

# Negative covariance between parasite load and body condition in a population of feral horses

LUCIE DEBEFFE<sup>1\*</sup>, PHILIP D. MCLOUGHLIN<sup>1</sup>, SARAH A. MEDILL<sup>1</sup>, KATHRINE STEWART<sup>1</sup>, DANIEL ANDRES<sup>1</sup>, TODD SHURY<sup>2</sup>, BRENT WAGNER<sup>3</sup>, EMILY JENKINS<sup>3</sup>, JOHN S. GILLEARD<sup>4</sup> and JOCELYN POISSANT<sup>4,5\*</sup>

<sup>1</sup> Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK S7N 5E2, Canada

<sup>2</sup> Parks Canada Agency, 52 Campus Drive, Saskatoon SK S7N 5B4, Canada

<sup>3</sup> Department of Veterinary Microbiology, University of Saskatchewan, 52 Campus drive, Saskatoon, SK S7N 5B4, Canada

<sup>4</sup> Department of Comparative Biology and Experimental Medicine, University of Calgary, 3330 Hospital Drive, Calgary, AB T2N 4N1, Canada

<sup>5</sup> College of Life and Environmental Sciences, University of Exeter, Cornwall Campus, Penryn TR10 9FE, UK

(Received 30 November 2015; revised 10 February 2016; accepted 11 February 2016; first published online 22 March 2016)

## SUMMARY

In wild and domestic animals, gastrointestinal parasites can have significant impacts on host development, condition, health, reproduction and longevity. Improving our understanding of the causes and consequences of individual-level variation in parasite load is therefore of prime interest. Here we investigated the relationship between strongyle fecal egg count (FEC) and body condition in a unique, naturalized population of horses that has never been exposed to anthelmintic drugs (Sable Island, Nova Scotia, Canada). We first quantified variation in FEC and condition for 447 individuals according to intrinsic (sex, age, reproductive status, social status) and extrinsic (group size, location, local density) variables. We then quantified the repeatability of measurements obtained over a field season and tested for covariance between FEC and condition. FECs were high relative to other horse populations (mean eggs per gram  $\pm$  SD = 1543.28  $\pm$  209.94). FECs generally decreased with age, were higher in lactating *vs* non-lactating females, and unexpectedly lower in males in some part of the island. FECs and condition were both spatially structured, with patterns depending on age, sex and reproductive status. FECs and condition were both repeatable. Most notably, FECs and condition were negatively correlated, especially in adult females.

Key words: gastrointestinal parasite, mammal, nematode, repeatability, strongyle, ungulate, wild population.

## INTRODUCTION

Parasites reduce host fitness through a number of mechanisms including behavioural modifications, diversion of resources, damage to tissues and the induction of energetically costly immune responses, with consequent impacts on host population dynamics (Bowman, 2003; Poulin, 2007). While negative impacts of parasites on host population fitness have been documented in several studies (Tompkins and Begon, 1999; Watson, 2013), expectations regarding the relationship between parasite load and individual fitness components within populations are unclear (Sheldon and Verhulst, 1996). In part, this is because phenotypic relationships between parasite load and fitness components can take various and somewhat unpredictable forms in response to both variation in host energy acquisition (Cressler *et al.* 2014) and trade-offs between immunity and life

history traits (Sheldon and Verhulst, 1996). As a consequence, relationships between parasite load and fitness components in wild populations are expected to be population-specific (Poulin and Forbes, 2012), and therefore best understood through detailed longitudinal individual-based research (Clutton-Brock and Sheldon, 2010; Hayward, 2013).

Variation in parasite load among wild animals generally stems from complex interactions among intrinsic (e.g. differences in sex, age, immune condition and genetic makeup) and extrinsic (e.g. exposure to parasites, density of conspecific and habitat quality) factors. For example, males are generally more parasitized than females (Poulin, 1996; Zuk and McKean, 1996; Moore and Wilson, 2002) and parasite load generally varies with age (Hayward, 2013). Furthermore, within each sex, individuals that are either dominant or nursing are usually more parasitized than subordinate and non-nursing individuals (Lloyd, 1983; Ezenwa *et al.* 2012; Habig and Archie, 2015), with these effects potentially varying across ontogeny. Extrinsic environmental and social variables such as local geo-physical characteristics, host density and group size can also

\* Corresponding authors: Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK S7N 5E2, Canada. E-mail: [lucie.debeffe@gmail.com](mailto:lucie.debeffe@gmail.com) and Centre for Ecology and Conservation, University of Exeter, Cornwall Campus, Penryn TR10 9FE, UK. E-mail: [j.poissant@exeter.ac.uk](mailto:j.poissant@exeter.ac.uk)

influence variance in parasite load (Body *et al.* 2011; Rifkin *et al.* 2012; Patterson and Ruckstuhl, 2013), in part through effects on levels of exposure to parasites, but also via effects on stress and energy balance. While numerous studies have investigated the influence of some of these variables in wild populations, relatively few have done so using data-rich, long-term individual-based population studies of vertebrates (but see Hayward *et al.* 2014 for an excellent example). While challenging to perform, such studies are essential to quantifying the relative influence and interaction of intrinsic and extrinsic variables on (co)variance in parasite load and fitness components under natural conditions (Clutton-Brock and Sheldon, 2010; Hayward, 2013).

Theory suggests that the relationship between parasite load and body condition may be hard to predict in the presence of variation in host energy acquisition because both hosts and parasites can benefit from an increase in host energy consumption (Cressler *et al.* 2014). However, in vertebrates high-quality diet is usually associated with reduced parasite load, perhaps because parasites in vertebrate hosts are energy saturated and mainly regulated by host immunity (Cressler *et al.* 2014). In that case, a negative relationship between condition and parasite load would generally be expected since individuals with low parasite load do not have to invest as much in mounting an immune response and tissue repair, while individuals in good condition can invest more heavily in parasite suppression. It is also commonly thought that parasites reduce host appetite and in turn impact body condition and growth rate (Stien *et al.* 2002). Social interactions can also affect the relationship between body condition and parasite load if access to high-quality habitat is determined by social dominance and habitat quality covaries with parasite contamination levels (Altizer *et al.* 2003; Ezenwa, 2004; Fairbanks *et al.* 2012). Subordinate individuals may be forced to feed in non-optimal, contaminated habitat or alternatively, subordinate individuals may be less susceptible to infection if contamination is higher in habitats dominated by higher-ranking individuals (Fairbanks *et al.* 2012). While negative relationships between the prevalence or intensity of parasitism and indices of body condition have been documented in numerous studies of vertebrates (e.g. Body *et al.* 2011 in roe deer *Capreolus capreolus*; Irvine *et al.* 2006 in red deer *Cervus elaphus*; Stien *et al.* 2002 in reindeer *Rangifer tarandus*; Rubenstein and Hohmann, 1989 in horses; Schulte-Hostedde and Elsasser, 2011 in American mink *Neovison vison*), the absence of relationship also appears to be common (e.g. Pilar Valdez-Cruz *et al.* 2013 in equids; Vatta *et al.* 2002 in goats, Scantlebury *et al.* 2010 in grey squirrel *Sciurus carolinensis*; Moretti *et al.* 2014 in toads *Rhinella icterica*;

Davidson *et al.* 2015 in moose *Alces alces*). Thus, more research on the link between parasite load and body condition in vertebrates seems warranted.

Horses are ubiquitously parasitized by a diversity of gastrointestinal parasitic nematodes (Lichtenfels *et al.* 2008). Of particular interest are strongyle species, which are known to cause severe pathologic issues (Nielsen *et al.* 2010a, b). In addition to generally hindering digestive function, larvae of large strongyle species such as *Strongylus vulgaris* create internal lesions by migrating through organs while the synchronised mass emergence of cyathostomins (small strongyle) encysted larvae can result in deadly larval cyathostomiasis (Love *et al.* 1999). Patterns of infection by strongyle nematodes in domestic horses have been studied extensively due to their importance to equine health (e.g. Osterman, 2005; Nielsen *et al.* 2010b; Carstensen *et al.* 2013; Flanagan *et al.* 2013; Wood *et al.* 2013). However, most of these studies were conducted on horses that have been exposed at one point or another to anthelmintic drugs and therefore do not depict 'natural' interactions between horses and parasitic strongyle communities. In addition, the combination of veterinary care, shelter and controlled diet limits opportunities to study the impacts of gastrointestinal parasites on performance and life history in domestic horses. In fact, while the pathogenic effects of equine strongyles are well established, evidence for (any) subclinical effects from high strongyle abundance in domestic horses remains equivocal (Fog *et al.* 2011). In contrast, in a study of feral horses from Shackelford Banks (North Carolina, USA), Rubenstein and Hohmann (1989) documented a clear negative relationship between body condition and the number of gastrointestinal parasite eggs per gram (EPG) of feces. This study highlighted the potential of feral horse populations for investigating the relationships between gastrointestinal parasites and fitness-related traits in unmanaged populations of animals, as well as testing hypotheses relevant to the management of gastrointestinal parasites in horses.

We examined inter-individual variation in gastrointestinal parasite load in the large feral horse population of Sable Island National Park Reserve, Nova Scotia, Canada. The population, which is the subject of a long-term individual-based monitoring programme initiated in 2008, has been unmanaged since 1960 and was never exposed to anthelmintic drugs. As such, it offers a unique opportunity to study natural variation in strongyle infections and their fitness correlates in horses. First, we explored how gastrointestinal parasite load [estimated by strongyle fecal egg count (FEC)] and body condition varied with intrinsic (sex, age, reproductive status, social status) and extrinsic (group size, location, local density) variables over a 2-month period in summer of 2014. To assess how reliable FEC and

body condition scores were across the sampling period, we estimated repeatability for both traits within a single summer period. Finally, we tested for the presence of a correlation between FEC and body condition. In line with the literature, we expected FEC to be higher in males (Poulin, 1996; Zuk and McKean, 1996; Turner and Getz, 2010), to decrease with increasing age (Relf *et al.* 2013; Wood *et al.* 2013), and to increase with increasing group size and local density (Rifkin *et al.* 2012; Patterson and Ruckstuhl, 2013). In contrast, we expected body condition to decrease with increasing density (Bonenfant *et al.* 2009). Because horse habitat quality decreases from west to east on Sable Island [forage quality and access to freshwater (Contasti *et al.* 2012; Rozen-Rechels *et al.* 2015)], we expected horses to be in better condition and less parasitized in the west after having accounted for the potential effect of density. Because dominant band stallions need to invest considerable energy into maintaining their social status (Habig and Archie, 2015) and can have depressed immune responses due to increases in testosterone (Ezenwa *et al.* 2012), we expected dominant band stallions to have higher FECs than bachelors. Due to the energy demands associated with producing and raising a foal, females are expected to reduce the energy allocated to immunity (Lloyd, 1983; Barger, 1993; Sheldon and Verhulst, 1996), and so we expected lactating females in Sable Island's resource limited environment to have higher FECs and lower body condition than non-lactating females (Festa-Bianchet *et al.* 1998; Clancey *et al.* 2012). Finally, we expected parasite load to increase with decreasing body condition.

## MATERIALS AND METHODS

### *Study area and population*

Sable Island National Park Reserve (43°55'N; -60°00'W) is a vegetated sandbar located 275 km southeast of Halifax, Nova Scotia (Fig. 1). It is 49 km long and 1.3 km wide at its broadest point. The climate is temperate oceanic with warm summers and cool, wet winters. Topography is characterized by sandy beaches, rolling heath meadows and sand dunes that reach heights of up to 30 m. The island's vegetation is dominated by marram grass (*Ammophila breviligulata*), sandwort (*Honckenya peploides*), beach pea (*Lathyrus maritimus*), and heath-type plants. There are several permanent freshwater ponds located in the western and central parts of the island which cover <1% of the island's surface area. The availability of freshwater as well as vegetation quality decreases from west to east (Contasti *et al.* 2012; Rozen-Rechels *et al.* 2015). Introduced to the island in the mid-1700s, the horses are now the island's only terrestrial mammals (apart from very limited

human presence). While removals and supplementation occurred periodically over the last centuries, the population became legally protected from human interference in 1960 and has been unmanaged since then (Christie, 1995). From 2008 to 2014, population size ranged from 380 individuals (in 2008) to 559 individuals (in 2013). In September 2014 there were 552 horses on the island.

### *Data collection*

*Population monitoring and life history.* Population and life history data collection began with a partial census in summer 2007. From 2008 onward, systematic yearly ground censuses were performed during the mid-late breeding season (July–September). Daily walking censuses focused on 1 of 7 sections, allowing complete coverage of the island in 1 week. The location of bands, bachelor groups and lone individuals were recorded to within 5 m using a hand-held Global Positioning System (GPS). At each encounter, several photographs were taken in addition to information on sex, age (foal, yearling, 2–3 years old or adult), coat colour and any other distinguishing features (e.g. leg and face markings, scars, etc.). Band membership (when applicable), reproductive status of females (lactating or not lactating, based on the presence or absence of a foal) and the social status of males (dominant band stallion, subordinate band member or bachelor) were also recorded. Photographs and observations made in the field were used to confirm the identity of each horse.

While the exact age of older individuals cannot be determined in the field, we were able to accurately age horses using birth records from the start of the research program in 2007 (and inferred to be 2006 for yearlings observed in 2007). Historical records and age determination using tooth cementum indicate that Sable Island horses can live beyond 20 years, but that very few individuals live past this age (3.5% of females live into the age 18+ category; 5.4% of males; Welsh, 1975). For the analyses presented herein, due to our length of study, age could only be considered as a continuous variable ranging from 0 to 9, with 9 including horses aged 9+ years (i.e. animals born in 2005 or earlier).

*Extrinsic variables.* We considered each individual's location, local density, and group size as extrinsic variables. These variables were estimated each year during the summer. Because equine strongyle nematodes can take multiple months to develop into sexually mature worms (although some small strongyles can complete their life cycle within a few weeks) (Khan *et al.* 2015), and because an animal's current body condition is generally determined by abiotic and biotic conditions experienced in the preceding months (Reading and Clarke,

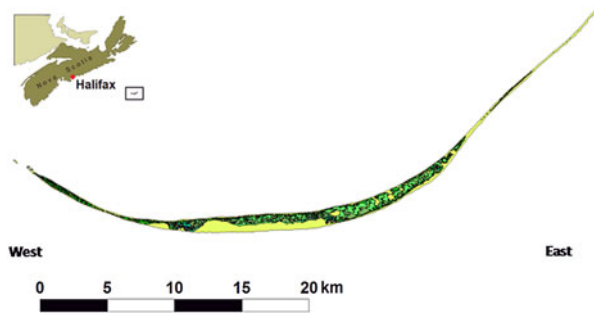


Fig. 1. Map of Sable Island, Nova Scotia, Canada.

1995), we considered environmental conditions experienced in the previous year (2013) rather than those observed during the 2014 field season. 2013 and 2014 variables were not included in the same models due to high collinearity, though it is worth noting that including 2014 instead of 2013 variables did not alter our conclusions (data not shown). Location, which is correlated with horse habitat quality on Sable Island (Contasti *et al.* 2012; Rozen-Rechels *et al.* 2015) and might influence exposure risk (strongyle larvae density), was calculated as the median longitude from census surveys. Local density was estimated as the number of individuals (excluding foals) within 8000 m of an individual's summer centroid location divided by the vegetated surface area within this buffer which is the area used by horses for foraging (see Marjamäki *et al.* 2013 for further details). Group size was estimated as the mean number of individuals (excluding foals) present in an individual's band or bachelor group during the summer of 2013.

**Strongyle FECs.** Sable Island horses are infected by a large diversity of gastrointestinal parasites. Necropsies performed in the early 1970s identified a tapeworm (*Paranoplocephala mamillana*), *Parascaris equorum*, 2 large strongyle (*S. vulgaris* and *Strongylus equinus*) and at least 9 cyathostomins (small strongyle) species (Welsh, 1975). Because the prevalence of species other than strongylids is generally low [Welsh (1975) and our own observations], we limited our analyses to strongyle species. Strongyle abundance was estimated by counting the number of eggs present in fecal samples (FEC). Because strongyle species cannot be differentiated based on egg morphology, our index represents an aggregate of all species present. FEC is a common non-invasive approach for estimating gastrointestinal parasitic nematode abundance in vertebrates (Raynaud, 1970; Turner and Getz, 2010). While strongyle FECs are known to be influenced by parasite life cycles and fecundity (Keymer and Hiorns, 1986; Nielsen *et al.* 2010b), they have nonetheless been shown to approximate total strongyle burden in horses (Nielsen *et al.* 2010b).

We collected freshly passed feces from the 22th of July to the 7th of September 2014, either opportunistically during daily census surveys, or by observing specific horses until they defecated. In total, we collected 670 samples from 447 yearling and adult individuals, for an average of  $1.5 \pm 0.69$  samples per individual (mean  $\pm 1$  standard deviation, SD). Foals were not considered in this study because they were generally too young to harbour a population of sexually mature strongyle nematodes (i.e. they all had very low FEC). Fecal samples were stored individually in tied-up nitrile gloves and whenever possible placed into a cooler containing ice packs immediately following collection (464 samples out of 670; the remaining samples were kept at ambient temperature for up to 7 h). Once in the laboratory, all samples were stored in a cooler filled with ice packs until conducting FECs on the same day.

We performed strongyle FECs using a modified McMaster protocol. For each sample, we homogenized 4 g of feces and 26 mL of Sheather's sugar solution (specific gravity of 1.27) in an 88 mL paper cup for 2 min using a tongue depressor. We then filtered the mixture through a funnel lined with cheesecloth into a second cup, mixed vigorously using a tongue depressor and immediately loaded in two 0.15 mL chambers of a McMaster slide (Chalex Corp., USA). After allowing eggs to float to the surface for at least a minute, we then counted strongyle eggs using a compound microscope. The number of EPG of feces was obtained by multiplying the total number of eggs observed by 25 (Sellon and Long, 2013).

The temperature at which fecal samples are stored and the time elapsed between sample collection and the FEC can bias results (Nielsen *et al.* 2010a). Specifically, at warm temperatures eggs may hatch before they can be counted, resulting in a downwardly biased FEC (Nielsen *et al.* 2010a). Strongyle FEC in horses is also known to show strong seasonality (e.g. Wood *et al.* 2013), which needs to be accounted for when present. To test for the presence of biases resulting from storage condition and seasonality, we modelled FEC as a function of storage temperature (i.e. whether samples were stored with icepacks in the field or not), time of collection, Julian date and their interaction (see Appendix 1 for details). As we found no evidence for FEC to be influenced by storage condition, time of collection or Julian date we did not consider these variables any further.

**Body condition.** We assessed body condition of horses from photographs using the index of Carroll and Huntington (1988). This index ranges from zero (very poor condition) to five (obese), with half points awarded when different regions of the body vary in score (Carroll and Huntington, 1988). The

score reflects the amount of subcutaneous fat deposition on the hips, ribs and spine of the animal. A total of 1607 condition scores we obtained for the 447 individuals for which FECs were conducted, for an average of  $3.6 \pm 0.9$  times per individuals (mean  $\pm$  1 SD). All condition scores were estimated in the laboratory after the field session by a single trained observer (L. Debeffe). Scores were only estimated when numerous clear photographs of the hips, ribs and spine were available. When obtaining repeated measurements (scores on different days for the same horse), the scorer was blind to previous measurements. Note also that members of the same band were generally not scored one after another. Joint FEC and condition estimates (i.e. measured on the same date) were obtained on 405 occasions from 322 individuals.

### Data analysis

*Intrinsic and extrinsic correlates of FEC and body condition.* We used univariate linear mixed models implemented in the R (R Development Core Team, 2010) lme4 package (Bates *et al.* 2015) to identify variables that significantly covaried with FEC and body condition at both the population level and within adults of each sex (4 years old and older). FECs had a skewed distribution (Fig. A1) and were therefore log-transformed prior to analysis [ $\log(X + 25)$ ]. Group (in summer 2013) and horse identity were included as random effects in all models to account for repeated measurements. For the overall population model, fixed effects included age (fitted as a continuous variable), sex, median location in summer (2013) (standardized to a mean of 0 and standard deviation of 1), group size (in summer 2013), local density (in summer 2013) and their two-way interactions. For body condition, fixed effects also included date (with 1st January as day 0) as well as corresponding two-way interactions.

Sex-specific models were the same as the model just described, but instead of the variable 'sex' they included a reproductive (females) or social status (males) variable. Female reproductive status was categorized as either 'lactating' or 'not lactating' during summer 2014. Male social status was categorized as either 'dominant band stallion' or 'bachelor'. To ensure that individuals were of reproductive age, we only included individuals of more than 3 years old in these analyses. These individuals are hereafter referred to as 'adults'. We tested for non-linear relationships between dependent and independent variables by fitting cubic splines with generalized additive models implemented in the R mgcv package (Wood, 2011).

We fitted the global models described above, as well as all simpler models, using the R AICcmodavg package (Mazerolle, 2015). The best overall and sex-specific models were then selected

using the Akaike Information Criterion corrected for small sample size (AICc), which reflects the best compromise between model precision and accuracy (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). According to the rule of parsimony, we selected the simplest model within 2 AICc of the top model (Burnham and Anderson, 2002). We also calculated evidence ratio (ER) and AICc weights (AICcWts) as a measure of the likelihood that a given model was the best among the set of fitted models. Using the sum of the AICcWts (termed the predictor weight), we estimated the relative importance of each variable and interactions according to Symonds and Moussalli (2011). The predictor weight can be interpreted as being equivalent to the probability that the predictor is a component of the best model.

*Repeatability of FEC and body condition.* We tested for the repeatability of FEC and body condition scores for the entire population, as well as for adults of each sex separately, using univariate linear mixed models and restricted maximum likelihood implemented in ASReml 3.0 (Gilmour *et al.* 2009). Specifically, phenotypic variance ( $V_p$ ) was partitioned into permanent variation among individuals ( $V_{ind}$ ), groups ( $V_{group}$ ) and residual variance ( $V_e$ ) by fitting individual ID and group as random effects. Repeatability was then calculated as  $V_{ind}/V_p$ , where  $V_p = V_{ind} + V_{group} + V_e$ . Significance of  $V_{ind}$  and  $V_{group}$  was tested using likelihood ratio tests. For hypotheses involving parameters on the boundary of parameter space, such as variances, the theoretical asymptotic distribution of the likelihood ratio is a mixture of  $\chi^2$  variates, where the mixing probabilities are 0.5, one with 0 degrees of freedom and the other with 1 degree of freedom (Self and Liang, 1987; Gilmour *et al.* 2009). P-values from  $\chi^2$  tests with 1 degree of freedom were therefore divided by 2. The number of individuals, records, and individuals with repeated records included in these analyses are presented in Table A2 in the Appendix.

Repeatability was first estimated by only including mean trait values as fixed effects. As commonly done in the quantitative genetics literature, we also estimated repeatability conditional on intrinsic and extrinsic causes of intra- and inter-individual variations (Wilson, 2008; Wilson *et al.* 2010). For FEC, these included sex, age, location on the island and the two-way interactions between age and location and sex and location. For body condition these included age, location on the island, sex, scoring date and the two-way interactions between age and location, sex and age, and sex and scoring date.

*Covariation of FEC and body condition.* We used bivariate linear mixed models in ASReml to estimate phenotypic covariance ( $COV_p$ ) and

correlations ( $r_p$ ) between FEC and body condition where  $\text{COV}_p = \text{COV}_{\text{ind}} + \text{COV}_{\text{group}} + \text{COV}_e$  and  $r_p = (\text{COV}_p / \sqrt{V_{\text{FEC}} + V_{\text{condition}}})$ . As for repeatability, these were estimated using models including trait means only, as well as with models that included known intrinsic and extrinsic sources of variation as fixed effects. The significance of covariance components was determined using likelihood ratio tests.

### Ethics statement

Sampling was performed under University of Saskatchewan Animal Care Protocol 20090032 in compliance with guidelines of the Canadian Council on Animal Care, and Parks Canada Agency Research and Collections Permit SINP-2013-14314. Note that raw data are available upon request.

## RESULTS

### Strongyle FECs

The mean number of eggs per gram (EPG)  $\pm 1$  SD was  $1543.28 \pm 1209.94$  ( $N_{\text{sample}} = 670$ ,  $N_{\text{horse}} = 447$ , range = 0 to 9200; see Table A2 in Appendix for details on each subsample). Non-linear relationships were observed between age and FEC, location and FEC and age and body condition (see Table A3 in Appendix). In all cases, non-linear relationships appeared to be well approximated by second-order polynomials and so these were used.

### Intrinsic and extrinsic correlates of FEC

Individual FEC was best described by the model including sex, age, location and two-way interactions between sex and location, and age and location (see Table A4a in Appendix for details on model selection). Note that for this model selection, as well as all other ones (below), the variables included in the selected model, had the highest predictor weights (Table 1). Individual FEC for females of reproductive age (i.e. of more than 3 years old) were best described by the effects of reproductive status and an interaction between age and location (see Table A4b in Appendix for details on model selection). FECs for adult males (i.e. of more than 3 years old) were best predicted by age, location, local density, social status, and interactions between age and location, local density and social status, age and social status, and location and social status (see Table A4c in Appendix for details on model selection).

Patterns of FECs were spatially structured according to the age and sex of individuals. On the east side of the island, middle-aged horses (between 3 and 6 years old) had higher FECs, while on the west

side, younger individuals (less than 4 years old) had higher FECs, especially in females (Table A6 in Appendix, Fig. 2a and b). In both sexes, FECs were higher in the centre of the island. However, in females FECs were higher in the east than in the west, while in males it was the opposite (Table A6 in Appendix, Fig. 2c). For both adult females and males, FECs were related to reproductive or social status. Within males, dominant band stallions inhabiting in the centre of the island had higher FECs than those occupying the west or east sections, while bachelors inhabiting the extreme ends of the island had higher FECs, especially in the west (Fig. 3b). Local density affected dominant band stallions and bachelors differently: while dominant band stallions showed an increase of FECs with increasing local density, bachelors showed the reverse trend (Fig. 3c). Overall, FECs were higher for dominant band stallions compare with bachelors, but this difference decreased over the summer. In both social categories, FECs were higher in younger individuals (Fig. 3a). Finally, nursing females had higher FECs than non-nursing females whatever their age or location on the island (Table A6 in Appendix; predicted log-transformed EPG  $\pm$  SE for nursing females and non-nursing females:  $7.56 \pm 0.22$  and  $6.95 \pm 0.24$ ).

### Intrinsic and extrinsic correlates of body condition

In order to analyse the covariation between FEC and body condition the same model selection procedure described above was performed for body condition (see Table A5 in Appendix for details on model selection). At the population level (i.e., for males and females combined), body condition was best predicted by sex, age, location, scoring date, as well as two-way interactions between sex and age, age and location, and sex and scoring date (Table A5a in Appendix). For adult females, body condition was best described by scoring date and the interaction between reproductive status and location, while for adult males, it was best described by location and interactions between social status and age, and social status and scoring date (Table A5b and c in Appendix).

As with FEC, body condition was spatially structured, with patterns depending on age and sex. Young males and females (i.e. <3 years old) both had higher body condition scores in the centre of the island; while females older than 5 had higher body condition scores towards the tips of the island (Table A6 in Appendix, Fig. 4a and b). Body condition of adult males increased from east to west (Table A6 in Appendix). With the exception of 2-year olds, body condition scores were higher for males than for females. In males, body condition scores increased with age until 6 years old and decreased thereafter. In females, middle-aged

Table 1. Predictor weights calculated as the sum of the Akaike weights for each model in which that variable appeared. Six datasets were used: strongyle fecal egg count (FEC) and body condition (BC) and for all individuals, adult females only and adult males only. Variables retained in the selected model are in bold. For the sex-specific datasets the variable 'status' (reproductive status for females and social status for males) replaced the variable 'sex'.

Predictor	Dataset					
	FEC all	FEC adult females	FEC adult males	BC all	BC adult females	BC adult males
Age	<b>1.00</b>	<b>1.00</b>	<b>0.99</b>	<b>1.00</b>	0.70	<b>0.95</b>
Sex or status	<b>0.90</b>	<b>1.00</b>	<b>0.96</b>	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>
Location	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>
Density	0.53	0.66	<b>0.86</b>	0.82	0.73	0.79
Group size	0.58	0.57	0.49	0.73	0.71	0.85
Date of scoring	–	–	–	<b>1.00</b>	<b>1.00</b>	<b>0.95</b>
Group size : Density	0.13	0.14	0.11	0.17	0.15	0.44
Group size : Age	0.06	0.05	0.05	0.29	0.06	0.16
Group size : Location	0.14	0.17	0.08	0.20	0.29	0.51
Group size : Sex or status	0.21	0.14	0.14	0.27	0.26	0.24
Density : Age	0.07	0.18	0.13	0.16	0.17	0.11
Density : Location	0.10	0.09	0.19	0.25	0.20	0.22
Density : Sex or status	0.15	0.31	<b>0.74</b>	0.31	0.20	0.30
Age : Location	<b>1.00</b>	<b>0.82</b>	<b>0.60</b>	<b>0.92</b>	0.24	0.19
Age : Sex or status	0.24	0.14	<b>0.71</b>	<b>1.00</b>	0.08	<b>0.61</b>
Location : Sex or status	<b>0.72</b>	0.30	<b>0.78</b>	0.43	<b>0.96</b>	0.60
Group size : Date	–	–	–	0.24	0.24	0.45
Density : Date	–	–	–	0.54	0.37	0.34
Location : Date	–	–	–	0.38	0.32	0.32
Age : Date	–	–	–	0.12	0.53	0.12
Sex or status : Date	–	–	–	<b>0.87</b>	0.55	<b>0.85</b>

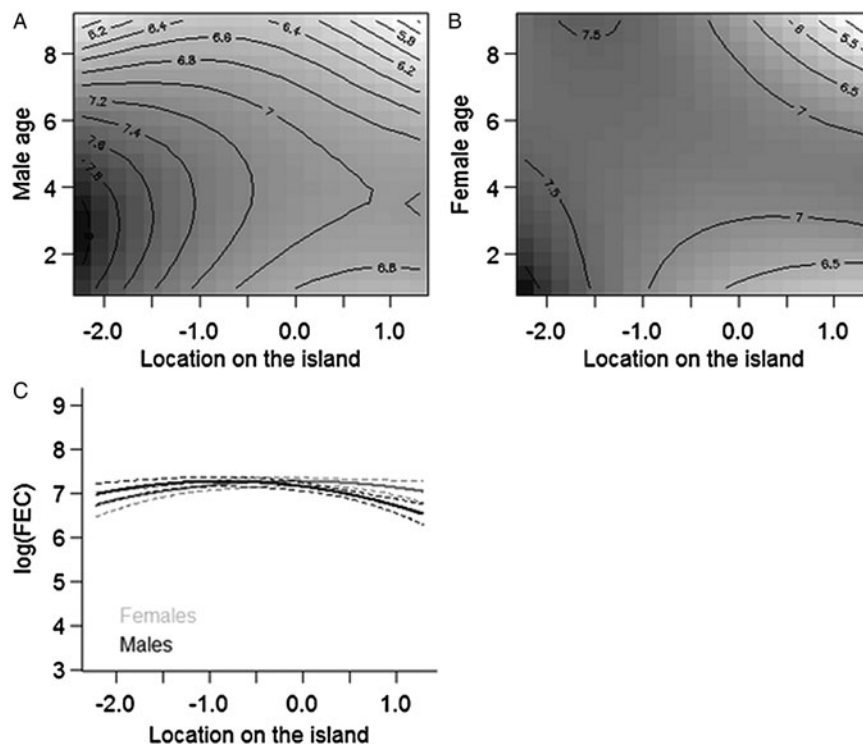


Fig. 2. Strongyle fecal egg count (FEC, expressed as the log-transformed number of eggs per gram of feces) in Sable Island horses ( $N_{\text{horse}} = 447$ ,  $N_{\text{sample}} = 670$ ) as a function of (A, B) the interaction between age and location in the previous year for males and females, respectively; (C) location on the island in the previous year (standardized UTM; lower values for western coordinates) with age set to its mean value. Isoclines and the grey shading in (A) and (B) represent the predicted FEC, with FEC increasing along the light to dark grey gradient. In (C) the solid and dashed lines depict relationships predicted by the selected model and their 95% confidence intervals, respectively.

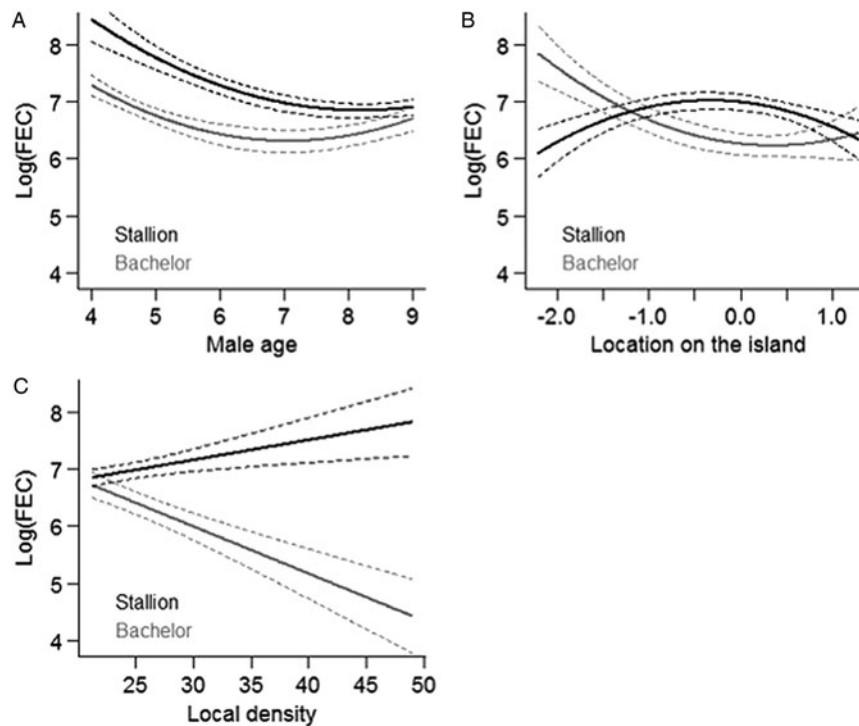


Fig. 3. Relationship between strongyle fecal egg count (FEC, expressed as the log-transformed number of eggs per gram of feces) and social status, age, location and local density in adult males (>3 years old,  $N_{\text{horse}} = 153$ ,  $N_{\text{sample}} = 242$ ). (A) Effect of age for each social category with location and density in the previous year set to their mean values. (B) Effect of location in the previous year (standardized UTM; lower values for western coordinates) on dominant stallions and bachelors with age and local density in the previous year set to their mean values. (C) Effect of local density in the previous year on dominant stallions and bachelors with age and location on the island in the previous year set to their mean values. The solid and dashed lines depict relationships predicted by the selected model and their 95% confidence intervals, respectively.

individuals had the lowest condition scores (Table A6 in Appendix, Fig. 4c) and body condition decreased over the field season (Table A6 in Appendix, predicted body condition  $\pm$ SE for males and females, respectively:  $2.87 \pm 0.04$  and  $2.41 \pm 0.04$  on the 22th of July,  $2.86 \pm 0.04$  and  $2.27 \pm 0.04$  on the 10th of September). Nursing females had lower body condition scores than non-nursing females except in the west end of the island, where body condition scores were comparable between nursing and non-nursing females (Table A6 in Appendix, Fig. 5). Adult males between 4 and 7 years old had similar body condition scores regardless of their social status; however body condition of dominant band stallions decreased sharply after 7 years old (Table A6 in Appendix; Fig. 6a). Across the sampling period, bachelor male body condition increased, whereas dominant band stallion body condition decreased (Table A6 in Appendix; Fig. 6b).

#### Repeatability and covariation

The repeatability of FEC and body condition scores was estimated by partitioning phenotypic variance into within- and among-individual components

(Table 2). When ignoring intrinsic and extrinsic sources of variation, repeatability of FEC ( $\pm 1$  SE) ranged from  $0.72 \pm 0.04$  at the population level to  $0.83 \pm 0.03$  when only considering adult males. Repeatability of body condition was relatively lower, ranging from  $0.44 \pm 0.09$  in adult males and adult females to  $0.57 \pm 0.03$  at the population level. In general, repeatability point estimates were only marginally reduced when conditioned on known sources of intrinsic and extrinsic variation (Table 2).

Group identity in the previous year (2013) explained a small but statistically significant amount of variation in both FEC and body condition at the population level when only fitting trait means as fixed effects (Table 2). In contrast, group ID explained a relatively large proportion of variation in body condition when only considering adult females (approximately 30%). However, phenotypic variation attributed to group effects was reduced and no longer statistically significant in all cases once known sources of intrinsic and extrinsic variation were accounted for.

We observed significant negative phenotypic covariance between FEC and body condition at the population level (Fig. 7) as well as within adult females, but not in adult males (Table 3). In



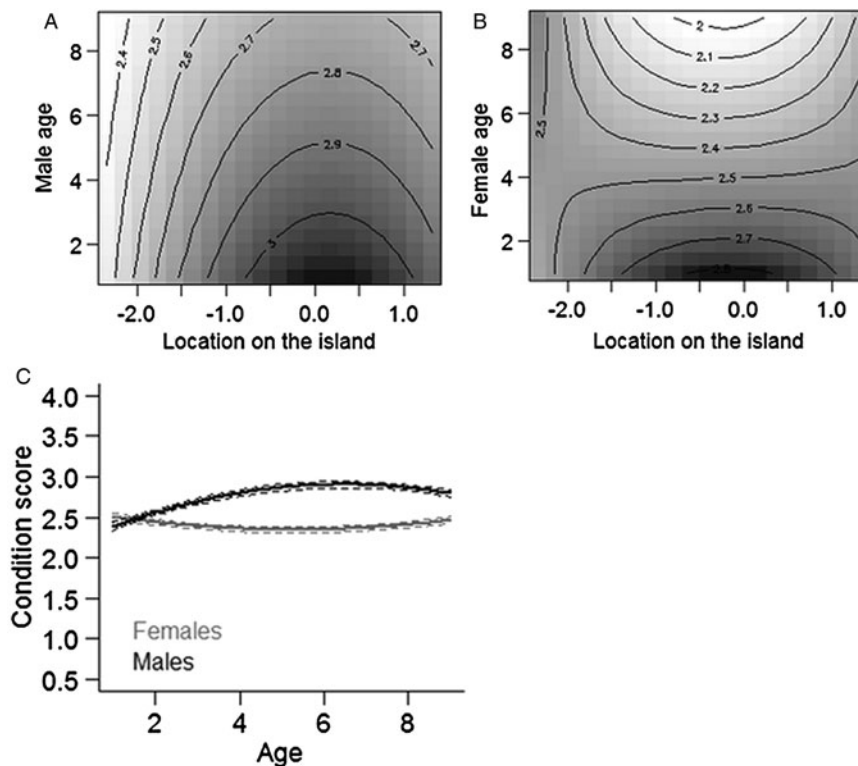


Fig. 4. Relationship between body condition and sex, age, location on the island and scoring date ( $N_{\text{horse}} = 447$ ,  $N_{\text{sample}} = 1607$ ) based on the prediction of the selected model. Panels (A, B) represent the two-way interaction effect between age and location on the island in the previous year (standardized UTM; lower values for western coordinates) for males and females, respectively; in (A) and (B) the isoclines and the grey shading represent the predicted condition score, with condition increasing along the light to dark grey gradient. Panel (C) represents the effect of age for each sex with location and scoring date set to their mean value; the line represents the relationship predicted by the selected model; dashed lines represent the 95% confidence interval around the predicted values.

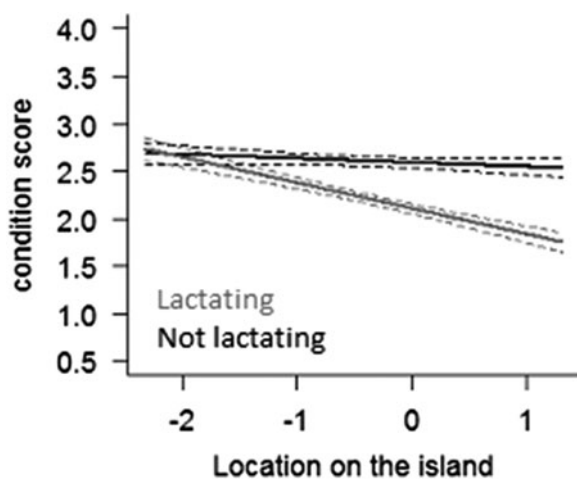


Fig. 5. Relationship between body condition and location on the island in the previous year (standardized UTM; lower values for western coordinates) for each reproductive status and with scoring date sets to its mean value for adult females ( $N_{\text{horse}} = 125$ ,  $N_{\text{sample}} = 439$ ); the line represents the relationship predicted by the selected model; dashed lines represent the 95% confidence interval around the predicted values.

general, fitting fixed effects in addition to trait means to account for known sources of variation did not influence covariance and correlation estimates

(Table 3). When including all relevant fixed effects, the phenotypic correlations at the population level and within adult females were  $-0.184 \pm 0.044$  and  $-0.333 \pm 0.081$ , respectively. Decomposing covariances into within and among individual components highlighted stronger negative correlations between repeatable components of variance ( $r_{\text{ind}}$ ,  $-0.289 \pm 0.063$  and  $-0.526 \pm 0.101$ ) coupled with an absence of significant correlation between residuals ( $r_e$ ).

#### DISCUSSION

We quantified variation in strongyle nematode egg shedding, as measured by FECs, in a naturalized population of horses with no history of exposure to anthelmintic drugs, and then explored the repeatability and covariation between FEC and body condition. Compared with previously published studies on domestic horses, the unmanaged Sable Island horse population appeared to shed high numbers of strongyle eggs (mean and range of eggs per gram:  $1543.28$ ,  $[0-9200]$  reported in this study; compared to  $442$ ,  $[0-3750]$  in Flanagan *et al.* 2013;  $NA$ ,  $[0-2420]$  in Carstensen *et al.* 2013;  $711$ ,  $[0-2900]$  in Pilar Valdez-Cruz *et al.* 2013 and  $1244$ ,  $[0-9730]$  in Nielsen *et al.* 2010b).

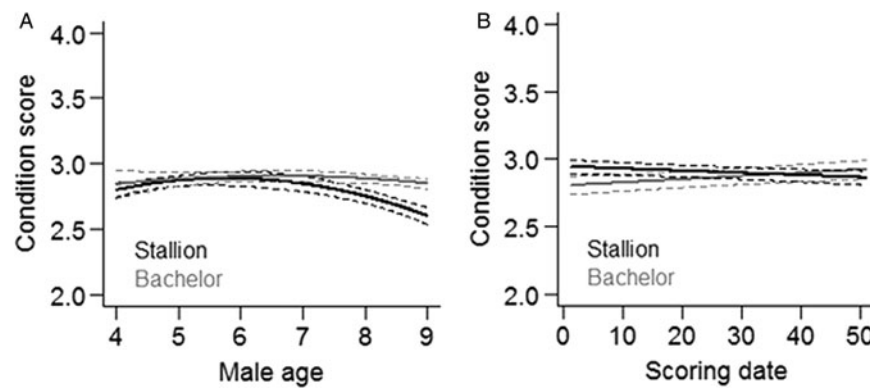


Fig. 6. Relationship between body condition and social status, age, location on the island and scoring date for adult males ( $N_{\text{horse}} = 153$ ,  $N_{\text{sample}} = 573$ ) based on the prediction of the selected model. Panel (A) represents the effect of age for each social status with location and scoring date set to its mean value; the line represents the relationship predicted by the selected model; dashed lines represent the 95% confidence interval around the predicted values. Panel (B) represents the effect of scoring date for each social status with location and age set to its mean value.

Both intrinsic (sex, age, reproductive status, social status) and extrinsic (group size, location, local density) factors were shown to affect FECs and body condition in the Sable Island horse population. Contrary to expectations (Poulin, 1996; Moore and Wilson, 2002), and the hypothesis that testosterone acts as an immune-suppressor (Ezenwa *et al.* 2012), males did not consistently display higher FECs than females. Females had higher FECs than males in the east, but the trend was reversed in the west (Fig. 2c). Still, patterns within each sex were consistent with expectations. As commonly observed in other species (Lloyd, 1983; Barger, 1993; Houdijk, 2008) such as bighorn sheep *Ovis Canadensis* (Festa-Bianchet, 1989) and European rabbit *Oryctolagus cuniculus* (Cattadori *et al.* 2005), nursing females had higher FECs than non-nursing females. Higher FECs in nursing females may be due to higher worm abundance and/or higher worm fecundity in response to host immune relaxation (Connan, 1976). Higher parasitism in nursing females potentially mediates costs of reproduction (Williams, 1966) in Sable Island's resource limited environment, and having access to good quality resources to maintain immunocompetence would be important for mitigating this cost (Hutchings *et al.* 2002). This appeared to be the case, as lactating females in the west part of the island, which has better forage and access to permanent freshwater ponds, generally had higher body condition scores than those in the east. Alternatively, as in the St Kilda Soay sheep population (Hutchings *et al.* 2002), nursing females may use the habitat differently and differ in their ranging behaviour to sustain higher energy and water demands in a way that increases exposure to gastrointestinal parasite larvae (i.e. by grazing in habitat with higher larvae densities) (Ezenwa, 2004). A similar pattern was observed in males, with dominant band stallions having higher FECs than bachelors. This pattern

may reflect the energetic costs associated with dominance behaviours including vigilance and band defence, as well as the immunosuppressive effects associated with high testosterone levels (Habig and Archie, 2015). The latter seems possible given that dominant band stallions are known to have higher testosterone levels than bachelors on Sable Island (S. Medill, unpublished data).

As expected, FECs decreased with age in both sexes. This result supports the hypothesis that immunity acquisition shapes FEC (Turner and Getz, 2010). Alternatively, decreases in FECs with age may be due to the selective disappearance of heavily infected individuals. In contrast to several other species (Santín-Durán *et al.* 2008; Hayward *et al.* 2009; Body *et al.* 2011), we did not observe an increase in FECs in older (senescent) individuals. This may not be observable in our dataset because individuals aged 9 years or older were pooled together.

An individual's location during the previous summer was found to be an important predictor of both FEC and condition with higher FECs found in the western part of island. Forage quality and access to water decrease along a west–east gradient on the island (Contasti *et al.* 2012; Rozen-Rechels *et al.* 2015), and median location probably acts as a proxy for these variables. Further analyses would be needed to better understand which particular factors are driving these relationships. We can speculate that the extreme difference in water availability between the west and the east part of the island might play a role in parasite ova viability through the landscape and contamination levels. Indeed, the west part is characterized by the presence of permanent freshwater ponds surrounded by pasture where numerous bands congregate, while in the east horses must drink at self-excavated wells (Rozen-Rechels *et al.* 2015).

While density did not explain FEC patterns at the population level, FECs unexpectedly decreased as

Table 2. Number of individuals, number of individuals with multiple phenotypic records (in parenthesis), number of observations, number of observations for individuals with multiple phenotypic records (in parenthesis), and raw trait means for strongyle fecal egg count (log transformed) and body condition in Sable Island horses. Phenotypic variances after having accounted for fixed effects ( $V_p$ ) as well variance attributed to variation among horses ( $V_{ind}$ ), groups ( $V_{group}$ ) and variation within individuals ( $V_e$ ) were estimated using linear mixed models. The proportion of phenotypic variation attributed to permanent variation among individuals (repeatability,  $ind^2 = V_{ind}/V_p$ ) and group identity ( $group^2 = V_{group}/V_p$ ) are also presented. Results are from models including only trait mean as a fixed effect or additional fixed effects identified using model selection (see text for details). Standard errors generated by ASReml are presented in parentheses. Statistical significance of  $V_{ind}$  and  $V_{group}$  was tested using likelihood ratio tests.

Trait	Dataset	Fixed effects	ind.	obs	mean (sd)	$V_p$	$V_{ind}$	$V_{group}$	$V_e$	$ind^2$	$group^2$	$e^2$
FEC	All individuals	Mean only	447 (178)	670 (401)	7.05 (0.87)	0.78 (0.05)	0.56 (0.05)***	0.05 (0.03)*	0.17 (0.02)	0.72 (0.04)	0.06 (0.04)	0.29 (0.03)
	Adult males	Mean only	153 (72)	242 (161)	6.81 (0.76)	0.59 (0.07)	0.49 (0.07)***	0 (-)	0.10 (0.02)	0.83 (0.03)	0 (-)	0.21 (0.04)
	Adult females	Mean only	125 (48)	183 (106)	6.93 (1.08)	1.14 (0.14)	0.88 (0.14)***	0 (-)	0.26 (0.05)	0.77 (0.05)	0 (-)	0.32 (0.07)
Condition	All individuals	Mean only	447 (446)	1607 (1606)	2.58 (0.45)	0.20 (0.01)	0.11 (0.01)***	0.02 (0.01)***	0.069 (0.003)	0.57 (0.03)	0.08 (0.03)	0.44 (0.02)
	Adult males	Mean only	153 (153)	573 (573)	2.84 (0.36)	0.13 (0.01)	0.06 (0.01)***	0 (-)	0.07 (0.01)	0.44 (0.05)	0 (-)	0.62 (0.05)
	Adult females	Mean only	125 (124)	439 (438)	2.39 (0.52)	0.28 (0.03)	0.12 (0.03)***	0.09 (0.03)***	0.06 (0.01)	0.44 (0.09)	0.32 (0.10)	0.32 (0.04)
FEC	All individuals	Mean + others <sup>a</sup>	447 (178)	670 (401)	7.05 (0.87)	0.59 (0.04)	0.40 (0.04)***	0.02 (0.02)	0.17 (0.02)	0.68 (0.04)	0.03 (0.03)	0.22 (0.02)
	Adult males	Mean + others <sup>a</sup>	153 (72)	242 (161)	6.81 (0.76)	0.48 (0.06)	0.38 (0.06)***	0.004 (0.026)	0.10 (0.02)	0.78 (0.06)	0.01 (0.05)	0.17 (0.03)
	Adult females	Mean + others <sup>a</sup>	125 (48)	183 (106)	6.93 (1.08)	0.83 (0.10)	0.57 (0.11)***	0 (-)	0.27 (0.05)	0.68 (0.07)	0 (-)	0.23 (0.05)
Condition	All individuals	Mean + others <sup>a</sup>	447 (446)	1607 (1606)	2.58 (0.45)	0.16 (0.01)	0.08 (0.01)***	0.005 (0.005)	0.070 (0.003)	0.52 (0.04)	0.04 (0.03)	0.35 (0.02)
	Adult males	Mean + others <sup>a</sup>	153 (153)	573 (573)	2.84 (0.36)	0.12 (0.01)	0.04 (0.07)***	0 (-)	0.07 (0.01)	0.38 (0.05)	0 (-)	0.56 (0.04)
	Adult females	Mean + others <sup>a</sup>	125 (124)	439 (438)	2.39 (0.52)	0.20 (0.02)	0.11 (0.03)***	0.02 (0.02)	0.07 (0.01)	0.57 (0.11)	0.11 (0.11)	0.24 (0.03)

<sup>a</sup> The complete list of fixed effects is presented in Table 1.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

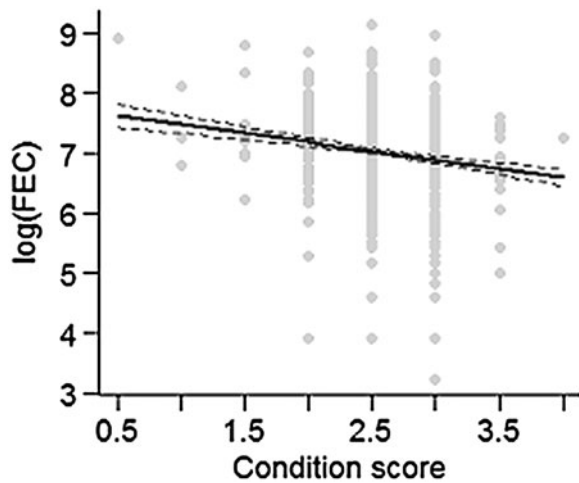


Fig. 7. Phenotypic relationship between strongyle fecal egg count (FEC, expressed as the log-transformed number of eggs per gram of feces) and body condition ( $N_{\text{horse}} = 322$ ,  $N_{\text{sample}} = 405$ ). The line represents the relationship predicted by a model including body condition as a fixed effect and horse identity as a random effects.

density increased in bachelor adult males, while the reverse was found in dominant stallions. We can speculate that these differences may result from social behaviour differences between the two groups. The pattern seen in dominant band stallions probably reflects the higher stress of maintaining a band in high density (and competitive) areas. The results in bachelors is less intuitive, and may indicate that bachelor males inhabiting densely populated areas may either be less stressed than individuals living with fewer conspecifics, perhaps as a result of sociality, and/or generally be more immunocompetent.

Few studies have investigated the repeatability of FEC in horse populations (Wood *et al.* 2013). In contrast with Wood *et al.* (2013) who observed very low repeatability for FEC in British horse populations, our FECs were highly repeatable (Table 2). This difference is likely due to the fact that Wood *et al.* (2013) studied repeatability across seasons and years while we focussed on a single season. The absence of temporal variation in FECs over the 2 months during which our study was conducted (see Appendix 1 for details) means that our repeatability estimates likely mainly reflected measurement error. In contrast, repeatability estimates obtained over several seasons and years will be influenced by changes in parasite number, fecundity and species composition. Though we only had repeated FECs measurements for 178 of the 447 sampled horses, the high repeatability observed in these individuals suggests that our FEC estimates are generally accurate.

Repeatability of body condition scores was noticeably lower than those for FECs (Table 2). As we do not expect condition to vary considerably within

Table 3. Phenotypic covariance between strongyle fecal egg count (log transformed) and body condition in Sable Island horses, its components, and associated correlations. Results are from bivariate models including only trait mean as a fixed effect for both traits or additional fixed effects accounting for important intrinsic and extrinsic variables identified using model selection (see text and Table 2 for details). Standard errors generated by ASReml are presented in parentheses. Statistical significance of covariances and correlations was tested using likelihood ratio tests.

Dataset	Fixed effects	COV <sub>p</sub>	COV <sub>ind</sub>	COV <sub>group</sub>	COV <sub>e</sub>	r <sub>p</sub>	r <sub>ind</sub>	r <sub>group</sub>	r <sub>e</sub>
All individuals	Mean	-0.075 (0.018)***	-0.078 (0.016)***	0.010 (0.010)	-0.006 (0.009)	-0.190 (0.045)***	-0.318 (0.058)***	0.292 (0.296)	-0.059 (0.080)
	Mean + others <sup>a</sup>	-0.056 (0.014)***	-0.052 (0.012)***	0.002 (0.007)	-0.006 (0.009)	-0.184 (0.044)***	-0.289 (0.063)***	0.182 (0.672)	-0.053 (0.077)
Adult males	Mean	0.017 (0.019)	0.018 (0.017)	-	-0.001 (0.011)	0.062 (0.070)	0.110 (0.101)	-	-0.015 (0.130)
	Mean + others <sup>a</sup>	-0.014 (0.017)	-0.008 (0.014)	-	-0.007 (0.011)	-0.062 (0.074)	-0.061 (0.112)	-	-0.079 (0.129)
Adult females	Mean	-0.175 (0.048)***	-0.181 (0.044)***	-	0.007 (0.021)	-0.314 (0.074)***	-0.535 (0.099)***	-	0.050 (0.157)
	Mean + others <sup>a</sup>	-0.134 (0.038)***	-0.136 (0.034)***	-	0.002 (0.019)	-0.333 (0.081)***	-0.526 (0.101)***	-	-0.015 (0.151)

<sup>a</sup> The complete list of fixed effects is presented in Table 1. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

individuals over a 2-month periods, this result suggests that measurement error was greater for body condition than FEC. Another notable difference between the repeatability of the 2 traits was that only body condition scores were similar among adult females of the same band (note that there is usually only one adult male present in each band, limiting power to detect such an effect in that sex). This suggests that habitat selection and access to resources have important impacts on female body condition. Alternatively, it may reflect differences in the quality of females that different males can acquire and maintain.

Demonstrating the negative impacts of parasites in unmanipulated free-living populations can be challenging (Tompkins and Begon, 1999). Body condition, which is often linked with fitness in ungulates (Barnett *et al.* 2015), was found to be negatively related to FEC. However, while the association was significant at the population level, it appeared to come mostly from a strong association in adult females (phenotypic correlation between FEC and condition =  $-0.333 \pm 0.081$  in adult females compared with  $-0.062 \pm 0.074$  in adult males; Table 3). A female horse has to trade-off available energy between growth, maintenance, immune function and, in the case of sexually mature mares, reproductive effort (Sheldon and Verhulst, 1996). The stronger relationship between FEC and body condition in adult females compared with adult males may reflect differences in trade-offs between the sexes. However, it could also be that our body condition index did not correlate well with energy reserves in males. Specifically, males are generally more muscular than females, and it could be that our visual index of body condition, when applied to males, mainly captured variation in musculature. Links between FEC and body condition have been found in other mammals (Stien *et al.* 2002; Yoseph *et al.* 2005; Irvine *et al.* 2006; Body *et al.* 2011), including horses (Rubenstein and Hohmann, 1989). These associations could stem from the fact that a high parasite burden can reduce body condition, or conversely, a lower body condition can predispose an individual to a higher parasite burden. Given its link with body condition in females, and the expectation that condition is related to future survival or reproduction, parasite load is likely to be under negative directional selection in females. We might also speculate that given the high overall FEC found in the population and the negative relationship between FEC and body condition, parasitism might be a limiting factor for Sable Island horses. However, further work will be needed to confirm these presumptions and assess if any such effects scale up to influence population dynamics. In the future, it would also be valuable to assess the extent to which changes in condition over time predict changes in parasitism or the

reverse and how these relate to social and reproductive status.

This study documents a complex interplay between intrinsic and extrinsic variables in determining individual variation in FECs in a natural population, with potentially important impacts on parasite transmission and population dynamics. Understanding disease processes in natural populations is crucial in the context of an increase in infectious diseases (Jolles and Ezenwa, 2015), and as noted by Jolles and Ezenwa (2015), wild ungulates are particularly good models for such research because they are a diverse and broadly distributed group of species, but also because they are closely related to domestic animals. Research on feral populations of farm animals or closely related species can benefit from well-developed toolbox such as genomic tools (Miller *et al.* 2012), while in return provide information that may be difficult to study in domestic animals. For example, in addition to improving our understanding of host–parasites interactions in the wild, our study offered a rare glimpse of how parasitism covaries with body condition in horses in the absence of confounding effects such as shelter, anthelmintic treatment and nutritional supplementation. Future studies on Sable Island could contribute much to our understanding of long-term relationships among FEC, body condition and other effects of parasite infections.

#### SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0031182016000408>.

#### ACKNOWLEDGEMENTS

We thank the numerous students, research assistants and volunteers who have contributed to the Sable Island horse project over the years. In kind and logistical support was provided by Fisheries and Oceans Canada (DFO), Canada Coast Guard, the Bedford Institute of Oceanography (DFO Science), Environment Canada, Parks Canada Agency, Maritime Air Charters Limited and Sable Island Station (Meteorological Service of Canada). We thank two anonymous referees and the editor for their constructive comments on an earlier version of the manuscript.

#### FINANCIAL SUPPORT

Funding was provided by the Ryan Dubé and Heather Ryan Veterinary and Research Fund, a Royal Society International Exchange grant, the Natural Sciences and Engineering Research Council of Canada (NSERC Discovery Grant to PDM, no. 371535-2009) and the Canada Foundation for Innovation (Leaders Opportunity grant to PDM, no. 25046). JP was supported by the University of Calgary NSERC-CREATE Host–Parasite Interactions programme and a Leverhulme Trust Early Career Research Fellowship.

## REFERENCES

- Altizer, S., Nunn, C. L., Thrall, P. H., Gittleman, J. L., Antonovics, J., Cunningham, A. A., Dobson, A. P., Ezenwa, V., Jones, K. E., Pedersen, A. B., Poss, M. and Pulliam, J. R. C. (2003). Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution and Systematics* **34**, 517–547.
- Barger, I. (1993). Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. *International Journal for Parasitology* **23**, 463–469.
- Barnett, C. A., Suzuki, T. N., Sakaluk, S. K. and Thompson, C. F. (2015). Mass-based condition measures and their relationship with fitness: in what condition is condition? *Journal of Zoology* **296**, 1–5.
- Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1–48. doi:10.18637/jss.v067.i01.
- Body, G., Ferte, H., Gaillard, J. M., Delorme, D., Klein, F. and Gilot-Fromont, E. (2011). Population density and phenotypic attributes influence the level of nematode parasitism in roe deer. *Oecologia* **167**, 635–646.
- Bonenfant, C., Gaillard, J.-M., Coulson, T., Festa-Bianchet, M., Loison, A., Garel, M., Loe, L. E., Blanchard, P., Pettorelli, N., Owen-Smith, N., Du Toit, J. and Duncan, P. (2009). Empirical evidence of density-dependence in populations of large herbivores. *Advances in Ecological Research* **41**, 313–357.
- Bowman, D. D. (2003). *Georgis' Parasitology for Veterinarians*, 8th Edn. W. B. Saunders, Philadelphia, Pennsylvania.
- Burnham, K. P. and Anderson, D. R. (2002). *Model Selection and Multimodel Inference – a Practical Information-Theoretic Approach*, 2nd Edn. Springer-Verlag, New York.
- Carroll, C. and Huntington, P. (1988). Body condition scoring and weight estimation of horses. *Equine Veterinary Journal* **20**, 41–45.
- Carstensen, H., Larsen, L., Ritz, C. and Nielsen, M. K. (2013). Daily variability of strongyle faecal egg counts in horses. *Journal of Equine Veterinary Science* **33**, 161–164.
- Cattadori, I., Boag, B., Bjornstad, O., Cornell, S. and Hudson, P. (2005). Peak shift and epidemiology in a seasonal host-nematode system. *Proceedings of the Royal Society B: Biological Sciences* **272**, 1163–1169.
- Christie, B. J. (1995). *The Horses of Sable Island*. Pottersfield Press, Lawrencestown, Nova Scotia, Canada.
- Clancey, E., Dunn, S. J. and Byers, J. A. (2012). Do single point condition measurements predict fitness in female pronghorn (*Antilocapra americana*)? *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **90**, 729–735.
- Clutton-Brock, T. and Sheldon, B. C. (2010). Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology & Evolution* **25**, 562–573.
- Connan, R. (1976). Effect of lactation on the immune response to gastrointestinal nematodes. *The Veterinary Record* **99**, 476–477.
- Contasti, A. L., Tissier, E. J., Johnstone, J. F. and McLoughlin, P. D. (2012). Explaining spatial heterogeneity in population dynamics and genetics from spatial variation in resources for a large herbivore. *PLoS ONE* **7**, e47858.
- Cressler, C. E., Nelson, W. A., Day, T. and McCauley, E. (2014). Disentangling the interaction among host resources, the immune system and pathogens. *Ecology Letters* **17**, 284–293.
- Davidson, R. K., Ličina, T., Gorini, L. and Milner, J. M. (2015). Endoparasites in a Norwegian moose (*Alces alces*) population – faunal diversity, abundance and body condition. (International Congress on Parasites of Wildlife, pp. 49–158) *International Journal for Parasitology: Parasites and wildlife* **4**, 29–36.
- Ezenwa, V. O. (2004). Host social behavior and parasitic infection: a multifactorial approach. *Behavioral Ecology* **15**, 446–454.
- Ezenwa, V. O., Ekernas, L. S. and Creel, S. (2012). Unravelling complex associations between testosterone and parasite infection in the wild. *Functional Ecology* **26**, 123–133.
- Fairbanks, B., Hawley, D. M., Demas, G. E. and Nelson, R. J. (2012). Interactions between host social behavior, physiology, and disease susceptibility. In *Ecoimmunology* (ed. Demas, G. and Nelson, R.), pp. 440–467. Oxford University Press, Oxford, UK.
- Festa-Bianchet, M. (1989). Individual differences, parasites, and the costs of reproduction for bighorn ewes (*Ovis canadensis*). *Journal of Animal Ecology* **58**, 785–795.
- Festa-Bianchet, M., Gaillard, J. and Jorgenson, J. (1998). Mass- and density-dependent reproductive success and reproductive costs in a capital breeder. *American Naturalist* **152**, 367–379.
- Flanagan, K. L., Morton, J. M. and Sandeman, R. M. (2013). Prevalence of infestation with gastrointestinal nematodes in Pony Club horses in Victoria. *Australian Veterinary Journal* **91**, 241–245.
- Fog, P., Vigre, H. and Nielsen, M. (2011). Strongyle egg counts in Standardbred trotters: are they associated with race performance? *Equine Veterinary Journal* **43**, 89–92.
- Gilmour, A. R., Gogel, B., Cullis, B., Thompson, R. and Butler, D. (2009). *ASReml User Guide Release 3.0*. VSN International Ltd, Hemel Hempstead, UK.
- Habig, B. and Archie, E. A. (2015). Social status, immune response and parasitism in males: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**, 20140109.
- Hayward, A. (2013). Causes and consequences of intra- and inter-host heterogeneity in defence against nematodes. *Parasite Immunology* **35**, 362–373.
- Hayward, A. D., Wilson, A. J., Pilkington, J. G., Pemberton, J. M. and Kruuk, L. E. B. (2009). Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep. *Proceedings of the Royal Society B: Biological Sciences* **276**, 3477–3485.
- Hayward, A. D., Garnier, R., Watt, K. A., Pilkington, J. G., Grenfell, B. T., Matthews, J. B., Pemberton, J. M., Nussey, D. H. and Graham, A. L. (2014). Heritable, heterogeneous, and costly resistance of sheep against nematodes and potential feedbacks to epidemiological dynamics. *The American Naturalist* **184**, S58–S76.
- Houdijk, J. (2008). Influence of periparturient nutritional demand on resistance to parasites in livestock. *Parasite Immunology* **30**, 113–121.
- Hutchings, M. R., Milner, J. M., Gordon, I. J., Kyriazakis, I. and Jackson, F. (2002). Grazing decisions of Soay sheep, *Ovis aries*, on St Kilda: a consequence of parasite distribution? *Oikos* **96**, 235–244.
- Irvine, R. J., Corbishley, H., Pilkington, J. G. and Albon, S. D. (2006). Low-level parasitic worm burdens may reduce body condition in free-ranging red deer (*Cervus elaphus*). *Parasitology* **133**, 465–475.
- Jolles, A. E. and Ezenwa, V. O. (2015). Ungulates as model systems for the study of disease processes in natural populations. *Journal of Mammalogy* **96**, 4–15.
- Keymer, A. and Hiorns, R. (1986). Faecal egg counts and nematode fecundity: *Heligmosomoides polygyrus* and laboratory mice. *Parasitology* **93**, 189–203.
- Khan, M., Roohi, N. and Rana, M. (2015). Strongylosis in Equines: a review. *Journal of Animal and Plant Sciences* **25**, 1–9.
- Lichtenfels, J. R., Kharchenko, V. A. and Dvojnos, G. M. (2008). Illustrated identification keys to strongylid parasites (strongylidae: Nematoda) of horses, zebras and asses (Equidae). *Identification Keys to Strongylid Nematode Parasites of Equids* **156**, 4–161.
- Lloyd, S. (1983). Immunosuppression during pregnancy and lactation. *Irish Veterinary Journal* **37**, 64–70.
- Love, S., Murphy, D. and Mellor, D. (1999). Pathogenicity of cyathostome infection. *Veterinary Parasitology* **85**, 113–122.
- Marjamäki, P. H., Contasti, A. L., Coulson, T. N. and McLoughlin, P. D. (2013). Local density and group size interacts with age and sex to determine direction and rate of social dispersal in a polygynous mammal. *Ecology and Evolution* **3**, 3073–3082.
- Mazerolle, M. J. (2015). AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). R package version 2.0–3. <http://CRAN.R-project.org/package=AICcmodavg>.
- Miller, J. M., Kijas, J. W., Heaton, M. P., McEwan, J. C. and Coltman, D. W. (2012). Consistent divergence times and allele sharing measured from cross-species application of SNP chips developed for three domestic species. *Molecular Ecology Resources* **12**, 1145–1150.
- Moore, S. L. and Wilson, K. (2002). Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* **297**, 2015–2018.
- Moretti, E. H., Madelaire, C. B., Silva, R. J., Mendonca, M. T. and Gomes, F. R. (2014). The relationships between parasite intensity, locomotor performance, and body condition in adult toads (*Rhinella icterica*) from the Wild. *Journal of Herpetology* **48**, 277–283.
- Nielsen, M. K., Vidyashankar, A. N., Andersen, U. V., DeLisi, K., Pilegaard, K. and Kaplan, R. M. (2010a). Effects of faecal collection and storage factors on strongylid egg counts in horses. *Veterinary Parasitology* **167**, 55–61.
- Nielsen, M. K., Baptiste, K. E., Tolliver, S. C., Collins, S. S. and Lyons, E. T. (2010b). Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of faeces are reliable indicators of numbers of strongyles and ascarids present. *EVPC 2009: Veterinary Parasitosis in the Mediterranean Area* **174**, 77–84.
- Osterman, L. E. (2005). Prevalence and control of strongyle nematode infections of horses in Sweden. *Doctoral thesis*. Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Patterson, J. E. H. and Ruckstuhl, K. E. (2013). Parasite infection and host group size: a meta-analytical review. *Parasitology* **140**, 803–813.

- Pilar Valdez-Cruz, M., Hernandez-Gil, M., Galindo-Rodríguez, L. and Angel Alonso-Díaz, M.** (2013). Gastrointestinal nematode burden in working equids from humid tropical areas of central Veracruz, Mexico, and its relationship with body condition and haematological values. *Tropical Animal Health and Production* **45**, 603–607.
- Poulin, R.** (1996). Helminth growth in vertebrate hosts: does host sex matter? *International Journal for Parasitology* **26**, 1311–1315.
- Poulin, R.** (2007). *Evolutionary Ecology of Parasites*. Princeton University Press, Princeton.
- Poulin, R. and Forbes, M. R.** (2012). Meta-analysis and research on host-parasite interactions: past and future. *Evolutionary Ecology* **26**, 1169–1185.
- R Development Core Team.** (2010). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Raynaud, J.** (1970). Etude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Annales de Parasitologie Humaine et Comparée* **45**, 321–342.
- Reading, C. and Clarke, R.** (1995). The effects of density, rainfall and environmental temperature on body condition and fecundity in the common toad, *Bufo bufo*. *Oecologia* **102**, 453–459.
- Relf, V. E., Morgan, R., Hodgkinson, J. E. and Matthews, J. B.** (2013). Helminth egg excretion with regard to age, gender and management practices on UK Thoroughbred studs. *Parasitology* **140**, 641–652.
- Rifkin, J. L., Nunn, C. L. and Garamszegi, L. Z.** (2012). Do animals living in larger groups experience greater parasitism? A meta-analysis. *American Naturalist* **180**, 70–82.
- Rozen-Rechels, D., van Beest, F. M., Richard, E., Uzal, A., Medill, S. A. and McLoughlin, P. D.** (2015). Density-dependent, central-place foraging in a grazing herbivore: competition and tradeoffs in time allocation near water. *Oikos* **124**, 1142–1150.
- Rubenstein, D. and Hohmann, M.** (1989). Parasites and social-behavior of Island feral horses. *Oikos* **55**, 312–320.
- Santín-Durán, M., Alunda, J. M., Hoberg, E. P. and de la Fuente, C.** (2008). Age distribution and seasonal dynamics of abomasal Helminths in wild red deer from central Spain. *Journal of Parasitology* **94**, 1031–1037.
- Scantlebury, M., McWilliams, M. M., Marks, N. J., Dick, J. T. A., Edgar, H. and Lutermann, H.** (2010). Effects of life-history traits on parasite load in grey squirrels. *Journal of Zoology* **282**, 246–255.
- Schulte-Hostedde, A. I. and Elsasser, S. C.** (2011). Spleen mass, body condition, and parasite load in male American mink (*Neovison vison*). *Journal of Mammalogy* **92**, 221–226.
- Self, S. G. and Liang, K. Y.** (1987). Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. *Journal of the American Statistical Association* **82**, 605–610.
- Sellon, D. C. and Long, M.** (2013). *Equine Infectious Diseases*, 2nd Edn. Elsevier Health Sciences, New York, pp. 454–455.
- Sheldon, B. and Verhulst, S.** (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* **11**, 317–321.
- Stien, A., Irvine, R. J., Ropstad, E., Halvorsen, O., Langvatn, R. and Albon, S. D.** (2002). The impact of gastrointestinal nematodes on wild reindeer: experimental and cross-sectional studies. *Journal of Animal Ecology* **71**, 937–945.
- Symonds, M. R. E. and Moussalli, A.** (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology* **65**, 13–21.
- Tompkins, D. and Begon, M.** (1999). Parasites can regulate wildlife populations. *Parasitology Today* **15**, 311–313.
- Turner, W. C. and Getz, W. M.** (2010). Seasonal and demographic factors influencing gastrointestinal parasitism. *Journal of Wildlife Diseases* **46**, 1108–1119.
- Vatta, A., Krecek, R., Letty, B., van der Linde, M., Motswatswe, P. and Hansen, J.** (2002). Effect of nematode burden as assessed by means of faecal egg counts on body condition in goats farmed under resource-poor conditions in South Africa. *Veterinary Parasitology* **108**, 247–254.
- Watson, M. J.** (2013). What drives population-level effects of parasites? Meta-analysis meets life-history. *International Journal for Parasitology: Parasites and Wildlife* **2**, 190–196.
- Welsh, D. A.** (1975). Population, behavioural and grazing ecology of the horses of Sable Island. PhD. Thesis, Dalhousie University, Canada.
- Williams, G. C.** (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *American Naturalist* **100**, 687–690.
- Wilson, A.** (2008). Why  $h^2$  does not always equal VA/VP? *Journal of Evolutionary Biology* **21**, 647–650.
- Wilson, A. J., Reale, D., Clements, M. N., Morrissey, M. M., Postma, E., Walling, C. A., Kruuk, L. E. and Nussey, D. H.** (2010). An ecologist's guide to the animal model. *Journal of Animal Ecology* **79**, 13–26.
- Wood, E. L. D., Matthews, J. B., Stephenson, S., Slotte, M. and Nussey, D. H.** (2013). Variation in faecal egg counts in horses managed for conservation purposes: individual egg shedding consistency, age effects and seasonal variation. *Parasitology* **140**, 115–128.
- Wood, S. N.** (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society Series B: Statistical Methodology* **73**, 3–36.
- Yoseph, S., Smith, D. G., Mengistu, A., Teklu, F., Firew, T. and Betere, Y.** (2005). Seasonal variation in the parasite burden and body condition of working donkeys in East Shewa and West Shewa regions of Ethiopia. *Tropical Animal Health and Production* **37**, 35–45.
- Zuk, M. and McKean, K. A.** (1996). Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology* **26**, 1009–1024.