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Parasite responses to large mammal loss in an African savanna

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Abstract. Biodiversity loss can alter disease transmission; however, the magnitude and direction of these effects vary widely across ecosystems, scales, and pathogens. Here we experimentally examine the effects of one of the most globally pervasive patterns of biodiversity decline, the selective loss of large wildlife, on infection probability, intensity and population size of a group of common rodent-borne parasites – macroparasitic helminths. Consistent with previous work on vector-borne pathogens, we found that large wildlife removal causes strong and systematic increases of rodent-borne parasites, largely due to increases in rodent density, as rodents are released from competition with larger herbivores. Although we predicted that increased host density would also increase per capita infection among all directly transmitted parasites, this additional amplification occurred for only two of three examined parasites. Furthermore, the actual effects of large mammal loss on per capita infection were mediated by the complex suite of abiotic and biotic factors that regulate parasite transmission. Thus, while these results strongly suggest that large wildlife loss will cause systematic increases in rodent parasite populations, they also underscore the difficulty of making more specific predictions for a given parasite based on simple attributes such as transmission mode or life history strategy. Instead, detailed information on the ecology of each parasite species would be necessary to make more accurate predictions of how biodiversity loss will affect infection.

Key words: defaunation; density-dependent transmission; exclosure; nematode; population regulation; wildlife decline.

Introduction

It is well-established that biodiversity loss can affect disease transmission, and several reviews and a recent meta-analysis have suggested a pervasive negative relationship between biodiversity and parasite abundance (Ostfeld and Keesing 2012, Civitello et al. 2015, Johnson et al. 2015). However, these findings are not consistent with other well-substantiated conclusions arguing that the magnitude and direction of the biodiversity and disease relationship is highly variable across infectious agents and ecosystems (Randolph and Dobson 2012, Salkeld et al. 2013, Wood et al. 2014). The relationship between biodiversity and disease thus remains contentious, with one of the few points of agreement being a need for more large-scale experimental studies examining mechanisms by which realistic patterns of biodiversity loss alter disease dynamics (Keesing et al. 2006, Gottdenker et al. 2014, Wood et al. 2014, Civitello et al. 2015, Johnson et al. 2015).

Among the many types of biodiversity loss, one of particular ecological consequence is the global decline of large-bodied species, due to their disproportionately strong and unique functional roles (Dirzo et al. 2014, Smith et al. 2015, Malhi et al. 2016). Megafauna (>15 kg) shape ecosystem structure and function in myriad ways. Large animals change vegetative structure and nutrient cycling (Schmitz et al. 2014, Asner et al. 2016), control energy flow and trophic cascades (Estes et al. 2011) and manipulate physical processes such as fire regimes and erosion rates (Van Langevelde et al. 2003, Kimuyu et al. 2014). Megafauna also regulate other species. For example, small mammals often increase in the absence of large mammals due to altered habitat structure (Putman et al. 1989, Buesching et al. 2011), decreased predation (Gilg et al. 2003, Packer et al. 2003) and reduced competition (Keesing 1998*a*, Steen et al. 2005, Previtali et al. 2009). Several studies have documented marked increases in small mammal abundance as megafauna decline (Leirs et al. 1997, Keesing 2000, Mills 2006, Previtali et al. 2009), and this rodent population release can also alter transmission of rodent-borne infectious agents (Young et al. 2014, Bordes et al. 2015).

Rodents are host to a diverse array of helminth, bacterial and viral pathogens, including at least 85 organisms that infect humans (Han et al. 2015, 2016). Any disturbance that increases rodent populations is likely to also increase their parasite populations simply because larger host populations can support more infected hosts (Packer et al. 2003, Keesing et al. 2006). Additionally, if parasite transmission is density-dependent (and increased rodent density increases contact rates), higher host density could

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lead to even larger parasite populations due to higher per capita infection (Anderson and May 1978, Arneberg et al. 1998). Density-dependent transmission is often assumed in disease models, and meta-analyses suggest that per capita infection and host density are often positively correlated for parasites transmitted via close proximity or contaminated feces (Côté and Poulin 1995, Arneberg 2001, Rifkin et al. 2012); however, experimental tests of these patterns in wildlife populations are rare (but see Caley et al. 1999, Gompper and Wright 2005, Woodroffe et al. 2006).

Although disease transmission should increase proportionally with host density when susceptible hosts move randomly and mix completely with parasite infective stages, these assumptions are rarely met in nature (Antonovics et al. 1995, McCallum et al. 2001, Fenton et al. 2002). Due to parasite life history and host behavior, transmission rates in some disease systems are independent of host density. This is most often the case for parasites that are sexually transmitted, vector borne, or have complex life cycles (De Jong et al. 1995) but it can also occur for directly transmitted parasites when contact between individual or infective stages remains constant regardless of host density (Bouma et al. 1995, Begon et al. 1999, Bjørnstad et al. 2002).

Furthermore, parasites that follow density-dependent transmission at low host density can have a threshold density above which all available hosts are infected and per capita infection no longer increases with increasing density (D'Amico et al. 1996, Dwyer et al. 1997, Knell et al. 1998). Thus, while contact might limit parasite populations at low host density, at high density, parasites might instead be regulated by intraspecific competition or host immunity (Bradley 1974). These contact and establishment rates might be further altered by abiotic factors and individual host differences in behavior and immunology. For example, elevated rainfall might increase contact rates by increasing infective stage survival (Pietrock and Marcogliese 2003, Weaver et al. 2010), larger hosts might ingest more infective stages due to greater foraging requirements (Combes 2004), and sex-based differences in behavior or immunocompetence might alter both contact and susceptibility (Zuk and McKean 1996, Krasnov et al. 2012). Thus, although many disease models assume a homogenous transmission function that increases with host density (Anderson and May 1978, McCallum et al. 2001), the complex interactions between host, parasite and environment suggest that the relationship between host density and per capita risk may be less predictable.

Understanding the relationship between rodent density and per capita infection is critical for predicting how parasites will respond to the rodent population increases that often follow large mammal loss. Higher parasite burden in rodents could reduce fitness and change behavior among these important consumers, and larger rodent parasite populations could also increase spillover risk to other species. Here, we used a large-scale, long-term, and replicated exclosure experiment in East Africa (the Kenya Long Term Exclosure Experiment, "KLEE") to explicitly test the effects of large herbivore loss and increased rodent density on the probability of infection, intensity, and population size of three nematode parasites in the East African pouched mouse (*Saccostomus mearnsi*). Traditional density-dependent transmission functions suggest that increased host density should increase per capita infection and total population size for all three parasites; however, life history differences among the parasites allow us to explore how environmental, host and parasite factors might drive deviations from predicted responses.

Methods

Study system

Although predator and megaherbivore populations are declining globally, parts of the East African savanna still retain robust populations of large mammals (Kinnaird and O'Brien 2012, but see Ogutu et al. 2016), including elephants (*Loxodonta africana*), giraffes (*Giraffa camelopardalis*), zebras (*Equus quagga* and *Equus grevyi*), buffalos (*Syncerus caffer*) and lions (*Panthera leo*), among others (Young et al. 1997). The Mpala Research Centre, which hosts the Kenya Long-Term Exclosure Experiment ("KLEE", Fig. 1A), is set in Laikipia County, Kenya (0°17′ N, 36°52′ E), which is one of the few Kenyan counties that has maintained or increased its wildlife biomass over the past 40 years (Ogutu et al. 2016). This long term experiment consists of a replicated series of wildlife exclosure plots that have been used extensively to examine the effects of defaunation (Young et al. 1997, Keesing and Young 2014), and it provides an ideal setting for experimentally testing the effects of large mammal loss and increased rodent densities on parasitism.

Established in 1992, KLEE uses a block design ($n = 3$ of each treatment) to exclude various groups of large mammals from replicated four hectare plots. Although KLEE includes multiple factorial treatments of wildlife and livestock removal, we used only the total exclosure sites that block access to all animals greater than approximately 15 kg and the control sites that allow open access to all wildlife and cattle (details on plot configuration available in Young et al. 1997). These plots support a relatively low-diversity small mammal community, with one rodent species, *S. mearnsi* (Fig. 1B), comprising 75–85% of all captures (Keesing 2000, McCauley et al. 2006, Young et al. 2015). Two decades of monitoring have established that densities of this rodent, and other less abundant small mammals, double in the absence of large mammals (Keesing and Young 2014, Young et al. 2015). Although small mammal density increases, the diversity and community composition of potential hosts for rodent helminths remain stable (Young et al. 2014), allowing us to examine the effects of host density without the potentially confounding effects

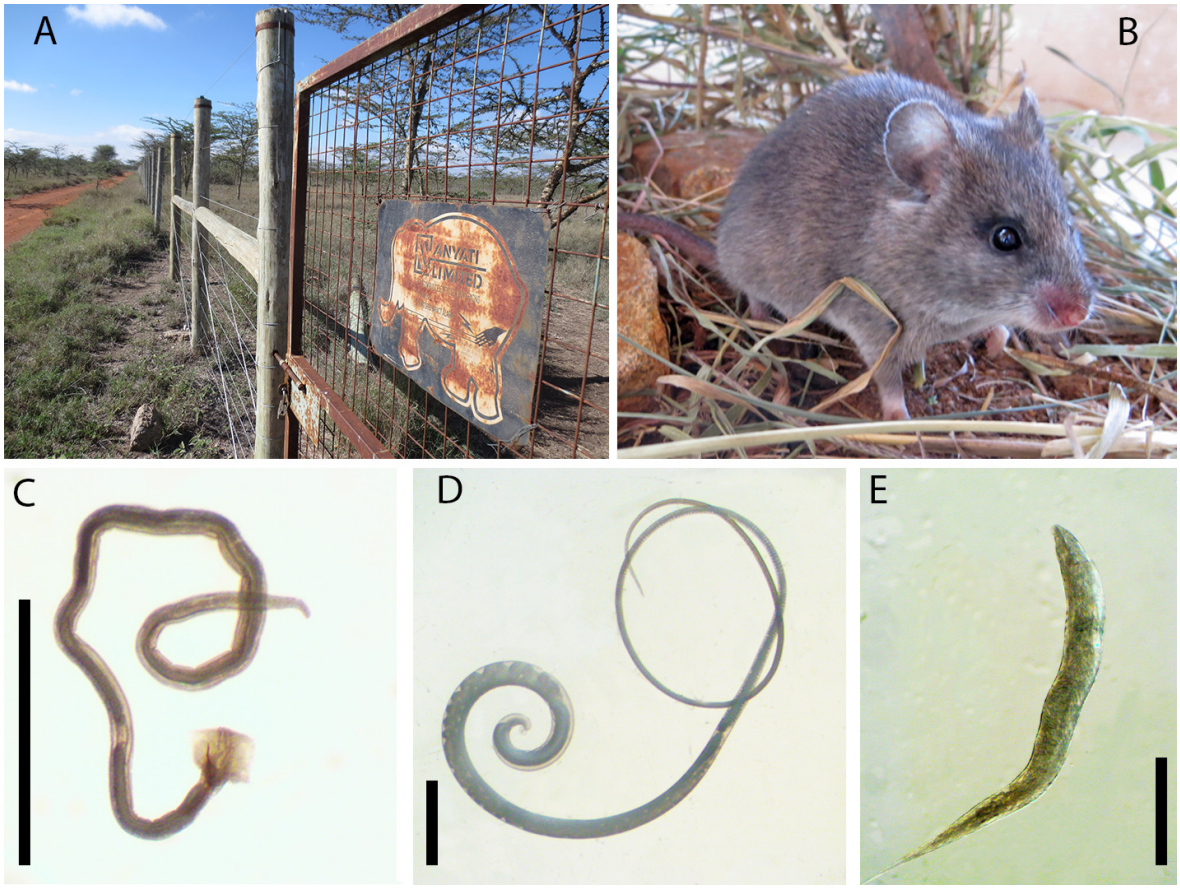

FIG. 1. Kenya Long Term Exclosure Experiment plot (A), *Saccostomus mearnsi* (B), and its three most common parasites *Neoheligmonella* sp. (C), *Trichuris* sp. (D), and *Syphacia muris* (E). Scale bars = 1 mm.

of transmission interference from changing small mammal community composition.

The hyperabundant *S. mearnsi* is host to multiple infectious agents including three directly transmitted nematodes: a trichostrongylid (*Neoheligmonella* sp., hereafter referred to as *Neoheligmonella*, Fig. 1C), a whipworm (*Trichuris* sp., hereafter referred to as *Trichuris*, Fig. 1D) and a pinworm (*Syphacia muris*, hereafter referred to as *Syphacia*, Fig. 1E). All three parasites develop in the host's gastrointestinal tract, and once mature, release eggs back into the environment (Anderson 2000). *Trichuris* and *Neoheligmonella* eggs always develop in the environment; however, *Syphacia* eggs frequently adhere to the host and can cause high rates of reinfection (Anderson 2000). *Trichuris* and *Syphacia* occur worldwide in a variety of rodents and produce eggs that can survive for months in the environment (Anderson 2000). Less is known about the biology of *Neoheligmonella*. Most species in the genus occur in East African rodents, exhibit high host specificity, and follow a typical trichostrongylid life cycle where eggs pass into environment and hatch into infective larvae that must penetrate the host's skin or mucosal membranes (Digiani and Durette-Desset 2013). Unlike many parasites with resistant eggs, infective *Neoheligmonella* larvae are relatively short-lived and sensitive to environmental conditions (Durette-Desset and Cassone 1987, Digiani and Durette-Desset 2013).

Rodent and parasite surveys

We trapped rodents monthly in KLEE from April through September 2014. Each month, a 100 Sherman trap grid was set in the inner one hectare of each exclosure ("North", "Central", "South") and control ("North", "Central", "South") plot using 10 × 10 m trap spacing. Traps were baited nightly with peanut butter and oats, and monthly trapping continued in each plot until at least three adult *S. mearnsi* were caught. Capturing three males per plot typically required one to three nights of trapping in exclosure plots and three to five nights in control plots. As the relationship between wildlife removal and small mammal density in these plots is well established and consistent over many seasons and years of sampling (Keesing and Young 2014, Young et al. 2014), we did not conduct mark recapture density estimates in this study.

To permit parasitological examination, captured *S. mearnsi* were euthanized and either frozen immediately

or refrigerated and processed within 48 hours. We measured, sexed, and dissected each animal, removing the gastrointestinal tract and separating stomach, small intestine, cecum and large intestine into separate dishes. Gut sections were opened lengthwise and contents washed into beakers of water. These contents were decanted until the supernatant was clear, and then contents were poured into petri dishes. Some parasites, particularly the smallest *Syphacia*, might have been lost during decanting; however, this error should be consistent across all samples. We identified and counted parasites under a dissecting scope and used a compound scope to identify smaller individuals. In the first 20 hosts we also examined heart, lungs, liver, kidneys, bladder, gonads and gut tissue under a dissecting microscope using squash plates to check for additional parasites in or adhering to the tissue. As no parasites were seen in the heart, kidneys, bladder, gonads, or the walls of the stomach, cecum, and large intestine, we did not examine these tissues in the remaining animals. We found larval cestodes and nematodes encysted in the liver, lungs, and small intestine, and we examined gastrointestinal contents and these tissues in every *S. mearnsi* (Appendix S1).

We restricted statistical analyses to the three most commonly encountered parasites, *Neoheligmonella*, *Trichuris*, and *Syphacia,* as other parasite taxa were too rare to permit robust analyses (Appendix S1: Table S1). We examined how increasing host density in exclosure plots affected the probability of being infected and the number of parasites per infected host (intensity) using binomial generalized linear models and negative binomial generalized linear models, respectively. For both presence and intensity models, we included treatment (large wildlife excluded or large wildlife allowed), host sex and weight, 30-d cumulative rainfall prior to the trapping date (accounting for a species specific lag, as below), and the two-way interaction between treatment and the other three factors. Rainfall data were collected from a nearby rain gauge and, to capture variation in parasite development rate, we used a 15 day lag for *Neoheligmonella* (Haley 1962) and *Syphacia* (Stahl 1963) and a 30 day rainfall lag for *Trichuris* (Behnke and Wakelin 1973). As *S. mearnsi* can substantially vary in weight (Keesing 1998*a*) and this could influence parasitism, we also verified that average mouse weight was similar between control and exclosure plots using a two sample *t*-test. We treat individual animals as replicates and do not include plot as a random effect as plots are within 1.3 km of each other and the standardized residuals of the fixed effect model were similar across plots (Zuur et al. 2009). Other parasites were not included as predictors in parasite abundance models as it is difficult to infer interspecific interactions from observational studies (Fenton et al. 2010, 2014) and, although infection with one parasite could facilitate or inhibit infection by another (Behnke et al. 2001), we found no evidence for an inhibitory effect of coinfection (Appendix S1: Fig. S1). Model selection was done by backwards stepwise selection, first dropping interaction terms and then main effects. Nested models were compared using *F*-statistic *P*-values with significance set at $P < 0.05$ (Zuur et al. 2009). Analyses were done using the "MASS" package in R version 3.30 (Venables and Ripley 2002, R Core Team 2016) and we report mean abundance, intensity and prevalence (as defined by Bush et al. 1997) for each plot type with standard error and 95% Bayesian confidence intervals estimated using the "prevalence" package (Devleesschauwer et al. 2014), respectively.

Results

We collected parasitological data from 91 *Saccostomus mearnsi*; 47 of these rodents were captured in exclosure plots (North: 16, Central: 17, South: 14) with no large herbivores and high small mammal density and 44 were captured in control plots (North: 15, Central: 14, South: 15) with large herbivores present and low small mammal density. Although we did not explicitly measure rodent density during this study, trapping the requisite three males per plot consistently required approximately twice as many trap nights in control compared to exclosure plots. This suggested that, consistent with past surveys (eg. Keesing and Young 2014, Young et al. 2015), rodent density was higher in exclosure plots than in controls. The three gastrointestinal nematodes, *Neoheligmonella*, *Trichuris* and *Syphacia* were the most commonly encountered parasites and together represented 99.2% of the 12,079 counted helminths. At least one of these nematodes was present in 100% and 95% of examined animals from exclosure and control plots, respectively.

Neoheligmonella

Wildlife removal and resulting increased small mammal density significantly increased per capita *Neoheligmonella* infection for all *S. mearnsi*. This parasite was more prevalent in hosts in exclosure (96%; CI: 87–99%) than in control (84%; CI: 71–93%) plots. Individual probability of infection was significantly lower in control plots, but increased with host weight, regardless of plot type (Table 1). Increased host density in exclosure plots had more complex effects on the number of parasites per *S. mearnsi*. Overall, the number of *Neoheligmonella* per

Table 1. GLM regression results for the best model for infection probability, including coefficient estimate (β), standard error (SE), Wald's *Z* score (*Z* value) and *P*-value. Significant factors are in bold.

Factor	β	SE	*Z* value	*P*-value
Neoheligmonella sp.				
Exclosure	**−1.89**	**0.93**	**−2.04**	**0.0412**
Weight	**0.05**	**0.02**	**2.34**	**0.0195**

Note: For *Trichuris* sp. and *Syphacia muris*, there were no significant predictors.

infected rodent nearly doubled in exclosure plots (57.1 ± 9.9 mean parasites per rodent ± standard error) compared to control plots (32.0 ± 5.8); however this pattern was characterized by strong interactions between wildlife exclosure, rainfall and host sex (Table 2). During the driest periods (<1 cm cumulative rainfall in the prior month), infected *S. mearnsi* in exclosure plots had over six times more *Neoheligmonella* than did individuals in control plots (89.5 ± 24.8, compared to 14.1 ± 7.7); however, this difference was less pronounced under wetter conditions (mean intensity in exclosure: 43.9 ± 8.8 and control: 36.1 ± 6.7, Fig. 2B). Furthermore, although both male and female *S. mearnsi* were infected, increased host density in exclosure plots only increased intensity in male rodents. Infected males hosted over twice as many parasites in exclosure than control plots (64.4 ± 11.9 vs. 30.9 ± 7.0 *Neoheligmonella* per infected male); whereas female intensity was similar in both plot types (31.3 ± 12.5 vs. 35.8 ± 8.4, Fig. 2A). In both exclosure and control plots, *Neoheligmonella* intensity increased with weight (Fig. 2C), with no weight differences seen between plot types (exclosure = 75.9 ± 2.6 g, control = 79.0 ± 2.2 g, two sample *t*-test (87.5) = −0.92, $P = 0.36$). Increased probability of infection for all *S. mearnsi* and increased intensity in males during dry periods led to more *Neoheligmonella* per rodent (Fig. 3A). Assuming that *S. mearnsi* populations doubled in the absence of large wildlife (as expected from long term population trends (Keesing and Young 2014, Young et al. 2014)), and accounting for the approximately 1:1 *S. mearnsi* sex ratio (H. Young, *unpublished*), suggests that *Neoheligmonella* populations are up to 3.5 times larger in exclosure plots than in controls.

Table 2. GLM regression results for the best model of parasite intensity, includes coefficient estimate (β), standard error (SE), Wald's *Z* score (*Z* value) and *P*-value. Significant factors are in bold. One rodent with 1889 pinworms was excluded from *Syphacia* intensity analyses; however a repeat analysis including this outlier is included in the supplementary material (Appendix S1: Table S2).

Factor	β	SE	*Z* value	*P*-value
Neoheligmonella sp.				
Exclosure	−0.68	0.68	−0.98	0.3268
Sex	**0.74**	**0.36**	**2.06**	**0.0394**
Rain	**0.52**	**0.22**	**−2.32**	**0.0201**
Weight	**0.04**	**0.01**	**5.67**	**1.4e-8**
Exclosure:Sex	**−1.94**	**0.52**	**−3.72**	**0.0002**
Exclosure:Rain	**1.07**	**0.38**	**2.85**	**0.0044**
Syphacia muris				
Exclosure	**−6.59**	**1.85**	**−3.56**	**0.0004**
Sex	**1.55**	**0.53**	**2.95**	**0.0031**
Rain	**−0.51**	**0.25**	**−2.03**	**0.0425**
Weight	−0.02	0.01	−1.58	0.1147
Exclosure:Sex	**−1.69**	**0.83**	**−2.02**	**0.0436**
Exclosure:Weight	**0.09**	**0.03**	**3.72**	**0.0002**

Note: For *Trichuris* sp., there were no significant predictors.

Trichuris

For *Trichuris*, wildlife exclusion had no significant effect on infection probability or intensity. *Trichuris* was found in 62% (CI: 47–75%) and 66% (CI: 51–79%) of *S. mearnsi* in exclosure and control plots respectively, and no examined factors significantly predicted probability of infection (Table 1). Intensity also did not differ between exclosure and control plots (5.5 ± 1.4 and 5.6 ± 1.1 per infected rodent, respectively), and no examined factors significantly influenced the number of *Trichuris* per infected rodent (Table 2). Although individual *S. mearnsi* in exclosure plots hosted, on average, as many *Trichuris* as those in control plots (abundance in exclosure was 3.4 ± 0.9 vs. 3.7 ± 0.8 in control, Fig. 3B), a doubling of small mammal abundance in the absence of large wildlife could lead to *Trichuris* populations up to 1.8 times larger.

Syphacia

The pinworm, *Syphacia*, was found in 79% (CI: 63–87%) and 66% (CI: 51–79%) of *S. mearnsi* in exclosure and control plots, respectively. Neither treatment, nor any other examined factor, significantly affected the probability of *Syphacia* infection (Table 1). Treatment effects on *Syphacia* intensity were more complex and varied based on the inclusion of an outlier. *Syphacia* were highly aggregated in rodents from both plot types (Fig. 2D, F, see Appendix S1: Table S2 for analyses with the outlier); however, one male *S. mearnsi* from a control plot hosted almost a quarter ($n = 1889$) of all counted pinworms. With this outlier, average intensity was higher in control plots than in exclosures (136.6 ± 70 vs. 105.4 ± 30.2). However, when this point was excluded, the pattern reversed and intensity was significantly lower in control plots (74.0 ± 30.9 vs. 105.4 ± 30.2, Table 2). Both with and without the outlier, increasing rainfall correlated with reduced *Syphacia* intensity in both plot types and larger *S. mearnsi* hosted more *Syphacia*, but only in control plots (Table 2, Fig. 2F, Appendix S1: Fig. S2). When the outlier was included, males hosted more *Syphacia* than females (140.2 ± 44.7 vs. 49.7 ± 20.0) with no interaction between sex and treatment. Excluding this outlier revealed a significant interaction between sex and treatment: *Syphacia* intensity was higher in exclosure plots, but only in male rodents (122.4 ± 39.1 vs. 82.6 ± 38.3), while intensity in females was similar in both treatments (54.7 ± 26.6 vs. 42.3 ± 32.8). This suggests that increasing small mammal density in exclosure plots might also increase per capita *Syphacia* infection in male rodents, mirroring patterns in *Neoheligmonella* intensity. Although large wildlife exclusion had no effect on the individual probability of infection and heterogeneous effects on infection intensity, in the absence of large wildlife (and excluding the outlier), higher small mammal density in exclosure plots could result in *Syphacia* populations up to 2.8 times larger than those in control plots.

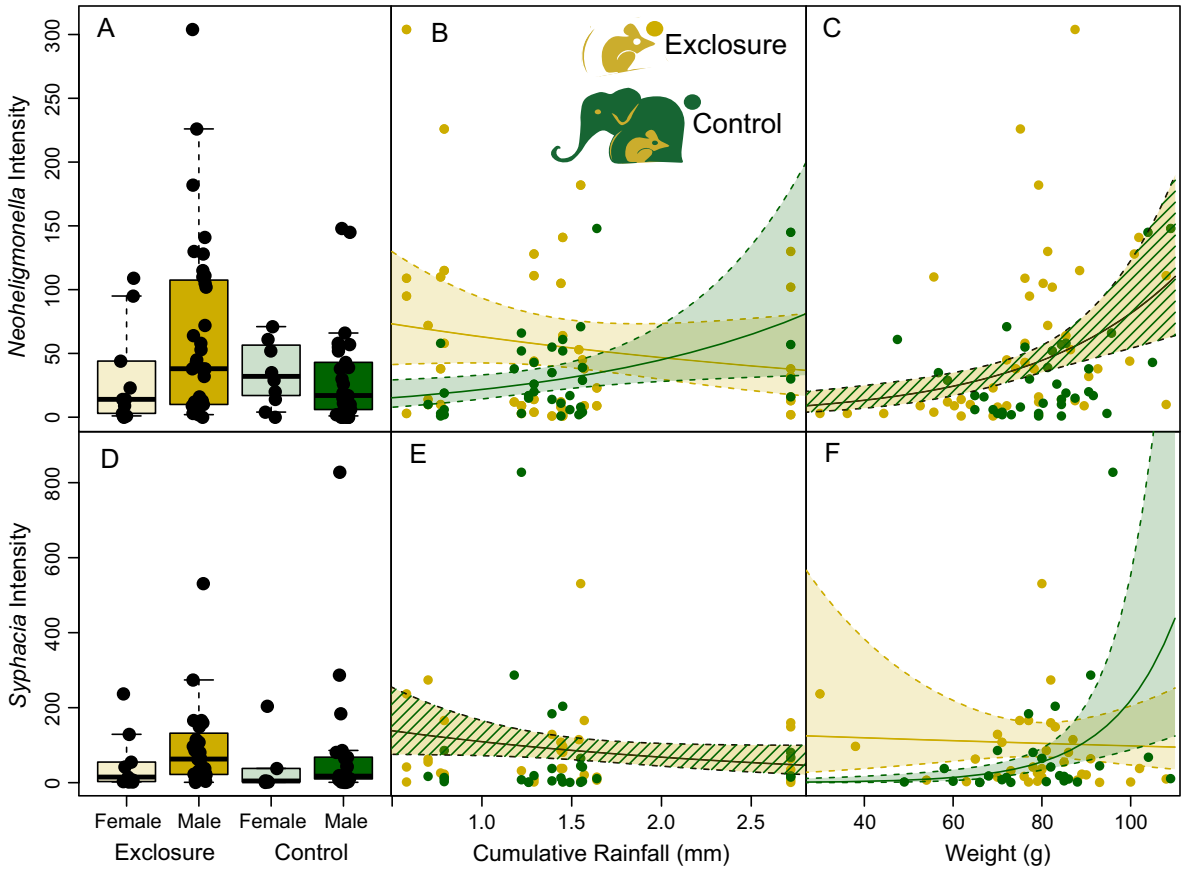

FIG. 2. Increasing host density in exclosure plots had species specific effects on parasite intensity. Increased *Neoheligmonella* and *Syphacia* intensity in exclosure plots was driven by increased per capita parasitism in male rodents (A, D). For *Neoheligmonella* these differences were most pronounced in the driest months when rodents in exclosure plots had intensities six times greater than rodents in control plots (B). *Neoheligmonella* intensity increased with host weight (C), *Syphacia* intensity was negatively correlated with rainfall (E), and, in control plots, *Syphacia* intensity increased with host weight (F). The single rodent that hosted 1,889 *Syphacia* is excluded in plots D-F; however, an additional figure including this host is included in the supplemental material (Appendix S1: Fig. S2).

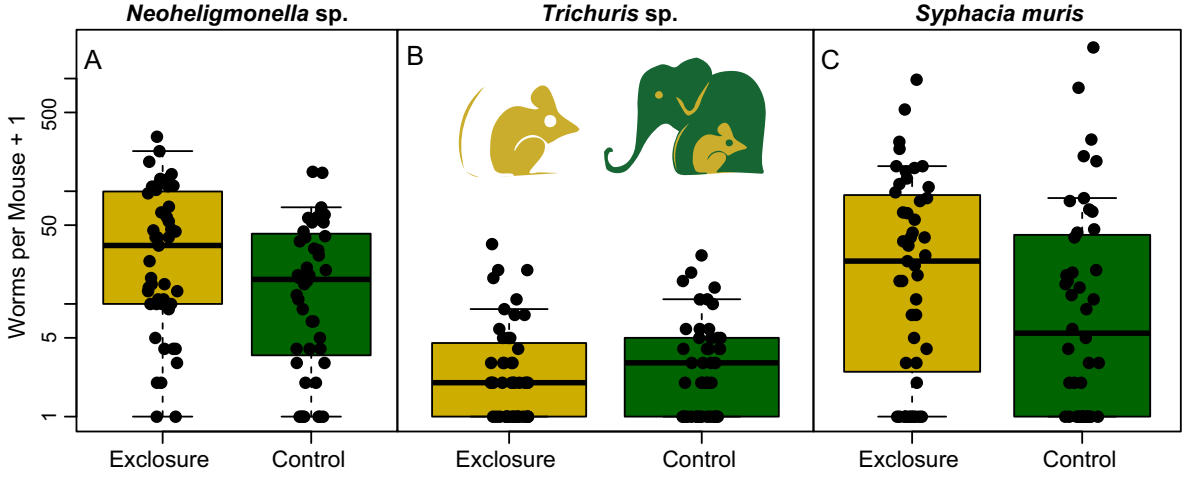

FIG. 3. Parasite responses to large mammal exclusion varied by species. Large mammal exclusion (and increased host density) increased the abundance of (A) *Neoheligmonella* sp. and *Syphacia muris* (C) but had no effects on the average number of *Trichuris* sp. (B) per rodent.

Discussion

When megafauna regulate reservoir host populations, the loss of these large-bodied species can amplify parasite populations by releasing controls on host populations, which in turn support more infected hosts. In our study system, large mammal exclusion increased parasite populations, mirroring past work that observed defaunation-driven increases in other rodent consumers, such as fleas and snakes (McCauley et al. 2006, 2008). Consistent population increases among these directly transmitted parasites and previously examined vector-borne pathogens (Young et al. 2014) suggest that this may be a general response for most rodent-borne infectious agents in this system. Although all *S. mearnsi* parasite populations increased, the magnitude of these increases varied substantially due to the complex interactions that determine per capita infection. Disease models often assume that per capita risk scales with host density (Anderson and May 1978, McCallum et al. 2001, 2017); however, our results suggest that this widely used assumption is a poor fit to the heterogeneous transmission patterns in wild hosts.

A key question arises from our results: why would parasites that are expected to exhibit density-dependent transmission not increase in per capita infection with increasing host density? One answer is that parasite exposure can only generate infection in *susceptible* hosts, and the proportion of susceptible hosts may be relatively unchanged by increased host density. When parasite infective stages are abundant in the environment, high contact rates with hosts might result in host populations that are ''saturated;" whereby host immune response or parasite competition limit additional parasite establishment (McCallum et al. 2001). Although there is limited information on parasite-specific immune responses in *S. mearnsi*, extensive work in laboratory rodents has shown that host immunity strongly regulates parasite populations (Paterson and Viney 2003, Hurst and Else 2013). For *Trichuris muris*, previous infection provides nearly complete immunity, and current infection substantially reduces additional parasite establishment (Wakelin 1967, Hurst and Else 2013). The apparent stability of per capita *Trichuris* infection suggests that, even in low density plots, this parasite might be regulated by host immune responses that limit parasite establishment. Although host immune responses would also be expected to regulate *Syphacia* and *Neoheligmonella* (Panter 1969, Ogilvie and Jones 1971, Jenkins and Phillipson 1972), increased per capita infection in exclosure plots suggests that transmission in low density control plots is still limited by contact with infective stages.

Neoheligmonella and *Syphacia* intensity increased at higher host density, but only in male rodents. Due to behavioral and physiological differences, male animals are often more heavily parasitized than females (Moore and Wilson 2002, Ferrari et al. 2004, Krasnov et al. 2012) and patterns observed here may be driven by differences in male and female territory size. In this system, male territories (which are larger than females') increasingly overlap as rodent density rises, while female territories remain small and non-overlapping (Keesing 1998*a,b*). Due to this increased overlap, males in exclosure plots are more likely to contact infective parasite stages, which, for contact-limited parasites like *Neoheligmonella*, and possibly *Syphacia*, could lead to increased per capita male parasitism.

Abiotic factors such as rainfall and soil moisture might also alter transmission rates by increasing parasite survival. Regardless of host density, increased vegetation in the absence of megafauna might create soil conditions that increase parasite egg and larval survival (Young et al. 2013). Larval stages of *Neoheligmonella* are particularly sensitive to desiccation (Durette-Desset and Cassone 1987), potentially contributing to the strong interactions between rainfall and exclosure treatment for this parasite. Although *Neoheligmonella* burdens were consistently elevated in exclosures, the greatest differences in parasite load occurred in the driest months when infected *S. mearnsi* from these exclosure plots had burdens six times greater than those in control plots. The impact of treatment and host density was less pronounced with increased rainfall, suggesting that higher larval parasite survival in wet periods may increase the pool of infective stages, and may shift the system from being contact-limited to establishment-limited. Precipitation in this region is irregular, and while the 6-month study period captured a range of rainfall that included the wettest part of the year, our sampling did not overlap with the prolonged dry period when high larval parasite mortality might generate even greater differences between exclosure and control plots. The warmer temperatures and less predictable rainfall expected under future climate scenarios for this region (Anyah and Qiu 2012) would likely further reduce egg and larval parasite survival, and could lead to even more pronounced effects of large mammal loss as infectious stages become increasingly limited.

Although moisture might directly influence parasite survival, rainfall effects could also reflect unmeasured fluctuations in host density or immune function driven by increased food during wet seasons (Keesing and Young 2014). We did not measure rodent density during this study, but it is well established that rainfall increases *S. mearnsi* recruitment in all plot types (Keesing 1998*a*, Keesing and Young 2014). Seasonal increases in *S. mearnsi* density would further increase *Neoheligmonella* contact rates and increased nutrition in wetter periods might explain the negative correlation between rainfall and *Syphacia* intensity. If *Syphacia* eggs are rare, higher *S. mearnsi* density could lead to an encounter dilution effect where increasing host density reduces per capita risk by "diluting" contacts across a larger number of individuals (Mooring and Hart 1992). However, given evidence for increased *S. mearnsi* immune function during wetter months (Young et al. 2016), it seems more likely

that rainfall might indirectly reduce *Syphacia* intensity by improving host immune status. Further study on the complex interactions between precipitation, host immune function, and parasite survival might be particularly valuable given that East African rainfall is expected to become increasingly variable (Anyah and Qiu 2012).

The effect of large wildlife loss and increasing host density may be more complex and difficult to detect for parasites like *Syphacia*, where autoinfection is common (Anderson 2000). For *Syphacia*, autoinfection can occur when grooming rodents re-infect themselves with eggs from their own parasites, potentially both decoupling intensity from environmental transmission and increasing parasite aggregation. Autoinfection can lead to high intensity in some animals and likely contributed to the extremely high *Syphacia* intensity observed in a few surveyed *S. mearnsi*. Estimating the proportion of worms that arise from autoinfection vs. the environment is challenging; however, in theory, high autoinfection rates would reduce the influence of mouse density or infective stage survival on parasite intensity.

Together these findings highlight both the challenges and promise of developing a broadly applicable and predictive framework for how large mammal loss and rodent release are likely to affect parasite intensity, prevalence, and population size. Consistent with the expectations of density-dependent transmission, some parasites exhibited substantial increases in per capita infection and may have more than tripled in population size in the absence of large wildlife. This suggests that large wildlife loss that increases rodent density can substantially amplify both per capita risk and total parasite population when transmission is limited by contact rates. However, our results also suggest that caution should be taken in predicting the effects of large mammal loss and increased rodent density on per capita infection because, due to heterogeneities imposed by the host, parasite and abiotic environment, transmission is not homogenous across the host population or over time. Ultimately, a nuanced understanding of what regulates each parasite species is critical to predicting the magnitude of change for any given parasite.

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