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Differential host responses to parasitism shape divergent fitness costs of infection

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Abstract

1. Fitness costs of infection are fundamental to understanding the ecology and evolution of host–parasite interactions. However, these costs, and particularly their underlying mechanisms, are challenging to evaluate in wild populations.
2. Here, we quantified total and species-specific costs of gastrointestinal worms on African buffalo, by combining the power of an anthelmintic treatment experiment that perturbed the entire worm community with a longitudinal study that tracked the two most dominant community members.
3. Reducing all worms improved buffalo body condition, which was strongly associated with increases in survival and reproduction. Species-specific analyses revealed that condition-mediated fitness costs of infection differed between parasite species. Hosts that gained the blood-sucking worm *Haemonchus*, lost condition, and this loss may have been mediated by reductions in forage intake. Hosts that resisted *Haemonchus* by elevating IL-4 and eosinophil immune defences were able to reduce their parasite loads and gain back condition.
4. Conversely, hosts that gained *Cooperia*, a less pathogenic worm, gained condition and had higher survival and reproductive success. Elevating immune defences had no effect on *Cooperia* abundance. Coupled with the positive relationship observed between *Cooperia* and host condition, our data suggest that hosts might benefit from tolerating *Cooperia* rather than incurring the costs of resistance.
5. Overall, our study reveals that differential host responses to parasites play a key role in mediating the costs of infection.

KEYWORDS
disease ecology, helminth infection, immune defence, resistance, tolerance

1 | INTRODUCTION

Quantifying the fitness costs of parasite infection is of fundamental importance for understanding the ecology and evolution of host–parasite interactions. The costs of infection to the host often result from the interplay among at least three factors: the parasite's direct negative effect on the host (parasite virulence), the host's ability to prevent or reduce parasite infection (host resistance), and the host's ability to mitigate immune- or parasite-inflicted damage (host tolerance) (Medzhitov, Schneider, & Soares, 2012; Råberg, Graham, & Read, 2009). Understanding how these three factors combine to shape parasite fitness costs in natural populations is challenging because interactions among these factors are dynamic, influenced by environmental variation, and differ among host individuals.

Within-species variation in resistance and tolerance to parasite infection may arise from differences in host genotype, reproductive status, age, or nutrition (Budischak, Sakamoto, et al., 2015; Medzhitov et al., 2012; Pedersen & Greives, 2008; Råberg, 2014). For example, under low resource conditions, laboratory mice were more likely to resist gastrointestinal (GI) helminths, whereas

well-fed mice tolerated infection (Budischak, Sakamoto, et al., 2015). Importantly, these differential responses of hosts to infection can lead to counterintuitive relationships between parasite burden and fitness. Resistant individuals may have low parasite loads, yet suffer significant fitness losses because immunological defences are energetically expensive, can damage host tissues (immunopathology), and may be misdirected or co-opted by parasites (Graham et al., 2011). Indeed, stimulation of immune defences can increase metabolic rates by 10%–50% in vertebrate species (Lochmiller & Deerenberg, 2000), and such a level of energetic investment may only have a net positive effect on host fitness if the target parasite is highly virulent. If the costs of host resistance exceed the physiological costs of parasite infection, selection can favour tolerance (Råberg et al., 2009). In such a case, tolerant individuals with high parasite loads could have higher fitness than individuals that effectively resist infection. Thus, neither the level of investment in immunological defences nor parasite loads alone can be used to judge the success of a host defence strategy or the fitness costs of infection (Graham et al., 2011).

Experimental approaches are often needed to distinguish fitness reductions attributable to parasite infection from other differences between infected and uninfected individuals (Pedersen & Fenton, 2015). In this study, we used a combination of an anthelmintic treatment experiment and longitudinal sampling to quantify the costs of GI worm infection in wild African buffalo (*Syncerus caffer*, Sparrman), and to assess the contribution of differential host responses to these costs. Our previous work has shown that anthelmintic treatment can improve body condition in buffalo (Ezenwa, Etienne, Luikart, Beja-Pereira, & Jolles, 2010). However, the two worm genera (*Cooperia* and *Haemonchus*) that dominate the GI parasite community of buffalo in our study population are known to have differential impacts on livestock hosts. *Haemonchus* are blood-feeders that cause greater pathology than do *Cooperia* (Bowman, 2009). In sheep and cattle, for example, infection by *Haemonchus* species is a well-known cause of anaemia, condition loss, decreased fecundity, and increased mortality risk (Le Jambre, 1995; West et al., 2009; Zarlenga, Hoberg, & Tuo, 2016). In contrast, infection by *Cooperia* species appears to have less severe effects, with some studies tying infection to significant reductions in nutrient uptake and weight gain (Alicata & Lynd, 1961; Armour et al., 1987; Stromberg et al., 2012), and others reporting weaker and more variable effects on cattle performance (Satrija & Nansen, 1992). Moreover, in buffalo these two worms are associated with haematology and host body condition (which may indicate variation in physiological traits such as aerobic capacity and oxygen binding (Jain, 1993)) in very distinct ways. For example, *Cooperia* abundance is positively associated with buffalo body condition and negatively associated with haematocrit. Contrastingly, *Haemonchus* abundance is negatively associated with body condition, haemoglobin concentration and red blood cell count (Budischak, Jolles, & Ezenwa, 2012), both of which are essential for oxygen binding and aerobic capacity (Jain, 1993). Therefore, to better understand if and why these two worms differentially affect host fitness, we: (1) examined the effect of these two worms on host body condition; (2) tested whether these condition effects translated to host fitness, quantified as differences in reproduction and survival; and (3) evaluated the host response to infection by quantifying innate and adaptive immune responses, infection-induced changes in forage intake rates, and age-intensity curves. For each of these questions, we compared the effects of experimental deworming versus total and species-specific worm burdens on host outcomes. In combination, this work provides rare insight into the relationship between host defences and fitness costs of infection in wild hosts.

2 | MATERIALS AND METHODS

2.1 | Animal sampling

African buffalo were sampled in Kruger National Park at approximately 6-month intervals between June 2008 and August 2012 (Ezenwa & Jolles, 2015). At the beginning of the study, 200 female buffalo were captured from two adjacent herds, the Lower Sabie (LS) and Crocodile Bridge (CB) herds, and then randomly assigned to an anthelmintic treatment or control group. Treated animals were given a slow-release fenbendazole bolus (Panacur, Intervet) to eliminate GI worm infections. At approximately 180-day intervals, each animal was recaptured and treated individuals were redosed with a fenbendazole bolus. Based upon recapture location, individuals were classified as belonging to the LS or CB herds, or Other (O) herds, if the animal had dispersed to a different location within the park. Individuals lost through mortality and emigration were replaced, and replacements were assigned the treatment status of the original buffalo. Overall, the anthelmintic bolus was given to 132 animals, while 168 were monitored as untreated controls. Initial ages ranged from 17 to 168 months, with a mean of 51 months and there was no difference in mean age between anthelmintic-treated and control groups (Ezenwa & Jolles, 2015). Buffalo were captured between 1 and 9 times ($M = 5.66 \pm 0.17$), and the treatment effectively reduced both the likelihood of being infected and the abundance of worms in infected hosts (Ezenwa & Jolles, 2015).

During each capture event, chemically immobilized buffalo were sampled to assess age, reproductive status, and body condition. Blood samples were collected via jugular venipuncture in EDTA or heparinized tubes and transported to the laboratory on ice. Age was estimated by incisor eruption and wear for young and old buffalo, respectively (Jolles, 2007). Condition was assessed using a body condition score on a 1 to 5 scale based on manual palpation and visual assessment of locations where buffalo store fat, with higher numbers representing greater fatness (Ezenwa, Jolles, & O'Brian, 2009). Pregnancy status was determined by rectal palpation and lactation was detected via manual milking (Jolles, Cooper, & Levin, 2005). For each capture, reproductive status was classified as a binomial response with hosts either being reproductive (i.e. pregnant and/or lactating) or non-reproductive. To examine age at first reproduction, we monitored all pre-reproductive animals under 60 months of age ($n = 87$) until the first capture where they were reproductive.

2.2 | Worm infection

Faecal samples were collected for each animal at each capture to measure the abundance and composition of GI worms. A modified McMaster faecal egg counting (FEC) protocol was used to quantify GI worms focusing on trichostrongyle nematodes (Ezenwa, 2003). Species-specific worm abundance was estimated by multiplying FEC by each species' relative abundance, which was determined via DNA barcoding of cultured larvae by Sanger sequencing (Budischak, Hoberg, Abrams, Jolles, & Ezenwa, 2015). Although 11 species were detected (Budischak, Hoberg, et al., 2015), only three were common (i.e. detected in >2.5% of captures): *Cooperia fuelleborni* (Hung), *Haemonchus placei* (Place), and *Haemonchus bedfordi* (Le Roux). The two *Haemonchus* species were combined for analyses based on evidence from livestock, suggesting that they have similar effects on host physiology (Le Jambre, 1995). Both *Cooperia* and *Haemonchus* worms are transmitted by the faecal oral route, and adults reside in the abomasum and small intestine.

2.3 | Host responses to infection

We assessed the host response to worm infection by quantifying immune responses and forage intake rates. As a measure of innate and adaptive defences against helminths, respectively, we quantified eosinophil and interleukin 4 (IL-4) concentrations. Eosinophil counts were performed manually from blood smears by a single observer. To measure IL-4, heparinized whole blood was incubated at 37°C for 28 hr, and supernatant plasma was collected and immediately frozen at −20°C for use in sandwiched ELISA as described in Nemzek, Siddiqui, & Remick (2001). Briefly, 96-well immunoplates were coated overnight with anti-bovine IL-4 antibodies (Abd Serotec), and blocked with 5% non-fat dry milk in phosphate buffered saline (PBS), before addition of plasma samples diluted 1:40 with PBS. After a 2 hr incubation at room temperature, detection antibody (mouse anti-bovine IL-4) was added to each well, and then plates were incubated at 37°C for 1 hr. Concentrations were determined by adding 1:10,000 Strepdavin:HRP in HRP-Stabliplus (Abd Serotec) to each well, stopping the reactions after 10 min with 100 µl of 0.2 M sulphuric acid, and reading optical densities at 405 nm with a microplate reader. All samples were run in duplicate, and cytokine concentrations (pg/ml) were calculated using standard curves generated by performing the above ELISA procedures on serial dilutions of recombinant bovine IL-4.

In livestock, reduced forage intake is a common behavioural response to GI worm infection, which is thought to be associated with the up-regulation of anti-worm immunity (Colditz, 2008; Coop & Holmes, 1996). A few studies have also found evidence of worm-induced anorexia in semi-captive and free-ranging hosts, suggesting that this response may be widespread in ungulates (Arneberg, Folstad, & Karter, 1996; Worsley-Tonks & Ezenwa, 2015). We used the nitrogen content of host faeces to quantify variation in buffalo intake rates since this measure has previously emerged as a key non-invasive indicator of intake rate in this species (Ryan et al., 2012). Faecal nitrogen analyses were performed by the Agricultural Research Council Laboratory (ARC-ITSC) Soil Laboratory in Nelspruit, South Africa, using a wet digestion and then quantifying ammonia concentrations with an Autoanalyzer (Coetsee, February, & Bond, 2008).

2.4 | Statistical analysis

We examined (1) the effects of worms on host body condition, (2) condition effects on host fitness, and (3) host immune and foraging responses to infection. For each of these questions, we combined inferences from experimental deworming and longitudinal datasets. For longitudinal analyses, change in focal variables from one capture session to the next were calculated by subtracting each individual's prior value from their current value (e.g. $FEC_t - FEC_{t-1}$ = Change in FEC_t). All statistical analyses summarized below were run in R (version 3.1.2) (R Core Team, 2014). The ADMB package (Fournier et al., 2012; Skaug, Fournier, Bolker, Magnusson, & Nielsen, 2014) was used to run binomial generalized linear mixed models, (GLMMs) and all other GLMMs (error = Gaussian, link = identity) were run using the LMER package (Bates, Maechler, Bolker, & Walker, 2004).

2.4.1 | Effects of worms on host body condition

First, we used a GLMM to test whether anthelmintic treatment increased host body condition. A random effect of buffalo identity was included to account for repeated captures. We included age, herd, and season (i.e. early wet, late wet, early dry, late dry) as covariates in this model based on previous work in this system showing that these factors can impact host immunity, infection, condition, and survival (Ezenwa & Jolles, 2015; Gorsich, Ezenwa, Cross, Bengis, & Jolles, 2015). To ensure that any treatment effect was not caused by initial differences in condition between the treatment and control groups we also tested for between-group differences in condition at the initial capture using a GLM (error = Gaussian).

Next, focusing on control (untreated) buffalo only, we used longitudinal data to evaluate whether changes in host body condition from one capture to the next were associated with changes in total worm abundance. To do this, we used a GLMM, with change in condition as the response variable, buffalo ID as a random effect, change in total worm abundance as the main predictor variable, and age, herd, season change (i.e. wet to dry, dry to wet, wet to wet, dry to dry) and capture interval (i.e. days between consecutive captures) as covariates. In this and all subsequent cross-time analyses, we excluded animals that were recaptured within 30 days to ensure that repeated measurements were independent, as well as intervals that were over 270 days to limit the potential for multiple fluctuations between captures. Finally, to distinguish species-specific worm effects, we repeated the longitudinal analysis above substituting the change in total worm abundance with the change in abundance of the two dominant worm species (*C. fuelleborni* and *Haemonchus* sp.).

TABLE 1 GLMMs testing the effects of anthelmintic treatment (treated vs. control buffalo, *n* = 300 individuals, 1,697 observations), total worm abundance (control buffalo only, *n* = 134, 687), and species-specific worm abundance (control buffalo only, *n* = 121, 426) on measures of body condition. For categorical parameters, results represent the comparisons of the listed level to the unlisted one (e.g. treated individuals, CB herd, early dry season, or dry-dry season change). Asterisks indicate significance: *$p < .05$, **$p < .01$, ***$p < .001$

Response	Factor	Estimate	*SE*	*df*	*t* value	*p* value
Body condition	Treatment (control)	−0.15	0.04	843.5	−3.96	<.0001***
	Age	−0.011	0.001	671.7	−15.9	<.0001***
	Herd (LS)	0.34	0.04	508.2	7.77	<.0001***
	Herd (O)	0.19	0.05	1,090	3.66	.0003***
	Season (early wet)	−0.65	0.03	1,448	−18.5	<.0001***
	Season (late dry)	−0.12	0.03	1,463	−3.73	<.0001***
	Season (late wet)	0.0078	0.038	1,490	0.21	.84
Δ Body condition	Δ Total abundance	−3.8E-06	1.3E-04	677.9	−0.030	.98
	Age	9.1E-04	0.0010	677.9	0.88	.38
	Herd (LS)	−0.092	0.068	677.9	−1.35	.18
	Herd (O)	0.066	0.075	677.9	0.89	.37
	Season Δ (dry − wet)	−0.30	0.09	677.9	−3.40	.0007***
	Season Δ (wet − dry)	0.20	0.08	677.9	2.47	.014*
	Season Δ (wet − wet)	0.17	0.11	677.9	1.55	.12
	Capture interval	0.0041	5.8E-04	677.9	7.13	<.0001***
Δ Body condition	Δ *Haemonchus*	−0.0042	0.0013	415.9	−3.34	.0009***
	Δ *Cooperia*	4.9E-04	1.8E-04	415.9	2.75	.0062**
	Age	0.0010	0.0013	415.9	0.77	.44
	Herd (LS)	−0.13	0.09	415.9	−1.54	.13
	Herd (O)	0.008	0.091	415.9	0.091	.93
	Season Δ (dry − wet)	−0.29	0.11	415.9	−2.72	.0068**
	Season Δ (wet − dry)	0.16	0.10	415.9	1.56	.12
	Season Δ (wet − wet)	0.15	0.13	415.9	1.17	.24
	Capture interval	0.0038	7.3E-04	415.9	5.28	<.0001***

2.4.2 | Condition effects on host fitness

To test whether observed effects of worms on host body condition translated into fitness outcomes, we used similar analytical approaches as above to test the effects of anthelmintic treatment, total worm abundance, and species-specific worm abundance on measures of buffalo survival and reproduction, including: (1) survival to the subsequent capture, (2) reproductive status at the subsequent capture, and (3) age of first reproduction. We tested the effects of anthelmintic treatment on survival of buffalo to the subsequent capture, using GLMMs (error = binomial, link = logit), with treatment or worm abundance as the main predictor, buffalo ID as a random effect, and condition, age, herd and season as covariates. For total and species-specific worm abundance, we ran identical models testing effects on survival of control buffalo. Next, we substituted reproductive status at subsequent capture for survival as the response variable and repeated the above analyses for treatment, total abundance, and species-specific abundance. Finally, we looked across the 4-year dataset to determine the age at which pre-reproductive individuals were first found to be reproductive (i.e. pregnant and/or lactating). We used a GLM (error = Gaussian) to test if anthelmintic treatment or condition at the capture prior to that initial reproductive bout were associated with the age of first reproduction. Age of first reproduction varied considerably by herd, so herd was included as a covariate in the analysis. We ran similar GLMs to test the relationship between total worm abundance, species-specific abundance, and age of first reproduction in control buffalo only. However, since treatment showed no effect on age of first reproduction, to increase the sample size for these analyses we also pooled the data from treated and control individuals. The final models for the effects of total and species-specific worm abundance on age of first reproduction therefore include data from control buffalo only as well as pooled data from control and treated buffalo.

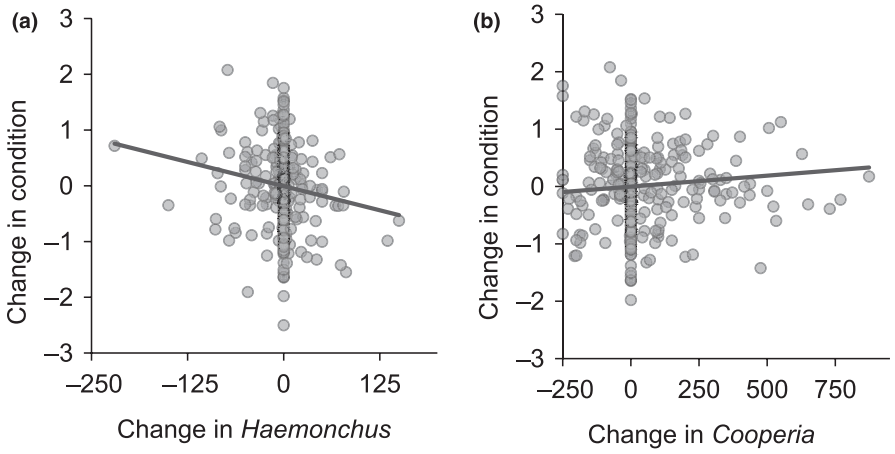

FIGURE 1 Associations between the change in *Haemonchus* sp. (a) and *Cooperia fuelleborni* (b) load and change in host body condition score in control buffalo

2.4.3 | Host immune and foraging responses to infection

To examine why the condition and fitness costs of infection may differ by worm species, we used control buffalo to explore the relationships between the two dominant worm species and host immunity and forage intake. Using GLMMs, we tested whether changes in species-specific worm abundance were associated with changes in eosinophil or IL-4 concentrations. Change in IL-4 level was square-root transformed to normalize model residuals. Buffalo ID was included as a random effect and body condition, herd, age, season change, and capture interval were included as covariates in these models. To examine whether hosts were able to acquire resistance to either worm species, we used GLMMs to test for relationships between age and parasite intensity (i.e. burden in infected hosts). Intensities were log transformed and each model also included herd, season, and a random effect of buffalo ID. Last, we used a GLMM with species-specific worm abundance, herd, age, and season as predictors to test for associations between worms and faecal nitrogen. Due to large effects of season on faecal nitrogen, we examined infection-faecal nitrogen relationships within time-points, rather than using the change in faecal nitrogen across captures.

3 | RESULTS

3.1 | *Cooperia* and *Haemonchus* have contrasting effects on host condition

Control buffalo were infected in 54% of captures, while anthelmintic-treated individuals were infected in 33% of captures. Total faecal egg count averaged (±*SE*) 108 ± 5.3 eggs/g in control buffalo, while treated buffalo had much lower egg counts (32.5 ± 2.9 eggs/g). Among control buffalo infected with worms, for whom we collected species-specific parasite data, *Cooperia* and *Haemonchus* were present in 94% and 64% of captures respectively. These buffalo were shedding a mean (±*SE*) of 9.1 ± 0.9 *Haemonchus* eggs per gram of faeces, while *Cooperia* shedding was nearly 10-fold higher at 85.5 ± 5.9 eggs/g.

Prior to anthelmintic treatment, body condition did not differ between anthelmintic-treated and control individuals (GLM: $n = 300$, *Treatment:* estimate ± *SE* (Control) = 0.031 ± 0.126, $t = 0.45$, $p = .65$; see Table S1). However, after treatment, treated individuals were in significantly better condition than controls (Table 1). Interestingly, among untreated control buffalo monitored through time, changes in total worm abundance between captures were not associated with changes in condition; however, when *Cooperia* and *Haemonchus* were considered separately, significant and opposing patterns emerged (Figure 1). Changes in *Haemonchus* abundance were negatively correlated with changes in condition, suggesting that hosts who gained *Haemonchus* lost condition; while changes in *Cooperia* abundance were positively associated with changes in body condition, suggesting that hosts who gained *Cooperia* gained body condition (Table 1). The magnitude of the *Haemonchus* association was such that the body condition of a host that experienced an increase in *Haemonchus* faecal egg count of 150 eggs per gram of faeces also dropped a full point on the condition scale between captures.

3.2 | Condition affects both survival and reproduction

Treated buffalo were no more likely to survive from one capture to the next than untreated controls (see Table S2). Among control buffalo, survival was also not related to total worm abundance. Likewise, neither the abundance of *Haemonchus* nor *Cooperia* was associated with survival between captures. However, in both the treatment and total worm abundance models, condition emerged as a significant predictor of survival probability to the next capture (Treatment model: $p = .0006$; Total abundance model: $p = .012$). This same effect fell just below significance when species identity was considered (Species-specific abundance model: $p = .052$, see Table S2).

Reproductive status was not affected by anthelmintic treatment (see Table S3). Likewise, among control buffalo the likelihood of being pregnant or lactating at a subsequent capture was not influenced by total worm abundance or species-specific worm

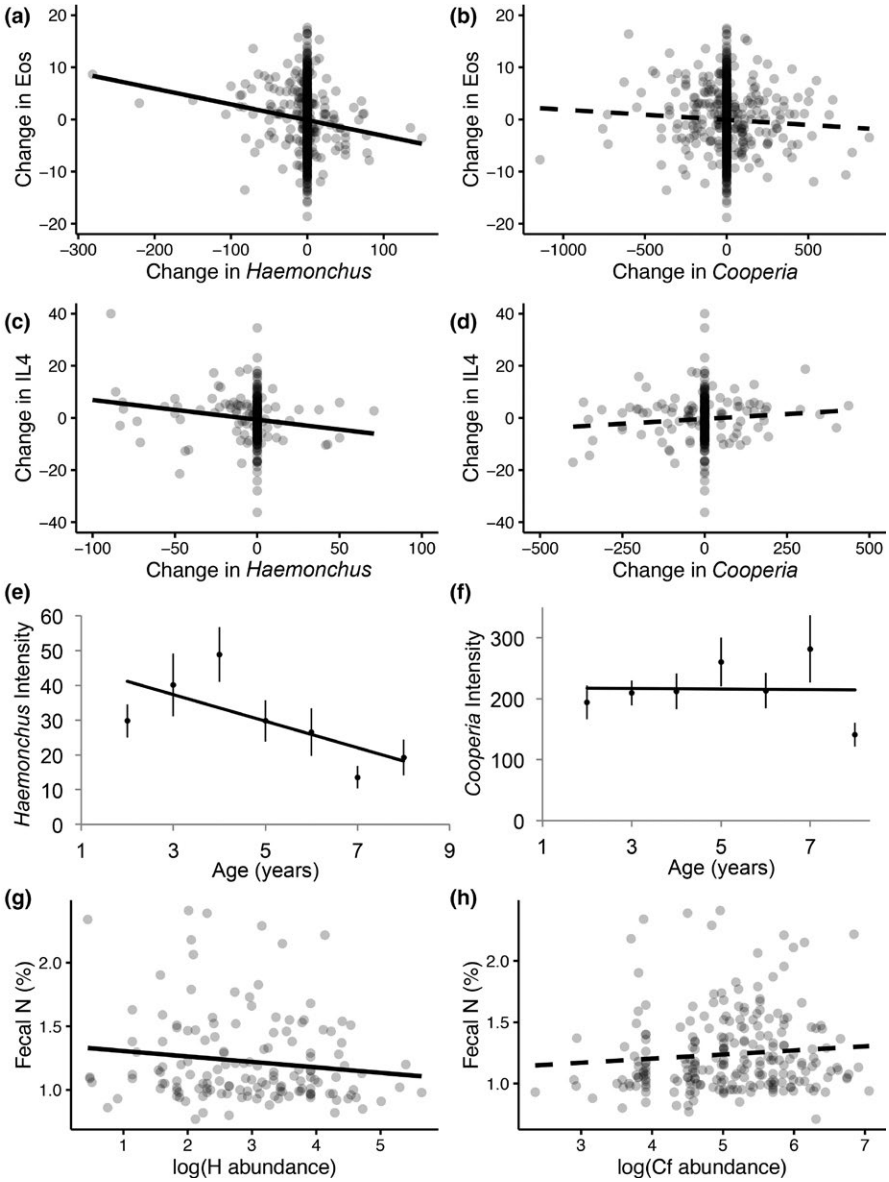

FIGURE 2 Host responses to infection varied by worm species including: (a, b) change in eosinophils (Eos), (c, d) change in IL-4, (e, f) intensity across host ages, and (g, h) faecal nitrogen (*Haemonchus* sp. [H], *Cooperia fuelleborni* [Cf]). Solid lines denote significant correlations, dashed lines denote non-significant relationships

abundance. However, once again, a significant condition effect emerged in the treatment model where buffalo in better condition were more likely to be reproductive at the next capture (p = .026), although this effect waned among the smaller subset of control individuals (Total abundance model: p = .28; Species-specific model: p = .91; see Table S3).

Age of first reproduction was not affected by anthelmintic treatment (see Table S4), but had a counterintuitive relationship with total and species-specific worm abundance. Total abundance was negatively associated with age at first reproduction, suggesting that individuals with higher worm burdens became reproductive at younger ages. The species-specific analyses suggest this relationship was driven by *Cooperia* abundance (see Figure S1). Condition was not associated with age of first reproduction in the treatment, total abundance, or species-specific abundance models (see Table S4).

3.3 | Differential fitness costs may result from variable host responses to infection

We found evidence that *Haemonchus*, but not *Cooperia*, infection was associated with host immune responses. Specifically, increases in both eosinophil and IL-4 concentrations were associated with declines in *Haemonchus* abundance (Eos: estimate ± *SE* = −0.045 ± 0.017, t = −2.66, p = .008; IL-4: est = −0.030 ± 0.013, t = −2.05, p = .042; Figure 2a,c), while changes in eosinophils and IL-4 were not correlated with changes in *Cooperia* abundance (Eos: est = 0.0003 ± 0.002, t = −0.12, p = .91; IL-4: est = 0.003 ± 0.002, t = 1.57, p = .12; Figure 2b,d; see Table S5). Changes in body condition were also positively associated with changes in eosinophil concentrations (est = 2.42 ± 0.65, t = 3.72, p = .0002; see Table S5, Figure S2), suggesting that the immune responses to *Haemonchus* might be effective at ameliorating the negative condition effect. The variable immune

responses to the two worms are further supported by differences in host age-parasite intensity profiles. From the ages of <1 year to 8 years of age, *Haemonchus* intensities declined significantly (est = −0.0073 ± 0.0034, t = −2.14, p = .035; Figure 2e; see Table S6) suggesting that there might be some degree of acquired immunity to this parasite. In contrast, there was no relationship between age and intensity for *Cooperia* (est = −0.0019 ± 0.0026, t = −0.72, p = .47; Figure 2f; see Table S6).

Finally, the two worm species showed different relationships with host forage intake as measured by faecal nitrogen levels. *Haemonchus* abundance was negatively associated with faecal nitrogen (est = −0.0016 ± 0.0006, t = −2.87, p = .0042; Figure 2g; see Table S5), whereas *Cooperia* abundance was not associated with faecal nitrogen (est = −0.000007 ± 0.00007, t = −0.095, p = 0.92; Figure 2h; see Table S5). Faecal nitrogen levels also varied seasonally with levels peaking in the late wet season and lowest in the late dry season (see Table S5, Figure S3), suggesting that faecal nitrogen tracked seasonal differences in forage availability and intake in a predictable manner.

4 | DISCUSSION

Our experimental and longitudinal studies suggest that GI worms pose significant fitness costs to free-ranging African buffalo. Importantly, these costs appear to be species-specific and driven, at least in part, by differential host responses to infection by two common worm species, *Haemonchus* spp. and *C. fuelleborni*. We found that experimental anthelmintic treatment increased host body condition suggesting that reducing all worm infections has a net positive effect on host condition. However, our species-specific analyses further revealed that individual worm species may have very different effects on host condition. Condition was negatively associated with changes in *Haemonchus* abundance, but positively associated with changes in *Cooperia* abundance. Since buffalo in better condition were more likely to survive and reproduce at the subsequent capture period, these distinct effects of the two worm species could translate into opposing condition-mediated effects on host fitness. Importantly, our work shows that differential host responses to the two parasites may contribute to these asymmetrical costs of infection. Both immune defences and forage intake rates were strongly associated with *Haemonchus*, but not *Cooperia* abundance. The tailoring of host defence strategies to parasite virulence may help explain these differential host responses and contrasting fitness effects.

Haemonchus is a highly virulent, blood-sucking parasite that is associated with anaemia, decreased condition and fecundity, and increased mortality risk in livestock (Le Jambre, 1995; West et al., 2009; Zarlenga et al., 2016). We found evidence that mixed *H. placei* and *H. bedfordi* infections also pose significant fitness costs to African buffalo; individuals that gained high numbers of *Haemonchus* from one capture to the next lost up to a full body condition score, a substantial effect size, given that mean condition only varied by 0.3 between the resource-abundant wet and resource-poor dry seasons. Importantly, condition was the strongest predictor of survival to the next capture, suggesting that *Haemonchus* infection could indirectly reduce survival odds. Since reproduction was also condition dependent, *Haemonchus* infection could further reduce individual fitness through indirect effects on host reproduction. Such condition-mediated effects of worm infections on host fitness are congruent with observations from other wild mammal-GI parasite studies. For example, a deworming experiment in reindeer showed that infection with the GI worm *Ostertagia gruehneri* reduces body weight and fecundity (Stien et al., 2002) to a degree that can impact host population dynamics (Albon et al., 2002). Importantly, the effect of deworming on reindeer fecundity was mediated by host condition; controlling for the effect of treatment on body weight removed the significant treatment effect on pregnancy. This result supports our conclusion that condition is a likely mediator of the fitness costs of *Haemonchus* infection in buffalo. Nevertheless, since we found no direct association between *Haemonchus* infection and either buffalo survival or reproduction, further work is needed to understand how potential effects of this parasite on host body condition translate into variation in fitness outcomes.

In direct contrast to *Haemonchus*, *C. fuelleborni* had no detectable fitness costs on buffalo. As a non-bloodsucking parasite, *Cooperia* is generally considered to be less virulent than *Haemonchus* in livestock (Idika, Chiejina, Mhomga, Nnadi, & Ngongeh, 2012; Stromberg et al., 2012), but known negative effects of *Cooperia* still include reduced growth, forage intake, and weight gain (Stromberg et al., 2012). However, counter to expectations, buffalo that gained *Cooperia* also gained body condition. In fact, the positive correlation between *Cooperia* and host condition was equivalent to gains of up to half a point in body condition score, which is larger than the effect imposed by seasonal extremes (i.e. wet vs. dry). Intriguingly, since both survival and reproduction were associated with condition, the associations between *Cooperia* and condition that we observed suggest that *Cooperia* infection could increase an individual buffalo's fitness. In support, we also found that *Cooperia* was associated with a younger age of first reproduction which, in many ungulates, enhances lifetime reproductive output (Green & Rothstein, 1991; Martin & Festa-Bianchet, 2012). Similarly, in humans, females with the non-bloodsucking roundworm *Ascaris lumbricoides* had a younger age of first pregnancy and higher total lifetime fertility than uninfected individuals, while those with blood-sucking hookworms (*Necator americanus* or *Ancylostoma duodenale*) became pregnant at older ages and had lower lifetime fertility (Blackwell et al., 2015). In the case of *Cooperia*, given the known negative effects of this worm on cattle health (Stromberg et al., 2012), it seems unlikely that it is a mutualist, although we cannot exclude this possibility. Rather, the associations we observed may be an indirect consequence of the host response to infection by this parasite.

Differential host responses to *Haemonchus* and *Cooperia* may help explain their contrasting relationships with fitness in African buffalo. First, in terms of the host immune response to worms, our analyses show that buffalo actively resist *Haemonchus* infection, but show no effective immune response to *Cooperia*. With respect to *Haemonchus* infection, increases in both eosinophil and IL-4 concentrations were associated with decreases in *Haemonchus* abundance suggesting that

both innate and adaptive immune responses are directed against this parasite by the host (Janeway, 2008). Although *Cooperia*-infected hosts altered their eosinophil and IL-4 concentrations to similar degrees (Figure 2a,b), investments in immunity did not correspond to decreases in worm abundance. Conversely, in cattle, eosinophilia is associated with expulsion of *Cooperia oncophora* (Kanobana, Ploeger, & Vervelde, 2002). Although our study examined only two aspects of immunity, these results are further supported by host age-parasite intensity profiles, which are often used to provide insight into how wild hosts respond immunologically to parasites (Raffel, Lloyd-Smith, Sessions, Hudson, & Rohr, 2010; Wilson et al., 2002). *Haemonchus* intensity decreased with age, whereas *Cooperia* intensity did not, which suggests that hosts may acquire protective immunity to *Haemonchus*, but not *Cooperia*, over time. These contrasting age-intensity profiles are similar to the distinct patterns reported for two GI parasites of European rabbits, *Trichostrongylus retortaefomis* and *Graphidium strigosum*. In wild rabbits, the intensity of *T. retortaefomis* decreases sharply with age whereas the intensity of *G. strigosum* increases with age (Cattadori, Boag, & Hudson, 2008); and controlled laboratory experiments confirmed that rabbits mount effective immune defences to T. *retortaefomis*, but not *G. strigosum* (Murphy, Nalpas, Stear, & Cattadori, 2011).

The immune responses that buffalo mount against *Haemonchus* appear to provide benefits, but likely also come with costs. Individuals that reduced their *Haemonchus* load gained body condition by the subsequent capture. We also observed a positive relationship between changes in eosinophils and condition. This pattern suggests that elevating eosinophils may have benefits for subsequent host condition. Although we cannot dismiss the alternative hypothesis that immune defences are condition dependent, it does not explain the negative relationship between host condition and *Haemonchus* infection. Host immune responses to *Haemonchus* also seem to come with important costs, one of which is reduced forage intake. In livestock, worm infection is associated with voluntary reductions in food intake (Coop & Kyriazakis, 1999), and the up-regulation of anti-worm immunity is considered to be an important cause of this anorexia response (Colditz, 2008; Greer, 2008; Greer, McAnulty, Stankiewicz, & Sykes, 2005). In buffalo, we found that *Haemonchus* abundance was negatively associated with faecal nitrogen, a result that provides preliminary support for the idea that the buffalo immune response to *Haemonchus* infection may be tied to reductions in forage intake. We used faecal nitrogen as a proxy for forage intake because this measure is strongly and positively correlated with dietary nitrogen in buffalo (Ryan et al., 2012). Use of this measure is also supported by our observation that faecal nitrogen varied seasonally in a predictable manner, with the lowest levels observed during the late dry season when forage is scarce and the highest levels observed during the late wet season when forage is abundant. Importantly, nematode-induced reductions in forage intake can contribute to between 40%–90% losses in production in livestock systems (Forbes, Huckle, Gibb, Rook, & Nuthall, 2000; Greer, 2008), and the fitness impacts may be of similar magnitude in natural systems. Thus in combination, our immunological and forage intake analyses paint a picture suggesting that when buffalo become infected with *Haemonchus*, they mount a protective immune response that can improve condition in the long-term (i.e. by subsequent capture), but this up-regulation may reduce their current body condition by reducing intake rates.

The positive relationship between *Cooperia* abundance and host fitness may also be driven by host responses. Rather than indicating the parasite itself has a positive effect on host condition and reproduction, this same pattern could arise if tolerating *Cooperia* (i.e. maintaining health despite a high parasite load) has a fitness advantage compared to resisting infection. Tolerance, can be identified by evaluating the relationship between parasite load and host health/fitness (Medzhitov et al., 2012; Råberg et al., 2009). Our results suggest that some buffalo maintain both survival and reproduction in the face of high *Cooperia* loads, implicating tolerance as a possible defence strategy. A tolerance strategy might be advantageous compared to a resistance strategy for at least two reasons. First, the immune defences to combat *Cooperia* may be more energetically expensive than the infection itself. Differences in body condition we observed between anthelmintic-treated and naturally uninfected hosts support the idea that immune defences to worms are energetically costly. For instance, we saw that experimental deworming imparted condition and survival benefits to buffalo, but changes in total worm abundance were not associated with these same aspects of fitness, indicating that buffalo with naturally low infection levels (i.e. those with a resistance strategy) did not receive the same benefits. This discrepancy suggests that having a naturally low worm load requires investment in defences that also impact host fitness. While *Haemonchus* likely contributes to this discrepancy, *Cooperia* is at far higher prevalence among infected individuals (94%) than *Haemonchus* (64%), and may thus contribute substantially to this pattern.

A second possible reason why tolerance may be advantageous for *Cooperia* defence is that in addition to being energetically expensive, immune defences to this parasite may be ineffective. In support, increases in eosinophils and IL-4 were not associated with reductions in *Cooperia* loads, suggesting that buffalo did not mount effective or protective responses to *Cooperia* infection. Both eosinophils and IL-4 are related to clearance of *Cooperia* species in cattle (Kanobana et al., 2002), suggesting that the two immune measures we examined have relevance for *Cooperia* defence, however, further studies assaying an expanded suite of immunological markers may provide additional insight into the mechanisms of *Cooperia* defence in buffalo. Nevertheless, worm-specific immune efficacy has been documented in other wildlife; rabbits elevate eosinophils and IL-4 in response to infections with *T. retortaeformis* and *G. strigosum*, but these defences are only effective at reducing burdens of *T. retortaeformis*, not *G. strigosum* (Murphy et al., 2011). Although not previously documented for *Cooperia*, many worms have evolved immune-evasion mechanisms that can block, interfere with, or divert host immune defences, rendering them ineffective (Maizels & Yazdanbakhsh, 2003). Moreover, even if efficacy and per-capita costs of resistance are identical for *Haemonchus* and *Cooperia*, average *Cooperia* burdens were over 10-fold higher than *Haemonchus* burdens in our buffalo. Therefore, simply due to the higher worm counts, total immune defence costs

could be significantly higher for resisting *Cooperia* when compared to *Haemonchus*. Given the potentially high cost of resistance and the positive associations between parasite intensity and fitness, for buffalo, a tolerance strategy appears to be a more effective defence against *Cooperia*. It is important to note, however, that not all buffalo appeared to tolerate *Cooperia* infection. The individuals that reduced their *Cooperia* loads also lost body condition and were less likely to be reproductive at the subsequent capture; thus it is likely that there is variation in tolerance with some individuals taking a less successful resistance strategy. Understanding the factors that maintain this variation is an interesting future direction.

Overall, our combination of experimental and longitudinal studies revealed that worms have significant direct and indirect fitness costs on hosts, and that host responses to infection play a considerable role in shaping these outcomes. Hosts resisted *Haemonchus* infection, and although immunological defences were effective at reducing worm abundance on the one hand, they may contribute to fitness losses on the other, via negative effects of condition and forage intake. By contrast, there was no detectable change in *Cooperia* burden relative to host immune responses, and those with more worms were in better condition, potentially indicating the advantages of a tolerance-type host response to this less-virulent parasite. In wild hosts, very little is known about how or when individuals adopt resistance or tolerance strategies. Our study thus illustrates how hosts can maximize lifetime reproductive success by tailoring their defence strategies to specific parasites, perhaps to balance parasite virulence, costs of immunity, and exposure.

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AUTHORS' CONTRIBUTIONS

S.A.B., A.E.J. and V.O.E. designed the study. All authors contributed to data collection. S.A.B. analysed data, and S.A.B. and V.O.E. wrote the manuscript. All authors contributed to drafts and approved the manuscript for publication.

DATA ACCESSIBILITY

Data can be accessed in the Dryad Digital Repository https://doi.org/10.5061/dryad.01v62 (Budischak, O'Neil, Jolles, & Ezenwa, 2017).

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