest that severe effects of mammalian reproduction on parasite infection are partly mediated by exposure as a result of constraints on resource acquisition, foraging selectivity and anti-parasite behaviours, in addition to increased immune susceptibility represented by antibody levels or additional unmeasured parameters.

Effects of foraging on exposure can profoundly affect epidemiological dynamics: for example, in the water flea *Daphnia dentifera*, temperature-induced increases in food intake can increase the magnitude of fungal pathogen epidemics (Shocket et al., 2018). Similar processes may act in the deer, if spatiotemporal variation in climatic conditions, deer density or food abundance modifies feeding behaviour or the threat of exposure. In particular, strongyle and *E. cervi* parasitism will be further exacerbated in years and areas of the study system where deer density is high and food availability is low (Wilson, Grenfell, Pilkington, Boyd, & Gulland, 2004). It is possible that higher parasitism in reproductive individuals will reduce their fitness, thereby producing lactation's fitness cost—and, by extension, gestation's lack of fitness cost—in this system (Clutton-Brock et al., 1989; Froy et al., 2016; Harshman & Zera, 2007; Williams, 1966). If exposure is determining parasitism and parasitism is reducing fitness, we would expect that parasite-mediated life history trade-offs would be exacerbated in years and areas of high deer density, as more deer will translate to higher levels of pasture contamination (Wilson et al., 2004). However, a previous study in this population determined that accounting for strong spatial autocorrelation in immunity and parasitism, fixed effects were not affected when this spatial dependence was accounted for (Albery, Becker, Kenyon, Nussey, & Pemberton, 2019). Therefore, we assert that these exposure effects are more likely to be mediated by individual drivers of variation such as individuals' forage intake rather than by environmental variation or density.

Reproductive trade-offs are a potential driver of seasonal dynamics of immunity and parasitism, in which periodic reproduction-associated relaxation of immunity leads to increased parasitism (Martin, Weil, & Nelson, 2008). Our results do not support this mechanism for several reasons: all status categories exhibited seasonality of antibodies, strongyles and *E. cervi* rather than only reproductive individuals; increased parasitism in reproductive females was not linked to lower immunity; and immunity did not correlate with resource availability, being highest in April, when the deer are in poor condition, having just survived the winter. In fact, antibody levels and strongyle counts correlated positively across seasons despite their negative correlation among individuals. This suggests that seasonality of propagule output is adaptive for helminths, facilitating highest transmission when environmental conditions are favourable and immunologically naïve calves are present, and leading to seasonal upregulation of immunity in warmer months to combat increased exposure (Møller, Erritzøe, & Saino, 2003; Wilson et al.,

2004). Our models revealed substantial inter-annual variation in all examined variables; although we did not have enough annual replicates to test the causal factors behind this variation, further data collection in this population may allow testing of whether, for example density-related competition effects or climatic variation are producing variation in immunity and parasitism.

This study describes unexpected and complex interrelationships between different components of mammalian reproduction, immunity and parasitism in the wild. We suggest that classical resource allocation mechanisms which are often hypothesized to underlie trade-offs with immunity (Deerenberg et al., 1997; French et al., 2007; Sheldon & Verhulst, 1996) are insufficient to explain many of the patterns seen in wild mammals, corroborating findings in other taxa (Stahlschmidt et al., 2013; Svensson et al., 1998). As such, studies examining such trade-offs in mammals should consider mechanistic links between reproduction and immunity, resource acquisition limitations, and exposure components of parasitism, particularly by quantifying both immunity and parasitism simultaneously (Bradley & Jackson, 2008; Graham et al., 2011). The potential complexity of such interrelationships may contribute to the relative rarity of conclusive evidence for reproduction-immunity-parasitism trade-offs in mammals.

ACKNOWLEDGEMENTS

The long-term red deer study is funded by the Natural Environment Research Council (grant number NE/L00688X/1), as is GFA's PhD studentship through the E3 Doctoral Training Partnership (grant number NE/L002558/1). FK receives funding from the Scottish Government, RESAS, Strategic Research Programmes 2016-21. We thank Scottish Natural Heritage for permission to work on the Isle of Rum NNR and for the support of the reserve management team on the island. Thanks to Dave McBean and Gillian Mitchell at the Moredun Research Institute for their help with parasitological methods. The *Teladorsagia circumcincta* antigen was received from Moredun Research Institute and was prepared by David Bartley, Alison Morrison, Leigh Andrews, David Frew and Tom McNeilly. Thanks also to Olly Gibb and all field assistants for their help in sample collection. Finally, thanks to Amy Sweeny, Eryn Macfarlane, and Adam Hayward and to two reviewers, Jean-Michel Gaillard and Marco Festa-Bianchet, for their helpful comments on the manuscript.

AUTHORS' CONTRIBUTIONS

G.F.A. collected the samples, conducted laboratory work, analysed the data and drafted the manuscript; K.A.W. designed and helped to carry out the ELISAs; R.K. carried out some antibody extractions and ELISAs; S.M. and A.M. helped with sample collection; F.K., D.H.N. and J.M.P. offered comments on methodology and theory throughout and helped draft the manuscript.

DATA AVAILABILITY STATEMENT

The data supporting this work are available at the Dryad Digital Repository: <https://doi.org/10.5061/dryad.8sf7m0ch8> (Albery et al., 2019)

ORCID

Gregory F. Albery  <https://orcid.org/0000-0001-6260-2662>

REFERENCES

- Albery, G. F., Watt, K. A., Keith, R., Morris, S., Morris, A., Kenyon, F., ... Pemberton, J. M. (2019). Data from: Reproduction has different costs for immunity and parasitism in a wild mammal, v3. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.8sf7m0ch8>
- Albery, G. F., Becker, D. J., Kenyon, F., Nussey, D. H., & Pemberton, J. M. (2019). The fine-scale landscape of immunity and parasitism in a wild ungulate population. *Integrative and Comparative Biology*, 1-11, icz016. <https://doi.org/10.1093/icb/icz016>
- Albery, G. F., Kenyon, F., Morris, A., Morris, S., Nussey, D. H., & Pemberton, J. M. (2018). Seasonality of helminth infection in wild red deer varies between individuals and between parasite taxa. *Parasitology*, 145(11), 1-11. <https://doi.org/10.1017/S0031182018000185>
- Beasley, A. M., Kahn, L. P., & Windon, R. G. (2010). The periparturient relaxation of immunity in Merino ewes infected with *Trichostrongylus colubriformis*: Parasitological and immunological responses. *Veterinary Parasitology*, 168(1-2), 60-70. <https://doi.org/10.1016/j.vetpar.2009.08.028>
- Becker, D. J., Czirják, G. Á., Volokhov, D. V., Bentz, A. B., Carrera, J. E., Camus, M. S., ... Streicker, D. G. (2018). Livestock abundance predicts vampire bat demography, immune profiles, and bacterial infection risk. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373, 20170089. <https://doi.org/10.1098/rstb.2017.0089>
- Bradley, J. E., & Jackson, J. A. (2008). Measuring immune system variation to help understand host-pathogen community dynamics. *Parasitology*, 135(7), 807-823. <https://doi.org/10.1017/S0031182008000322>
- Butler, J. E. (1969). Bovine immunoglobulins: A review. *Journal of Dairy Science*, 52(12), 1895-1909. [https://doi.org/10.3168/JDS.S0022-0302\(69\)86871-2](https://doi.org/10.3168/JDS.S0022-0302(69)86871-2)
- Christe, P., Arlettaz, R., & Vogel, P. (2000). Variation in intensity of a parasitic mite (*Spinturnix myoti*) in relation to the reproductive cycle and immunocompetence of its bat host (*Myotis myotis*). *Ecology Letters*, 3(3), 207-212. <https://doi.org/10.1046/j.1461-0248.2000.00142.x>
- Clutton-Brock, T. H., Albon, S. D., & Guinness, F. E. (1989). Fitness costs of gestation and lactation in wild mammals. *Nature*, 337(6204), 260-262. <https://doi.org/10.1038/337260a0>
- Clutton-Brock, T. H., Guinness, F. E., & Albon, S. D. (1982). *Red deer: Behavior and ecology of two sexes* (Vol. 15). University of Chicago Press. Retrieved from https://books.google.co.uk/books/about/Red_Deer.html?xml:id=x4SGuA3t-NoC&pgis=1
- Cressler, C. E., Nelson, W. A., Day, T., & Mccauley, E. (2014). Disentangling the interaction among host resources, the immune system and pathogens. *Ecology Letters*, 17(3), 284-293. <https://doi.org/10.1111/ele.12229>
- Deerenberg, C., Arpanius, V., Daan, S., & Bos, N. (1997). Reproductive effort decreases antibody responsiveness. *Proceedings of the Royal Society B: Biological Sciences*, 264(1384), 1021-1029. <https://doi.org/10.1098/rspb.1997.0141>
- Eraud, C., Duriez, O., Chastel, O., & Faivre, B. (2005). The energetic cost of humoral immunity in the Collared Dove, *Streptopelia decaocto*: Is the magnitude sufficient to force energy-based trade-offs? *Functional Ecology*, 19(1), 110-118. <https://doi.org/10.1111/j.0269-8463.2005.00934.x>
- Festa-Bianchet, M. (1989). Individual differences, parasites, and the costs of reproduction for bighorn ewes (*Ovis canadensis*). *Journal of Animal Ecology*, 58(3), 785-795. <https://doi.org/10.2307/5124>
- Foo, Y. Z., Nakagawa, S., Rhodes, G., & Simmons, L. W. (2017). The effects of sex hormones on immune function: A meta-analysis. *Biological Reviews*, 92(1), 551-571. <https://doi.org/10.1111/brv.12243>

- French, S. S., Denardo, D. F., & Moore, M. C. (2007). Trade-offs between the reproductive and immune systems: Facultative responses to resources or obligate responses to reproduction? *American Naturalist*, 170(1), 79–89. <https://doi.org/10.1086/518569>
- Froy, H., Sparks, A. M., Watt, K., Sinclair, R., Bach, F., Pilkington, J. G., ... Nussey, D. H. (2019). Senescence in immunity against helminth parasites predicts adult mortality in a wild mammal. *Science*, 365, 1296–1298.
- Froy, H., Walling, C. A., Pemberton, J. M., Clutton-brock, T. H., & Kruuk, L. E. B. (2016). Relative costs of offspring sex and offspring survival in a polygynous mammal. *Biology Letters*, 12, 20160417. <https://doi.org/10.1098/rsbl.2016.0417>
- Garnier, R., & Graham, A. L. (2014). Insights from parasite-specific serological tools in eco-immunology. *Integrative and Comparative Biology*, 54(3), 363–376. <https://doi.org/10.1093/icb/icu022>
- Graham, A. L., Shuker, D. M., Pollitt, L. C., Auld, S. K. J. R., Wilson, A. J., & Little, T. J. (2011). Fitness consequences of immune responses: Strengthening the empirical framework for eco-immunology. *Functional Ecology*, 25(1), 5–17. <https://doi.org/10.1111/j.1365-2435.2010.01777.x>
- Grindstaff, J. L., Brodie, E. D., & Ketterson, E. D. (2003). Immune function across generations: Integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings of the Royal Society B: Biological Sciences*, 270(1531), 2309–2319. <https://doi.org/10.1098/rspb.2003.2485>
- Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software*, 33(2), 1–22. <https://doi.org/10.1002/ana.22635>
- Hamel, S., & Côté, S. D. (2008). Trade-offs in activity budget in an alpine ungulate: Contrasting lactating and nonlactating females. *Animal Behaviour*, 75(1), 217–227. <https://doi.org/10.1016/j.anbehav.2007.04.028>
- Hamel, S., & Côté, S. D. (2009). Foraging decisions in a capital breeder: Trade-offs between mass gain and lactation. *Oecologia*, 161(2), 421–432. <https://doi.org/10.1007/s00442-009-1377-y>
- Harshman, L. G., & Zera, A. J. (2007). The cost of reproduction: The devil in the details. *Trends in Ecology and Evolution*, 22(2), 80–86. <https://doi.org/10.1016/j.tree.2006.10.008>
- Hasselquist, D., & Nilsson, J.-A. (2009). Maternal transfer of antibodies in vertebrates: Trans-generational effects on offspring immunity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1513), 51–60. <https://doi.org/10.1098/rstb.2008.0137>
- Hurley, W. L., & Theil, P. K. (2011). Perspectives on immunoglobulins in colostrum and milk. *Nutrients*, 3(4), 442–474. <https://doi.org/10.3390/nu3040442>
- Hutchings, M. R., Judge, J., Gordon, I. J., Athanasiadou, S., & Kyriazakis, I. (2006). Use of trade-off theory to advance understanding of herbivore-parasite interactions. *Mammal Review*, 36(1), 1–16. <https://doi.org/10.1111/j.1365-2907.2006.00080.x>
- Irvine, R. J., Corbishley, H., Pilkington, J. G., & Albon, S. D. (2006). Low-level parasitic worm burdens may reduce body condition in free-ranging red deer (*Cervus elaphus*). *Parasitology*, 133(Pt 4), 465–475. <https://doi.org/10.1017/S0031182006000606>
- Jeffcoate, I. A., Wedrychowicz, H., Fishwick, G., Dunlop, E. M., Duncan, J. L., & Holmes, P. H. (1992). Pathophysiology of the periparturient egg rise in sheep: A possible role for IgA. *Research in Veterinary Science*, 53(2), 212–218. [https://doi.org/10.1016/0034-5288\(92\)90112-F](https://doi.org/10.1016/0034-5288(92)90112-F)
- Jones, L. A., Sakkas, P., Houdijk, J. G. M., Knox, D. P., & Kyriazakis, I. (2012). Amelioration of the periparturient relaxation of immunity to parasites through a reduction in mammalian reproductive effort. *International Journal for Parasitology*, 42(13–14), 1127–1134. <https://doi.org/10.1016/j.ijpara.2012.09.010>
- Knowles, S. C. L., Nakagawa, S., & Sheldon, B. C. (2009). Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: A meta-regression approach. *Functional Ecology*, 23(2), 405–415. <https://doi.org/10.1111/j.1365-2435.2008.01507.x>
- Langer, P. (2008). The phases of maternal investment in eutherian mammals. *Zoology*, 111(2), 148–162. <https://doi.org/10.1016/j.zool.2007.06.007>
- Maestripietri, D., & Mateo, J. M. (2009). The role of maternal effects in mammalian evolution and adaptation. In D. Maestripietri & J. M. Mateo (Eds.), *Maternal effects in mammals* (pp. 1–10). Chicago, IL: University of Chicago Press. <https://doi.org/10.1016/j.anbehav.2010.03.020>
- Martin, L. B., Weil, Z. M., & Nelson, R. J. (2008). Seasonal changes in vertebrate immune activity: Mediation by physiological trade-offs. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1490), 321–339. <https://doi.org/10.1098/rstb.2007.2142>
- McRae, K. M., Stear, M. J., Good, B., & Keane, O. M. (2015). The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunology*, 37(12), 605–613. <https://doi.org/10.1111/pim.12290>
- Møller, A. P., Erritzøe, J., & Saino, N. (2003). Seasonal changes in immune response and parasite impact on hosts. *American Naturalist*, 161(4), 657–671. <https://doi.org/10.1086/367879>
- R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.r-project.org/>
- Rödel, H. G., Zapka, M., Stefanski, V., & von Holst, D. (2016). Reproductive effort alters immune parameters measured post-partum in European rabbits under semi-natural conditions. *Functional Ecology*, 30(11), 1800–1809. <https://doi.org/10.1111/1365-2435.12663>
- Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology – Costly parasite defenses and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, 11(96), 317–321. [https://doi.org/10.1016/0169-5347\(96\)10039-2](https://doi.org/10.1016/0169-5347(96)10039-2)
- Sheldrake, R. F., Husband, A. J., Watson, D. L., & Cripps, A. W. (1984). Selective transport of serum-derived IgA into mucosal secretions. *Journal of Immunology*, 132(1), 363–368.
- Shocket, M. S., Strauss, A. T., Hite, J. L., Šiljivar, M., Civitello, D. J., Duffy, M. A., ... Hall, S. R. (2018). Temperature drives epidemics in a zooplankton-fungus disease system: A trait-driven approach points to transmission via host foraging. *American Naturalist*, 191(4), 435–451. <https://doi.org/10.1086/696096>
- Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1490), 375–398. <https://doi.org/10.1098/rstb.2007.2145>
- Stahlschmidt, Z. R., Rollinson, N., Acker, M., & Adamo, S. A. (2013). Are all eggs created equal? Food availability and the fitness trade-off between reproduction and immunity. *Functional Ecology*, 27(3), 800–806. <https://doi.org/10.1111/1365-2435.12071>
- Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional Ecology*, 3(3), 259–268. <https://doi.org/10.2307/2389364>
- Svensson, E., Råberg, L., Koch, C., & Hasselquist, D. (1998). Energetic stress, immunosuppression and the costs of an antibody response. *Functional Ecology*, 12(6), 912–919. <https://doi.org/10.1046/j.1365-2435.1998.00271.x>
- Watt, K. A., Nussey, D. H., Maclellan, R., Pilkington, J. G., & McNeilly, T. N. (2016). Fecal antibody levels as a noninvasive method for measuring immunity to gastrointestinal nematodes in ecological studies. *Ecology and Evolution*, 6(1), 56–67. <https://doi.org/10.1002/ece3.1858>
- Weinberg, E. D. (1984). Pregnancy-associated depression of cell-mediated immunity. *Reviews of Infectious Diseases*, 6(6), 814–831. <https://doi.org/10.2307/4453516>
- Weinberg, E. D. (1987). Pregnancy-associated immune suppression: Risks and mechanisms. *Microbial Pathogenesis*, 3(6), 393–397. [https://doi.org/10.1016/0882-4010\(87\)90009-X](https://doi.org/10.1016/0882-4010(87)90009-X)

- Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *American Naturalist*, 100(916), 687–690. <https://doi.org/10.1086/282461>
- Wilson, K., Grenfell, B. T., Pilkington, J. G., Boyd, H. E. G., & Gulland, F. M. D. (2004). Parasites and their impact. In T. Clutton-Brock & J. Pemberton (Eds.), *Soay sheep: Dynamics and selection in an island population* (pp. 113–165). Cambridge, UK: Cambridge University Press.
- Woodroffe, R., & Macdonald, D. W. (1995). Costs of breeding status in the European badger, *Meles meles*. *Journal of Zoology London*, 235, 237–245. <https://doi.org/10.1111/j.1469-7998.1995.tb05140.x>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Albery GF, Watt KA, Keith R, et al. Reproduction has different costs for immunity and parasitism in a wild mammal. *Funct Ecol*. 2019;00:1–11. <https://doi.org/10.1111/1365-2435.13475>