
THE UNIVERSITY
*of* EDINBURGH

# The Impacts of Nutrition Quality on Host-Parasite Dynamics in Wild Wood Mice

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Submitted for the degree of Doctor of Philosophy

Institute of Evolutionary Biology

University of Edinburgh

2020

# Authorship Declaration

I declare that I am the sole author of this thesis. All writing and analyses within represent my own work, with input from my supervisors and co-authors.

Data for Chapter 2 is comprised of a field experiment inclusive of two replicates in 2015-16 and one laboratory experiment conducted in 2016. Field and laboratory data for the 2015 replicate was collected by Melanie Clerc and Paulina Pontifes. I carried out fieldwork and laboratory work for the 2016 replicate. Saudamini Venkatesan helped with ELISA assays for the 2016 replicate. I carried out all laboratory work for the laboratory experiment presented in this chapter, with sampling help from SV.

Data and samples for chapter 3 analysis is from 2015-2016 field experiments with credit as described above. DNA extraction and PCR for blood-borne parasites was carried out by myself and Madison MacLean as part of an honours project co-supervised by me. All statistical analyses were completed by me.

Chapter 4 is based primarily on data from a 2017 field experiment and laboratory. I conducted all fieldwork with help from SV. All laboratory and statistical analyses were completed by me, save for some faecal egg counts from this experiment which Jess Hall and SV assisted with.

Chapter 5 is based on data from field experiments designed by Amy Pedersen and Andy Fenton. Susan Withenshaw, Godefroy Devevey, and many others were involved in fieldwork which contributed to data represented in this chapter. Data on mast events presented in this chapter was provided by the Woodland Trust.

Chapters 2, 3, and 5 are in late-stage preparation for submission, and Chapter 4 represents an in-progress manuscript. Co-authors for Chapter 2 are MC, PP, SV, SAB, and ABP. Co-authors for Chapter 3 are SAB and ABP. Co-authors for Chapter 4 are SV, SAB, and ABP. Co-authors for Chapter 5 are Greg Albery, SV, AF, and ABP. Co-authors provided input into writing and analyses. I use ‘we’ throughout my data chapters because they were written as papers.

Many volunteers and students played a role in field data collection and sample processing for fieldwork represented in this thesis.

# Abstract

Factors from the environmental, host, and parasite community levels can all determine helminth burden in natural populations. In particular, the nutritional resources available to the host have long been associated with helminths; a large body of work in the laboratory has shown that both macro-and micro-nutrients play an important role in host response to infection. However, the relationship among nutrition, immunity, and helminth infection can depend upon several factors in the wild including season, host condition, and co-infecting parasites. Co-infection is the norm in natural populations, and the many parasites present may each have unique and contradictory relationships with nutrition quality. Recent increase in anthropogenic influences to the food available to wild animals –either accidental through urban waste or intentional through supplemental feeders—has therefore generated a crucial need for understanding the short- and long-term effects of changes to nutrition quality on disease outcome in natural host-parasite systems. To date, however, experimental, empirical data is still lacking in these areas particularly in regards to naturally co-infected populations.

This thesis comprises a combination of statistical analysis and experimental work in the field and laboratory in a wood mouse (*A. sylvaticus*) system. I carried out diet supplementation manipulations for one laboratory and two field experiments designed to investigate how experimental perturbation to host environment in the context of resource availability influence the dynamics of both a highly prevalent nematode, *Heligmosomoides polygyrus,* and co-infecting parasites within the system. Making use of historical wood mouse trapping data, I further designed statistical approaches to determine how much the natural variation in environmental context affects host-parasite relationships

Using experimental diet supplementation in both a wild and a captive population of *A.sylvaticus*, I found that supplemented nutrition quality increased both natural resistance to *H. polygyrus* and the efficacy of anthelminthic treatment via increased host condition and both general and *H. polygyrus*-specific immune investment. These

results have important consequences for the control of disease and transmission of helminth infections in natural populations.

I screened wood mouse populations in the wild following diet supplementation for an additional >10 parasite species including several other gastrointestinal helminths, gastrointestinal protozoans, ectoparasites, and blood-borne protozoans, bacteria, and viruses. I show that although supplemented nutrition decreased infection with helminths and ectoparasites via increased investment in immunity and condition, it unexpectedly increased infection risk and burden of some blood-borne and intestinal microparasites. This gives important insight into how nutrition may shape parasite communities and host fitness in wild populations where co-infection is the norm.

I carried out a long-term field experiment with ongoing nutrition supplementation to investigate the effects of nutrition supplementation for host infection, reproduction, and survival over multiple seasons. I found that beyond short-term effects on parasite infection dynamics, supplemented nutrition drastically alters population dynamics for wood mouse populations, and the effects of nutrition on immunity within the population were both season- and cohort- dependent.

Finally, through statistical analysis of six years of trapping data across multiple sites and seasons, I first show that there were significant drivers of helminth infection intensity at both the environment and host level. However, by accounting for spatiotemporal variation, I show further that these drivers varied significantly in magnitude and direction according to environmental context (i.e. across-years), and that sampling regime is key for the estimation of biological variation in *H. polygyrus* dynamics in a natural population.

These results represent important experimental and statistical insights into the role of resource availability and environmental context for host-parasite dynamics in the wild. I discuss these findings and their implications for the study of nutrition quality and infection dynamics in disease ecology. I also present several avenues of ongoing and future work to complement insights provided by these experiments

# Lay Summary

Parasites are present in all populations of humans, animals, and wildlife. Most individuals in natural populations will therefore experience parasite infection at some point in their life. The quality of food that an individual consumes plays a large role in how well they will respond to parasite infection. For human populations where malnutrition is common, or wildlife populations where times are hard and resources are scarce, this can have serious impacts on risk of infection and individual health. This has been well studied in the laboratory, but is very hard to study in the wild, where animals face a number of environmental stressors, a number of parasites, and food availability varies over time and space. However, understanding these relationships can give important insights into the use of nutrition as a means to combat disease in humans or manage wildlife populations.

In this thesis, I experimentally tested the relationship between nutrition quality and infection with a well-known gastrointestinal parasitic worm in wild wood mice (*A. sylvaticus*). By adding high-quality supplemental food to natural populations of wood mice in the wild and alongside a deworming drug, I showed that nutritional supplemental can dramatically reduce infection and increase the effectiveness of a common drug. By replicating this in a laboratory setting I was able to show that these benefits are due to increased host condition and immunity. I next took advantage of this mouse population which is typically infected with multiple parasites to ask: is extra nutrition always good for the host during infection? By monitoring multiple parasites at the same time, I showed that surprisingly some parasite infections became worse after in populations after food was added, and that often this was due to relationships between the parasites themselves. These results suggest that nutrition is a viable option for infection control for parasitic worms, but that the complexity of the wild, where many parasites co-exist, may alter expected outcomes of food quality.

I next tested how variation in time and space influences the role of food quality for wild animals. I found that additional food in the wild increases condition, immunity, and reproduction most strikingly in the summer when wood mice are breeding

frequently, but that in years of plentiful natural food, effects on parasitic worms were non-existent. Finally, I used data from many years of wood mice trapping carried out by my research group to ask how predictable patterns in this gastrointestinal parasite are in wild populations and find that there is a tremendous amount of variation over time and space in which groups of individuals and what season are likely to have the most severe infections. These results explore how dynamic these relationships are and give important context to studying the role of nutrition quality in the wild.

Overall, my thesis represents a collection of statistical and experimental work in the field and laboratory which advances current understanding of how the potential benefits of improved nutrition quality are influenced by environmental and host diversity in the wild.

# Acknowledgements

I have worked with an incredible group of people during my PhD. First and foremost, I am thankful for the patience, enthusiasm, and sincere support of my mentors. I am particularly grateful to my supervisor Amy Pedersen. Since day one, she has encouraged me to pursue my own interests within our study system, celebrated my successes, and commiserated with the difficult stretches of a PhD with me. She has treated me as both a mentee and colleague, and supported not just my thesis but broader development as a scientist and for this I'm very appreciative. Our broader collaborative group has also been invaluable throughout my PhD. Thanks to Simon Babayan for thoughtful guidance on my writing and calm energy during my the most chaotic days of my first field season and to Andy Fenton for his good humour and insights into my analyses. It has been a pleasure to work with the entire EGLIDE group during my PhD and I look forward to working with them in the future.

I am very grateful to everyone in the Pedersen group and beyond who helped with the various demands of running field and laboratory experiments. Many thanks to Alex Morris, and all of my incredible field assistants and students who spent many hours in the lab and field with me in good spirits. Thanks to Melanie Clerc for wisdom imparted during my first experiments and to Jess Hall for being unfailingly organised and helpful this year. In particular, I would like to thank Saudamini Venkatesan. Thank you for reminding me often of the joy of scientific curiosity, for drives to the field that were a bright spot during a tough season, and geeking out about stats or experiments with me regularly. Thanks as well to all members of the Animal Staff for their help and genuine care for our adorable colony mice.

It really takes a community for a successful PhD, and I lucked out with mine. Thank you to Greg Albery, as brilliant a friend as he is a scientist, for years of friendship, flatemateship, and always having time for my drafts and code bugs. Thanks to Eevi Savola for her indispensable companionship and for being there for many smiles and tears since our very first reality show binge. To my OG hedgehog Mary who always seems to be on my wavelength and my fellow hedgies Megan, and Verity, thank you for the constant wit and for being absolutely dynamite women in science peers. To Alicia who is always a Skype away, to Lewis for the laughs and commiseration in our final weeks, and to Jess and Kat for always having time for a glass of wine or a sympathetic office chat. I have learned a lot about friendship during my PhD because of the calibre of those I'm proud to call my friends.

A big thank you to my rowing families near and far. Rowing continues to remind me of the value of investment in the sum over the parts and the joy and balance of working toward non-academic goals, but it would be nothing without the people it has introduced me to. Thanks to my Capital forever teammates who welcomed me on thesis breaks, to the B'wings group for Dog House catch-ups and the platform for support, and my SABC group who have been supportive of my write-up months during our countless hours of training together. I have never met a rower who doesn't face a challenge head-on, and I have been inspired by all of you these last four years.

Thank you to Gretchen for always reminding me of the bigger picture and believing in me more than I did at times. Finally, I will be forever grateful to my parents Howard and Barbara Sweeny. I learned everything about hard work through their tireless examples. They have never wanted anything less than the best for me, and they have supported me chasing my dreams wherever they have taken me.

Thank you all from the bottom of my heart. It has been such a fulfilling four years.

# Contents

# Chapter 1 General Introduction

## 1.1 Overview: An experimental approach to disease ecology

Parasites, defined broadly here to include both macroparasites (i.e. helminths, ectoparasites, etc.) and microparasites or pathogens (i.e. viruses, bacteria, protozoans, etc.), are ubiquitous in humans, livestock, and wildlife. Over the past few decades both ecologists and evolutionary biologists have begun to use apply tools to better understand the role that infectious diseases, caused by parasites, can play in natural populations (May and Anderson, 1979; Anderson and May, 1985; Grenfell and Dobson, 1995). The resultant field of 'disease ecology' has advanced the study of infectious disease in many novel and important ways, for example by highlighting the impacts that even sublethal parasites can have on individual health and population dynamics and that these are not static relationships, and by conducting studies of naturally infected hosts in the wild to assess the abiotic and biotic factors which drive these processes.

For the last several decades, a large focus in this field has been the cost of immunity for wild animals and variation in the outcome of parasite infection due to limited resources to allocate among other processes, such as reproduction (Sheldon and Verhulst, 1996). Several hypotheses about possible trade-offs arising from these costly activities have been developed, such as individuals with access to higher quality resources should have reduced parasite infections, and/ or reproductively active individuals will suffer higher parasite burdens (number of parasites) due to a compromised immune response. However, in practice there is a high degree of variation found in these relationships and it is often difficult to determine specific

mechanisms governing the interactions between resources, immunity, and parasitism. These heterogeneities can arise from several factors, including a high degree of inter-individual variation, spatial and temporal variation, abiotic and biotic effects, and interactions between multiple parasite species. For example, temporal variation in both resource availability and energetic demands can interact with host factors such as sex or body condition influencing infection. Seasonal or other cyclical patterns in environmental conditions and host behaviour may also have important consequences for host-parasite relationships, such as changing the transmission potential of the parasites or the susceptibility of the host (Nelson and Demas, 1996; Altizer *et al.,* 2006), however, many studies are unable to account for this variation over time, due to sampling or monetary constraints.

A further layer of complexity for disease ecology studies that aim to understand the causes and consequences of parasitism is simultaneous infection with multiple parasites. Co-infection is the norm in wild populations, humans and animals alike, and the resulting interactions among parasites that can be analogous to what happens between free living species within an ecosystem. These within-host parasite interactions can alter the outcome of each parasite species infection and transmission and impact host fitness (Cox, 2001; Pedersen and Fenton, 2007). Diverse parasite communities can therefore fundamentally change the dynamics of infectious diseases in a population, but often are ignored in many studies, which tend to focus on a single parasite of interest. Experiments using anti-parasite treatments as a way to reduce or eliminate one parasite taxa or group, have shown significant effects on non-target parasite species and host condition (Pedersen and Greives, 2008; Knowles

*et al.*, 2013; Pedersen and Antonovics, 2013; Pedersen and Fenton, 2015; Budischak *et al.*, 2018). These perturbations to the within-host parasite community have provided important advances for understanding both the costs of parasitism and the dynamic relationships that can occur within the parasite community due to either direct or indirect interactions mediated by a shared immune response or host resources (Pedersen and Fenton 2007, Graham 2008). However, effectiveness of anti-parasite treatments can vary in magnitude over time (Hudson *et al.*, 1992), and the outcome of within-host parasite interactions may be dependent on environmentally variable factors such as the host's resource availability (Budischak *et al.*, 2015b).

This thesis aims to address the intersection of how environmental variation can impact host-parasite interactions using a combination of field experiments, laboratory experiments, long-term observational studies, and statistical analysis. Using experimental resource supplementation and anti-parasite drug treatments in a natural host-helminth worm system (*Apodemus sylvaticus* & *Heligmosomoides polygyrus*) in both the wild and laboratory, I specifically test the short-term effects of resource availability for host condition, immunity, and resistance to *H. polygyrus*. I next explored the consequences of resource supplementation and anti-parasite treatment for the broader parasite community of wild *A. sylvaticus* to test for possible within-host interactions that could be mediated by resource availability. I further conducted an intensive, longitudinal (capture-mark-recapture) experiment where I manipulated both resource supplementation and the parasite community through drug treatment to investigate the longer terms impacts of increased availability of high-quality resources. Finally, I use a newly available six-year observational dataset

from a collection of *A. sylvaticus* populations to explore how natural spatiotemporal variation in seasonality, host characteristics, and the broader parasite community can drive *H. polygyrus* infection. In this introduction, I present background and knowledge on the environmental and individual factors governing helminth infections, with a specific focus on advances made by experimental work in wild rodents and the suitability of *H. polygyrus* and co-infecting parasite community ecology in *A. sylvaticus* as an ideal system in which to address the above aims.

## 1.2 Part 2. Gastrointestinal helminths

### *1.2.1 Ecology*

Helminthic worms, which include parasitic trematodes, cestodes and nematodes, are ubiquitous parasites and among the most common causes of chronic infections in wildlife mammal, domestic animal and human populations (Brooker, 2010). Among wildlife, helminths typically are highly prevalent, and play an important role in population dynamics by reducing survival and reproduction (Dobson et al., 1992; Dobson and Hudson, 1992). This has been demonstrated experimentally in both wild red grouse (Hudson et al., 1998) and deer mice (Pedersen and Greives, 2008), where helminth removal through anthelmintic drug treatment prevented population crashes.

Many of the most problematic species of helminths for domestic and wild animals are gastrointestinal (GI) nematodes. Much of the current knowledge regarding GI nematode lifecycles, infection, and host immune responses come from controlled rodent studies conducted in the laboratory. Wild small mammals also harbour a diverse array of GI nematode species, such as *Strongyloides* spp., *Nippstrongylus*

*brasiensis, Trichuris* spp., *Syphacia* spp., and *Heligmosomoides polygyrus* (Morand et al., 2007). *H. polygyrus* in particular, is a well-studied model for chronic human GI nematode infections such as *Ascaris spp.* and hookworms (Behnke *et al.*, 2009b). The lifecycle of these soil-transmitted GI nematodes, *H. polygyrus* included, is typically comprised of two phases—an adult stage of males and females whom reside, mate, and reproduces within the host and a free-living egg and four larval stages phase which occurs in the environment or in some cases an intermediate host (Morand et al., 2007):Figure 1.1)). Transmission of nematodes is driven by an infected host shedding eggs produced by adult female worms within the host into the environment via faeces followed by ingestion of infectious larval stages by susceptible hosts through environmental contact or via contaminated food or water (Brooker et al., 2006).

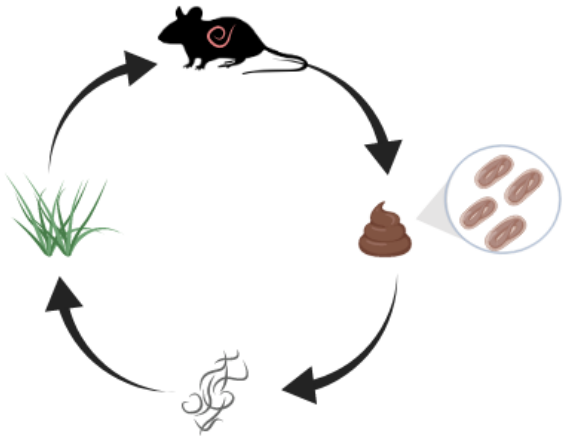


**Figure 1.1.The lifecycle of many soil-transmitted gastrointestinal nematodes, including *H. polygyrus*.**

Adult worms live in the host's small or large intestine (upper duodenum is the preferred site for *H. polygyrus*). Worms mate and shed eggs via the faeces, where they develop through several larval stages in the soil. Hosts encounter these infectious larval stages in the environment and infection occurs upon ingestion of larvae. Once in the host, 4$^{th}$ stage *H. polygyrus* larvae embed in the gut epithelium, mature into adult worms, and then emerge in the gut lumen to infect the small intestine. *H. polygyrus* goes from egg to egg (full lifecycle) within 9-12 days, although the specific site of infection and lifecycle duration with vary across different GI nematode species.

### *1.2.2 Human Helminths and Control Programmes*

Several of the most important human helminth infections are caused by soil-transmitted GI nematode species, such as *Ascaris lumbricoides, Trichuris trichiura,* and hookworm (Hotez et al., 2008). Although helminth infection often does not present overt clinical symptoms, (Hotez et al., 2008), infections can negatively impact the daily life of the approximately one third of humans harbouring one or more species (Hotez et al., 2008); specifically leading to diminished educational performance due to impaired memory and cognition, nutritional deficits, stunted growth, and impaired physical fitness in children, and pregnancy complications, nutritional deficits, and decreased worker productivity in adults (Nokes and Bundy, 1994; Hotez et al., 2008; Taylor-Robinson et al., 2012).

To reduce the burden (number of worms per individual) and morbidity (sublethal pathology) of helminth infections, the standard practice in humans and livestock is anthelmintic drug therapy, using one of several currently effective, low cost pharmaceuticals (Anderson and Medley, 1985; Bundy and de Silva, 1998; Jamison *et al.*, 2006; Keiser and Utzinger, 2008). However, despite the high availability and low cost of these drugs, morbidity from helminth infections remains high (Hotez et al., 2008) and effective control faces several hurdles (Brooker *et al.*, 2006). On specific concern is the increasing rise in anthelmintic drug resistance for both human and domestic populations where the drugs are commonly used (Geerts *et al.*, 1997). Development of resistance in livestock has motivated alternative approaches which take a more holistic approach to helminth control, including both measures to reduce primary and secondary (after the initial infection/exposure) helminth exposure

(Barger et al., 1994; Krecek and Waller, 2006). This is particularly important, as re-infection following anthelmintic treatment can occur very rapidly and is a widespread problem in endemic areas (Anderson and Medley, 1985). Within one year of treatment in endemic populations, *A. lumbricoides* prevalence can reach nearly 100% of pre-treatment levels, while *T. trichiura* and hookworm can reach 50% of pre-treatment prevalence (Anderson and Medley, 1985; Jia et al., 2012). Public health programmes have therefore aimed to integrate measures drug treatment with hygiene and sanitation improvement to reduce re-exposure rates (Strunz et al., 2014); but re-infection remains a significant problem. Altogether, challenges surrounding our current reliance of repeated use of anthelmintic drugs, and the fact that there are no currently effective helminth vaccines available, suggest that a more holistic view is needed for effective control. This requires understanding both the environmental conditions that contribute to high helminth prevalence and burdens and mechanistic details about host-helminth interactions, specifically those that drive successful infection and onward transmission. While most previous work has focused on laboratory models, I believe that a combined laboratory – wild host – helminth approach will provide the needed, tractable model to better understand the environmental and host drivers of helminth infection, immunity and transmission.

### *1.2.3 Anti-helminth immunity*

While many environmental factors and host behaviour and movement patterns may dictate exposure, once helminths are able to successful infected a host, these infections are typically chronic, possibly driven by the fact that helminths have evolved to evade host immune responses and persist within the host for long periods of time (Mulcahy *et al.*, 2004; Maizels *et al.*, 2004). These immunomodulatory

helminths can manipulate the induction of the T-helper cell 2 (Th2; anti-parasitic response) and T-helper cell regulatory (Treg; regulatory response) arm of the immune system (Maizels et al., 2004). These responses are suited to minimise damage to the host, rather than directly clearing parasites, and involve a suite of Th2-related cytokines, including the following interleukins (IL): IL-3, IL-4, IL-9, IL-13, and Treg cytokine IL-10 (Maizels & Yazdanbakhsh 2003). The latter two cytokines are particularly important mediators for controlling inflammatory responses of the T helper cell 1 (Th1; anti-pathogen response) arm of the immune system (Graham et al., 2005). As a result, many helminth infections are not associated with overt symptoms and present with little clinical disease (Mulcahy et al., 2004). However, when symptoms occur, they are often gastrointestinal in nature, including diarrhoea or impaired nutrient absorption related to protective immune responses which affect gut physiology to increase motility and facilitate worm expulsion (Mulcahy et al., 2004).

Clearing or killing adult worms living with the GI tract can be very damaging to the host leading to tissue damage, so many hosts have evolved immune responses to other stages of helminth development, such as impairing development from larval to adult stages or reducing fecundity of adult worms to limit onward transmission (Maizels *et al*., 2004). For example, activation of IL-5 can results in high numbers of eosinophils (eosinophilia) which target and kill larval stages of helminths such as *N. brasiliensis* and *Strongyloides stercoralis*, and immunoglobulin class switching to Immunoglobulin A (IgA) (Harrison *et al.*, 2008), IgE (Obata-Ninomiya *et al.*, 2013; Fitzsimmons *et al.*, 2014), or IgG (Hewitson et al., 2015). During secondary

challenge with *H. polygyrus* larvae, a combination of IgG1 antibodies and IL-4 dependent effector molecules are responsible for trapping larvae in tissues and blocking maturation to adult worms, which is a key resistance mechanism in this system (Hewitson et al., 2015).

While our knowledge of the anti-helminth immune response is mostly known form the laboratory mouse model where extensive immunological tools have been developed, it remains a pressing challenge for wild studies, where these tools are not currently available (Pedersen & Babayan 2011). One common approach in ecological studies of disease to assess immunity is to measure total and parasite-specific antibodies, but the use of such techniques for non-model systems and for individual in the wild still face many limitations (Gilbert et al., 2013). First, reagents for common serological assays such as enzyme-linked immunosorbent assay (ELISA) have been developed for model systems in the laboratory, and therefore adaptation to wild species is not always straightforward (Bradley and Jackson, 2008; Pedersen and Babayan, 2011). Data from these assays which have been validated for use on non-model species, still raises practical or interpretation issues from wild populations, as serological assays rely on blood sampling, which can be impractical for many systems, particularly large mammals (Gilbert et al., 2013). Serological methods for measuring the most common mucosal antibody (IgA) –which is found in epithelial surfaces—using non-invasive faecal samples have been developed for investigation of anti-helminth immunity in Soay sheep (Watt et al., 2016) and adapted to other species (Clerc et al., 2018). However, typically the range of immune molecules quantifiable for a wild species is limited; although there have been significant recent advances in characterization of the immune responses in wild

rodents, using both standard immunological tools and genomic techniques (Abolins et al., 2011; Babayan et al., 2018; Wanelik et al., 2019). The resolution of the sampling regime also presents common challenges, where longitudinal sampling with repeated captures per individual is logistically difficult for many systems, but can be key for interpretation in the relationship of variation of immunity across and within individuals with infection burden (Pedersen and Babayan, 2011). Importantly this type of data even when accurately measured can be difficult to interpret, as a high antibody measure may represent either high exposure to a parasitic antigen or a strong, effective immune response, but these are difficult processes to separate in the wild, especially without repeated sampling and/or experimental perturbations (Gilbert et al., 2013).

A myriad of factors can influence both exposure and susceptibility in the wild (Lazzaro and Little, 2009; Pedersen and Babayan, 2011). This is particularly true for helminths as transmission depends both on environmental exposure and within-host success. As a result, helminth infection patterns within individual in natural populations is typically characterised by a high degree of variation in burdens, where infections are often highly aggregated, with 20% of individuals responsible for 80% of disease transmission (Woolhouse *et al.*, 1997). More specifically, exposure to nematodes is often determined by host behaviour which can influence contact rates with contaminated material in the environment and extrinsic factors (e.g. geographical location, climate, season) which can affect the success of environmental stages of the parasites (Keymer and Dobson, 1987; Appleton and Gouws, 1996; Brooker et al., 2003). Host susceptibility, meanwhile, is influenced

primarily by intrinsic host variables (e.g. sex, age, nutrition status, host microbiota, genetics) (Gregory et al., 1990; Woolhouse et al., 1991; Chan et al., 1996; Curtale et al., 1999; Stephenson et al., 2000; Bethony et al., 2002; Quinnell, 2003; Quihui-Cota et al., 2004).

### *1.2.4 Extrinsic variables*

The complex, soil-transmitted life cycle of many GI helminths includes free-living stages which persist in the environment for long periods of time. Microhabitat characteristics, such as climate (i.e. temperature and rainfall), topography (altitude and landscape and soil-type) are therefore key determinants of free-living helminth larval survival and subsequent transmission (Hotez et al., 2008). These environmental characteristics explain, in part, the consistent high prevalence of soil-transmitted helminths in the tropics and sub-tropics where temperatures are higher and soil is moist (de Silva et al., 2003) and higher prevalence in warmer months, as these conditions are optimal for survival of many helminth species (Stromberg, 1997).

Environmental factors can also influence parasites indirectly via changes within individual hosts and/or the host population, including both spatial (the broad geographical area) and temporal (seasonal changes, especially in species with large changes in population size) factors. For example, many helminths that infect small mammal populations tend to exhibit seasonality, whereby infection is highest over winter when the population age structure is at its oldest (Keymer and Dobson, 1987).

### *1.2.5 Intrinsic host characteristics*

#### Host demographic traits

Host traits, such as age, sex and condition, can influence on helminth infection success and burdens through both behavioural or physiological mechanisms. The age of an animal is an important determinant of helminth burden in humans (Bundy, 1988; Maizels *et al.*, 1993), large (Gulland and Fox, 1992) and small mammals (Behnke *et al.*, 1999). This is often hypothesised to be driven by variation in susceptibility due to the delayed development and maturity of the immune system in young animals and/or immunosenesence (a reduction in immune response efficacy with ageing), which predisposes the youngest and oldest individuals in a population to the highest burdens of infection (Grenfell et al., 1995). However, for hosts which remain susceptible to reinfection as is the case for many small mammals, age effects are often manifested as an increase in burden over time due to an increase in exposure as an individual ages (Keymer and Dobson, 1987; Gregory, 1992).

Sex bias in parasitic infection is common (Moore and Wilson, 2002) and can stem from both ecological factors (i.e. behaviour) and/or physiological ones (i.e. hormones) (Zuk and McKean, 1996). Typically, males are generally expected to have higher levels of parasitism due to stress from engaging in more competitive and aggressive behaviours, because androgens, such as testosterone, can act as a depressor of the immune system, and because secondary sexual traits, such as deer antlers, can be testosterone dependent (Poulin, 1996; Zuk and McKean, 1996; Schalk and Forbes, 1997). In free-living gazelles, negative associations were found between testosterone and adaptive immunity and positive relationships between territoriality

in males and nematode burden (Ezenwa, 2004; Ezenwa et al., 2012; Ezenwa and Snider, 2016).

For both sexes, the costs of reproduction can have costs for immunity, with a general expectation of a corresponding reduction in immunity and/or increase in infection burden during periods of reproduction (Sheldon and Verhulst, 1996); however there are many exceptions (Schwanz, 2008). For example, lactation was associated with increased lungworm larvae counts in in bighorn ewes (Festa-Bianchet, 1989), and lactation has been associated with costs for both anti-strongyle immunity and strongyle count in red deer (Albery *et al.*, 2018b). Similar trade-offs between macroparasites and female reproductive effort have been shown in African ground squirrels (Hillegass et al., 2010), red squirrels (Gooderham and Schulte-Hostedde, 2011), flycatchers (Nordling et al., 1998), and humans (Blackwell et al., 2015). Importantly, many of the observed effects of reproductive effort on parasitism (or vice versa) in the wild are sex-dependent, either occurring only in one sex (Norris *et al.*, 1994; Ezenwa, 2004; Gooderham and Schulte-Hostedde, 2011; Ezenwa *et al.*, 2012; Ezenwa and Snider, 2016) or more strongly in males than females (Hayward *et al.*, 2014a).These sex-biased differences suggest that host factors do not operate independently in driving variation in parasitism, and that relative costs of reproduction between sexes should be considered within each system.

### Host condition & nutrition

Proper high quality whole-diet nutrition is crucial for an effective immune system. However, the relationship between nutrition, immunity, and infection is complex, dynamic, and can operate through effects on multiple components of the immune

response (Chandra, 1997). Inadequate nutrition/ malnutrition (defined by insufficient macro-and micro-nutrients) can impair effective and protective immune responses and increase infection risk. The most common form of malnutrition considered in relation to immunity is due to inadequate protein-energy, which can significantly impair the majority of host defence mechanisms, including cellular and humoral immunity (Chandra, 1997). Specific micronutrients (i.e. vitamin A, Zinc, Iron) also play an important role in immune function, and deficiencies in these nutrients can therefore increase severity of infection (Calder and Jackson, 2000).

Impaired immunity due to malnutrition is particularly important for helminth infections, and there is a high degree of geographical overlap in human populations of malnutrition and high helminth prevalence (Strunz *et al.*, 2016). This may be driven by the nutritional symptoms associated with helminth infection, creating a positive feedback loops in these areas, where malnutrition predisposes individuals to high burdens of infection, and high burdens of infection exacerbate malnutrition, increasing susceptibility to reinfection later (Koski and Scott, 2001). Likewise, wildlife typically face limited and variable resources and stressful environmental conditions, but are concurrently challenged with many parasites including helminths (Cox, 2001). Though nutrition plays a key role for helminth dynamics in these natural populations, noise from multiple environmental and host characteristics often confound interpretation of nutrition-infection relationships in nature (but see (Pedersen and Greives, 2008; Forbes *et al.*, 2014)).

Much of our knowledge on the mechanisms of nutritional impacts on infection is from controlled laboratory studies, particularly in birds and rodents (Lochmiller et al., 1993; Lochmiller and Deerenberg, 2000). Protein deficiencies were found to significantly impair development of the spleen and lymphocyte yield in Northern Bobwhite (*Colinus virginianus*) chicks (Lochmiller et al., 1993), and deficiencies of both protein (Slater and Keymer, 1986a; Ing *et al.*, 2000) and micronutrients such as Zinc (Shi et al., 1997; Boulay et al., 1998) impair host immune response and increase susceptibility to the helminth *H. polygyrus bakeri* in laboratory mice. Effects of nutritional supplements on the clinical symptoms of helminth infection have been explored in humans (Long et al., 2007; Nga et al., 2009; Rajagopal et al., 2014; Al-Mekhlafi et al., 2014), but results overall have been equivocal (Yap et al., 2014). In the wild, increasing attention is being paid to the influence of nutrition on infectious disease dynamics (Becker *et al.*, 2015); however, interpretation of these relationships are affected by factors from the environment and host, which are often not available in many studies.

## 1.3 Part 4. Complexities for studying parasitism in the wild: interactions across scales

### *1.3.1 Interactions between abiotic & biotic factors*

Host-parasite dynamics in the wild are highly context-dependent, as extrinsic and intrinsic factors often act simultaneously within a population. Laboratory studies provide controlled opportunities to study the effect intrinsic variables such as sex, nutrition, and age on helminth infection, but laboratory conditions do not represent ecologically realistic settings. Fluctuating resource availability provides a good example of how extrinsic and intrinsic variables can interact to affect host-parasite

relationships. A notable example of variable resource availability in the wild is mast events (synchronous crops of specific tree species providing very high levels of food) for small mammal populations (Ostfeld et al., 1996). For populations of deer mice in the US (*Peromyscus spp*.) and wood mice in the UK (*Apodemus sylvaticus*) the additional resources available to individuals from periodic beech and acorn masts increase host reproduction and abundance (Flowerdew, 1972; Montgomery *et al.*, 1991). However, while higher quantities and qualities of food may have benefits at the individual level, increased population density driven by more breeding may actually facilitate increased parasite exposure and transmission, leading to possible negative feedback loops (Pedersen & Grieves 2008).

The complex effects of resource availability on immunity and parasitism have become increasingly investigated in the last five years, in part to better understand and address the rising incidence of anthropogenic activities impacting wildlife food sources (Becker and Hall, 2014; Becker et al., 2015; Civitello et al., 2018; Strandin et al., 2018). Both theoretical and empirical work has highlighted both the potential positive and negative effects of resource provisioning on parasitism within wildlife populations as a result of indirect and direct effects on within- and between-host factors (Becker et al., 2015), suggesting laboratory studies or wild studies which account for only individual-level differences may miss important population-level effects and feedbacks. Seasonality of both parasitism and host processes (Altizer et al., 2006) and alteration of the host immunity as a result of exposure to co-circulating parasites (Cox, 2001) are examples of conditions which represent important deviations from laboratory populations, but which are common place in the wild.

### *1.3.2 Multiparasite communities*

For natural populations in which helminth infections are common, co-infection with other macro- and micro-parasite species is the norm (Graham, 2008a; Poulin, 2011; Ezenwa, 2016). Co-infection (defined here as concomitant infection of multiple parasite species) can change both the magnitude and type of immune response elicited either directly (*i.e.* occupying the same niche or using the same resources) or indirectly (via immune molecules) by interfering with the response the host would mount against the other, co-infecting pathogen, particularly in the case of helminth-microparasite infections (Graham, 2008b). Chronic activation of immunosuppressive Th2 responses by helminths can change the immune response to concomitant microparasite (positively) or additional macroparasites (negatively) infections (Lello *et al.*, 2004; Pedersen and Fenton, 2007; Graham, 2008b; Griffiths *et al.*, 2015). A community ecology perspective to frame the within-host parasite community as analogous to free-living community dynamics can provide import insight into understanding the variation observed in patterns of infection in naturally co-infected populations (Pedersen and Fenton, 2007). However, cross-sectional data (single samples per individual) has been found to fail to provide information on parasite interactions (Lello et al. 2010, Fenton et al. 2015), which poses a limitation for the study of such interactions in the wild. However, controlled co-infection experiments in the laboratory or studies in systems where longitudinal or time-series data is available can circumvent this problem. Importantly, drug treatment experiments, defined previously, which remove one parasite taxa and measure the impacts on other co-infecting parasite which are not targeted by the drug, can reveal potential negative or positive within-host parasite interactions (Pedersen & Fenton 2015). Host

–parasite laboratory models have shown that interactions between parasites can alter host susceptibility and infection length (Graham, 2008b), and time-series data from wild field voles (*Microtus agrestis*) showed that parasite interaction effects exerted even greater effects on infection risk in the population than did host susceptibility or exposure (Telfer et al., 2010). Notably, the environmental context, such as the resource availability or microclimate, can alter the outcome of parasite species interactions. In laboratory mice, experimental resource limitation altered showed that outcome of co-infection between helminths (*N. brasiliensis* or *H. p. bakeri*) and a microparasite (*M. bovis*) was dependent on protein levels, where limited protein increased fecundity of *N. brasiliensis* during co-infection (Budischak *et al.*, 2015b).

## 1.4 Experimental approaches in wild rodent populations

Wild rodent populations represent an ideal system for the investigation of the drivers of helminth infections in a natural population. Helminth infections are common among wild rodents (Keymer and Dobson, 1987; Behnke *et al.*, 2009a), populations are usually large which enables large sample sizes, recapture rates are high facilitating longitudinal studies, and they are tractable for field perturbations at both the individual and population level. However, much of the existing knowledge on immunity to helminths comes for laboratory mouse systems, which are typically associated within unlimited food, minimal genetic variation, and minimal environmental stress (Finkelman *et al.*, 1997; Pedersen and Babayan, 2011). Wild mouse systems can benefit from tools developed for laboratory mice (Abolins *et al.,* 2017; Pedersen & Babayan, 2001). but also represent ecologically realistic settings

and therefore may be an ideal system to carry out experimental work in investigate both cause and effect in natural host-helminth interactions.

### *1.4.1 Anti-parasite perturbations*

Assessing the cost of parasitism in a natural population is difficult by observation alone, as there may be many confounding factors (i.e. exposure, co-infection, host condition, etc.) which are either difficult to measure or control. Several landmark studies in disease ecology have used anthelminthic treatment to experimentally investigate the role of parasites in wild host population dynamics (Hudson et al., 1992; 1998; Vandegrift et al., 2008). In the first such study published, Hudson and colleagues removed/reduced the nematode *Trichostrongylus tenuis* in red grouse (*Lagopus lagopus scotius*) populations, which prevented dramatic periodic population crashes (Hudson et al., 1998) and improved reproductive output (Hudson et al., 1992). Many studies have subsequently followed this approach (see Pedersen & Fenton 2015), and have shown a myriad of responses, including an increase in the probability of reproduction in the year following removal of the nematode *Ostertagia grueneri* in reindeer (*Rangifer tarandus plathyrynchus*) (Albon et al. 2002).

Anthelmintic treatment experiments have been used frequently in small mammal population dues to high trapping success and ability to collect longitudinal data (Pedersen and Fenton, 2015). Sex-specific removal of *H. polygyrus* in yellow-necked mice in Europe (*Apodemus flavicolis*) showed that males contribute disproportionately more than females to transmission (Ferrari *et al.*, 2004). Anthelmintic treatment in an ecologically similar white-footed mice (*Peromyscus leucopus*) in the US eliminated the summer breeding hiatus typical of this species

(Vandegrift et al., 2008). In addition to identifying costs of parasitism, parasite removal experiments can provide insights into the relationships of helminths with other factors in the wild such as co-infecting parasites. Previous work by our research group in UK wood mice (*Apodemus sylvaticus)* has shown that removal of *H. polygyrus* increases intensity of infection by over fifteen-fold for *Eimeria hungaryensis*, a gastrointestinal protozoan which resides in the same portion of the gut as *H. polygyrus* and may share resources with the nematode (Knowles *et al.*, 2013; Rynkiewicz *et al.*, 2015).

### *1.4.2 Resource supplementation*

The role of fluctuating food sources in rodent populations has been reasonably well-studied, often showing that increased availability to resources is associated with increases in individual survival and reproduction (Wolff, 1996; Ostfeld and Keesing, 2000). The relationship between resources, parasites, and population dynamics has also been explored experimentally in wild mouse systems using experimental resource supplementation to mimic mast events either alone or in conjunction with anthelmintic treatment (Flowerdew, 1972; Diaz and Alonso, 2003; Pedersen and Greives, 2008; Shaner et al., 2018). These studies have found that increased food availability alone resulted in increased reproductive activity (Flowerdew, 1972) and reduction of some nematode species within the population (Diaz and Alonso, 2003). However, when combined synergistically with anthelmintic treatment, resulted in removal of annual population crashes, suggesting that an interaction between resource availability and helminth infection drove population dynamics (Pedersen and Greives, 2008).

Such interactions between resources and helminths are complex (Figure 1.2), and can be mediated by both host processes (i.e. demography, behaviour, condition, and immunity) which are subject to natural variation or influence from other parasites in the community and environmental factors. In this thesis I use experimental perturbations of both resource availability and helminth infection alongside longitudinal live-trapping in wild *Apodemus sylvaticus* (wood mice) populations commonly infected with *H. polygyrus* to experimentally investigate the how nutrition and helminth infections interact and the consequences of these interactions for both host and parasite fitness.

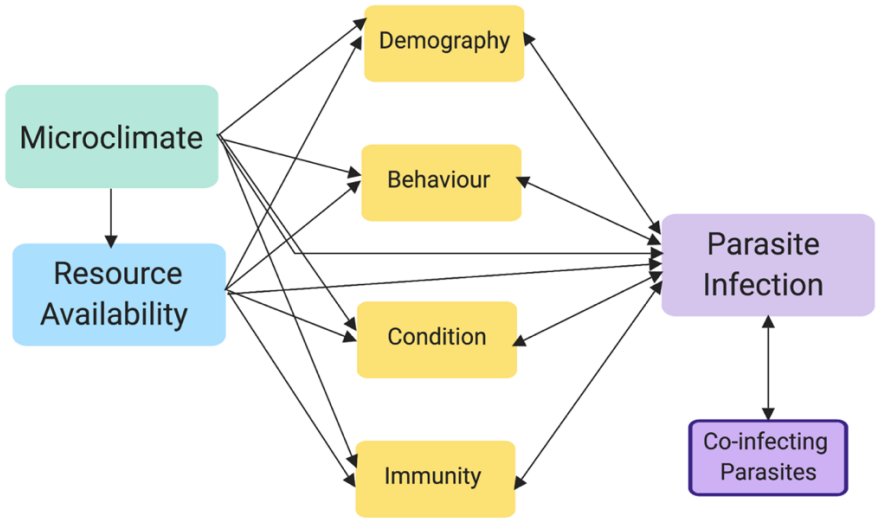



**Figure 1.2. A network of possible relationships between environment (green) and host (yellow) factors that could impact helminth parasite infection/co-infection (purple) and resource availability (blue) in a wild mammal population infected with helminths.**

## *1.5 Apodemus sylvaticus & Heligmosomoides polygyrus*

The wood mouse (*Apodemus sylvaticus*) is one of the most common species of small mammals in the United Kingdom. These granivorous, nocturnal rodents have distinct

seasonal population cycles, with the peak breeding season from June-September. Individuals born during this time will survive for approximately a year, overwintering and becoming the breeding adults in the following year (Flowerdew 1985). Among the diverse community of parasites identified in wild wood mice, the gastrointestinal nematode *H. polygyrus* is a very common and important species (Gregory et al. 1990; Knowles et al. 2013). *H. polygyrus* has a direct life cycle typical of GI nematodes, with eggs passed via host faeces into the environment, where infective larval stages develop and then are ingested by hosts (Figure 1.1). *Heligmosomoides bakeri* (a sister taxa to wild *Heligmosomoides polygyrus* of wood mice (Cable et al., 2006)) is one of the best laboratory models of human and domestic animal gastrointestinal nematodes, specifically for understanding immunosuppression during chronic infection and modification of host behaviour (Behnke 1987; Behnke et al. 2009).

The population biology and ecology of *H. polygyrus* in wild wood mice has been well-documented, and exhibits typical endemic helminth infection patterns (i.e. temporal persistence, high prevalence, uneven, aggregated distribution among hosts (Anderson & May 1982; Gregory et al. 1990). Extensive observational studies of *H. polygyrus* infection in wood mice across different geographic areas and time periods (Montgomery and Montgomery, 1988; 1989; Abu-Madi *et al.*, 2000; Eira *et al.*, 2006) support differential exposure (i.e. season- and site-specific effects) as a driver of variation in prevalence and burden. Previous observational work has also suggested that host characteristics such as sex and age (intrinsic factors) affect

susceptibility and infection risk (Keymer and Dobson, 1987; Gregory *et al.*, 1990; Behnke *et al.*, 1999).

Although much of this work has been observational, *A. sylvaticus* provides a valuable experimental study system because (1) helminths (and specifically *H. polygyrus*) are highly prevalent and found at high burdens (40- 90%; up to 100+ worms per mouse (Keymer and Dobson, 1987; Gregory *et al.*, 1990; 1992; Knowles *et al.*, 2013) (2) they have high repeatability of trapping thus individuals can be followed longitudinally, (3) anthelmintic treatment can be conducted effectively at a large-scale in wild populations (Clerc *et al.*, 2019a), (4) co-infection is common (~70% (Knowles et al. 2013)). Previous work from the Pedersen research group has established a long-running trapping systems of wood mice in England and Scotland inclusive of 10 years of trapping with extensive information about the environment, host demographic characteristics, >30 species of parasites and pathogen species, and individual effects of anthelmintic treatment (Knowles et al., 2012; 2013; Clerc et al., 2018; 2019a).

In addition to the suitability of wild wood mouse populations for field experiments, we also maintain the only current wild-derived colony of wood mice in the UK. Over the last 5 years, we have begun pairing wild experiments to correlates in the laboratory using the same host and parasites species as we study in the wild (Clerc *et al.*, 2019b). These types of direct lab to wild comparisons are extremely rare, but invaluable for overcoming some common limitations of wild populations such as heterogeneity in parasite exposure, immunity, and behaviour. Some exciting and complementary studies by Graham and colleagues have recently focused on taken

inbred laboratory mice and moving them to enclosures in the wild to determine the impact on infection and immunity (Budischak *et al.*, 2017; Leung *et al.*, 2018). Both of these approaches can provide exciting possibilities for disentangle these complex, but important host-parasite interactions. We currently maintain *H. polygyrus* and *Eimeria hungaryensis* (a common gastrointestinal coccidian protozoan) parasite isolates collected from our local Scottish field sites, via regular lifecycle experiments using colony mice, which enables experimental infection studies in controlled environments. For example, foundational work from our wood mouse colony (Clerc *et al.*, 2019b) has shown key advances to our understanding of the *H. polygyrus – E. hungaryensis* relationship previously observed in the wild.

## 1.6 Parasitology and nutrition manipulation

### *1.6.1 Wood mice parasites and within-host parasite interactions*

Parasites of wood mice in the UK have been previously shown to be a diverse group of taxa, including both micro- and macro-parasitic species (Knowles *et al.*, 2013). Although *H. polygyrus* is by far the most common parasite of wood mice (~50% prevalence; Knowles *et al.* 2013), additional GI helminths have also been identified, including the nematode *Capillaria murissylvatici,* pinworms (*Syphacia* spp.), and cestodes (i.e. *Hymenolepid* spp). Several species (up to 5) of the coccidian protozoan genus *Eimeria* also commonly inhabit the gut, including *E. hungariensis* and *E. apiodonis*. In addition, we commonly find mice infected with several ectoparasites including ticks (*Ixodes* spp.), mites, and fleas, as well as are several species of microparasites, including the blood-borne, flea transmitted bacteria *Bartonella* spp., infections of chronic Wood Mouse Herpes Virus (WMHV), the blood-borne, flea

transmitted protozoan *Trypanasoma* grosi, and Cowpox virus have all been identified previously through wood mouse trapping within our research group (Knowles *et al.*, 2012; 2013)

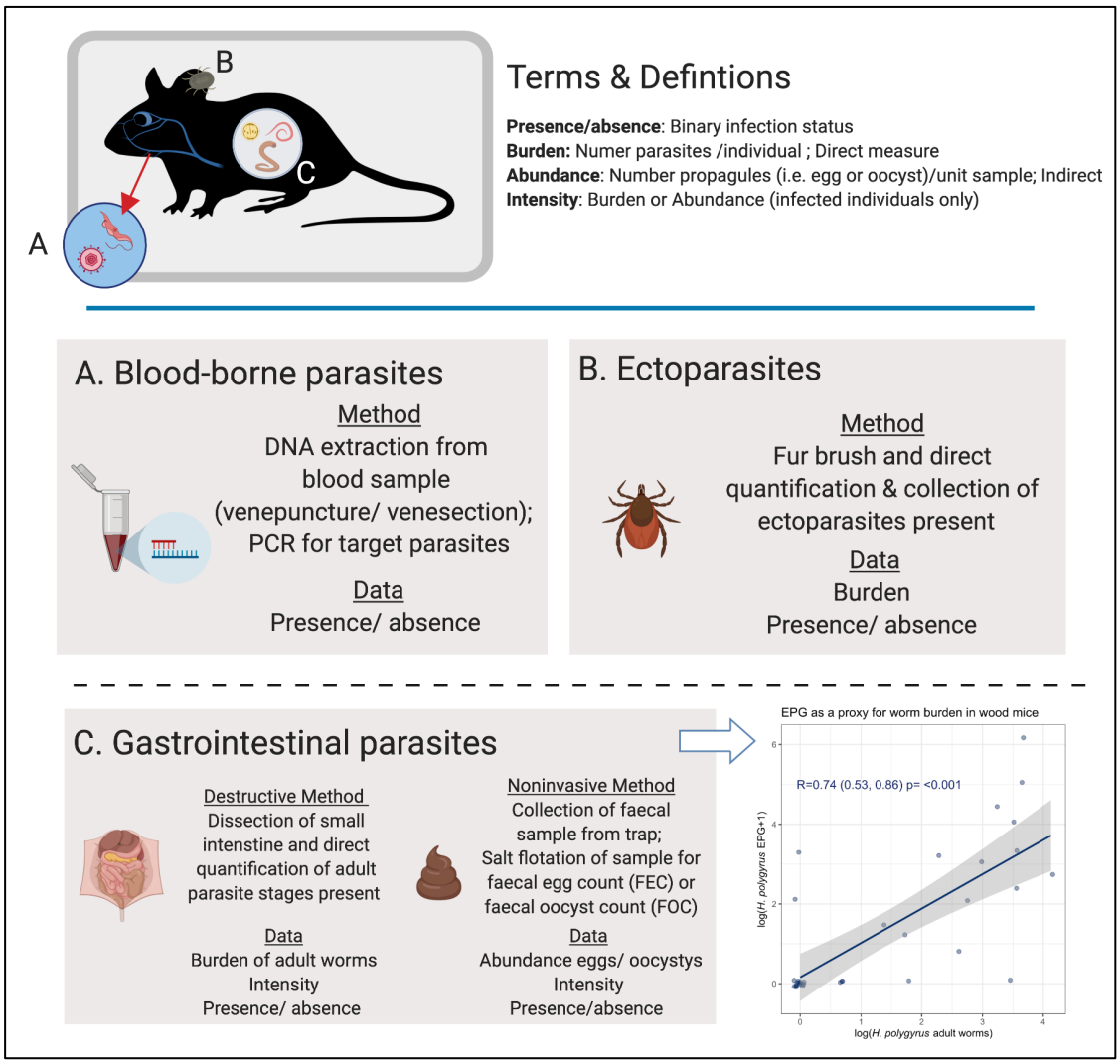
Accurately assessing parasite infection can be challenging in the wild due to limited sampling opportunities and methodological limitations. Within the wild wood mouse populations, I am able to longitudinally monitor this diverse community of blood-borne, ectoparasites, and gastrointestinal macro and micro-parasites (Box 1.1). Each mouse within our experimental framework is tagged with a microchip at first capture, which enables me to follow individuals longitudinally and sample for parasite infection at each capture. Across my field experiments, to assess blood-borne parasites, I collected blood samples with minimal stress from the animal from either venipuncture of the cheek or venesection of the tail, separated blood pellets from the sera, and used DNA extraction and parasite-specific PCR for presence/absence data. At each capture, I also surveyed individuals for ectoparasites by brushing the fur and counting individual ticks, fleas and mites. I quantified the gastrointestinal parasites species infections in two ways: (i) by lethal sampling at the end of experiments and counting the number of adult worms (worm burden) in the small intestine, or (2) by counting the number of helminth eggs in the faeces (abundance) of an animal; which can be measured throughout an individual's life through repeated sampling. Egg burdens, which are generally standardised to 1g of faeces (egg/gram- EPG), is often used as a proxy for number of adult worms in the gut. Though the correlation of EPG with adult worms in the host can vary (Budischak *et al.*, 2015a), within the populations that I studied in this thesis I find a highly significant correlation between egg abundance and worm burden (Pearson's

R= 0.74, p<0.001; Box 1.1), indicating that EPG is a suitable proxy for adult *H. polygyrus* burdens, as well as an indicator of egg shedding and onward transmission,

Throughout this thesis I use the following guidelines for parasitological terms. I describe an individual's infection status as presence/ absence data. Statistical models using binary infection data (infection:1/uninfected:0) as a response can be interpreted as giving insights into the factors that determine the probability of infection with a specific parasite taxa. I refer to indirect measures of parasites according to Bush *et al.*, 1997. Accordingly, I use 'abundance' (all individuals) or 'intensity' (infected individuals only) for EPG data (Box 1.1). To differentiate direct from indirect measures, I use 'burden' to refer to the number of parasites when directly measured (Box 1.1). Models using abundance, intensity, or burden of infection as a response can be interpreted as insights into the mean within the groups considered.

**Box 1.1 A description of parasite diagnostics and terms used to describe various infection with common parasite groups of mice.**

A. blood-borne B. ectoparasites C. gastrointestinal parasites. Top row illustrates locations of infections for groups depicted and a glossary of parasitological terms used throughout. Boxes A-C detail sample types, method of quantification associated, and the resultant data. Bottom right depicts the correlation between adult worm burden and EPG abundance in Scotland wood mouse populations (n=36), with Pearson's R and 95% credibility intervals.




## *1.6.2 Nutrition supplementation*

Dietary manipulation experiments are common in laboratory mouse systems (Calder and Jackson, 2000). Investigating the impacts of nutrition on infection or immunity typically involves extreme manipulations (either restriction or addition) or specific

nutrients such as protein, vitamins, or trace elements (Slater and Keymer, 1986a; Carman *et al.*, 1992; Shi *et al.*, 1997; Boulay *et al.*, 1998; Ing *et al.*, 2000) Diet supplementation experiments have likewise been conducted in wild mouse populations, but in contrast commonly mimic natural variation in wild resources by using supplements such as seeds (Diaz and Alonso, 2003; Pedersen and Greives, 2008; Shaner *et al.*, 2018). For all experimental nutrition supplementations that I employed in this thesis, I sought to enrich the diet of wood mice with a high-quality resource consisting of high levels of both macro- and micro-nutrients. This chow (diet) is high-nutrient, standard veterinary feed which is formulated for optimum breeding performance in laboratory mice (Table 1.1).

**Table 1.1 Nutrition content of the mouse chow used in both the field and laboratory experiments.**

Transbreed™ was used in the wild as the supplemental resources and as the high-quality resource, while Rat Mouse 1 (RM1™) was used at the standard diet . Both diets are made by Special Diet Services (SDS). Micronutrients included are selected elements and vitamins which have previously been implicated in host response to helminth infection, however full calculated analysis of RM1™ and Transbreed™ nutritional content can be found at http://www.sdsdiets.com/pdfs/RM1P-E-FG.pdf &http://www.sdsdiets.com/pdfs/TransBreed.pdf.

| Diet | Macronutrients (% by weight) | | | Micronutrients (quanity/ kg) | | | | |
|---|---|---|---|---|---|---|---|---|
| | Protein | Fat | Starch | Zn | Fe | Se | Vit A | Vit E |
| RM1 | 14.38 | 2.7 | 44.97 | 35.75 mg | 159.30 mg | 298.99 µg | 8554.27 iu | 84.10 iu |
| Transbreed | 20.07 | 10.05 | 38.48 | 80.42 mg | 216.0 mg | 429.81 µg | 18368.9 iu | 267.4 iu |

### *1.6.3 Field sites*

All experimental work in this thesis was carried out in 100ha broadleaf woodland in Falkirk, Scotland (Callendar Wood, 55.990470, -3.766636). This woodland has wood mouse (*A. sylvaticus*) populations that are naturally infected with *H. polygyrus* (Clerc *et al.* 2019a). Each year experimental grids as appropriate to experimental

design were set-up prior to trapping. This thesis contains experimental fieldwork carried out in Callendar Wood from three separate years. The study area was consistent year-to-year, but the grids were set each year dependent on area available and experimental design (Figure 1.2). Between years, grids that were designated as supplemented were not kept constant to avoid confounding of experimental results with grid effects. Within each trapping grid, trapping stations were set every 10m. In 2015, grids consisted of 3 plots of 8x8 trapping station. In 2016, this was increased to 4, 6x5 grids, and in 2017, 4 grids of 7x5 trapping stations. Each trapping station was set with 2 traps.


**Figure 1.2 Schematic of trapping grids in Callendar Park spanning three years.** Full study area is outlined in top panel, while approximate location of experimental grids set in each year are indicated in bottom panels. Grids designated as supplemented for each year are indicated in blue, while control grids are indicated in white.

## 1.7 Study aims

The primary research question of this thesis was: What are the impacts of nutrition quality on host-parasite dynamics in a wild rodent? My specific aims were:

1. To characterise the effect of an experimentally enriched diet on the infection dynamics of the gastrointestinal nematode *H. polygyrus* and the hosts response to treatment in wild wood mouse populations in a controlled laboratory setting (Chapter 2).
2. To examine the effects of increased resource availability on the entire parasite community of wild wood mice by extending the analysis of the field experiments presented in Chapter 2 to include addition parasite diagnostics of helminths, ectoparasites, and microparasites (protozoan & blood-borne) (Chapter 3).
3. To use both a long-term single year nutrition supplementation experiment and collation of data from multiple years of supplementation to investigate the long-term effects of resource supplementation and how these effects may be mediated by season, host age, birth cohort, and possible other environmental changes (Chapter 4).
4. To use a large-scale data set including multiple years and sites of observational trapping data for wild populations to investigate the natural drivers of *H. polygyrus* infection and how these drivers vary over space and time (Chapter 5).

In Chapter 6 (Discussion), I synthesise the results of my thesis in the context of current disease ecology research and policy implications beyond ecological insight.

Specifically, I discuss the results of Chapter 2 in relation to previous knowledge on the mechanisms underlying the relationship between nutrition and helminth infection and the use of nutrition supplements alongside anthelmintic treatment. I then link findings of Chapter 3 to a growing body of work exploring the effects of resource provision on wildlife infectious disease, and further discuss considerations of interpreting these studies using results from Chapter 4. I next discuss the implications of Chapter 5 for considerations for biological interpretation and practice design for studies of parasitism in the wild. Finally, I address limitations of this study and address areas of ongoing and future work to complement the results of this thesis and discuss how greater understanding of the processes underlying the relationship of nutrition to host-parasite dynamics can inform the design of treatment programmes in both wild and human populations.

# Chapter 2 Supplemented nutrition increases immunity and drug efficacy in a natural host-helminth system

## 2.1 Summary

Gastrointestinal helminths are among the most common parasites of humans, wildlife, and livestock. Despite the availability of effective anthelminthic drugs, high prevalence and chronic infections still persist in large parts of the world. Further, the overlapping incidence of malnutrition and helminth infections can predispose individuals to higher burdens of infection and reduced anthelmintic efficacy due to compromised immunity in malnourished individuals. While the relationship between nutrition and helminth immunity and infection has been well-studied in controlled laboratory models, the documented benefits of supplemented nutrition have typically focused on large-scale alterations of specific macro- or micro-nutrients – an approach which is unlikely to scale to heterogeneous populations. However, much less is known about the benefits of a whole-diet enrichment in natural host-helminth systems, and whether these effects increase protective immune responses and control infection across all individuals. Here, we experimentally supplemented the diet of wood mice (*Apodemus sylvaticus)* and measured anthelmintic treatment efficacy and resistance to infection with the gastrointestinal nematode *Heligmosomoides polygyrus* in both natural and captive populations. In both settings, wood mice given a supplemented diet, even for just a few weeks, were more resistant to *H. polygyrus* infection, cleared adult worms more efficiently after treatment, had better body condition and higher general and parasite-specific immune responses. In addition, supplemented nutrition in conjunction with anthelminthic treatment significantly reduced *H. polygyrus* egg shedding, nearly eliminating transmission into the environment. These effects were observed in all individuals and were strongest in the reproductive adults. These rapid, large-scale effects of supplemented nutrition across

all metrics measured in controlled and wild environments show the dramatic benefits of switching to an enriched diet. These findings have important implications for the feasibility of diet interventions to improve host condition, immunity and treatment efficacy.

## 2.2 Introduction

Gastrointestinal (GI) helminth parasite infections are ubiquitous and one of the most common causes of chronic disease in wildlife, livestock, and human populations (Bethony *et al.*, 2006). Globally, helminths infect one in three humans (Hotez *et al.*, 2008), often causing stunted growth and development, impaired physical fitness and cognition in children, pregnancy complications, and decreased productivity in livestock (Nokes and Bundy, 1994; Bethony *et al.*, 2006; Hotez *et al.*, 2008; Charlier *et al.*, 2009; Taylor-Robinson *et al.*, 2012). Among wildlife, helminth infections are very common and can significantly impact host survival and reproduction, while also playing a key role in regulating population cycles and dynamics (Grenfell *et al.*, 1995; Hudson *et al.*, 1998; Pedersen and Greives, 2008). To reduce the burden (number of worms per individual) and morbidity of helminth infections, standard practice in humans and livestock is anthelmintic drug therapy, using one of several currently effective pharmaceuticals (Bundy and de Silva, 1998; Jamison *et al.*, 2006). However, despite the high availability and low cost of these drugs, morbidity from helminth infections remains high (Hotez *et al.*, 2008).

Further, reinfection post treatment is rapid (Speich *et al.*, 2016). Several of the most common human helminth infections are caused by gastrointestinal nematodes (Hotez *et al.*, 2008), whose transmission cycles includes infective larvae shed via faeces,

after which they can persist for long periods of time, especially in areas with poor sanitation (de Silva *et al.*, 2003). For example, within one year of treatment in populations where worms are endemic, *A. lumbricoides* prevalence can reach nearly 100% of pre-treatment levels, while *T. trichiura* and hookworm can reach 50% of pre-treatment prevalence (Anderson and Medley, 1985; Jia *et al.*, 2012). Effective helminth control therefore depends not only on reducing burdens within all individuals, but also reducing exposure and susceptibility to reinfection. To achieve this, a better understanding of both the environmental and host factors that drive reinfection is needed (Brooker *et al.*, 2006).

Limiting environmental exposure by integration of such measures as sanitation and hygiene improvement (Strunz *et al.*, 2014) or grazing management in livestock (Barger *et al.*, 1994; Krecek and Waller, 2006) can be an effective strategy for nematode control, but these methods do not address underlying susceptibility to infection or treatment efficacy. Individual differences in response to infection and treatment are largely mediated by immune and nutritional status (Bundy and Golden, 1987; Koski and Scott, 2001). It is well-established that micronutrient, macronutrient, and overall energy deficiencies impair the immune system (Calder and Jackson, 2000) and given that mounting an immune response is costly, insufficient resources can worsen the impact of nematode infection (Sheldon and Verhulst, 1996). Additionally, pre-existing malnutrition can also increase susceptibility to infection and/or compromise immune responses (Koski and Scott, 2001). These relationships have largely been studied in humans in areas with endemic nematode infections and malnutrition (Bundy and Golden, 1987; Strunz *et*

*al.*, 2016), but also exist in other organisms. In livestock, the increased nutrition demand of late pregnancy and lactation is associated with a substantial increase in GI nematode burdens (Houdijk, 2008) and  in wild animal where resource availability is typically limited, trade-offs between the immune response and other energetically costly processes mean that individuals must divert resources between different physiological demands (Sheldon and Verhulst, 1996).

Integration of nutritional supplementation with standard drug therapies has been explored to address the problem of reinfection after treatment (van Houtert and Sykes, 1996; Houdijk *et al.*, 2012), and clinical trials in humans have measured the impact of micronutrient supplements (Vitamin A (Donnen *et al.*, 1998; Al-Mekhlafi *et al.*, 2014) Zinc (Friis *et al.*, 1997), Iron (Casey *et al.*, 2017), or multiple micronutrients (Nga *et al.*, 2009)) on the reduction of *Ascaris, Trichuris,* and hookworm burdens. However, a recent meta-analysis found mixed results, including even negative impacts of supplemented nutrition on nematode infection (Yap *et al.*, 2014). Laboratory mouse models have shown that both macro- and micro-nutrients play a key role in host immunity to nematodes and susceptibility to infection (Slater and Keymer, 1986a; Slater, 1988; Shi *et al.*, 1997; Boulay *et al.*, 1998; Ing *et al.*, 2000). For example, deficiencies in protein (Ing *et al.*, 2000) and zinc (Shi *et al.*, 1997; Boulay *et al.*, 1998) have been shown to increase worm burdens and reduce eosinophilia and parasite-specific IgG1 response (Boulay *et al.*, 1998) to *Heligmosomoides polygyrus bakeri,* a well-studied model nematode. Laboratory conditions, however, are highly controlled and unlikely to mimic life in the wild, and most laboratory house mice (*Mus musculus domesticus*) models are not natural hosts to the helminths, as in the case of *H. polygyrus bakeri* (Behnke *et al.*, 2009b).

Although effects of experimental supplementation have been investigated in wild mouse models (Diaz and Alonso, 2003; Pedersen and Greives, 2008; Shaner *et al.*, 2018), these studies have typically used supplements to mimic natural variation in food supply.

Here we experimentally enriched nutrition, by supplementing mice with a well-balanced diet, to test the impacts on immunity to *H. polygyrus* and anthelmintic treatment efficacy in wood mice (*Apodemus sylvaticus*). Wood mice live in woodlands across much of Europe and are commonly, chronically infected with *H. polygyrus* (prevalence 20-100%) (Keymer and Dobson, 1987; Gregory *et al.*, 1990), a sister taxa to *H. polygyrus bakerii* (Cable *et al.*, 2006). Importantly, while anthelmintics are effective for wood mice, reinfection rapidly occurs, with mice returning to pre-treatment burdens within 2-3 weeks of treatment (Knowles *et al.*, 2013; Clerc *et al.*, 2019a). Further, wild wood mice have significant energetic demands for reproduction, foraging, and survival, and are exposed to marked seasonal changes (Pedersen and Babayan, 2011; Maurice *et al.*, 2015); conditions which laboratory settings cannot replicate, but which are likely very important drivers of infection, immunity and nutritional status. Crucially, here we have the unique ability to test same host-helminth system in both the wild and controlled laboratory conditions in order to control infection/reinfection, exposure, coinfection and other important factors, as we have a wild-derived, but now laboratory reared, colony of wood mice and a wild-collected *H. polygyrus* isolate (Clerc *et al.*, 2019b). We experimentally tested the effects of supplemented nutrition and anthelmintic treatment on (i) *H. polygyrus* burden and egg shedding in the wild (ii) egg shedding,

susceptibility to infection and reinfection in the laboratory and (iii) body condition and immune responses in both settings. We find strong evidence of rapid and broad impacts of supplemented nutrition for host condition, helminth resistance and treatment efficacy; suggestion that a balanced diet supplementation could provide significant benefits for helminth control by increasing the host's ability to respond to infection and reducing the probability of reinfection.

## 2.3 Methods

### *2.3.1 Field experiment*

We conducted the field experiment in a 100ha broadleaf woodland in Falkirk, Scotland (Callendar Wood, 55.990470, -3.766636). This woodland has wood mouse (*Apodemus sylvaticus*) populations that are naturally infected with *H. polygyrus* (Clerc *et al.*, 2019a). We conducted two 8-week experiments (2 temporal replicates) during the wood mouse breeding season, when host energetic demands are highest: (i) May - July 2015 and (ii) June - August 2016. We used a 2 x 2 factorial design, where supplemented nutrition was manipulated using high-quality, whole-diet food pellets (hereafter simply "diet") at the population level (unit – trapping grid; control (unmanipulated) vs. supplemented) and anthelmintic treatment (hereafter simply "treatment") was manipulated at the individual level (unit – mouse; control (water) vs. treatment; Fig. 1). In 2015, we trapped three grids (1 supplemented grid, 2 control grids, 49 trapping stations per grid with 2 traps/station, 10m between each trap, for a total area of 3600m$^2$), while in 2016, we trapped four grids (2 supplementation and 2 control grids; with each grid set up as a 6x5 array of 30 trapping stations with 2 traps/station, 10m between each trap, for a total area of 2000m$^2$). All grids in both years were spaced a minimum of 50m from each other to minimise mouse movement

between grids, and grids were randomly assigned to nutrition regimes prior to the start of the experiment.

We diet-supplemented for three weeks before and then throughout the 8-week experiment twice per week with 2kg/ 1000m$^2$ of sterilised, TransBreed™ mouse chow pellets, scattered at regular intervals across the grids to ensure an even spatial distribution. TransBreed™ is a high-nutrient, standard veterinary feed which is formulated for optimum breeding performance in laboratory mice and offers whole-diet nutrition to the wild mice in this study (20% protein, 10% fat, 38% starch, high content of micronutrients, see Chapter 1, Table 1.1 for full details), therefore our supplementation complemented natural food availability. We live-trapped mice for 3 nights/week using Sherman live traps (H.B. Sherman 2x2.5x6.5-inch folding trap, Tallahassee, FL, USA). Each trap contained cotton wool bedding, and was baited with seeds, carrot, mealworms, and TransBreed™ pellets (on supplemented grids only), set in the early evening (16.00-18.00) and then checked early the following morning. All wood mice weighing >10g at first capture were tagged with a subcutaneous microchip transponder for identification (Friend Chip, AVID2028, Norco, CA, USA). On both control and diet-supplemented grids, all mice at first capture were rotationally assigned within each sex to either control or drug treatment groups. We administered a single 2ml/g dose of Pyrantel pamoate (Strongid-P, 100 mg/kg) and Ivermectin (Eqvalan, 9.4mg/kg) to each mouse allocated to the anthelminthic group. Ivermectin and Pyrantel pamoate are broad-spectrum anthelmintics which target adult and larval stages (Ivermerctin) and adult stages (Pyrantel) of *H. polygyrus* in both laboratory and wild mice (Wahid *et al.*, 1989;

Ferrari *et al.*, 2004; 2009). Previous work in wild *A. sylvaticus* found that the combination of Ivermectin and Pyrantel at 9.4mg/kg and 100 mg/kg, respectively, efficiently cleared *H. polygyrus* infection for ~14 days (Clerc et al. *2019).*

For each mouse at every capture we measured: sex, age, and host condition including body mass, length, fat scores, and reproductive status. Age of mice (classed as: juvenile, subadult, adult) was determined by weight and coat colour based on juvenile moulting patterns. Generally, juveniles weigh 10g or less, subadults weigh between 10-15g, and adults are 15g or heavier. Juveniles have a distinctly different coat colour (grey, compared to brown colour of adults), while subadults have an intermediate colour coat (grey/brown). Sex and reproductive status were assigned by visual examination of the genitals as male *A. sylvaticus* have a greater urogenital distance than females. Males were classed into the following reproductive categories: Abdominal (testes non-visible); Descended, or Scrotal. Females were classed into the following reproductive categories: Non-perforate vagina; Perforate vagina; Pregnant or Lactating. Animals which are scrotal, pregnant, or lactating are considered reproductive for binary reproductive status assignment. Weight in grams and length in mm was measured for each individual. Body fat scores were assessed on a scale of 1-5 (emaciated-obese) by palpating the sacroiliac bones (back and pubic bones) (Ullman-Cullere and Foltz, 2011). Blood samples were collected via mandibular bleed (first capture) or tail snip (subsequent captures) a maximum of once per week from which serum was separated by centrifugation at 12,000 rpm for 10 minutes and then stored at -80°C. Faecal samples were collected for each mouse at every capture from previously sterilised traps and preserved in 10% formalin. In addition, 2-3

pellets from each faecal sample were stored at -80°C for faecal IgA antibody measures.

Mice were sacrificed 12-16 days after first capture, corresponding to the period of efficacy for these drugs in wild wood mice [52,55]. Mice caught outside of this date range, or those pregnant or lactating at capture, were released. Eyes were collected and stored in 10% formalin for dissection of eye lenses for assessment of animal age. Eye lens mass has been shown to strongly correlate with age in many species (rodents and others), and has successfully been used to distinguish age classes for both laboratory and wild mice (Rowe *et al.*, 1985; Augusteyn, 2014). Eyes collected from sacrificed animals were removed from their container and left at room temperature for 5-10 minutes to allow the formalin to evaporate. Eye lenses were then extracted and dried at 70°C overnight. They were then weighed to the nearest mg using a precision balance. The combined weight of both eye lenses (log-transformed) for each individual were used as a proxy for age. We calculated the relationship between age and eye lens weight using wood mice of known ages from our colony to be:

$$Age\ (weeks) = \frac{eye\ lens\ weight\ (mg) - 0.043 * mouse\ weight\ (g) - 5.34}{0.152}$$

Small intestine, caecum, and colon of each individual were stored in 1X phosphate-buffered saline and dissected on the same day for counts of adult *H. polygyrus* worms present.

### 2.3.2 Laboratory experiment

We maintain a formerly-wild, but now lab-reared wood mouse colony in standard laboratory conditions at the University of Edinburgh. The colony has been in captivity for many generations, but the wood mice are purposely outbred to maintain genetic diversity. In this experiment, all mice were housed individually in ventilated cages (Techniplast, 1285L) with food and water ad libitum. *H. polygyrus* L3 larvae were isolated from the same Callendar Wood wild wood mouse population and were screened using PCR diagnostics to ensure the isolate was not contaminated with any other known mouse parasites or pathogens (IDEXX Bioresearch, Germany), and then passaged several times through colony-housed wood mice (Clerc *et al.*, 2019b).

#### Experimental design

We conducted a 2x2 factorial design in the lab, parallel to our field experiment: (i) diet (standard vs. supplemented, both Special Diet Services (SDS) pelleted rodent chow) and (ii) anthelmintic treatment (control (water) vs. treatment); both implemented at the individual level (Figure 1.1). As in the field experiment, Transbreed™ was used for the diet supplementation. Rat Mouse 1 (RM1™) was used as the control diet, as it is a maintenance chow with lower nutrient content, but is not considered a restrictive diet (Table 1.1). Mice on both diets were fed *ad libitum* and were given a 32-day diet acclimatisation period and we included both primary and secondary *H. polygyrus* infections to mimic the high level of exposure found in wild wood mice. Sixteen wood mice from our colony, aged 15-21 weeks (median 18 weeks), were randomly assigned to 4 experimental groups (n = 4/group; Figure 1.1): (i) supplemented nutrition, treated, (ii) supplemented nutrition, control, (iii) standard nutrition, treated, and (iv) standard nutrition, control. Eight mice were designated as

controls and placed on the same standard and supplemented mice as experimental mice (n = 4 per group; Figure 1.1). After diet acclimatisation, all experimental mice were infected with 200 wild-derived *H. polygyrus* L3 in 150uL $dH_2O$ via oral gavage. Third stage larvae (L3) of *H. polygyrus* were originally derived from wild *A. sylvaticus* that were infected with *H. polygyrus* (Clerc *et al.*, 2019b). Since then they have been passaged approximately ten times through colony-housed *A. sylvaticus* at the University of Edinburgh. In order to extract *H. polygyrus* eggs from faecal samples, the pellets were broken up and mixed with inactivated charcoal to mimic soil. The charcoal-soil mix was spread thinly on moist filter paper maintained in petri dishes at 17C. Larvae started to hatch after 9-12 days after culture and were collected into dH2O and kept at 4°C until use. Prior to infections, larvae concentrations were adjusted to a final concentration of 200 L3/ 150uL. Infective doses were administered to mice via oral gavage.

On day 46, 14 days post-infection, half of the male and female mice challenged with *H. polygyrus* were randomly assigned to treatment groups and were given either anthelmintic drug treatment (identical combinations and doses as in field experiment) or a control dose of water via oral gavage. Control animals received equivalent water control on day 32 and 46 experimental primary infection and treatment timepoints. On day 53, all experimental mice were re-infected with 200 *H. polygyrus* L3 in 150uL $H_2O$ via oral gavage to act as a secondary challenge, and control mice were given a primary challenge with 200 *H. polygyrus* L3. On day 67 (14 days post-secondary challenge) all mice were culled and adult *H. polygyrus* in the small intestine were counted.

## Sampling

Once per week, both weight (grams) and fat scores were recorded. From the day of primary infection (day 32) we collected a weekly blood sample for each individual; on days 32, 39, and 53 the samples were taken via venesection (tail bleed), while on day 46, the samples were taken via venepuncture (cheek bleed). On day 67, individuals were sacrificed and sampled as described above. Faecal samples were collected three times/week starting at primary infection and then continued throughout the course of the experiment, by changing the cage bedding ~12 hr prior to each collection and then collecting faecal pellets from the freshly-used bedding and then preserving the samples in 10% buffered formalin. A small sample of 2-3 pellets was also collected to measure faecal IgA. Over the course of this experiment, 5 mice exhibited weight loss over the threshold for our experimental protocol, not related to the diet supplementation or *H. polygyrus* infection and were culled and removed from further analysis.

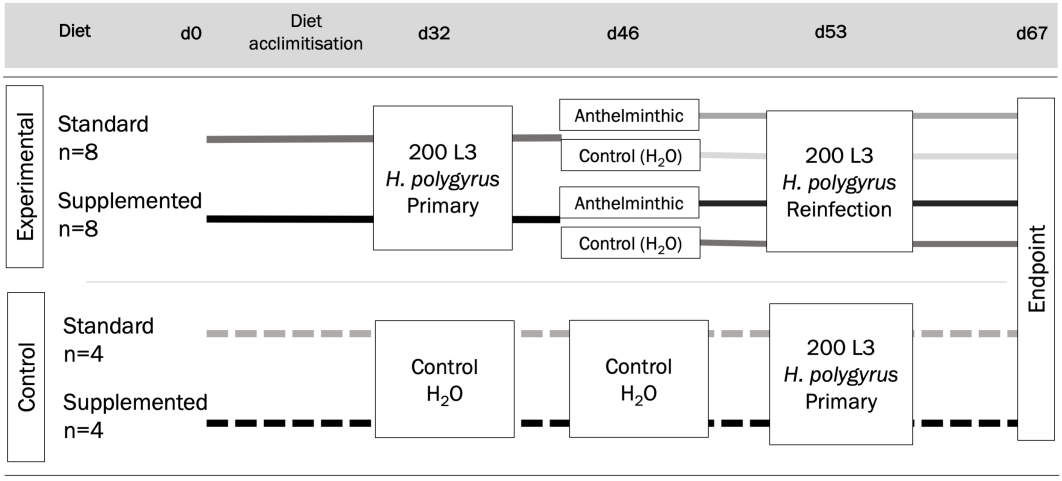



**Figure 2.1 Diagram of supplemented diet- H. polygyrus infection laboratory experimental design.**

All wood mice were assigned to diet groups at day 0 (d0). After 32 days of diet acclimatisation (d32), all experimental (n=16; solid lines) mice were given a primary challenge of H. polygyrus. On day 46, 14 days post infection (dpi), the experimental mice were randomly assigned within diet groups to either receive an anthelminthic treatment (darker lines) or a control dose of water (lighter lines; n=4/ group). On day 53, all experimental mice received a secondary challenge of H. polygyrus, and were culled on day 67, 14 days post-secondary challenge. On days 32 and 46, control mice (dashed lines, n=4/ group) received equivalent volumes of water, and on day 53 received a primary challenge with H. polygyrus. Control mice were culled on day 67, 14 days post primary challenge.

### *2.3.3 Laboratory assays for both field and laboratory experiments*

*H. polygyrus* egg shedding was measured as eggs per gram of faeces (EPG). using salt flotation and microscopy following Knowles et al. (2013). Briefly, saturated salt solution was added to formalin-preserved faecal samples to concentrate eggs on a coverslip, counted at 10X magnification, and adjusted by sample weight to EPG. Adult *H. polygyrus* were counted from PBS-preserved small intestine sections within 5 hours of dissection.

We used ELISA assays to measure (1) total faecal IgA concentration and (2) sera *H. polygyrus*-specific IgG1 antibody titres for each mouse at each capture/sampling point using protocols optimised for wood mouse samples [56]

For IgA ELISA, a 3:1 volume of protease inhibitor solution (Complete Mini Protease Inhibitor Tablets, Roche) was added each faecal sample and then homogenised. These faecal extractions were then incubated for 1hr at room temperature and then centrifuged at 12,000 rpm for 5min. Supernatants were separated from faecal pellets and stored at -80C. 96-well microplates (Nunc™ MicroWell™, ThermoScientifc™) were coated with goat anti-mouse IgA (Southern Biotech 1040-01, 2ug/ml) diluted in carbonate buffer overnight at 4°C. Capture antibody was then flicked off plates and

4% BSA-TBS was added and incubated for 2hr at 37°C to block non-specific binding sites. Faecal extracts were diluted 1:50 in 1% BSA TBS in triplicate and added to plates and incubated overnight at 4°C. Two serial dilutions of IgA standard (BD Bioscience 3039828) were included on each plate as positive controls and to obtain curves for calculation of IgA concentration. Following incubation, plates were washed 3 times with TBS-Tween and 50uL of goat anti-mouse IgA-HRP (Southern Biotech 1040-05) diluted 1:4000 in 1% BSA TBS was added to each well. Plates were incubated for 1hr and washed 4x with TBS-Tween and 2x with ddH20. 50ul TMB substrate was added to each well and plates were developed for 7 minutes protected from light. After that, 50uL of 0.18M sulphuric acid was added to each well to stop the enzymatic reaction. Plates were read at 450nm and sample concentrations were determined by fitting a 4-parameter logistic regression to standard curves.

To determine *H. polygyrus*-specific IgG1 titres from blood sera, 96-well microplates were coated with *H. polygyrus* excretory-secretory antigen (HES, 1.0ug/ml; obtained from Amy Buck, University of Edinburgh, and Rick Maizels, University of Glasgow) diluted in carbonate buffer overnight at 4°C. Capture antigen was then flicked off plates and 4% BSA-TBS was added and incubated for 2hr at 37°C to block non-specific binding sites. Sera samples were prepared as twofold serial dilutions with a starting concentration of 1:100in 1% BSA TBS and added to plates, and incubated overnight at 4°C. Two serial dilutions of sera from infected colony mice were included on each plate as positive controls. Following incubation, plates were washed 3x with TBS-Tween and 50uL of goat anti-mouse IgG1-HRP (Southern

Biotech 1070-05) diluted 1:2000 in 1% BSA TBS was added to each well. Plates were incubated for 1hr and washed 4x with TBS-Tween and 2x with ddH20. 50ul TMB substrate was added to each well and plates were developed for 7min protected from light, at which point 50uL of 0.18M sulphuric acid was added to each well to stop the enzymatic reaction. *H. polygyrus* specific IgG1 was calculated as a relative concentration to a positive reference sample consisting of sera from *Mus musculus* experimentally infected with *H. polygyrus* in the laboratory. Plates were prepared with serial dilutions of reference and experimental samples, and a dilution factor of 1:200 was selected for calculation of relative antibody concentrations. Standardised IgG1 concentrations were calculated by plate as follows:

$$IgG1\ Standardised\ Concentration = \frac{Sample\ OD - Mean\ Plate\ Blanks}{Plate\ Positive\ Control\ OD - Mean\ Plate\ Blanks}$$

We assigned a value of 0 to samples for which the OD did not exceed 3x SD of control blanks as we considered them indistinguishable from no IgG1 response. We refer to both IgA and IgG1 values as 'antibody concentration'.

### *2.3.4 Statistical Analysis*

We carried out all statistical analysis using R v 3.5.1 (R Core Team, 2019). All models were fit using the package 'glmmTMB' [1]. All model components are listed in Appendix A, Table S2.1, and additional information on model terms in Appendix A, Table S2.2. Year (factor; two levels) was included in condition and immunity models to account for variation across replicates, but was dropped for models of infection in the wild as we no observed no significant differences between years in infection variables.

*H. polygyrus* infection

***Wild experiment****:* To investigate the impact of supplemented nutrition on *H. polygyrus* infection, we used Generalized Linear Models (GLMs) and Mixed Models (GLMMs) for the following response variables (i) intensity of infection (EPG) at first capture (before treatment) (ii) mean EPG per individual for subsequent post-treatment captures and (iii) infection burden (adult worm count) at final capture. The distribution of EPG abundance and worm burden were highly over-dispersed with a high number of zero counts, which is typical for many helminth infections (Grenfell *et al.*, 1995), thus we fit the models with negative binomial (NB) error distributions. Fixed effects in all models included diet and host characteristic variables (i.e. sex, reproductive status, age full details in Table S2.1). A random effect of grid:year (7 levels) was also included in all models to account for spatiotemporal variation in *H. polygyrus* infection. Drug treatment and a treatment - supplement interaction were included in models of data after first capture. Age was only included as an explanatory variable in the model examining worm burden, where lethal sampling allowed the use of eye lens weight as a proxy for age (Rowe *et al.* 1985). Mice were classified as 'supplemented' if they were captured > 50% of the time on supplemented grids and as 'control' otherwise. Only 18% (n = 16) of mice were captured on both grid types, but to test the possibility that effects of supplemented nutrition could be dependent on the time spent on these grids, we fit another set of 3 models, identical to those described above, but including three levels of supplemented nutrition as an explanatory variable (control, mix, supplemented), where 'mix' represented sixteen mice that were found on both control and supplement grids across the experiment (see Appendix A).

***Laboratory experiment**:* We investigated the impact of supplemented nutrition and treatment on *H. polygyrus* infection in the laboratory using GLMs with NB errors to the following response variables to primary infection: (i) peak EPG shed, (ii) total EPG shed, and (iii) adult worm burden. Although all experimental mice were infected and re-infected, only two individuals had EPG values >0 after reinfection, and models could not be reliably fit to this dataset. Therefore, only adult worm burden was used as a response variable for secondary infection. Explanatory variables for all models are listed in Table S2.1, and included diet, host characteristics, and day of experiment as fixed effects. Although anthelmintic drugs were administered to half of the experimental group before secondary challenge, there was no difference in worm clearance (as indicated by EPG) between drug-treated and control mice (Appendix A, Figure S2.7B) and thus they were combined for these analyses.

### Body condition

At each capture, weight in grams and length in mm was measured for each individual. Body fat scores were assessed on a scale of 1-5 (emaciated-obese) by palpating the sacroiliac bones (back and pubic bones) as detailed in (Ullman-Cullere and Foltz, 2011). After field data collection, body condition index was calculated by obtaining the residuals of an OLS regression of Mass against Length (Peig and Green, 2009). Because we expected less variable body length in the laboratory than in the wild, we did not take regular length measurements, therefore we only tested body mass (g) (not weight/ length residuals) and total fat score (as measured in the wild, details in Appendix A) as response variables.

***Wild experiment:*** We fit GLMMs to two metrics of body condition to determine the effect of supplemented nutrition (i) body condition index (BCI, weight vs. length regression residuals; (Peig & Green, 2009)) and (ii) total fat score (FS, sum of dorsal and pelvic fat scores (Ullman-Cullere and Foltz, 2011), both of which were normally distributed. Diet and drug treatment, host characteristisc, and time were included as fixed effects, as well as *H. polygyrus* infection abundance (log of egg/gram+1; Table 1). We also fit a supplement-by-day interaction to investigate differences in the slope of the body condition-diet relationship over the 2-week period. Due to variation in weight change due to growth and gestation, we excluded pregnant females from base body condition models, and fit separate models for these individuals. Explanatory variables included in models for pregnant females were as above, except that sex and reproductive condition were excluded.

***Laboratory experiment:*** We fit linear mixed models (LMM) to determine the effect of supplemented nutrition on body condition. Because we expected less variable body length in the laboratory than in the wild, we did not take regular length measurements, therefore we only tested body mass (g) (not weight/ length residuals) and total fat score (as measured in the wild, details in SI) as response variables. We selected terms to investigate the same relationships as in the wild (Table S2.1).

### General and specific antibody measures

We tested for the effect of supplemented nutrition and *H. polygyrus* infection on general and specific antibody response in the wild and laboratory, by fitting GLMMs with either total non-specific IgA or *H. polygyrus*-specific IgG1 as the response variable with gaussian error distributions. Fixed effects included host characteristics,

year and day of experiment, and experimental manipulations (Table S2.1) and all mixed models included mouse ID as a random effect.

## 2.4 Results

Our field experiment included 310 captures of 91 individual mice (2015: n = 49 and 2016: n = 42), 61 of which were captured > 1 time (mean number of captures = 3.42 +/- 0.26). Of these, 36 mice were sacrificed after two weeks to measure *H. polygyrus* adult worm burdens; and for all other captures *H. polygyrus* eggs/gram was used as a proxy of adult worm burden (Chapter 1, Box 1.1). Adult mice comprised 87.5% of all captures in our dataset (juveniles = 2.4%, subadults = 10.1%). At the start of field and colony experiments, wood mice in the colony compared to wild wood mice had higher body mass (Colony mean weight = 23.88g; Wild mean weight = 20.32g; T-test, $t = -2.99$ $p = 0.005$) and better body condition (Colony mean total fat score = 9.08/10; Wild mean total fat score = 5.7/10, Wilcoxon Rank-Sum test, $W = 127$, $p < 0.001$).

### *2.4.1 Supplemented nutrition decreased* H. polygyrus *worm burdens and egg shedding and improved anthelmintic drug efficacy*

Field experiment

On average, mice spent ~30 days on supplemented grids (SEM = 1.5 days), but the range was between 12–63 days. We found that mice caught for the first time on supplemented grids had significantly lower *H. polygyrus* EPG than mice on control grids (Figure 2.2A and 2.3, $\beta = -1.42$, $p = 0.018$); resulting in ~50% reduction in egg shedding (23.55 vs 43.68 EPG, Appendix A Table S4). By 12–16 days after first capture, mice on supplemented grids also had 60% fewer adult worms compared to

mice on control grids (Figures 2.2C and 2.3, $\beta = -1.2$, $p = 0.045$; Appendix A Table S2.3).

In addition to main effects of diet supplementation alone, we also found a significant interaction between diet and anthelminthic treatment following assignment to treatment groups at first capture (EPG; $\beta = -4.51$, $p = 0.01$, Figure 2.3A). Notably, treatment reduced shedding to < 1 *H. polygyrus* egg per gram faeces in diet-supplemented mice for two weeks following treatment, while the mice on control grids still shed ~29 eggs/gram faeces during the same period ($\beta = -7.62$, $p < 0.001$, Figure 2.2B, Appendix A Table S2.3) compared to control grids (Tukey post-hoc test: $\beta = -6.06$, $p < 0.001$, Figure 2.2B). Likewise, for adult worms, anthelmintic treatment efficacy was highest in mice on supplemented grids ($\beta = -2.74$, $p < 0.001$, Figures 2.2C and Figure 2.3); resulting in complete worm clearance for all but one mouse, who had a single worm (Figure 2.2C, Table S2.3).

For all models of *H. polygyrus* EPG in the wild, diet and a diet-by-treatment interaction were the only significant predictors (Figure 2.3). For *H. polygyrus* worm burden, we found body mass and age to be additional predictors of infection, where larger ($\beta = 0.21$, $p = 0.019$) and older ($\beta = 2.22$, $p = 0.016$) individuals carried higher worm burdens (Figure 2.3). Including a factor level accounting for a mixed amount of supplemented nutrition did not significantly improve the fit for models of EPG at first capture, post-treatment, or at end point ($\Delta AIC = 1.62$, $\Delta AIC = -1.15$, $\Delta AIC = 1.28$, respectively; Fig S2.1) and accounting for levels of supplementation (time spent on supplemented grids) did not change any of the results (See Appendix A and Figures S2.1 and S2.3)

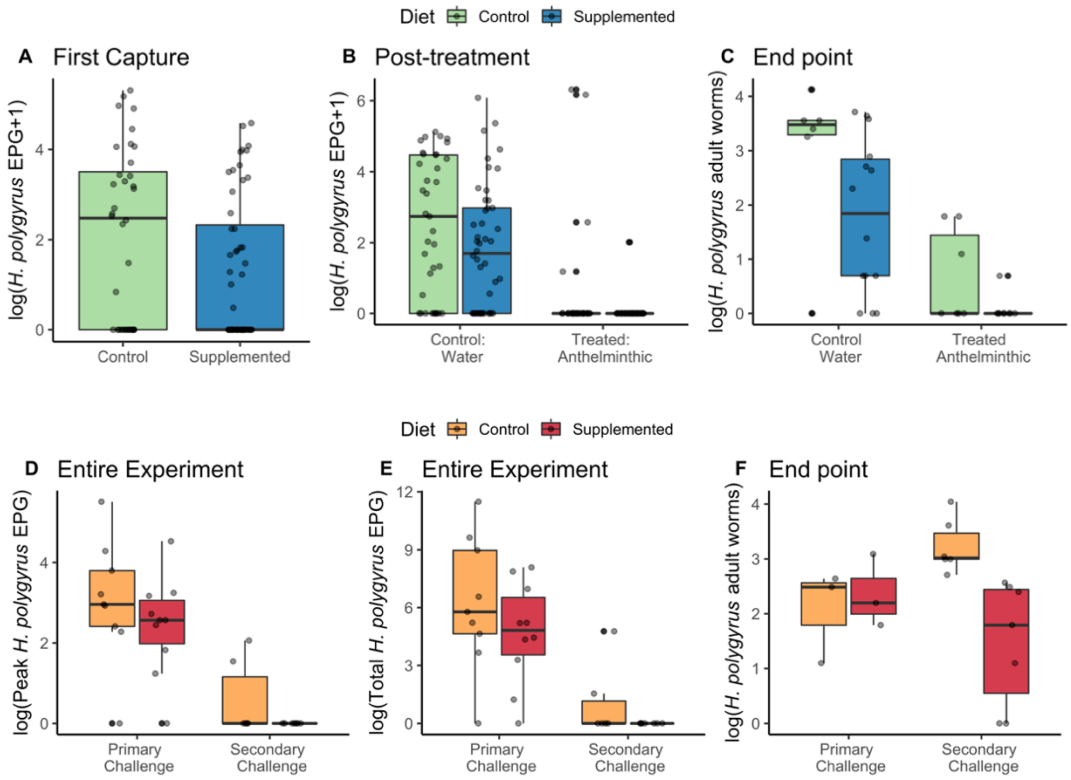



**Figure 2.2. Effect of a supplemented nutrition diet on *H. polygyrus* infection in wild and laboratory wood mice.**

Top row- wild; bottom row-laboratory. A. Infection abundance (log EPG) at first capture, N=88 individuals B. Mean EPG for all individuals captured beyond first capture and after assignment to treatment categories, N=62 individuals; 166 captures C. Burden (log adult worms) at end point for culled individuals, N=36. D. Peak EPG shed E. Total EPG shed. F. Effect of nutrition supplementation on *H. polygyrus* burden in the laboratory in the presence and absence of anthelminthic treatment. Burdens represent log of adult worm counts from gut dissections 14 days post primary or secondary infection with 200L3, n=19 mice (Primary only control group, n=6, Primary +Secondary group, n=13).

## Laboratory experiment

In the controlled laboratory experiment, supplemented diet reduced both peak ($\beta$ = -1.96, p = 0.017) and total *H. polygyrus* EPG ($\beta$ = -1.07, p < 0.001) compared to mice on the standard 'control' diet, and, importantly mice receiving the diet supplement shed no eggs during reinfection (Figures 2.2D-E and 4; Appendix A, Table S2.4). While there was no difference in adult worm burdens between mice on supplemented or control diets after primary infection, mice on the supplemented diet were

significantly less susceptible to secondary challenge, with a 75% lower adult worm burden (β = -1.76, p = 0.002, Figures 2.2F and 4; Appendix A, Table S2.3).

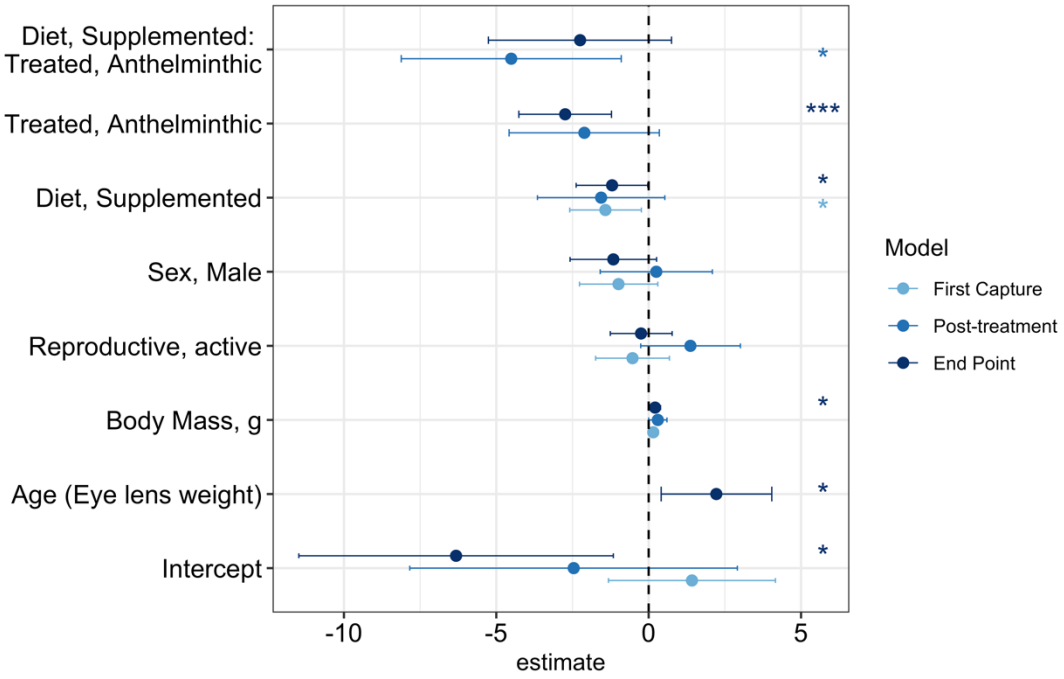



**Figure 2.3. Effect size estimates from models investigating the effect of supplemented nutrition on *H. polygyrus* infection in the wild.**

Response variable and data used in each model is indicated by colour. Models represent faecal egg counts (EPG) for (i) first capture (light blue, GLM) and (ii) mean post-treatment captures (blue; GLM) and adult worm burdens for (iii) the experimental end point for culled animals (~ 12-16 days post treatment; dark blue; GLM) . Points and ranges represent model estimates and 95% credibility estimates for each model. Asterisks indicate the significance of variables: ***, ** and * indicate P<0.001, P<0.01 and P<0.05 respectively. Eye lens mass (log-transformed) was included as a proxy for age in final capture model only where samples were available.

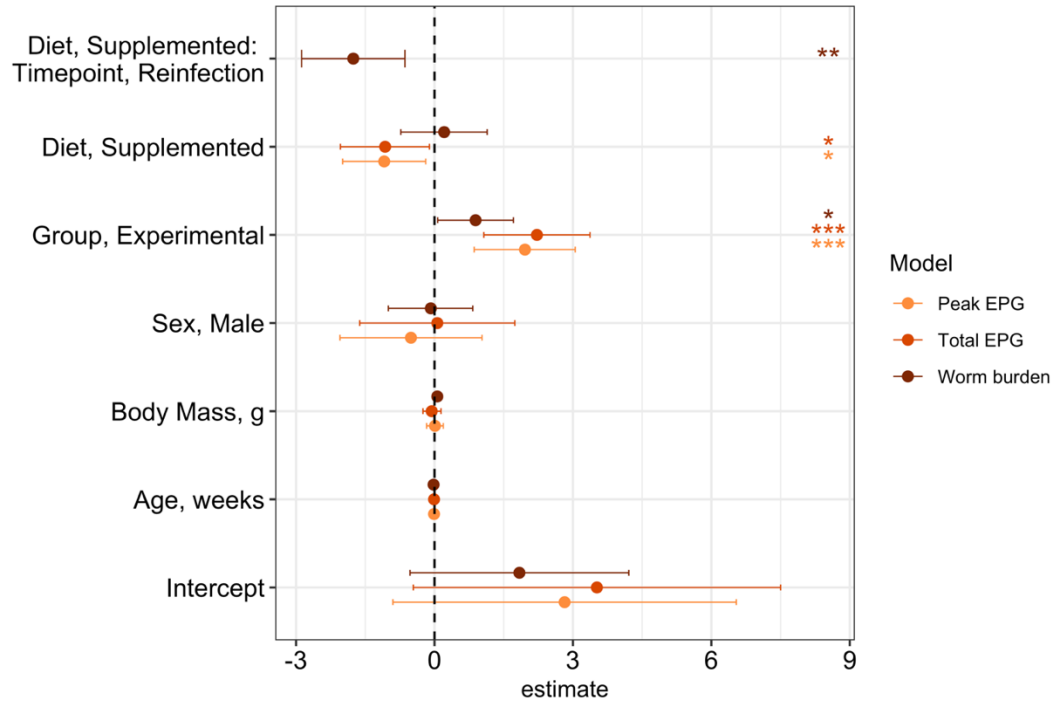



**Figure 2.4. Effect size estimates from models investigating the effect of supplemented nutrition on *H. polygyrus* infection in the laboratory.**

Colours represent separate models for egg shedding or adult worm data collected during primary infection (EPG, GLM) or at endpoint of the experiment (Worm burden, GLM). 'Group' represents experimental and control mice, where controls received only a primary treatment and no secondary challenge. Diet*timepoint interaction was included in the endpoint models to investigate potential differences in effects of supplemented diet on primary and reinfection. Points and ranges represent model estimates and 95% credibility estimates for each model. Asterisks indicate the significance of variables: ***, ** and * indicate P<0.001, P<0.01 and P<0.05 respectively. 'Group' represents experimental and control mice, where controls received only a primary treatment and no secondary challenge.

### *2.4.2 Supplemented nutrition increased wood mouse body condition*

Wild wood mice on supplemented grids had higher body condition (BCI) and total fat scores (FS) compared to mice on control grids (BCI $\beta = 1.46$, $p = 0.011$; FS $\beta = 0.64$, $p = 0.006$; Figures 2.4; Appendix A, S2.4); however, we also found significant interactions between diet and day of experiment, suggesting that supplemented mice lost weight over time despite overall greater condition (BCI: diet,

supplemented*day β = -0.16, p = 0.011; FS: diet, supplemented*day β = -0.09, p = 0.002; Appendix A, Figure S2.4). In addition, we found that reproductively active mice had higher BCI scores (β = 1.53, p < 0.001); however, the relationship with FS was non-significant (β = -0.22, p = 0.215) (Appendix A, Figure S2.4). Among pregnant females, supplemented diet was also associated with significantly higher BCI and total FS (BCI β = 8.32, p < 0.001, FS β = 1.98, p = 0.005, Figures 2.5; Appendix A, S2.4). In general, males had lower FS (β = -0.44, p = 0.02) compared to females, and mice from 2016 had higher BCI (β = 1.09, p = 0.038) but lower FS (β = -0.63, p < 0.001) compared to 2015 (Figure S2.4). There were no effects of treatment detected on either metric of body condition, however among pregnant females, higher *H. polygyrus* infection abundance was associated with lower BCI scores (β = -0.64, p < 0.001) (Figure S2.4).

In the laboratory, supplemented diet resulted in higher total FS compared to control mice (β = 0.85, p = 0.019, but did not affect body mass (Figures S2.5-S2.6). Males had both higher mass (β = 8.56, p < 0.001) and FS (β = 2.24, p < 0.001) compared to females (Figure S2.6). Lastly, higher *H. polygyrus* infection abundance was associated with overall decreased body mass (β = -1.40, p = 0.012, Figure S2.6).

### *2.4.3 Supplemented nutrition increased total faecal IgA and* H. *polygyrus-specific IgG1*

Total faecal IgA antibody concentrations differed between the years in the field experiment, with lower levels found in 2016 (β = -5.14, p = 0.0008). In 2016, wood mice on supplemented grids had significantly higher total faecal IgA antibody concentrations (β = 5.31 p = 0.007, no difference in 2015; Figure 2.6A; Appendix A

Table S2.7) and anthelminthic treatment was also associated with higher total faecal IgA in both years (β = 2.19, p = 0.031; Appendix A, Table S2.7). Body condition (BCI) was also found to be positively associated with higher concentrations of IgA (Figure 2.6A-B; Appendix A, Table S2.7) and was the only significant predictor of *H. polygyrus*-specific IgG1; where better body condition was associated with higher *H. polygyrus*-specific IgG1 concentration (β = 0.02, p = 0.01, Figure 2.6C; Appendix A, Table S2.8). Following controlled *H. polygyrus* infection in the laboratory, mice on a supplemented diet had significantly higher total faecal IgA 2-4 weeks post-infection (β = 2.40, p = 0.018) and *H. polygyrus*-specific IgG1 3 weeks post-infection (Figure 2.6D-E; Appendix A, Table S2.7).

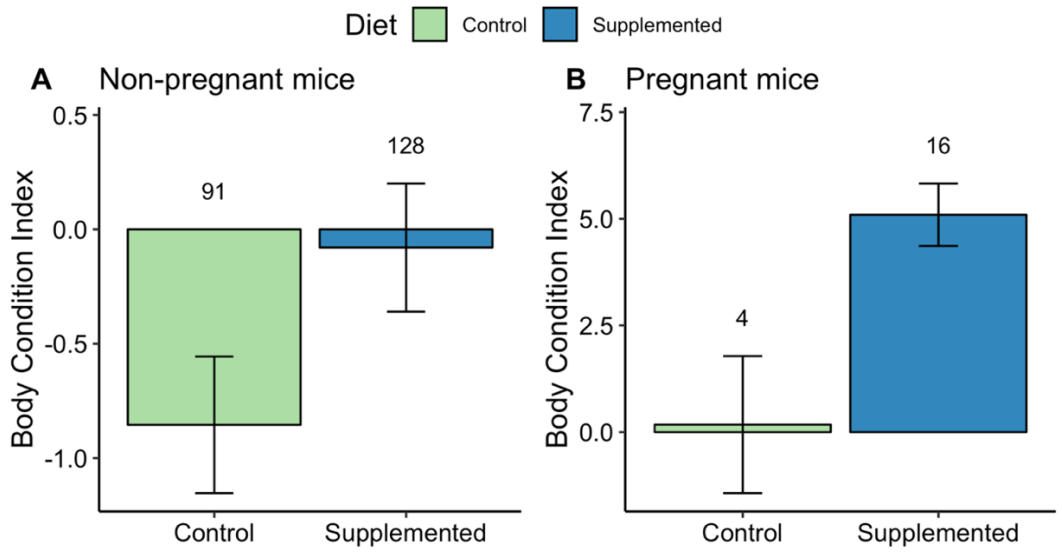



**Figure 2.5. Effect of supplemented diet on body condition metrics.**

Figure represents raw means +/- SEM for the metric indicated. Numbers above bars indicate number of observations per group. Body condition index was higher in supplemented individuals for both A. Non-pregnant mice B. Mice pregnant at the time of body condition measurement

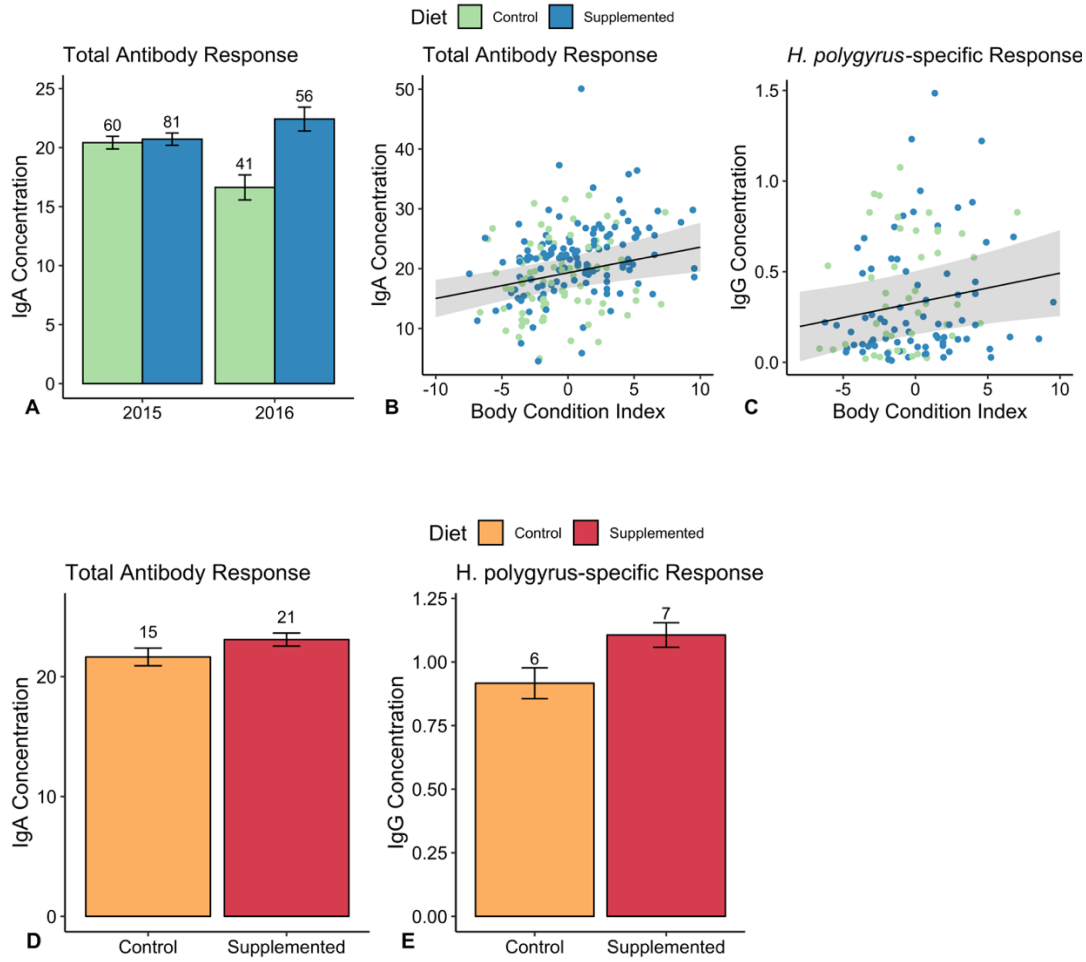



**Figure 2.6. The impact of diet supplementation on wood mouse antibody responses, wild: top row; laboratory: bottom row.**

IgA Concentration (absolute, ng/uL) at all captures **(A)** across years and (B) compared to the body condition index (BCI). **(C)** *H. polygyrus* specific IgG1 concentration (standardised) at all captures compared to BCI. **(D)** Mean IgA Concentration (absolute, ng/uL) and (E) mean *H. polygyrus* specific IgG1 concentration (standardised) at days 14, 21, 28 post-primary challenge E. IgG1 Concentration d21 post-primary challenge. Bar plots represent raw means +/- SEM for the metric indicated. Numbers above bars indicate number of observations per group. Point-line plots represent raw data points and model-predicted regression slopes with 95% credibility interval ribbons.

## 2.5 Discussion

We investigated the impact of supplementing with diet enriched with micro- and macro-nutrients on helminth infection and immunity in a natural host-parasite relationship in both controlled laboratory and wild conditions. Our results demonstrate dramatic and surprisingly fast-acting benefits of supplemented nutrition for host resistance, immunity, treatment efficacy and body condition within just a few weeks in both the wild and laboratory. By conducting a parallel experiment with the same host and nematode species in controlled laboratory conditions we overcame many of the limitations of field experiments by allowing us to control for variation among individuals in parasite exposure, demographic characteristics and nutritional status. We demonstrate that an enriched diet directly reduces both infection and onward transmission of *H. polygyrus* in its natural host via increased host condition and immunity and suggest that this type of integrated approach could be important for successful helminth management.

While the relationship between nutrition and gastrointestinal nematode infections have been extensively studied in the laboratory, their impacts in natural populations are less well understood. A previous field experiment on wood mice during winter found that supplemented diet with grass seeds and found a reduction in pinworms (*Syphacia stroma* and *Syphacia frederici*) but not cestodes (*Gallegoides arfaai* and *Hymenolepis diminuta*) or other nematodes (*Gongylonema neoplasticum*) (Diaz and Alonso, 2003). Similarly, a recent study in a semi-wild mouse system (laboratory strain C57BL/6) found no effect of restricted resource availability on *Trichuris muris* burden despite documenting effects of increased host feeding and lower IL-13 and *T.*

*muris*-specific IgG1 in restricted animals (Budischak *et al.*, 2017). In addition, most previous studies in wild systems investigating benefits of supplementation in wild mouse populations (e.g. *Peromyscus spp.* (Pedersen and Greives, 2008) and *A. sylvaticus* (Diaz and Alonso, 2003) mimicked food that was naturally available. Here, we found that supplementing with well-balanced diet in order to enhance nutrition beyond their naturally available resources had quick and significant impacts on *H. polygyrus* infection in the wild. Wood mice had markedly lower worm burdens and transmission potential (as measured by egg shedding) compared to mice on control grids. The positive impacts of supplementation were observed after just 14 days, and were detected in transient mice, who were not residents on the supplemented grids. This suggests resources are naturally limiting in wild wood mice populations, hampering their ability to produce a protective immune response to *H. polygyrus*.

Interestingly, we find very similar results of supplemented nutrition reducing worm burdens and egg shedding in the controlled laboratory experiment, suggesting that the benefits of whole-diet supplementation are driven by physiological and/or immunological changes and not due to differences in wild wood mice behaviour or foraging patterns. The enriched diet reduced both *H. polygyrus* egg shedding during primary infection and susceptibility to reinfection compared to mice fed the standard, maintenance lab chow – the latter being similar to the diet used in many lab mouse studies. Protein and zinc levels have been previously found to impact susceptibility and protective immunity to *H. polygyrus* in lab mice (Slater and Keymer, 1986a; Shi *et al.*, 1997; Boulay *et al.*, 1998). However, much of this previous work was conducted using diet restrictions regimes (i.e. protein restriction ( 2-3% protein)

compared to high protein (16-24% protein)) (Slater and Keymer, 1986b; Slater, 1988; Keymer and Tarlton, 1991; Boulay *et al.*, 1998; Clough *et al.*, 2016). We find similar and compelling effects of diet on infection in the laboratory, but using more modest differences in macro-and micro-nutrients (i.e. 20% vs 14% protein, 80mg/kg vs 36mg/kg zinc), and importantly no difference in caloric intake. Our results suggest that diet-induced changes to nematode infection dynamics are not limited to significant malnutrition, or cases where there is a severe deficit in a specific nutrient. We hypothesise that the balance of multiple micro-and macro-nutrients contained in the enriched diet increased the host's ability to produce specific molecules and effectors in response to infection, and resulted in a more protective response. However, more research is needed to determine if benefits of supplementation on condition and immunity to *H. polygyrus* are due to net effects of our additional resources, or specific macro-and micro-nutrients contained in the supplemented diet.

Adequate levels of macro- and micro-nutrients are vital to the function of cellular and humoral components of the immune system(Chandra, 1997; Calder and Jackson, 2000). We found that our supplemented diet increased adaptive immunity in both wild and laboratory settings, specifically total faecal IgA and *H. polygyrus*-specific IgG1 levels were higher in supplemented individuals in the laboratory and total faecal IgA was higher in supplemented mice in 2016. Faecal IgA is an important component of resistance to gastrointestinal nematodes and has been used as an indicator of general gut health (Macpherson *et al.*, 2012; Watt *et al.*, 2016), whereas parasite-specific IgG1 has a key role in the strong Th2 immune response induced by H. polygyrus (Reynolds *et al.*, 2012). These antibodies specifically play an important

function in blocking the maturation of larvae into adult worms within the host intestinal tissue and reducing worm fecundity (Hewitson *et al.*, 2015). Our findings align with previous work suggesting that inadequate levels of nutrients (e.g. protein and zinc) compromise both general and specific host immune response (Shi *et al.*, 1997; Boulay *et al.*, 1998; Ing *et al.*, 2000; Budischak *et al.*, 2017). Although we saw weaker evidence for a direct effect of supplemented nutrition on immune expression in the wild, we found positive associations between the body condition index (BCI) and both total faecal IgA and the *H. polygyrus* -specific immune response. Therefore, the increase in body condition for supplemented individuals may indicate an indirect effect of supplementation on antibody levels and increased resistance. Typically, immune measures in the wild are difficult to interpret due to the context-dependency of immune phenotypes in the wild, and our limited ability to relate immune measures to exposure (Pedersen and Babayan, 2011). Thus, our results from our exposure-controlled colony experiment may be a more reliable indicator of how supplemented nutrition can impact both specific and general immune responses and impact helminth resistance.

Hosts in the wild are typically resource-limited, with finite energy to invest in immunity, reproduction, and other processes (Sheldon and Verhulst, 1996). In addition to improving immunity, we found that a supplemented diet improves body condition and investment in reproduction. Mice, particularly pregnant females, on supplemented grids had increased host body condition scores (BCI; body condition index and FS; fat scores). Higher body condition during pregnancy, particularly for BCI which represents a body mass/ body length relative score, may indicate increased allocation to more or larger offspring. These results agree with previous

research in which females in *A. sylvaticus* populations supplemented with grass seeds during the winter bred earlier and had larger litter sizes (Diaz and Alonso, 2003). In our laboratory experiment, supplementation had only a weak positive effect on fat scores, and did not affect BCI, which is contrary to previous laboratory studies which found that animals given lower protein had significantly lower weight, while our standard diet group did not lose weight over the experiment (Boulay *et al.*, 1998). However, this may not be surprising, as wood mice in our laboratory colony are able to feed *ad libitum* and have higher body mass and better body condition than those in the wild whom are likely to be chronically under some degree of dietary restriction. Due to the relatively short nature of this field experiment within one season, it is unclear whether these condition and reproductive effects in the wild may alter host survival or density and have important population-level consequences for disease transmission in this system. Future work sustaining nutrition supplementation for a longer duration can help to provide complementary insights to the short-term individual effects of *H. polygyrus* infection dynamics and treatment efficacy investigated in the present study.

Effective helminth control in endemic areas is difficult because even with readily available anthelmintic drugs, reinfection rates are usually high (Speich *et al.*, 2016). Limiting infection in a population requires (1) lowering worm burdens, (2) reducing onward transmission, and (3) preventing reinfection. While our previous research in wild rodents has shown additive benefits of anthelmintic treatment and nutrition supplementation for mouse fitness and population dynamics, we were not able to measure the direct implications on nematode infection prevalence, worm burdens or

reinfection rates (Pedersen and Greives, 2008). In addition, evidence for additive effects of these interventions in human populations remains equivocal (Yap *et al.*, 2014). In our wild population, we found that supplementation synergistically enhanced effects of anthelmintic treatment, reducing *H. polygyrus* adult worm burdens and egg shedding to almost zero following treatment for mice on supplemented grids. Notably, supplemented individuals in the laboratory also shed zero eggs during secondary challenge, even when harbouring adult worms. Our results demonstrate the significant impact of nutrition on reducing onward transmission; an important component to limiting exposure and reinfection. In addition, we found that increased resistance to reinfection in supplemented individuals in the laboratory highlights an additional benefit of supplemented nutrition in managing nematodes in natural populations where reinfection after treatment is problematic. Given varied results from human trials investigating the addition of supplements with different nutrients (Yap *et al.*, 2014) and the range of macro-and micro-nutrients implicated in immunity to gastrointestinal nematodes (Michael and Bundy, 1992; Koski and Scott, 2003; Clough *et al.*, 2016), our study presents key experimental results regarding the role of nutrition as a viable option for complementing helminth control interventions.

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# Chapter 3 Diverse impacts of resource provisioning on parasite infection in a naturally co-infected host

## 3.1 Summary

Resource availability plays a key role in determining exposure and resistance to parasites in the wild. Increasingly, anthropogenic activities alter food sources available to wildlife, either directly or indirect; resulting in important impacts on host-parasite dynamics. However, much of our knowledge is from single-host, single-parasite systems, yet co-infection with multiple species is the norm in nature. In a wild population of wood mice (*Apodemus sylvaticus)*, we experimentally supplemented resources to improve nutrition and treated individuals with an anthelmintic drug to remove nematodes to measure the direct and synergistic impacts on the broader parasite community of >10 species. We found that the impact of supplemental resources on parasite infection was incredibly variable and dependent on parasite biology and transmission mode. Resource supplementation reduced susceptibility to gastrointestinal nematodes and some ectoparasites, but actually increased infection risk and burden for several blood-borne and intestinal microparasites. Furthermore, some effects of resource supplementation were impacted by anthelmintic drugs and the subsequent reduction of nematodes, suggesting that within-host interactions between coinfecting parasites can determine their response to resource supplementation. Importantly, resource supplementation impacted host condition, immunity, reproduction and population size, highlighting the complexity of both direct and indirect impacts of resource provisioning in a wild population. Our results represent an important advance for understanding the outcomes of disease at the human-wild interface and suggests that measuring the whole parasite community is crucial for understanding and predicting the response of parasites to resource availability in the wild.

## 3.2 Introduction

Resource availability plays a crucial role in determining both individual- and population-level dynamics of infectious diseases (Randolph and Storey, 1999; Calder and Jackson, 2000; Clotfelter *et al.*, 2007; Bogdziewicz and Szymkowiak, 2016). Increasingly, anthropogenic activities, whether intentional or accidental, are altering the quality and quantity of naturally resources available to wildlife (Oro *et al.*, 2013). These supplemental resources can have significant impacts in wild populations, such as changing individual body condition and nutritional status. Although it is expected that these changes to the host might result in increased allocation of energy to the immune system (Calder and Jackson, 2000) there are several exceptions to this outcome (Becker *et al.*, 2015), such as poor quality food from urban waste instead reducing immune function in coyotes (Murray *et al.*, 2015). Furthermore, effects of resource provisioning in a population extend beyond host condition and affect host demography and behavior as well as condition, processes which have substantial implications for parasite transmission (Becker and Hall, 2014). The outcome of resource provisioning for infectious disease will therefore depend on the quality of food source, effects at the host-level, and the effects at the population-level (Becker *et al.*, 2015).

There have been an increasing number of theoretical and empirical studies that have highlighted the diversity of outcomes possible following resource provisioning, with highly varied results across systems and parasites investigated. It is becoming increasingly clear that effects of resource availability on host demography and movement patterns often indirectly alter parasite infection dynamics. For example, a recent experiment that increased the density of bird feeders resulted in significantly

increased *Mycoplasma gallisepticum* transmission within house finches (Moyers *et al.*, 2018), and intentional resource provisioning among Elk populations resulted in an increases infection with *Brucella abortis,* both likely due to increased host densities and contacts (Cross *et al.*, 2007). Meanwhile, recent theoretical frameworks have likewise highlighted the varied outcomes of resource availability according to heterogeneity of within-host response to infection (Cressler *et al.*, 2014), and population-level consequences (Becker and Hall, 2014). These results have advanced our understanding of diverse effects across a broad range of host-parasite systems, but to date how this complexity is manifest in a single host population with multiple parasites is poorly understand.

Co-infection is ubiquitous in wild populations (Petney and Andrews, 1998; Cox, 2001) and mammals typically host a diverse community of parasites throughout the course of their life. Simultaneous or subsequent infection with multiple parasite species can change both the magnitude and type of immune response elicited either directly (*i.e.* occupying the same niche or utilising the same resources) or indirectly by interfering with response to the other, co-infecting pathogen; particularly in the case of helminth-microparasite infections (Graham, 2008b). Specifically, chronic activation of immunosuppressive T helper-2 (Th2) responses during helminth infection can suppress the immune response to co-infecting microparasites or reduce additional macroparasites via cross-reactivity of immune responses (Lello *et al.*, 2004; Pedersen and Fenton, 2007; Graham, 2008b; Griffiths *et al.*, 2015). Viewing the host as an ecosystem for a community of pathogens has helped inform our understanding of why the infections in wildlife have significant variation across

hosts and locations, where the direct and indirect relationships among co-infecting parasites can alter host health and infection (Pedersen and Fenton, 2007). The effects of resource provisioning on individual parasites in a host population can therefore have repercussions for the entire parasite community. However, dynamics of multiple pathogens in a single host population are difficult to study and typically require experimental perturbation of the parasite community and ability to measure downstream effects (Pedersen & Fenton 2015; Hellard *et al.*, 2015). Here we use both experimental resource supplementation and anti-parasite treatment in a naturally co-infected small mammal population to ask whether resource provisioning alters parasite community dynamics in a single host population.

Wild wood mice (*Apodemus sylvaticus*) are found throughout Europe and are the natural host to *Heligmosomoides polygyrus*, an extensively-studied gastrointestinal nematode (Maizels *et al.*, 2012), but are also commonly infected with a diverse and abundant community of other parasites (Knowles *et al.*, 2013). We have previously shown that experimental supplementation of resources increases resistance and lowers *H. polygyrus* worm burdens while also positively impacting anthelmintic drug efficacy (Sweeny *et al.* 2019; Chapter 2). Here we investigate the impact of supplementation of a high-quality resource on the broader parasite community, including both micro- (e.g. protozoans, viruses, bacteria) and macro-parasites (e.g. helminths, fleas, ticks, etc). We conducted a large-scale population-level nutritional supplementation experiment, where we also treated individual mice with an anthelmintic drug to remove/reduce nematode infection and perturb the parasite community. We quantified the probability of infection and parasite intensity (when available) for gastrointestinal helminths and protozoa, ectoparasites, and blood-borne

viruses and protozoa. We investigated a. the main effects of resource supplementation on parasite community and b. the interaction between anthelmintic treatment and resources. We found that food supplementation significantly impacted the parasite community; with some parasite species decreasing in infection probability and intensity while others remained unaffected, and importantly, some parasites species increased. The experimental supplementation of resources, while only conducted over a short period of time, had substantial impacts on host condition, demography, and contact networks. Importantly, we also find found strong evidence of how within-host parasite interactions may have mediated the outcomes of co-infecting parasites. Overall, we found that supplementing resources can have diverse and complex impacts across the parasite community of a single host population, highlighting the importance of understanding both the direct and indirect impacts of wildlife resource provisioning.

## 3.3 Methods

### *3.3.1 Field Experiment*

We conducted a field experiment in Callendar Wood (55.990470, -3.766636; Falkirk, Scotland), a 100ha broadleaf woodland, which contain a populations of wood mice, which are naturally exposed to and infected with a wide range of parasites and pathogens (Clerc *et al.*, 2019a). The experiment had two, 8-week long, temporal replicates; both of which took place during the wood mouse breeding season: (i) May - July 2015 and (ii) June - August 2016. We used a 2 x 2 factorial design, where (i) nutrition was manipulated at the population level by supplementing resources (unit – trapping grid; control (unmanipulated) vs. supplemented nutrition) and (ii)

anthelmintic treatment was manipulated at the individual level (unit – individual mouse; control (water) vs. anthelminthic treatment). Full details of the methods and trapping grids can be found in Chapter 2. Briefly, we carried out resource supplementation for three weeks prior to the beginning of the trapping and then continued throughout the 8-week period. We supplemented grids twice per week with 2kg/ 1000m$^2$ of sterilized, TransBreed$^{TM}$ mouse chow pellets, scattered at regular intervals across the grids to ensure an even spatial distribution. TransBreed$^{TM}$ is a high-nutrient, standard veterinary feed which is formulated for optimum breeding performance in laboratory mice and offers whole-diet nutrition to the wild mice in this study (20% protein, 10% fat, 38% starch, high content of micronutrients, full details in Table S1 of Chapter 2). Following this 3-week period of supplemented nutrition, we live-trapped mice for 3 nights/week using Sherman live traps (H.B. Sherman 2x2.5x6.5 inch folding trap, Tallahassee, FL, USA). Traps were baited with cotton wool bedding, seeds, carrot, mealworms, and TransBreed pellets (on supplemented nutrition grids only), set in the early evening (16.00-18.00) and then checked early the following morning. All wood mice weighing >10g were tagged with a subcutaneous microchip transponder for identification (Friend Chip, AVID2028, Norco, CA, USA). On both control and nutritional supplementation grids mice at first capture were rotationally assigned within each sex to either control or anthelminthic treatment groups. We used a weight-adjusted dose (2ml/g) of both Pyrantel pamoate (Strongid-P, 100 mg/kg) and Ivermectin (Eqvalan, 9.4mg/kg) (full details in Chapter 2).

Each tagged individual was followed for a period of 12-16 days. During this time, we collected the following morphometric data at every capture: sex, age, measures of

host condition, including body mass, length, fat scores, and reproductive status (as described in Chapter 2). Blood samples were collected via mandibular bleed (first capture) or tail snip (subsequent captures) a maximum of once per week; faecal samples were collected for each mouse at every capture from previously sterilised traps and preserved in 10% formalin. Mice were sacrificed 12-16 days after their first capture.

### *3.3.2 Laboratory Assays*

Gastrointestinal helminths were detected by counting adult worms in the gut following dissection at days 12-16, and both GI helminths and coccidian were assessed as eggs per gram (EPG) of faeces through salt flotation methods and microscopy taken from samples collected throughout the experiment (Knowles *et al.*, 2013). Ectoparasites (mites, fleas, and ticks) were counted following fur-brushing in the field. We also screened for blood-borne parasites (Mouse Herpes Virus, and Trypanosomes) using diagnostic PCR (details in Appendix B). We previously quantified the absolute concentration of total faecal IgA and relative, standardised concentration of *H. polygyrus*-specific IgG1 and report those results here for illustration of the effects of resource supplementation (details in Chapter 2).

### *3.3.3 Statistical Analysis*

All statistical analysis was run using R v 3.6.0 (R Core Team 2019). All models were run using the package 'glmmTMB' [1]. Edge lists for network construction were generated using the package 'spatsoc' [3] and networks were constructed using the package 'igraph' [4].

### *Parasite community*

We first calculated infection intensity (where possible) and prevalence for all parasite species that we identified within this wood mouse population (Table 1). We then investigated whether nutritional supplementation impacted (i) total (gastrointestinal (GI), ectoparasites, blood-borne) parasite species richness and (ii) GI parasite species richness by comparing the mean number of co-infecting parasite species using a Wilcoxon Rank-Sum Test. We restricted further analyses to those species/genera with 10% or higher prevalence in the population to prevent fitting models to unsuitable sample sizes for convergence. When the data was available for specific parasite taxa we used intensity of infection (log (eggs/ oocysts per gram of faeces +1) or log(ectoparasite count+1); infected individuals only) as the response variable. For the parasite species where intensity was not measured, we used probability of infection (presence/absence) as the response variable. For flea and mite infection data, although we did collect count data, a very high percentage (85% in mites and 99% in fleas) were 0 or 1 counts; so we instead investigated the probability of infection (presence/absence) for both groups.

We fit the following the following fixed effects to each model of the probability of infection or intensity of infection for models for each parasite considered: supplementation (control or supplemented), sex (male or female), body mass (continuous, g) and reproductive status (active or inactive). To account for the possibility that the effects of supplementation may be mediated by the changes to the parasite community via anthelminthic treatment and subsequent nematode removal; we also ran another set of model for each parasite species/group, just using the trapping data beyond first capture (when treatment would be effective) with the same

fixed effects as described above, but including a supplement-by-treatment interaction. All models included individual ID as a random effect (Paterson and Lello, 2003) and grid:year (7 levels). Error distributions for parasite intensity models were gaussian and binomial for the probability of infection models. To compare the effect sizes across all parasites investigated, we calculated effect size using Hedges' g for a standardised measure of nutrition impacts on each parasite for each intensity model (Hedge & Olkin 1985). Hedges' g was calculated from estimated means from supplemented and control resource groups ($Y_1$ and $Y_2$) as follows:

$$g = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}} J$$

Where:

$$J = 1 - \frac{3}{4(n_1 + n_2 - 2) - 1}$$

With variance:

$$v_g = \frac{n_1 + n_2}{n_1 n_2} + \frac{g^2}{2(n_1 + n_2)}$$

For probability models, we calculated odds ratio (OR) values for predictors and Hedges' g was derived from OR values to facilitate comparison across intensity and probability models. Hedges' g was derived from OR values as follows:

$$g = \log(OR) * \frac{\sqrt{3}}{\pi} J$$

When there was no interaction between supplementation and treatment retained after model selection, effect sizes are reported for the effect of supplementation alone. However, where a significant interaction of supplementation and treatment was detected, the effect size is reported for both anthelmintic treated and control levels of the interaction in addition to the main effect.

### *Host demography, contacts, and behaviour*

To investigate further effects of the supplemental resources on population-level traits we measured host demography, by estimating the number of new individuals born on supplemented or control grids over the trapping period, and then testing the differences in new cohort population size between resource groups with a GLMM with resources as a fixed effect and week of the experiment ('time') as a random effect. To investigate the impacts of resource supplementation on host behaviour and the number and distribution of contacts between individuals we constructed social contact networks for both grid supplemented and control types. We first constructed edge lists, where a contact or 'edge' was defined as two individuals occurring at the same or adjacent trapping station within the same trap session (one night). We next constructed undirected social networks for each grid, where each edge was drawn as an unweighted (thickness constant, not weighted by the number of contacts between nodes where n>1 contact) line between the appropriate nodes (individual mice of each grid). Individuals with no contacts during the trapping duration were included in networks as unconnected nodes. Node size was set as proportionate to the number of total captures per individual. We extracted the degree distributions (distributions of number of contacts per node) and used a Wilcoxon rank-sum test to test the mean

differences in degree (number of contacts) within a grids across the supplemented and control resource grid groups.

## 3.4 Results

### *3.4.1 Diverse impacts of resource supplementation on parasite community*

Throughout the course of the experiment, we identified a diverse parasite community harboured by the wild wood mouse populations (Table 3.1). The six most common genera of parasite species/groups were *Heligmosomoides polygyrus* (70% prevalence), *Capillaria murissylvatici* (26%), *Eimeria spp.* (67%), *Ixodes spp.* (100%), mites (29%), *Trypanasoma grosi* (29%), and Wood Mouse Herpes Virus (WMHV; 10%). Individual mice were commonly co-infected with multiple parasite species/groups (median of 2; maximum of 6). Gastrointestinal parasite species richness was significantly different between mice on resource supplemented vs. control grids, where supplemented mice had average of 1.17 parasites species, while control mice have 0.82 (Figure 3.1; Wilcoxon rank-sum test: $p=0.001$). We found no significant difference in ectoparasite ($p = 0.623$), blood-borne ($p = 0.495$) or overall parasite species richness (Figure 3.1; $p=0.108$).

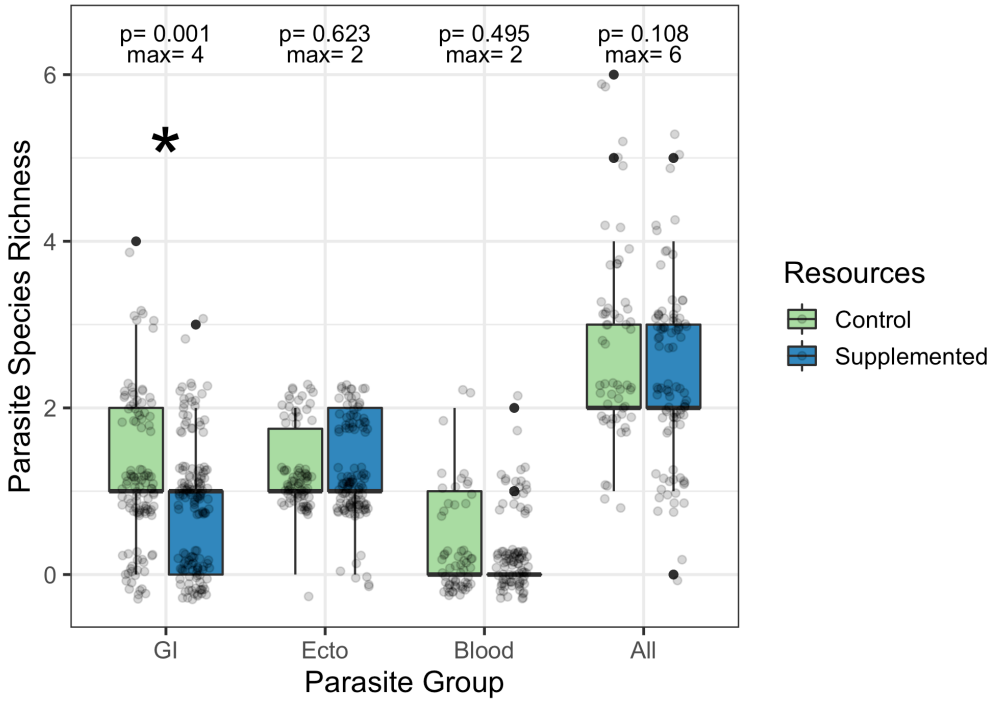


**Figure 3.1. Parasite species richness for parasite groups surveyed in wood mice populations.**

Supplemented mice had lower gastrointestinal parasite species richness than mice on control grids; however, there were no differences in parasite richness for ectoparasites, blood-borne parasites, or for all parasites combined. Boxplots and points represent raw data describing the number of parasites species found within an individual mouse at a single timepoint. The maximum number of species found in a wood mouse and the p-values from Wilcoxon-rank sum tests are reported above each boxplot.

**Table 3.1. The infection prevalence and intensity of the parasite community in the wood mice population.**

| Group | Parasite type | | Method of transmission | Species | Prevalence | Intensity (eggs or oocysts /gram) |
|---|---|---|---|---|---|---|
| Gastro-intestinal | Macroparasites | Helminth | Environmental | *Heligmosomoides polygyrus* | 70% | 44.3 (0 – 1,829) |
| | | | | *Syphacia stroma* | 6.6% | 0.32 (0 – 70) |
| | | | | *Capillaria murissylvatici* | 26% | 90.74 (0 – 8,744) |
| | Acute, microparasite | Protozoan | Environmental | *Eimeria spp.* | 67% | 910.5 (0 -127,185) |
| Ectoparasite | | | Environmental | *Ixodes spp.* | 100% | 5 (0-30) |
| | | | Direct | Fleas | 15.0% | 0.10 (0-6) |
| | | | Direct | Mites | 28.0% | 1 (0-10) |
| Blood-borne | Acute, microparasite | Bacteria | Flea-borne | *Bartonella spp.* | NA | NA |
| | Chronic, microparasite | Protozoan | Flea-borne | *Trypanasoma grosi* | 29% | NA |
| | | Virus | Direct | Wood mouse herpes virus | 10% | NA |

Resource supplementation had significant, diverse effects on the parasite community. The main effect of resource supplementation varied considerably across the parasite taxa found in this study; with food supplementation found to have positive, negative, and even null relationships with probability and/or intensity of infection for a suite of parasites (Figure 3.2; Appendix B, Table S3.1). Specifically, wood mice on supplemented grids had significantly lower mean infection intensity of *H. polygyrus* (EPG; $\beta = -0.73$, SE = 0.30, $p = 0.015$, Figures 3.2, 3.3A) and this effect was independent of anthelminthic treatment. In contrast, there was no main effect of resources on intensity of *C. murissylvatici* infection (intensity: $\beta = 1.41$, SE=0.98, $p = 0.15$, Figures 3.2, 3.3B). We also found that mice on resource-supplemented grids had lower rates of infection with some ectoparasites, but not others. Specifically, tick intensity for individuals on supplemented grids were significantly lower ($\beta = -0.46$, SE = 0.11, $p < 0.001$, Figures 3.2, 3.3G), however, there was a trend (though non-significant) of higher mite infection for supplemented individuals (Probability: $\beta = 0.64$, SE = 0.33 $p = 0.053$) and no effect of resources on fleas (Probability: $\beta = -0.55$, SE = 0.60, $p = 0.355$, Figures 3.2, 3.3H&I).

For the micro-parasites (both gastrointestinal and blood-borne), we found contrasting effects of resource supplementation on infection. We found no main effect of resource supplementation on the intensity of the GI protozoan *Eimeria hungaryensis* (Intensity: $\beta = -0.08$, SE = 1.1, $p = 0.944$, Figure 3.3C) or *Eimeria uptoni* (Intensity: $\beta = -0.66$, SE = 1.2, $p = 0.580$, Figure 3.3D). For blood-borne parasites we found no main effects of resource supplementation on either *T. grosi* or MHV (Figure 3.3E-F; Appendix B, Table S3.1)

### 3.4.2 Resource supplementation interacts with drug treatment to impact non-target parasite infection

We found a resource supplementation-by-anthelmintic drug treatment interaction for several parasite species, when treated individuals responded differently than control mice, based on whether they were on a resource supplemented or control grid. In some instances, there was a significant effect of resources in conjunction with treatment where there were no main effects of resources alone (Figures 3.2 & 3.3, bottom panels). For example, with regard to the gastrointestinal helminth *C. murissylvatici*, individuals who were both supplemented and treated had significantly lower intensity of infection ($\beta$ = -4.99, SE =1.84, p = 0.007, Figures 3.2-3.3K). Similarly, there was a significant interaction of supplemented resources and treatment for *E. uptoni* infection ($\beta$ = -4.99, SE=1.84, p=0.007, Figures 3.2-3.3M). In contrast, the same group had slightly higher *E. hungaryensis* infection intensity after treatment, although this interaction was not significant (Figure 3.2, Appendix B-Table S3.1). Despite a negative main effect of resources on ticks (where infection was reduced by supplemented resources), we found a significant interaction of resources and treatment, where only untreated individuals on supplemented grids showed this reduction, and there was no difference between supplement groups for treated individuals (Figure 3.3P, $\beta$ = 0.57, SE = 0.28, p=0.044). In addition, for the blood-borne parasite *T. grosi,* mice on resource supplemented grids had lower probability of infections if anthelmintic-treated, and higher probability of infection in the control treatment group (Figure 3.3N, probability: $\beta$ = -2.32, SE=1.03, p=0.024).

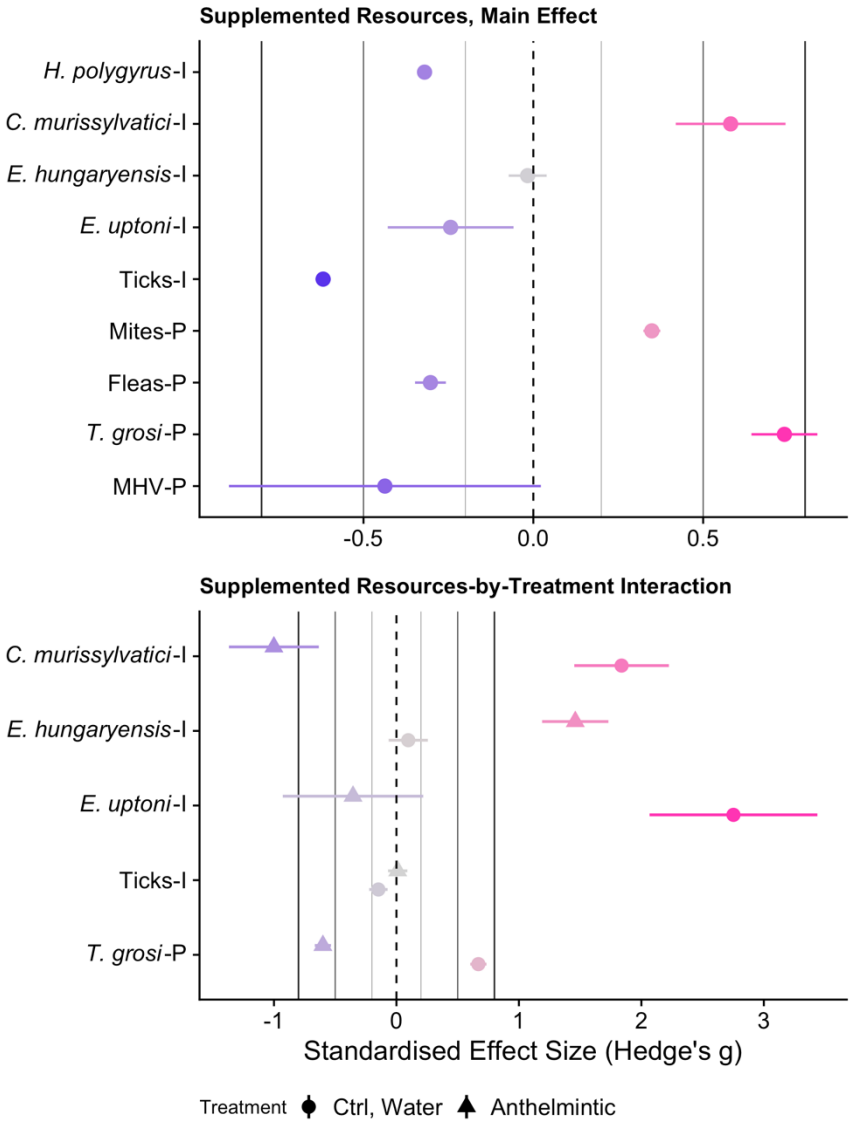


**Figure 3.2. Standardised mean differences (Hedges' g) representing the effect size of resource supplementation (supplemented vs. control).**

Top panel represents resources fit as a main effect in the model and bottom panels represents a resource-by-treatment interaction in a model that only included data beyond first capture. For intensity models (I), Hedges' g was calculated using estimated means from GLMMs, while the for probability of infection (P) models Hedges' g was derived from lnOR values from binomial GLMMs to facilitate comparison across response types. In the top panel, Hedges' g values below zero (dashed vertical line) indicate that the resource supplemented group had lower intensity or probability of infection than mice on control grids, while values above zero represent cases where mice on supplemented grids had higher infection probability or intensity. For the interaction effects (bottom panel), mean resource differences are represented from model estimates for both untreated (water control; circles) and

anthelmintic-treated (triangles) individuals for comparison. Shading gradient indicates the magnitude of the effect, where dark blue represents the most negative, dark pink the most positive, and grey the null effects. The three vertical lines on either side of the dashed zero line represent suggested guidelines for interpretation of Hedges' g, where 0.2 is considered a small effect, 0.5 a moderate effect and 0.8 a large effect, respectively (Lakens, 2013).

### *3.4.3 Resource supplementation alters host condition, behaviour, and demography*

Resource supplementation altered both within- and between-host dynamics in the wood mouse population (Figure 3.4). Specifically, individual mice on supplemented grids had significantly better body condition (body condition index; BCI) than those on control grids ($\beta = 1.87$, SE = 0.63, $p = 0.003$; see Chapter 2). In addition, better body condition was associated with significantly higher concentration of both total and parasite-specific antibodies (total faecal IgA: $\beta = 0.46$, SE = 0.15, $p = 0.003$; *H. polygyrus*-specific IgG1: $\beta = 0.02$, SE = 0.01, $p = 0.01$; see Chapter 2).

We also found evidence for increased investment in reproduction in mice on resource supplemented grids (Figure 3.4). Female mice on supplemented grids had significantly higher body mass (g) during pregnancy ($\beta$=8.51, SE=1.93, $p<0.001$). Furthermore, significantly more juvenile mice were found on resource supplemented grids, compared to manipulated control grids, over the course of the experiments ($\beta = 3.33$ , SE=1.04, p=.001). We also found that resource supplementation altered the contacts between mice within a population (Figure 3.4); contact networks that were constructed for both grid types showed that mice on resource supplemented grids had a higher average degree (number of contacts; median=2.00, max=4.00) versus unmanipulated, control grids (median =1.00, max=3.00; Wilcoxon rank sum, W=1423, p=0.003).

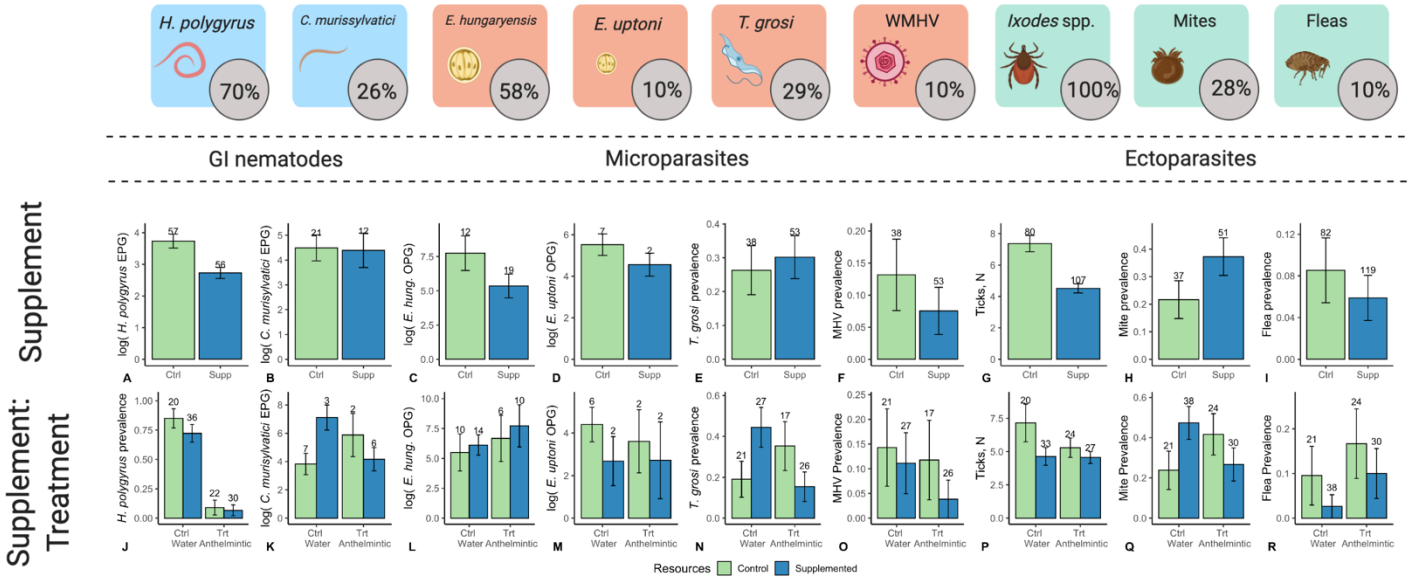


**Figure 3.3. Effects of resource supplementation on gastrointestinal, micro-, and ectoparasites in wild wood mice.**

Each figure (A-O) represents the results of a model run for a specific parasite taxa, where the figures A-I represent models with resource supplementation as a main effect (top panel); while the bottom plan represent effects of resource supplementation according to anthelmintic treatment (J-R) on the dynamics of the wood mouse parasite community. The effects of resource supplementation (blue) versus control (green mice) on parasite infection probability and intensity of infection were highly variable and the bars represent raw means (as indicated) ± SEM. Numbers above bars indicate sample size for each group.

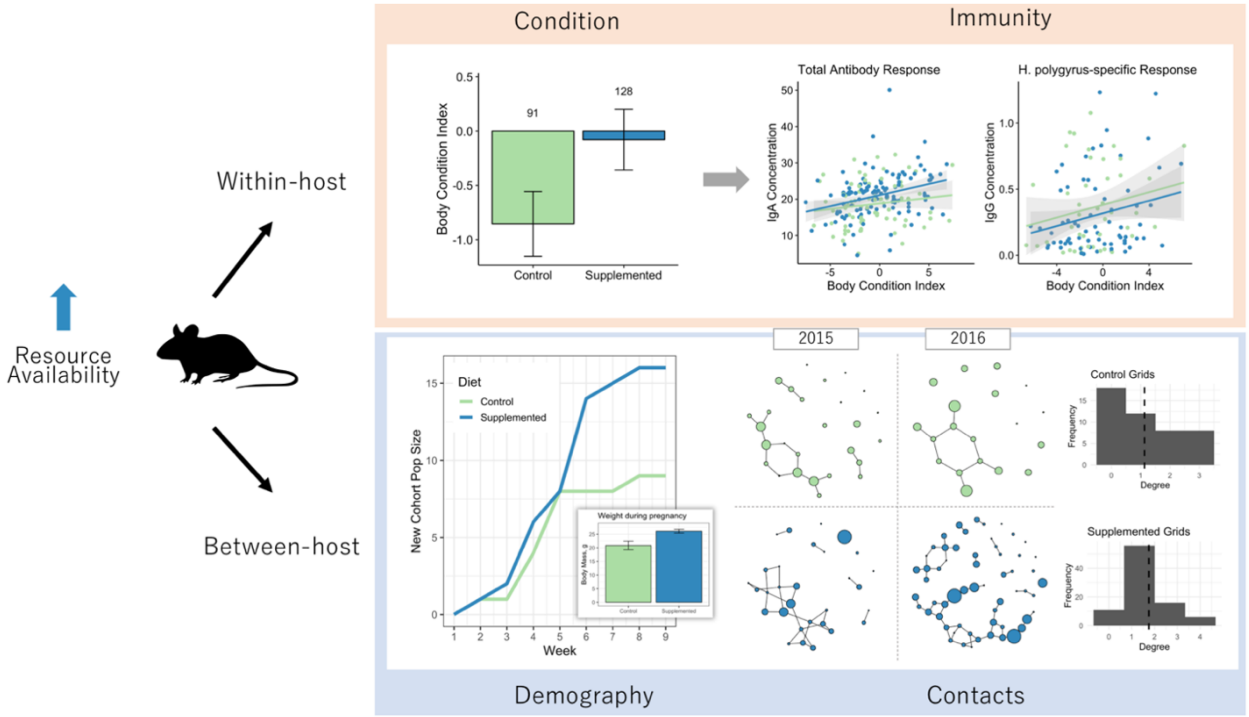


**Figure 3.4. Resource supplementation impacts both within-host (top panel) and between host (bottom panel) processes.**

Body condition index (BCI; weight vs length residuals, raw data ± SEM; top panel, eft) was significantly higher for mice on resource supplemented plots. Total faecal IgA (ng/mL) and standardised H. polygyrus-specific IgG1 had a positive association with body condition index (Model fit regression lines and 95% credibility intervals presented with raw data points, (see Chapter 2, Condition & Immunity shown here for comparison). The main demography plots represents the number of new mice born on control & supplemented grids throughout the trapping period. The inset demography plot represents raw data ± SEM for weight of pregnant females. Contact plots represent contact networks constructed from trapping locations across the experiment and the corresponding degree distributions for the numbers of contacts within both resource groups.

## 3.5 Discussion

By experimentally manipulating resource availability, we found that supplemental resources altered the parasite community dynamics in a naturally co-infected population; but not always in the same direction. Following only a short period of resource supplementation with a high-quality diet, we found positive, negative, and null effects across the 9 most common parasite species in the wood mouse population. Surprisingly, for several parasites the effect of supplemented resources was altered when anthelmintic treatment was administered, indicating that the interaction among parasites themselves may mediate the outcome of resource provisioning in co-infected populations. In addition to the diverse impacts on the probability and intensity of parasite infection, we previously found that resource supplementation impacts body condition and immunity (Sweeny *et al.* 2019; Chapter 2) and reproductive effort, population demography, and the number of contacts between individuals. Broadly, these results imply that that resource provisioning impacts both within- and between-host processes and may be impacting parasite infection both directly and indirectly. Overall, these results suggest that considering the parasite community as a whole is integral to understanding the outcome of how resources and altered nutrition are likely to impact parasites. Importantly, as both accidental and purposeful resource provisioning of wildlife populations is being increasingly common, understanding the response of the parasite community, as opposed to a single parasite of interest, will be important for making appropriate and effective management decisions.

Within a diverse parasite community, we found that mice on resource supplemented grids had lower species richness of co-infecting gastrointestinal parasite species,

however there were no differences in richness of ectoparasites, blood-borne parasites or across the whole parasite community. For specific parasite species' intensity and probability of infection, we found that mice on resource supplemented grids had lower intensities of the gastrointestinal helminth *H. polygyrus*, but that *C. murissylvatici* intensity was lowered in response to supplementation only in conjunction with anthelmintic treatment. However, due to a low prevalence of *C. murissylvatici,* very few individuals were infected post-treatment and results for this parasite should be interpreted with caution. We have previously reported both the effectiveness of anthelmintic treatment and supplemented resources for reduction of *H. polygyrus* intensity as well as the synergistic effects of supplementation with anthelmintic treatment in this field study, and confirmed these results using the same host and parasite in a controlled laboratory study (Sweeny *et al.* 2019; Chapter 2). Other mouse laboratory systems (Slater and Keymer, 1986b; Boulay *et al.*, 1998; Ing *et al.*, 2000) and other experimental work in the wild have shown the benefits of adequate nutrition for resistance to helminths (Diaz and Alonso, 2003; Budischak *et al.*, 2017), however laboratory studies typically manipulate specific nutrients, and those in the wild often mimic natural variation in food sources available in the wild, and therefore likely are not comparable to anthropogenic-sourced food. Results from this experiment therefore support the hypothesis that higher-quality diet enriched with multiple nutrients has a negative effect on helminth infection, but that this may depend on the species of helminth.

Among the broader parasite community, we found a high degree of variation in the both the direction and magnitude of the effect of resource supplementation on the

intensity and probability. For example, among the ectoparasites, we found that mice on resource-supplemented grids had fewer ticks, a trend of higher rate of infection with mites and the same rate of infection with fleas as mice on control grids. Additionally, effects observed post-first capture were altered according to whether the mouse has received anthelminthic treatment or a placebo. After assignment to treatment groups only untreated mice had significantly lower tick infection intensities on resource supplemented grids, while there was no difference in treated mice. This may be because there was a main effect of anthelmintic treatment itself on tick intensity and this was stronger than effects of resources. We have previously shown that this drug treatment is highly effective at removing gastrointestinal nematodes for 12-16 days (Knowles *et al.* 2013, Clerc *et al*. 2018), and confirm this within this population, so within this experiment treated individuals effectively represent an absence of *H. polygyrus,* by far the most prevalent endoparasite in this population.

The complex effects of provisioning also extended to blood-borne microparasites, dependent on whether *H. polygyrus* had been removed. For the flea-transmitted blood-borne protozoan, *T. grosi,* we found contrasting direction of effect in control and anthelmintic treatment groups. Among anthelmintic-treated individuals, probability of infection for supplemented grids is approximately half that of control grids. We find exactly the opposite for the control treatment group (probability of infection for supplemented grids is greater than twice that of controls). Although we saw no effect of supplementation on fleas (the vector of *T. grosi*) in this experiment, fleas of *A. sylvaticus* are often located in the nests (Langley and Fairley, 1982). Our counts are conducted by investigation of the fur to identify ectoparasites after mice

have been trapped overnight. It is therefore possible that we are not detecting the full extent of flea infestation in this population, and the mechanism of interaction observed in this study requires further investigation. These results suggest important and unexpected indirect effects of resource supplementation potentially caused by relationships among the parasite community.

Previous work from our group has shown evidence for a negative relationship netween *H. polygyrus* and the gut protozoan *E. hungaryensis* (Knowles *et al.*, 2013; Clerc *et al.*, 2019b). An experimental perturbation study showed that anthelmintic treatment reveals a negative relationship between *H. polygyrus* and *E. hungaryensis* (Knowles *et al.*, 2013), where the highly effective removal of the GI nematode facilitates increased intensity of the protozoan. Tests of this relationship in the laboratory showed that *H. polygyrus* can suppress *E. hungaryensis* infection intensity (Clerc *et al.*, 2019b). *E. hungaryensis* is notable among *Eimeria spp.* in that it shares an infection niche with *H. polygyrus* and therefore competes for space and resources (Rynkiewicz *et al.*, 2015). In this population *E. hungaryensis* and *E. uptoni* were the two most common *Eimeria* species. We saw no main effect of supplemented resources on *E. hungaryensis* or *E. uptoni,* but contrasting effects of resources on the intensity of infection following treatment, where *E. uptoni* decreased significantly & *E. hungaryensis* had a trend of increasing in supplemented individuals. Previous work from this experimental design has shown that anthelmintic treatment in supplemented individuals clears worms more effectively than for control resource groups (Sweeny *et al.* 2019). The suggestion of treatment-dependent increases in *E. hungaryensis* in this experiment, alongside decreased intensity for a parasite of the

same genus which does not share the same infection niche (*E. uptoni*) (Nowell and Higgs, 1989), may indicate that more effective helminth removal in supplemented individuals is exacerbating a negative relationship between *H. polygyrus* and *E. hungaryensis.* This mechanism would support previous work from the laboratory which show that resource availability dictates the outcome of coinfection between helminths and the microparasite *M. bovis* (Budischak *et al.*, 2015b) and highlights an important example of resource provisioning exacerbating existing relationships within a natural parasite community. Such interactions may be crucial considerations for predicting the outcome of altered resource availability in the wild.

However, infection with *Eimeria spp.* is extremely acute and can be cleared in only a few days, so that intensity of infection can vary dramatically in a short time (Clerc *et al.*, 2019b). Consequently, intensity data from the wild will have a large degree of noise – particularly in this study, where samples are on average one week apart. The acute nature of *Eimeria spp.* infections may further result in spatial as well as temporal noise in infection data. Although we attempted to address variation of this nature by accounting for grid and year in our models, the representativeness of each sample is reduced when exposure is unknown and estimations of *Eimeria spp.* infections in this population show large confidence intervals. Possible relationships between nutrition quality*, H.* polygyrus and *E. hungaryensis* in wood mice would therefore benefit from investigation of these two parasites in a controlled laboratory system may help to elucidate their interaction and the role of resource supplementation in influencing their dynamics.

The conceptual framework for understanding the impacts or resource provisioning on host health, introduced by Becker *et al.* (2015) highlights the potential for changes to both resource quantity and quality to influence parasite infection by both direct effects of altered host condition and immunity on parasites and indirect effects of altered host demography and behaviour to parasite transmission within the population. We found broad effects of experimental resource supplementation on host condition (Sweeny *et al.* 2019 & see Chapter 2), demography, and social contact degree. Given that we find evidence of the impacts supplementation on both within- and between-host processes, our results suggest that impacts of infection may be occurring through both direct and indirect effects on parasites present. Importantly, the outcomes of resource provisioning will likely vary across parasite groups, both in terms of biology and transmission mode, and according to the nature of their impacts to host health and population dynamics (Becker *et al.*, 2015).

Although we highlight effects beyond the individual in this study, there are limitations on the metrics of demography and social contacts used. For example, the number of new individuals recruited to supplemented and non-supplemented grids provides some information on reproductive activity within each grid type, but given the short duration of trapping regimes, it is not clear whether all individuals recruited stay on the same grid or immigrate elsewhere. This can have important implications for density-mediated mechanisms of disease. Likewise, weight gained during pregnancy provides only a rough estimate of reproductive output. Number of offspring or morphological qualities off offspring would provide a more complete picture, but were not feasible to collect in this experiment. Future work will be

needed to monitor larger populations over a longer trapping duration to partition effects of supplementation on demographic and population-level processes for better understanding of mechanism. Future monitoring multiple parasites simultaneously in a single host population longitudinally will provide important complementary knowledge to previous work documenting the interplay between individual- and population- level processes in multiple single host- single parasite species studies or theoretically (Becker and Hall, 2014; Becker *et al.*, 2015). Although we suggest only preliminary explanations for mechanism, to our knowledge this is the first study to consider this complexity within a naturally co-infected host and documented varied infection outcomes for a diverse parasite community in a single host population.

In this study we show high heterogeneity in the outcome of resource provisioning dependent on parasite considered and the presence of specific co-infecting parasites present. Parasite interactions are difficult to study in the wild, and often require perturbation experiments to detect, and disentangle complex direct and indirect relationships (Pedersen and Fenton, 2015). Importantly, we show the potential for within-host interactions to be an important, and understudied, indirect effect that can mediate the outcome of resource provision, often in unpredicted, and negative ways. The possible within-host parasite interactions that we detected within anthelmintic drug treatment and resource supplementation would benefit from further investigation in controlled laboratory settings or within a theoretical framework, but our study serves to highlight the potential role of direct and indirect relationships between parasites in multi-parasite community in mediating the outcome of resource provisioning. Overall, this study is a novel experimental demonstration of the ability of resource availability to shape parasite community dynamics. These results suggest

that the context of the entire parasite community is a crucial consideration for understanding and predicting the impact of resource provisioning on wildlife populations.

# Chapter 4 Long-term effects of resource supplement for wild wood mice and their gastrointestinal parasites

## 4.1 Summary

Resource availability and nutritional quality can play a central role in driving the dynamics of wildlife populations, with impacts on both individual- and population-level factors. Increasingly, the influence of anthropogenic activities has led to new food sources becoming available to wildlife, either intentionally, as part of management programs or accidentally. While there has been empirical research that highlights the impacts of resource provisioning on wildlife, recently further attention has focused on both the direct and indirect implications that changes in resource availability can have for the outcomes of infectious diseases. Previous resource supplementation experiments have been conducted in small mammal populations, and results have provided important, but sometimes contradictory evidence for the benefits of increased nutrition. Several studies show resource supplementation can improve body condition, immunity, and reproduction; however, these benefits are not always found. We have previously shown that in wood mice, supplementing resources with a high-quality diet during peak breeding season can reduce gastrointestinal helminth burdens and increase anthelminthic drug treatment efficacy. However, there is naturally a high degree of variation in energetic demands for individuals throughout the course of their life, and resource availability across seasons and years, both of which will likely influence the outcome of the interaction between resources and infectious disease. Because of limitations in the duration of many field experiments, it is often difficult to determine if the benefits of supplementation are dependent on the characteristics of the individual, population, or site. To address the longer-term impacts of resource supplementation, we conducted a five-month longitudinal supplementation and anthelmintic treatment experiment in

a wild wood mouse population to investigate how these perturbations impact host individual and population factors, and the dynamics of their key gastrointestinal nematode (*Heligomosomoides polygyrus*). We find that the benefits of supplementation on *H. polygyrus*-specific immunity and circulating protein levels were strongest during the peak breeding months (late summer). Additionally, we find evidence that supplemental resources are associated with lower host survival and can alter population-level demographic patterns. Furthermore, previously documented short-term benefits of supplementation on *H. polygyrus* infection were not clear in the longer-term experiment. We analysed data from three years of resource supplementation experiments conducted in wild wood mice populations to investigate the interannual variation in these short-term responses to high-quality diet supplementation. We found that our resource supplementation had strongest effects on reducing helminth infections in the year with the lowest average tree fruit (mast) score, but short-term effects on body condition were consistent across all years. Overall, these results suggest that naturally fluctuating resources and energetic demands can fundamentally change the host responses to experimentally altered resource availability. Understanding this complex interaction will be crucial for considering supplementation strategies for effective wildlife management.

## 4.2 Introduction

Resource availability is a central factor determining wild populations dynamics (Clotfelter *et al.*, 2007) and infectious disease (Johnson *et al.*, 2010). Resource limitation plays a key role in the regulation of wild animal populations due to the energy requirements of reproduction, and increases in resource availability can have important consequences for reproductive activity and demography—processes which

play a key role in disease transmission in the wild (Becker *et al.*, 2015). Mast events—where species synchronise their breeding or emergence by producing very abundant, synchronised crops—can provide an excellent, transient resource pulse that can impact both individual and population dynamics for those species that consume this resource (Ostfeld and Keesing, 2000). Every 3-5 years in the eastern United States, white oaks (*Quercus alba)* and red oaks (*Q. rubra)* produce a synchronised acorn mast across a ~10km area. These mast events can have direct, positive impacts on small mammal populations, where mice tend to extend their breeding cycle throughout the winter and have higher over-wintering survival (Smyth, 1966; Ostfeld *et al.*, 1996; Wolff, 1996). Previous extensive observational studies have shown that the populations dynamics of deer and white-footed mice (*Peromyscus maniculatus* and *P. leucopus*) in the US (Wolff, 1996) and wood mice (*Apodemus sylvaticus*) in the UK (Montgomery 1989) are, at least in part, driven by oak and beech mast events, respectively. Our research group previously demonstrated that experimental resource supplementation to wild *Peromyscus* populations can limit seasonal population crashes (Pedersen and Greives, 2008). Importantly in this study, we found that when resource supplementation was combined with anthelmintic drug treatment that reduces nematode infections, the benefits to the mouse population were enhanced. This suggests that parasites and resources interact in their effects on population dynamics, but the exact mechanism of the effects at the host and population level that mediate these relationships have not often been explored experimentally in the wild.

While there is increasing evidence of the impacts of resource supplementation on both host and parasite dynamics (Becker *et al.*, 2015) it is often difficult to determine whether the changes in infection patterns are mediated through individual or population level responses. A key component of anti-parasite defence is the immune response, but this is a costly process, potentially requiring the diversion of resources from other processes (Sheldon and Verhulst, 1996). There is strong evidence from controlled laboratory populations that resource quality can significantly benefit host immunity, reproduction, and resistance to infection (French et al. 2007, Jones et al. 2012, Cox et al. 2010). For example, in female tree lizards, experimentally increasing reproductive investment in resource-limited conditions resulted in a suppressed immune response; however this result was only detected during energetically demanding reproductive periods (French et al. 2007). Similar reductions in immune investment have been documented in ground crickets when their mating activity was increased (Fedorka *et al.*, 2004). Such trade-offs are often difficult to detect in wild populations, due to variation within and between hosts in acquisition of resources, where individuals that have access to a high quality diet may not exhibit trade-offs because they do not experience any constraints for allocating to multiple costly processes (van Noordwijk and de Jong, 1986). However, several wild studies have demonstrated trade-offs associated with periods of high energetic demand. A recent meta-analysis of wild birds showed increased parasitaemia in four common blood parasites following experimental increases in reproductive effort (Knowles *et al.*, 2009), and in wild red deer the significant costs of lactation have negative associations with strongyle nematode infection and immunity (Albery *et al.*, 2018b). Reliably estimating resource partitioning among

immunity, reproduction, and other processes like survival in the wild is difficult, but using experimental perturbations of both resource availability and parasite infection combined with intense longitudinal sampling of individuals, can enable robust investigation of these complex relationships.

The implications for changes in food availability for parasite infection in small mammal populations have been investigated by experimentally supplementing food with ecologically relevant food sources and assessing the impact on nematode infections, host condition, immunity, and breeding (Diaz and Alonso, 2003; Pedersen and Greives, 2008; Shaner *et al.*, 2018). For example, supplementing *Apodemus sylvaticus* with canary grass seeds increased winter breeding and reduced infection with some gastrointestinal nematodes (*Syphacia stroma* and *Syphacia frederici* pinworms) but not cestodes (*Gallegoides arfaai* and *Hymenolepis diminuta*) or other nematodes (*Gongylonema neoplasticum*) (Diaz and Alonso, 2003), while supplementary sorghum seeds were found to increased litter size in Taiwan field mice (*Apodemus semotus*) (Shaner *et al.*, 2018). In addition, we previously showed that supplementation with a high-quality diet (laboratory mouse chow) in wild wood mice populations increased resistance to an important gastrointestinal nematode, *Heligosomoides polygyrus,* while improving mouse body condition and the adaptive immune response (Chapter 2). However, because these studies are often conducted over a short period of time, occur in only one site, or have a single temporal replicate, it is difficult to interpret the longer term impacts to host individuals and the population dynamics, as well as to the parasite community (but see (Pedersen and Greives, 2008). It is therefore often unclear if the results of supplementation

experiments occur only in the context of very limited natural resources, or what role parasites play in the effects of resource availability on population dynamics.

Understanding these complex interactions is becoming increasingly important, as anthropogenic influences are rapidly altering the quality and quantity of food sources available to wildlife (Oro et al. 2013). Despite a large body of work dedicated to understanding and predicting the outcome of these changes for infectious disease in the wild (Becker and Hall, 2014; Becker *et al.*, 2015; Civitello *et al.*, 2018; Strandin *et al.*, 2018), very few of these studies are able to simultaneously consider natural and artificial fluctuations in resources. In *A. sylvaticus*, significant effects of temporal and spatial variation in tree seed availability have been found to impact dispersal patterns in populations studies, where the relationship between seed availability and dispersal declined within year from winter to summer and was weakest in years of high population size (Montgomery *et al.*, 1991). However, it is unclear how natural spatiotemporal variation in food availability may influence the impacts of experimental resource provisioning. In addition to differences across years, within-year seasonality can have broad consequences for host and parasite community dynamics (Altizer *et al.*, 2006) For example, seasonal changes in temperature, humidity and precipitation can directly affect exposure to parasites via climatic effects on survival of transmission stages in the environment (Altizer *et al.*, 2006). Seasonal fluctuations in resource availability and the seasonal demands of reproduction can also impair host susceptibility (Nelson:1996hn; Martin *et al.*, 2008). Overall parasite dynamics will be shaped both by direct impacts on individual exposure and changes in susceptibility, as well as seasonal changes and population traits, such as changes to population size and transmission potential for parasites

following recruitment of new individuals after breeding periods (Montgomery and Montgomery, 1988; Altizer *et al.*, 2004; Begon *et al.*, 2009). However, how seasonality will affect the outcome of resource provisioning on host and parasite dynamics in the wild is still poorly understood (Becker *et al.*, 2015). Untangling the possible confounding between these processes and resource availability requires either experimental perturbation or longitudinal sampling of both parasitological and immunological outputs.

Here we investigate the long-term effects of experimental resource supplementation in wild wood mouse (*Apodemus sylvaticus*) populations in Falkirk, Scotland, in order to investigate how the effects of supplementation may be dependent on dynamic processes such as reproductive cycles, age structure of populations, and naturally fluctuating resource availability. Wood mice have a seasonal breeding pattern, beginning in the spring and continuing throughout the autumn. Increased population sizes following the breeding season can face a survival bottleneck in the winter, when resources are scarce. Those that survival to the next year become the first group of breeding adults. New individuals born in the spring or early summer will mature and beginning reproducing the same year, but those born in the autumn typically delay reproduction until the following year. These patterns suggest the benefits of resource supplementation on mouse body condition and reproduction, and their subsequent knock on effects on parasites, may be dependent on the time of the year and the breeding condition and sex of the mouse.

Wood mice are exposed to and infected/co-infected with a diverse community of macro- and micro-parasites, including gastrointestinal parasites, bacteria, protozoans, viruses, and ectoparasites (see Chapter 3). Importantly, wood mice have a high prevalence of a well-studied, immunomodulatory gastrointestinal nematode, *Heligmosomoides polygyrus,* and within this population we have previously shown rapid effects of high-quality resource supplementation, including increasing *H. polygyrus* resistance, severe reductions in *H. polygyrus* transmission potential, increasing anthelmintic drug efficacy, improving host condition and adaptive immunity (Chapter 2), changing population demography through increasing reproductive investment, and unpredictable changes to the rest of the parasite community (Chapter 3). Here we build on our previous work, which was focused on the short-term implications of supplementation and anthelmintic treatment to address three questions: (1) What are the longer-term impacts of resource supplementation and anthelmintic drug treatment on parasitism, immunity, host condition, and demography? (2) Are the effects of resource supplementation within a year dependent on season, host age, or parasite infection/coinfection? And (3) How do inter-annual fluctuations of natural resource availability alter the impacts of experimental supplementation on host dynamics and *H. polygyrus* infection? We find that in this longer-term experiment, supplemented resources did not significantly impact *H. polygyrus* abundance, though transient anthelmintic treatment was highly effective at lowering abundance for the entire trapping duration. However, we provide evidence that supplemented resources still have significant impacts on host condition, immunity, and demography, resulting in short-term positive effects on host condition and increase reproduction, but lower overall survival. In addition, by

collating three years of data from resource supplementation experiments, we find that there is interannual variation in the effects of increased resource availability on *H. polygyrus* infection; and this interaction may be dependent on the naturally available food abundance and quality. These results provide important evidence that the outcome of experimental resource supplementation is linked to natural biological variation in the wild – which may impact how resource supplementation can impact host-parasite dynamics and immunity, and that short-term effects of supplementation do not necessarily represent the full picture for host populations.

## 4.3 Methods

### *4.3.1 Field Experiments*

We conducted field experiments in a 100ha broodleaf woodland in Falkirk, Scotland (Callendar Wood, 55.990470, -3.766636). We trapped wild populations of wood mice across three years in 2015, 2016, & 2017. In 2015 and 2016 we trapped an 8-week experimental period from June-August (detailed in Chapter 2 and 3). In 2017, we extended the trapping season to 17 weeks from July-November. The 2015 and 2016 replicates represented 'short-term' experiments in which all animals were followed for 2 weeks and then sacrificed at 12-16 days after first capture for destructive sampling for parasitology. However, the 2017 field experiment represented the 'longer-term' dynamics; in which all animals were followed for as long as possible within the full 17 weeks of trapping and were not sacrificed to allow investigation of host reproduction and survival.

For all three experimental replicates, we used a 2 x 2 factorial design, where resource supplementation was manipulated using a high-quality, whole-diet food chow (hereafter simply "resources") at the population level (unit – trapping grid; control (unmanipulated) vs. supplemented), while anthelmintic treatment (hereafter simply "treatment") was manipulated at the individual level (unit – mouse; control (water) vs. treatment; Fig. 4.1). We trapped three grids in 2015 (1 supplemented and 2 control) and four grids in both 2016 and 2017 (2 supplemented and 2 control). Grids were set up as rectangular arrays with 2 traps/station, 10m between each trap (2015: 7x7 array, 2016: 6x5, 2017: 7x5). In all years, grids were spaced a minimum of 50m from each other to minimise mouse movement between grids, and grids were randomly assigned to resource regimes groups. New grids were formed, prior to the start of each annual experiment to minimise the effects of the previous years experiment.

We supplemented each grid for three weeks prior to the start of trapping, and then throughout the experiment - twice per week with 2kg/ 1000$m^2$ of sterilised, TransBreed™ mouse chow pellets, scattered at regular intervals across the grids to ensure an even spatial distribution. TransBreed™ is a high-nutrient, standard veterinary feed which is formulated for optimum breeding performance in laboratory mice and offers whole-diet nutrition to the wild mice in this study (20% protein, 10% fat, 38% starch, high content of micronutrients, full details in Table 1.1), therefore our supplementation complemented natural food availability. We live-trapped mice for 3 nights/week in 2015-16 and 2 nights per week in 2017 using Sherman live traps (H.B. Sherman 2x2.5x6.5-inch folding trap, Tallahassee, FL, USA). Each trap contained cotton wool bedding, and was baited with seeds, carrot, mealworms, and

TransBreed™ pellets (on supplemented grids only), set in the early evening (16.00-18.00) and then checked early the following morning. All wood mice above 13 grams were tagged at first capture with a subcutaneous microchip transponder for identification (Friend Chip, AVID2028, Norco, CA, USA). On both control and resource-supplemented grids, all mice at first capture were rotationally assigned within each sex to either control or drug treatment groups. We administered a single 2ml/g dose of Pyrantel pamoate (Strongid-P, 100 mg/kg) and Ivermectin (Eqvalan, 9.4mg/kg) to each mouse allocated to the anthelminthic group, a combination dose shown to be highly effective at removing both adult and larval *H. polygyrus* from wood mouse for 12-16 days in our previous work (Clerc *et al.*, 2019a). In short-term replicates of 2015-16, mice were sacrificed 12-16 days post first capture for destructive sampling to count *H. polygyrus* worms in the small intestine. In our long-term replicate (2017), treatment was re-administered at the same dosage 4 weeks after first capture/treatment and mice were followed for as long as they were recaptured within our 17-week trapping period.

For each mouse at every capture we measured: sex, age, and host condition including body mass, length, fat scores, and reproductive status (see Chapter 2 for more details). Blood samples were collected via mandibular bleed (first capture; maximum of once per 4 weeks) or tail snip (subsequent captures; a maximum of once per week) from which serum was separated by centrifugation at 12,000 rpm for 10 minutes and then stored at -80°C. Faecal samples were collected for each mouse at every capture from previously sterilised traps and preserved in 10% formalin. In addition, 2-3

pellets from each faecal sample were stored at -80°C for faecal IgA antibody measures.

### 4.3.2 Mast data

'Mast' describes the fruit of trees which can serve as a food source for small mammals and other wildlife. Years of above-average seed crops are termed a 'mast-year', and these pulses of resources have widespread consequences in an ecosystem (Ostfeld *et al.*, 1996). We used data provided by Nature's Calendar to quantify mean tree fruit score of oaks and beech, two common species within Callendar Park for each year (details in Appendix D and at: https://naturescalendar.woodlandtrust.org.uk). We fit general linear models (GLM) using mean fruit score as a response variable, with year and tree species as explanatory variables. Tree fruit score was ranked on a scale of 1-5 (1-no fruit, 2-meagre, 3-moderate, 4-good crop, 5-exceptional).

### 4.3.3 Laboratory Analysis

*H. polygyrus* abundance was measured as eggs per gram of faeces (EPG). using salt flotation and microscopy as described in Chapter 2. Briefly, saturated salt solution was added to formalin-preserved faecal samples to concentrate eggs on a coverslip, which were counted at 10X magnification, and the abundance adjusted by sample weight to give EPG.

We used Enzyme-Linked Immunosorbence Assays (ELISAs) to measure (1) total faecal IgA concentration and (2) serum *H. polygyrus*-specific IgG1 antibody titres for each mouse at each capture/sampling point as previously described (Chapter 2).

We calculated total faecal IgA concentration by extrapolation from a standard curve of known concentrations from a synthetically manufactured standard antibody. H. polygyrus-specific IgG1 was calculated as a relative concentration to a positive reference sample consisting of sera from *Mus musculus* experimentally infected with *H. polygyrus* in the laboratory. Plates were prepared with serial dilutions of reference and experimental samples, and a dilution factor of 1:200 was selected for calculation of relative antibody concentrations. Standardised IgG1 concentrations were calculated by plate as follows: (Sample OD1:200- Mean Blanks)/ (Positive reference OD1:200-Mean Blanks). We assigned a value of 0 to samples for which the OD did not exceed 3x SD of control blanks. We refer to both IgA and IgG1 values as 'antibody concentration'.

Nutritional status was assessed by quantifying circulating serum albumin concentration from the serum samples. Serum albumin is a dynamic and long-lived plasma protein which has important functions for multiple physiological roles (Garnier *et al.*, 2017). Assays were optimized from (Garnier *et al.*, 2017) for use on mouse samples using samples from our University of Edinburgh colony of formerly-wild wood mice. Samples for the serum albumin assays were diluted 1:4 in Mill-Q water and 5uL was added to each well and adjusted to a total volume of 50uL with albumin buffer. 100uL of bromocresol green reagent (prepared according to kit guidelines, Biovision Albumin (BCG) Colorimetric Assay Kit) was added to each well. Plates were shaken for 45", incubated at RT for 20' and read at 650nm. Samples for total protein assays were diluted 1:60 in Milli-Q water and 10uL of sample was added to each well. 300uL of Coomassie Reagent (Thermoscientific

Coomassie Plus Bradford Assay) was added to each well. Plates were shaken for 45", incubated at RT for 10' and read at 570nm. Samples were run in duplicate for both assays. Two standard dilutions of known concentrations of BSA were included for each plate in both assays. Standard curves were fit using a 4-parameter logistic regression and sample concentrations were determined by plate using the standard curves. Concentrations were corrected by sample dilution factors and expressed as ug/uL for analyses.

### *4.3.4 Statistical Analysis*

All statistical analysis was carried out in R Version 3.6.0 (R Core Team, 2019). All models were fit using the package 'glmmTMB' [1], model selection was carried out using the package 'buildmer' [2], and post-hoc tests were carried out with package 'emmeans'[5]. We used two Model Sets to investigate: 1) intra-annual variation of resource supplementation effects on parasite infection, host condition, and immunity within a single year (2017) in which individuals were followed over a 120-day period and 2) inter-annual consistency of short-term resource supplementation effects on *H. polygyrus* count EPG from 2015-2017. Additionally, using 2017 data we investigated the effects of supplementation on observation period length as a proxy for survival. Model sets are described below and in Table 4.1.

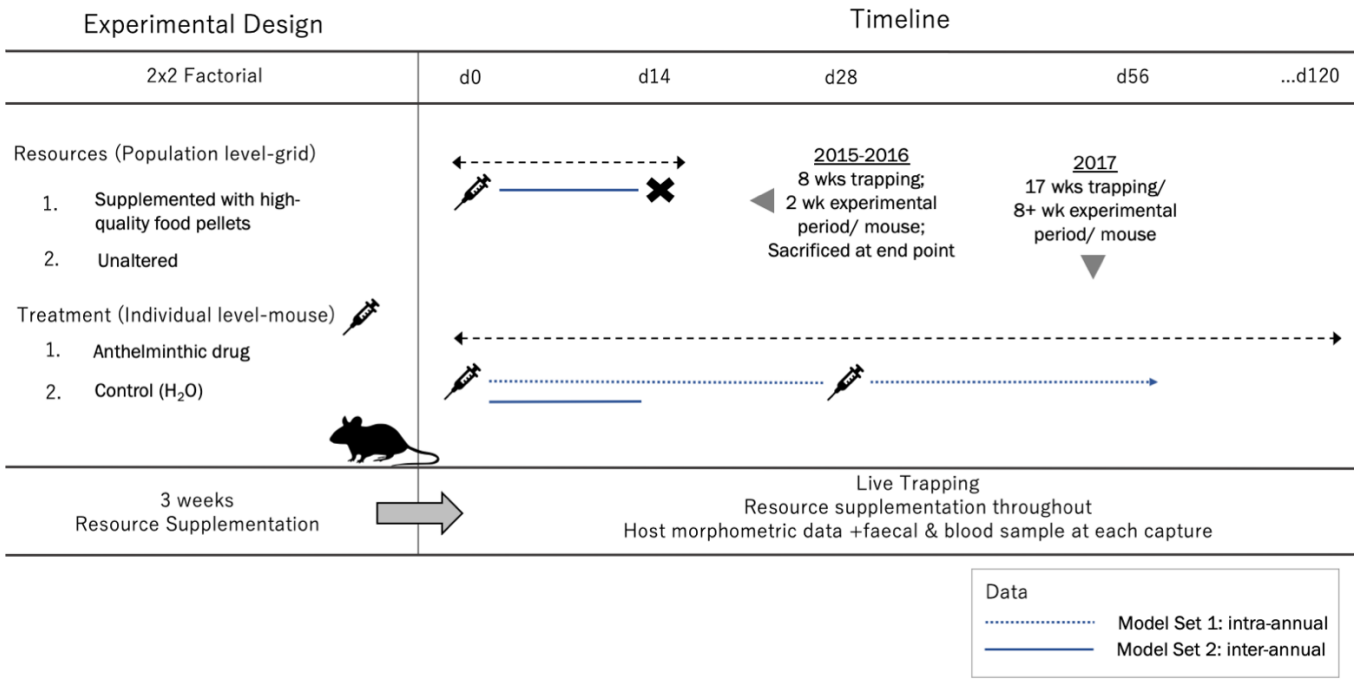


**Figure 4.1. Experimental design of resource supplementation experiments from 2015-2017.**

Briefly, we used a 2x2 factorial design (two resource groups at the population level; two treatment groups at the individual level) in all years.. In years 2015-16, treatment or water control was administered once upon initial capture (day 0; d0), and individuals were followed for two weeks (±2 days) and sacrificed at the end of the experimental period. In 2017, treatment or water control was administered at first capture (d0) and then again four weeks later (±2 days), and were trapped as long as possible or until the end of the trapping season (d120). Data from the longer-term field experiment of 2017 was analysed in Model Set 1, and data from all three annual field experiments was analysed in Model Set 2.

### *4.3.5 Model Set 1 – Long-term effects of resource supplementation*

Within our 2017 field experiment, we trapped mice from July-November which covered two 'seasons' (Summer: July & August; Autumn: September, October, November). In the spring of each year, *A. sylvaticus* populations are comprised primary of adults who have survived the winter and represent the breeding adults for the new year. Breeding typically begins in late spring and continues through the Autumn, with the highest population sizes occurring in early Autumn. In our experimental populations in 2017, the numbers of new individuals recruited to the population began to rise from week 35. Analysis of capture history and body size indicates that from week 35 onward, individuals entered into the experiment were of substantially lower body weight at first capture (mean 15g versus mean 21g prior to week 35). Therefore, we suggest that data from this experiment represents two birth 'cohorts', a 'first cohort' of older wood mice that survived the winter and are breeding the following spring/summer, and a 'second cohort' of young mice, born in the summer, who are breeding in the same year as their birth. These two cohorts will differ in many ways in the wild, but specifically the first cohort will only have had supplemental resources available for one part of their life, where the second cohort of mice may have experienced supplemented resources from birth. We classed individuals entered into the experiment from week 35 on as the second cohort, resulting in a total of 54 individuals in the first and 55 in the second cohort (Appendix C, Figure S4.1).

Using data from our 2017 field experiment, we investigated intra-annual variation in resource supplementation effects according to season, cohort, and anthelminthic treatment (109 individual wood mice captured 479 times). We fit 7 total models to

investigate resource supplementation on to response variables of: parasite infection (*H. polygyrus,* abundance), host condition (body condition index (BCI), weight/length residual), immunity (*H. polygyrus*-specific IgG1 (standardised) and total faecal IgA (ng/mL, square-root transformed)) ,host nutritional status (serum albumin (μg/μL, log-transformed)), and host demography (survival (observation length), and reproduction (new births)). For parasite, immunity, and nutritional status models we first performed forward model selection adding fixed effects and interactions to a minimal model with only resources (control-unaltered/ supplemented), treatment (water control /anthelminthic drug), and an individual ID random effect. Fixed effects were sex (female/ male), reproductive status (binary, inactive/active body condition index (BCI, scaled), body length (mm, scaled) and year (2015, 2016, 2017). The exceptions were condition models, which were not fit with BCI or body length as explanatory variables, and immunity models, which were fit with additional fixed effects of *H. polygyrus* infection status and the ELISA block (replicate, n=2) in which plates were run (Table 4.1). Continuous fixed effects were scaled to have a mean of zero and a SD of 1 to aid interpretability. Interactions tested were: resources by season (summer or autumn), resources by cohort (first or second cohort), and resources by treatment. Cohort and season were included as fixed effects as well. Each term was added one-by-one to obtain an order of effects and evaluated by the change in AIC to the model. A reduction of >2AIC was taken as the threshold to improve model fit. In each additional model addition, the fixed effect or interaction (if any) which improved model fit most was retained in the model, and this was repeated until no interactions improved the model.

**Table 4.1. Model structure for short- and long-term effects of resource supplementation.**

| Model Set | Data Replicate | Response | Transformation | Experiment period represented | Model Class | Model Family | Fixed Effects | Interactions Tested | Random Effects |
|---|---|---|---|---|---|---|---|---|---|
| 1 Long-term | 2017 | *H. polygyrus* abundance EPG | rounded to nearest integer | Full trapping period 17 weeks | GLMM | Negative Binomial | Sex + Body condition index + Body length + Reproductive status +Treatment+ Resources + Season | resources:season resources:cohort resources:treatment | ID |
| | | *E. hungaryensis* abundance EPG | | | | | | | |
| | | *Ixodes spp.* Burden | | | | | | | |
| | | Body condition index | | | | Gaussian | Sex + Reproductive status + Treatment + Resources + Season | | |
| | | *H. polygyrus*-specific IgG1 | | | | | Sex + Age + Body condition index + H. polygyrus infection + Experiment Timepoint + Resources + Season + ELISA Block | | |
| | | Total Faecal IgA | square-root | | | | | | |
| | | Serum albumin | log-transformed | | | | Sex + Age + Body condition index + H. polygyrus infection + Experiment Timepoint + Resources + Season | | |
| | | New individuals recruited | | | GLM | Negative Binomial | Resources + Trapping week | | |
| | | Survival Days observed | | | | | Sex + Age + log(*H. polygyrus* EPG) + Resources + Day of First Capture + Treatment | resources:treated | |
| 2 Short-term | 2015-2017 | *H. polygyrus* abundance EPG | rounded to nearest integer | First Capture + 12-16 days | GLMM | Negative Binomial | Body Mass + Reproductive status + Sex +Resources + Year | resources:year | ID |
| | | Body condition index | | | GLMM | Gaussian | Reproductive status + Sex +Resources + Year | | |

Demographic models did not represent longitudinal sampling but rather absolute numbers of either new individuals recruited over the trapping period or survival (observation length). We therefore did not approach these models with the same interaction testing. We fit a GLM for each demographic response variable with terms as detailed in Table 4.1. We included resources and the week of trapping season as fixed effects for recruited individuals and sex,

Age, *H. polygyrus* abundance (log EPG+1), day of first capture (continuous, scaled), treatment, and resources. We also tested a resource by treatment interaction based on previous evidence of synergistic effects of these two perturbations (Chapter 2).

### *4.3.6 Model Set 2 – Short-term effects of resource supplementation*

Because individuals were sacrificed 12-16 days post first capture in 2015-16, we used data from first capture through a maximum of 16 days post capture from all three-year replicates to investigate variation in short-term supplementation effects on *H, polygyrus* infection. This dataset was comprised of 159 individuals captured 481 captures over 16 days. We fit two models using *H. polygyrus* EPG (abundance) and body condition index (BCI; weight/ length residuals, scaled) as a response variable negative binomial and gaussian error structure respectively. We included the following fixed effects as explanatory variables: sex, reproductive status, treatment, BCI, resources—all as detailed above—year (factor: 2015, 2016, 2017), and a year-by-resources interaction. Where a significant interaction with year was found, we used a Tukey post-hoc comparison test to investigate the differences between multiple levels of the interaction.

## 4.4 Results

### *4.4.1 Supplemented resources have both short- and long-term effects on host condition, immunity, and demography*

Throughout our 2017 longer-term field experiment, we captured a total of 109 individuals, with first capture incidences that spanned the full trapping period, and these wood mice were captured 479 times over 17 weeks. We found that reproductive activity varied across the experiment, with the number of reproductively active (scrotal) males and pregnant or lactating females peaking in the late summer (August) and then declining throughout the remainder of the experiment (Appendix C, Figure S4.2).

For Model Set 1 (intra-annual variation), we found that for 4/5 response variables (parasitism, immunity and condition), interactions of supplementation with either season, cohort, or treatment (or multiple interactions) significantly improved model fits, $\Delta$AIC>2, and were retained in final models of infection and host dynamics over our long-term dataset (Table 4.2).

In our short-term models and in contrast to our previous research (Chapters 2 & 3), we found no main effect of supplemental resources on *H. polygyrus* EPG for our longer-term 2017 field experiment (GLMM: $\beta$= 0.80, SE=0.62, p=0.20). However, a resource supplementation-by-season interaction significantly improved model fit ($\beta$= -1.74, SE=0.73, -0.017), and we found that while wood mice on control grids did not have significantly different *H. polygyrus* abundance across seasons (Tukey post-hoc comparison, p=0.73), individuals on supplemented grids had lower *H. polygyrus* EPG abundance in the autumn than in the summer (Tukey post-hoc comparison,

p=0.018, Figure 4.2A). We detected several other significant predictors of *H. polygyrus* EPG infection (Table 4.2). As expected, anthelmintic treated individuals had significantly lower *H. polygyrus* abundance (β= -2.67, SE = 057, p <0.001; Appendix C Figure S4.2). Additionally, the second cohort of mice had lower abundance than those in the first cohort (β= -1.76, SE = 0.71, p=0.014). There was a significant positive main effect of resource supplementation on body condition index (β= 0.22, SE=0.09, p=0.02), and these effects were not dependent on season, cohort, or treatment. Additionally, reproductively active individuals had significantly better body condition than inactive (β= 0.44, SE=0.11, p <0.001), and the mice in the second cohort had significantly worse body condition than the first cohort (β= -0.48, SE=0.15, p = 0.001).

Models of *H. polgyrus*-specific IgG1 and total faecal IgA were significantly improved by resource supplementation-by-season and resource supplementation-by-cohort interactions, respectively (Figure 4.2B-C). There was a trend of higher IgG1 concentration for supplemented grids in the summer (Tukey post-hoc comparison test, p=0.064), but this trend was not supported in the autumn (Tukey post-hoc comparison test, p=0.71). There was no significant differences among mice on resource supplementation and control grids for either cohort for total faecal IgA. However, the difference between control and supplemented grids had a very modest change in direction across cohorts, where the second cohort had higher IgA on supplemented grids and the first cohort had slightly lower IgA on supplemented grids (Figure 4.3C). We also found higher concentrations of IgG1 in autumn compared to summer for all mice (β= 0.43, SE=0.13, p<0.001), and a positive correlation of IgG1

with both higher body condition (β= 0.27, SE=0.06, p<0.001) and larger (longer body length) individuals (β= 0.40, SE=0.07, p<0.001). However, anthelminthic-treated individuals (β= -0.37, SE=0.14, p=0.009) and reproductively active individuals (β= -0.25, SE=0.10, p=0.016) had lower concentrations of IgG1. Total faecal IgA was likewise significantly higher in larger (longer body length) individuals (β= 0.18, SE=0.06, p=0.03) and significantly higher in autumn compared to summer (β= -0.31, SE=0.11, p=0.006). There was also significant variation across blocks of the ELISA assay runs for IgA (β=1.43, SE=0.1, p<0.001), and block was therefore retained in models to account for this variation.

An interaction of resource supplementation with season significantly improved the model fit for serum albumin, used here as a marker of nutrition. As with total faecal IgA and cohort, marginal means indicate that although there is no significant difference among mice on either resource supplemented or control grids in either season, estimated mean differences changed direction from summer to autumn. Mice on supplemented grids went from slightly higher to slightly lower levels of circulating serum albumin compared to control grids (Tukey post-hoc comparison test, summer: β = -0.41, SE=0.36, p = 0.216; autumn: β = 0.34, SE=0.35, p = 0.33; Figure 4.3D). Additionally, individuals in the second cohort compared to the first cohort had higher levels of circulating albumin (β=0.82, SE=0.32, P=0.011).

Supplemented resources significantly increased reproduction as measured by the number of new births observed on supplemented compared to control grids (Figure 4.4B, β = 0.92, SE = 0.21, p <0.001). New births also increased significantly as the

field experiment progressed (Figure 4.3B, week number (scaled): β = 0.95, SE = 0.15, p <0.001). However, we also found negative effects of supplemented resources for wood mouse survival over the course of the experiment, when using total length of time observed (log, days) accounting for first capture date as a proxy for survival (Figure 4.3A, β=-1.66, SE=0.64, p=0.009). No other fixed effects were significant predictors of survival.

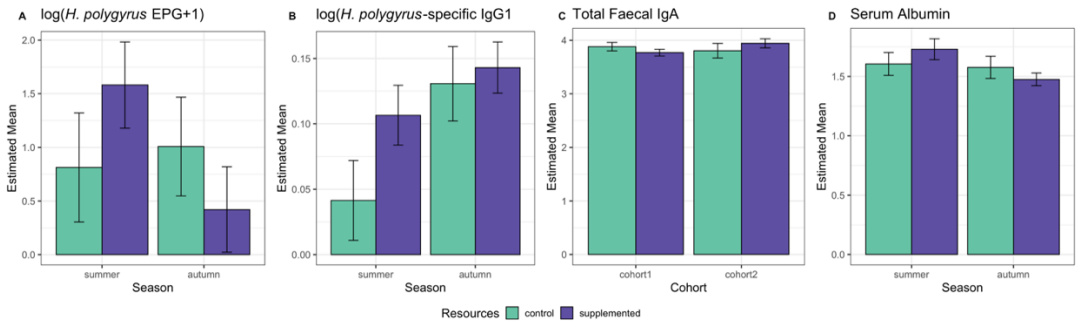


**Figure 4.2. Estimated means for interactions with resources which improved model fit.**

Interactions which improved model fit for GLMMs fit to the response variables A. *H. polygyrus* abundance, B. *H. polygyrus*-specific IgG1, C. Total faecal IgA D. Serum albumin. Bar plots represent estimated marginal means ±SE.

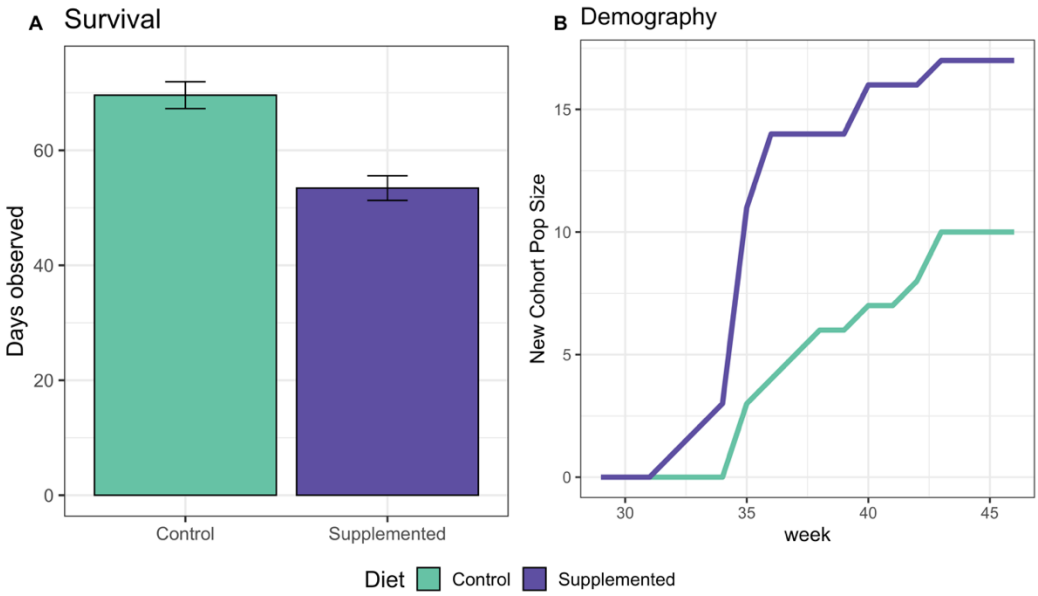


**Figure 4.3. Effects of resource supplementation on host survival (A) and demography (B).**

(A) Survival is represented using a proxy of the maximum length known alive (days observed) during trapping, with raw means ±SEM. (B) New cohort population size is representing the number of new individuals recruited on grids of both resource types per week over the full 17 weeks of the experiment.

**Table 4.2. Model estimates from final models following forward selection.** Blank cells represent cases where a term was not retained in the final model; 'na' cells represent cases where a term was not fit in a given model. Bolded p-values indicates significance at p<0.05.

| | ***H. polygyrus* EPG** | | **Body Condition Index** | | ***H. polgyrys*-specific IgG1** | | **Total Faecal IgA** | | **Serum Albumin** | |
|---|---|---|---|---|---|---|---|---|---|---|
| Term | Estimate | p | Estimate | p | Estimate | p | Estimate | p | Estimate | p |
| Resources, Supplemented: Season, Autumn | -1.74 (-3.16 to -0.32) | **0.017** | | | -0.05 (-0.12 - 0.02) | 0.133 | | | -0.23 (-0.52 - 0.07) | 0.133 |
| Resources, Supplemented: Cohort, New | | | | | | | 0.25 (-0.08 - 0.58) | 0.138 | | |
| Resources, Supplemented | 0.8 (-0.42 - 2.02) | 0.197 | 0.22 (0.03 - 0.4) | **0.02** | 0.07 (0 - 0.13) | 0.063 | -0.11 (-0.29 - 0.07) | 0.234 | 0.12 (-0.09 - 0.34) | 0.252 |
| Treated, Anthelminthic | -2.67 (-3.79 - -1.54) | **<0.001** | 0.01 (-0.19 - 0.21) | 0.931 | -0.08 (-0.14 - -0.02) | **0.009** | 0.06 (-0.1 - 0.21) | 0.464 | -0.04 (-0.18 - 0.1) | 0.583 |
| Season, Autumn | 1.03 (-0.09 - 2.16) | 0.072 | -0.11 (-0.55 - 0.32) | 0.61 | 0.09 (0.03 - 0.14) | **0.001** | -0.19 (-0.32 - -0.05) | **0.006** | -0.03 (-0.27 - 0.21) | 0.814 |
| Cohort, New | -1.76 (-3.16 - -0.36) | **0.014** | -0.48 (-0.76 - -0.19) | **<0.001** | -0.07 (-0.15 - 0) | **0.059** | -0.08 (-0.39 - 0.24) | 0.639 | 0.25 (0.06 - 0.44) | **0.011** |
| BCI (scaled) | 0.35 (0 - 0.7) | 0.052 | na | na | 0.06 (0.03 - 0.08) | **<0.001** | | | | |
| Body length (scaled) | | | na | na | 0.08 (0.06 - 0.11) | **<0.001** | 0.11 (0.04 - 0.19) | **0.003** | | |
| Sex, Male | | | | | | | | | | |
| Reproductive, active | | | 0.44 (0.23 - 0.66) | **<0.001** | -0.05 (-0.09 - -0.01) | **0.016** | -0.05 (-0.17 - 0.08) | 0.478 | | |
| ELISA Block | na | na | na | na | | | 0.86 (0.74 - 0.99) | **<0.001** | | |
| Intercept | 1.92 (0.89 - 2.94) | **<0.001** | -0.16 (-0.39 - 0.08) | 0.188 | 0.14 (0.08 - 0.21) | **<0.001** | 3.53 (3.35 - 3.72) | **<0.001** | 1.5 (1.34 - 1.67) | **<0.001** |

### *4.4.2 Short-term effects of supplemented resources on H. polygyrus EPG show high inter-annual variation*

Over the three years sampled in this experiment, mean tree fruit scores varied significantly across years (Figure 4.4A, GLM observation year 2017: β= 0.98, SE= 0.35, p=0.0046; observation year 2016: β= 0.49, SE= 0.27, p=0.071). Mean fruit score in 2017 was significantly higher than in 2015 but not 2016 (Figure 4.4A, Tukey post-hoc comparison, p= 0.016).

Across our short-term, multi-year dataset, we found that effects of resource supplementation on *H. polygyrus* EPG abundance varied according to year (Figure 4.4B GLMM supplement-by-year - 2017: β = 2.16, SE = 0.92, p = 0.019), and there was significantly lower estimated mean EPG in 2017 (β = -2.01, SE = 0.73, p = 0.0057). Notably, means of *H. polygyrus* abundance for control grids in 2017 were comparable to supplemented grids in 2015-16 (Figure 4.5A). We also found significant main effects of treatment and reproductive status across the entire three years, where anthelminthic-treated individuals had significantly lower EPG than individuals given water controls (β = -1.75, SE = 0.38, p<0.001), and reproductively active individuals had consistently higher *H. polygyrus* EPG than reproductively inactive mice (β =0.66, SE = 0.10, p < 0.001). Short-term effects of supplemented resources were consistent across years (resources (β = 0.30, SE = 0.12, p= 0.015). There were additional main effects of sex, where males had significantly lower BCI compared to females (β = -0.33, SE = 0.12, p= 0.007) and reproductive status, where actively reproductive individuals had higher body condition compared to inactive (β = 0.66, SE = 0.10, p < 0.001).

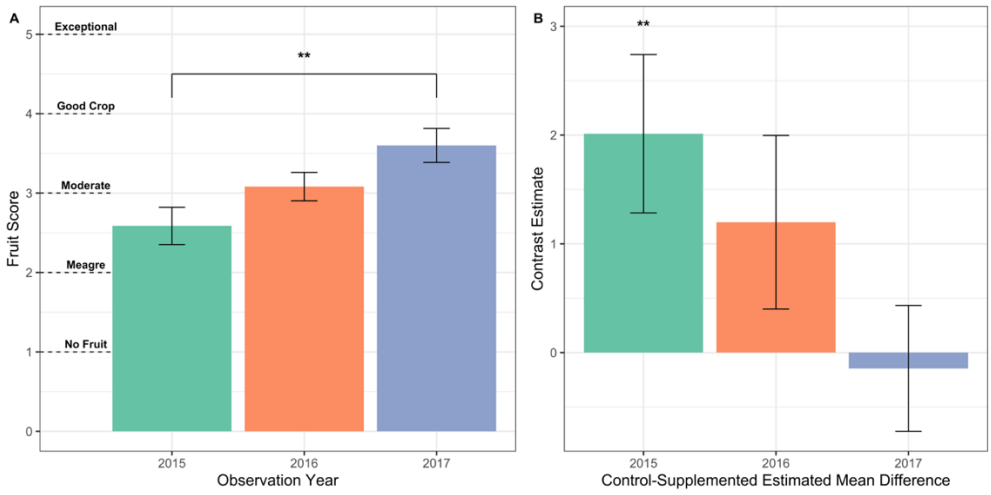


**Figure 4.4. Variation of naturally available resources and effects of experimentally supplemented resources over three, year replicates.**

A. Mean fruit scores within Scotland, UK from 2015-2017. Bars represent raw mean data ± SEM. Comparison bar and asterisks designate significance of Tukey post-hoc comparison tests for all pairs of years. B. GLMM estimated mean differences between *H. polygyrys* abundance for control and supplemented resource grids, where zero would equal no effect of supplemented resources. Asterisks designate significance between contrasts from Tukey post-hoc comparison test for resource groups at each year level.

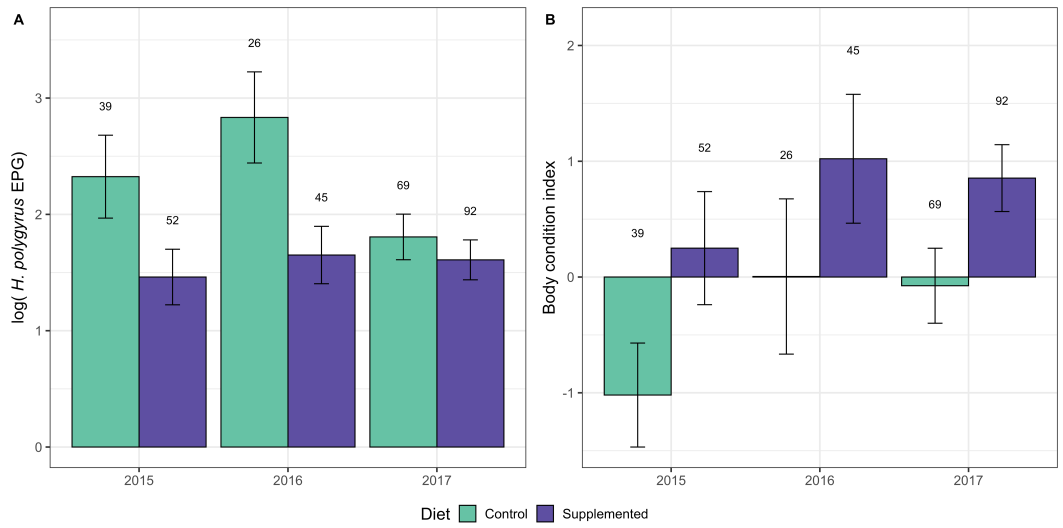


**Figure 4.5. Short-term effects of supplemented resources on (A) *H. polygyrus* abundance and (B) host body condition (raw data).**

(A) Barplots represent raw means ± SEM for A. *H. polygyrus* abundance (log(EPG+1), infected only), which exhibited inter-annual variation in effects of supplementation and (B) host body condition (BCI; weight/length regression residuals) which exhibited only a main, positive effect of supplementation.

## 4.5 Discussion

We carried out a long-term resource supplementation and anthelmintic treatment field experiment which incorporated intensive longitudinal sampling of individual wood mice during and after the peak breeding season in a Scottish woodland. We found that our perturbations impacted key host demographic patterns and gastrointestinal nematodes, which supports previous studies in small mammal populations (Flowerdew, 1972; Diaz and Alonso, 2003; Pedersen and Greives, 2008;

Forbes *et al.*, 2014; Shaner *et al.*, 2018), but here we pair investigation of demographic effects with finer scale host effects. Anthropogenic resource provisioning in wild populations likewise impacts infectious disease through population- and host-level processes (Becker and Hall, 2014; Civitello *et al.*, 2018), but it is unclear how natural variation in resource availability and spatiotemporal fluctuations in host population processes might influence the outcome of additional resource provisioning. Here we show that within a single year, the effects of sustained longer-term supplementation with a high-quality diet are not always positive, but rather are dependent on season and the age of the individual. Importantly, the impact of supplementation can actually switch directions from beneficial to apparently harmful, dependent on these extrinsic and intrinsic variables. Furthermore, we show that the effects of experimental resource supplementation in a wild wood mouse population can vary dependent on naturally occurring food availability. Specifically, we found that the benefits of short-term supplementation on *H. polygyrus* infection varied across years, and was correlated with the tree fruit score, such that when natural resources may be plentiful, the impacts of supplementation on hosts and their parasites may be reduced. These results suggest primarily that individual traits such as age and environment traits such as season and tree fruit abundance can mediate, and ultimately, determine the broader parasitological, immunological, and demographic effects of resource provisioning for wild population.

Our field experiment which simultaneously perturbed both resource availability and nematode infection suggests that the outcome of these changes is dependent on

sometimes complex relationships between multiple variables. Specifically, we found that by fitting interactions of resource group with season, cohort, and treatment, we show that both season and host age mediate the effects of resource supplementation in wood mice. Both *H. polygyrus* abundance and *H. polygyrus*-specific IgG1 were higher for individuals on supplemented grids in summer, however in autumn, mice in both supplemented and control grids were comparable. We have previously demonstrated that supplementation with a high-quality supplement increases adaptive immunity, body condition, and resistance to the nematode *H. polygyrus* (Sweeny *et al.* 2019; Chapter 2). Importantly, we detected these effects rapidly, within a relatively short timeframe of approximately two weeks, and only in summer. Surprisingly, the results of this current experiment only found a positive impact of supplementation on reducing *H. polygyrus* in autumn, but not summer, which is contradictory to our previous supplementation experiments (Sweeny *et al.* 2019; Chapter 2). One hypothesis for this discrepancy among year replicates is that the degree to which the supplemental resources offer benefits for resistance may vary according to how the level of resource-limited in the population. This hypothesis is supported, in part, by our interannual results which showed that the differences in *H. polygyrus* abundance between mice on control and supplemented grids was greatest in the year of lowest tree fruit score (2015), and lowest in the year of highest tree fruit score (2017).

Higher *H. polygyrus*-specific IgG1 on supplemented grids observed in this long-term season in summer, but not autumn, does not have an immediately clear explanation. Antibody measures in the wild are difficult to interpret because without exposure history, it is not possible to determine whether a high antibody response represents

simply a host forced to respond to repeated parasite challenge or whether host is eliciting a stronger, more protective immune response (Pedersen and Babayan, 2011; Gilbert *et al.*, 2013). In a wild ungulate, parasite-specific IgA responses were found to correlative negatively with abundance of the gastrointestinal nematode, *Teladorsagia circumcincta* (Albery *et al.*, 2018b); however previous work by our group in wood mice has shown a positive correlation between *H. polygyrus*-specific IgG1 titre and probability of infection, which we had hypothesised indicated that these antibodies may be best suited as makers of exposure/infection (Clerc *et al.*, 2018). Further work using both wild and controlled laboratory studies of wood mice and *H. polygrus* infection may provide a better indication of whether these antibodies are playing a protective role.

There are a few possible explanations as to why supplemented individuals have higher IgG1 in summer only, when *H. polygyrus* abundance was higher for supplemented individuals. Seasonal variation in immune responses is a common phenomenon for wild animals (Nelson and Demas, 1996; Dowell and Ho, 2004). Cyclic variation in immune activity can be driven by environment (Nwaogu *et al.*, 2019), seasonal reproductive activity (Martin *et al.*, 2008), or may be indicative of seasonal variations in exposure to parasites (Stromberg, 1997; Altizer *et al.*, 2006). We suggest two primary mechanisms that may explain our observed patterns of *H. polygyrus* abundance and *H. polygyrus*-specific IgG1 and why the impact of resource supplementation may dependent on the season. The first hypothesis is that *H. polygyrus* specific IgG1 levels simply reflect *H. polygyrus* abundance for mice on both grid types and in both seasons. This is partially supported by our results, given

that in the summer we find both higher levels of *H. polygyrus* abundance of *H. polygyrus* specific IgG1. In this scenario, overall increases of parasite-specific IgG1 from summer to autumn may simply reflect increasing exposure as time goes on. The significant negative effect of treatment on *H. polygyrus*-specific IgG1 further supports this hypothesis that antibody levels reflect the abundance of *H. polygyrus,* as we have found that our drug treatment was highly effective at reducing infection in this population.

The second possible hypothesis is that there is an underlying mechanism that antibody concentrations in summer are indicative of *H. polygyrus* abundance patterns in the autumn. It is possible that despite higher *H. polygyrus* abundance on resource supplemented grids earlier in the year (summer), that the increased resources during the peak of the breeding season enable individuals to mount a stronger immune response to *H. polygyrus,* which is reflected in the significantly lower abundance from summer to autumn on supplemented grids. The changes in peak IgG1 is not instantaneous, and occurs approximately 21 days after infection (Clerc *et al*., 2019b). Furthermore, *H. polygyrus*-specific IgG1 acts on larval stages of the parasite by blocking maturation to adult stages (Hewitson *et al.*, 2015). This may mean that the effects of increased IgG1 levels on *H. polygyrus* abundance would have a time lag before a reduction in egg shedding of adult worms would be documented, which would support this second possibility. Additional work will be required to resolve the mechanism underlying these results, particularly given the addition of effects found in this study for reproduction and demography.

We observed significantly increased reproductive activity on resource supplemented grids in our longitudinal sampling, as indicated by the birth of new mice on supplemented grids. Previous work investigating the longer-term effects of resource availability have typically focused on broader population dynamics; including reduced population crashes (Pedersen and Greives, 2008), and the recruitment of new individuals (Flowerdew, 1972). Interestingly, in contrast to common findings of alleviation of population crashes (Pedersen and Greives, 2008; Forbes *et al.*, 2014) and increased population size following supplemented food (Flowerdew, 1972), we found that individuals on supplemented grids had significantly lower observation length, suggesting that supplementation had a negative effect on survival. This may be indicative of a cost or trade-off incurred from increased reproductive or immune activity during summer for supplemented individuals. However, observation length may be influenced by trapping probability as well as survival. Behavioral responses to resource supplementation may alter trapping probability, so observation length results should be regarded as preliminary and further work incorporating additional longitudinal trapping to estimate both capture probability and survival is warranted. Though the mechanism is not clear, this preliminary result does highlight the important of considering both individual- and population-level processes.

Evidence from empirical and theoretical work investigating the effects of anthropogenic provisioning to wildlife highlight the importance of considering both the individual and population level effects, as population level effects can 'overwhelm' individual-level benefits in condition or immunity and result in higher infection rates due to increased aggregation or population densities (Becker and Hall,

2014; Becker *et al.*, 2015; Moyers *et al.*, 2018). At the within-host level, mice that are in an actively reproductive state had lower *H. polygyrus*-specific IgG1, suggestive of a general cost of reproduction for the adaptive immune response. Increased IgG1 levels during summer (which was peak breeding time during this trapping season) may therefore reflect a relaxation in the limited resources to be allocated between immunity and reproduction. Though we cannot conclusively infer this from out data, host level effects of increased condition and immunity may play a role in costs to survival and downstream population effects.

Increases in population size observed in this study will have fundamental effects on the age structure of the population. *H. polygyrus* typically shows consistent seasonal patterns, where infection abundance is often lowest when population sizes are largest, which is due to the fact that there are a high proportion of young mice that are less likely to be exposed or infected (Gregory *et al.*, 1990). These seasonal changes after breeding and altered age structure may have played a role in the lower *H. polygyrus* abundance found in the autumn in mice on supplemented grids. Furthermore, throughout the longer trapping season of 2017 individuals were regularly recruited to the population, but not all were tagged. Because our data and interpretation in this study is restricted to data collected from tagged individuals on trapping occasions, there may be population-level movement and transmission processes contributing to *H. polygyrus,* immunity, and condition results observed in this experiment.

Population-level changes to age structure and density may have additional important consequences for non-target parasites beyond *H. polygyrus* as well, so it is possible

that the interaction of individual- and population-level effects over seasons will result in outcomes of co-infecting parasites that disagree with those seen in the short-term (Chapter 3). We suggest that this type of longer-term experimental resource supplementation while monitoring the broader parasite community after an anti-parasite perturbation paired with longitudinal monitoring is an ideal extension to advance understanding of fine-scale relationships between resource availability and host-parasite dynamics in the wild.

In addition to the complex predictors of *H. polygyrus* -specific IgG1, both non-specific total faecal IgA and serum albumin measures were determined by resource supplementation interactions with cohort and season, respectively. However, differences among the levels were more modest compared to *H. polygyrus* abundance and IgG1, and these interactions may represent spurious improvements to model fit. IgA is a common antibody of mucosal surfaces (Mulcahy *et al.*, 2004; Macpherson *et al.*, 2012) and ,is likely to indicate overall immune activation. The only significant predictors of total faecal IgA concentration and serum albumin were wood mouse body length and cohort; where longer individuals had higher total IgA and second cohort mice had higher levels of circulating albumin, respectively. This is most likely indicative of older individuals accumulating exposure to a number of antigens which may have stimulated an IgA response. Previous work in Soay sheep has shown age-dependent relationships with the allocation of plasma proteins, where older individuals benefit from maintaining higher nutritional plane (plasma protein levels) and younger individuals benefit from investing in immunity (Garnier *et al.*, 2017) . Wood mice represent a very different system to ungulates, with much faster

pace of life and differences in helminth epidemiology within the populations. The second cohort of wood mice in this system represents younger individuals, and therefore higher circulating proteins in these individuals may be indicative of an age-specific priority, but this is speculative and will require addition timepoints of sampling for serum albumin.

Building on previous work investigating the effects of resource supplementation on *H. polygyrus* infection and host condition, we have carried out both experimental resource supplementation and sustained removal of a key nematode and monitored parasitological, immunological, and demographic responses. We have shown that although supplemented resources improved host body condition consistently, effects on *H. polygyrus* infection vary intra- and even interannually. Furthermore, long-term monitoring revealed that benefits of supplementation for parasite-specific immunity was limited to the summer. In conjunction with observed effects of increased reproductive activity and decreased survival in our long-term experiment, these results suggest that short-term benefits of condition can have downstream costs and implications for population structure driven contributions to parasite transmission. Overall, this study highlights that the responses to resource manipulation in the wild are subject to variation from both biological variation within a population environmental conditions and understanding these complex relationships will be important if we are to predict the implications for wildlife and their parasite community when subjected to intentional or accidental resource supplementation.

# Chapter 5 A long—term wild study reveals important spatiotemporal variation in the drivers of parasitism

## 5.1 Abstract

Host-parasite interactions in nature are highly context-dependent, with parasite infection dynamics being driven by a wide range of factors occurring across several ecological scales, from individual to ecosystem. The importance of each of these drivers, in isolation and combination, are unclear because they can vary profoundly across space and time, and practical limitations to sampling designs can bias inferences. Here, we used a long-term longitudinal (repeated captures) dataset of >1000 individual wood mice (*Apodemus sylvaticus*) spanning 6 years of sampling across 5 different woodland field sites. Using this extensive dataset which followed mice throughout their lives, we aimed to determine how both intrinsic and extrinsic factors drive infection intensity of a highly prevalent and important gastrointestinal nematode *Heligmosomoides polygyrus*. Season, host body condition, and sex were the three most important determinants of infection intensity, but notably the strength and even direction of their effects varied in time, but not in space. We also show that using longitudinal (repeated-sample points) datasets, in which we could control for within-individual variation provided better estimates of the drivers of parasite infection intensity, then when restricted to using cross-sectional (single-sample point). These results highlight the importance of sampling regime design in ecological studies. Furthermore, they suggest that embracing rather than simply controlling for spatiotemporal variation can reveal important insight into host-parasite relationships in the wild.

## 5.2 Introduction

Host-parasite interactions in the wild are highly context-dependent, with a wide range of intrinsic and extrinsic factors driving the dynamics. Potential drivers range from large-scale environmental factors such as seasonal fluctuations (Nelson and Demas, 1996; Dowell, 2001; Altizer *et al.*, 2013) or geographic variation (Davies and Pedersen, 2008; Tompkins *et al.*, 2011) to host-level factors such as sex (Zuk and McKean, 1996), age (Plowright *et al.*, 2017), or nutritional status (van Noordwijk and de Jong, 1986; Sheldon and Verhulst, 1996) and even include within-host effects such as co-infection (Cox, 2001; Fenton and Pedersen, 2005). Many ecological studies of infectious disease are limited in their spatiotemporal replication and sampling breadth, so such variation is difficult to detect and ability to determine whether the drivers of infection are consistent across space and time is limited. These limitations can have important consequences on understanding infection disease dynamics, but this area remains understudied (Becker et al., 2019).

Disease ecologists generally seek to understand drivers of parasite dynamics, yet results are often equivocal across studies, systems, and temporal or spatial replicates. For example, host body condition – a widely used metric suggested to be a proxy of fitness—is typically thought to be negatively correlated with parasite infection. However, a recent meta-analysis of >500 body condition - parasite infection relationships demonstrated high heterogeneity in both strength and direction, with a high proportion of null patterns (Sánchez *et al.*, 2018). Anti-parasite perturbation experiments (e.g. via drug treatment) have provided insight into the dynamics of host-parasite relationships, but the results have also been shown to be context-dependent. Impacts of parasitism and treatment efficacy have been found to vary

from year to year, between host groups (e.g. sex, age), and according to presence of co-infecting parasites (Pedersen and Fenton, 2015). In a ten-year study of red grouse (*Lagopus lagopus*), survival and reproduction (clutch size and hatching success) were greater in animals with experimentally reduced helminth burdens from drug treatment, but the magnitude of these effects varied in magnitude from year to year and the effects were only detected as statistically significant in 2 of 6 years sampled for survival and 3 or 4 years for reproduction (clutch size and hatching success respectively (Hudson *et al.*, 1992). These examples highlight important, common problems in the study of disease ecology: specifically, low detection ability of, or mixed evidence for, hypothesised relationships in natural populations, and spatial or temporal variation across sampling replicates. Thus, quantifying this spatiotemporal variation in the drivers of parasitism allows us to address two fundamental questions: (i) what are the key factors driving host-parasite variation over space and time, and (ii) how should we best sample within these populations to detect important biological variation within a system?

Few studies have systematically addressed the above questions simultaneously. Many ecological studies face practical sampling limitations, precluding simultaneous investigation of spatiotemporal factors such as seasonality, inter-annual variation, and geographical location. Consequently, it is not clear how often disease ecology studies experience biased inference emerging from the chosen sampling resolution. Investigating the relative contributions from multiple ecological scales, such as environmental and host factors, typically requires multiple years and multiple sites. For example, data collected from dace (*Leuciscus leuciscus*) across 3 years and 8

sites demonstrated that host and environmental effects differentially influence ectoparasite burden and pathogenicity, and that relationships were inconsistent across years (Cardon *et al.*, 2011). Similarly, sampling across four annual cycles of 530 tropical Common Bulbuls (*Pyconotus barbatus*) revealed that occurrence of rainfall, and not breeding stage, drive seasonal differences immune function (Nwaogu *et al.*, 2019). These studies make a strong case for the use of carefully structured statistical approaches and well-replicated sampling to disentangling the complex drivers of parasitism. However, while both studies collected multiple samples within a population over time, they focused on single samples per individual and single parasite species. There is therefore still a need for studies involving longitudinal sampling at the individual level to investigate the roles of environmental, host, and parasite interaction factors in shaping parasitism over spatiotemporal scales in wild systems (Clutton-Brock and Sheldon, 2010).

Helminths—large parasitic worms including nematodes, cestodes and trematodes—are important parasites of humans, domestic and wild animals and livestock. Helminths of wild mammals represent a valuable study system for addressing these complex relationships. The ecology of helminths has been extensively studied in many wild systems (Anderson and May, 1982; Grenfell *et al.*, 1995). Most gastrointestinal helminths live within their hosts, but typically have developmental stages of eggs and/or larvae which are shed into the environment via the faeces where these infectious stages are encountered by susceptible hosts (Keymer, 1982). Infection is typically chronic, morbidity is often related to the number of worms (infection intensity) within the host, and importantly patterns of prevalence and intensity can be driven by both environmental factors (i.e. season, weather,

geography (Stromberg, 1997; Abu-Madi *et al.*, 2000)) and host traits (i.e. sex, age, condition(Behnke *et al.*, 1999; Ferrari *et al.*, 2004)) . For example, helminth infection often peaks in more temperate months (Keymer and Dobson, 1987; Stromberg, 1997), but this can vary considerably across species (Albery *et al.*, 2018a). Similarly, helminth burdens have also been shown to vary with host traits such as sex common for helminths in natural populations (Poulin, 1996), and higher body condition (in this instance slower weight loss) has been shown to increase reproductive fitness in the face of high worm burdens (Hayward *et al.*, 2014b).

Wild wood mice (*Apodemus sylvaticus*) are widely distributed European rodents with distinct seasonal population cycles that are commonly infected with *Heligmosomoides polygyrus*, an extensively studied gastrointestinal helminth (Gregory 1992). Numerous previous studies have demonstrated variation in *H. polygyrus* parasitism according to environmental factors (Langley & Fairley 1982; Montgomery & Montgomery 1988; Gregory 1992; Brown *et al.* 1994; Abu-Madi *et al.* 2000; Eira *et al.* 2006), host factors (Gregory *et al.* 1990; Ferrari *et al.* 2004), and parasite interactions (Behnke *et al.* 2005; Knowles *et al.* 2013). Many of these studies, however, are limited in temporal replication, and focus on either a single year or single sampling points per indiviudal over multiple years, and to the best of our knowledge no studies simultaneously measure the impact of factors across three scales (environment, host, within-host).

Here we use a 6-year, 5-woodland site wood mouse dataset to investigate determinants of *H. polygyrus* infection intensity among environmental factors

(season), host factors (body condition, sex, age, reproductive status) and co-infecting parasites (gastrointestinal parasites). We first use the full aggregated dataset (which includes both singly- and multiply-sampled individuals) to investigate average effects of selected fixed effects on *H. polygyrus* intensity. We next fit year- and site-level interactions to ask whether the magnitudes and/or directions of the focal effects varied over space and time, and whether there are significant differences in the slopes of effects across spatiotemporal environments. Next, we sub-sampled the dataset to spatial 'single site' and temporal 'single year' replicates to investigate the variation in what conclusions we would make about the drivers of infection, if each replicate was a stand-alone study, as is often the case for wild host-parasite studies. Lastly, we tested how robust our conclusions were from cross sectional (single sample per individual) datasets, specifically to determine if multiply sampling individuals improves the ability to detect biological relationships in the wild. We predicted that infection intensity would be considerably variable between years and sites, and that the slopes of the effects would be dependent on the spatiotemporal environment, especially when limited to cross-sectional data. We found considerable spatial and temporal variation in *H. polygyrus* infection intensity, and clearly demonstrate that the key drivers of infection (season, sex, and body condition) vary in magnitude and direction across space and time, and that repeated sampling can increase confidence in interpretation of these results as biological (rather than methodological) variation. This highlights the importance of accounting for spatiotemporal variation for understanding ecological processes in the wild and when designing sampling regimes.

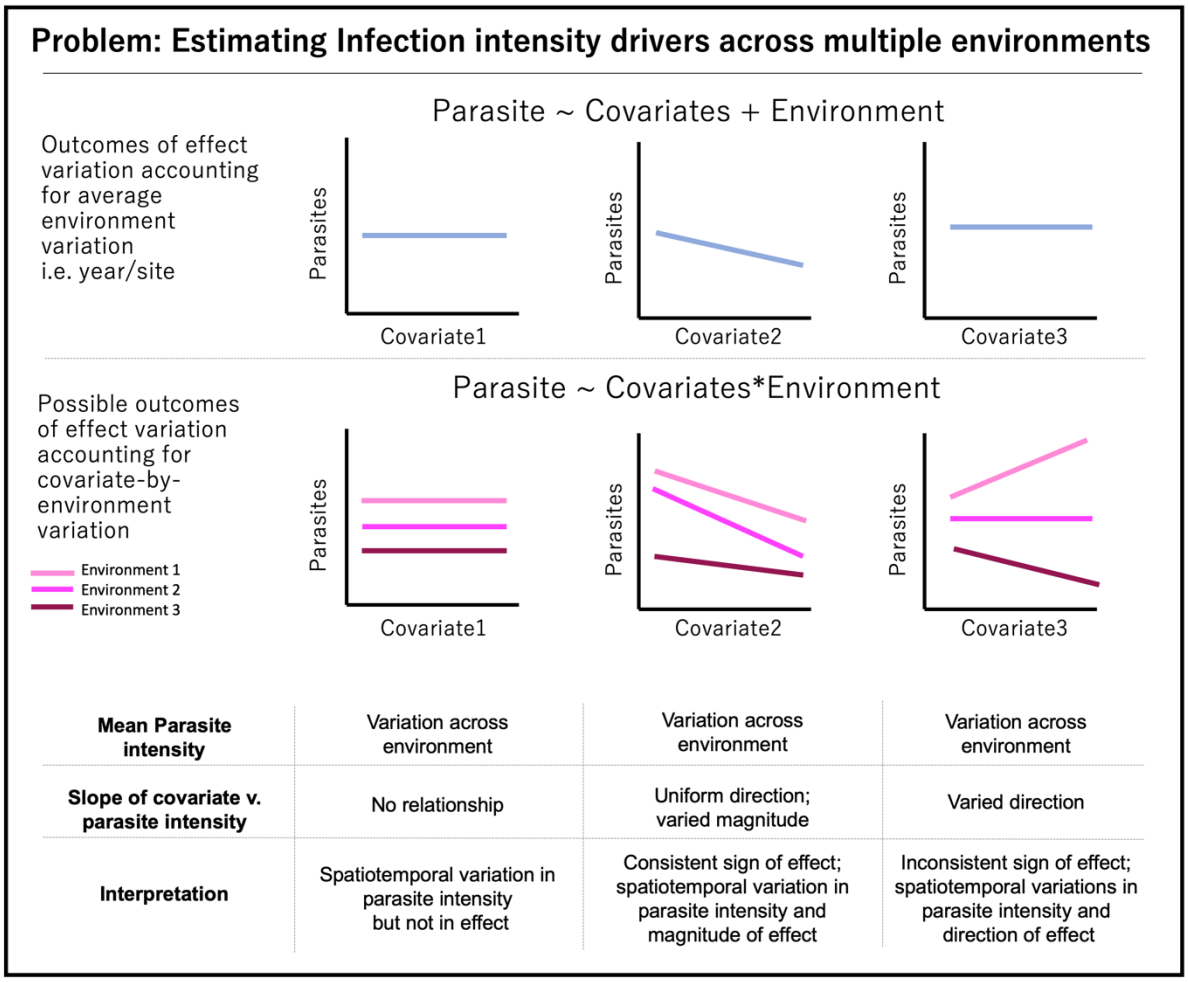


**Figure 5.1 Statistical approaches for analysis of the drivers of infection intensity across multiple environments.**

## 5.3 Methods

### *5.3.1 Data Collection*

Wild wood mouse populations located near Liverpool, UK were trapped regularly between May-December for six consecutive years (2009-2014). We sampled 19 distinct trapping grids ranging in size from 2500m$^2$-10,000m$^2$, spread across six different woodland sites (Appendix D, Table S5.1; Figure S5.1). On each grid, trapping stations were placed every 10m apart in a grid, with two live traps (H.B. Sherman 2x2.5x6.5 in. folding traps, Tallahassee, FL, USA) at each station baited with grains and bedding material. We sampled each grid every 4 weeks from 2009-2011 and every 3 weeks between 2012-2014) with each grid trapped 3-4 nights per week. Traps were set in the late afternoon and checked the following morning. At first capture, we tagged each mouse with a subcutaneous microchip transponder (AVID, PIT tag), and then for all captures, we recorded morphometric data (age, sex, weight, body length, reproductive condition; details below), collected a faecal and small volume blood sample, and examined the fur to record ectoparasite (ticks, fleas and mites) presence and intensity. Faecal samples were collected from the pre-sterilised traps, weighed and stored in 10% buffered formalin at 4°C until parasite identification.

We quantified gastrointestinal parasites (including *H. polygyrus*) via faecal egg counts using a salt flotation to obtain number of eggs or oocysts per gram (EPG/OPG) (Knowles *et al.*, 2013). Within *A. sylvaticus* EPG correlates highly to adult worm burdens for *H. polygyrus* (Chapter 1, Figure 1.3). Briefly, saturated salt solution was added to the faecal samples and eggs/oocysts floated to the top collected on a coverslip and examined at 10X magnification (identified to species at

40X magnification). We identified *Eimeria* to species according to unsporulated oocyst morphology (Nowell and Higgs, 1989). Raw counts were divided by the weight of the faecal sample to obtain a standardised value used in subsequent analyses.

### *5.3.2 Statistical Analysis*

#### Defining model variables and dataset

We investigated how host-, environmental- and parasite coinfection-related factor drive *H. polygyrus* intensity (EPG from infected animals only). In four of the six years of sampling, we conducted experiments in which anthelminthic treatments and controls (given an equal dose of water) were carried out on randomly selected mice to remove/reduce gastrointestinal nematodes, such as *H. polygyrus* (see Knowles et al. 2013). Because this can affect *H. polygyrus* infection intensity, we restricted our analyses to only those individual mice which had not been anthelminthic-treated; in addition, we have tested for and never detected any knock-on effects or reduced transmission or infection in untreated animals when in the presence of treated animals on the same grid. Sample sizes from all years and woodland sites included in final analyses can be found in Table 1. One woodland site (Mudhouse) was excluded from analyses due to low mouse captures and low *H. polygyrus* prevalence (41 infected mice).

In all of the statistical models, we included the following terms as fixed effects: environmental factors: season (categorical, 3 levels: spring, summer, autumn). host characteristics: sex (categorical, 2 levels: male/ female); scaled mass in grams as a

measure of body condition (continuous, (Peig and Green, 2009)); reproductive status (categorical, 2 levels: active [males- descended or scrotal testes, females- lactating or gestating]; inactive [males- abdominal testes, females- perforate or non-perforate vagina]). Previous work from this system has demonstrated negative interactions between two coinfecting parasites (*Eimeria hungaryensis* and *H. polygyrus* (Knowles *et al.*, 2013)), thus to investigate this, and other gastrointestinal (GI) parasite interactions, we included the presence/absence of the two most common GI parasites: the coccidian *Eimeria hungaryensis* and a Hymenolepid cestode (both categorical, 2 levels: present/absent).

### Model Structure

Statistical analysis was carried out in R version 3.6.0, in the Bayesian linear modelling package MCMCglmm [6] (Hadfield, 2010). MCMC methods produce a distribution of estimates of effect sizes for a given variable. The proportional overlap of these estimates can be used to give a measure of significance for the difference between effects (pMCMC) as well as an estimate of the mean and 95% credible intervals of the difference, without the use of posthoc tests (Hadfield 2010). In order to investigate spatiotemporal variation in *H. polygyrus* intensity and the detection of biological variation through different ecological sampling regimes, we constructed three sets of models, two that use the full dataset which includes multiple captures for most individual mice, and one with the full dataset, and one using data subsampled within sampling replicates (year and site). Specifically, we had three aims in this analysis, which correlate to model sets described below: (1) investigation of predictors of *H. polygyrus* intensity when year and site variation are controlled for (2) estimation of variation in fixed effects across years and sites (3) the benefits of

different levels of spatiotemporal resolution in sampling for estimating drivers of infection intensity.

First ('Model Set 1'), we used the full dataset to investigate the effects present across the entire study system using a base model, with site and year included as fixed effects in addition to season, host, and parasite community effects previously described. For each level of site, and year we examined the proportional overlaps among the estimated posterior distributions of each fixed effect to determine which sites and years differed in terms of *H. polygyrus* intensity. Additionally, we fit an alternative model formula with year, site, site:year, grid, and grid:year as random effects to estimate the proportion of variance explained by each spatiotemporally relevant term. For the second aim ('Model Set 2'), we then added two-way interaction terms between year or site and the other fixed effects into the full models to investigate whether such interactions could reveal spatiotemporal interactions in the host- and parasite community-related drivers of *H. polygyrus* infection intensity, which the base model does not account for. We carried out a model addition approach using INLA [7] to determine the importance of the interactions without overloading the model. Starting with the base model including all fixed effects, we added interaction terms (e.g. season:year) one at a time. Each round, the interaction which lowered the DIC of the model the most (improving model fit) was kept, and the process was repeated with the remaining interaction terms. This was repeated until the model was optimised, and could not be improved in fit with the addition of any further interaction terms (decrease DIC by >2). We then ran all final model formulae in MCMCglmm [6]. We again examined the posterior distributions of the

effect estimates for interactions derived from MCMCglmm in the optimal model, which gave an estimate for pairwise distribution overlaps for each year or site, demonstrating which years and sites differed in terms of their effect sizes for each fixed effect (e.g., were seasonal effects greater in 2010 than in 2011?).

Next in ('Model Set 3'), we explored whether different spatial and temporal sampling regimes impacted estimation of the effects driving infection intensity, by running a series of models for either each site (N=5; all years combined) or each year (N=6; all sites combined), to investigate whether the results were consistent across site or year sampling resolutions. Finally, we compared the merits of cross-sectional (single capture per individual) versus longitudinal (repeat captures) sampling of individuals (Clutton-Brock and Sheldon, 2010). All analyses were repeated with the cross-sectional dataset which includes only the first capture of each mouse ($N_i$=$N_c$=783) and with the longitudinal data, which includes all captures ($N_i$= 926, $N_c$=1609, max captures per individual=28, median captures per individual=4; Fig S2). All longitudinal models included individual ID as a random effect (Paterson & Lello, 2003). All models were run for 130,000 iterations, with a 100-iteration thinning interval and a 30,000-iteration burnin period, for a total of 10,000 stored iterations.

## 5.4 Results

### *5.4.1 Model Set 1 – Longitudinal dataset with fixed spatiotemporal effects*

Within our full, longitudinal (repeat capture) dataset, we found environment (season) and a co-infecting parasite (Hymenolepid infection) were the most important

predictors of *H. polygyrus* infection intensity (Figure 5.2; Appendix D, Table S5.2). Summer and Autumn both showed significantly lower infection intensity compared to Spring (Figure 5.2, pMCMC:Summer <0.001, pMCMC:Autumn <0.001). Hymenolepid presence was associated with higher intensities of *H. polygyrus* infection (Figure 5.2, pMCMC=0.018). We found no main effects of host characteristics on intensity of infection in these analyses without spatiotemporal interactions. In addition, there was significant spatial variation in mean *H. polygyrus* intensities across the five woodland sites, and 6 years (Figure 5.3). Across sites, we found significantly higher infection intensities in Haddon Wood compared to all other woodlands (Figure 5.3B&D). There were also significant between-year differences detectable across many pairs of years (Figure 5.3B&D); notably, *H. polygyrus* infection intensity was higher in 2010 than in any other year, and intensities in 2012-2014 were lower than each year from 2009-11 (Figure 5.3D). Alternative full models with spatiotemporal terms (year, site, site-by-year, and site-by-grid) specified as random effects alongside individual ID showed that year (7.9%; 95% CI = 0.031% - 33.99%), individual ID (11.0%; 95% CI = 4.70%-17.70%), and site (15.9%; 95% CI = 0.96% - 54.07%) explained the highest proportion of variance for *H. polygyrus* intensity, while site:year (1.9%; 95% CI = 0.0011% - 9.87%), grid:year (2.4%; 95% CI = 0.055% - 7.29%), and grid (0.8%; 95% CI = 0.00064% - 4.25%) explained relatively much lower proportions of variance.

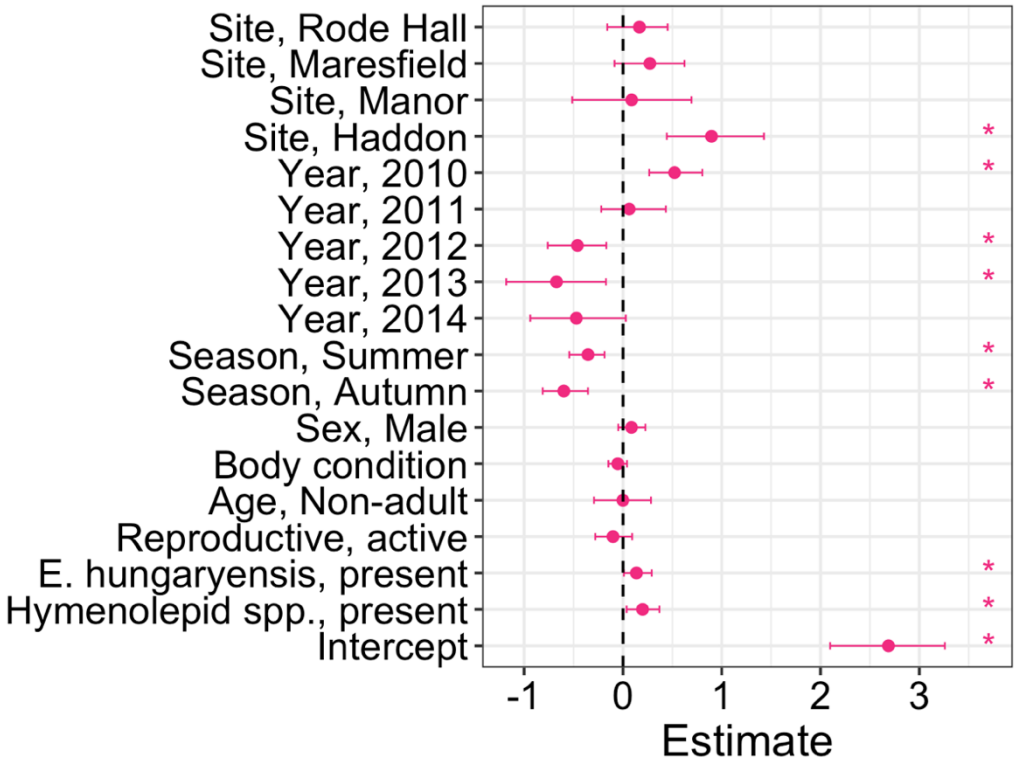


**Figure 5.2. Full model output for data from all years and site collected using longitudinal samplings.**

Points and ranges represent model estimates and 95% credibility estimates for each model. Asterisks indicate the significance of variables with a $p_{MCMC}$ <0.05 threshold.

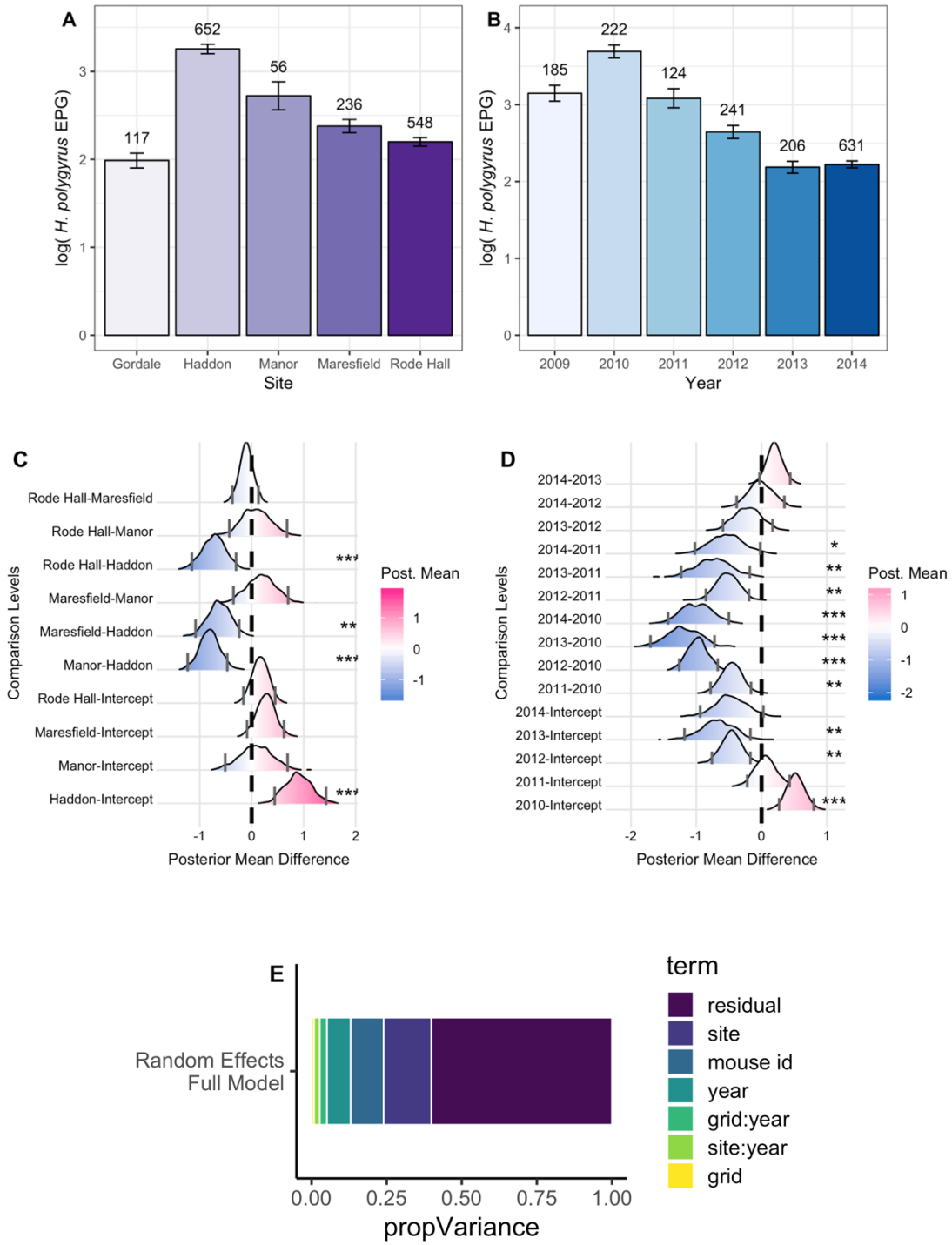


**Figure 5.3. Spatiotemporal variation in mean *H. polygyrus* intensity across full dataset.**

Top row: raw data for significant spatiotemporal main effects from base model: Bars represent mean intensity (± SE) for A. site, and C. year. Middle row: ridge plots below bar graphs (C-D) represent pair-wise comparisons for base model output for fixed effect factors.

Density ridges represent distributions drawn from the differences between the posterior means of the indicated comparison levels [a-b] for each iteration ($N_{iterations}$=130000). Blue shading denotes that the mean of effect estimates from the x-axis is lower than that on the y-axis. Differences between effects can be interpreted by comparison of the density ridges to zero; grey lines for each ridge indicate the 95% credibility intervals for these distributions. Blue shading denotes that the mean of effect estimates for [a] is lower than that of [b] for a given interaction. Pink shading denotes that mean of effect estimates from [a] is higher than that of [b]. If credibility intervals do not cross zero, this is considered a significant difference in effects between [a-b]. Significant differences between effects are indicates by ***, ** and * for P<0.001, P<0.01 and P<0.05 respectively. 'Intercept' represents the baseline year of the model (2009) in all panels. 'Intercept' represents spring for season, Gordale for site effect levels, and '2009' for year. Bottom row: E. Proportion variance explained by each spatiotemporal random effect in an alternate model.

### *5.4.2 Model Set 2: Variation of ecological and demographic drivers of parasitism across year and site*

Including spatiotemporal interactions in models of the full, longitudinal dataset revealed substantial variation in the fixed effect estimates across years (Figure 5.4). Three by-year interactions with fixed effects significantly improved model fit for longitudinal models (Appendix D, Table S5.3-5.4; Figure S5.5). Proportional overlap for the interactions which remained in the optimal model revealed significant change across interaction levels for all 3 interactions (season ΔDIC=-58.92, body condition ΔDIC=-14.61, sex ΔDIC=-5.42) which remained in the final longitudinal model (Figure 5.4). All levels of season-by-year interaction effects in longitudinal models consistently agreed in direction of effect (Appendix D, Fig S5.3); however, proportional overlaps for effects still showed significant variation in magnitude and direction of effect (Fig 4; Fig S7A). Effects of season for Summer were greatest in 2011 and 2012 and associated with significantly lower infection intensities compared to all other years (Fig 4; Fig S7A). Effects of season for Autumn for all years 2011-14 were greater (showing lower infection intensities) than 2009 and 2010 (Fig 4; Fig S7A). Interactions of year with body condition improved models by the second-largest DIC decrease (Table S4). Longitudinal models indicated a high degree of

variation for the effect of body condition-by-year, where the effect in each year from 2011-14 was greater (higher scaled mass associated with lower intensity of infection) than in 2009, and effects in both 2011 and 2014 were greater than in 2010 (Figure 5.4; Appendix D, Figure S5.7C). Proportional overlap for sex-by-year interaction effects indicated a significantly higher male bias of infection intensity in 2012-14 compared to 2010, and a greater male bias in 2013 compared to 2009 (Figure 5.4, Figure S5.7B). Furthermore, variation in the effect of sex on infection intensity represents a change of direction in effect (from female bias to male bias) between the years 2010 and 2011 (Appendix D, Figure S5.7B).

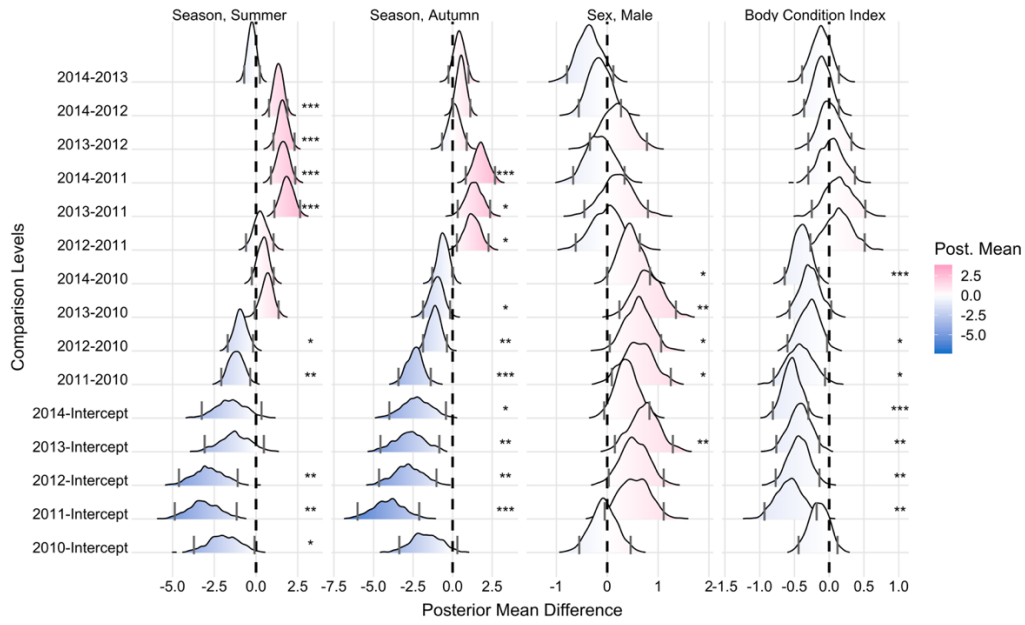


**Figure 5.4. Differences across estimated effects (with 95% credible intervals) for interaction levels which improved full model fit, longitudinal models.**

Density ridges represent distributions drawn from the differences between the posterior means of the indicated comparison levels [a-b] for each iteration ($N_{iterations}$=130000). Blue shading denotes that the slope of effect from the x-axis is lower than that on the y-axis. Differences between effects can be interpreted by comparison of the density ridges to zero; grey lines for each ridge indicate the 95% credibility intervals for these distributions. Blue shading denotes that the slope of effect for [a] is lower than that of [b] for a given

interaction. Pink shading denotes that slope of effect from [a] is higher than that of [b]. If credibility intervals do not cross zero, this is considered a significant difference in effect slope of [a-b]. Significant differences between effects are indicates by ***, ** and * for P<0.001, P<0.01 and P<0.05 respectively. 'Intercept' represents the baseline year of the model (2009) in all panels.

### 5.4.3 Model Set 3 - Consistency of effect estimation across spatial and temporal sampling regimes

In models from subsampling for year- and site-specific replicates, estimates of drivers of parasite infection varied considerably across sampling contexts, both in time and space (Figure 5.5; Appendix D, Table S5.5-5.6). Across year-specific models, season was the most consistent effect in both direction and detection. Summer was associated with significantly lower intensity of *H. polygyrus* infection in 2/6 years for longitudinal models (Figure 5.5; Appendix D, Table S5.5). Autumn was likewise associated with lower intensity compared to Spring in in the majority (4/6) of longitudinal models (Figure 5.5; Appendix D, Table S5.5). Models within years revealed main effects of host characteristics were less consistent than environmental (season) main effects (Figure 5.5; Appendix D, Table S5.5). Males were associated with a higher intensity of infection compared to females in one year only, and show contrasting direction of effect over the years. Body condition had a significant association with *H. polygyrus* in two years, and this association was likewise either positive or negative dependent on the year (Figure 5.5; Appendix D, Table S5.5). The presence of both co-infecting parasites examined increased the intensity of *H. polygyrus* infection in one longitudinal model each (Figure 5.5; Appendix D, Table S5).

Our models which investigating sampling within single sites showed similar trends to the year-specific models, with seasonal effects being the most consistent and

frequently detected (Figure 5.5; Appendix D, Table S6). We found significantly lower intensity of infection in Summer compared to Spring for 2/5 sites and for Autumn compared to Spring in 4/5 sites. A host effect of body condition was detected for two sites, where individuals in better condition were associated with lower intensity of infection. Males had higher intensity of infection compared to females only in the Rode Hall woodland. Lastly, we found positive associations between the presence of both Hymenolepid cestodes and *E. hungaryensis* and *H. polygyrus* intensity for one woodland site only each in longitudinal models (Figure 5.2; Appendix D,Table S5.2).

### *5.4.4 Comparison of cross-sectional versus longitudinal sampling*

Across all three model sets, models fit to longitudinal sampling data estimated the effects of the drivers influencing *H. polygyrus* with higher confidence than the cross-sectional analyses. For models of the full dataset without interactions (Model Set 1), all effects save reproductive status were estimated with tighter 95% credibility intervals (Appendix D, Figure S5.3). For Model Set 2, cross-sectional and longitudinal models differed in regard to which year and site interactions improved model fit (Appendix D, Figure S5.5, Table S5.4). As with longitudinal models, cross-sectional models were improved significantly by season-by-year ($\Delta$DIC=-56.79), body condition-by-year ($\Delta$DIC= -23.41), and sex-by-year ($\Delta$DIC= -7.85) interactions; posterior overlaps for interaction levels from the optimal cross-sectional model showed significant variation across years, but detected less variation than those from the corresponding longitudinal optimal model (Figures 5.3; Appendix D, S5.6). Furthermore, cross-sectional models were also improved by three additional

interactions compared to the corresponding longitudinal model (Appendix D, Table S5.4): sex-by-site, reproductive status-by-year, and Hymenolepid presence-by-year. However, there was very little variation across these interaction levels (Appendix D, Figure S5.6); only the effect of Hymenolepid presence varied significantly across interaction levels, where Hymenolepid presence in 2011 had a stronger positive association with *H. polygyrus* intensity than in all other years.

Among models fit to data subsampled within each year or site (Model Set 3), cross-sectional models largely agreed with the directions of the drivers of infection intensity effect compared to longitudinal models, but detected fewer significant effects for all ecological scales (environment, host, and parasite community) (Figure 5.5). As with longitudinal sampling models, effects of season were largely consistent in cross-sectional models, but effects of host factors and co-infecting parasite presence were detected less frequently, and variations detected by interactions in optimal models for the full dataset were estimated with lower confidence (wider 95% credibility intervals; Figure 5.5).

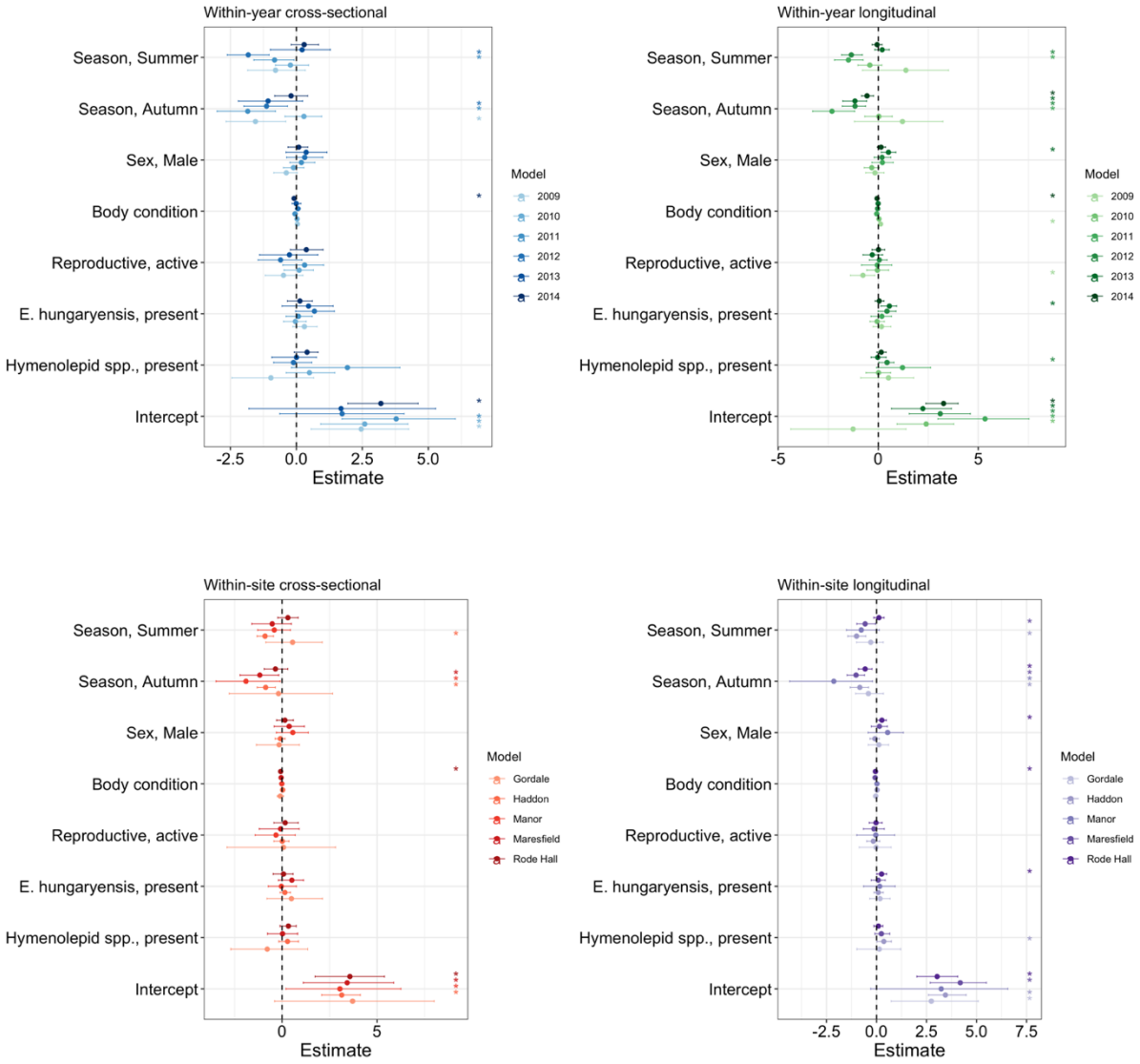


**Figure 5.5. Effect size estimates from models investigating the effect environment, host, and parasite community-level predictors of *H. polygyrus* intensity within sampling resolutions.**

Points and ranges represent model estimates and 95% credibility estimates for each model. Asterisks indicate significance at a threshold of $p<0.05$. Intercepts for categorical covariates are as follows: Season, Spring; Sex, Female; Reproductive status, Inactive; Age, Adult; *E. hungaryensis* & Hymenolpeid, absent.

## 5.5 Discussion

Using a highly spatially- and temporally- replicated data set, we demonstrate that fitting interactions with year and site with common environmental and host drivers of helminth infection demonstrates support for both environment (season) and host factors (body condition and sex) varying biologically in their relationship with *H. polygyrus* over six years. Methodologically, we then used our dataset to approximate temporal and spatial replicates of sampling and show the utility of a large sampling scale for estimating factors driving parasitism in the wild. These results suggest that caution should be taken when generalising interpretation of the effects from analyses of drivers of parasitism in the wild that are limited in their spatial or temporal replication. Overall, we highlight the importance of inclusion of spatiotemporal context in estimating the effects of factors across ecological scales across parasitism in the wild.

We found that among all fixed effects considered trapping sites, year, and seasons were the most important predictors of variation in *H. polygyrus* intensity (Figure 5.2), and that there was a high degree of variation in mean intensity across both space and time (Figure 5.3). These results support previous work on helminth infection dynamics in the wild (Anderson, 1986; Keymer and Dobson, 1987). For example, it is well-established in human (Anderson, 1986), large mammal (Albery *et al.*, 2018a), and small mammal populations that helminths exhibit a high-degree of seasonality—with higher transmissibility in warmer, wetter temperatures and inter-annual and geographic variation in prevalence and infection intensity (Montgomery and Montgomery, 1988; 1989; Abu-Madi *et al.*, 2000; Eira *et al.*, 2006). Despite great interest in understanding the relationship between hosts and helminths, and

what factors are likely to determine heterogeneity in burden (e.g., highest number of worms/host (Anderson, 1986)]), there is little knowledge on how the effects of intrinsic and extrinsic drivers of helminth infection can vary over space and time.

We selected potential drivers of infection which have been previously investigated in helminth disease ecology. Our results from the base model suggest that, aside from weak positive correlations between both *Eimeria hungaryensis* and Hymenolepid presence and infection intensity (Figure 5.2), no host characteristics had significant effects across all years and sites. This goes somewhat against a number of studies investigating the impact of host factors such as age, sex, reproductive status, and body condition, as well as interactions from co-infecting pathogens on helminth infection intensity. Indeed, several studies of *H. polygyrus* in *Apodemus spp.* populations have documented male-biased transmission (Ferrari *et al.*, 2004) and burden (Langley and Fairley, 1982; Gregory *et al.*, 1990). Similarly, age-dependent burdens and response to helminths (Behnke *et al.*, 1999; Clerc *et al.*, 2019a), costs of reproduction (Albery *et al.*, 2018b), impacts of body condition (add ref), and interactions between co-infecting parasites (Behnke *et al.*, 2005; Clerc *et al.*, 2018) are well-documented in mammal-helminth systems. Importantly, however, these relationships are highly inconsistent across studies, and there has been little systematic investigation into the true extent of variation.

Model fit was drastically increased by the addition of interactions which accounted for spatiotemporal variation in the slope of the relationship between fixed effects and *H. polygyrus* intensity. Overall, interactions by year were associated with much

larger reductions in DIC than interactions by site (Table S4), suggesting that allowing temporal variation via interaction effects is important for confident estimation of the factors driving parasitism in this dataset. As noted above, the three fixed effects included in interactions which remained in the optimal model after selection (season-by-year, sex-by-year, and body condition-by-year) have all previously associated with helminth infections in wild populations. Common patterns for helminth ecology suggest that burdens are highest in the spring and decline throughout the winter (Stromberg, 1997), and these patterns are aligned with the results from our base model (Figures 5.2-5.3). Our interaction models, however, show that the magnitude and direction of this effect changes significantly across years, and that in some years intensities in Spring are in fact lower than in Summer or Autumn, for example (Figures 5.4; Appendix D, S5.7). Seasonality in helminth dynamics can be related to either microclimate effects on survival of infectious lifecycle stages (Stromberg, 1997) but is also linked to changing age structures according to reproductive seasons in small mammals (Keymer and Dobson, 1987). Within this study, there was large variation in the age structures over time across years (Appendix D, Figure S5.9), indicating potentially important underlying demographic context for variation in seasonal infection dynamics.

Despite no mean effect of either sex or body condition in our base model, our interaction models revealed that the magnitude and direction of the slope varied across years (Figure 5.4, Appendix D, S5.7). This finding indicates some plasticity in the relationship between host characteristics and helminth infection according to environmental context. Sex differences have been extensively studied in relation to infection, with many responsible processes playing a role (Zuk and McKean, 1996).

Likewise, the relationship of body condition and infection may be positive, negative, or null dependent on the mechanism involved (i.e. clearance versus tolerance) (Sánchez *et al.*, 2018). Meta-analyses of both sex bias (Moore and Wilson, 2002) and body condition effects (Sánchez *et al.*, 2018) have shown that despite prevailing hypotheses of positive slopes for male effects and negative slopes for body condition effects on parasitism, effects can vary by host system and/ or the method of sampling. Meta-analyses, however, typically consider a wide range of host species. In contrast, our results indicate important temporal variation in sex and body condition effects within a single host species. Applying statistical analyses to the posterior distributions from these interaction models further allowed us to make inferences on specific pair-wise differences in these effect slopes across years. Overall, these differences suggest that the relationship between parasitism and both seasonality and host characteristics may be dependent on environmental context.

Results from our models applied to subsampled datasets from single years and sites suggest that interpretation of drivers of parasitism from limited spatiotemporal replicates are often not generalisable across years and sites (Figure 5.5). However, encouragingly, our longitudinally subsampled models do approximate the trends revealed by the interaction effects using the full dataset in Model Set 1 (Figure 5.4 B&D vs Appendix D, Figure S5.5; Table 2). Though this does not alleviate the lack of reproducibility across replicates, it does suggest confidence in the estimates of main effects of interest within a given sampling replicate when multiple samples per individual are taken.

For all model sets in this study, there was universal support for longitudinal (repeat sample) models outperforming cross-sectional (single sample). In our base model with no interactions, cross-sectional effect estimates largely follow the trends of longitudinal (Appendix D, Figure S5.3). This encouragingly indicates that cross-sectional sampling does provide insight into data trends, but longitudinal models provided smaller credibility intervals and higher confidence in estimation (Appendix D, Figure S5.3). For models including interaction effects, longitudinal models differed in both the interactions which remained in the optimal model (Appendix D, Table S5.4) and the detection of variation in effect slopes across interaction levels (Appendix D, Figure S5.6 vs Figure 5.4). Cross-sectional models featured more retained interaction effects, but fewer of these effects featured significant difference across interaction levels; this lack of significant differences may indicate spurious retained effects and/or less power to detect important spatiotemporal variation. These differences between cross-sectional and longitudinal models are notable given that the median number of observations per individual was low (4) and very few animals approach the maximum number recorded (28; Fig S1), so the benefit of longitudinal sampling for estimating factor driving parasitism is conferred even with very few additional data points per individual. These findings generally agree with findings showing that longitudinal versus cross-sectional sampling is more robust for detecting parasite interactions (Fenton *et al.*, 2014), and although we were asking different statistical questions, highlight the broad benefits of longitudinal sampling for multiple areas of interest within wildlife ecological studies.

This study revealed substantial temporal variation in effects of season, sex, and body condition on *H. polygyrus* intensity. We focused our aims exclusively on those factors which influence infection intensity and their spatiotemporal variation. Though our response variable infection intensity (measured as EPG) is a common proxy for infection burden (Budischak *et al.*, 2015a), prevalence probability of infection is also a key metric of disease for many study systems and may be influenced by different processes than intensity. It is well-known that helminth burdens are over-dispersed in populations, with heterogeneity in predisposition to heavier infections for some individuals in the population (Anderson, 1986). Factors which increase exposure and infection probability may therefore differ from those which dictate infection burden, and this should be kept in mind for interpretation of these results.

Both model sets indicated a higher degree of temporal than spatial variation in this dataset. There was only one spatial interaction effect (sex-by-site) retained in cross-sectional interaction models, and this effect did not actually show any significant variation across sites (Appendix D, Figure S5.6). In line with these interaction effect results, models on single-year and -site data subsets' confidence interval estimates for within-site main effects largely spanned zero (Figure 5.5). This may suggest either that temporal variation is more common, or that our dataset cannot adequately partition spatial variation. Year and site combinations in this dataset were not perfectly independent, and there is a degree of confounding between the two terms (Table 1). It is possible therefore that some apparent temporal variation is attributable to spatial site changes, and *vice versa*. It is also possible that despite

vegetation differences across sites considered here, these differences are not drastic enough for significant variation in environmental context shaping drivers of parasitism. Previous work has found significant differences in the seasonality & species compositions of helminth infections of *A. sylvaticus* over highly different habitats (i.e. sand dunes versus lake margins) (Eira *et al.*, 2006). Further application of this approach to datasets across varied habitats would therefore be useful.

It was not in the scope of this study to determine specific mechanisms behind variation in magnitude or direction of slope for the effects considered here, however, it is likely that there are important ecological mechanisms behind and implications for the variation detected here. (Additionally, despite weakly significant positive associations between *H. polygyrus* and coinfecting parasites in both model sets, we do not consider these to be interpretable as interactions between these parasites. Commonly, untangling the relationships among parasites requires perturbation of the parasite community (Pedersen and Fenton, 2015), and we view these results as suggestive of either co-exposure patterns or tentative associations warranting further investigation.

### *5.5.1 Inferences and Practical Takeaways*

By incorporating model structures that allow for spatiotemporal variation in factors influencing parasitism in the wild, we have provided evidence for important temporal changes in seasonality, sex, and body condition effects on helminth infection dynamics in a wild wood mouse population. These findings add important insight to previous knowledge that mean intensity of infection varies substantially over space and time. Furthermore, they suggest that varied support for hypotheses regarding

factors influencing parasitism may represent important biological variation rather than simply variation in detection or limited statistical power across different populations and replicates. Given practical limitations of many ecological studies it is typically of interest to investigate which sampling resolution in a wild system can provide reliable estimates of main effects of interest. Our results suggest that longitudinal sampling within limited replicates provides reliable estimates of effects, but highlight that caution should be applied in extrapolating these results beyond the context of the study. Overall, we hope that more studies will investigate and control for spatiotemporal variation in effects driving wildlife disease ecology.

# Chapter 6 Discussion

## 6.1 Thesis summary

This thesis aimed to explore how variation in the environment of the host influences parasite infection, with a particular focus on how resource quality and availability drives dynamics of *H. polygyrus* and co-infecting parasites in wood mice. I used a combination of paired experimental and laboratory experiments, longitudinal sampling, and statistical analysis to investigate the impacts of supplemented nutrition in *A. sylvaticus* populations of Scotland, and how natural drivers of *H. polygyrus* parasitism vary in time and space.

In Chapter 2, I used a combination of experimental supplementation with an enriched diet and anthelmintic treatment in both a wild and laboratory population of *A. sylvaticus.* I found that in both wild and laboratory setting, an enriched diet improved host condition and increased expression of adaptive immune response. These physiological benefits resulted in increased resistance to *H. polygyrus,* higher drug efficacy, and lower egg shedding in supplemented mice. By using our laboratory population to control for variation in exposure and susceptibility in the wild, I show important evidence for the role of nutrition quality in host response to infection with a natural helminth. Results from this chapter also indicate the potential for nutrition supplements as a beneficial complement to traditional anthelmintic treatment.

In Chapter 3, I expand upon the study of supplementation effects on *H. polygyrus* in a wood mouse population and characterise the effects on the broader parasite community. Through a combination of field data collection, faecal egg count analysis, and diagnostic PCR, I show that supplemented resources generally reduce

infection with macroparasites—both the gastrointestinal helminths *H. polygyrus* and *C. murissylvatici* and *Ixodes spp.* ectoparasites. Unexpectedly, supplementation increased infection with some gastrointestinal and blood-borne microparasites, but these effects were dependent on whether anthelminthic treatment had been administered. Along with evidence of within- and between-host processes affected by supplemented resources, this study suggests experimentally that the outcome of altered resources is dependent on parasite biology, host effects, and presence of co-infecting parasites. My results imply that measuring co-infection is crucial for predicting the outcome of resource provisioning in wildlife.

In Chapter 4, I combine data from three years of experimental resource supplementation and show the short-term effects of nutrition on *H. polygyrus* infection are greatly diminished when the quality of resources naturally available to the population during supplementation is high. I next used data from a long-term experiment I conducted over 5 months of supplementation to show that even within a year of high food availability, additional resources can increase host immunity and reproduction, but that these effects vary by season and according to the age of mice at supplementation. In addition, I combined data from three years of experimental resource supplementation and show the short-term effects of nutrition on *H. polygyrus* infection are greatly diminished when the quality of resources naturally available to the population is high. These results demonstrate the importance of understanding the host and environmental context in determining the outcome of perturbations to resources and parasites of wildlife.

In Chapter 5, I used six years of data from an intensively sampled *A. sylvaticus* populations from woodlands near Liverpool, UK and showed that season, body condition, and host sex were the most important drivers of *H. polygyrus* infection intensity. Importantly, however, the relationship between these factors and *H. polygyrus* varied in both magnitude and direction over time and space. These results suggest that accounting for spatiotemporal variation should be embraced, rather than merely controlled for, when investigating determinants of parasite infection in the wild.

In this final discussion chapter, I will discuss the broader implications of my research for the role of understanding how resources impact natural host-parasite systems (Figure 6.1) and how these insights advance current knowledge within disease ecology and human helminth control. I will discuss limitations of the research presented, and ongoing and future avenues of work following from this thesis to complement results to date.

## 6.2 Nutrition-nematode relationships

The relationship of host nutrition and gastrointestinal helminths is of high priority given its clinical significance in areas with high geographical overlap of malnutrition and helminth presence (Koski and Scott, 2001). This has resulted in extensively studied effects of baseline nutritional status impacts on helminth infection (Hagel et al., 1995; Payne et al., 2007; Long et al., 2007; Al-Mekhlafi et al., 2014) and the effects of nutrition supplements alongside anthelminthic treatment in humans (Hall, 2007; Casey et al., 2009; Yap et al., 2014; Rajagopal et al., 2014; Casey et al., 2017).

Although many studies suggested benefits of supplementation, interpretation from these studies within clinical interventions are often equivocal due to small sample size, confounding in the data, and a range of nutrients considered for supplementation (Yap et al., 2014). Furthermore, few have considered the synergistic effects of nutrition supplements as a complement to anthelmintic treatment (but see (Nga *et al.*, 2009; Wieringa *et al.*, 2011)).

Controlled laboratory studies in model systems have provided much clarification on the mechanism of the relationship between macro- and micro-nutrients, providing key evidence that deficiencies in protein (Slater and Keymer, 1986b; Michael and Bundy, 1992; Ing *et al.*, 2000) and micro-nutrients such as zinc (Shi et al., 1997; Boulay et al., 1998) and vitamin A (Carman et al., 1992) impair parasite-specific adaptive immune responses in laboratory mouse strains. However, hosts facing helminth challenges in natural populations will face markedly different conditions—high genetic diversity, challenge with multiple parasites, and highly variable resources (Pedersen and Babayan, 2011) —and relationships may not be as straightforward.

In Chapter 2, I aimed to combine benefits of ecologically realistic conditions and controlled exposure and timeseries possible in the laboratory. To the best of my knowledge, results presented in this Chapter represent the first experimental demonstration of a paired investigation of the effects of supplemented nutrition on nematode infection in a laboratory and natural population using the same host and nematode species. In contrast to previous laboratory studies which primarily focus on severe differences in nutrient levels, I enriched nutrition using the same multi-

nutrient supplemented diet in the laboratory and wild. This supplement is similar to multi-micronutrient biscuits which have been used in clinical trials in areas of endemic helminths (Nga et al., 2009; Hieu *et al.*, 2012; de Gier *et al.*, 2014).

Investigation of the benefits of such fortified biscuits have found encouraging results, such as increased efficacy of deworming and improved cognition (Nga *et al.*, 2009; Wieringa *et al.*, 2011), but typically have not simultaneously measured adaptive immune responses and rely solely on EPG as a proxy for infection, meaning the mechanism and direct effects on worm burden were not well understood. I measured a very broad range of outputs in both my wild and laboratory experiments inclusive of immune measures, condition indices, egg shedding, and adult worm burden. This showed conclusively that a modest increases of a well-balanced array of nutrients can dramatically reduce egg shedding and burden of *H. polygyrus* in both the laboratory and wild wood mice and that the mechanism of this reduction was an increase in body condition and increased adaptive immunity.

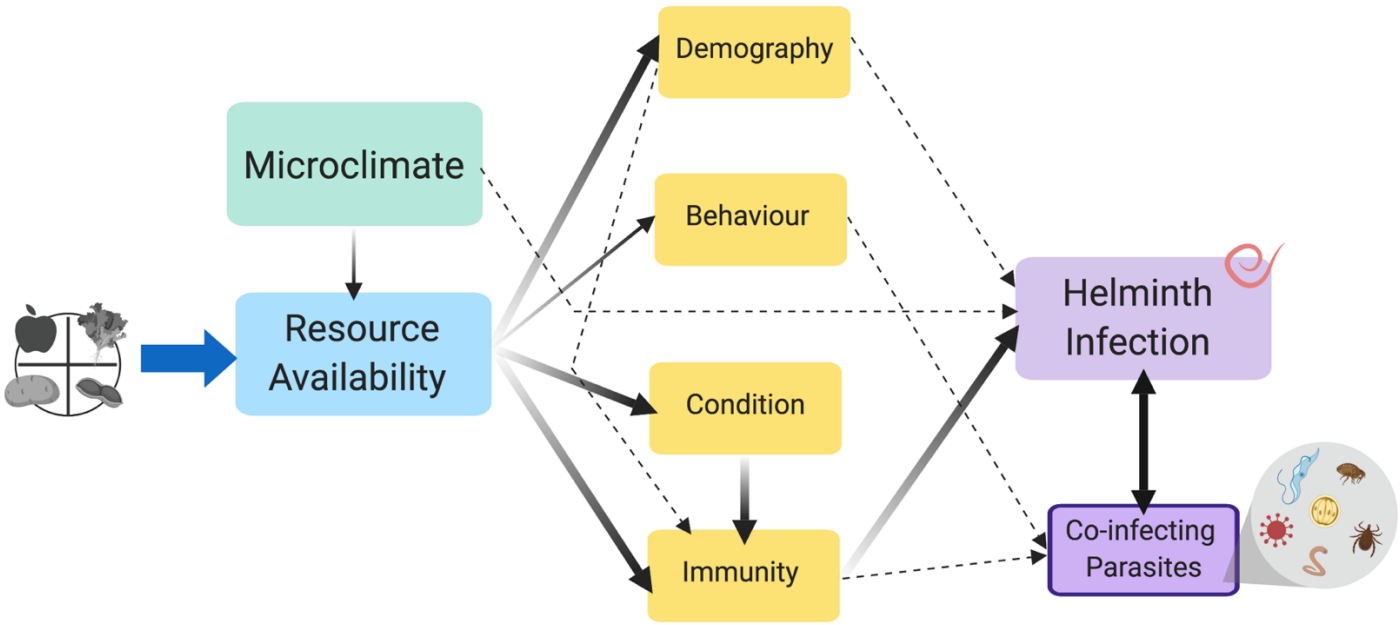


**Figure 6.1. Illustration of results from three years of resource supplementation experiments in this thesis.**

Lines represent support for the relationship indicated. Relationships detected statistically are indicated by solid lines, where thick lines indicate an effect detected across both supplementation experiments and thin black lines indicate an effect seen in only one year or season. Dashed lines indicate relationships that are suggested but not statistically supported by results.

The consistent results of Chapter 2 for laboratory and wild populations are an important finding for model helminth systems given the gap that typically exists between the observational nature and/or limited samples of wild or human systems and the contrived setting of the laboratory. However, within this experiment, we found evidence for increased reproductive investment in individuals on a higher quality diet. This was not surprising, as reproduction is well known to be a costly process, and the addition of resources and reduction of parasites should alleviate some strain of resource allocation. However, it suggested potential population-level effects that were not addressed in my specific investigation of nematode infections. Furthermore, I expected that wood mice populations in this woodland would be host to a number of parasites beyond *H. polygyrus,* and so my next two chapters were dedicated to understanding how far-reaching effects of supplemented nutrition might be in this population.

## 6.3 Experimental insight into broad effects of resources and parasites in the wild

This thesis bridges several somewhat disparate areas through experimental approaches investigating common focal areas of resource limitation and co-infection in wild disease studies. Many classical ecological studies of wild populations have identified resource availability as a key determinant of population cycles and demography (Wolff, 1996; Ostfeld and Keesing, 2000; Clotfelter *et al.*, 2007). Eco-immunological studies have classically considered the dependence of investment in immunity, reproduction, and survival (Sheldon and Verhulst, 1996; Svensson *et al.*, 1998). Several key advances in this area experimentally demonstrating costs of

reproduction for immunity and infection have been made by either measuring immunological and fitness measures simultaneously and longitudinally (Graham *et al.*, 2011; Ezenwa *et al.*, 2012; Nussey *et al.*, 2014; Hayward *et al.*, 2014b; Albery *et al.*, 2018b), but this is not always possible in the wild.

It is likewise difficult to interpret data on multiple parasites in the wild to make conclusions regarding co-infection dynamics without longitudinal sampling, perturbation of the parasite community, or robust statistical approaches (Pedersen and Fenton, 2007; Telfer *et al.*, 2010; Vaumourin *et al.*, 2014; Carver *et al.*, 2015). There is substantial evidence that co-infection can alter the outcome of infection in the wild (Telfer *et al.*, 2010; Knowles *et al.*, 2013; Clark *et al.*, 2016; Abbate *et al.*, 2018; Gorsich *et al.*, 2018; Tołkacz *et al.*, 2018). However, the role of resources has rarely been investigated as a determinant of co-infection in the wild, despite evidence from the laboratory (Budischak *et al.*, 2015b), humans (Budischak *et al.*, 2018), mathematical energy budget models (Cressler *et al.*, 2014), and theoretical frameworks (Pedersen and Fenton, 2007; Graham, 2008a; Rynkiewicz *et al.*, 2015) which suggest that resources are a key factor governing the relationships between parasite species within a host.

The recent focus on increasing access to anthropogenic food sources as a driver of wildlife disease (Oro *et al.*, 2013; Becker *et al.*, 2018) highlights the need for a synthesis of understanding of these responses to artificially altered food sources, mechanisms underlying resource allocation between costly processes in the wild, and the outcomes for infection in the context of co-infection with multiple parasites. A key framework for understanding the processes which govern the outcome of

anthropogenic provisioning was established by Becker and colleagues (2015). Within this theoretical framework, parasitism may increase, decrease, or have a null response to changes to food sources dependent on the effects on host condition weighed up against the effects on patterns influencing transmission such as aggregation or behaviour. Empirical examples of several of these scenarios are presented both along this framework (Wright and Gompper, 2005; Cross *et al.*, 2007; Murray *et al.*, 2015; Moyers *et al.*, 2018) and since (Hwang *et al.*, 2018; Strandin *et al.*, 2018), but few empirical studies have been able to manipulate both resources and parasites and monitor immunological, demographic, and a suite of parasitological responses longitudinally. By doing exactly this in Chapter 3, I built upon the detailed analysis of supplemental nutrition and nematode relationships and further documents that supplemented resources affects both population-level (host contacts, reproductive activity, and population size) and individual-level (host condition and immunity) consistently, and these effects seem to have diverse consequences for the parasite community of wood mice. To my knowledge this is the first time that the heterogeneity that has been observed across multiple, single host-parasite systems has been demonstrated in a single, co-infected host population, which provides important evidence that predicting the impact of supplementation on different parasite species is difficult, even when the host, environmental context, and type of supplementation is controlled.

In Chapter 4, I further explored another often-ignored aspect of resource provisioning by investigating the longer-term effects of resource supplementation to determine how dynamic the host responses to increased food availability, both across

seasons within a single year and across multiple years. These analyses provided important context for understanding the consequence of resource supplementation in this system. One major benefit of the non-destructive sampling and longitudinal trapping used in this experiment was that we detected a cost to survival for individuals on supplemented grids, highlighting an important effect I was not able to measure in the short-term, destructively sampled experiments; but which may represent a trade-off as a result of rapid increased condition, immunity, and reproduction following supplementation. Additionally, I found that although increases in host condition were fairly consistent, the effects on *H. polygyrus* varied across years and seasons and that the impact of supplemental resources on all other parasitological and immunological or condition measures also varied according to season or host cohort. Although I present some hypotheses as to why we may see specific patterns in Chapter 4, overall these results suggest that outcomes of provisioning may be governed by the energetic demands or natural resources available.

### *6.3.1 Spatiotemporal variation and drivers of parasitism*

Results from Chapter 4 highlighted the value of embracing spatiotemporal variation in longitudinal sampling data for greater understanding of biological relationships between hosts and parasites. In my final data chapter, I sought how observational data from an intensively sampled wood mouse population would enable investigation of the possibility that the natural drivers of parasitism in the wild vary spatiotemporally. We expect, and often find, spatiotemporal variation in mean helminth parasitism across spatiotemporal scales such as season (Montgomery and Montgomery, 1988; Stromberg, 1997; Albery *et al.*, 2018a), year (Montgomery and

Montgomery, 1988; Behnke *et al.*, 1999), and site (Abu-Madi *et al.*, 2000; Eira *et al.*, 2006), but variation in the slope of helminth parasitism versus commonly hypothesized drivers of helminth infection such as sex, body condition, or reproductive status have rarely been examined systematically. A modelling approach which included interactions with year and site in this dataset with high numbers of temporal and spatial replicates detected important biological variation in the relationships between sex, body condition, and seasonality with helminth infection intensity. Although we did not specifically investigate it within this Chapter, fluctuating resources and microclimate are often a key component of seasonal, geographic, and interannual variation (Stromberg, 1997; Altizer *et al.*, 2006). Results across my data chapters suggest that a general trend of spatiotemporal and resource availability context as crucial determinants of host-parasite dynamics in the wild, and furthermore suggest that focus on a focal parasite (even the most prevalent in a population) or single temporal or spatial replicate may lead to misleading results masking important indirect processes.

## 6.4 Methodological limitations and caveats

Although I sought to design experimental perturbations, sampling and laboratory work, and statistical analysis to allow for robust conclusions, there were several limitations in this work that should be considered when interpreting results.

### *6.4.1 Causal processes in the wild*

In my field sampling, the primary parasitological and immunological outputs I used were abundance or intensity of infection (EPG) and total and parasite-specific

antibody responses. Although the use of EPG as a proxy for worm burden was verified in these populations (Chapter 1, Box 1.1), as discussed in Chapters 2 & 4, the interpretation of antibody responses in the wild is complex due to lack of exposure history and comparatively high activation compared to laboratory correlates (Pedersen and Babayan, 2011; Gilbert *et al.*, 2013; Abolins *et al.*, 2017). In Chapter 2, I addressed this issue by carrying out paired laboratory and wild experiments. This was crucial to my interpretation as my strongest results in regard to supplementation effects on immunity were observed in the colony, where exposure was controlled and timepoints of sampling were highly regular. From this experiment, I would conclude that a higher level of *H. polygyrus* -specific IgG1 represents a more immunocompetent individual, as these individuals later had significantly lower adult worm burdens and zero egg shedding during reinfection. However, it is possible that these results are not representative of the dynamics of the wild. Dramatic effects on condition in the wild and the positive associations of body condition with higher immunity suggest that supplemented nutrition is still contributing to an increased ability to mount general and parasite-specific immune responses. Paired experiments were not possible or appropriate for the analyses in Chapters 3 and 4, however, as we are not able to study the interaction of parasites, reproduction, and survival in the colony. We maintain lifecycles of both *H. polygyrus* and *E. hungaryensis,* and have recently added Wood Mouse Herpes Virus to the parasites we are able to experimentally infect with in the colony. Further research could experimentally consider the resource effects on co-infection outcome among combinations of the above parasites where possible for additional

clarification on whether outcomes within the parasite community were driven by host-level immune or condition or population-level densities or contacts.

The relative contribution of the population-level responses such increased population densities observed on supplemented grids throughout supplementation experiments compared to host condition and immune responses for infection metrics cannot reliably be partitioned in the analyses shown here. I typically considered strictly individual level traits in models as explanatory variables for my experimental supplementation studies, however results from Chapter 5 which indicate significant spatiotemporal variation likely involves a degree of influence from changing age structures across years and seasons. Empirical data from these or similar experiments would be ideal to use for mathematical models similar to those used by Becker *et al.* (2014) to theoretically explore the impact of the population-level effects of supplementation.

### *6.4.2 Experimental design*

Experimental design for work presented in this thesis was designed to maximise replicates for experimental manipulations of interest and, where possible, repeated samples for individuals over time. This entailed the set-up of grids for 'population-level' perturbations (resource supplementation) and randomisation within grids for individualised perturbations (anthelmintic treatment). Though most analyses contained in this thesis control for grid to account for variation due to this blocking set-up, there may be finer scale spatial variation in either naturally available resources or parasite presence which influence results from experimental field work.

Spatiotemporal models from Chapter 5 highlight the substantial impact that site and year can have on host-parasite relationships, and suggest that underlying spatial distributions of parasites investigated and/or temporal variation in host density or other environmental factors could play a non-trivial role in host-parasite dynamics observed. In addition to intrinsic variance which may not be fully captured by models, field experiments were carried out in the same study area for three years in a row. Although I designed experimental manipulations to avoid cumulative effects on the same grids from year to year, trapping was carried out for only a portion of the year, so my interpretation of effects is limited to a portion of each year. This may leave out important data on population dynamics and individual movement within or outside of the study area that would further inform conclusions drawn from this thesis. Though the focus of analyses presented here were largely focused on the individual host, future work using this data could explicitly link longitudinal population-level data available to individual parasite, condition, and immunity measures to investigate possible confounding effects. Additionally, spatially explicit models could be applied to all years and grids for investigation of underlying variation in the landscape of parasite and host traits.

### 6.4.3 *Current limitations and prospects for* A. sylvaticus *as a model system*

Although *A. sylvaticus* is an excellent system for experimental manipulations and longitudinal sampling of tagged individuals, it does have drawbacks for the study of immunological and parasitological processes. Unlike longer lived observational populations of large ungulates were individual identity is known and reproduction events are documented and offspring remain associated with parents (i.e. in the Soay

sheep (Hayward *et al*.2014b; Graham *et al.* 2010), genetic pedigree and specific reproductive metrics such as number of offspring or offspring size are generally unknown as birthing events occur in nests, and offspring are typically only caught after weaning. A few wild studies have used microsatellites for genotyping to circumvent unknown parentage (Selkoe and Toonen, 2006; Makova:1998uy; Pemberton, 2008), but pedigrees constructed by microsatellites may be less reliable than single-nucleotide polymorphisms (Pemberton, 2008; Bérénos *et al.*, 2014). During my PhD I tested nest boxes on my field sites to attempt identification of offspring directly, however I did not have success in wood mice using the nest boxes for breeding. Still, overcoming this limitation in wood mice in the future within our system is very possible.

An additional limitation is related to the unknown birth date of the wild wood mice, as we are only able to use crude estimates of age in our field sampling. Age according to coat colour and morphology was described in Chapter 2. This typically results in highly skewed group sizes, where up to 90% of individuals eligible for microchip tags by weights are typically classed as 'adults,' which makes including age as an explanatory variable difficult due to uneven group sizes. Other variables such as weight and body length can give rough proxies of the age of an individual, but are confounded with measures of host body condition and reproduction; both of which are typically associated with larger animals and can be related to reproductive status. As indicated in Appendix A, eye lens weight from sacrificed animals is highly correlated with known age in wood mice and other small mammals (Rowe *et al.* 1985; Clerc *et al.*, 2019a), but this requires destructive sampling. When eye lens

weight was available, it indicated that older wood mice in our populations have higher burdens of *H. polygyrus,* but interests in longitudinal sampling for subsequent experiments meant that we did not have access to this information. It is therefore possible that there is a degree body condition and reproductive variables may be confounded with age. Generally, for models using data from non-destructive samples, I used body length (mm) as a proxy for age under the assumption that it would be the least subject to variation from its confounding relationship with condition. However, there are exciting prospects for overcoming this limitation in the future. An 'epigenetic clock' has been developed using multiple tissue samples and DNA methylation data in laboratory (Han *et al.*, 2018). Recently Tom Little & Amy Pedersen have optimized this method for *A. sylvaticus* using tissue, blood and faecal samples, with a predictive power of within two weeks of known age (unpublished data). This offers a robust addition to the wood mouse system for considering ageing related immunological and fitness processes.

## 6.5 Ongoing and future avenues of nutrition quality research in *A. sylvaticus*

### 6.5.1 Natural versus laboratory host

Work from my thesis has led to several ongoing and planned avenues of complementary research. Results from my paired field and laboratory experiment and previous work showing variation in resistant to *H. polygyrus bakeri* in inbred laboratory strains (Filbey *et al.*, 2014) led to the development of an honours project in collaboration with Matthew Taylor co-supervised by myself and Amy Pedersen investigating the mechanism of immune response to *H. polygyrus bakeri* in laboratory (BALB/c strains) versus our captive wood mice. We found promising

preliminary results indicating that methods of resistance were fundamentally different in the strains, where BALB/c resistance to secondary infection was mediated by granuloma formulation and wood mice, although also resistant to secondary infection, did not develop any granulomas. This line of work was carried on to masters' student projects which investigated this comparison of laboratory and wild captive mouse *H. polygyrus bakeri* resistance in the context of the diet groups used in my thesis and will be an ongoing area of research to determine the mechanism of nutrition-dependent immune variation across mouse strains.

### 6.5.2 Diet quality and distribution

Preliminary data from Chapters 2 and 3 contributed to the successful award of a large NERC grant. This grant project, already underway, will address the gap between individual-level and population-level responses to resource supplementation by comparison high- and poor-quality diets and homogenous (as used in my thesis) versus aggregated resources. Insights from explicit tests of these conditions will provide important mechanistic understanding to the observation of diverse impacts on the parasite community of *A. sylvaticus* in Chapter 3. I will continue to work with my advisor, Amy Pedersen, who leads this grant and colleagues to help decipher the results of the paired laboratory and field experiments.

### 6.5.3 Deciphering the complex interactions within the mammalian gut

Microbiota community analysis has been surging in recent years in relation to its impact on helminth infections (Walk *et al.*, 2010; Lee *et al.*, 2014; Kreisinger *et al.*, 2015; Newbold *et al.*, 2017), diet (Muegge *et al.*, 2011; Kau *et al.*, 2011), and host

and environmental variation (Maurice *et al.*, 2015). I received a small grant to sequence 16S V4 regions of individual time series from both field and laboratory experiments after diet supplementation and experimental helminth removal (or experimental infection and reinfection). These data will be analysed for investigation of the microbiota responses to experimental perturbations in diet and helminth infection across a controlled and wild environment. These experimental and observational insights may provide key insights into the within-host responses of mammals to artificially or naturally fluctuating resources in relationship to outcome of parasite infection.

## 6.6 Broader implications

Embracing the role of environmental variation in shaping host-parasite dynamics in the wild can have important implications beyond biological understanding. Results from laboratory and wild experiments in Chapter 2 suggest that nutritional supplementation can serve as a complement to deworming programmes to address the common problem of rapid reinfection after treatment by both limiting onward transmission by reducing egg shedding of *H. polygyrus* and by increasing host resistance to parasites they are exposed to, which significantly disrupts multiple phases of the transmission cycle (Figure 6.2). Evidence from supplement plus treatment groups in Chapter 2, and treatment outcome in Chapter 4, which was carried out only transiently, but in a year of high tree-fruit, also suggest that the synergistic effects of supplementation with anthelmintic treatment can increase the efficacy of these commonly used drugs. The co-occurrence of malnutrition with helminths is regarded as a serious problem (Koski and Scott, 2001). Multi-nutrient biscuits have been used in clinical trials previously for this aim, but conclusive

results have been difficult to reach with observational data and without specific quantification of both parasitological and immunological responses (Yap *et al.*, 2014). Wood mice and *H. polygyrus* are considered a model system for chronic helminths in humans (Behnke *et al.*, 2009b). Work from this thesis therefore represents important experimental tests of this intervention.

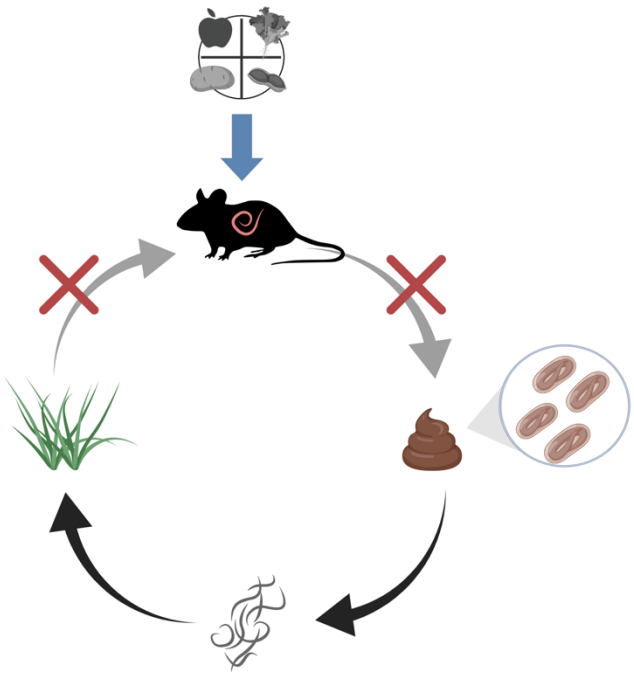

**Figure 6.3 Illustration of disruption of *H. polygyrus* transmission cycle following nutrition supplementation.**

Results from Chapter 3 and Chapter 4 may have key implications for predicting the outcome of altered wildlife food sources as a result of increasing anthropogenic-wildlife overlap. Primarily, they show that sole focus on even the most prevalent

parasite of a population may overlook numerous other altered infectious disease dynamics. Chapter 4 additionally shows that there are important longer-term effects of resource provisioning which are not suggested by short-term assessment only. These suggest that co-infection and host population cycles are crucial context for predicting the outcome of access to anthropogenic food sources. Although the sampling required to consistently account for these factors is typically prohibitive in the wild, consideration of the mechanisms underlying these potential relationships for theoretical work could also be considered.

Finally, in addition to highlighting that the relationships of season and host characteristics with *H. polygyrus* intensity vary fundamentally over time, results from Chapter 5 highlight the need for robust sampling designs for disease studies in the wild. Single temporal or spatial replicates, even if high-powered, can result in misleading inferences. This chapter also suggests substantial benefits of longitudinal sampling for investigating the drivers of parasitism in the wild.

## 6.7 Concluding remarks

Using a naturally co-infected, wild wood mouse system I was able to combine experimental perturbations, longitudinal sampling, and statistical analysis to investigate the relationships among nutrition quality, host immunity, and a diverse parasite community. Experimental perturbations in a wild natural host-helminth system enabled me to test the ecological role of resources within the population, while similarities of *H. polygyrus* epidemiology to human helminth infections and of experiment nutritional supplementation with anthropogenic provisioning to wildlife provide extensions to considerations for policy. This work represents to the best of

my knowledge the first demonstration of the synergistic effects of nutrition supplementation and anthelmintic treatment in a wild and laboratory study in the same natural host-helminth system and the first demonstration of diverse effects of resource supplementation on both host traits and parasite infection in a naturally co-infected host population. Statistical approaches designed for the investigation of long-term effects of nutrition supplementation and spatiotemporal variation in can be applied to other focal questions and datasets for investigation of the influence of time and space on the determinants of parasitism in the wild. Overall, the results from this thesis in addition to continued ongoing and planned work provides both mechanistic and broad insights into the role of nutrition quality in wild wood mouse parasite dynamics.

## R Packages Used


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## Image Credits

1. *Apodemus sylvaticus* silhouette (throughout): Dr. Anthony Caravaggi.

   Phylopic

2. Infographics throughout created on Biorender.com

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# Appendix A: Supplementary Material for Chapter 2

Table S2.1. Model formulae for analyses of wild and laboratory experiments. EPG stands for eggs per gram faeces; dpi for days post-infection

| MODEL GROUP | SYSTEM | RESPONSE | EXPERIMENT TIMEPOINT | MODEL CLASS | MODEL FAMILY | FIXED EFFECTS* | INTERACTIONS | RANDOM EFFECTS |
|---|---|---|---|---|---|---|---|---|
| ***H. POLYGYRUS* INFECTION** | Wild | EPG | First capture | GLM | Negative Binomial | Body Mass + Reproductive status + Sex +Diet | | Grid*Year |
| | | EPG (mean) | Trapping duration | GLM | | as above + Treatment | Diet:Treatment | Grid*Year |
| | | Adult worm | End point | GLM | | as above + Age | Diet:Treatment | Grid*Year |
| | Lab | EPG (peak) | Primary challenge | GLM | Negative Binomial | Body Mass+ Sex + Age + Group+ Diet | | |
| | | EPG (total) | Primary challenge | | | as above | | |
| | | Adult worm | End point | | | as above | Diet:timepoint | |
| **ANTIBODY RESPONSE** | Wild | IgA (total) | All captures | GLMM | Gaussian | Body condition index + Reproductive status+ Sex, H. polygyrus infection + Diet + Year | Diet:Year | ID + Grid*Year |
| | | IgG1 (specific) | | | | as above | | Grid*Year |
| | Lab | IgA (total) | 14 & 21 dpi | GLMM | Gaussian | Sex + Age + H. polygyrus infection + Experiment Timepoint + Diet | Diet:timepoint | ID |
| | | IgG1 (specific) | 21 dpi | GLM | | Sex + Age + H. polygyrus infection (n worms) + Diet | | |
| **BODY CONDITION** | Wild (a) non-pregnant | BCI | All captures | LMM | Gaussian | Reproductive status + Sex + H. polygyrus infection (Log, EPG) + Year + Day + Diet + Treatment | Diet:Day | ID + Grid*Year |
| | | Total fat score | | | | as above | Diet:Day | ID + Grid*Year |
| | Wild (b)pregnant | BCI | | | | H. polygyrus infection (Log, EPG) + Year + Day + Diet + Treatment | Diet:Day | ID + Grid*Year |
| | | Total fat score | | | | as above | Diet:Day | ID + Grid*Year |
| | Lab | Body mass (g) | All captures | LMM | Gaussian | Sex + Age + H. polygyrus infection (Log, EPG) + Day + Diet | Diet:Day | ID |
| | | Total fat score | | | | as above | Diet:Day | ID |

**Table S2.2. Description of fixed effects included in models**

| Term | System | Class | Description |
|---|---|---|---|
| Diet | Wild | Factor | Control (grids not supplemented); Supplemented (High quality pellets added to grids) |
| | Laboratory | | Control (standard chow); Supplemented (high-quality chow used in wild) |
| Treatment | Wild & Laboratory | Factor | Control (water); Treated (anthelminthic) |
| *H. polygyrus* infection | Wild & Laboratory | Continuous | Endpoint: N worms; Other timepoints: Log, EPG |
| Body Mass | Wild & Laboratory | Continuous | Mass in g |
| Body Condition Index (BCI) | Wild | Continuous | Residuals of weight ~ length regression |
| Age | Wild | Continuous | Age proxy: Log, paired eye lenses mass) |
| | Laboratory | | Age in weeks |
| Reproductive status | Wild | Factor | Inactive (Males- Abdominal or Descended Testes; Females- nonperforate or perforate vagina); Active (Males- Scrotal; Females - Pregnant or Lactating); |
| Year | Wild | Factor | 2015 Replicate; 2016 Replicate |
| Day | Wild & Laboratory | Continuous | Day of experiment |
| Timepoint | Laboratory | Factor | Day 14 or 21 post-infection |
| Group | Laboratory | Factor | Control (primary challenge only group); Experimental (primary and secondary challenge) |
| Grid | Wild | Factor | Spatial replicates: 2015 (Grids 1-3) or 2016 (Grids 1-4) |

## Raw Data Summary, Full Model Output & Model Variation Output

This section includes raw data summaries and full model output for further interpretation of data presented in the main text. Additionally, we describe the models using a 3-level factor for supplementation in the wild to more accurately classify mice who were captured on both supplemented and control grids. Model output for full main models is shown in Figure 2.3. For model variation described, fixed effects are held the same as in the main models, with the exception of the addition of Supplement category ('Mix') (Figure S2.1). Estimates for fixed effects are compared between main models and their variations.

For both models where an effect of supplemented nutrition was detected, both levels of supplemented nutrition (mix and supplemented) lowered *H. polygyrus* EPG and worm burden, and the magnitude and direction of other fixed effects closely mirrored those of the base model of comparison (Figures S2.1& S2.3).

**Table S2.3 Raw data H. polygyrus summary. Data represents mean egg/gram (EPG) (± SE) and sample sized for the indicated group.**

| | Group | Control (dH2O) | | Treated Anthelmintic | |
|---|---|---|---|---|---|
| | | Control | Supplemented | Control | Supplemented |
| *Wild* | First Capture (mean EPG) | 23.55 (± 8.72); n=36 | 12.41 (± 3.20); n=52 | na | na |
| | Trapping During (mean EPG) | 43.68 (± 8.78); n=37 | 29.96 (± 11.07); n=46 | 29.02 (± 19.99); n=36 | 0.136 (± 0.136); N=48 |
| | End point (N worms) | 30.50 (± 7.99); n=6 | 12.21 (± 3.96); n=14 | 1.71 (± 0.89); n=7 | 0.11 (± 0.11); n=9 |

| | Group | Primary Challenge | | Secondary Challenge | |
|---|---|---|---|---|---|
| | | Control | Supplemented | Control | Supplemented |
| *Colony* | Peak EPG | 48.03(± 25.79); n=9 | 19.59 (± 8.42); n=10 | 1.76 (± 1.19); n=6 | 0.00 (± 0.00); n=7 |
| | Sum EPG | 63.86 (± 26.82); n=9 | 28.26 (± 11.38); n=10 | 2.72 (± 2.07); n=6 | 0.00 (± 0.00); n=7 |
| | End point (N worms) | 9.67 (± 3.38)' n=3 | 12.33 (±4.91); n=3 | 28.33 (±6.50); n=6 | 6.71 (± 1.98); n=7 |

**Table S2.4. Model estimates for fixed effects on wild H. polygyrus infection**

| | First Capture EPG | | | Trapping Duration EPG | | | End Point Number of adult worms | | |
|---|---|---|---|---|---|---|---|---|---|
| Term | Estimate (CI) | Std.error | p.value | Estimate (CI) | Std.error | p.value | Estimate (CI) | Std.error | p.value |
| Diet, Supplemented: Treated, Anthelminthic | | | | -4.51 (-8.12 - -0.9) | 1.84 | **0.014** | -2.25 (-5.26 - 0.75) | 1.53 | 0.142 |
| Treated, Anthelminthic | | | | -2.11 (-4.58 - 0.35) | 1.26 | 0.092 | -2.74 (-4.26 - -1.22) | 0.78 | **< 0.001** |
| Diet, Supplemented | -1.42 (-2.59 - -0.24) | 0.6 | **0.018** | -1.56 (-3.65 - 0.53) | 1.07 | 0.145 | -1.2 (-2.38 - -0.03) | 0.6 | **0.045** |
| Sex, Male | -0.99 (-2.27 - 0.3) | 0.66 | 0.132 | 0.25 (-1.59 - 2.09) | 0.94 | 0.787 | -1.16 (-2.58 - 0.26) | 0.72 | 0.109 |
| Reproductive, active | -0.53 (-1.74 - 0.68) | 0.62 | 0.387 | 1.37 (-0.26 - 3.01) | 0.83 | 0.100 | -0.25 (-1.26 - 0.77) | 0.52 | 0.632 |
| Body Mass, g | 0.15 (-0.01 - 0.32) | 0.08 | 0.065 | 0.3 (0 - 0.6) | 0.15 | 0.053 | 0.21 (0.03 - 0.39) | 0.09 | **0.019** |
| Age (Eye lens weight, mg) | | | | | | | 2.22 (0.41 - 4.04) | 0.93 | **0.016** |
| (Intercept) | 1.42 (-1.32 - 4.16) | 1.4 | 0.309 | -2.46 (-7.84 - 2.91) | 2.74 | 0.369 | -6.32 (-11.48 - -1.16) | 2.63 | **0.016** |

**Table S2.5. Model estimates for fixed effects on laboratory H. polygyrus infection**

| | **Primary Challenge Peak EPG** | | | **Primary Challenge Sum EPG** | | | **End Point (Primary & Secondary Challenge) N worms** | | |
|---|---|---|---|---|---|---|---|---|---|
| Term | Estimate (CI) | Std.error | p.value | Estimate (CI) | Std.error | p.value | Estimate (CI) | Std.error | p.value |
| Diet, Supplemented: Secondary Challenge | | | | | | | -1.76 (-2.88 - -0.64) | 0.57 | **0.002** |
| Diet, Supplemented | -1.09 (-1.99 - -0.19) | 0.46 | **0.017** | -1.07 (-2.04 - -0.11) | 0.49 | **0.03** | 0.21 (-0.73 - 1.14) | 0.48 | 0.663 |
| Group, Experimental | 1.96 (0.86 - 3.05) | 0.56 | **<0.001** | 2.22 (1.07 - 3.37) | 0.59 | **<0.001** | 0.89 (0.07 - 1.71) | 0.42 | **0.033** |
| Sex, Male | -0.51 (-2.05 - 1.03) | 0.79 | 0.515 | 0.06 (-1.62 - 1.74) | 0.86 | 0.945 | -0.08 (-1 - 0.83) | 0.47 | 0.857 |
| Body Mass, g | 0.01 (-0.17 - 0.19) | 0.09 | 0.902 | -0.06 (-0.25 - 0.14) | 0.1 | 0.57 | 0.06 (-0.04 - 0.16) | 0.05 | 0.248 |
| Age (Weeks) | -0.01 (-0.09 - 0.08) | 0.04 | 0.875 | -0.01 (-0.1 - 0.08) | 0.05 | 0.81 | -0.02 (-0.08 - 0.04) | 0.03 | 0.428 |
| (Intercept) | 2.82 (-0.9 - 6.54) | 1.9 | 0.138 | 3.52 (-0.46 - 7.5) | 2.03 | 0.083 | 1.84 (-0.53 - 4.21) | 1.21 | 0.127 |

**Table S2.6. Model estimates for fixed effects on body condition in the wild**

| | All Adults | | | | | | Pregnant Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Body Condition Index | | | Total Fat Score | | | Body Condition Index | | | Total Fat Score | | |
| **Term** | **Estimate (CI)** | **Std. error** | **p value** | **Estimate (CI)** | **Std. error** | **p value** | **Estimate (CI)** | **Std. error** | **p value** | **Estimate (CI)** | **Std. error** | **p value** |
| Diet, Supplemented: Day | -0.16 (-0.28 - -0.04) | 0.06 | **0.011** | -0.09 (-0.15 - -0.02) | 0.03 | **0.007** | -0.61 (-0.76 - -0.45) | 0.08 | **<0.001** | -0.15 (-0.3 - 0) | 0.08 | **0.045** |
| Diet, Supplemented | 1.46 (0.34 - 2.59) | 0.57 | **0.011** | 0.64 (0.18 - 1.11) | 0.24 | **0.006** | 8.32 (5.5 - 11.15) | 1.44 | **<0.001** | 1.98 (0.6 - 3.37) | 0.71 | **0.005** |
| Day | -0.03 (-0.13 - 0.07) | 0.05 | 0.525 | 0.01 (-0.04 - 0.07) | 0.03 | 0.582 | 0 (-0.11 - 0.12) | 0.06 | 0.969 | 0.11 (-0.01 - 0.22) | 0.06 | **0.062** |
| Year, 2016 | 1.02 (0.04 - 1.99) | 0.5 | **0.041** | -0.81 (-1.14 - -0.47) | 0.17 | **<0.001** | 1.57 (-0.8 - 3.95) | 1.21 | 0.194 | -0.83 (-1.72 - 0.06) | 0.46 | **0.069** |
| Log, *H.polygyrus* EPG | 0.08 (-0.14 - 0.29) | 0.11 | 0.49 | 0.03 (-0.07 - 0.12) | 0.05 | 0.583 | -0.64 (-0.87 - -0.41) | 0.12 | **<0.001** | 0.06 (-0.2 - 0.32) | 0.13 | 0.64 |
| Treated, Anthelminthic | 0.28 (-0.71 - 1.26) | 0.5 | 0.582 | -0.14 (-0.49 - 0.21) | 0.18 | 0.429 | 0.26 (-2.27 - 2.79) | 1.29 | 0.838 | 0.16 (-0.81 - 1.13) | 0.5 | 0.746 |
| Sex, Male | 0.57 (-0.42 - 1.55) | 0.5 | 0.258 | -0.25 (-0.59 - 0.09) | 0.17 | **0.15** | | | | | | |
| Reproductive, active | 1.53 (0.74 - 2.33) | 0.4 | **<0.001** | -0.22 (-0.57 - 0.13) | 0.18 | **0.215** | | | | | | |
| Intercept | -2.6 (-4.03 - -1.17) | 0.73 | **<0.001** | 5.98 (5.41 - 6.56) | 0.29 | **<0.001** | -0.46 (-4.24 -3.32) | 1.93 | 0.81 | 4.57 (2.81 - 6.34) | 0.9 | **<0.001** |

**Table S2.7. Model estimates for fixed effects on wild and laboratory immune responses**

| | Wild | | | | | | Laboratory | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IgA, Total | | | IgG1, Specific | | | IgA, Total | | | IgG1, Specific | | |
| Term | Estimate (CI) | Std. error | p.value | Estimate (CI) | Std. error | p.value | Estimate (CI) | Std. error | p.value | Estimate (CI) | Std. error | p.value |
| Diet, Supplemented: Year, 2016 (wild) Time point (Lab) | 5.31 (1.46 - 9.17) | 1.97 | **0.007** | 0.2 (-0.11 - 0.52) | 0.16 | 0.204 | -2.39 (-5.68 - 0.9) | 1.68 | 0.155 | | | |
| Diet, Supplemented | -0.08 (-2.69 - 2.53) | 1.33 | 0.953 | -0.12 (-0.29 - 0.06) | 0.09 | 0.204 | 2.4 (0.42 - 4.38) | 1.01 | 0.018 | 0.2 (0.11 - 0.3) | 0.05 | **<0.001** |
| Year, 2016 (wild) Timepoint, d21 (Lab) | -5.14 (-8.13 - -2.15) | 1.52 | **0.001** | -0.05 (-0.3 - 0.2) | 0.13 | 0.689 | 1.53 (-1.4 - 4.46) | 1.49 | 0.306 | | | |
| Body condition Index | 0.46 (0.16 - 0.76) | 0.15 | **0.003** | 0.02 (0 - 0.03) | 0.01 | **0.01** | | | | | | |
| Log, *H.polygyrus EPG* | 0.43 (-0.07 - 0.93) | 0.25 | 0.089 | -0.01 (-0.03 - 0.01) | 0.01 | 0.32 | -0.2 (-0.82 - 0.43) | 0.32 | 0.538 | 0 (0 - 0) | 0 | 0.442 |
| Treated, Anthelminthic | 2.19 (0.2 - 4.18) | 1.02 | **0.031** | 0.07 (-0.08 - 0.21) | 0.07 | 0.366 | | | | 0.16 (-0.81 - 1.13) | 0.5 | 0.746 |
| Sex, Male | 0 (-1.87 - 1.87) | 0.96 | 1 | 0 (-0.15 - 0.14) | 0.07 | 0.981 | 0.42 (-1.72 - 2.57) | 1.09 | 0.699 | -0.23 (-0.31 - -0.15) | 0.04 | **<0.001** |
| Reproductive, active | 1.18 (-0.65 - 3.01) | 0.93 | 0.205 | 0 (-0.08 - 0.08) | 0.04 | 0.974 | | | | | | |
| Age, weeks | | | | | | | -0.42 (-0.9 - 0.05) | 0.24 | 0.082 | 0 (-0.02 - 0.02) | 0.01 | 0.804 |
| Intercept | 18.71 (15.72 - 21.71) | 1.53 | **<0.001** | 0.38 (0.18 - 0.57) | 0.1 | **<0.001** | 31.63 (20.88 - 42.38) | 5.48 | <0.001 | 0.93 (0.39 - 1.47) | 0.28 | **0.001** |

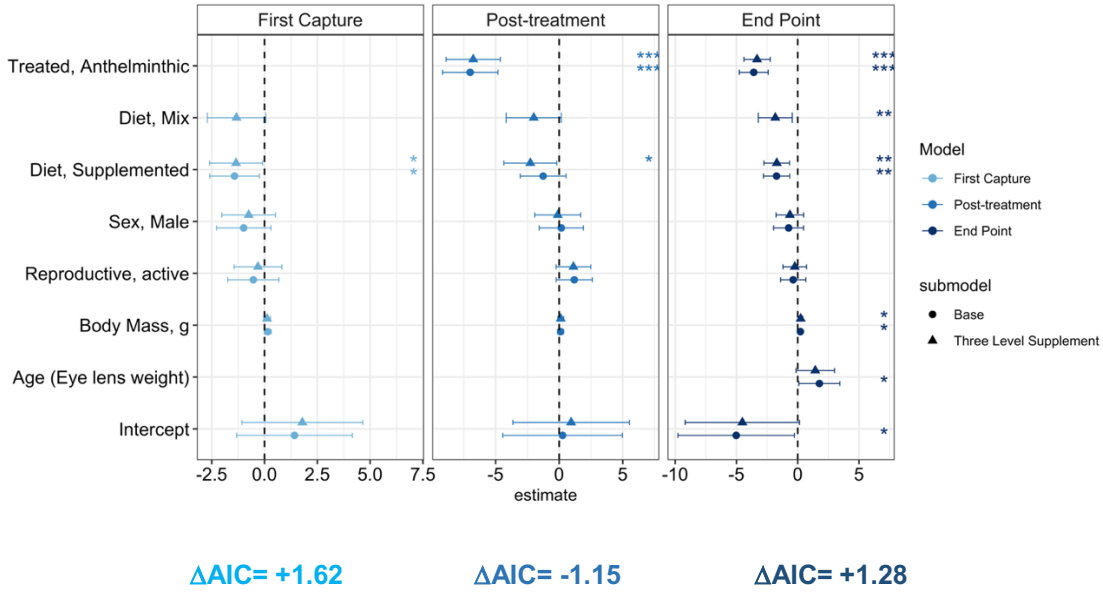


**Figure S2.1. Effect size estimates from models investigating the effect of supplemented nutrition on *H. polygyrus* infection, accounting for individuals captured only a portion of time on supplemented grids (mix).** Panels represent separate models for first capture, infection abundance, (EPG infected individuals only) and risk; average abundance (EPG) across two weeks (infected individuals only) and risk for individuals captured beyond first capture and after assignment to treatment categories; end point burden (adult worm count) for individuals culled 12-16 days post first capture. Models represented are identical to those included in Fig 3 with the exception of the inclusion of an additional factor level in the supplemented nutrition explanatory variable. Points and ranges represent model estimates and 95% credibility estimates for each model. Asterisks indicate the significance of variables: ***, ** and * indicate P<0.001, P<0.01 and P<0.05 respectively. Change in AIC from main models is included in the bottom left of each panel. Only risk models including two supplemented categories represent a superior fit to models including only category for supplemented individuals.

## Additional figures

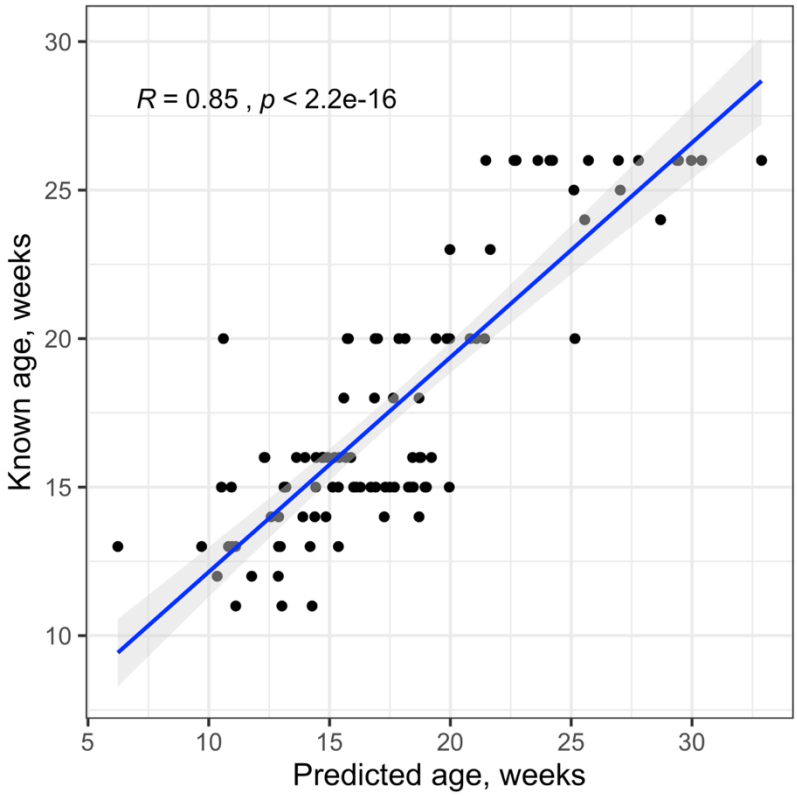


**Figure S2.2. Correlation of mouse age predicted from eye lens weight and known age (in weeks).** Pearson's R with 95% credibility intervals and significance of the correlation is included for both 2015 and 2016 data. Points have been jittered by 10% of raw values to aid in visualisation.

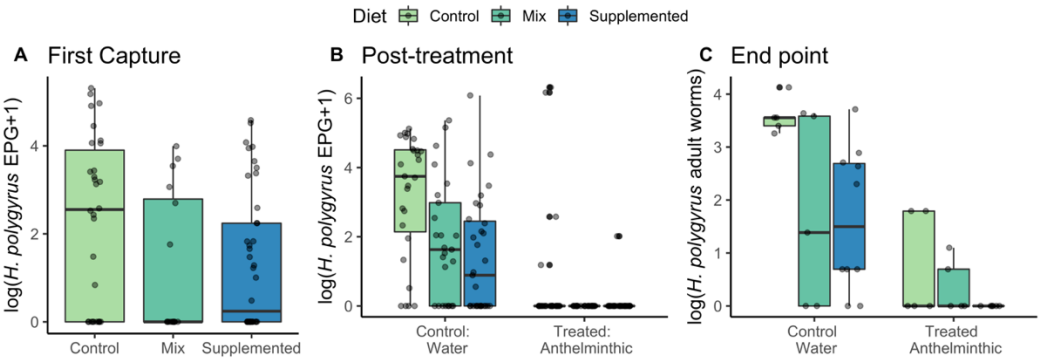


**Figure S2.3. Effect of supplemented nutrition on *H. polygyrus* infection in wild wood mice, accounting for both individuals who were found exclusively on supplemented grids or individuals who were found on supplemented grids only a portion of the time ('Mix")**. A. Infection abundance (EPG) at first capture, N=88 individuals. B. Mean EPG for all individuals captured beyond first capture and after assignment to treatment categories, N=62 individuals; 166 captures C. Adult worm burden at end point for culled individuals, N=36. Data represent log means and SE for raw EPG data. Labels above bars indicate the number of observations for each group.

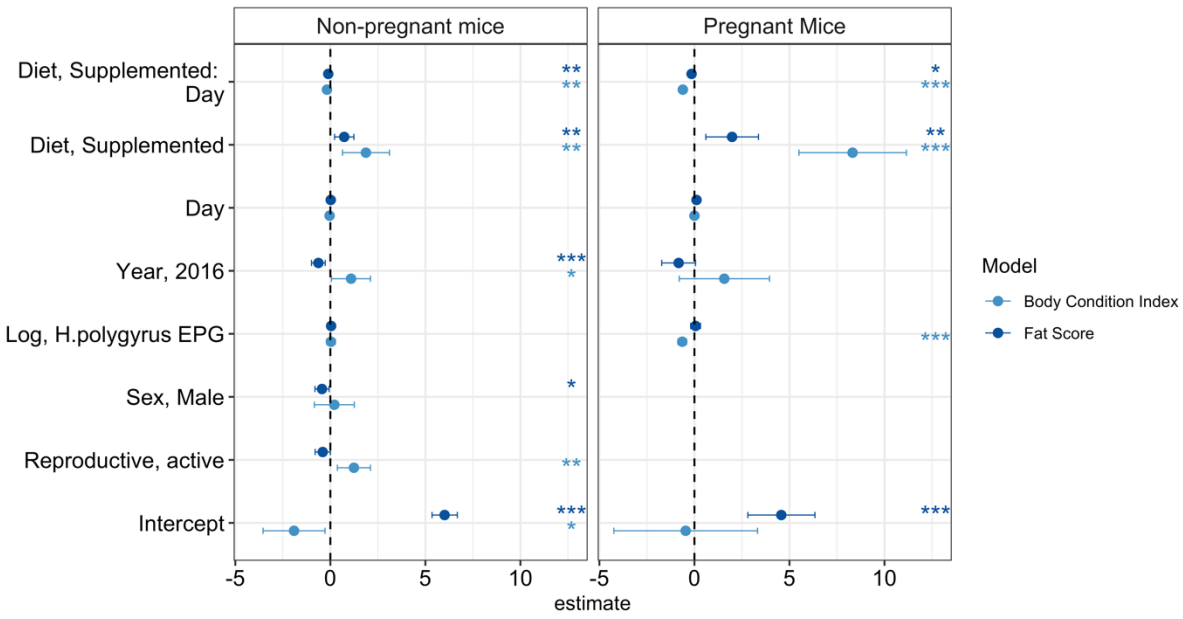


**Figure S2.4. Effect size estimates from the models investigating the effect of supplemented nutrition on wood mouse body condition in the wild, measured by body condition index (residuals of weight against length regression) and total fat score**. Points and ranges represent model estimates and 95% confidence intervals. Asterisks indicate the significance of variables: ***, ** and * indicate P<0.001, P<0.01 and P<0.05 respectively. .A. Models including all adult mice excepting pregnant females; N=79 individuals, 178 captures. B. Models on pregnant females, N=15 individuals and 32 captures

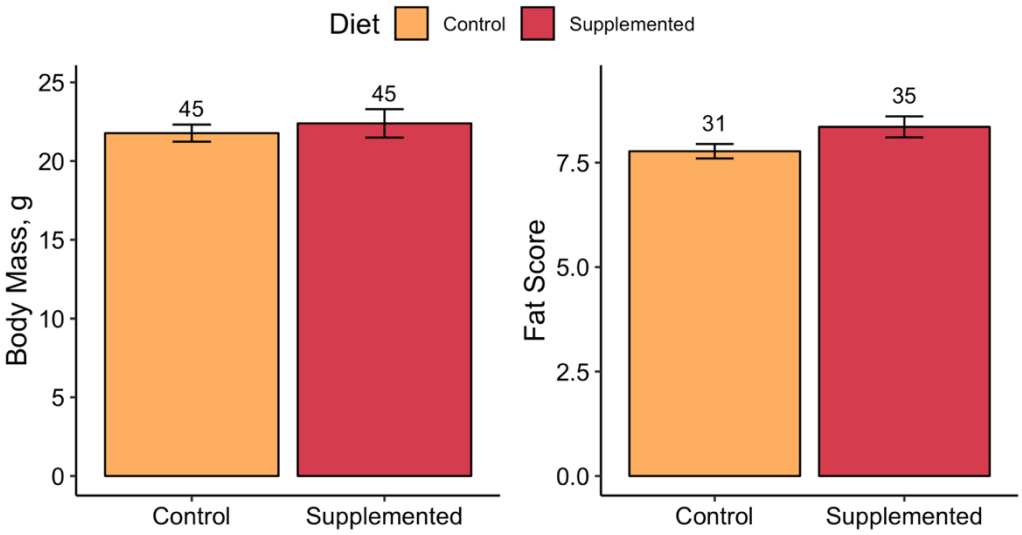


**Figure S2.5. Effect of supplemented diet on body condition in the laboratory.**

**Bar plots represent raw data means ± SEM.**

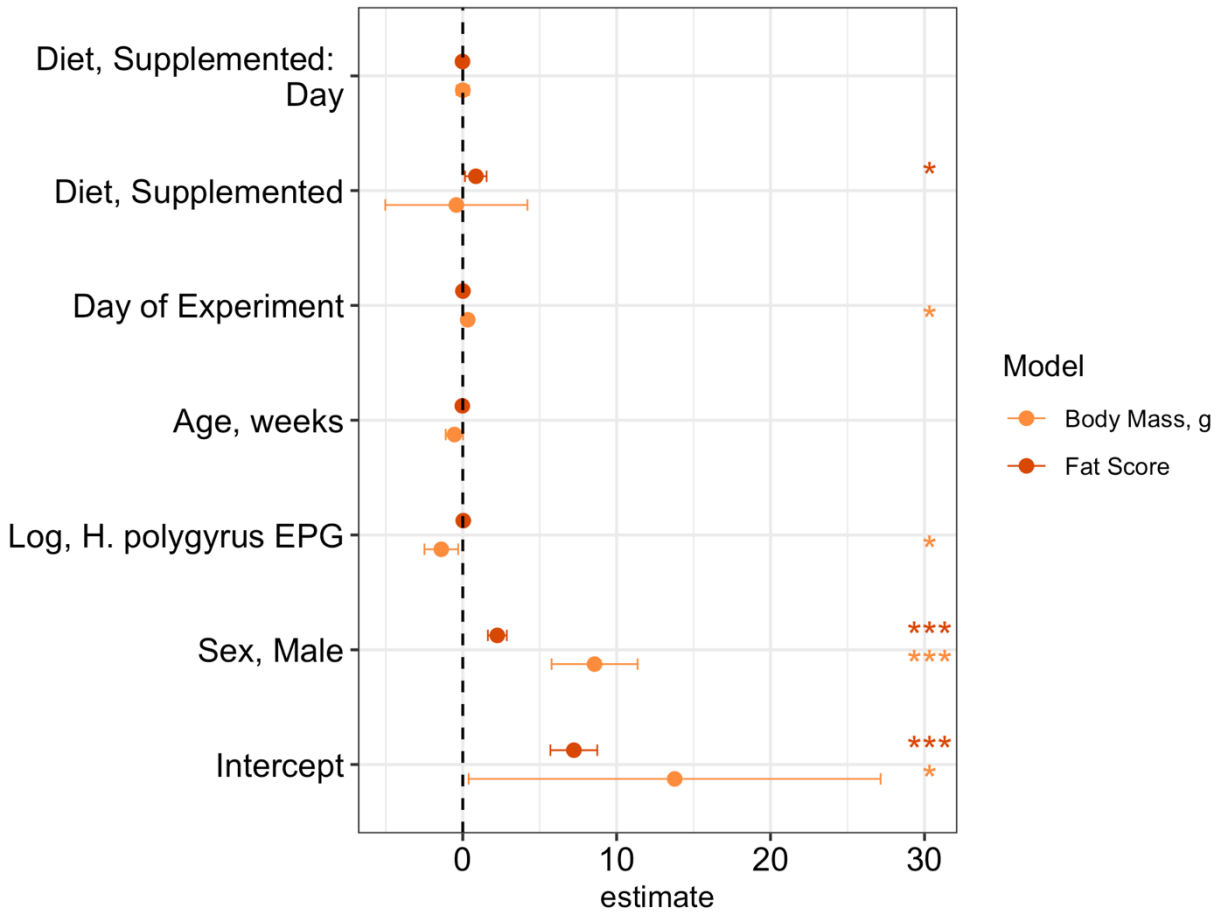


**Figure S2.6. Effect size estimates from the models investigating the effect of supplemented nutrition on wood mouse body condition in the laboratory, measured by mass and total fat score**. Points and ranges represent model estimates and 95% confidence intervals. Asterisks indicate the significance of variables: ***, ** and * indicate P<0.001, P<0.01 and P<0.05 respectively.

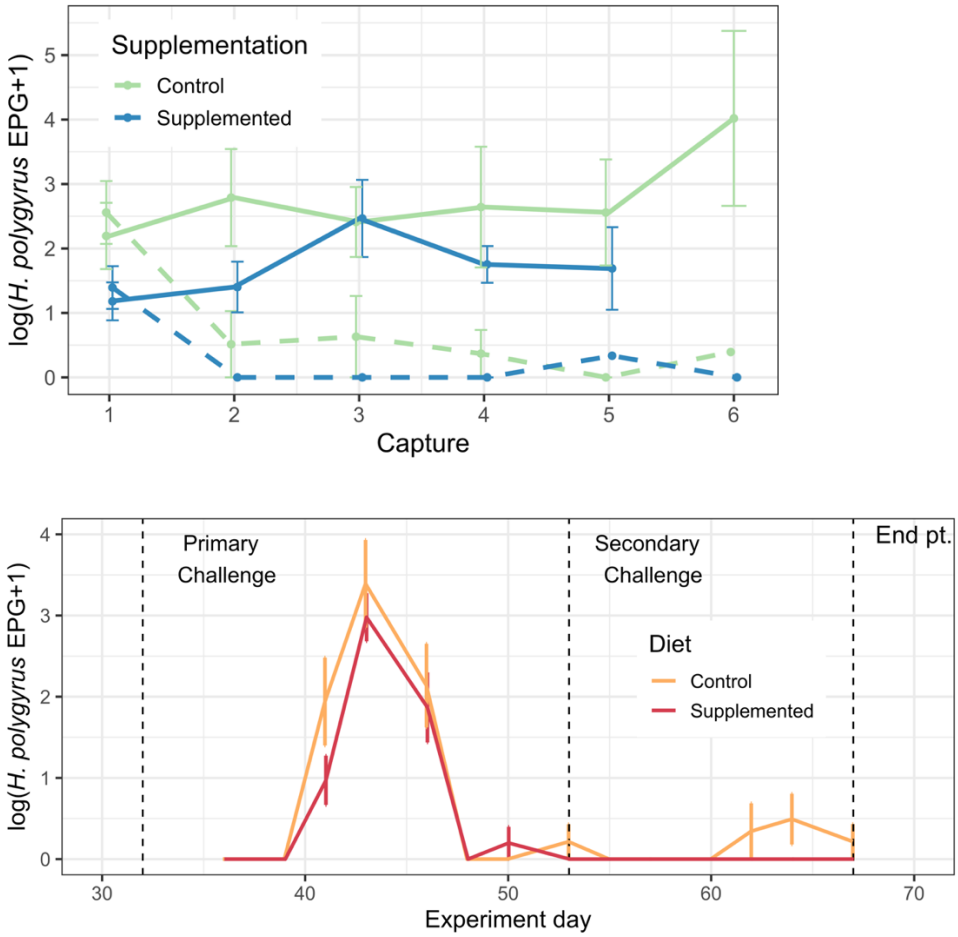


**Figure S2.7. H. polygyrus abundance dynamics, as measured by eggs/gram over the course of A. field experiment and B. colony experiment.** Data represent the log of EPG+1 and SE. Dashed lines in A represent individuals who were treated with anthelminthics. Dashed lines in B indicate infection and end timepoints of experiment.

# Appendix B: Supplementary Material for Chapter 3

**PCR protocols**

DNA was extracted from blood samples using a MagMAX™-96 DNA Multi-Sample Kit (ThermoFisher Scientific) on a KingFisher™ Flex Purification System optimised for small blood volumes and mouse samples according to manufacturer instructions. This kit is designed for high throughput purification of DNA from animal tissue and blood and uses magnetic bead-based isolation and extractions were carried out according to manufacturer's instructions. Briefly, 10 µL of $dH_2O$ was added to whole blood pellets and homogenised. Samples were lysed with proteinase K followed by a treatment with guanidinium thiocyanate-based solution. Following lysis, samples were mixed with isopropanol and combined with paramagnetic beads with a nucleic acid binding surface. The beads, with bound nucleic acid, are immobilized on magnets and washed to remove proteins and other contaminants. A second wash solution is used to remove residual binding solution and then the nucleic acid is eluted using a low-salt buffer.

Trypanosomes were detected using a nested PCR targeting a 530bp section of the 18S rRNA gene (1). Primers TRY927F 5'-GAAACAAGAAACACGGGAG and TRY927R 5'-CTACTGGGCAGCTTGGA were used in the first round, and SSU561F 5'-TGGGATAACAAAGGAGCA and SSU561R 5'-CTGAGACTGTAACCTCAAAGC in the second round. Each reaction contained 2µl genomic DNA, 0.1mM each dNTP, 0.2µM each primer, 0.8mM $MgCl_2$, 0.5U Platinum Taq DNA Polymerase (Invitrogen) with the accompanying buffer at 1x concentration. A touchdown PCR profile was used, with initial denaturation at 96°C for 3min, followed by 20 cycles of 96°C for 30s, 60°C for 1min (decreasing by 0.5°C each cycle until 55°C), and 72°C for 90s, followed by a final extension at 72°C for 10min. In the event that any negative control was positive, the entire PCR plate was repeated. 5µl of all PCR products were run on 2% agarose gels stained with ethidium bromide and visualized under UV light. For the trypanosome PCR, samples showing an approx. 500bp band were scored as positive. All positive samples were sequenced

and across the 502bp sequence obtained (excluding primers) all were 100% identical to *Trypanosoma grosi* (Genbank accession: AB175624). Wood Mouse Herpes Virus (WMHV) was detected using pan-herpesvirus primers targeting a 160 bp region of the DPOL gene: ILK+ 5′-ATAAACAACAGCTGGCCATCAA-3′ and KG1+ 5′-CTGACCAGATCCACCCCTTT-3′ in the first round, followed by TGV+ 5′-TGTAATTCTGTCTATGGCTTCACAGGAGT-3′ and IGY+ 5′-AAGAGAATCTGTGTCTCCATAAAT-3′ in the second round (2). Reactions were run in 25 µl volumes, containing 0.4 µM each primer (Metabion), 0.2 mM dNTPs, 3 mM $MgCl_2$, one unit GoTaq DNA Polymerase (Promega), 5 µl 5× GoTaq Flexi Buffer (Promega) and 2 µl template DNA. The following PCR conditions were used for either 25 cycles (first round) or 35 cycles (second round): initial denaturation at 95 °C for 3 min, cycles of 95 °C for 20 s, 61 °C for 30 s and 72 °C for 30 s, and a final extension of 72 °C for 10 mins.

**Appendix B, References**


1. Noyes H, Stevens J, Teixeira M, Phelan J, Holz P (1999) A nested PCR for the ssrRNA gene detects *Trypanosoma binneyi* in the platypus and *Trypanosoma* sp. in wombats and kangaroos in Australia. *Int J Parasitol* 29:331–339.
2. Knowles, SCL, Fenton, A, Pedersen, AB (2012). Epidemiology and fitness effects of wood mouse herpesvirus in a natural host population. *Journal of General Virology* 93: 2447-2456

Table S3.1 Model output for parasite-specific models presented in Chapter 3

| parasite | term | estimate | std.error | p.value | response | model |
|---|---|---|---|---|---|---|
| *C. murissylvatici* | Intercept | 9.87 (4.24 - 15.5) | 2.87 | **0.001** | intensity | main |
| *C. murissylvatici* | Resources, supplemented | 1.41 (-0.51 - 3.33) | 0.98 | 0.15 | intensity | main |
| *C. murissylvatici* | Body Mass, g | -0.3 (-0.59 - -0.01) | 0.15 | **0.041** | intensity | main |
| *C. murissylvatici* | Sex, M | 1.4 (-0.12 - 2.93) | 0.78 | 0.071 | intensity | main |
| *C. murissylvatici* | Year, 2016 | -1.86 (-4.62 - 0.91) | 1.41 | 0.188 | intensity | main |
| *C. murissylvatici* | Intercept | 3.83 (2.56 - 5.11) | 0.65 | **<0.001** | intensity | post-treatment |
| *C. murissylvatici* | Resources, supplemented | 3.28 (0.95 - 5.61) | 1.19 | **0.006** | intensity | post-treatment |
| *C. murissylvatici* | Treatment, drug | 2.04 (-0.66 - 4.75) | 1.38 | 0.139 | intensity | post-treatment |
| *C. murissylvatici* | Resources, supplemented: Treatment, drug | -4.99 (-8.6 - -1.38) | 1.84 | **0.007** | intensity | post-treatment |
| *E. hungaryensis* | Intercept | 8.03 (3.36 - 12.71) | 2.39 | **0.001** | intensity | main |
| *E. hungaryensis* | Resources, supplemented | -0.08 (-2.23 - 2.08) | 1.1 | 0.944 | intensity | main |
| *E. hungaryensis* | Body Mass, g | -0.17 (-0.41 - 0.08) | 0.13 | 0.185 | intensity | main |
| *E. hungaryensis* | Sex, M | 0.77 (-1.37 - 2.92) | 1.1 | 0.481 | intensity | main |
| *E. hungaryensis* | Year, 2016 | 3 (0.96 - 5.05) | 1.04 | **0.004** | intensity | main |
| *E. hungaryensis* | Intercept | 11.98 (4.38 - 19.59) | 3.88 | **0.002** | intensity | post-treatment |
| *E. hungaryensis* | Resources, supplemented | 0.23 (-3.32 - 3.79) | 1.81 | 0.897 | intensity | post-treatment |
| *E. hungaryensis* | Body Mass, g | -0.41 (-0.81 - -0.02) | 0.2 | **0.042** | intensity | post-treatment |
| *E. hungaryensis* | Sex, M | 2.35 (-0.52 - 5.22) | 1.46 | 0.108 | intensity | post-treatment |
| *E. hungaryensis* | Year, 2016 | 3.36 (0.59 - 6.12) | 1.41 | **0.017** | intensity | post-treatment |
| *E. hungaryensis* | Treatment, drug | -0.69 (-5.11 - 3.73) | 2.25 | 0.76 | intensity | post-treatment |
| *E. hungaryensis* | Resources, supplemented: Treatment, drug | 2.65 (-3.02 - 8.33) | 2.9 | 0.359 | intensity | post-treatment |
| *E. uptoni* | Intercept | 8.97 (2.92 - 15.01) | 3.08 | **0.004** | intensity | main |
| *E. uptoni* | Resources, supplemented | -0.66 (-3.02 - 1.69) | 1.2 | 0.58 | intensity | main |
| *E. uptoni* | Body Mass, g | -0.23 (-0.54 - 0.08) | 0.16 | 0.153 | intensity | main |
| *E. uptoni* | Sex, M | 0.44 (-0.96 - 1.84) | 0.71 | 0.537 | intensity | main |
| *E. uptoni* | Year, 2016 | 0.17 (-1.71 - 2.04) | 0.96 | 0.862 | intensity | main |
| *E. uptoni* | Intercept | 12.88 (8.43 - 17.34) | 2.27 | **<0.001** | intensity | post-treatment |
| *E. uptoni* | Resources, supplemented | 3.88 (0.28 - 7.47) | 1.83 | **0.035** | intensity | post-treatment |
| *E. uptoni* | Body Mass, g | -0.39 (-0.6 - -0.19) | 0.1 | **<0.001** | intensity | post-treatment |
| *E. uptoni* | Sex, M | -2.92 (-4.16 - -1.68) | 0.63 | **<0.001** | intensity | post-treatment |
| *E. uptoni* | Year, 2016 | -3.24 (-6.52 - 0.03) | 1.67 | 0.052 | intensity | post-treatment |
| *E. uptoni* | Treatment, drug | -1.22 (-2.34 - -0.1) | 0.57 | **0.033** | intensity | post-treatment |
| *E. uptoni* | Resources, supplemented: Treatment, drug | -4.31 (-8.48 - -0.15) | 2.13 | **0.042** | intensity | post-treatment |

| | | | | | | |
|---|---|---|---|---|---|---|
| Fleas | Intercept | -3.95 (-7.23 - -0.68) | 1.67 | **0.018** | probability | main |
| Fleas | Resources, supplemented | -0.55 (-1.72 - 0.62) | 0.6 | 0.355 | probability | main |
| Fleas | Body Mass, g | 0.09 (-0.07 - 0.25) | 0.08 | 0.261 | probability | main |
| Fleas | Sex, M | 0.34 (-0.82 - 1.5) | 0.59 | 0.566 | probability | main |
| Fleas | Year, 2016 | -1.13 (-2.37 - 0.1) | 0.63 | 0.073 | probability | main |
| Fleas | Intercept | -3.71 (-8.16 - 0.74) | 2.27 | 0.103 | probability | post-treatment |
| Fleas | Resources, supplemented | -1.23 (-3.75 - 1.28) | 1.28 | 0.337 | probability | post-treatment |
| Fleas | Body Mass, g | 0.09 (-0.13 - 0.31) | 0.11 | 0.425 | probability | post-treatment |
| Fleas | Sex, M | -0.22 (-1.65 - 1.2) | 0.73 | 0.758 | probability | post-treatment |
| Fleas | Year, 2016 | -0.71 (-2.17 - 0.75) | 0.74 | 0.341 | probability | post-treatment |
| Fleas | Treatment, drug | 0.96 (-0.97 - 2.9) | 0.99 | 0.33 | probability | post-treatment |
| Fleas | Resources, supplemented: Treatment, drug | 0.14 (-3.06 - 3.33) | 1.63 | 0.934 | probability | post-treatment |
| *H. polygyrus* | Intercept | 4.46 (2.7 - 6.22) | 0.9 | **<0.001** | intensity | main |
| *H. polygyrus* | Resources, supplemented | -0.73 (-1.32 - -0.14) | 0.3 | **0.015** | intensity | main |
| *H. polygyrus* | Body Mass, g | -0.05 (-0.14 - 0.04) | 0.05 | 0.24 | intensity | main |
| *H. polygyrus* | Sex, M | 0.5 (-0.09 - 1.08) | 0.3 | 0.096 | intensity | main |
| *H. polygyrus* | Year, 2016 | 0.04 (-0.52 - 0.59) | 0.28 | 0.901 | intensity | main |
| *H. polygyrus* | Intercept | 3.68 (0.19 - 7.16) | 1.78 | **0.039** | intensity | post-treatment |
| *H. polygyrus* | Resources, supplemented | -0.64 (-1.7 - 0.41) | 0.54 | 0.231 | intensity | post-treatment |
| *H. polygyrus* | Body Mass, g | -0.03 (-0.21 - 0.15) | 0.09 | 0.748 | intensity | post-treatment |
| *H. polygyrus* | Sex, M | 0.64 (-0.45 - 1.73) | 0.56 | 0.246 | intensity | post-treatment |
| *H. polygyrus* | Year, 2016 | 0.24 (-0.79 - 1.26) | 0.52 | 0.652 | intensity | post-treatment |
| *H. polygyrus* | Treatment, drug | -0.07 (-2.61 - 2.48) | 1.3 | 0.96 | intensity | post-treatment |
| *H. polygyrus* | Resources, supplemented: Treatment, drug | -0.42 (-4.64 - 3.81) | 2.15 | 0.847 | intensity | post-treatment |
| MHV | Intercept | -12.08 (-33.49 - 9.33) | 10.92 | 0.269 | probability | main |
| MHV | Resources, supplemented | -0.8 (-8.81 - 7.21) | 4.09 | 0.845 | probability | main |
| MHV | Body Mass, g | 0.02 (-0.95 - 0.99) | 0.49 | 0.967 | probability | main |
| MHV | Sex, M | 0.06 (-8.22 - 8.33) | 4.22 | 0.989 | probability | main |
| MHV | Year, 2016 | -2.23 (-14.1 - 9.63) | 6.05 | 0.712 | probability | main |
| MHV | Intercept | -12.18 (-32.79 - 8.42) | 10.51 | 0.247 | probability | post-treatment |
| MHV | Resources, supplemented | -0.2 (-9.48 - 9.08) | 4.73 | 0.967 | probability | post-treatment |
| MHV | Body Mass, g | 0.03 (-0.88 - 0.95) | 0.47 | 0.944 | probability | post-treatment |
| MHV | Sex, M | -0.11 (-8.33 - 8.11) | 4.19 | 0.979 | probability | post-treatment |
| MHV | Year, 2016 | -2.37 (-14.28 - 9.54) | 6.08 | 0.696 | probability | post-treatment |
| MHV | Treatment, drug | 0.13 (-10.15 - 10.42) | 5.25 | 0.98 | probability | post-treatment |

| | | | | | | |
|---|---|---|---|---|---|---|
| MHV | Resources, supplemented: Treatment, drug | -1.65 (-18.18 - 14.89) | 8.44 | 0.845 | probability | post-treatment |
| Mites | Intercept | -0.15 (-1.64 - 1.35) | 0.76 | 0.847 | probability | main |
| Mites | Resources, supplemented | 0.64 (-0.01 - 1.28) | 0.33 | 0.053 | probability | main |
| Mites | Body Mass, g | -0.08 (-0.15 - 0) | **0.04** | 0.052 | probability | main |
| Mites | Sex, M | 0.62 (-0.01 - 1.25) | 0.32 | 0.054 | probability | main |
| Mites | Year, 2016 | 0.55 (-0.07 - 1.17) | 0.32 | 0.08 | probability | main |
| Mites | Intercept | -0.25 (-2.7 - 2.2) | 1.25 | 0.84 | probability | post-treatment |
| Mites | Resources, supplemented | 0.93 (-0.3 - 2.16) | 0.63 | 0.137 | probability | post-treatment |
| Mites | Body Mass, g | -0.07 (-0.19 - 0.05) | 0.06 | 0.264 | probability | post-treatment |
| Mites | Sex, M | 0.77 (-0.09 - 1.62) | 0.43 | 0.078 | probability | post-treatment |
| Mites | Year, 2016 | 0.34 (-0.53 - 1.21) | 0.44 | 0.448 | probability | post-treatment |
| Mites | Treatment, drug | 0.51 (-0.85 - 1.86) | 0.69 | 0.464 | probability | post-treatment |
| Mites | Resources, supplemented: Treatment, drug | -1.16 (-2.96 - 0.64) | 0.92 | 0.206 | probability | post-treatment |
| *T. grosi* | Intercept | 0.55 (-1.99 - 3.09) | 1.29 | 0.67 | probability | main |
| *T. grosi* | Resources, supplemented | 1.35 (-0.34 - 3.04) | 0.86 | 0.117 | probability | main |
| *T. grosi* | Body Mass, g | -0.12 (-0.24 - -0.01) | 0.06 | **0.039** | probability | main |
| *T. grosi* | Sex, M | 0.15 (-0.94 - 1.24) | 0.55 | 0.788 | probability | main |
| *T. grosi* | treatedY | 1.15 (-0.53 - 2.84) | 0.86 | 0.18 | probability | main |
| *T. grosi* | Resources, supplemented: treatedY | -2.77 (-5.05 - -0.49) | 1.16 | **0.017** | probability | post-treatment |
| *T. grosi* | Intercept | 0.74 (-1.56 - 3.04) | 1.17 | 0.529 | probability | post-treatment |
| *T. grosi* | Resources, supplemented | 1.38 (0 - 2.76) | 0.7 | 0.051 | probability | post-treatment |
| *T. grosi* | Body Mass, g | -0.12 (-0.23 - -0.01) | 0.06 | **0.029** | probability | post-treatment |
| *T. grosi* | Sex, M | 0.25 (-0.79 - 1.29) | 0.53 | 0.636 | probability | post-treatment |
| *T. grosi* | Treatment, drug | 0.84 (-0.66 - 2.35) | 0.77 | 0.271 | probability | post-treatment |
| *T. grosi* | Resources, supplemented: Treatment, drug | -2.3 (-4.33 - -0.26) | 1.04 | **0.027** | probability | post-treatment |
| Ticks | Intercept | 1.59 (1.12 - 2.06) | 0.24 | **<0.001** | intensity | main |
| Ticks | Resources, supplemented | -0.46 (-0.67 - -0.24) | 0.11 | **<0.001** | intensity | main |
| Ticks | Body Mass, g | 0.02 (-0.01 - 0.04) | **0.01** | 0.175 | intensity | main |
| Ticks | Sex, M | 0.16 (-0.03 - 0.35) | 0.1 | 0.091 | intensity | main |
| Ticks | Year, 2016 | 0 (-0.21 - 0.2) | 0.11 | 0.972 | intensity | main |
| Ticks | Intercept | 1.53 (0.75 - 2.31) | 0.4 | **<0.001** | intensity | post-treatment |
| Ticks | Resources, supplemented | -0.53 (-0.96 - -0.11) | 0.22 | **0.014** | intensity | post-treatment |
| Ticks | Body Mass, g | 0.01 (-0.02 - 0.05) | **0.02** | 0.479 | intensity | post-treatment |
| Ticks | Sex, M | 0.31 (0.04 - 0.59) | 0.14 | **0.026** | intensity | post-treatment |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ticks | Year, 2016 | -0.02 (-0.29 - 0.26) | 0.14 | 0.897 | intensity | post-treatment |
| Ticks | Treatment, drug | -0.36 (-0.77 - 0.05) | 0.21 | 0.088 | intensity | post-treatment |
| Ticks | Resources, supplemented: Treatment, drug | 0.57 (0.01 - 1.13) | 0.28 | **0.044** | intensity | post-treatment |

# Appendix C: Supplementary Material for Chapter 4

## Mast data

Detailed information an all recording species and categories can be found at: https://naturescalendar.woodlandtrust.org.uk/what-we-record-and-why/species-we-record/

Nature's Calendar is a citizen science project run by the Woodlan Trust and Centre for Ecology & Hydrology. Recorders may enter sightings on the website for a number of events of interest. One such event is flowering events, both date of flowering events and a tree fruit quality score. Species recorded for Nature's Calendar includes Beech (*Fagus sylvatica*) and Oak (*Quercus robur* & *Q. petraea*), fruit-beering trees. 'Amount of fruit' is a subjective assessment of fruit crop used in this analysis and has been recorded as a score of 1-5, where 1 represents no fruit, 2-meagre fruit, 3- moderate, 4- good crop, and 5- exceptional.

For every entry recorded, a thorough list of information is recorded including: Species name, Latin name, observation year, observation date, season, the event recorded (i.e. 'amount of fruit), easting, northing, whether the recorder is a new recorder, how current the recorders last entry was, the county and region of observation.

Nature's Calendar kindly provided us with complete records of the mast-associated events 'amount of fruit' and 'date of first flower.' We used the species, easting, northing, year, and amount of fruit categories to calculate average fruit scores for Oak and Beech trees per year of experimental resource supplementation for the region in which trapping was conducted.

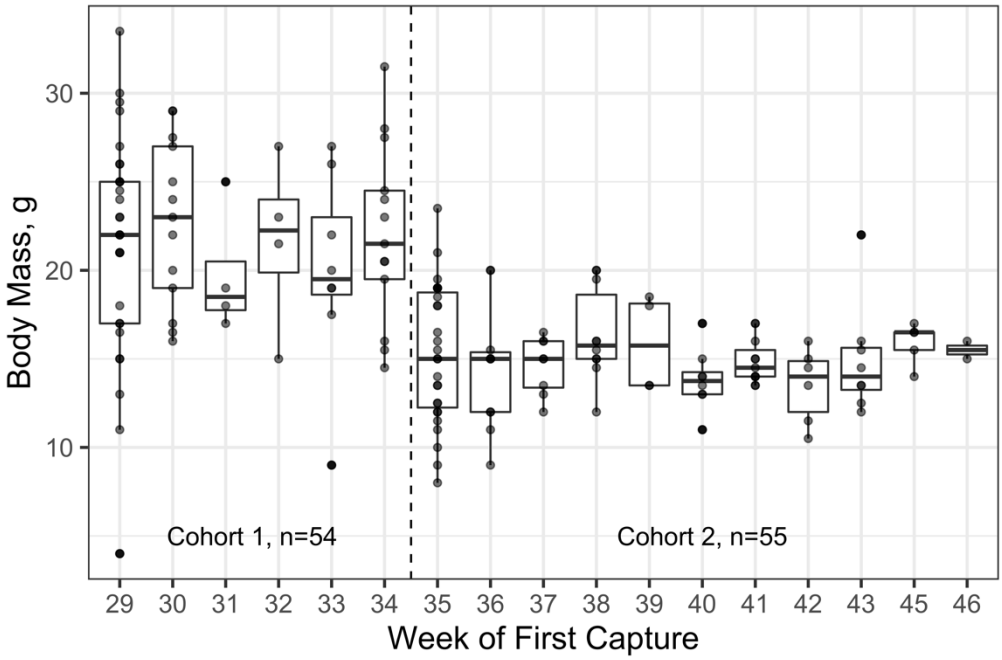


**Figure S4.1. Body mass at first capture across experimental period.**

From week 29-35, all first captured mice were of a larger boy mass and many were reproductively active; using these criteria we defined these mice as ’cohort one’; which would have survived the winter. However, from week 35 onward, individual mice first captured into the experiment were primarily in lower weight classes, associated with younger individuals, and we proposed that these mice represented individuals recruited to the population within the trapping season and were defined as ‘cohort two’.

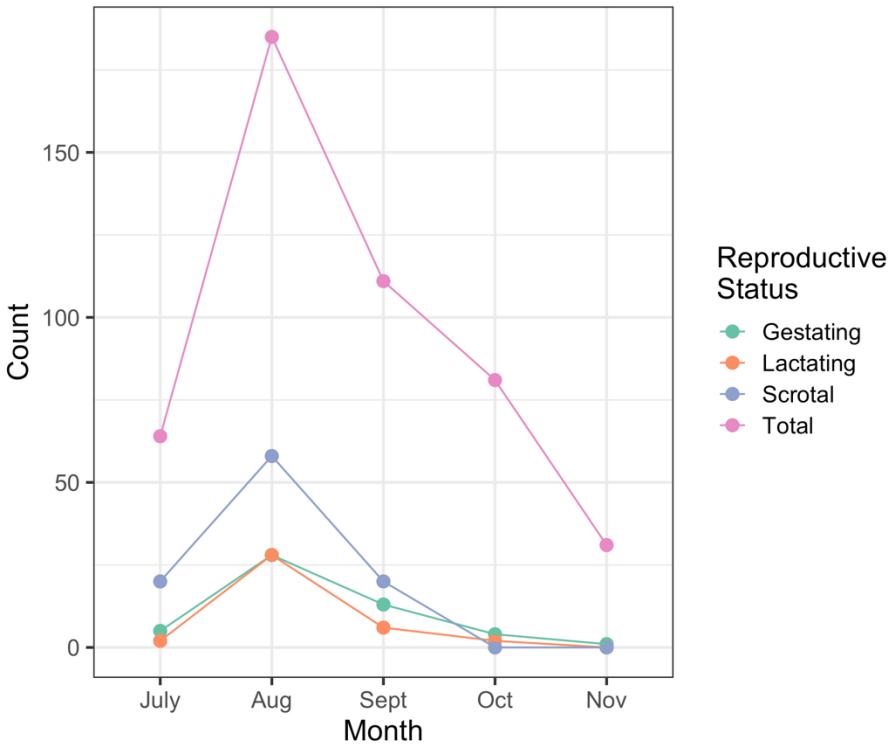


**Figure S4.2. Number of reproductively active individuals throughout 2017 trapping season.**

Points represent the number of captures per month belonging to either gestating, lactating, or scrotal individuals. Pink points and lines running along the top of the graph indicates the total number of captures per month.

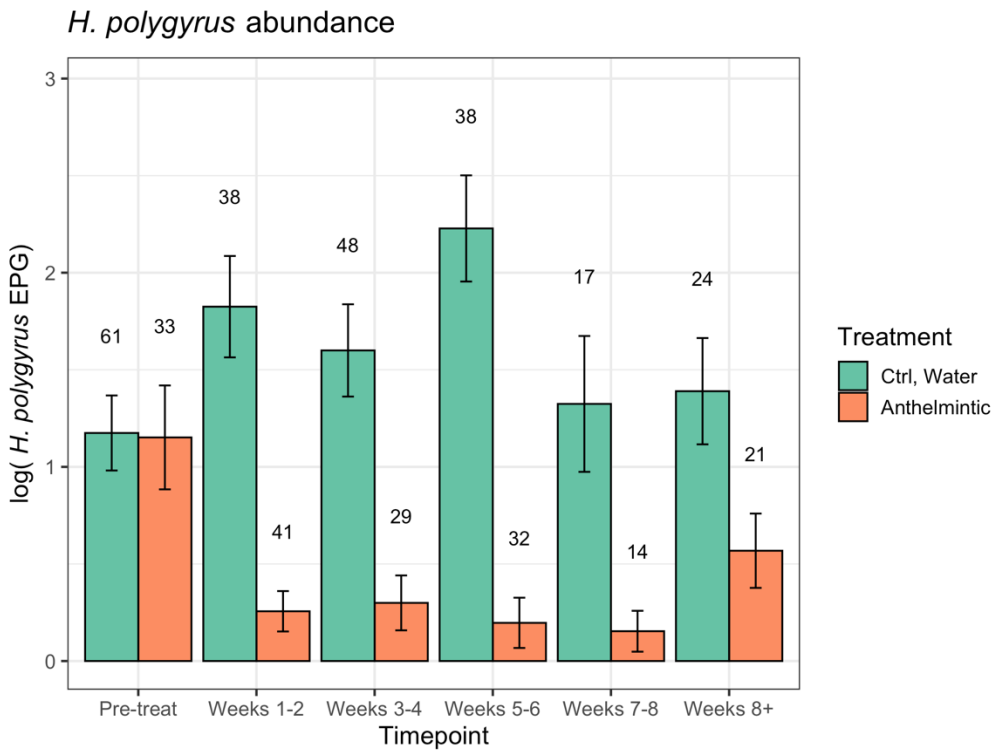


**Figure S4.3 Anthelmintic treatment effects over the course of the experiment.**

Sustained anthelmintic treatment (every 28 days) lowered *H. polygyrys* abundance throughout the trapping season. Barplots represent log-transformed EPG data means ± SEM.

# Appendix D: Supplementary Material for Chapter 5

## Dataset information


Figure S5.1. Sites included in dataset A. Gordale B. Haddon Wood C. Manor Wood D. Maresfield Farm E. Rode Hall F. Mudhouse

Table S5.1. Dimensions of grids included in analysis

| Years | Sites | Grids | Dimensions (m) |
|---|---|---|---|
| 2009 | Haddon Wood | HW1, HW2, HW3, HW4 | 70 x 70 |
| | Manor Wood | MW1, MW2 | 70 x 70 |
| 2010 | Haddon Wood | HW1, HW2, HW3, HW4 | 70 x 70 |
| | Manor Wood | MW1, MW2 | 70 x 70 |
| 2011 | Haddon Wood | HW1, HW2, HW3, HW4 | 70 X 70 |
| | Manor Wood | MW1, MW2 | 70 x 70 |
| | | MW3, MW4 | 50 x 50 |
| 2012 | Haddon Wood | HA1 | 100 x 100 |
| | | HA2, HA3 | 50 x 50 |
| | Maresfield | MF1, MF2 | 50 x 50 |
| | Rode Hall | RH2, RH3, RH4 | 50 x 50 |
| 2013 | Gordale | GOR | 50 x 50 |
| | Maresfield | MF1, MF2 | 50 x 50 |
| | Rode Hall | RH1 | 20 x 120 |
| | | RH2, RH3, RH4 | 50 x 50 |
| 2014 | Gordale | GOR | 50 x 50 |
| | Maresfield | MF1, MF2 | 50 x 50 |
| | Rode Hall | RH1 | 20 x 120 |
| | | RH2, RH3, RH4 | 50 x 50 |

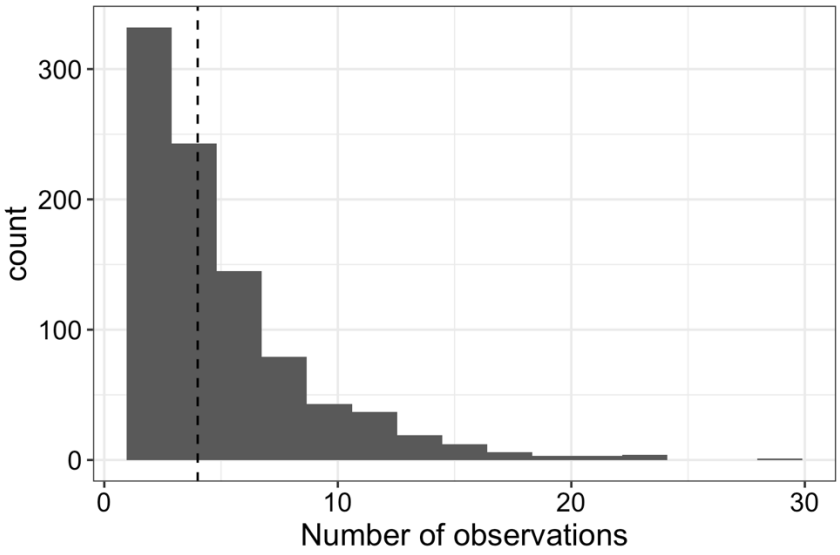


Figure S5.2. Histogram of observations per individual included in longitudinal models. $N_i$=927; $N_c$=1611. Dashed line represents the median number of observations.

Table S5.2. Full Model Output, without interactions

| | Cross-sectional | | Longitudinal | |
|---|---|---|---|---|
| variable | Estimate | pMCMC | Estimate | pMCMC |
| Site, Rode Hall | 0.13 (-0.43 - 0.67) | 0.646 | 0.17 (-0.16 - 0.45) | 0.294 |
| Site, Maresfield | 0.31 (-0.28 - 0.93) | 0.342 | 0.27 (-0.09 - 0.62) | 0.118 |
| Site, Manor | 0.05 (-0.92 - 1.1) | 0.924 | 0.09 (-0.51 - 0.69) | 0.778 |
| Site, Haddon | 0.83 (-0.04 - 1.78) | 0.09 | 0.9 (0.44 - 1.43) | 0.001 |
| Year, 2010 | 0.63 (0.35 - 0.93) | 0.001 | 0.52 (0.27 - 0.8) | 0.001 |
| Year, 2011 | 0.23 (-0.12 - 0.56) | 0.188 | 0.06 (-0.22 - 0.43) | 0.698 |
| Year, 2012 | -0.26 (-0.69 - 0.13) | 0.22 | -0.46 (-0.76 - - 0.17) | 0.004 |
| Year, 2013 | -0.67 (-1.65 - 0.09) | 0.122 | -0.67 (-1.18 - - 0.17) | 0.01 |
| Year, 2014 | -0.37 (-1.23 - 0.41) | 0.362 | -0.47 (-0.94 - 0.03) | 0.058 |
| Season, Summer | -0.32 (-0.57 - - 0.02) | 0.02 | -0.35 (-0.54 - - 0.19) | 0.001 |
| Season, Autumn | -0.49 (-0.83 - - 0.15) | 0.006 | -0.6 (-0.81 - - 0.35) | 0.001 |
| Sex, Male | -0.04 (-0.25 - 0.13) | 0.666 | 0.09 (-0.05 - 0.23) | 0.23 |
| Body condition | 0.02 (-0.11 - 0.18) | 0.788 | -0.05 (-0.15 - 0.04) | 0.288 |
| Age, Non-adult | 0.28 (-0.03 - 0.56) | 0.056 | 0 (-0.29 - 0.28) | 0.982 |
| Reproductive, active | 0.11 (-0.18 - 0.41) | 0.472 | -0.1 (-0.28 - 0.09) | 0.306 |
| E. hungaryensis, present | 0.11 (-0.1 - 0.32) | 0.282 | 0.14 (0.01 - 0.29) | 0.058 |
| Hymenolepid, present | 0.2 (-0.13 - 0.51) | 0.224 | 0.2 (0.04 - 0.37) | 0.018 |
| Intercept | 2.36 (1.39 - 3.37) | 0.001 | 2.69 (2.1 - 3.26) | 0.001 |

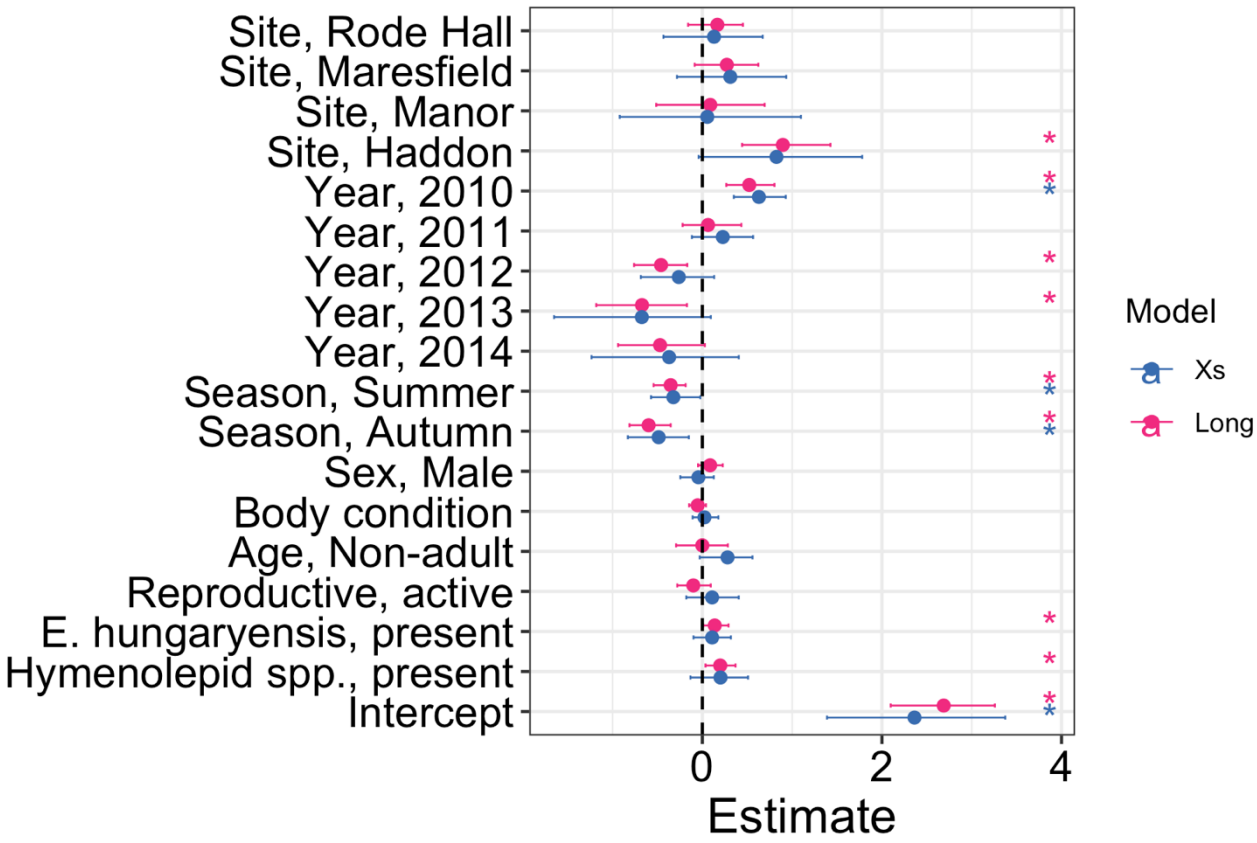


**Figure S5.3.** Full model output for cross-sectional and longitudinal models on data from all years and sites. Points and ranges represent model estimates and 95% credibility estimates for each model. Asterisks indicate the significance of variables with a $p_{MCMC}$<0.05 threshold.

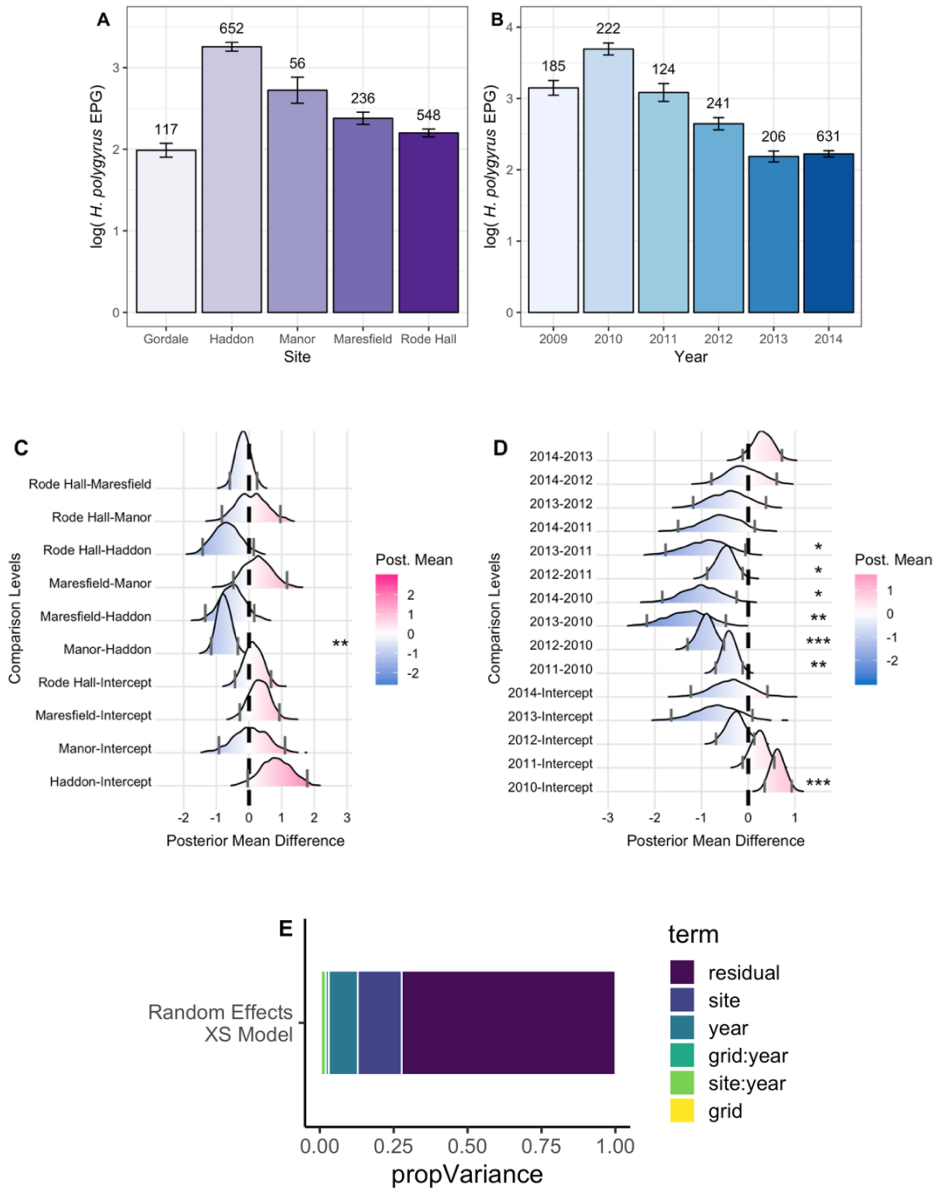


**Figure S5.4. Spatiotemporal variation in mean *H. polygyrus* intensity in** cross-sectional base model: Bars represent mean intensity (± SE) for A. site and B. year. Ridge plots below bar graphs (C-D) represent the pair-wise comparisons for base model output for fixed effect factors. Density ridges represent distributions drawn from the differences between the posterior means of the indicated comparison levels [a-b] for each iteration ($N_{iterations}$=130000). Blue shading denotes that the mean of effect estimates from the x-axis is lower than that on the y-axis. Differences between effects can be interpreted by comparison of the density ridges to zero; grey lines for each ridge indicate the 95% credibility intervals for these distributions. Blue shading denotes that the mean of effect estimates for [a] is lower than that of [b] for a given interaction. Pink shading denotes that mean of effect estimates from [a] is higher than that of [b]. If credibility intervals do not cross zero, this is considered a significant difference in effects between [a-b]. Significant differences between effects are indicates by ***, ** and * for P<0.001, P<0.01 and P<0.05 respectively. 'Intercept' represents the baseline year of the model (2009) in all panels. 'Intercept' represents spring for season, Gordale for site effect levels, and '2009' for year. E. Proportion variance explained by each spatiotemporal random effect in an alternate model.

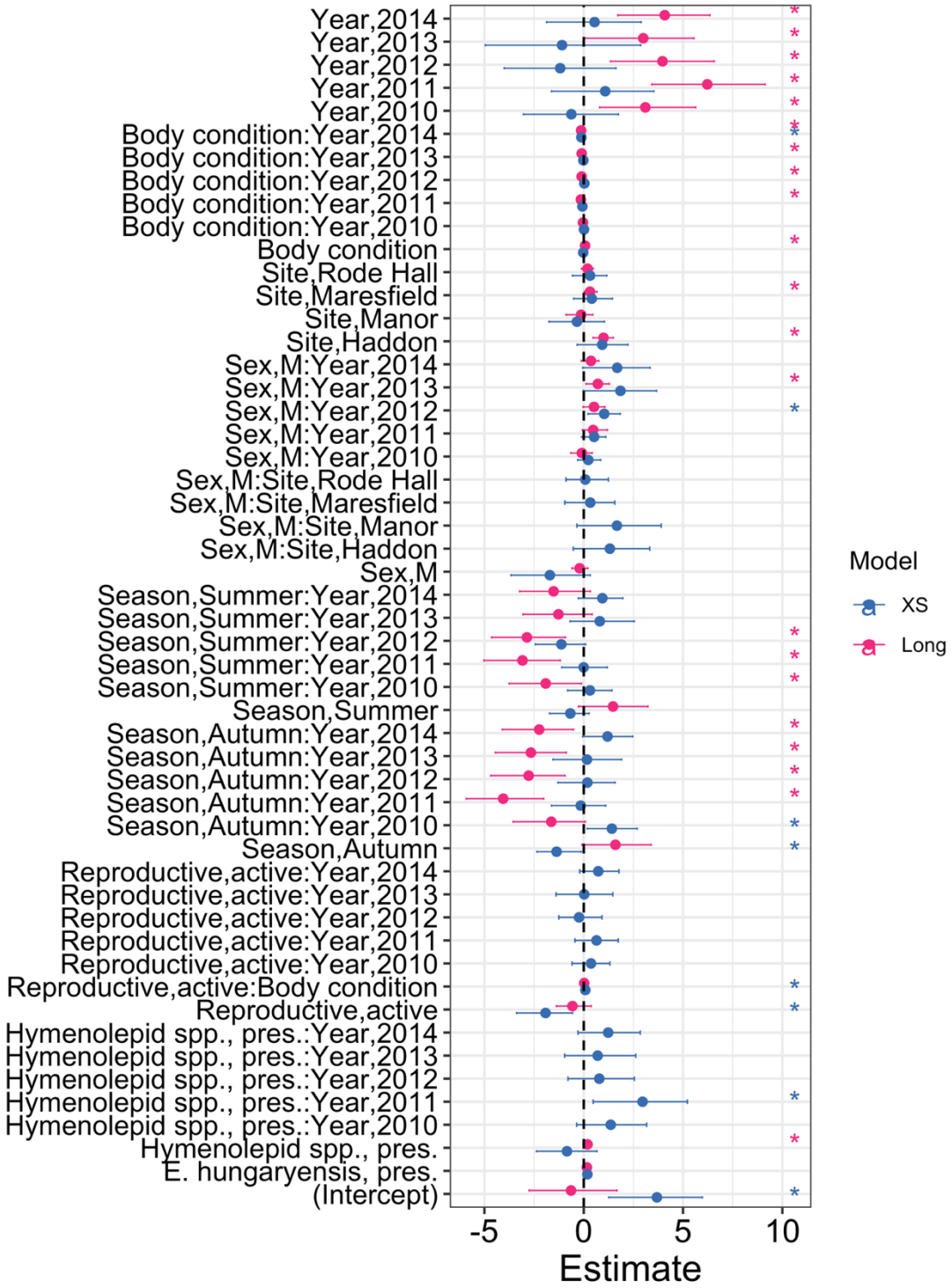


**Figure S5.5.** Interactions remaining in final model after selection. Interactions remaining represent those which had ΔDIC >2. Points and ranges represent model estimates and 95% credibility estimates for each model. Asterisks indicate the significance of variables: ***, ** and * indicate P<0.001, P<0.01 and P<0.05 respectively.

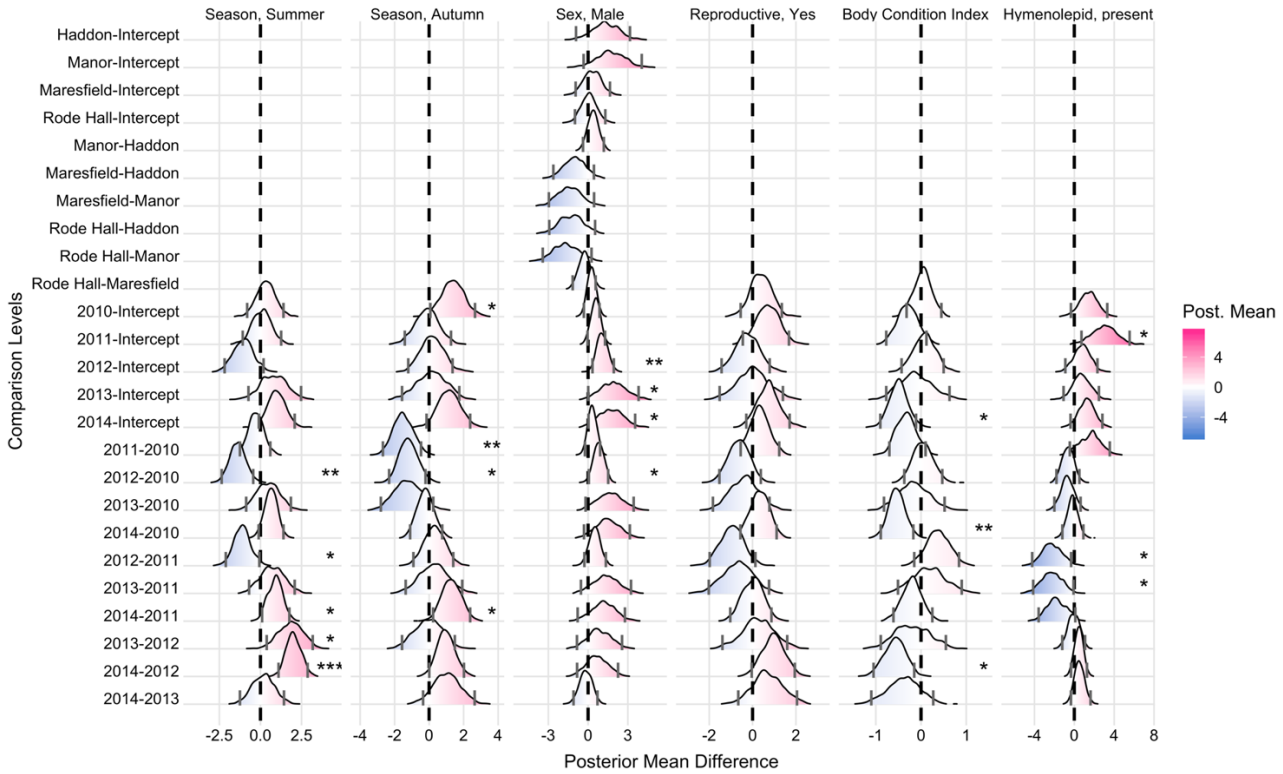


**Figure S5.6.** Differences across estimated effects (with 95% credible intervals) for interaction levels which improved full model fit, cross-sectional models. Density ridges represent distributions drawn from the differences between the posterior means of the indicated comparison levels [a-b] for each iteration ($N_{iterations}$=130000). Blue shading denotes that the slope of effect from the x-axis is lower than that on the y-axis. Differences between effects can be interpreted by comparison of the density ridges to zero; grey lines for each ridge indicate the 95% credibility intervals for these distributions. Blue shading denotes that the slope of effect for [a] is lower than that of [b] for a given interaction. Pink shading denotes that slope of effect from [a] is higher than that of [b]. If credibility intervals do not cross zero, this is considered a significant difference in effect slope of [a-b]. Significant differences between effects are indicates by ***, ** and * for P<0.001, P<0.01 and P<0.05 respectively. 'Intercept' represents the baseline year of the model (2009) in all panels.

Table S5.3. Full Model Output, with interactions

| | Cross-sectional | | Longitudinal | |
|---|---|---|---|---|
| variable | Estimate | pMCMC | Estimate | pMCMC |
| (Intercept) | 3.21 (1.42 - 4.79) | 0.001 | -0.64 (-2.75 - 1.68) | 0.608 |
| Season,Summer | -0.7 (-1.66 - 0.29) | 0.156 | 1.47 (-0.19 - 3.28) | 0.098 |
| Season,Autumn | -1.3 (-2.36 - -0.24) | 0.016 | 1.64 (-0.26 - 3.29) | 0.072 |
| Sex,M | -1.76 (-3.68 - 0.4) | 0.092 | -0.22 (-0.6 - 0.14) | 0.262 |
| Reproductive,active | -0.32 (-1.07 - 0.44) | 0.424 | -0.14 (-0.32 - 0.04) | 0.152 |
| Age, Non-adult | 0.2 (-0.13 - 0.52) | 0.24 | 0.13 (-0.17 - 0.43) | 0.374 |
| Body condition | -0.01 (-0.38 - 0.38) | 0.954 | 0.29 (0.03 - 0.53) | 0.026 |
| E. hungaryensis, pres. | 0.18 (-0.01 - 0.38) | 0.068 | 0.17 (0.03 - 0.3) | 0.012 |
| Hymenolepid spp., pres. | -0.86 (-2.47 - 0.62) | 0.256 | 0.18 (0.01 - 0.35) | 0.05 |
| Year,2010 | -0.45 (-1.68 - 0.8) | 0.458 | 2.34 (0.54 - 4.25) | 0.01 |
| Year,2011 | -0.32 (-1.51 - 1) | 0.638 | 3.3 (1.46 - 5.35) | 0.002 |
| Year,2012 | -0.65 (-1.99 - 0.61) | 0.294 | 1.91 (0.08 - 3.73) | 0.036 |
| Year,2013 | -1.55 (-3.27 - 0.39) | 0.09 | 0.98 (-0.81 - 2.85) | 0.306 |
| Year,2014 | -1.88 (-3.54 - -0.42) | 0.018 | 1.47 (-0.25 - 3.46) | 0.128 |
| Site,Haddon | 0.85 (-0.4 - 2.13) | 0.19 | 1.01 (0.49 - 1.5) | 0.001 |
| Site,Manor | -0.43 (-1.85 - 1.07) | 0.562 | -0.15 (-0.83 - 0.5) | 0.694 |
| Site,Maresfield | 0.42 (-0.54 - 1.46) | 0.43 | 0.33 (-0.01 - 0.66) | 0.064 |
| Site,Rode Hall | 0.32 (-0.52 - 1.24) | 0.5 | 0.2 (-0.08 - 0.53) | 0.164 |
| Reproductive,active:Body condition | 0.27 (0.01 - 0.54) | 0.046 | 0.08 (-0.11 - 0.24) | 0.382 |
| Season,Summer:Year,2010 | 0.34 (-0.81 - 1.4) | 0.54 | -1.93 (-3.72 - -0.09) | 0.03 |
| Season,Autumn:Year,2010 | 1.38 (0.07 - 2.66) | 0.03 | -1.68 (-3.37 - 0.3) | 0.074 |
| Season,Summer:Year,2011 | 0.08 (-1.08 - 1.26) | 0.888 | -3.12 (-4.87 - -1.16) | 0.002 |
| Season,Autumn:Year,2011 | -0.13 (-1.41 - 1.27) | 0.86 | -4.06 (-6.01 - -2.11) | 0.001 |
| Season,Summer:Year,2012 | -1.04 (-2.18 - 0.19) | 0.11 | -2.85 (-4.62 - -1.1) | 0.006 |
| Season,Autumn:Year,2012 | 0.19 (-1.21 - 1.36) | 0.8 | -2.82 (-4.65 - -1.02) | 0.002 |
| Season,Summer:Year,2013 | 0.81 (-0.73 - 2.47) | 0.294 | -1.26 (-3.08 - 0.47) | 0.162 |
| Season,Autumn:Year,2013 | 0.15 (-1.57 - 1.75) | 0.838 | -2.7 (-4.56 - -0.84) | 0.004 |
| Season,Summer:Year,2014 | 0.98 (-0.1 - 2.08) | 0.078 | -1.51 (-3.25 - 0.33) | 0.094 |
| Season,Autumn:Year,2014 | 1.17 (-0.15 - 2.38) | 0.066 | -2.28 (-4 - -0.42) | 0.012 |
| Sex,M:Year,2010 | 0.26 (-0.32 - 0.86) | 0.412 | -0.07 (-0.55 - 0.46) | 0.768 |
| Sex,M:Year,2011 | 0.57 (-0.04 - 1.28) | 0.09 | 0.53 (-0.05 - 1.11) | 0.08 |
| Sex,M:Year,2012 | 1.07 (0.33 - 1.94) | 0.008 | 0.54 (0.03 - 1.11) | 0.054 |
| Sex,M:Year,2013 | 1.93 (0.01 - 3.81) | 0.038 | 0.73 (0.15 - 1.29) | 0.006 |
| Sex,M:Year,2014 | 1.75 (0.03 - 3.55) | 0.05 | 0.38 (-0.06 - 0.83) | 0.098 |
| Reproductive,active:Year,2010 | 0.39 (-0.55 - 1.35) | 0.428 | | |

| | | | | |
|---|---|---|---|---|
| Reproductive,active:Year,2011 | 0.7 (-0.45 - 1.68) | 0.196 | | |
| Reproductive,active:Year,2012 | -0.24 (-1.43 - 0.79) | 0.674 | | |
| Reproductive,active:Year,2013 | 0.02 (-1.52 - 1.4) | 0.982 | | |
| Reproductive,active:Year,2014 | 0.72 (-0.29 - 1.71) | 0.154 | | |
| Body condition:Year,2010 | 0.04 (-0.32 - 0.45) | 0.808 | -0.15 (-0.44 - 0.12) | 0.31 |
| Body condition:Year,2011 | -0.3 (-0.77 - 0.12) | 0.204 | -0.57 (-0.93 - -0.18) | 0.006 |
| Body condition:Year,2012 | 0.08 (-0.43 - 0.5) | 0.746 | -0.44 (-0.77 - -0.14) | 0.008 |
| Body condition:Year,2013 | -0.12 (-0.77 - 0.63) | 0.714 | -0.42 (-0.76 - -0.14) | 0.002 |
| Body condition:Year,2014 | -0.5 (-0.9 - -0.11) | 0.016 | -0.54 (-0.81 - -0.3) | 0.001 |
| Hymenolepid spp., pres.:Year,2010 | 1.42 (-0.34 - 3.31) | 0.132 | | |
| Hymenolepid spp., pres.:Year,2011 | 3.02 (0.72 - 5.55) | 0.012 | | |
| Hymenolepid spp., pres.:Year,2012 | 0.79 (-0.9 - 2.32) | 0.328 | | |
| Hymenolepid spp., pres.:Year,2013 | 0.73 (-1.08 - 2.47) | 0.4 | | |
| Hymenolepid spp., pres.:Year,2014 | 1.25 (-0.38 - 2.82) | 0.126 | | |
| Sex,M:Site,Haddon | 1.33 (-0.91 - 3.16) | 0.194 | | |
| Sex,M:Site,Manor | 1.71 (-0.34 - 4.05) | 0.126 | | |
| Sex,M:Site,Maresfield | 0.32 (-0.94 - 1.65) | 0.642 | | |
| Sex,M:Site,Rode Hall | 0.06 (-0.99 - 1.3) | 0.906 | | |

Blank cells represent instances where interactions did not remain in longitudinal models after selection.

Table S4. ΔDIC for interactions added to full models for each run of model selection.

**Model Additions, Cross-Sectional**

| **variable** | dDIC 1 | dDIC 2 | dDIC 3 | dDIC 4 | dDIC 5 | dDIC 6 | dDIC 7 |
|---|---|---|---|---|---|---|---|
| **season:year** | -57.316 | | | | | | |
| **body condition:year** | -31.553 | -22.568 | | | | | |
| **reproductive status:year** | 1.04 | 6.447 | -7.3 | | | | |
| **sex:year** | -10.84 | -6.664 | -4.92 | -7.919 | | | |
| **sex:site** | -3.863 | -3.95 | -1.139 | -1.589 | -5.614 | | |
| **Hymenolepid presence:year** | -1.818 | -3.713 | -5.805 | -6.951 | -5.173 | -5.113 | |
| body condition:site | -27.794 | -21.218 | 2.162 | 1.793 | 1.189 | 4.174 | 3.432 |
| season:site | -25.587 | 3.844 | 4.126 | 3.535 | 5.68 | 3.93 | 4.509 |
| *E. hungayrensis* presence:year | 1.464 | 4.765 | 2.588 | 3.282 | 2.66 | 2.772 | 4.677 |
| reproductive status:site | 3.906 | -1.099 | 5.661 | 5.975 | 5.752 | 7.362 | 7.314 |
| age:site | 3.315 | 5.368 | 3.551 | 3.056 | 1.863 | 4.822 | 5.359 |
| *E. hungayrensis* presence:site | 4.642 | 5.818 | 7.148 | 6.51 | 6.712 | 6.332 | 5.754 |
| age:year | 6.505 | 2.352 | 6.985 | 7.176 | 8.247 | 8.976 | 8.575 |

**Model Additions, Longitudinal**

| **variable** | dDIC 1 | dDIC 2 | dDIC 3 | dDIC 4 |
|---|---|---|---|---|
| **season:year** | -58.916 | | | |
| **body condition:year** | -0.034 | -14.608 | | |
| **sex:year** | 1.298 | -0.198 | -5.42 | |
| *E. hungayrensis* presence:site | 8.023 | 6.64 | 0.734 | 10.928 |
| *E. hungayrensis* presence:year | 6.651 | 7.325 | -1.951 | 5.673 |
| Hymenolepid presence:year | 7.298 | -2.678 | -0.567 | -0.039 |
| reproductive status:site | -4.259 | -5.582 | 2.511 | 8.718 |
| reproductive status:year | 0.685 | -2.435 | -3.443 | -0.666 |
| season:site | -24.45 | 7.01 | 6.285 | 13.197 |
| age:site | 8.848 | 1.915 | 3.201 | 3.827 |
| sex:site | 15.569 | 0.622 | -4.72 | 1.682 |
| body condition:site | 2.785 | -4.34 | 5.548 | 6.737 |
| age:year | -5.11 | -3.22 | 2.067 | 7.796 |

Table S5.6. Model output from within-year models.

| Cross-Sectional | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Factor** | **Estimate** | **pMCMC** | **Model** | **Factor** | **Estimate** | **pMCMC** | **Model** |
| Site, Rode Hall | 0.29 (-0.89 - 1.67) | 0.666 | 2013 | | -0.47 (-1.15 - 0.34) | 0.238 | 2009 |
| | 0.26 (-0.28 - 0.9) | 0.4 | 2014 | | 0.11 (-0.49 - 0.63) | 0.686 | 2010 |
| Site, Maresfield | -0.21 (-1.65 - 1.15) | 0.806 | 2012 | | 0.29 (-0.47 - 1.05) | 0.446 | 2011 |
| | 0.75 (-0.67 - 2.18) | 0.314 | 2013 | | -0.61 (-1.47 - 0.22) | 0.15 | 2012 |
| | 0.49 (-0.21 - 1.15) | 0.164 | 2014 | Reproductive, active | -0.26 (-1.41 - 0.8) | 0.66 | 2013 |
| Site, Haddon | 1.16 (0.17 - 2.07) | **0.024** | 2009 | | 0.37 (-0.29 - 0.93) | 0.246 | 2014 |
| | 0.49 (-0.21 - 1.13) | 0.156 | 2010 | | 0.3 (-0.15 - 0.74) | 0.204 | 2009 |
| | 1.53 (0.82 - 2.29) | **0.001** | 2011 | | -0.04 (-0.41 - 0.36) | 0.824 | 2010 |
| | 0.82 (-0.54 - 2.11) | 0.226 | 2012 | | 0.07 (-0.46 - 0.57) | 0.77 | 2011 |
| Season, Summer | -0.8 (-1.87 - 0.28) | **0.154** | 2009 | | 0.66 (-0.17 - 1.43) | 0.112 | 2012 |
| | -0.21 (-0.81 - 0.38) | 0.464 | 2010 | E. hungaryensis, present | 0.47 (-0.43 - 1.46) | 0.302 | 2013 |
| | -0.84 (-1.69 - -0.06) | 0.058 | 2011 | | 0.11 (-0.32 - 0.56) | 0.668 | 2014 |
| | -1.85 (-2.7 - -1.01) | **0.001** | 2012 | | -0.96 (-2.5 - 0.67) | 0.246 | 2009 |
| | 0.2 (-0.9 - 1.25) | 0.75 | 2013 | | 0.51 (-0.53 - 1.34) | 0.31 | 2010 |
| | 0.26 (-0.33 - 0.73) | 0.332 | 2014 | | 1.85 (-0.18 - 3.86) | 0.074 | 2011 |
| Season, Autumn | -1.55 (-2.68 - -0.45) | **0.01** | 2009 | | -0.11 (-0.85 - 0.59) | 0.796 | 2012 |
| | 0.29 (-0.41 - 0.98) | 0.412 | 2010 | Hymenolepid spp., present | -0.01 (-0.9 - 0.81) | 0.98 | 2013 |
| | -1.87 (-3 - -0.8) | **0.001** | 2011 | | 0.43 (0.01 - 0.87) | 0.058 | 2014 |
| | -1.14 (-2 - -0.27) | **0.006** | 2012 | | 2.46 (0.63 - 4.36) | 0.014 | 2009 |
| | -1.08 (-2.36 - 0.15) | 0.096 | 2013 | | 2.65 (1.15 - 4.42) | **0.002** | 2010 |
| | -0.23 (-0.9 - 0.35) | 0.514 | 2014 | | 3.8 (1.83 - 6.04) | **0.002** | 2011 |
| Sex, Male | -0.4 (-0.89 - 0.01) | 0.076 | 2009 | | 1.67 (-0.7 - 4.14) | 0.176 | 2012 |
| | -0.11 (-0.51 - 0.26) | 0.562 | 2010 | | 1.78 (-1.45 - 5.21) | 0.282 | 2013 |
| | 0.2 (-0.29 - 0.68) | 0.408 | 2011 | Intercept | 3.29 (2 - 4.59) | **0.001** | 2014 |
| | 0.33 (-0.37 - 1.07) | 0.364 | 2012 | | | | |
| | 0.39 (-0.32 - 1.06) | 0.26 | 2013 | | | | |
| | 0.1 (-0.27 - 0.52) | 0.628 | 2014 | | | | |
| Body condition | 0.04 (-0.02 - 0.12) | 0.31 | 2009 | | | | |
| | 0.02 (-0.04 - 0.08) | 0.518 | 2010 | | | | |
| | -0.05 (-0.15 - 0.02) | 0.242 | 2011 | | | | |
| | 0.06 (-0.05 - 0.15) | 0.256 | 2012 | | | | |
| | -0.01 (-0.15 - 0.15) | 0.89 | 2013 | | | | |
| | -0.09 (-0.15 - -0.02) | **0.002** | 2014 | | | | |

| Longitudinal | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Factor** | **Estimate** | **pMCMC** | **Model** | **Factor** | **Estimate** | **pMCMC** | **Model** |
| Site, Rode Hall | 0 (-0.75 - 0.78) | 0.982 | 2013 | | -0.78 (-1.34 - -0.17) | 0.014 | 2009 |
| | 0.24 (-0.06 - 0.55) | 0.142 | 2014 | | -0.08 (-0.59 - 0.45) | 0.752 | 2010 |
| Site, Maresfield | 0.37 (-0.35 - 1.12) | 0.33 | 2012 | | -0.04 (-0.81 - 0.68) | 0.914 | 2011 |
| | 0.09 (-0.8 - 0.93) | 0.816 | 2013 | | 0.06 (-0.43 - 0.53) | 0.812 | 2012 |
| | 0.35 (0.02 - 0.71) | **0.042** | 2014 | Reproductive, active | -0.29 (-0.78 - 0.2) | 0.25 | 2013 |
| Site, Haddon | 1.22 (-0.08 - 2.29) | 0.054 | 2009 | | 0 (-0.31 - 0.34) | 0.974 | 2014 |
| | 0.97 (0.12 - 1.75) | **0.018** | 2010 | | 0.15 (-0.31 - 0.56) | 0.534 | 2009 |
| | 1.04 (0.2 - 1.74) | **0.002** | 2011 | | -0.07 (-0.47 - 0.3) | 0.746 | 2010 |
| | 1.02 (0.4 - 1.68) | **0.002** | 2012 | | 0.18 (-0.31 - 0.7) | 0.478 | 2011 |
| Season, Summer | 1.35 (-0.82 - 3.63) | 0.242 | 2009 | | 0.44 (0 - 0.95) | 0.084 | 2012 |
| | -0.43 (-1.07 - 0.19) | 0.17 | 2010 | E. hungaryensis, present | 0.56 (0.18 - 0.94) | **0.008** | 2013 |
| | -1.51 (-2.29 - -0.74) | **0.001** | 2011 | | 0.05 (-0.15 - 0.25) | 0.66 | 2014 |
| | -1.36 (-1.85 - -0.76) | **0.001** | 2012 | | 0.55 (-0.81 - 1.79) | 0.41 | 2009 |
| | 0.19 (-0.23 - 0.52) | 0.32 | 2013 | | -0.03 (-0.74 - 0.6) | 0.946 | 2010 |
| | -0.08 (-0.34 - 0.18) | 0.576 | 2014 | | 1.18 (-0.08 - 2.57) | 0.092 | 2011 |
| Season, Autumn | 1.17 (-0.94 - 3.47) | 0.296 | 2009 | | 0.43 (-0.03 - 0.82) | 0.072 | 2012 |
| | 0 (-0.72 - 0.65) | 0.99 | 2010 | Hymenolepid spp., present | -0.03 (-0.41 - 0.3) | 0.882 | 2013 |
| | -2.32 (-3.45 - -1.32) | **0.001** | 2011 | | 0.15 (-0.09 - 0.38) | 0.21 | 2014 |
| | -1.15 (-1.7 - -0.57) | **0.001** | 2012 | | -1.24 (-4.2 - 1.91) | 0.44 | 2009 |
| | -1.17 (-1.77 - -0.65) | **0.001** | 2013 | | 2.33 (1.01 - 3.76) | **0.002** | 2010 |
| | -0.57 (-0.87 - -0.26) | **0.001** | 2014 | | 5.32 (3.25 - 7.65) | **0.001** | 2011 |
| Sex, Male | -0.17 (-0.61 - 0.29) | 0.422 | 2009 | | 3.06 (1.43 - 4.62) | **0.001** | 2012 |
| | -0.32 (-0.7 - 0.08) | 0.1 | 2010 | | 2.21 (0.76 - 3.73) | **0.001** | 2013 |
| | 0.21 (-0.34 - 0.75) | 0.418 | 2011 | Intercept | 3.29 (2.42 - 4.02) | **0.001** | 2014 |
| | 0.18 (-0.23 - 0.58) | 0.394 | 2012 | | | | |
| | 0.52 (0.15 - 0.88) | **0.01** | 2013 | | | | |
| | 0.14 (-0.09 - 0.35) | 0.268 | 2014 | | | | |
| Body condition | 0.11 (0.05 - 0.19) | **0.001** | 2009 | | | | |
| | 0.04 (-0.01 - 0.09) | 0.144 | 2010 | | | | |
| | -0.07 (-0.17 - 0.01) | 0.104 | 2011 | | | | |
| | -0.03 (-0.1 - 0.03) | 0.32 | 2012 | | | | |
| | -0.01 (-0.07 - 0.06) | 0.782 | 2013 | | | | |
| | -0.06 (-0.1 - -0.02) | **0.002** | 2014 | | | | |

Table S5.6. Model output from within-site models

| | Cross-Sectional | | | | | | Longitudinal | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Factor** | **Estimate** | **pMCMC** | **Model** | **Factor** | **Estimate** | **pMCMC** | **Model** | **Factor** | **Estimate** | **pMCMC** | **Model** | **Factor** | **Estimate** | **pMCMC** | **Model** |
| Year, 2014 | -0.09 (-1.37 - 1.29) | 0.88 | Gordale | Reproductive, active | -0.02 (-2.68 - 2.6) | 0.986 | Gordale | Year, 2014 | 0.06 (-0.8 - 0.93) | 0.878 | Gordale | Reproductive, active | -0.06 (-0.9 - 0.77) | 0.912 | Gordale |
| | -0.23 (-1.11 - 0.58) | 0.612 | Maresfield | | 0.01 (-0.39 - 0.39) | 0.962 | Haddon | | -0.21 (-0.72 - 0.23) | 0.396 | Maresfield | | -0.16 (-0.47 - 0.15) | 0.31 | Haddon |
| | -0.22 (-1.46 - 0.97) | 0.734 | Rode Hall | | -0.29 (-1.31 - 0.76) | 0.596 | Manor | | 0.14 (-0.37 - 0.67) | 0.63 | Rode Hall | | -0.04 (-0.91 - 0.87) | 0.942 | Manor |
| Year, 2013 | -0.21 (-1.43 - 0.91) | 0.76 | Maresfield | | -0.03 (-1.07 - 1.16) | 0.942 | Maresfield | Year, 2013 | -0.65 (-1.27 - -0.03) | **0.04** | Maresfield | | -0.12 (-0.61 - 0.38) | 0.626 | Maresfield |
| | -0.59 (-1.75 - 0.77) | 0.35 | Rode Hall | | 0.16 (-0.51 - 0.78) | 0.632 | Rode Hall | | -0.06 (-0.63 - 0.5) | 0.79 | Rode Hall | | -0.02 (-0.36 - 0.29) | 0.93 | Rode Hall |
| Year, 2012 | -0.47 (-0.87 - -0.06) | **0.024** | Haddon | E. hungaryensis, present | 0.47 (-1.01 - 1.83) | 0.502 | Gordale | Year, 2012 | -0.63 (-0.96 - -0.3) | **0.001** | Haddon | E. hungaryensis, present | 0.19 (-0.3 - 0.68) | 0.446 | Gordale |
| Year, 2011 | 0.12 (-0.26 - 0.47) | 0.534 | Haddon | | 0.15 (-0.11 - 0.44) | 0.286 | Haddon | Year, 2011 | -0.07 (-0.47 - 0.29) | 0.686 | Haddon | | 0.09 (-0.12 - 0.35) | 0.466 | Haddon |
| | -0.12 (-1.38 - 1.11) | 0.83 | Manor | | -0.03 (-0.75 - 0.73) | 0.936 | Manor | | -0.96 (-3.1 - 1.19) | 0.384 | Manor | | 0.15 (-0.58 - 0.92) | 0.702 | Manor |
| Year, 2010 | 0.61 (0.29 - 0.92) | **0.001** | Haddon | | 0.52 (-0.2 - 1.18) | 0.124 | Maresfield | Year, 2010 | 0.47 (0.14 - 0.76) | **0.002** | Haddon | | 0.09 (-0.26 - 0.46) | 0.626 | Maresfield |
| | 0.62 (-0.63 - 1.79) | 0.32 | Manor | | 0.09 (-0.38 - 0.63) | 0.724 | Rode Hall | | -0.96 (-3.14 - 1.17) | 0.394 | Manor | | 0.26 (0.03 - 0.53) | 0.036 | Rode Hall |
| Season, Summer | 0.52 (-0.89 - 2.02) | 0.476 | Gordale | Hymenolepid spp., present | -0.72 (-2.9 - 1.22) | 0.464 | Gordale | Season, Summer | -0.29 (-0.99 - 0.44) | 0.434 | Gordale | Hymenolepid spp., present | 0.15 (-0.97 - 1.2) | 0.78 | Gordale |
| | -0.9 (-1.34 - -0.44) | **0.001** | Haddon | | 0.28 (-0.29 - 0.78) | 0.306 | Haddon | | -1 (-1.46 - -0.6) | **0.001** | Haddon | | 0.38 (0.01 - 0.73) | **0.038** | Haddon |
| | -0.38 (-1.28 - 0.48) | 0.424 | Manor | | 0.03 (-0.77 - 0.73) | 0.92 | Maresfield | | -0.77 (-1.55 - 0.05) | 0.058 | Manor | | 0.24 (-0.08 - 0.63) | 0.19 | Maresfield |
| | -0.53 (-1.55 - 0.56) | 0.336 | Maresfield | | 0.33 (-0.07 - 0.74) | 0.128 | Rode Hall | | -0.56 (-1.02 - -0.11) | **0.012** | Maresfield | | 0.1 (-0.12 - 0.34) | 0.392 | Rode Hall |
| | 0.29 (-0.24 - 0.75) | 0.27 | Rode Hall | Intercept | 3.73 (-0.21 - 8.01) | 0.076 | Gordale | | 0.13 (-0.16 - 0.37) | 0.318 | Rode Hall | Intercept | 2.75 (0.77 - 4.84) | **0.01** | Gordale |
| Season, Autumn | -0.25 (-2.95 - 2.14) | 0.844 | Gordale | | 3.15 (2.13 - 4.18) | **0.001** | Haddon | Season, Autumn | -0.44 (-1.14 - 0.28) | 0.216 | Gordale | | 3.46 (2.71 - 4.38) | **0.001** | Haddon |
| | -0.87 (-1.33 - -0.35) | **0.001** | Haddon | | 3.16 (0.23 - 6.12) | **0.048** | Manor | | -0.84 (-1.28 - -0.4) | **0.001** | Haddon | | 3.37 (-0.17 - 6.82) | **0.056** | Manor |
| | -1.92 (-3.64 - -0.26) | **0.018** | Manor | | 3.49 (0.98 - 5.78) | **0.004** | Maresfield | | -2.24 (-4.26 - -0.08) | **0.038** | Manor | | 4.2 (2.8 - 5.63) | **0.001** | Maresfield |
| | -1.16 (-2.13 - -0.18) | **0.028** | Maresfield | | 3.59 (1.59 - 5.5) | **0.001** | Rode Hall | | -1.02 (-1.47 - -0.6) | **0.001** | Maresfield | | 3.02 (1.99 - 4) | **0.001** | Rode Hall |
| | -0.36 (-1.04 - 0.29) | 0.3 | Rode Hall | | | | | | -0.56 (-0.91 - -0.23) | **0.004** | Rode Hall | | | | |
| Sex, Male | -0.16 (-1.21 - 0.97) | 0.802 | Gordale | | | | | Sex, Male | 0.12 (-0.29 - 0.66) | 0.636 | Gordale | | | | |
| | -0.08 (-0.32 - 0.2) | 0.54 | Haddon | | | | | | -0.08 (-0.3 - 0.18) | 0.538 | Haddon | | | | |
| | 0.54 (-0.33 - 1.42) | 0.232 | Manor | | | | | | 0.59 (-0.36 - 1.45) | 0.174 | Manor | | | | |
| | 0.34 (-0.48 - 1.21) | 0.426 | Maresfield | | | | | | 0.15 (-0.21 - 0.57) | 0.446 | Maresfield | | | | |
| | 0.15 (-0.25 - 0.6) | 0.524 | Rode Hall | | | | | | 0.27 (0.05 - 0.51) | **0.024** | Rode Hall | | | | |
| Body condition | -0.09 (-0.28 - 0.06) | 0.23 | Gordale | | | | | Body condition | -0.04 (-0.13 - 0.05) | 0.412 | Gordale | | | | |
| | 0.03 (-0.01 - 0.07) | 0.148 | Haddon | | | | | | 0.03 (0 - 0.07) | 0.06 | Haddon | | | | |
| | -0.02 (-0.13 - 0.1) | 0.754 | Manor | | | | | | 0.02 (-0.09 - 0.14) | 0.68 | Manor | | | | |
| | -0.05 (-0.16 - 0.07) | 0.332 | Maresfield | | | | | | -0.07 (-0.13 - 0) | 0.056 | Maresfield | | | | |
| | -0.07 (-0.15 - -0.01) | 0.056 | Rode Hall | | | | | | -0.05 (-0.09 - -0.01) | **0.008** | Rode Hall | | | | |

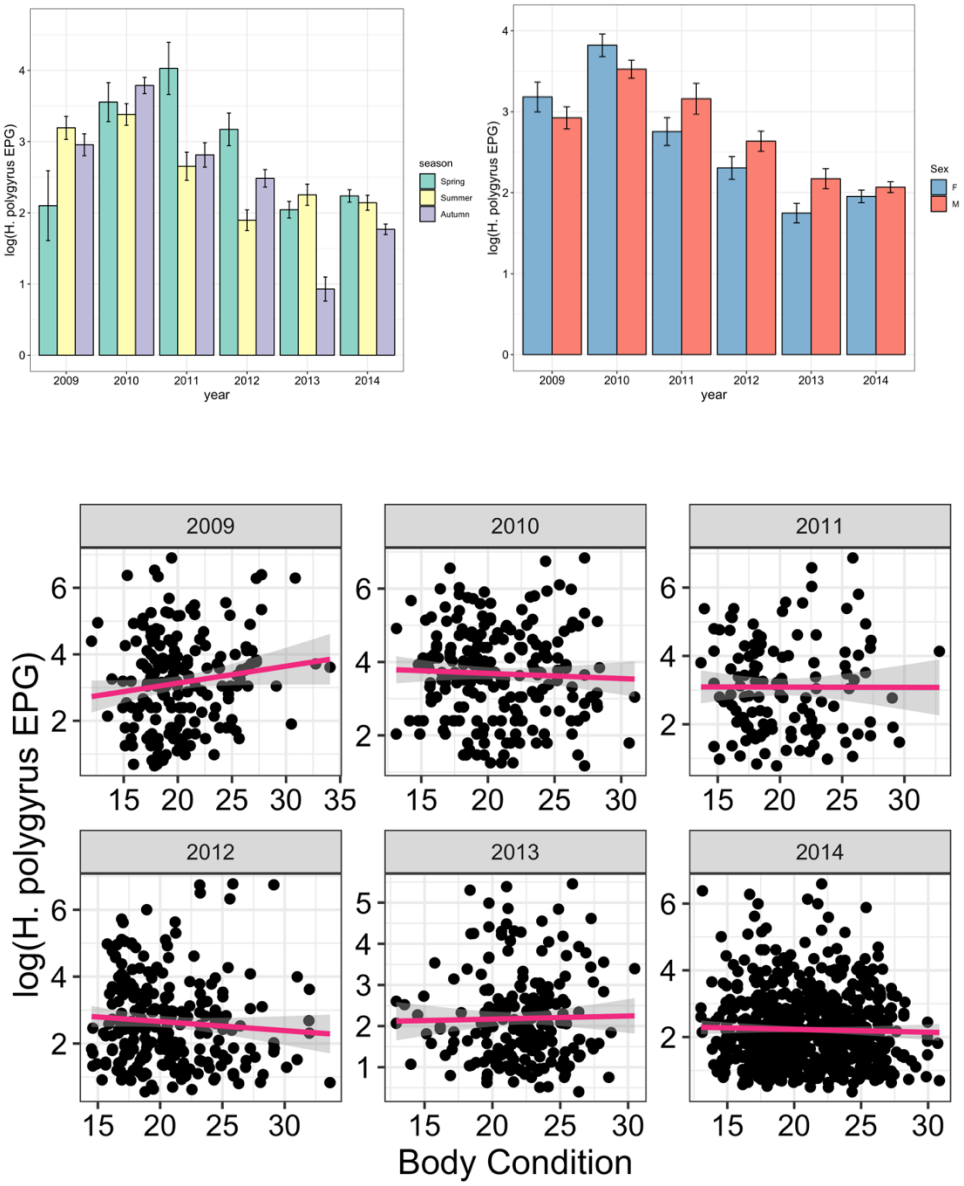


**Figure S5.7.** Raw data, interactions selected for final longitudinal models A. Season:year B. Sex:year C. Body condition:year**.** Bar graphs represent mean +/- SE of raw *H. polygyrus* intensity per category; Points represent raw values with regression slopes.

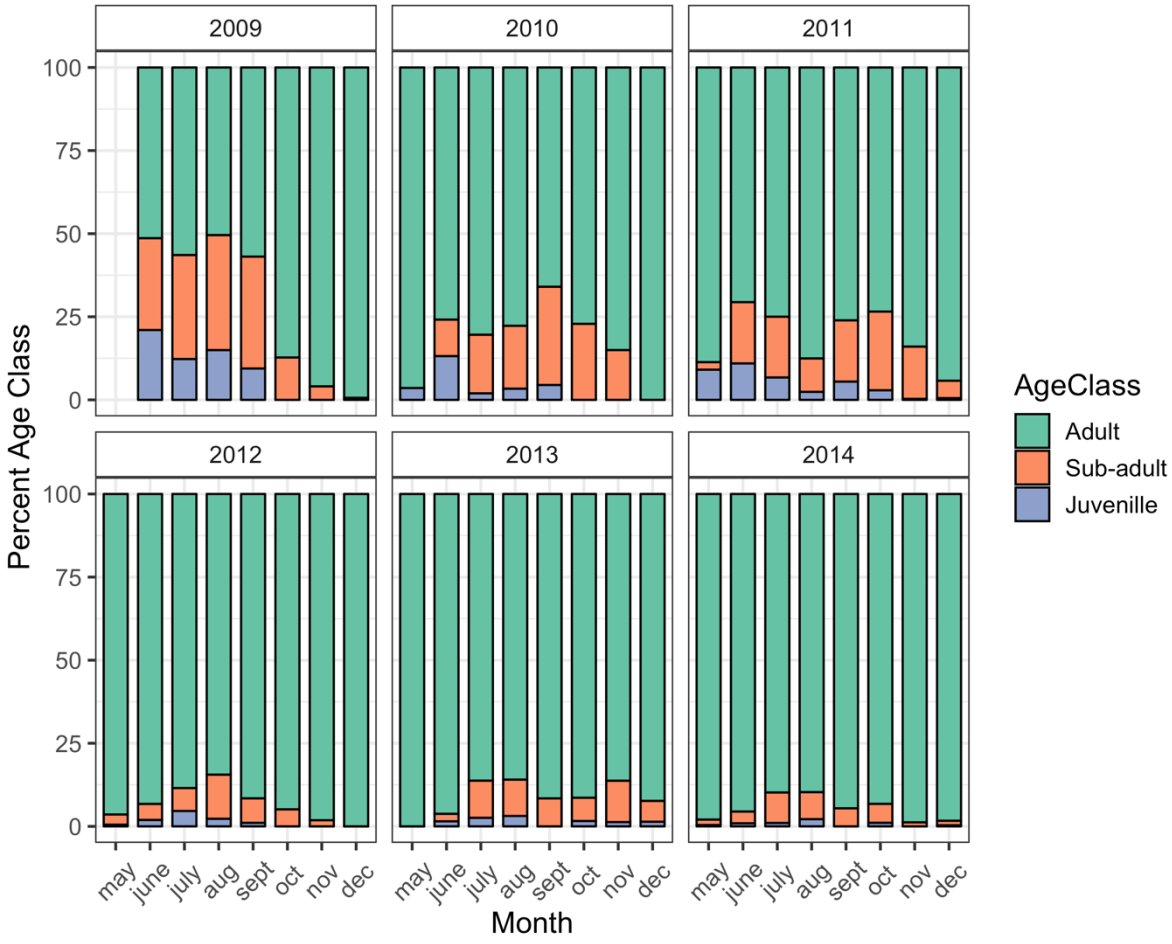


**Figure S5.8**. Age structure of populations by months across years included in this study. Stacked bars represent the percentage of captures during the month and year indicated which represent given age classes.