

Ectoparasitism of rodent hosts in Algonquin Provincial Park, Ontario, Canada: Infestation patterns, host glucocorticoids, and species co-occurrence

by

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## Abstract

Examining multiple parasite taxa across host species presents an opportunity to assess the biology of host-parasite systems. This study investigated: 1) factors associated with ectoparasite prevalence on deer mice (*Peromyscus maniculatus*), southern red-backed voles (*Myodes gapperi*) and woodland jumping mice (*Napaeozapus insignis*); 2) relationship between ectoparasites and glucocorticoid levels of deer mice, and; 3) whether ectoparasites of deer mice and North American red squirrels (*Tamiasciurus hudsonicus*) form structured assemblages. I examined data from fleas, mites, and botflies on these hosts in Algonquin Provincial Park, Ontario, Canada. Ectoparasite prevalence varied with host traits and date. Ectoparasites had no relationship with deer mouse glucocorticoid production. Lastly, ectoparasites of deer mice, but not red squirrels, had exhibited non-random co-occurrence patterns. Parasites play an important role in population regulation and thus, these findings provide a better understanding on the effect of ectoparasites on their hosts, on each other, and consequently on their ecosystem.

Keywords: ectoparasites; rodents; fecal glucocorticoid metabolites; corticosterone; species co-occurrence

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# General Introduction

## Ecology of parasites

Almost half of all animal species on Earth can be considered parasitic (Price 1980; Poulin & Morand 2000) based on the broad definition of parasitism as the obligate feeding of one organism on another with limited mortality to the host. Parasites also represent significant proportions of biomass and comprise a large component of species interactions and biodiversity in natural ecosystems (Mouritsen & Poulin 2005; Preston et al. 2013). Thus, given the growing importance of preserving global biodiversity (Barbier et al. 2019; Di Marco 2019), parasites are a critical component that demand serious consideration (Dougherty et al. 2016). Historically, the ecological importance of parasites has generally been oversimplified and understudied, and yet parasites play key roles in any ecosystem (Marcogliese 2005; Dougherty et al. 2016). Parasites can equally stabilize or destabilize an ecosystem, depending on factors such as host susceptibility and ecosystem size (Combes 1996). Furthermore, parasites influence the biodiversity of free-living species through manipulation of a variety of processes, including migration, competition, predation, and speciation (Altizer et al. 2000; Hatcher et al. 2006; Karvonen & Seehausen 2012).

Wildlife populations are often exposed to a large diversity of parasites, with simultaneous co-infections being the rule rather than the exception (Bordes & Morand 2011; Thumbi et al. 2014). Parasite co-infections of single host individuals have been detected in early records of life, such as helminths in mummies and coprolites of humans from pre-historic times (Araujo et al. 2008). In fact, up to 30% of parasite infestations may be co-infections (Petney & Andrews 1998). Given the co-infectious nature of parasites, hosts can be considered ecosystems for parasites to colonize, where significant ecological and immunological interactions can occur (Zelmer & Seed 2004). Individual hosts are discretely bounded habitat patches for parasites with

easily defined resource availabilities (host size and abundance) and histories (host phylogeny) (Sousa 1994; Morand & Poulin 1998). Thus, host-parasite systems provide great ecological models at multiple scales to address patterns that are usually difficult to investigate in natural populations (Zelmer & Seed 2004). Furthermore, the majority of research on biodiversity in host-parasite systems has focused on host rather than parasite diversity (Dargent et al. 2017). Given the ecological importance of parasites in natural systems, studies on parasites of wild populations can connect data and ecological theory and improve our knowledge of parasite interactions and the dynamics of co-infections (Ezenwa 2016).

#### Important topics in parasite ecology and epidemiology

Emerging infectious diseases have been a substantial global threat to human health for centuries and instances of outbreaks are increasing over time (Oaks et al. 1992; Jones et al. 2008). Consequently, there has been a recent focus on furthering our understanding of disease transmission and factors associated with host susceptibility (Byers et al. 2008; Lucius et al. 2017). Parasite infestation patterns vary among individual hosts, among host populations or locations, and among host species (Poulin & Mouritsen 2003; Bagge et al. 2004). Thus, identification of host and environmental factors influencing parasite communities are principal concerns in parasitology (Vignon & Sasal 2010). Furthermore, parasite prevalence can affect crucial ecological and evolutionary processes of both hosts and parasites (Byers et al. 2008). Examination of factors related to parasite prevalence may be essential to understand patterns of host susceptibility and the broader effects of parasites on host ecology and evolution (Bordes & Morand 2009; Benavides et al. 2012).

Prevalence and intensity of parasite infestations often vary with environmental factors, as well as the biology of the parasite and host (Beldomenico & Begon 2016). Consequently, effects

of parasites on a host population are generally context-dependent. Parasite prevalence and intensity can have a large effect on hosts, such as significantly reducing host fitness (Arnold & Anja 1993; Hatcher et al. 2006; Cornet et al. 2009). However, host defence mechanisms can reduce the negative consequences of parasites. For instance, an increase in grooming activity or immune response can reduce ectoparasite burdens and collateral damage (Hawlena et al. 2007; Råberg et al. 2008). Such parasite defences can be costly, leading to increased trade-offs with other life-history traits such as survival, growth, and reproduction (Giorgi et al. 2001; Zuk & Stoehr 2002). For the majority of wildlife, our understanding of the effects of parasitism is unclear (Monello et al. 2010). Therefore, investigation of effects of parasite infestations on hosts is a prominent question in infectious disease ecology.

It is not only parasites themselves, but also the interactions between co-infecting parasite species on individual hosts that can influence disease severity and spread, as well as host fitness (Gorsich et al. 2014; Clark et al. 2016). Thus, parasite communities can ultimately shape disease epidemiology, with implications for wildlife conservation and management (Graham 2008; Dargent et al. 2017). Parasites can interact with one another either directly through competition for resources or indirectly through modulation of the host immune system (Lello et al. 2004). However, parasite communities in natural populations may form random assemblages without strong pair-wise interactions (Gotelli & Rhode 2002; Krasnov et al. 2006; 2010). Weak species interactions may promote community stability against small perturbations, although larger changes can still be destabilizing (Pedersen & Fenton 2007). Overall, parasite co-infections can have far-reaching effects on parasite transmission, the host population, and the influence of disease management efforts (Brooker & Clements 2009; Vaumourin et al. 2015). Therefore, it is

important to consider potentially complex dynamics of host-parasite systems containing multiple parasite and host species to improve control of infectious diseases.

### Thesis description

In this thesis, I examine correlations of ectoparasite prevalence with host traits and date, relationships between glucocorticoid responses by hosts and ectoparasites, and whether ectoparasites form random or structured assemblages. The first chapter is a description of factors associated with ectoparasite prevalence across different ectoparasites (fleas, trombiculid mites, botflies) and small mammal host species (deer mice (*Peromyscus maniculatus*), southern red-backed voles (*Myodes gapperi*), woodland jumping mice (*Napaeozapus insignis*)). Broadly, this chapter examines how ectoparasite prevalence varies with host traits and date. The second chapter investigates relationships between fecal glucocorticoid metabolites of deer mice and their associated ectoparasites. I examine the association of ectoparasite prevalence, intensity and species richness and the effect of experimental removal of fleas (Siphonaptera) on glucocorticoid production. The third chapter explores patterns of ectoparasite co-occurrence on deer mice and North American red squirrels (*Tamiasciurus hudsonicus*). This chapter has two objectives. The first is to describe whether ectoparasite assemblages exhibit non-random patterns. The second objective of this study is to examine the effect of targeted removal of a flea species on the ectoparasite community.

### Study Species

There are a variety of rodent species involved in the propagation of diseases to humans, wildlife, or domesticated species, and rodents are established hosts of at least 60 severe zoonotic diseases (Kruse et al. 2004). This is unsurprising because Rodentia are the most diverse order of mammals, with 2,277 extant species in 481 genera currently described (Musser & Carleton 2005)

and new genera described each year (Emmons & Fabre 2018; Candela et al. 2019). Furthermore, rodents make up roughly 45% of all known living mammalian species, representing the most numerous, pervasive, and diverse group of mammals on Earth (Hafner 1998; Nowak & Walker 1999). Arthropods are common vectors of disease transmission from rodents to other organisms and are culpable for millions of disease cases each year (Gubler 2009). Disease surveillance of wildlife populations can allow for early detection and control of disease outbreaks (Morner et al. 2002). To develop control methods for parasitic arthropods of rodents, we need to expand our understanding of these host-parasite systems in an ecological context (Johnson et al. 2015).

This thesis focuses on three main ectoparasite groups commonly observed on rodent hosts in Algonquin Provincial Park, Ontario, Canada (45°54' N, 78°26' W); fleas, trombiculid mites, and botflies. Fleas (Siphonaptera) are a common haematophagous ectoparasite of mammalian species, and a great abundance and diversity of fleas are on small- and medium-sized species (Krasnov et al. 2005). Host specificity varies from highly host-specific to host opportunistic (Marshall 1981). Fleas tend to alternate between the time spent on a host's body and within a burrow, with larvae feeding on organic material in a burrow (Krasnov et al. 2004). Prior to reaching the adult stage, development of fleas is exclusively off-host for almost all species.

Trombiculid mites (also called chiggers) are larval parasites of small mammals that feed on host tissue fragments and fluid (Schumacher & Hoeppli 1963). These mites tend to be associated with specific habitat types rather than host species or characteristics (Domrow 1967). In fact, host specificity is poorly developed in this group, with mites observed across a variety of mammalian hosts, and trombiculid mites frequently occurring in high numbers (Jameson & Brennan 1957). Chiggers are free-living, soil-dwelling predators in post-larval stages (Shatrov &

Kudryashova 2006). They are located principally on the ears of rodent hosts, and occasionally around the base of the tail, the anus, and genital region (Worth 1950).

Cuterebrid botflies are obligate larval parasites of many rodent and lagomorph species in the Americas that feed on digested tissue, tissue fluid, and white blood cells (Catts 1982; Colwell 2001). Botflies are very host-specific and larvae obtain all the energy needed for adult maintenance and reproduction (Catts 1982). Females lay eggs in vegetation near burrows; then larvae hatch and adhere to a passing host. Newly hatched larvae enter a host through the eyes, nose, or mouth and migrate subcutaneously until they settle in enclosed pockets called warbles (Catts 1982).

While many studies have focused on single-host and single-parasite systems, this approach is limited in scope due to indirect effects that parasites can have in their community (Mouritsen & Poulin 2005). Furthermore, parasite co-infections can have profound effects on host infection risk and survival (Telfer et al. 2010; Thumbi et al. 2014). Therefore, it is important to broaden our views and investigate multi-host and multi-parasite systems. Studies investigating co-infections can provide greater comprehension of parasite dynamics, with implications for disease management (Pedersen & Fenton 2007). Additionally, the majority of studies on host-parasite systems tend to focus on endoparasites rather than ectoparasites, and aquatic rather than terrestrial hosts (Morand et al. 2007). Fleas, trombiculid mites, and cuterebrid botflies represent very different parasitic groups, though they share similarities in space (fleas and mites) and permanence (mites and botflies). By examining different ectoparasite taxa across different mammalian host species, I can further our understanding on a host-parasite system that has received less attention in the scientific literature.

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# **Chapter 1 – Ectoparasite Prevalence Patterns on Rodent Hosts in Algonquin Provincial Park, Ontario, Canada**

## 1.1 Abstract

Although parasites often play influential roles in their ecosystems, factors associated with parasite prevalence are still poorly understood in many host-parasite systems. I studied prevalence patterns of ectoparasitic arthropods (fleas, trombiculid mites, cuterebrid botflies) on three small mammal host species (deer mice, *Peromyscus maniculatus*; southern red-backed voles, *Myodes gapperi*; woodland jumping mice, *Napaeozapus insignis*) with a focus on variation with host traits and date. Prevalence of the mite (*Neotrombicula microti*) had a positive correlation with date over the summer study period and was observed primarily on reproductively active individuals of all three host species. Additionally, there was a positive association of the combined population density of all three hosts with mite prevalence on deer mice and a negative association on red-backed voles, suggesting mites were being transmitted from one host to another. I also observed that mite prevalence had a negative relationship with deer mice population density, reflecting a potential parasite-mediated reduction in deer mouse reproduction or survival. Prevalence of the flea (*Orchopeas leucopus*) varied with deer mouse sex, age, and body mass, and flea intensity varied with body mass and deer mouse population density. Botfly (*Cuterebra* sp.) prevalence varied with host reproductive status and age, where prevalence was higher on reproductively inactive and subadult hosts. Botfly prevalence also varied negatively with deer mouse population density, and positively with date. Ectoparasite species richness also varied positively with date. These findings suggest that there are a variety of factors that can potentially vary with ectoparasite prevalence, and that ectoparasites may play an influential role in structuring small mammal communities.

## 1.2 Introduction

Incidence of parasitic infestations and levels of immunity can have a high degree of variation within populations and among individuals, especially in wild organisms where parasite assemblages are highly dynamic over time and space (Behnke et al. 1999; Schmidt Hempel 2011; Poulin 2013). Variability in host susceptibility can influence aspects of host-parasite systems, such as host-parasite coevolution, host mortality rates, and parasite dynamics, transmission, and distribution (Carius et al. 2001; Moore and Wilson 2002; Ferrari et al. 2004; Beldomenico and Begon 2010). Furthermore, parasites often play influential roles in regulation of host populations through substantial effects on host energetic requirements, growth, body condition, behaviour, survival, and reproduction (Khokhlova et al. 2002; Perez-Orella & Schulte-Hostedde 2005; Hakkarainen et al. 2007; Hawlena et al. 2007; Lo & Shaner 2015). There is a growing focus on predicting patterns of parasite prevalence in host species due to an increasing emergence of numerous infectious diseases, which are best detected and mitigated through surveillance of natural populations (Daszak et al. 2000; Morner et al. 2002). Parasite ecology is a rapidly growing field, and examination of parasite infestation patterns still remains an important topic that requires further investigation (Matthee & Krasnov 2005).

Parasite prevalence may vary over time, as well as with host characteristics (Podmokła et al. 2014). Temporal patterns of parasite infestations have been previously demonstrated in different taxa, even within a single year (Weatherhead & Bennett 1992; Altizer et al. 2000). Furthermore, susceptibility to parasite infestations can vary with host traits, such as sex and age (Baird 1995; Smallridge & Bull 2000; Tschirren et al. 2003). For example, juvenile gerbils (*Gerbillus andersoni*) are more susceptible than adults to a flea species (*Synosternus cleopatrae*) compared to adults (Hawlena et al. 2006). Male northern flying squirrels also have a higher flea

and mite loads compared to females (Perez-Orella & Schulte-Hostedde 2005). Host characteristics can play a large role in determining susceptibility to parasites and immunocompetence (Esparza et al. 2004). Thus, there is likely individual and temporal variation in immune responses and parasite infestations, and it is important to examine how both date and host intrinsic factors influence parasitism.

I examined patterns of ectoparasite prevalence and intensity of a flea, trombiculid mite, and botfly species on various small mammal hosts (*Peromyscus maniculatus*, *Myodes gapperi*, and *Napaeozapus insignus*). I collected data on host sex, reproductive status, age, body mass, an index of population density (number of host individuals in a given area) and date to investigate which variables may vary with ectoparasite infestations. The objective of the study was to assess prevalence and explore relationships of host traits and date with ectoparasite prevalence on small mammals in Algonquin Provincial Park, Ontario, Canada.

### **1.3 Materials and Methods**

#### Study area and trapping

This study was conducted in Algonquin Provincial Park, Ontario, Canada (45°54' N, 78°26' W) in 2017 and 2018. In 2017, deer mice (*Peromyscus maniculatus*), southern red-backed voles (*Myodes gapperi*), and woodland jumping mice (*Napaeozapus insignus*) were sampled from May – August across 17 100-metre traplines with two Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) placed at stations 10 m apart (detailed methods in Fryxell et al. 1998). In 2018, deer mice, red-backed voles, and woodland jumping mice were sampled from May – August across 8 of the previous 17 traplines sampled in 2017. The dataset was restricted to after July 8<sup>th</sup>, when all ectoparasite species had emerged and were abundant.

Captured individuals were transferred to a handling bag, sexed and assessed for reproductive status (non-reproductive, scrotal, perforated, pregnant, lactating). Individuals were weighed using a Pesola scale and age (juvenile, subadult, adult) was categorically determined from body mass and from fur colour (Schmidt et al. 2019). Individuals received one or two metal ear tags with unique alphanumeric codes (National Band and Tag Co., Newport, Kentucky, USA). Red-backed voles and woodland jumping mice received a single tag. Deer mice received an additional ear tag (one on each ear), as tags can be removed by this species (Kuenzi et al. 2005). All methods used in these studies were reviewed and approved by the Animal Care Committee (ACC) at Laurentian University, Sudbury, Ontario, Canada, protocol number 2017-06-01.

### Parasites

Observers spent 60 seconds examining the dorsal and ventral side of each capture for fleas, trombiculid mites and cuterebrid botflies while gently combing or blowing on the fur to expose the skin (Patterson et al. 2013). A subset of ectoparasites (specifically fleas and mites) were combed from the fur and collected using tweezers to identify to species. Specimens were stored in 70% ethanol. Recaptures of individuals with ectoparasites removed were not included in the dataset unless at least a week had passed since ectoparasite removal because fleas and mites take ~3-4 days to recolonize a host after parasite removal (data not shown).

Representative subsamples of fleas from deer mice were prepared for detailed morphological examination and identification using balsam mounts (Richards 1964). The rest of the flea samples from deer mice were examined using a dissecting microscope (Olympus SZ61). All flea samples were identified to species rank using Siphonaptera keys (Holland 1985; Lewis 2000) and with the assistance of T. Galloway at the University of Manitoba, Canada. The mite

species was identified by H. Proctor at the University of Alberta, Canada (Bobbie et al. 2016). I was unable to collect a botfly specimen to identify the botfly larva to the species level, but given the geographical range and host preference, it is likely *Cuterebra fontinella* or *americana* (Catts 1982; Stewart 2011).

### Statistical analysis

The ectoparasite screening method determined infestation status (yes or no) at the individual level, and so I considered these results as multiple observations on the same individual. Statistical models focused on observations as units of analysis accordingly, while incorporating higher-level hierarchical effects to account for host individuals. Therefore, I used generalized linear mixed-effects models to examine biotic (host biology) and abiotic (external environment) properties. All analyses were conducted using statistical software package R version 3.5.2 (R Core Team 2018).

Statistical analysis of these models was completed using the ‘glmmTMB’ package version 0.2.3.0 (Magnusson et al. 2017). A Bernoulli response variable with a logit link function was used for prevalence models. A quasi-Poisson response variable and logit link function was used for the intensity model, and Poisson response variable with logit link function for the species richness model. Separate models were run for each host and parasite species. The focal response variable was individual infestation status (prevalence). The fixed effects were a population density index of target and additional host species (deer mice (*Peromyscus maniculatus*), red-backed voles (*Myodes gapperi*) and woodland jumping mice (*Napaeozapus insignis*)), host sex, reproductive status (non-reproductive, reproductively active), age (juvenile, subadult or adult for deer mice, juvenile or adult for red-backed voles and woodland jumping mice), body mass and date. An index of population density was measured as captures per

hundred trap-nights at each trapline over two-week periods, as used by Stewart et al. (2014). A combined small mammal population density index was calculated as the sum of each species population density measure. The random effects were sampling year and individual ID number. Two-level categorical fixed effects were coded as binary continuous variables to improve model simplicity. Analyses including deer mouse age, a multi-level categorical variable, had a zero-sum contrast incorporated. Continuous fixed effects were centered and scaled by their mean and standard deviation. P-values were calculated using likelihood ratio chi-squared tests with the ‘drop1’ function in the ‘stats’ package.

#### **1.4 Results**

The final dataset included 229 deer mice (*Peromyscus maniculatus*), 49 southern red-backed voles (*Myodes gapperi*), and 37 woodland jumping mice (*Napaeozapus insignis*). A trombiculid mite species (*Neotrombicula microti*) was identified on all three host species, and two additional ectoparasites (*Orchopeas leucopus* flea and *Cuterebra* botfly) were identified on deer mice (Table 1.1). An index of combined population density of deer mice, red-backed voles, and woodland jumping mice had a positive relationship with mite prevalence on deer mice, but negative relationship on red-backed voles (Figure 1.1; Table 1.2). Furthermore, host reproductive status and date exhibited a correlation with mite prevalence across all hosts, with prevalence increasing on reproductively active individuals and over sampling periods (Table 1.2). The host population density index correlated with mite prevalence in deer mice only. Specifically, deer mice were less likely to have mites at higher population densities. Flea prevalence varied with host sex, age, and body mass, with females, subadults, and larger deer mice having lower flea prevalence. In comparison, flea intensity had a positive correlation with host population density and negative correlation with host body mass. Botfly (*Cuterebra* sp.) prevalence varied with host

reproductive status and age, where prevalence was higher on reproductively inactive and subadult hosts. Botfly prevalence also varied negatively with deer mouse population density, and positively with date. Lastly, ectoparasite species richness had a positive association with date.

## **1.5 Discussion**

Mite prevalence demonstrated a relationship with an index of combined population density of deer mice (*Peromyscus maniculatus*), southern red-backed voles (*Myodes gapperi*), and woodland jumping mice (*Napaeozapus insignis*), where a positive pattern was observed on deer mice but a negative pattern on red-backed voles. Mite (*Neotrombicula microti*) prevalence was also higher on reproductively active hosts and increased with date across all host species. For deer mice, there was a negative correlation between host population density and mite prevalence. Flea (*Orchopeas leucopus*) prevalence varied with deer mice sex (highest on females), age (highest on subadults) and body mass (positive relationship). Flea intensity had a positive association with the host population density index and negative association with body mass. Botfly (*Cuterebra* sp.) prevalence varied with host reproductive status and age, where prevalence was higher on reproductively inactive and subadult hosts. Botfly prevalence was also negatively correlated with population density, and positively correlated with date. Ectoparasite species richness also had a positive correlation with date.

### Host population density

Combined population density of deer mice, red-backed voles, and woodland jumping exhibited a positive correlation with mite prevalence on deer mice, but a negative correlation with mite prevalence on red-backed voles. When re-running the model using a population density index of each species separately for the deer mouse analysis, the same positive pattern occurs (data not shown). A model with separate species population densities could not be run for the red-backed

vole analysis because the sample size was too low. It is not unexpected that mite prevalence would vary with population density, as it has been previously demonstrated that generalist parasites, such as *N. microti* species, are often influenced by host population density (Lindenfors et al. 2004). Furthermore, trombiculid mites, such as *N. microti*, are only parasitic in the larval stage, and are expected to be associated with soil and habitat types rather than specific hosts (Timm 1985). As this is a group of soil-dwelling mites that are often found in decaying logs during nymph and adult stages (Wrenn 1974; Santibáñez et al. 2015), it may not be surprising that ectoparasite prevalence was highest on red-backed voles, who rely on fallen logs as an important habitat component and moisture reservoir (Craig et al. 2014). Thus, red-backed voles may be initial primary hosts when *N. microti* mites are emerging and looking for hosts. However, as population density of both red-backed voles and other host species such as deer mice increase, mites are principally observed on deer mice instead. Deer mice and red-backed voles have highly similar ecological profiles, including similar habitat preferences (Armstrong 1977; Harper & Austad 2004). This would allow for potential transmission of *N. microti* from red-backed voles to deer mice. Ectoparasite transmission in mammalian hosts has been previously demonstrated, though primarily between individuals of the same species and in social animals (Côté & Poulin 1995; Zohdy et al. 2012; Webber et al. 2015). Parasites may select for higher quality or more suitable hosts, or simply infest hosts with higher parasite encounter rates (Christe et al. 2003; Krasnov et al. 2003; Presley 2011). Deer mice are the more abundant species, which may make them easier targets for parasite transmission (Rigaud et al. 2010). Furthermore, different small mammal species vary in parasite defence, including both immune response and physical/behavioural characteristics (skin structure, fur density, grooming behaviour) (Krasnov et al. 2003; Gouy de Bellocq et al. 2006). Therefore, deer mice may provide superior habitat to

mites or may simply be more abundant and easier to infest compared to red-backed voles. However, further experimentation on host selection of the mite *N. microti* is required to test these hypotheses.

Population density of deer mice alone had a negative relationship with mite prevalence. As previously noted, red-backed voles are likely a primary host for mites, which suggests that red-backed voles may transmit mites to deer mice. However, a negative relationship between parasite prevalence and host population density may be attributed to a lower rate of parasite reproduction and transmission compared to host reproduction and dispersal (Stanko et al. 2006). Therefore, at high densities, many deer mice may remain uninfested simply because the mite species cannot establish themselves fast enough on young or dispersing hosts.

Another explanation for a negative relationship between mite prevalence and the deer mouse population density index is that the mite species may increase host mortality or reduce host reproduction. Parasites can increase host vulnerability to predation and promote host species exclusion or coexistence (Hatcher et al. 2006). Furthermore, parasites can decrease host reproductive success by slowing development or increasing mortality of host offspring (Proctor & Owens 2000). This is in line with observed results, as mites were observed to have higher prevalence on reproductively active deer mice and may then have negative effects on host reproduction. If red-backed voles transmit mite infestations to deer mice at higher combined small mammal population density, and the mite species has a negative relationship with population density of deer mice, then red-backed voles may act as potential “biological weapons” to deer mice populations, effectively reducing the latter’s numbers (Strauss et al. 2012). This would be a case of apparent competition, where a superior competitor (southern red-backed vole) carries and passes on a parasite to a more vulnerable competitor (deer mouse)

(Hudson & Greenman 1998). Therefore, the mite species in may affect the structure of the small mammal community through apparent competition, leading to a reduced population of the vulnerable host (Tompkins et al. 2001). For parasites to reduce their host's population, parasite growth rate must be higher than that of its host, and a parasite must reduce fecundity or increase mortality rates, leading to a reduction of a host population at high densities (Anderson & May 1978; May & Anderson 1978). However, it is difficult to confirm population regulation by parasites without a perturbation experiment (Tompkins et al. 2002). Therefore, it is possible that red-backed voles may transmit mites to deer mice, and that mites may reduce deer mouse population density. Nonetheless, further experimentation is needed to determine the presence of host population regulation and apparent competition in this system.

A negative relationship was also identified between botfly prevalence and deer mouse population density. This can be attributed to the same explanations given for the relationship with mite prevalence. Botflies lay their eggs at entrances of small mammal burrows, and body heat enhances egg hatching (Catts 1982). At a high population density, this method of transmission and reproduction may not be able to keep up with reproduction and dispersal of deer mice (Stanko et al. 2006). Botflies may also reduce deer mouse population densities through effects on host reproduction or survival. For instance, botfly prevalence can reduce reproductive organ size and may disturb regular breeding cycles (Wecker 1962; Smith 1977; Timm & Cook 1979). Botflies may also reduce survival rates in *Peromyscus* mice (Miller & Getz 1969). However, it is important to consider that botflies do not always demonstrate a negative effect on breeding or survival of mice (Munger & Karasov 1991; Cramer & Cameron 2006). Therefore, a negative trend between botfly prevalence and deer mouse population density may be related to

effectiveness of parasite reproduction and transmission, or due to detrimental effects of botflies on deer mouse reproduction or survival.

In contrast, a positive correlation was observed between flea intensity and deer mouse population density. There is a minimum number of host individuals needed for parasite establishment and transmission within a host population (Bagge et al. 2004). As host population density increases, so does the likelihood of parasite transmission, as hosts are more likely to come into contact with one another (Beldomenico & Begon 2010). Therefore, at a high population density of deer mice, hosts may be more likely to transmit fleas to one another, leading to higher flea intensities.

### Reproductive status

Reproductively active individuals of each host species exhibited higher mite prevalence compared to reproductively inactive hosts. The relationship between host traits and parasite prevalence is often linked to host life-history trade-offs. For instance, restrictions to resource availability can lead to trade-offs between reproduction and parasite defence (Forbes 1993; Zuk & Stoehr 2002; Šimková et al. 2008; Warburton et al. 2016). During reproduction, energy is often redirected, leaving hosts with increased parasite susceptibility (Cattadori et al. 2005). Specifically, host immune function, and thus parasite susceptibility, can change during reproductive stages due to changes in hormone profiles (Christe et al. 2000). For example, mite species can increase their infestation rates based on host pregnancy hormones (Lourenço & Palmeirim 2007; Zhang et al. 2010). Overall, reproductively active hosts experience higher energy costs compared to their non-reproductive counterparts. This may influence strength of an immune system response and subsequent susceptibility of small mammal hosts to the mite species.

In comparison, non-reproductive deer mice had higher botfly prevalence. Parasites may delay development or breeding (Agnew et al. 2000; Telfer et al. 2005; Møller 2010), leading to a negative relationship between parasite prevalence and reproductive condition. Previous studies have reported that *Peromyscus* mice with botfly infestations had smaller reproductive organs and may have irregular breeding cycles (Wecker 1962; Smith 1977; Timm & Cook 1979). However, not all studies find a negative effect of botflies on reproduction of mice (Munger & Karasov 1991). Generally, parasite resistance is costly, and highly susceptible hosts may not reproduce if they are already under tight energy constraints (Allen & Little 2011). It is possible that botfly prevalence may lead to higher energy costs that influence deer mouse reproduction.

#### Host sex

Flea prevalence was higher on male than female deer mice, a common observation (Klein 2004; but see McCurdy et al. 1998). There are a variety of explanations for this pattern, including sex differences in behaviour, body size and immunity (Folstad & Karter 1992). For example, compared to females, male deer mice tend to engage in aggression, disperse farther distances and have larger home ranges, behaviours that increase likelihood of parasite infestations (Fairbairn 1977; Møller & Erritzøe 2001; Morand et al. 2004). Furthermore, male deer mice tend to have larger body mass compared to females, which may provide greater nutritional sources, space and range of niches for parasites (Poulin 1995; Schulte-Hostedde et al. 2001). Along with sex differences in behaviour or size, there are also immunological differences that lead to increased parasitism of males. For instance, females generally have stronger immune responses compared to males (Zuk & McKean 1996). Additionally, testosterone may increase parasite susceptibility in males by reducing immune function (Klein 2004). Therefore, behaviour, morphology and physiology may contribute to higher flea prevalence in males compared to female deer mice.

### Host age

On deer mice, prevalence was lower on subadults compared to other age classes. Subadults may be more susceptible to ectoparasites, as they are the most likely age class to disperse from their parents' home range to establish their own (King 1968). Since these hosts are unlikely to occupy permanent burrows, which fleas rely on for reproduction and development, subadults may represent poor habitat for flea infestations (Krasnov et al. 2002). In comparison, juveniles are less likely to leave burrows and disperse to new areas, and adults will likely not need to disperse if they already have established home ranges (Lyman et al. 2001). Therefore, resident hosts (such as juveniles and adults) that possess burrows will be more suitable hosts to fleas compared to dispersing hosts (such as subadults). This may explain lower prevalence of fleas on subadult deer mice.

In comparison, botfly prevalence was highest on subadults compared to other age classes. This may be related to dispersal, as increased host movement may lead to exposure to a wider variety of habitats (which may host different parasite species), as well as more host individuals to encounter (allowing for greater parasite transmission) (Bordes et al. 2009). Subadult deer mice are the most likely age class to disperse from their home range (King 1968; Cross et al. 2009). Thus, they may have an increased exposure/encounter rate with botfly larvae. Furthermore, many parasite populations rely on host movement for propagation as they are often less mobile compared to their host species (Morgan et al. 2004). Specifically, botfly acquisition requires hosts to be in close proximity after their eggs are laid (Catts 1982). Therefore, subadult deer mice, which are the most likely age class to disperse, may be more likely to come into contact with botflies and subsequently become infested.

### Host body mass

There was a negative correlation of both flea prevalence and intensity with deer mouse body mass, a common observation that is usually due to harmful effects of a parasite (Burger et al. 2012). Specifically, flea parasitism often leads to blood loss and energetic costs of defences against parasites, which may result in a decrease in body mass (Hawlana et al. 2006). Infested hosts may also experience a reduction in body condition, leading to a lower body mass (Neuhaus 2003). However, this pattern is usually only observed in cases with hosts that are heavily parasitized (Khokhlova et al. 2002; Neuhaus 2003), while the majority of hosts that were infested carried a single flea. Regardless, it is important to note that while only fleas on bodies of hosts were accounted for, there is often a much higher level of flea parasitism in a host's nest that is positively correlated with host body infestations (Krasnov et al. 2004). Thus, flea-infested hosts in this study may be heavily parasitized within a nest. Flea infestations may lead to a decrease in host body mass either due to costly defences against parasites or due to hosts with reduced a body condition experiencing greater flea susceptibility.

#### Date

Mite and botfly prevalence, as well as ectoparasite species richness, increased from early July – late August in 2017 and 2018. This is expected, as previous studies have noted that *N. microti* and *Cuterebra* species tend to emerge in July and reach high prevalence within a host population in late summer or fall (for *N. microti*) and late July or early August (for *Cuterebra* sp.) (Timm 1985; Cramer & Cameron 2006; Ott-Conn et al. 2015). Thus, the sampling period falls within time periods where infestation rates should be increasing, as chigger mites and botflies are only active during warm months in northern temperate areas (Nichols 1994; Dietsch 2005). This also explains the positive trend of ectoparasite species richness with date, as two of the three species had increasing prevalence between early July and late August. Overall, the results confirm

previously established temporal trends in the probability of individual infestation of mites and botflies on various small mammal hosts.

### Conclusion

There were a variety of factors strongly related to ectoparasite prevalence in at least one parasite species. These factors included host population density, sex, body mass, age, reproductive status and date. Host ecological traits can influence parasite exposure and susceptibility, leading to non-random parasite distributions across hosts (Alzaga et al. 2009). These results suggest a shift in host infestations from red-backed voles to deer mice with increasing small mammal population densities, where mites appear to infest deer mice preferentially. However, transmission of mites from red-backed voles to deer mice may reduce a deer mouse population. Thus, this study presents further evidence that ectoparasites of rodents may potentially be involved in regulating host populations and shaping small mammal communities. Infestations of *N. microti* can lead to severe clinical trombiculiasis, leaving hosts with serious lesions and deformities, which can reduce reproductive success and survival (Foley et al. 2013). Therefore, interactions between red-backed voles and deer mice through their shared ectoparasite, *N. microti*, may lead to changes in composition of small mammal communities. Overall, these findings demonstrate that there are a variety of host-specific factors that must be investigated to understand patterns of ectoparasite prevalence. Furthermore, ectoparasites of small mammals can influence structuring of small mammal communities.

## 1.6 Literature Cited

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Table 1.1 Proportion of deer mice (n = 229 individuals), southern red-backed voles (n = 49 individuals), and woodland jumping mice (n = 37 individuals) infested by ectoparasite species.

Host species	Parasite species	Prevalence (%)
Deer mouse ( <i>Peromyscus maniculatus</i> )	Mite ( <i>Neotrombicula microti</i> )	24.9
	Flea ( <i>Orchopeas leucopus</i> )	20.1
	Botfly ( <i>Cuterebra</i> sp.)	05.7
		43.7
Southern red-backed vole ( <i>Myodes gapperi</i> )	Mite ( <i>Neotrombicula microti</i> )	85.7
Woodland jumping mouse ( <i>Napaeozapus insignis</i> )	Mite ( <i>Neotrombicula microti</i> )	35.1

Table 1.2 Summary of factors varying with prevalence, intensity, and species richness of ectoparasite species for deer mice (n = 229 individuals), southern red-backed voles (n = 59 individuals), and woodland jumping mice (n = 37 individuals) based on linear mixed models (package ‘glmmTMB’). Factors included have  $p < 0.05$  or  $\beta > 0.8$ .

Host species	Response variable	Covariate	$\beta$	SE	$p$
Deer mouse	Mite prevalence	Host population density	-0.938	0.336	0.003
		Combined small mammal population density <sup>1</sup>	0.741	0.303	0.01
		Reproductive status	1.240	0.579	0.03
		Date	1.063	0.193	<0.001
	Flea prevalence	Sex <sub>M</sub>	1.234	1.136	0.54
		Age <sub>Adult</sub>			0.01
		Juvenile	0.349	0.720	
		Subadult	-1.354	0.620	
	Flea intensity	Body mass	-1.211	0.553	0.02
		Host population density	0.673	0.372	0.07
	Botfly prevalence	Body mass	-0.516	0.277	0.06
		Host population density	-1.078	1.716	0.53
		Reproductive status	-0.992	2.926	0.73
		Age <sub>Adult</sub>			0.53
		Juvenile	-1.172	1.778	
Subadult		1.049	1.152		
Ectoparasite species richness	Date	0.976	0.652	0.11	
	Date	0.391	0.088	<0.001	
Southern red-backed vole	Mite prevalence	Combined small mammal population density <sup>1</sup>	-0.870	0.357	0.004
		Reproductive status	1.675	0.821	0.04
		Date	0.838	0.310	0.003
Woodland jumping mouse	Mite prevalence	Reproductive status	0.254	0.535	0.10
		Date	1.440	0.422	<0.001

<sup>1</sup>Includes combined population density of deer mice, southern red-backed voles and woodland jumping mice.

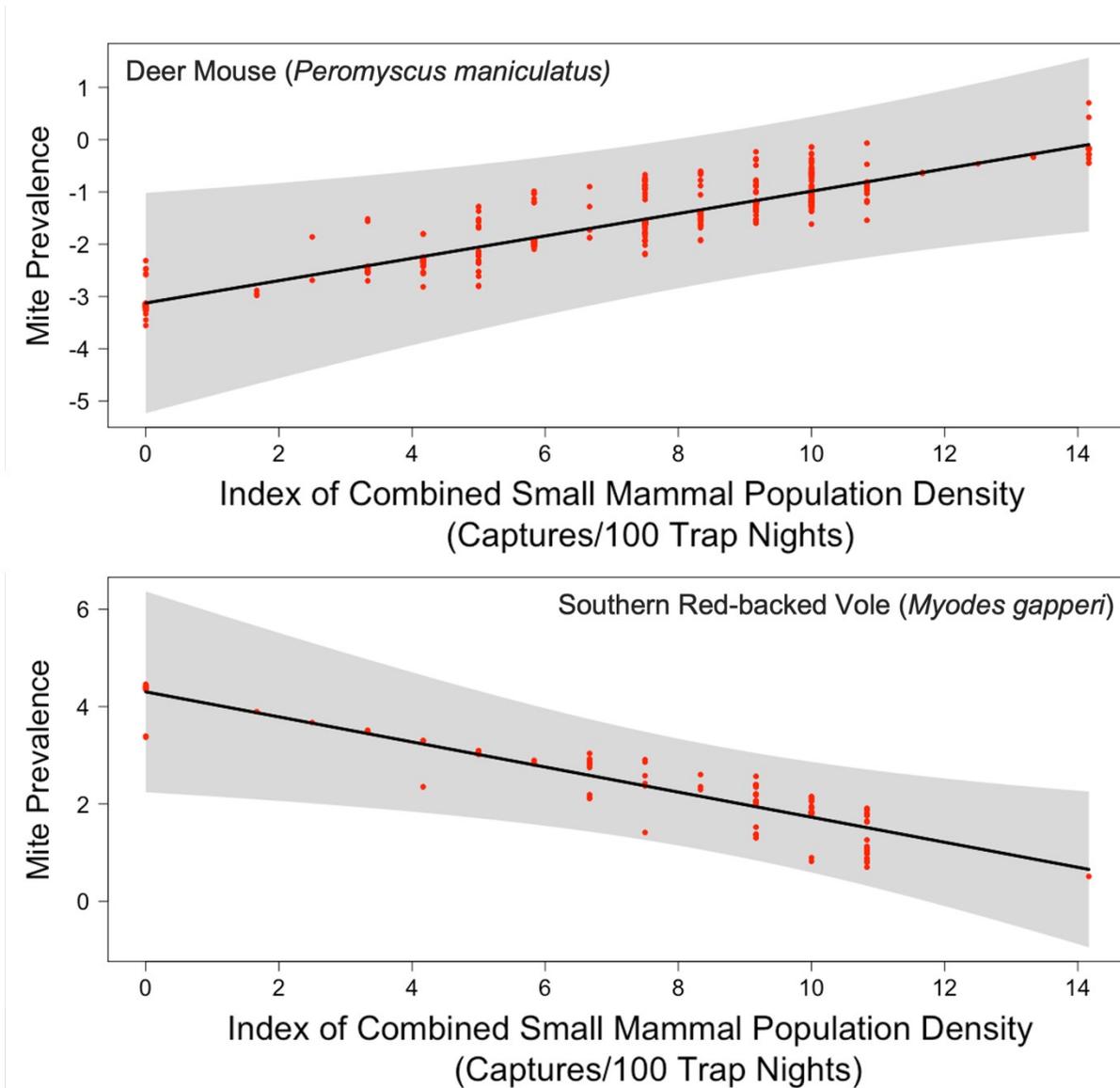


Figure 1.1 Relationship between mite (*Neotrombicula microti*) prevalence for deer mice (n = 229 individuals) and southern red-backed voles (n = 59 individuals) and combined population density of deer mice, southern red-backed voles, and woodland jumping mice. Shown are partial residuals (dots) and the 95% confidence interval (shading) extracted from generalized linear mixed models (package ‘glmmTMB’).

**Chapter 2 – Ectoparasites and Fecal Glucocorticoid Metabolites of  
Deer Mice (*Peromyscus maniculatus*)**

## 2.1 Abstract

Parasites can have a variety of harmful effects on their hosts, and a common form of parasite defence is increased production of glucocorticoid hormones, which promotes immune function. I examined relationships between ectoparasite prevalence, intensity, and species richness with fecal glucocorticoid metabolites of deer mice (*Peromyscus maniculatus*). Furthermore, I experimentally removed fleas to determine if reductions in ectoparasites affected glucocorticoid production. Lastly, I examined the relationship between ectoparasites and intra-individual variation in glucocorticoid concentrations. Individuals were assigned to control (no flea removal) or treatment (anti-parasite application, physical combing) and individuals were recaptured to assess changes in glucocorticoid concentrations. There was no association of experimental removal of the flea (*Orchopeas leucopus*), ectoparasite species richness, and prevalence or intensity of any of the ectoparasite species with fecal glucocorticoid metabolites of deer mice. There was also no significant relationship of ectoparasites with intra-individual variation in glucocorticoid production. However, there was a significant relationship with date, where glucocorticoid production decreased towards the end of the breeding season. Therefore, adverse events associated with reproduction, rather than ectoparasites, may be more important to changes in glucocorticoids of deer mice.

## **2.2 Introduction**

Parasites are an integral component of ecological systems; they can reshape host physiology, life history, and behaviour, and thus influence the role that their hosts play within an ecological community (Pederson & Fenton 2007; Wood et al. 2007). By definition, the effect of parasites on a host is deleterious. These effects can be direct through consequences of parasitic feeding, including tissue damage, toxins from saliva, and allergic reactions (Steen et al. 2006; Raissey & Ansari 2011; Palm et al. 2012). However, parasites can also have indirect effects on their hosts, including reduced fecundity, body condition, and survivorship (Brown et al. 1995; Newey & Thirgood 2004; Irvine et al. 2006).

Given the effects that parasites can have on their hosts, many studies have focused on trade-offs between parasite defence and life history traits (Lochmiller & Deerenberg 2000; Branson 2003; Sandland & Minchella 2003). Hosts often have limited availability of resources such as energy, protein or nutrients (Norris & Evans 2000). Furthermore, parasite resistance is often expected to play a demanding role in host life history, and an associated immune defence is generally accepted as a costly investment by a host (Langand et al. 1998; Zuk & Stoehr 2002). Thus, further consumption of host resources by a parasite or requisite immune defence response by a host can lead to fewer resources available for other functions, such as host maintenance and reproduction (Branson 2003).

It is common for studies to focus on the host immune system, but it is a combination of host systems that interact together as a response to parasitism (Modha et al. 1996; Morales-Montor et al. 2001). For instance, a stimulus (such as a parasite) can generate production of a collection of cytokines, hormones, neurotransmitters, and neuropeptides expressed from

receptors of immune, endocrine, and nervous cells (Besedovsky & del Rey 1996; Corrêa-de-Santana et al. 2006). One of the main components of this complex network is the HPA (hypothalamic-pituitary-adrenal) axis, which modulates immune response (Buckingham et al. 1996). At the level of the hypothalamus, pituitary, and adrenal glands, corticotropin-releasing hormone (CRH), adrenocorticotropin (ACTH), and glucocorticoids are secreted, respectively (Corrêa-de-Santana et al. 2006).

Glucocorticoids play an especially vital role in the immune-neuro-endocrine system, as they regulate immune system functioning (Weil et al. 2006). Additionally, high levels of glucocorticoids are often associated with a stressor, and these hormones are a critical component of the adaptive biological stress response (St. Juliana et al. 2014; Romero & Wingfield 2015). Acute stressors can lead to an increase in glucocorticoid levels that promote immune function. However, chronic exposure to stressors and associated glucocorticoids can lead to immunosuppression (Dhabar & McEwen 1997; Padgett & Glaser 2003). Parasites are chronic stressors, leaving hosts with reduced resistance abilities, facilitating further parasite infestations and limited energy to expel them (Dwyer & Bornett 2004; Lobato et al. 2008). Parasite infestations can lead to an increase in host glucocorticoid levels either through parasite defense or a parasite's manipulation of glucocorticoids to favour their own survival (Defolie et al. 2019). Therefore, an increase in glucocorticoids is often expected as a common host response to parasite infestations.

Studies examining relationships between parasites and glucocorticoids in mammals have had inconsistent results, with some finding an increase in glucocorticoids with parasite prevalence (Brown & Fuller 2006; Martínez-Mota et al. 2017; Seguel et al. 2019) and others finding no relationship (Monello et al. 2010; Carlsson et al. 2016; Trevisan et a. 2017). Overall,

reviews of relationships between parasites and host glucocorticoids demonstrate that a positive relationship is the most common trend (Defolie et al. 2019; O’Dwyer et al. 2019). However, this elevation of glucocorticoids may be delayed over the course of infestation (O’Dwyer et al. 2019). Furthermore, there is variation in how different hosts respond to parasites due to biotic (host) and abiotic (environmental) factors (Pedersen & Greives 2008; Gaitan & Millien 2016; Puehringer-Sturmayer et al. 2018).

Thus, I evaluate how deer mice (*Peromyscus maniculatus*) hosts respond to ectoparasite prevalence, intensity, and species richness in terms of glucocorticoid production. I experimentally removed fleas, expecting that hosts would experience a decrease in glucocorticoids while statistically controlling for host and environmental factors. I also expected that deer mice with a greater ectoparasite prevalence, intensity or species richness present would have heightened glucocorticoid production.

## **2.3 Materials and Methods**

### Field methods

Deer mice (*Peromyscus maniculatus*) were sampled in Algonquin Provincial Park, Ontario, Canada (45°54’ N, 78°26’ W) from May – August 2018 across 17 100-metre traplines with two Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) placed at stations 10 m apart (detailed methods in Fryxell et al. 1998). Traps were baited with water-soaked sunflower seeds at dusk and checked at dawn over 3 consecutive nights on alternate weeks. Captured individuals were transferred to a handling bag, sexed and assessed for reproductive status (non-reproductive, scrotal, perforated, pregnant, lactating). Individuals were weighed using a Pesola scale and age (juvenile, subadult, adult) was categorically determined from body mass and from

fur colour (Schmidt et al. 2019). Individuals received two metal ear tags with unique alphanumeric codes (National Band and Tag Co., Newport, Kentucky, USA).

Traplines were paired by habitat type (sugar maple hardwood, cut-over mixed-wood, dense mixed-wood, conifer, white pine and white spruce, black spruce and aspen, white pine and red pine) and were assigned to a control or treatment group. In the treatment group, fleas were removed from mice through combing and application of 0.8 uL of Frontline Plus (Merial Limited, Duluth, GA, USA, main active ingredient: fipronil 0.29%). Treatment was reapplied to recaptures that had not been treated within 26 days, as the labels indicated that reapplication is necessary after 30 days. Flea specimens were stored in 70% ethanol for future identification. In the control group, mice were visually examined for ectoparasites, did not have their fleas removed, and did not receive Frontline Plus. For visual assessment of ectoparasites, observers spent 60 seconds examining the dorsal and the ventral side of the mouse for ectoparasites such as fleas, trombiculid mites, and cuterebrid botflies while gently combing and blowing on the fur to expose the skin (Patterson et al. 2013). Since Frontline Plus is only effective at preventing infestation of fleas, no other ectoparasites were removed, only quantified through visual assessments. Juvenile deer mice were not treated with Frontline Plus and were excluded from the ectoparasite removal experiment. All methods used in this study were reviewed and approved by the Animal Care Committee (ACC) at Laurentian University, Sudbury, Ontario, Canada, protocol number 2018-03-04.

### Taxonomic identification

Representative subsamples of fleas from deer mice were prepared for detailed morphological examination and identification using balsam mounts (Richards 1964). The rest of the flea samples from deer mice were examined using a dissecting microscope (Olympus SZ61). All flea

samples were identified to species using keys to Siphonaptera (Holland 1985; Lewis 2000) and with the assistance of T. Galloway at the University of Manitoba, Canada. The mite species was identified to species by H. Proctor at the University of Alberta, Canada (Bobbie et al. 2016). I was unable to collect a botfly specimen to identify a botfly larva in this study to the species level, but given geographical range and host preference, it is likely *Cuterebra fontinella* or *americana* (Catts 1982; Stewart 2011).

#### Collection and processing of fecal glucocorticoid metabolites

Feces can be used as a non-invasive sample that represents an integrated average measure of circulating hormone levels that are less affected by short-term fluctuations compared to blood or saliva samples (Dantzer et al. 2010). Fecal glucocorticoid metabolites are a common method to examine glucocorticoid concentrations and have been used previously to examine glucocorticoid levels in deer mice (Hayssen et al. 2002; Harper & Austad 2004). Feces were collected from all traps containing deer mice (no later than 19 hours after defecation) as well as any fresh samples that could be collected from captures. Feces were placed in individual Eppendorf tubes with 80% methanol, put on ice packs in the field and then stored at -20°C until analyzed. Concentrations of corticosterone, the main circulating glucocorticoid in mice (Pedersen & Greives 2008), were determined from hormone extraction and enzyme immunoassays conducted at the Endocrinology Laboratory at the Toronto Zoo (detailed methods in Baxter-Gilbert et al. 2014).

Fecal corticosterone metabolites of mice in the treatment and control group were examined for ~two-week periods. Fecal samples were then labelled as “pre-” or “post-treatment”. For treatment individuals, “pre-treatment” was before application of Frontline Plus and then roughly two weeks later at subsequent capture. For control individuals, “pre-” and

“post-treatment” are simply ~two weeks apart. In total, 184 fecal samples collected from 59 deer mice over two months (June 20<sup>th</sup> to August 15<sup>th</sup>, 2018) were included in the statistical analysis.

### Population density

An index of population density of three nocturnal small mammal species (deer mice (*Peromyscus maniculatus*), southern red-backed voles (*Myodes gapperi*), and woodland jumping mice (*Napaeozapus insignis*)) was measured as captures per hundred trap-nights for each trapline, as used by Stewart et al. (2014). Southern red-backed voles and woodland jumping mice were included in the analysis, as they are known to share similar space or food preferences (Vickery 1979; Schulte-Hostedde & Brooks 1997; Boonstra & Krebs 2012). I calculated density estimates over two-week periods at each trapline, to determine overall population density (number of host individuals in a given area) of all three small mammal species and individual species’ population density. While many studies have used Spatially Explicit Capture-Recapture (SECR) models, this was an unsuitable approach for this dataset. Small mammal live traps can only catch and hold a single individual, and competition between individuals for traps creates a complex dependence structure that violates assumptions of independence in Bayesian SECR models (Royle 2013; Gerber & Parmenter 2015). Furthermore, frequentist SECR models do not perform well for two reasons. First, the multi-catch trap estimator is typically used for analysis of single-catch trap data; high levels of trap saturation, as in this study, violate multi-catch trap estimator assumptions. Second, the current method for examining density from single-catch traps is unavailable for data with unknown capture times, which is the case for this study (Distiller & Borchers 2015).

### Statistical analysis

All analyses were conducted using statistical software package R version 3.5.2 (R Core Team 2018). Prior to main analyses, I tested for variation in corticosterone concentrations due to intrinsic and extrinsic factors using a linear mixed-effects model with host age (subadult or adult), sex, reproductive status (non-reproductive, reproductively active), body mass, date, predator presence/absence, population density, and habitat type as fixed effects and individual ID number as a random effect (Model 1). I also tested for variation in deer mouse corticosterone concentrations with flea prevalence, intensity, mite prevalence, the interaction between mite and flea prevalence, ectoparasite species richness, and the interaction of treatment group (control or treatment) and occasion (pre- or post-treatment), and number of Frontline applications received as fixed effects and individual ID number as a random effect (Model 2). A log transformation was applied to the corticosterone variable in all models to normalize distribution of data. Two-level categorical variables were coded as binary continuous variables to improve model simplicity. Continuous predictors were centered and scaled by their mean and standard deviation.

To fit these models and the subsequent final model, I used the ‘lme4’ package version 1.1-21. (Bates et al. 2014). Model averaging was performed using the ‘MuMIn’ package version 1.43.6 (Barton 2009). For initial models (Model 1, 2) used in these analyses, only variables from the best submodels (with  $\Delta AICc < 2$ ) were included in the final model (Model 3) (Grueber et al. 2011). A separate model testing how ectoparasites influenced intra-individual corticosterone variability was built where residual variance from the final model (Model 3) was allowed to vary with the variables from Model 2 (detailed methods in Montiglio et al. 2015). I used the ‘nlme’ package version 3.1-141 to fit this model (Pinheiro et al. 2012). Unbiased 95% confidence intervals were calculated for the final model (Model 3) using the ‘confint’ function in the ‘MuMIn’ package.

## 2.4 Results

The final dataset included 59 mice: 27 in the treatment group and 32 in the control group, where 21 mice were infested with the flea *Orchopeas leucopus*, 14 mice were infested with a trombiculid mite (*Neotrombicula microti*), and 4 mice were infested with a botfly (*Cuterebra* sp.). Frontline Plus was an effective treatment against fleas, with only a single individual in the treatment group infested with a flea after being treated. In contrast, Frontline treatment had no effect on mite or botfly infestations. Individuals without fleas were still included in the statistical analysis as they may still come into contact with fleas in their burrows (van der Mescht et al. 2018). Mode and average number of days between fecal collections per individual was 14 days.

A total of 14 top models within 2  $\Delta$ AICc of the best supported model were produced after model averaging (Table 2.1). Number of treatment applications, mite prevalence, host sex, body mass, date, and indices of population density of deer mice (*Peromyscus maniculatus*), woodland jumping mice (*Napaeoapus insignus*), and southern red-backed voles (*Myodes gapperi*) were included in at least one of the top 14 models. There was no significant relationship between ectoparasite variables and intra-individual variability in corticosterone concentrations. In general, there was a high degree of individual variation in deer mice corticosterone concentrations (Figure 2.1). All of the 95% confidence intervals for predictor variables included in the top models contained zero except for date, suggesting that the majority of predictors included in this analysis did not explain a significant proportion of variance in corticosterone of deer mice (Table 2.2). However, date did have a significant and negative relationship with corticosterone of deer mice (Figure 2.2; Table 2.2).

## 2.5 Discussion

Generally, I observed a high degree of individual variation in deer mice corticosterone concentrations. There was no association of experimental removal of the flea (*Orchopeas leucopus*), ectoparasite species richness, prevalence or intensity with fecal corticosterone metabolites of deer mice. There was also no relationship of these variables on intra-individual variation in corticosterone concentrations. Thus, predictions on the relationship between fecal corticosterone concentrations and ectoparasite species richness or experimental removal of fleas were not supported by the findings in this study. However, there was a significant relationship of date with fecal corticosterone concentrations, demonstrating that there is variation in glucocorticoids on a temporal scale.

### Variation in corticosterone

There was a high degree of variation in corticosterone concentrations of deer mice, a trend that has been previously established (Sarabdjitsingh et al. 2012; Stedman et al. 2017). This large amount of variation may make it difficult to discern factors that influence glucocorticoid production and may explain the relatively large number of top models, low effect sizes, and lack of relationship of ectoparasites with glucocorticoid levels. However, it is important to note that the majority of ectoparasite variables were not in the final model, suggesting that ectoparasites are very poor predictors of glucocorticoid concentrations. I did find a significant temporal trend of glucocorticoid concentrations, which highlights how important a role date may play. Considering the potential difficulty in identifying predictors of corticosterone variation, date is likely an important component when considering corticosterone concentrations in deer mice.

### Ectoparasites

There was no effect of experimental removal of fleas and fecal corticosterone in deer mice. This is surprising, since fleas can negatively affect a host directly, not only through blood loss, but also skin irritation, injection of salivary toxins, and introduction of harmful pathogens (Khoklova et al. 2002). Furthermore, fleas are associated with indirect adverse effects on growth, survival, fitness, and high energetic costs (Lehmann 1992; Van Vuren 1996; Khoklova et al. 2002). Since the ectoparasite removal treatment was effective at removing fleas, one can infer that *Orchopeas leucopus* does not have a significant effect on deer mouse corticosterone concentrations.

There was also no relationship of ectoparasite prevalence, intensity or species richness with deer mice fecal corticosterone metabolites. Hosts can develop a range of defence strategies against parasites, which are generally categorized as resistance (ability to limit parasite burden) or tolerance (ability to limit damage of parasite burden) (Råberg et al. 2008). A high tolerance can reduce fitness losses associated with parasite infestations, rather than eliciting a strong immune response (Graham et al. 2012). A variety of organisms have tolerance mechanisms against parasite infestations, including house finches (Adelman et al. 2013), soy sheep (Hayward et al. 2014), and field voles (Jackson et al. 2014). There is also evidence that deer mice are capable of developing tolerance to parasite species, such as the microparasite *Toxoplasma gondii* (Rejmanek et al. 2010). Thus, deer mice may not exhibit a change in corticosterone production due to ectoparasites if they have a tolerance for ectoparasite infestations.

Another possible explanation for a lack of a relationship between ectoparasites and deer mice corticosterone concentrations is that glucocorticoid levels influence ectoparasite infestations. In this study, I examined the effect of ectoparasites on host glucocorticoid production, but it is also possible that chronic elevation of corticosterone can promote survival, feeding, and reproduction of parasites through immunosuppression (St. Juliana et al. 2014). The

immune response can act indirectly or directly on parasites, and chronic production of glucocorticoids can weaken the immune system, consequently influencing parasite population growth (Munck et al. 1984; Cox 2001; Owen et al. 2009). For instance, skin inflammation can block ectoparasites from feeding on host blood and impair ectoparasite development and survival (Owen et al. 2009). Chronic elevation of glucocorticoids, such as corticosterone, can compromise hosts through increased susceptibility to parasites (Belden & Kiesecker 2005). Therefore, we may not observe an increase in corticosterone production with ectoparasite infestations because it may be the case that host susceptibility is inversely influenced by glucocorticoid production.

There may also be a lack of trends in corticosterone concentrations of deer mice from ectoparasites because I was unable to quantify nest ectoparasite loads. Many ectoparasites are often located in burrows or nests of their hosts more often than on a host's body (van der Mescht et al. 2018). Live-trapping is a common method to examine ectoparasites of small mammals (Guo 1999; Morand et al. 2004; Oguge et al. 2009; Tsai et al. 2010; Sponchiado et al. 2017), but this method only allowed us to account for ectoparasites located on a host's body, which may not provide an accurate representation of the full number or species of ectoparasites infesting that individual. As an example, *Ctenophthalmus* and *Rhadinopsylla* flea species make up roughly 60% of fleas on bodies of *Apodemus* and *Microtus* hosts, but over 80% of fleas in nests (Soshina 1973; Krasnov 2008). However, it is important to note at least for small mammals that host body infestation of fleas is generally a reliable indicator of flea population size (Krasnov et al. 2004), suggesting that nest ectoparasites may not be a concern for this study.

Date

A significant and negative trend was observed between date and deer mice fecal corticosterone metabolites, suggesting that glucocorticoid production varied on a temporal scale. Surprisingly, this was not the same trend in Harper & Austad (2001), where deer mice corticosterone excretion increased between July and August. Mammals in temperate, compared to tropical, areas often demonstrate a high degree of variation in their endocrine profile as a response to their environment (Harper & Austad 2001). For instance, corticosterone levels in deer mice vary with temperature (Demas & Nelson 1996). Predictable changes in corticosterone production have evolved as a response to environmental conditions to help individuals adjust their energetic demands over different seasons (Reeder & Kramer 2005). Therefore, there may be some seasonal or environmental component to changes in corticosterone secretion observed here.

One explanation for a relationship between date and corticosterone concentrations is related to breeding season of deer mice (May to early August), with females producing multiple litters within a season (Fairbairn 1977a). While I did not find a relationship between deer mice reproductive status and corticosterone, there may be variation due to mate competition, territory defence and variation in mortality risk. This is in line with the preparative hypothesis, which states that glucocorticoid concentrations prime cardiovascular, immune, cognitive, and metabolic systems during time periods with an increased frequency of adverse events (Romero 2002). During breeding seasons, young deer mouse males are often highly aggressive in spring towards both sexes; there is an increase in territory defence, and females experience greater mortality due to energy demands of breeding (Petticrew & Sadleir 1974; Fairbairn 1977b). We may expect that in June, males would still be engaging in aggression and females would still be experiencing reproduction-related energetic costs compared to August when the breeding season has ended. This pattern has also been observed in flying squirrels, where cortisol levels were higher during

the breeding season compared to the non-breeding season (Desantis et al. 2018). Therefore, an observed decrease in corticosterone metabolites over the course of this study may then be related to adverse events associated with the reproductive season.

### Conclusion

In conclusion, corticosterone production of deer mice did not vary with ectoparasite presence, intensity, species richness or experimental removal of a flea species (*Orchopeas leucopus*). Thus, ectoparasites may not represent a physiological stressor to deer mice. Ectoparasites were not an important factor in explaining corticosterone variation, and very few variables examined had much of an effect, other than date. Thus, there is still much to investigate in deer mice glucocorticoids to gain a better understanding of what biotic and abiotic characteristics demonstrate a relationship with corticosterone production. However, I did find a clear temporal trend in glucocorticoid production, suggesting deer mouse corticosterone concentrations may differ between breeding and non-breeding seasons.

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Table 2.3 Summary of top linear mixed models ( $\Delta\text{AICc} < 2$ ) predicting log<sub>10</sub>-transformed values of fecal corticosterone metabolites in deer mice through model averaging selection. Individual ID is included as a random effect (n = 59 individuals).

Parameters	K	AIC <sub>c</sub>	$\Delta\text{AIC}_c$	$w_i$
Body Mass + Date + WJM PD	6	277.89	0.00	0.13
Sex + Body Mass + Date + WJM PD	7	277.92	0.03	0.13
Body Mass + Date	5	278.85	0.96	0.08
Application + Body Mass + Date + WJM PD	7	279.15	1.26	0.07
Sex + Body Mass + Date	6	279.18	1.29	0.07
Application + Sex + Body Mass + Date + WJM PD	8	279.20	1.31	0.07
Age + Sex + Body Mass + Date + WJM PD	8	279.29	1.40	0.06
Age + Sex + Date + WJM PD	7	279.34	1.45	0.06
Age + Date + WJM PD	6	279.39	1.50	0.06
Age + Body Mass + Date + WJM PD	7	279.51	1.62	0.06
Sex + Body Mass + Date + DM PD + WJM PD	8	279.51	1.62	0.06
Body Mass + Date + DM PD + WJM PD	7	279.65	1.76	0.05
Body Mass + Date + RBV PD + WJM PD	7	279.74	1.85	0.05
Mite Prevalence + Body Mass + Date + WJM PD	7	279.85	1.96	0.05

PD, index of population density; WJM, woodland jumping mice; DM, deer mice; RBV, southern red-backed vole; Application, number of applications of Frontline Plus; number of estimated parameters; AIC<sub>c</sub>, Akaike information criterion for small sample sizes;  $\Delta\text{AIC}_c$ , difference in AIC<sub>c</sub> to the best model;  $w_i$ , relative support of a particular model compared to other models.

Table 2.4 Summary of standardized fixed effects of top linear mixed models ( $\Delta AIC_c < 2$ ) predicting log10-transformed values of fecal corticosterone metabolites in deer mice after model averaging of top models (Table 2.1). Individual ID is included as a random effect (n = 59 individuals). Bolded variables are those with 95% confidence intervals that do not include zero (considered to have strong support).

Predictor variable	Estimate	SE	Lower 95% CI	Upper 95% CI	$\Sigma$
Application	-0.052	0.063	-0.176	0.073	0.14
Mite Prevalence	-0.015	0.052	-0.117	0.087	0.05
Sex <sub>M</sub>	-0.205	0.128	-0.458	0.050	0.45
Age	0.065	0.080	-0.093	0.225	0.25
Body Mass	-0.112	0.076	-0.266	0.044	0.88
<b>Date</b>	<b>-0.179</b>	<b>0.062</b>	<b>-0.302</b>	<b>-0.056</b>	<b>1.00</b>
DM PD	-0.050	0.053	-0.156	0.056	0.11
RBV PD	-0.035	0.056	-0.146	0.075	0.05
WJM PD	-0.103	0.054	-0.209	0.003	0.85

PD, population density; WJM, woodland jumping mice; DM, deer mice; RBV, southern red-backed vole; Application, number of applications of Frontline Plus; SE, standard error; CI, confidence interval;  $\Sigma$ , relative variable importance.

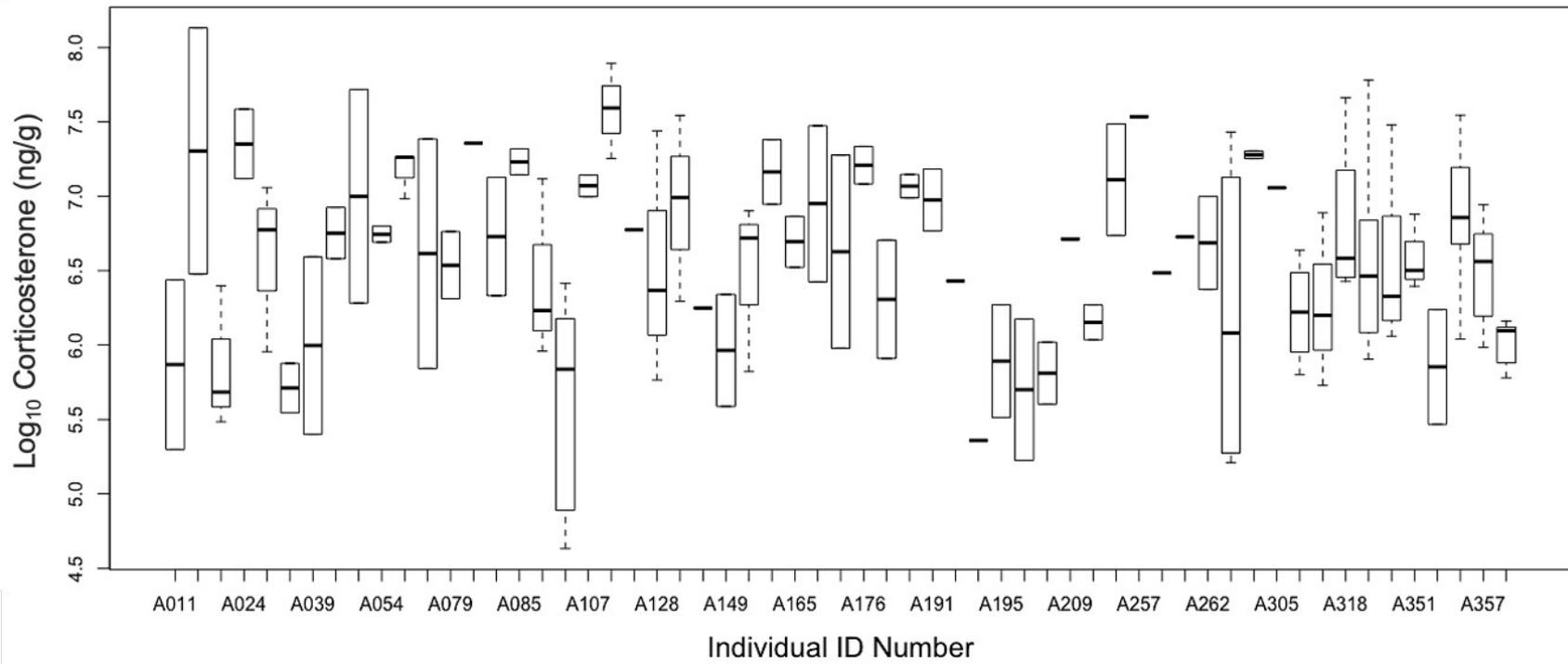


Figure 2.2 Relationship between log<sub>10</sub>-transformed values of fecal corticosterone metabolites in deer mice and individual ID number (n = 59 individuals). Shown are the median (black line), interquartile range (box) and minimum and maximum values (bars).

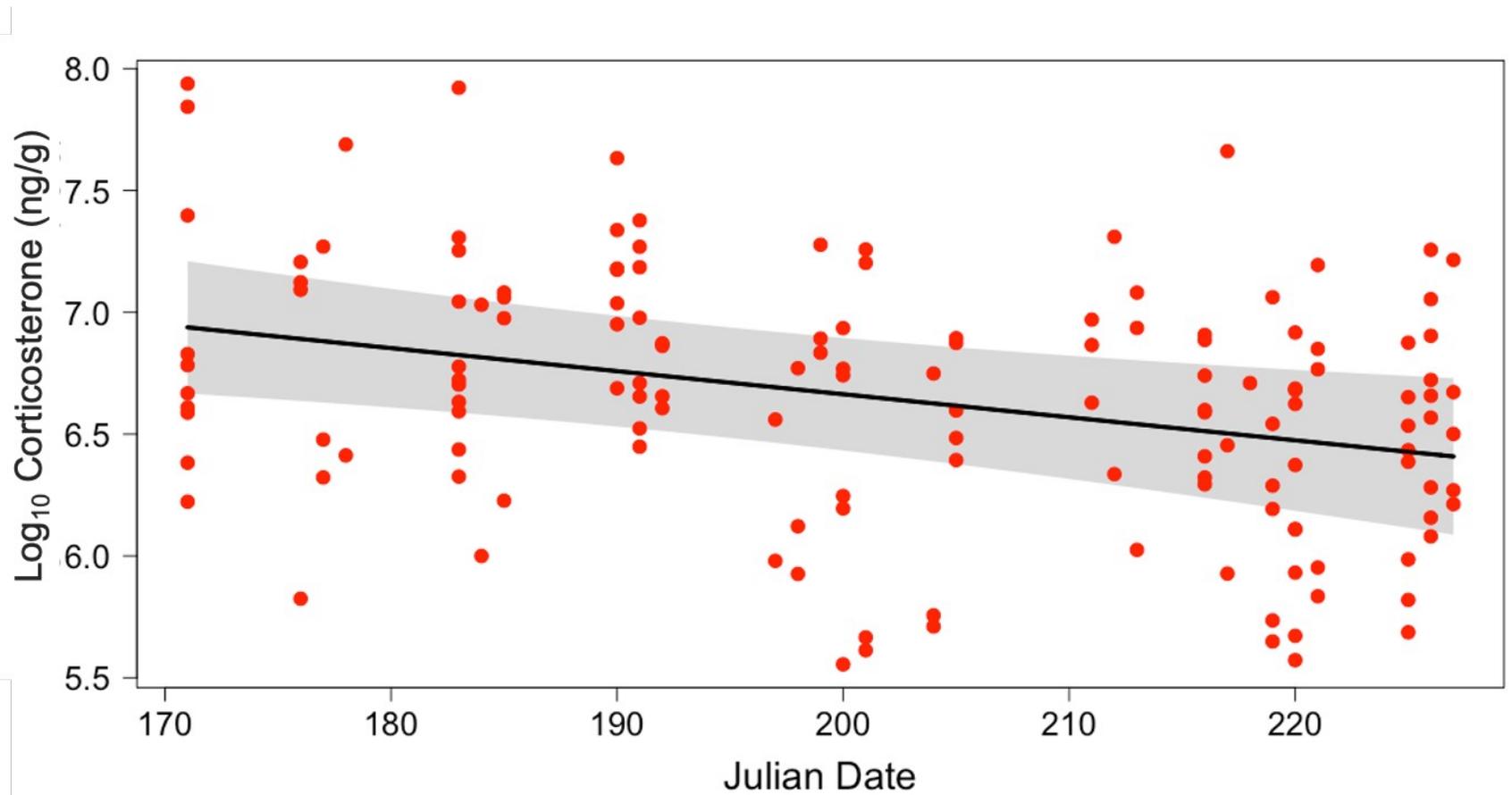


Figure 2.3 Relationship between  $\log_{10}$ -transformed values of fecal corticosterone metabolites in deer mice and Julian date ( $n = 59$  individuals). Shown are partial residuals (dots) and the 95% confidence interval (shading) extracted from linear mixed effects model (package 'lme4').

## **Chapter 3 – Ectoparasite Species Co-occurrence Patterns on Rodent Hosts in Algonquin Provincial Park, Ontario, Canada**

### 3.1 Abstract

Hosts are often infested by multiple parasite species, but it is often unclear whether patterns of parasite co-occurrence are driven by parasite habitat requirements or species interactions. Using datasets on infestation patterns of ectoparasitic arthropods (fleas, trombiculid mites, cuterebrid botflies) from deer mice (*Peromyscus maniculatus*) and North American red squirrels (*Tamiasciurus hudsonicus*), I analyzed species associations using joint species distribution modelling. I also experimentally removed a primary ectoparasite species (*Orchopeas leucopus*) from a subset of deer mice to examine the effect on other common ectoparasite species. Significant associations were identified between ectoparasite species on deer mice, but not North American red squirrels. Specifically, the mite (*Neotrombicula microti*) and botfly (*Cuterebra* sp.) species had a negative relationship that is likely a true biotic species interaction. The flea (*Orchopeas leucopus*) had a negative association with the mite and a positive association with the botfly species, both of which appear to be influenced by parasite habitat requirements, specifically host traits. Furthermore, experimental removal of the flea species did not have a significant effect on ectoparasite prevalence of another species. Overall, these findings suggest that complex parasite species associations can be present among multiple parasite taxa, and that aggregation is not always the rule for ectoparasite communities of small mammals.

### **3.2 Introduction**

Ecological communities include a complex network of biotic interactions such as predation, parasitism, competition, mutualism and facilitation (Fontaine et al. 2011). Parasites play a fundamental role in these communities, in part because parasites represent a substantial portion of worldwide biodiversity and biomass (Toft 1986; Poulin 2014), but also due to influences parasitism can have in shaping biological communities and maintaining biodiversity (Poulin 1999; Ricklefs 2010). It is also important to note community dynamics at a smaller scale. For parasites, hosts can be considered an ecosystem that is discretely bound and has easily defined resource availabilities (host size and abundance) and histories (host phylogeny) (Morand & Poulin 1998; Rynkiewicz et al. 2015). Hosts are often infested by numerous ectoparasite species and it is important to assess relationships of multiple parasite species simultaneously (Rynkiewicz et al. 2015; Dargent et al. 2017). Thus, parasites can interact either directly (through competition for resources) or indirectly (through modulation of a host's immune system) (Graham 2008).

When investigating parasite assemblages among individual hosts, two patterns might result: (1) species co-occur as often as expected by chance, or (2) species co-occur more or less often than expected by chance (Krasnov et al. 2006a). Random patterns of species co-occurrence adhere to the unified neutral theory of biodiversity, predicting that distributions of species abundances are mainly controlled by ecological drift (i.e. random variation of species abundances where each species has an equal chance of reproduction or death) (Norris 2003; Ricklefs 2006). Population dynamics are mainly influenced by birth, death, immigration, and emigration; however, this may not be the case if there are large differences in functional equivalence of parasite species (Volkov et al. 2003; Hubbell 2005). If the demographics of

parasite species are distinct on a per capita basis, then we can expect divergence from unified neutral theory in a parasite community (Hubbell 2006).

Non-random patterns in parasite species assemblages are generally due to interspecific competition or environmental conditions (either host environment or external environment) (Mouillot et al. 2005). Species that are more similar tend to have a greater magnitude of competition, leading to negative species interactions or competitive exclusion (e.g. Kuris & Lafferty 1994). However, ectoparasite assemblages of small mammal hosts tend to demonstrate aggregation rather than segregation (Krasnov et al. 2010; Nava & Lareschi 2014). External environmental factors can create a small window through which only species that can successfully thrive under those similar conditions will co-exist with one another, leading to positive species interactions (Krasnov et al. 2006a). A similar pattern can be seen with a host environment, such as when hosts with reduced anti-parasitic responses are more susceptible to multiple different parasite species, facilitating co-existence and positive interactions (Krasnov et al. 2005). These mechanisms are not mutually exclusive (Mouillot et al. 2005), though it is not yet clear which mechanism has the largest effect on community structure in ectoparasites of small mammals.

While many studies have explored community dynamics of parasites through observational data, investigations into ramifications of removing a species on parasite community dynamics is rarely undertaken (Pedersen & Antonovics 2013; Fenton et al. 2014). Experimental addition or removal of species can provide insight into effects of a species on community structure (Tilman et al. 2001). For parasite communities, this can be explored through perturbation experiments using an insecticide treatment on a host to reduce or remove a target parasite species (Rynkiewicz et al. 2015). Perturbation experiments are the most reliable

method to assess species interactions and are essential to determine critical parasite species interactions that may affect drug treatments and host health (Fenton 2013; Pedersen & Fenton 2015). Therefore, targeted removal of a parasite species will provide a clear understanding on parasite species interactions.

I examined patterns of co-occurrence in ectoparasitic arthropods (fleas, trombiculid mites, cuterebrid botflies) of small mammal hosts (*Peromyscus maniculatus*, *Tamiasciurus hudsonicus*) to determine whether ectoparasite species co-occur more or less often than expected by chance. Specifically, I tested whether ectoparasite species form non-random assemblages on different host species. Furthermore, if they do exhibit non-random patterns, I examined whether parasite species associations are positive or negative. I expected that non-random patterns would follow an aggregative structure with positive associations, consistent with previous studies on ectoparasites of rodent hosts (Krasnov et al. 2010; Nava & Lareschi 2014). I also expected that if non-random co-occurrences were observed with target ectoparasite taxa (fleas), experimental removal would significantly alter prevalence of other ectoparasite species.

### **3.3 Materials and Methods**

#### Study area and trapping

This study was conducted in Algonquin Provincial Park, Ontario, Canada (45°54' N, 78°26' W) across four field seasons (May to August) of 2013, 2015, 2017, 2018. In 2013 and 2015, North American red squirrels (*Tamiasciurus hudsonicus*) were sampled in a ~21-ha grid in an area of hardwood forest using Tomahawk live traps (Tomahawk Live Trap Co., Hazelhurst, Wisconsin, USA) (detailed methods in Bobbie et al. 2017). In 2017, deer mice (*Peromyscus maniculatus*), were sampled across 17 100-metre traplines with two Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) placed at each station, 10 m apart (detailed methods in Fryxell et al.

1998). In 2018, deer mice were sampled across 8 of the previous 17 traplines sampled in 2017. Sampling of deer mice ectoparasites in 2017 and 2018 was conducted throughout a variety of habitats, including hardwood forests, mixed forests, and low-lying bog areas.

Captured individuals were transferred to a handling bag, sexed and assessed for reproductive status (non-reproductive, scrotal, perforated, pregnant, lactating). Individuals were weighed using a Pesola scale. Age of North American red squirrels (juvenile, adult) and deer mice (juvenile, subadult, adult) was categorically determined from body mass (for both species) and from fur colour (for deer mice) (Gooderham & Schulte-Hostedde 2011; Schmidt et al. 2019). Individuals received one or two metal ear tags with unique alphanumeric codes (National Band and Tag Co., Newport, Kentucky, USA). North American red squirrels received a single tag. Deer mice received an additional ear tag (one on each ear), as tags can be removed by this species (Kuenzi et al. 2005). All methods used in these studies were reviewed and approved by the Animal Care Committee (ACC) at Laurentian University, Sudbury, Ontario, Canada, protocols number 2017-06-01 and 2013-05-01.

#### Parasite specimen collection

Captured animals were visually examined for fleas, trombiculid mites, and cuterebrid botflies. A subset of ectoparasites (specifically fleas and mites) were combed from hosts' fur and collected using tweezers to identify to species. Specimens were stored in 70% ethanol. Recaptures of individuals with ectoparasites removed were not included in the dataset unless at least a week had passed since ectoparasite removal because fleas and mites take ~3-4 days to recolonize a host after parasite removal (data not shown).

### Experimental parasite removal

In 2018, fleas (Siphonaptera) were experimentally removed from deer mice. Traplines were paired by habitat type (sugar maple hardwood, cut-over mixed-wood, dense mixed-wood, conifer, white pine and white spruce, black spruce and aspen, white pine and red pine) and were assigned to a control or treatment group. In the treatment group, fleas were removed from mice through combing and application of 0.8 uL of Frontline Plus (Merial Limited, Duluth, GA, USA, main active ingredient: fipronil 0.29%). All flea specimens were stored in 70% ethanol for future identification. In the control group, mice were visually examined for ectoparasites and did not have their fleas removed and did not receive Frontline Plus. Since Frontline Plus is only effective at preventing infestation of fleas, no other ectoparasites were removed, only quantified through visual assessments. Juvenile deer mice were not treated with Frontline Plus and were excluded from the ectoparasite removal experiment.

### Taxonomic identification

Representative subsamples of fleas from deer mice were prepared for detailed morphological examination and identification using balsam mounts (Richards 1964). The rest of the flea samples from deer mice were examined using a dissecting microscope (Olympus SZ61). All flea samples were identified to species using Siphonaptera keys (Holland 1985; Lewis 2000) and with the assistance of T. Galloway at the University of Manitoba, Canada. The flea specimens from North American red squirrels sequenced at the Canadian Centre for DNA Barcoding (CCDB), University of Guelph, Ontario, Canada. The mite species was identified to species by H. Proctor at the University of Alberta, Canada (Bobbie et al. 2016). I was unable to collect a

botfly specimen to identify botfly larva to the species level, but given geographical range and host preference, it is likely *Cuterebra fontinella* or *americana* (Catts 1982; Stewart 2011).

### Statistical analysis

Many questions in community ecology, including presence and direction of species co-occurrence patterns, requires a joint analysis of species presence or abundance data gathered concurrently across different taxonomic groups (such as different species) (Warton et al. 2015; Aivelo & Norberg 2018). These kinds of datasets often include a large number of zeros, such as studies examining parasite counts (Denwood et al. 2008; Pulosof et al. 2012; Warton et al. 2015). Furthermore, individual samples may not contain a great deal of information. Joint species distribution models integrate the joint distribution of species, as well as zero-inflated data, granting investigation of species co-occurrence patterns (Clark et al. 2014). By examining distributions of multiple species simultaneously, these models can be used to study relationships between species that occur due to and regardless of environmental responses (Pollock et al. 2014).

The ectoparasite screening method determined infestation status (yes or no) at the individual level, and so I considered these results as multiple observations on the same individual. I also determined intensity measures for flea species, but subsequent analyses did not produce area under the receiver operating characteristic (AUC) values, suggesting model performance was poor. Therefore, models presented here use only prevalence data instead of intensity. Only ectoparasites infesting more than 5% of the host population were included. Statistical models focused on observations as units of analysis accordingly, while incorporating higher-level hierarchical effects to account for host individuals. Consequently, I used multivariate joint species distribution models to control for biotic (host biology) and abiotic (external environment)

factors while assessing species co-occurrence patterns. I fitted all models with a Bayesian inference by applying the hierarchical modelling of species communities (HMSC) approach (Ovaskainen et al. 2017). Separate models were run for each host species and associated common ectoparasite species (i.e. >10 host individuals infested). All analyses were conducted using statistical software package R version 3.5.2 (R Core Team 2018).

For each analysis, I modelled the response parasite co-occurrence matrix (Y matrix) using a probit model. I included host age (juvenile or adult), sex, reproductive status (non-reproductive, reproductively active), body mass, an index of host population density (number of host individuals in a given area), and date as fixed effects (X dataframe) and specified individual ID number and year as random effects. Analyses for deer mice also included habitat type and an index of population density for additional host species (southern red-backed voles (*Myodes gapperi*), woodland jumping mice (*Napaeozapus insignis*)) that are known to share similar space or food preferences (Vickery 1979; Schulte-Hostedde & Brooks 1997; Boonstra & Krebs 2012). This index of population density was measured as captures per hundred trap-nights at each trapline over two-week periods, as used by Stewart et al. (2014).

I fitted four different models for each host species: (1) a model constrained by fixed and random effects, (2) an unconstrained model with only random effects included, (3) a subsetted constrained model including only hosts that are infested by at least one of the ectoparasite species (i.e. excluding “empty” hosts), and (4) a subsetted unconstrained model also restricted to exclude empty hosts. By comparing results from constrained and unconstrained models, I can investigate which non-random ectoparasite species co-occurrence patterns are exclusively due to habitat (host and/or external environment) conditions, hidden by these habitat conditions, or unaffected by habitat predictor variables (Aivelo & Norberg 2018). I also investigated inclusion

and exclusion of empty, or unparasitized, hosts. Empty hosts may represent unsuitability for parasite colonization, non-colonization by chance (stochasticity of assembly), or spatial dynamics of parasite populations (Gotelli & Rohde 2002; Tello et al. 2008). Thus, excluding empty hosts treats such individuals as unusable, whereas including empty hosts considers them random absences or subjects of parasite spatial dynamics (Gotelli & Rohde 2002). By including models with and without empty hosts, we can further our understanding on assembly of parasite communities (Tello et al. 2008).

Models were fitted by running two MCMC chains with 15,000 iterations (first 5,000 being burn-in) and thinning remaining iterations by 10. The model was cross-validated using a two-fold cross validation. All effective sample sizes (ESS) of the parameters were >2,000. MCMC convergence was assessed through distributions of potential scale reduction factors (PSRF) for model parameters. Model performance was examined using Tjur's  $R^2$  and area under the receiver operating characteristic (AUC).

Variance partitioning can be used to assess explanatory power of various predictor variables, therefore highlighting their relative importance in structuring an ectoparasite community (Ovaskainen et al. 2017). The variance in the constrained models (1 & 3 for each species) was partitioned based on fixed and random effects. To provide insights into relative differences in the influence of fixed and random effects, I separated the variance explained among all of the fixed and random effects.

I also used generalized linear mixed-effects models to examine positive or negative relationships of ectoparasite prevalence with important biotic (host biology) and abiotic (external environment) factors from variance partitioning to provide insights into differences between

constrained and unconstrained models. The focal response variable was individual infestation status (prevalence). Separate models were run for each host species, associated common ectoparasite species (i.e. infested >5% of the host population). Statistical analysis of these models was completed using the ‘glmmTMB’ package version 0.2.3.0 with a Bernoulli response variable and logit link function (Magnusson et al. 2017). Two-level categorical variables were coded as binary continuous variables to improve model simplicity. Analyses including host age, a multi-level categorical variable, had a zero-sum contrast incorporated. Continuous predictors were centered and scaled by their mean and standard deviation.

A separate generalized linear mixed-effects model was used to examine effect of flea removal on the mite species. Similar to the aforementioned mixed models, the focal response variable was individual infestation status (prevalence), and statistical analysis was completed using the ‘glmmTMB’ package version 0.2.3.0 with a Bernoulli response variable and logit link function (Magnusson et al. 2017). Main effects and interaction of treatment group (control or treatment) and occasion (pre- or post-treatment) were included as fixed effects and individual ID number as a random effect. A P-value was calculated using a likelihood ratio chi-squared test with the ‘drop1’ function in the ‘stats’ package. There were not enough host individuals infested with the botfly species in this study to examine effect of flea removal.

### **3.4 Results**

The final dataset included 229 deer mice (*Peromyscus maniculatus*) and 56 North American red squirrels (*Tamiasciurus hudsonicus*). When restricting the dataset to remove “empty hosts”, the subsetted data included 100 deer mice and 28 North American red squirrels. For the perturbation experiment removing the flea species (*Orchopeas leucopus*), the dataset included 59 deer mice. Prevalence of parasite species varied from 5.7% to 39.3% (Table 3.1). For deer mice, there was

one prominent flea species (*O. leucopus*), and for North American red squirrels, there were two distinct species (*Orchopeas caedens* and *Ceratophyllus vison*). There was one trombiculid mite species on both hosts (*Neotrombicula microti*), and a botfly (*Cuterebra* sp.) on deer mice.

Significant non-random co-occurrence patterns were identified between ectoparasites of deer mice, but not of North American red squirrels. Specifically, from the full constrained joint species distribution model, there was only a negative species relationship between the mite and botfly on deer mice (Figure 3.1). When examining associations in the unconstrained models, the mite and botfly still had a negative relationship; however, there are also associations of the flea with each of the other two ectoparasites of deer mice. A negative association was observed between the flea and mite species, as well as a positive association between the flea and botfly. When removing empty hosts, the negative relationship between the mite and botfly disappears and no species associations are identified in the full model. However, associations in the unconstrained models remain identical regardless of whether empty hosts are included.

Variance partitioning of the full constrained model for deer mice demonstrated that different ectoparasite species have unique responses to fixed (host age, sex, body mass, reproductive status, date, target and additional host population densities, habitat type) and random (individual, year) effects included in the study (Figure 3.2). While relative importance of variables changed with ectoparasite species, model performance was generally high, except for the unconstrained models for North American red squirrels (Table 3.1, SI Appendix Table 3.3). Host age, reproductive status, and sex were the most influential fixed effects for the flea species in the full constrained model (Figure 3.2). Host age and sex were also of relatively high importance (explained more than 10% of variance) for mite prevalence, while host age and reproductive status were associated with botfly prevalence. The flea and mite species had

difference prevalence patterns with host age, where adults and juveniles were the most infested class of the flea species, but adults were the least infested class of the mite species (Table 3.2). Furthermore, fleas infested more males than females, while the opposite pattern was observed for the mite species. There were no similarities for infestation of host age classes between the flea and botfly; however, both species infested reproductively inactive individuals more often than hosts that were reproductively active. Lastly, removal of the flea species did not significantly influence mite prevalence ( $P=0.225$ ; Figure 3.3).

### **3.5 Discussion**

This study demonstrated that associations between ectoparasites of deer mice varied based on whether parasite habitat requirements or empty hosts were accounted for. In comparison, ectoparasites of North American red squirrels demonstrated no significant associations regardless of controlling for habitat requirements or empty hosts. I identified a negative association between the mite (*Neotrombicula microti*) and botfly (*Cuterebra* sp.) in the full constrained and unconstrained models for deer mice. There was also a negative relationship between the flea (*Orchopeas leucopus*) and mite and a positive relationship with the flea and botfly species in the full unconstrained model. When comparing analyses with or without empty hosts, the only change was between the constrained models for deer mice, with a negative association between the mite and botfly species only in the full model. Host age and sex, and host age and reproductive status appeared to be shared factors for prevalence of the flea and mite, and flea and botfly species, respectively. Finally, the experimental removal of fleas had no effect on mite prevalence.

Ectoparasite co-occurrence patterns of North American red squirrels

Significant associations were identified between ectoparasites of deer mice, but not between ectoparasites of North American red squirrels. There was poor model performance for the unconstrained models of red squirrels; however, there were still no significant associations in the constrained models. Therefore, the models at least demonstrate that there are no parasite species interactions for ectoparasites of red squirrels. Consequently, ectoparasite assemblages on red squirrels may follow a random pattern. This is consistent with the unified theory of biodiversity suggesting that species distributions are primarily driven by stochastic processes (Norris 2003; Ricklefs 2006). Furthermore, random assemblages of parasites may promote community stability by increasing robustness against small perturbations (Pedersen & Fenton 2007). However, this result is surprising, as North American red squirrels are larger, live longer, and travel greater distances than deer mice (McNab 1963; Wyse et al. 2010), factors that usually contribute to structured parasite communities (Kuris & Lafferty 1994; Lareschi & Krasnov 2010). While ectoparasites communities are often structured, there is temporal and geographic variation, as well as differences among parasite taxa and host species (Krasnov 2013). Thus, further sampling of ectoparasite assemblages on North American red squirrels is required to determine if a random pattern is consistent across time and space.

#### Model comparison of ectoparasite co-occurrence patterns of deer mice

A negative association was identified between the mite and botfly species in both the full unconstrained and constrained models, while associations of these two ectoparasite species with the flea species were only present in the unconstrained model. Thus, the relationship between the mite and botfly can be considered potentially a biotic species interaction, as a non-random co-occurrence pattern exists even after accounting for host and external environmental factors (Aivelo & Norberg 2018). In contrast, relationships of the mite and botfly with the flea species

appear to be restricted to complementary or contradictory habitat requirements. These associations are not strictly biotic species interactions, but rather shaped by their environment.

An antagonistic relationship between the mite and botfly disappeared in the constrained model excluding empty hosts (hosts without ectoparasites) compared to all other models. This brings into question whether the observed pattern is a true biotic species interaction, since it is not in one of the unconstrained models. Through multiple live-captures of the same host individuals, I was able to observe that some uninfested hosts did become infested at later captures, or vice versa (data not shown). This suggests that the full models are better suited here, and that a segregative pattern between the mite and botfly species is a true species interaction. However, examining subsetted models highlights the importance of examining inclusion and exclusion of empty hosts in analyses, as many studies have historically ignored empty hosts in presence-absence matrices (Gotelli & Graves 1996; Gotelli & Rohde 2002). Ignoring empty hosts and assuming they represent unsuitable sites would have led to a likely false interpretation that the mite and botfly relationship is not a biotic species interaction, but rather a result of differing habitat requirements.

#### Ectoparasite species interactions

The mite and botfly species had a significant and negative species association. This is surprising, as non-random patterns of ectoparasite co-occurrence on small mammals are frequently aggregative (Krasnov et al. 2010). Antagonistic interactions tend to be due to some form of interspecific competition, leading to exclusion of one species by another (Mouillot et al. 2005). This can be influenced by host resources, location (where parasites occur on a host), and immunology (Pedersen & Fenton 2007). For instance, both the mite and botfly feed on host tissue and tissue fluid (Slansky 2007; Foley et al. 2013). Parasites, compared to free-living

organisms, may have very limited resource availability, restricted to size and age of their host (Lagrue & Poulin 2008). Thus, niche overlap, such as parasite species sharing the same food resource, can lead to competition for access to host resources (Mideo 2009). In contrast, the mite species is mainly located on hosts' ear pinnae, and occasionally on the mammae and genitalia (Ott-Conn et al. 2015), while the botfly predominantly appears in the inguinal region beneath the skin (Grundmann & Frandsen 1960; Cogley 1991). Therefore, it is unlikely that a segregative pattern between the mite and botfly is due to infestation location. However, another explanation for this antagonistic relationship between parasites species is that parasites may interact via a host's immune system. For example, a low-density parasite species such as the botfly, where only a single warble was observed per deer mouse host (data not shown), could experience excessive damage due to co-infection of a high-density species, such as the mite, where clusters of mites on hosts were often observed (Bobbie et al. 2016). High-density parasite species may generate a severe, non-specific immune response that has a greater effect on low-density species (Råberg et al. 2006; Ulrich & Schmid-Hempel 2012). In parasite networks, bottom-up (host resources) and top-down (host immune system) effects are difficult to distinguish as they share the same source (host individual) (Pedersen & Fenton 2007). Consequently, a negative interaction between the mite and botfly may be a result of resource competition, immune system modulation, or a combination of both mechanisms.

#### Ectoparasite species associations

A negative association between the flea and mite was observed in the unconstrained full and subsetted models. Since a negative association was not observed in the constrained models, this suggests that these non-random co-occurrence patterns are due to contrasting habitat requirements (Aivelo & Norberg 2018). This is further supported by results from experimental

removal of the flea species, which had no effect on mite prevalence. From variance partitioning and generalized linear mixed-effect models, it is clear that host age and sex are likely to play a role in an antagonistic association between the flea and mite species. While the flea primarily infested juveniles and adults, the mite species was less likely to infest adults. Furthermore, flea prevalence was highest on male deer mice, while mite prevalence was highest on females. Thus, this differentiation in host infestation patterns of each ectoparasite species is likely due to intrinsic factors linked to host susceptibility rather than extrinsic factors tied to host exposure (Cattadori et al. 2006). Divergent susceptibilities of host individuals to one ectoparasite species or another may explain a negative pattern between the flea and mite species in the unconstrained models.

There was also a positive association observed between the flea and botfly that was only observed in the unconstrained full and subsetted models. From variance partitioning and generalized linear mixed-effect models, host age and reproductive status were suggested to potentially influence the relationship between the flea and botfly. There was not a common pattern between these two species for infestation of different host age classes; however, host reproductive status may play a role in this positive relationship. Both the flea and botfly prevalence were higher on reproductively inactive hosts than reproductively active hosts. Nonetheless, it is important to note that parasites are generally expected to delay development or breeding production (Agnew et al. 2000; Telfer et al. 2005; Møller 2010), rather than reproductively inactive individuals being more susceptible. Parasite resistance is costly, and highly susceptible hosts may simply not reproduce because they are already under tight energy constraints (Allen & Little 2011). Thus, while a positive association between the flea and botfly species is likely due to habitat requirements, it is difficult to discern specific explanations given

the investigated factors. The relationship may be related to factors that were not examined in this study, such as external habitat components, a component known to influence both flea and botfly infestation patterns (Krasnov et al. 1998; Bowman 2000).

### Conclusion

In conclusion, I identified a negative species association that is likely a biotic species interaction between the mite (*Neotrombicula microti*) and botfly (*Cuterebra* sp.), as well as species associations of the flea (*Orchopeas leucopus*) with the mite and botfly that are likely due to habitat requirements, such as host characteristics. The lack of many parasite species interactions on deer mice and North American red squirrels may be vital for the stability of these ectoparasite assemblages, leading to more robust communities that are less likely to be influenced by small perturbations (Pedersen & Fenton 2007). However, these results are surprising, as I expected to find mainly positive species interactions. Aggregative patterns have been much more common in studies of ectoparasite assemblages on small mammals (Krasnov et al. 2006b; 2010; Presley 2011). Antagonistic relationships, such as that between the mite and botfly species, can have implications for disease management programs, as targeted reduction of one ectoparasite species could lead to unanticipated increases in the other (Telfer et al. 2010). Thus, knowledge on dynamics of parasite communities can be crucial to predict and manipulate parasites and associated diseases in wild populations. Another implication is that artificial infestation of a parasite species with minimal effects to a host could be used to generate a cross-immunological response and prevent future infestation of a more harmful species without turning to costly medication (Lello et al. 2004). Therefore, the predicted models in this study may provide insight into patterns of ectoparasite prevalence with changes in host trait distributions, as well as complex dynamics between ectoparasite species that can have ramifications for disease and

parasite management. Future research should focus on experimental manipulation of the mite or botfly species on deer mice to provide further insights into this ectoparasite community and whether the observed negative relationship is a true species interaction.

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Table 3.5 Number and proportion of deer mice (n = 229 individuals) and North American red squirrels (n = 56 individuals) infested by ectoparasite species.

Host species	Parasite species	Infested individuals	Prevalence (%)
Deer mouse ( <i>Peromyscus maniculatus</i> )	Mite ( <i>Neotrombicula microti</i> )	57/229	24.9
	Flea ( <i>Orchopeas leucopus</i> )	46/229	20.1
	Botfly ( <i>Cuterebra</i> sp.)	13/229	05.7
		100/229	43.7
North American red squirrel ( <i>Tamiasciurus hudsonicus</i> )	Mite ( <i>Neotrombicula microti</i> )	08/56	14.3
	Flea ( <i>Orchopeas caedens</i> )	19/56	33.9
	Flea ( <i>Ceratophyllus vison</i> )	22/56	39.3
		27/56	48.2

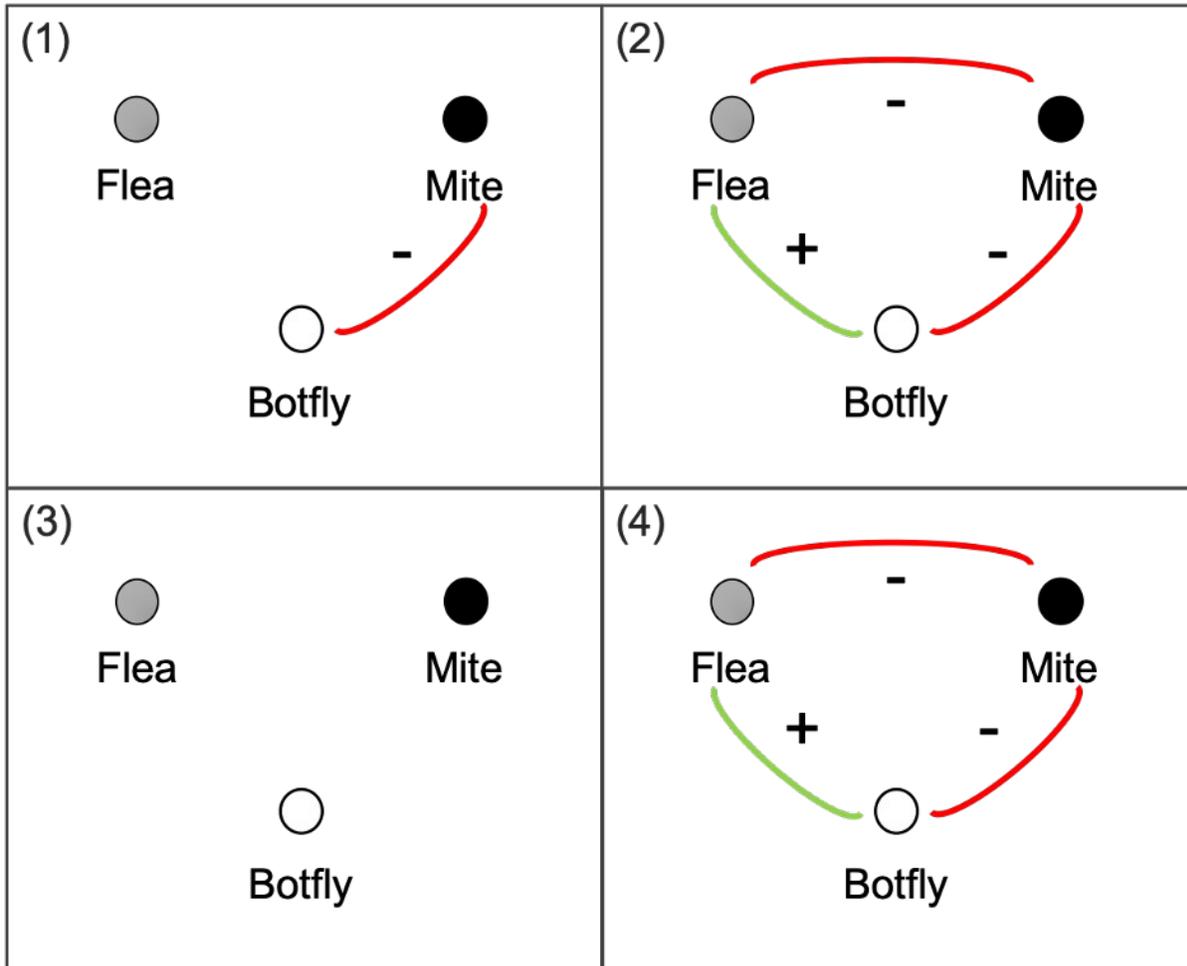


Figure 3.4 Summary of positive (green, +) and negative (red, -) associations between ectoparasite species (flea (*Orchopeas leucopus*), mite (*Neotrombicula microti*), botfly (*Cuterebra* sp.)) on deer mice with statistical support of at least 95% posterior probability (n = 229 individuals). Results presented are from a constrained model (1) or an unconstrained model with the full dataset (2) or constrained model excluding empty hosts (3) and an unconstrained model excluding empty hosts (4).

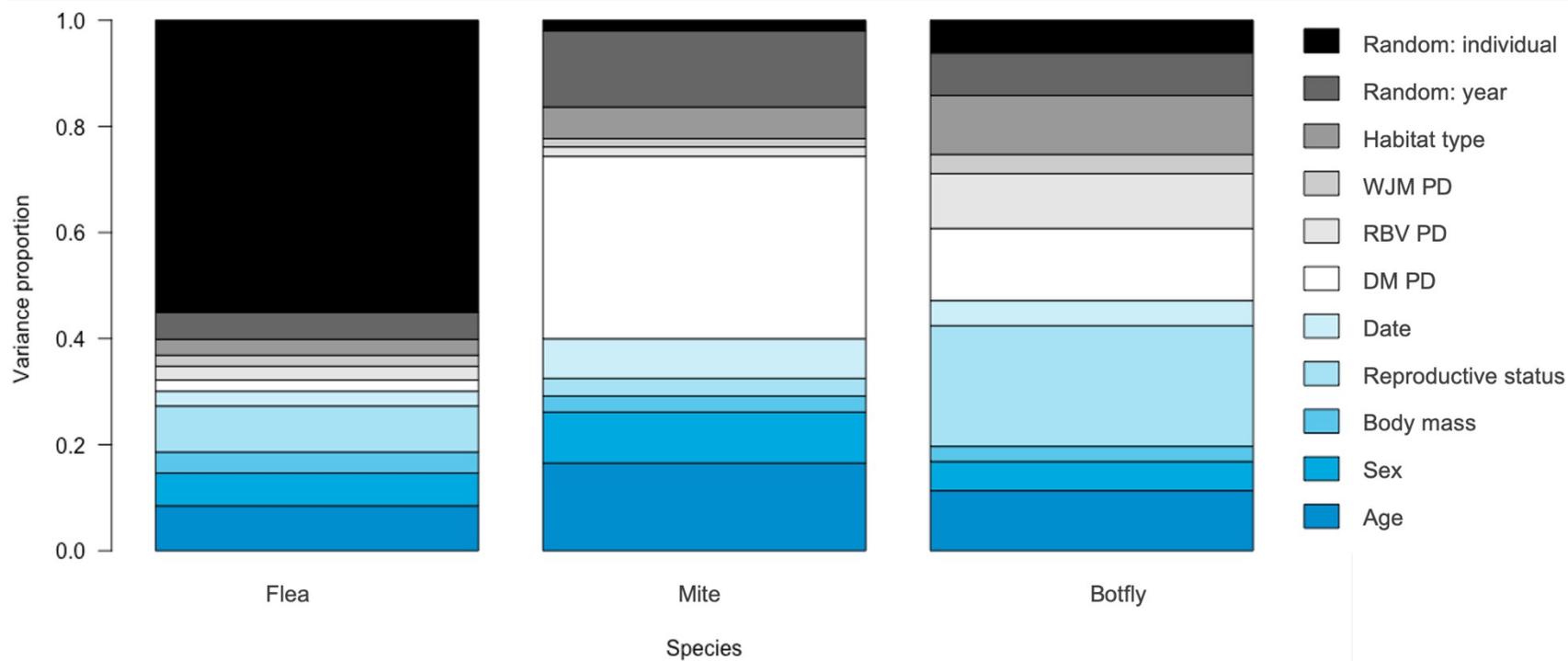


Figure 3.5 Results of variance partitioning for variation in ectoparasite prevalence explained by fixed and random effects for each ectoparasite species (columns). Explained variance presented for the constrained model with the full dataset for deer mice ( $n = 229$  individuals). PD represents index of population density; WJM, woodland jumping mice; DM, deer mice; RBV, southern red-backed vole.

Table 3.6 Relationship between prevalence of flea (*Orchopeas leucopus*), mite (*Neotrombicula microti*) or botfly (*Cuterebra* sp.) on deer mice and influential fixed effects (host age, sex, reproductive status) (n = 229 individuals).

Response variable	Covariate	$\beta$	SE	<i>p</i>
Flea prevalence	Age <sub>Adult</sub>			0.01
	Juvenile	0.349	0.720	
	Subadult	-1.354	0.621	
	Sex <sub>M</sub>	1.234	1.137	0.26
	Reproductive status	-0.064	1.017	0.95
Mite prevalence	Age <sub>Adult</sub>			0.75
	Juvenile	0.229	0.404	
	Subadult	0.040	0.260	
	Sex <sub>M</sub>	-0.277	0.448	0.54
Botfly prevalence	Age <sub>Adult</sub>			0.53
	Juvenile	-1.172	1.778	
	Subadult	1.049	1.152	
	Reproductive status	-0.992	2.926	0.73

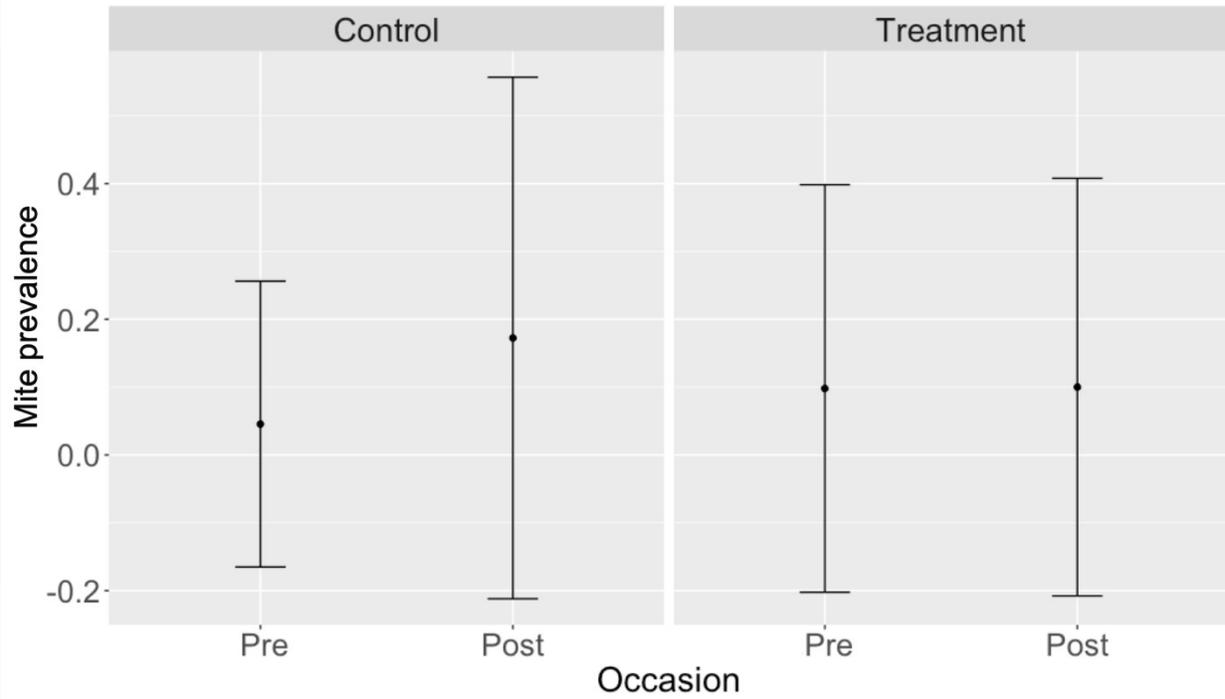


Figure 3.6 Effect of experimental removal of fleas (treatment) on mean mite prevalence in a deer mouse population (n = 229 individuals). For the control group, pre- and post-treatment represents a ~two-week period. For the treatment group, pre-treatment represents before experimental removal of the flea (*Orchopeas leucopus*) and post-treatment represents hosts sampled ~two weeks after experimental removal of fleas. Error bars denote standard error.

### 3.7 Supplemental Information Appendix

Table 3.7 Model performance presented as Tjur's  $R^2$  and area under the receiver operating characteristic (AUC) for all models of deer mice (n = 229 individuals) and North American red squirrels (n = 56 individuals).

Host	Model	Parasite	AUC	Tjur $R^2$
Deer Mouse ( <i>Peromyscus maniculatus</i> )	Full Constrained	Flea	0.5989	0.0222
		Mite	0.8159	0.2468
		Botfly	0.8327	0.0608
	Full Unconstrained	Flea	0.6146	0.0154
		Mite	0.6885	0.0546
		Botfly	0.6842	0.0071
	Subsetted Constrained	Flea	0.8235	0.2882
		Mite	0.8552	0.3818
		Botfly	0.7755	0.1954
	Subsetted Unconstrained	Flea	0.7764	0.1641
		Mite	0.8733	0.3210
		Botfly	0.7245	0.0560
North American Red Squirrel ( <i>Tamiasciurus hudsonicus</i> )	Full Constrained	<i>Orchopeas</i> flea	0.7300	0.0875
		<i>Ceratophyllus</i> flea	0.7016	0.0851
		Mite	0.9363	0.2101
	Full Unconstrained	<i>Orchopeas</i> flea	0.5206	-0.0019
		<i>Ceratophyllus</i> flea	0.5087	-0.0004
		Mite	0.6017	-0.0050
	Subsetted Constrained	<i>Orchopeas</i> flea	0.8575	0.3261
		<i>Ceratophyllus</i> flea	0.6298	0.0387
		Mite	0.9545	0.4810
	Subsetted Unconstrained	<i>Orchopeas</i> flea	0.5983	0.0085
		<i>Ceratophyllus</i> flea	0.6369	-0.0629
		Mite	0.6468	-0.0467

## General Discussion

Parasites represent an immense proportion of worldwide biodiversity, with ~40% of all known species being parasitic (Poulin & Morand 2005; Dobson et al. 2008). Additionally, parasites play a considerable role in maintaining ecosystem stability by increasing connectedness and nestedness (Lafferty et al. 2006). Therefore, research has begun to recognize the importance of parasites in natural ecosystems. While many studies have focused on single host and parasite systems, hosts are typically infested by multiple parasite species (Cox 2001). The predominant objectives of this thesis were to identify 1) determinants of ectoparasite prevalence on deer mice (*Peromyscus maniculatus*), southern red-backed voles (*Myodes gapperi*) and woodland jumping mice (*Napaeozapus insignis*); 2) how deer mice respond to ectoparasite infestations through glucocorticoid production, and; 3) whether ectoparasites of deer mice and North American red squirrels (*Tamiasciurus hudsonicus*) form random or structured assemblages.

### Determinants of ectoparasite prevalence

Prevalence of the mite species (*Neotrombicula microti*) varied with combined population density of deer mice (*Peromyscus maniculatus*), southern red-backed voles (*Myodes gapperi*), and woodland jumping mice (*Napaeozapus insignis*), population density of deer mice only, host reproductive status and date. Prevalence of the flea species (*Orchopeas leucopus*) varied with host age, sex, and body mass, while flea intensity varied with deer mouse population density and body mass. Prevalence of the botfly species (*Cuterebra* sp.) varied with deer mouse population density, reproductive status, age, and date. Lastly, date was the sole factor that had a relationship with ectoparasite species richness.

Combined population density of deer mice, red-backed voles, and woodland jumping mice positively correlated with mite prevalence on deer mice and negatively correlated with mite prevalence on red-backed voles. Red-backed voles likely act as a primary host for mites, initially becoming infested when combined population density of small mammals is low. Both the mite and vole species rely on decaying logs as an important habitat component (Wrenn 1974; Craig et al. 2014), which may lead to initial attachment of mites onto voles. On the other hand, at higher combined small mammal population densities, there are more opportunities for parasite transmission. Deer mice, a more abundant small mammal species, may be easier targets for mites or may represent more suitable habitat (Krasnov et al. 2003; Rigaud et al. 2010).

There was also a negative relationship between mite prevalence and deer mouse population density index. Rate of mite reproduction and establishment may be too low to keep up with deer mouse reproduction and dispersal, leading to lower mite prevalence when a deer mouse population is large (Stanko et al. 2006). However, the mite species may also regulate deer mouse population density. Parasites can negatively affect host reproduction and/or survival, leading to reductions in a host population (Møller 2005). This host-parasite system may demonstrate apparent competition, where a red-backed vole population (no relationship between population density and mite prevalence) transmits the mite to deer mice (negative relationship between population density and mite prevalence) (Hudson & Greenman 1998). Therefore, the mite species may play a key role in structuring a small mammal community.

#### Ectoparasites and fecal glucocorticoid metabolites

Deer mice demonstrated no significant change in glucocorticoid production in response to ectoparasite prevalence, intensity, species richness, or experimental removal of a main ectoparasite species, a flea (*Orchopeas leucopus*). There was also no relationship between

ectoparasite infestations and intra-individual variation in glucocorticoid concentrations. One explanation is that deer mice may have developed tolerance mechanisms against ectoparasites, which would minimize negative effects without needing to increase glucocorticoid production (Graham et al. 2012). Another explanation is that host susceptibility to ectoparasites is influenced by glucocorticoid levels rather than the other way around (St. Juliana et al. 2014). Overall, this suggests that ectoparasites do not induce a change in glucocorticoid levels of deer mice.

While there was no relationship between ectoparasites and deer mice glucocorticoid concentrations, there was a significant and negative relationship between date and glucocorticoids. The time period of this study extended from early in the breeding season to the end of the breeding season. Therefore, adverse events associated with breeding such as aggression, territory defence, and energetic costs of reproduction may lead to an increase in glucocorticoid production during the breeding season (Petticrew & Sadleir 1974; Fairbairn 1977; Romero 2002). Directed examination of changes in glucocorticoid levels of deer mice during breeding and non-breeding seasons would illuminate the relationship between glucocorticoid production and adverse stressors associated with reproduction. Overall, negative stimuli associated with the breeding season, rather than ectoparasites, may have influenced deer mouse glucocorticoid levels.

#### Ectoparasite species co-occurrence patterns

Ectoparasites of North America red squirrels (*Tamiasciurus hudsonicus*) exhibited a random assemblage, while ectoparasites of deer mice exhibited a structured assemblage. Specifically, the mite species (*Neotrombicula microti*) and botfly species (*Cuterebra* sp.) on deer mice exhibited a negative species interaction with one another. Furthermore, the flea species (*Orchopeas*

*leucopus*) had a negative association with the mite species and positive association with the botfly species. These associations were likely due to habitat requirements and variation in parasite infestation patterns due to host characteristics. The absence of many species interactions between ectoparasites on both deer mice and red squirrels may promote ecosystem stability, as small perturbations are less likely to have critical impacts (Pedersen & Fenton 2007). However, negative species interactions between the mite and botfly can help to inform disease management strategies, as removal of one species could lead to an increase in the other (Telfer et al. 2010).

#### Future directions

While this thesis has uncovered a lot of questions about the ecology of ectoparasites on small mammals, there is still more to investigate. For instance, I identified a potential case of apparent competition mediated by a mite species on southern red-backed voles and deer mice. Since data in this thesis are observational, perturbation experiments are required to identify deer mouse population regulation and apparent competition. Apparent competition can be a structuring force in natural communities and can potentially lead to local extinctions of wildlife populations (Tompkins et al. 2000). Future research should aim to identify whether there is apparent competition in this host-parasite system.

I also observed a lack of a relationship between ectoparasites and deer mice glucocorticoid levels. Conversely, chronic elevation of glucocorticoids can increase host susceptibility to parasites through immunosuppressive effects (St. Juliana et al. 2014). Changes in deer mice glucocorticoid concentrations may not be observed by removing fleas if this is the case. However, I was also unable to account for nest ectoparasites that spend the majority of their time in burrows rather than a host's body. Inclusion of nest ectoparasites through use of nest boxes could further our understanding on the relationship between ectoparasites and host

glucocorticoid production. While measures of host body infestation of fleas were a reliable indicator of nest fleas on California voles (*Microtus californicus*) (Krasnov et al. 2004), nest ectoparasites of deer mice have not yet been examined. Therefore, further experimentation should focus on the effect of glucocorticoids on ectoparasite susceptibility as well as the inclusion of nest ectoparasites.

Lastly, I identified a negative species interaction between a mite and botfly species. Targeted removal of one of these parasite species would be required to test whether this is a true species interaction. Parasites are an integral part of ecological communities and management efforts can run into “ecological surprises” when parasite interactions are not accounted for (Doak et al. 2008; Hellard et al. 2015). It is therefore important to study parasite species community ecology to make informed management decisions (Pedersen & Fenton 2007).

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