Ixodid Tick Effects on Deer Mice (*Peromyscus maniculatus)* Hematology and Ectoparasite Community Assemblages Across Populations of Varying Tick Exposure

by

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General Abstract

The ranges of the blacklegged tick (*Ixodes scapularis*) and the wood tick (*Dermacentor variabilis*) are expanding northward in Ontario, Canada in response to climate warming, reaching naïve or inexperienced host populations of deer mice (*Peromyscus maniculatus*) in more northern areas. As hematophagous parasites, ticks can affect their host's hematology and differing levels of hemoglobin in mice populations where tick exposure varies, may be due to the difference in exposure to ticks. If a naïve mouse population does not cohabitate with an established tick population, the mice should then have higher hemoglobin levels since they are not being affected by ticks. The parasite community structures of deer mouse hosts should also differ when ticks are prevalent at varying exposure levels, as the prevalence of these ticks is expected to decrease the likelihood of other ectoparasite species co-occurring with them on the host. Blood samples were collected from individual mice from populations where: 1) both blacklegged ticks and wood ticks were prevalent, 2) only wood ticks were prevalent, or 3) where both tick species were absent in order to assess hemoglobin levels. Ectoparasites were collected from these same mice to determine parasite loads and species co-occurrences. Both my hypotheses were supported as hemoglobin levels were found to be higher in naïve mice compared to infested mice, particularly those with high tick infestations, and heterospecific parasite prevalence appeared to be higher when ticks were absent. As the ticks' ranges expand, it is important to understand the differences between naïve and experienced hosts when the prevalence of these ticks can potentially alter the physiology and community assemblages of naïve mice.

Key Words: Community ecology, hematology, naïve hosts, parasite-host interactions, tick range expansion

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Table of Contents

List of Figures

General Introduction

Chapter Two

List of Tables

General Introduction

Host-parasite interactions are dynamic, as each participant influences the survival of the other. Parasites are dependent on their hosts for food and shelter and can be categorized as internal (endoparasites) or external (ectoparasites) to the host (Dougherty *et al*., 2015). Some ectoparasites are hematophagous, whereby they negatively impact their host via blood-feeding (Bordes & Morand, 2011; Godinho *et al*., 2013). Although this can reduce the host's fitness and can lead to host fatality, it also indirectly aids in trophic regulation and provides carrion for scavenger species (Dougherty *et al*., 2015). When a parasite is present, the effect on the host's physiology can impact reproductive success, as less energy can be allocated to reproduction, so the number of offspring that can be produced is reduced (Fitze *et al*., 2004). Hosts of parasites may be more susceptible to pathogenic infections (Mabbott, 2018), and may need to alter their behaviour or physiology to cope with multiple parasite infections (Thompson & Kavaliers, 1994). These energetic demands associated with parasitism can drive hosts to increase their movements, whereby the parasite manipulates the host's behaviour to favour their transmission to other potential hosts (Poulin, 2010; Thomas *et al*., 2010; Binning *et al*., 2017). At the same time, hosts may decrease their movements as an adaptive strategy to conserve energy (Binning *et al*., 2014; Binning *et al*., 2017).

Peromyscus mice are hosts to several ecto- and endoparasites, including ticks (Gaitan & Millien, 2016), fleas (Krasnov *et al*., 2002), mites (Rynkiewicz *et al*., 2013), botflies (Wolf & Baltzi, 2001), and internal nematodes (Pedersen & Greives, 2008). The parasite they host that is arguably of the greatest public health concern in North America is the blacklegged tick (*Ixodes scapularis*), which can transmit numerous zoonotic pathogens to humans (Kocan *et al*., 2015). Despite the fact that the blacklegged tick has approximately 125 different host species that it can

feed from (Keirans *et al*., 1996; Halsey *et al*., 2018), *Peromyscus* mice play an essential role as a reservoir host of larval and nymph ticks (Ostfeld *et al*., 1996). Due to their dispersal and colonizing abilities (Cummings & Vessey, 1994), *Peromyscus* mice promote the establishment of tick populations as they expand their own geographical ranges at a rate of approximately 15 km a year (Myers *et al*., 2009). *Peromyscus* mice contract and carry the spirochaete *Borrelia burgdorferi* (the causal agent of Lyme disease) from infected blacklegged ticks and transmit the bacteria to initially uninfected blacklegged ticks when the parasites feed from them (Ostfeld *et al*., 1996). Other tick species, such as the wood tick (*Dermacentor variabilis*) also use *Peromyscus* as a host (Minigan *et al*, 2018). Although it has been suggested that larval ticks do not affect the survival of *Peromyscus* mice (Hersh *et al*., 2014), physiological metrics that may be affected by tick blood-feeding, such as blood loss and iron deficiency, have not been examined for this genus.

The blood consumed by ticks contains iron-rich hemoglobin (Toh *et al*., 2010; Pishchany & Skaar, 2012). Hemoglobin is important for oxygen transport, aiding in motor skills and immune response (Nicolas e*t al*., 2002). Little is known about the frequency of iron deficiency in wild populations of mice, although many studies have been conducted on mice in a laboratory setting with applications for human-related iron deficiency (Baggs & Miller, 1973; Nicolas *et al*., 2002; Grant *et al.*, 2003). Baggs & Miller (1973) showed that when rats were iron deficient, they were more susceptible to bacterial infections. This pattern may also exist in natural populations that experience iron deficiency – perhaps due to the blood-feeding habits of ticks. Ticks typically feed from mice at the larval or nymph stage, and then move on to larger hosts once they mature (Estrada-Peña & de la Fuente, 2014). The cost of parasitism may be particularly high during periods that are energetically costly to mice (pregnancy, lactation), leading to insufficient

immune responses to tick infestations that cause the mice to be vulnerable to other infections (Rosales *et al*., 1999; Dlugosz *et al*., 2014). In a study comparing cattle (*Bos taurus*) calves infested with cattle ticks (*Rhipicephalus microplus*) and non-infested calves, there was a significant reduction in hematological parameters, including hemoglobin, leucocytes, and erythrocytes (Kaur *et al*., 2017). Heavy parasite loads may be related to significant effects on host hematology. In several studies on avian hosts, hematocrit decreased as parasite loads increased (Møller, 1991; Whitworth & Bennet 1992; Hurtrez *et al*., 1997). Although few experiments of this nature have been conducted on small mammals in the wild, Pfäffle *et al*. (2009) found that tick feeding on European hedgehogs (*Erinaceus europaeus*) reduced blood parameters including hemoglobin, hematocrit, and erythrocytes. Results may be similar in deer mice (*P. maniculatus*) whereby infested individuals have reduced blood parameters, and those infested with greater parasite loads have further reductions to their hematological condition.

Several studies have been conducted on how tick prevalence may affect the survival of their hosts, but physiological metrics, such as hemoglobin levels, are rarely considered. Similarly, despite many studies conducted on birds (Wheeler & Threlfall, 1986; Mallory *et al.*, 2006), mammals (Thompson *et al.,* 1998; Galloway, 2012; Webber *et al*., 2014), and fish (Poulin, 1991; Poulin & Guegan, 2000) comparing differences in host populations where ectoparasite prevalence and intensity differ, there are few, if any, studies on mouse populations. *Peromyscus* mice are common hosts of several zoonotic pathogens that are transmitted through ticks, including *Borrelia burgdorferi, B. miyamotoi* (Talagrand-Reboul *et al*., 2018), *B. bissettii* (DeNatale *et al*., 2002), *Babesia microti* (Westblade *et al*., 2017), and Powassan virus (Mlera *et al*., 2018). Therefore, recognizing how *Peromyscus* hosts are physiologically affected by blood-feeding may help to make better predictions on how a host's mobility is affected by a parasite.

This provides a more accurate prediction on the ecology of the host (*i.e.*, range expansion, dispersal, mating; Binning *et al*., 2017), which can potentially help track the transmission of pathogens they carry (Salkeld *et al*., 2013).

Ectoparasites such as fleas (Insecta: Siphonaptera) or hematophagous mites (Acari: Mesostigmata) can co-exist with ticks (Rynkiewicz, et al., 2013; Gómez-Rodríguez *et al*., 2015). Since these ectoparasites are also blood feeders, they tend to prefer areas of exposed skin on their hosts as their micro-habitat niche (Anderson *et al*., 2017), such as the ears (Ostfeld *et al*., 1993; Bobbie *et al*., 2016), which can lead to competition between species (Baer-Lehman *et al.*, 2012). Micro-habitat partitioning on a host (whereby the host is the habitat) can occur, particularly when species richness is high in an ectoparasite community and several species fit into the same niche (Gómez-Rodríguez *et al*., 2015). However, if one species consistently outcompetes another in the micro-habitat on a host (*i.e.*, in an area both ectoparasites prefer), then prevalence or intensity differences between species may occur (Baer-Lehman *et al.*, 2012). These differences can then affect parasite species diversity on a host, which in turn can alter the effects of parasitism on the ecology and evolution of a host (Bordes & Morand , 2011).

Differences in macro-habitats or landscape ecosystems can affect the diversity of ectoparasites (Gómez-Rodríguez *et al*., 2015). Specific geography and associated ecological factors have been shown to influence inter-individual variation in the microbiota of wild mice (Weldon *et al*., 2015), and can potentially affect tick microbiota as well (Clow *et al*., 2018). Latitudinal differences in parasite species richness and community structure have been studied in fish (Rohde & Heap, 1997), primates (Nunn *et al*., 2005), and rodents (Bordes *et al*., 2011; Preisser, 2019), where there is a trend in increasing parasite species richness with decreasing latitudes. It is also likely that there are systematic differences in genetics, habitat, and infection

status between host population sites that can affect parasite loads (Weldon *et al*., 2015; Carbayo *et al*., 2019). This can in turn affect interspecific competition between ectoparasite species, whereby one parasite reduces the host's susceptibility to another co-infecting parasite (Ashby & King, 2017). Ectoparasites are also able to partition their host's body, allowing for these species to co-exist across micro-habitat niches (Gómez-Rodríguez *et al*., 2015). The influence of ticks on ectoparasite community assemblages has been widely studied and patterns of species co-occurrences tend to vary across host species depending on their habitat and ecology (Krasnov *et al*., 2007; Krasnov *et al*., 2009; Lareschi & Krasnov, 2010; Esser *et al*., 2016; Krasnov *et al*., 2020). It is important to understand how differences in macro and micro-habitats can affect parasite-host relationships when examining topics that may be affected by these parameters.

The objective of this study is to determine differences in hemoglobin levels and ectoparasite community assemblages of deer mice populations when individuals are exposed to varying established levels of two tick species; blacklegged ticks and wood ticks. Establishment levels differ by lengths of time the ticks have been established in an area, as well as the number of tick species established there. In tick established areas, deer mice have increased their grooming efficiency to counteract tick infestations (Ostfeld *et al*., 1996; Shaw *et al*., 2003; Hersh *et al*., 2014), however the extent of a tick's hematological impact on *Peromyscus* hosts is still unknown. This includes differences between mice that inhabit an area where ticks have long established and less experienced mice that have not cohabited with ticks for very long – or at all. Additionally, interactions between ticks and other ectoparasites that may affect the host's hematological composition have also not been well studied. Therefore, I hypothesized that if mice are parasitized by a greater intensity of ticks, then they will have lower hemoglobin levels compared to mice infested with ticks at low intensities. Concurrently, if a naïve mouse

population has not cohabitated with an established tick population, then this mouse population should have higher hemoglobin levels relative to mice that are cohabiting with ticks since they are not afflicted by the effects of blood-feeding by these species.

The parasite community structures of the *Peromyscus* hosts may also differ when blacklegged ticks or wood ticks are prevalent, as the prevalence of these ticks are predicted to lead to a competitive relationship with other co-occurring parasites (Baer-Lehman *et al*., 2012). This may result in a decrease in the abundance of other ectoparasite species co-occurring on the host, especially those species that congregate in the same areas on the mouse's body - *i.e.* the ears, where ticks (Ostfeld *et al*., 1993) and mites (Bobbie *et al*., 2016) are commonly found. I predicted that if ticks are prevalent in an ectoparasite community, then there will be fewer other ectoparasites since ticks will exclude them from the micro-habitat (the host), as they tend to prefer to feed from the same exposed areas, such as the ears. I predicted that mice parasitized by ticks would have fewer other ectoparasite species on them, as the ticks are larger and require larger blood meals (McKenzie, 1998), suggesting they are more competitive than the other common ectoparasite species found on North American small mammals that share their micro-habitat niches on the host (*i.e*. mites on ears; Bobbie *et al*., 2016).

Study Host Species: *Peromyscus maniculatus* (deer mouse)

The deer mouse is one of the widest ranging mice in North America (Figure 0.1; Bedford and Hoekstra, 2015; Leo & Milien, 2017). This mouse tends to inhabit conifer and deciduous forests alike, as well as grasslands (Garcia-Elfring *et al*., 2017). Their diets mainly consist of seeds and nuts (Cramer, 2014). Female deer mice have been documented nesting alone or in kin groups on occasion (Wolff, 1994), although parental care is exclusively maternal (Wolff, 1985).

It is during encounters with conspecifics in nests that *Peromyscus* mice typically transfer ectoparasites (such as fleas, lice, mites; Mize *et al*., 2011; Maaz *et al*., 2018) from one individual to another (Glicken & Schwab, 1980), however, ticks rely on one host per life stage. Ticks will emerge from the grass or forest leaf litter to make contact with mice rather than opportunistically jumping from one host to the next (Arsnoe *et al*., 2015).

Study Parasite Species: *Ixodes scapularis* (blacklegged tick) and *Dermacentor variabilis* (wood tick)

Hard bodied ticks (Acari: Ixodidae) are obligate parasites that rely on vertebrate hosts for blood meals to complete the three stages in their life cycles (Figure 0.2; Brunner *et al*., 2011; Kada *et* al., 2017; Halsey & Miller, 2018). Several millilitres of blood are taken from the host by the tick (Brunner *et al*., 2011), which will typically feed for three to six days as a larva or nymph (Estrada-Peña & de la Fuente, 2014). Digestion of blood starts hours after feeding, and can take several months, until moulting is complete (Estrada-Peña & de la Fuente, 2014). Since they are small and wingless, ticks rely on their hosts to move within and between habitats or to expand their geographical ranges. Therefore, ticks are constrained by the geographic barriers imposed on their hosts (Falco & Fish, 1991; Estrada-Peña & de la Fuente, 2014; Kada *et al*., 2017). Northward migrating birds aid blacklegged ticks in expanding their geographic ranges during the spring (Scott *et al*., 2012; Khatchikian *et al*., 2015). Some bird migrations can carry ticks to northern Ontario (including arctic ecoregions), beyond suitable living conditions for ticks (Ogden *et al*., 2015). However, it is possible that as the climate warms, these areas will become adequate habitat and the ticks' range will further expand. The rate of range expansion for blacklegged ticks has been predicted to be approximately 46 km/year, with the rate of tick

colonization being slower and more variable (Leighton *et al*., 2012; Clow *et al*., 2017). The majority of the southern Ontario landscape is composed of deciduous ecosystems, and therefore suitable habitat for blacklegged ticks (Ogden *et al.*, 2006). As mean temperatures increase due to climate change, more of northern Ontario's terrain will become similar to the southern landscape and will be suitable for this species to inhabit (Ogden *et al.*, 2006; Leighton *et al*., 2012). Leighton *et al*. (2012) suggested that by 2030 blacklegged tick populations will be established across Ontario, exceeding beyond the current geographical range (Public Health Ontario 2019; Figure 0.3). This expansion will potentially lead to new interactions between blacklegged ticks and populations of deer mice that have never interacted with them before (*i.e.* naïve to this interaction), leading to the spread of multiple infections that can be transmitted by these ticks.

The wood tick (*Dermacentor variabilis*) is another medically important species abundantly found in brush field and forest habitats across the eastern United States and in several regions in Canada, including southern Ontario (Sonenshine, 2018; Minigan *et al*., 2018). There are many ecological similarities between blacklegged ticks and wood ticks. *Peromyscus* mice are fundamental for the development of the larval and nymph stages of this species (Sonenshine, 2018), much like they are for blacklegged ticks (Ostfeld *et al*., 1996; Estrada-Peña & de la Fuente, 2014). They have been found in similar habitats (Morshed *et al*., 2003; J Curtis & E Fellin, pers. obs.), and are active at similar times of year (between May and September; blacklegged ticks; Ferreri *et al*., 2014; wood ticks; Minigan *et al*., 2018), although wood ticks have been found in areas farther north than blacklegged ticks (Figure 0.3: Public Health Ontario, 2019; Figure 0.4: Minigan *et al.*, 2018). The wood tick's range is also expanding with climate change and is expected to have a northwards expansion as more habitat becomes suitable (Minigan *et al*., 2018). Wood ticks are the primary vector for the bacterium *Rickettsia rickettsii*,

which can cause Rocky Mountain spotted fever in humans and domestic animals, although it is also capable of transmitting *Coxiella burnetii* and *Francisella tularensis* at extremely low frequencies in Ontario (Wood *et al*., 2016). It is expected, however, that as this tick's range expands, their population dynamics will change, altering the exposure of the pathogens they carry to humans and other animals (Minigan *et al*., 2018).

Study Sites

The sites chosen for this research overlap with the progression of the geographic expansion of blacklegged ticks and wood ticks into Ontario from both its southern and eastern populations (Hamer *et al.*, 2014; Clow *et al*., 2016; 2017; Minigan *et al*., 2018), and are based off of range expansion predictions of Leighton *et al*. (2012), Ogden *et al*. (2013) and Minigan *et al*. (2018; Figure 0.4). Three different sites in Ontario were visited for data and specimen collection. Each specific location was chosen to encompass the different levels of tick establishment. The most southern site (Long Point Provincial Park) has well established populations of blacklegged ticks and wood ticks (Watson & Anderson, 1976; Lindsay *et al*., 1991). The other sites (Queen Elizabeth II Wildlands Provincial Park and Algonquin Provincial Park) are areas with no established populations of blacklegged ticks, although Queen Elizabeth II Wildlands Provincial Park is geographically close to Orillia, Ontario, which is a documented area where blacklegged ticks are prevalent (Public Health Ontario, 2019), and has known established American dog or wood tick (*Dermacentor variabilis*) populations (Minigan *et al.*, 2018; P. Careless, 2019, pers. comm.). All three areas are within the geographical habitat range of deer mice (Figure 0.1: Bedford and Hoekstra, 2015). I travelled from the most southern field

site (Long Point Provincial Park) northwards towards Algonquin Provincial Park, up a latitudinal gradient (Figure 0.5).

Long Point Provincial Park (LP; 42.5817° N, -80.3952° W) is a small park, covering a land area of just 1.50 km^2 and located in Port Rowan, Ontario (Long Point, 2019). LP hosts the oldest known established blacklegged tick population in Ontario (Watson & Anderson, 1976). The study site in Long Point was approximately 1.11 km^2 (Figure 0.5), encapsulating the forest space of the entire park. It should be noted that Long Point has been faced with seasonal flooding for several decades (Rasid *et al*., 1992), and during this field season parts of the forest floor were submerged in water, providing a higher *Peromyscus* population density in the small land area than expected.

Queen Elizabeth II Wildlands Provincial Park (QEW; 44.7534° N, -78.7844° W) is located in Kawartha Lakes, near Orillia, Ontario. It is 335.05 km^2 (Queen Elizabeth II Wildlands, 2019). Leighton *et al*. (2012) predicted this area to have blacklegged tick populations establish by 2020. As of 2019, Orillia is the northernmost area in southwestern Ontario where there is a risk of Lyme's disease due to blacklegged tick presence (Public Health Ontario, 2019). Despite a large land area size, a small sample area in the southern region was used as a study site, similar to Long Point (Figure 0.5). Although blacklegged ticks are not prevalent here, this site offers a unique circumstance where there is a high prevalence of wood ticks (P. Careless, 2019, pers. comm.).

Algonquin Provincial Park (AP; 45.3402° N, -78.2618° W) is located between Georgian Bay and the Otter River in Ontario. Algonquin is Ontario's largest green space, bridging the gap between Southern and Northern Ontario, where ticks are expected to establish populations by 2022 (Leighton *et al*., 2012). Currently, there are no documented sightings of either wood ticks

or blacklegged ticks in Algonquin, and so the Algonquin population of deer mice acted as a control group. The land area of AP is 7723 km^2 (Algonquin, 2019), however, I conducted my research in a smaller concentrated area at the Algonquin Wildlife Research Station (WRS). There are established traplines set up at the WRS for the long-term small mammal study that I used to collect data and specimens (Falls, 1953; Fryxell *et al.* 1998; Falls *et al*., 2007; Bobbie *et al*., 2016). During my data collection, these traplines were exclusively used for my study, despite typically being included in the long-term study. However, no mice found at this site were ear-tagged from previous studies, indicating these were all new individuals, avoiding any potential bias in capture effort or sampling as these mice had not been handled before. The ectoparasite communities on these mice were used as a comparison to determine any differences in parasite community assemblage when ticks are present or absent. The traplines here were set farther apart from each other than the other field sites (Figure 0.5), but were all 90 m long, so the overall trapping effort was the same.

Although it is not entirely clear when wood ticks initially established in LP or QEW, wood ticks have been found in Ontario "south of 44 degrees latitude" since the 1960s (Wilkinson, 1967), and the first documentation (according to Public Health Ontario) of wood ticks in LP was by Barker *et al*. (1988). Wood ticks have also been considered a common species in Canada since 1967, whereas blacklegged ticks were considered non-endemic at this time (Scholten, 1977), with their first establishment being documented in LP in 1976 (Watson & Anderson, 1976). There is no published data of the first record of wood ticks in QEW, although they are prevalent. Blacklegged ticks were also expected to be in this area (Leighton *et al*., 2012), although none were found in this study. It can be concluded, however, that wood ticks established in LP before QEW, due to the northward expansion of both species (Clow *et al*.,

2017; Minigan *et al*., 2018). While other tick species, such as the squirrel tick (*Ixodes angustus*), have been found in AP at low abundance and prevalence, neither blacklegged ticks nor wood ticks have established this far north.

It is also important to note the difference in habitat between the northern and southern sites. Southern sites are deciduous, and the landscape composition gradually changes to coniferous, moving northwards (Ontario Biodiversity Council, 2015). In the south, the forested areas where tick populations are established tend to be smaller provincial or national parks, between dense urban settlements. In northern Ontario, there tend to be larger landscapes dedicated as conservation territories, allowing for greater unfragmented areas where tick hosts can move around more easily. In general, the prevalence of blacklegged ticks has been shown to be greater in larger land fragments compared to small areas (Wilder & Meikle, 2004). Although all sites are a part of different ecoregions with somewhat differing habitats (Figure 0.6), LP and QEW are from the same ecozone (Mixedwood Plains). Similarly, despite AP being in the Canadian Shield ecozone, this site is in the south of this ecozone, and close in proximity to QEW. Although there are differences in habitats across these sites, the flora is similar, having mixed forests of coniferous and deciduous trees (Crins *et al*., 2009). Trap sites were set in areas of mixed wood forests at each location, having both deciduous and coniferous trees, as well as shrubs, ferns, and both dense and sparse canopy cover in order to have as similar habitat as possible across sites that differed in ecozones.

Literature Cited:

Algonquin. (2019). *Ontario Parks*. Retrieved from https://www.ontarioparks.com/park/algonquin

Anderson, J, Moore, I, Nagata, B, Ribeiro, J, Valenzuela, J, Sonenshine, D. (2017). Ticks, *Ixodes scapularis*, Feed Repeatedly on White-Footed Mice despite Strong Inflammatory Response: An Expanding Paradigm for Understanding Tick-Host Interactions. *Frontiers in. Immunology*.8(1784): 1-15. doi: 10.3389/fimmu.2017.01784

Ashby, B, King, KC. (2017). Friendly Foes: The Evolution of Host Protection by a Parasite. *Evolution Letters*. 1(4): 211-221. doi: 10.1002/evl3.19

Arsnoe, I, Hickling, G, Ginsberg, H, McElreath, R, Tsao, J. (2015). Different Populations of Blacklegged Tick Nymphs Exhibit Differences in Questing Behavior That Have Implications for Human Lyme Disease Risk. *PLoS ONE*. 10(5): e0127450. doi:10.1371/journal.pone.0127450

Baer-Lehman, M, Light, T, Fuller, N, Barry-Landis, K, Kindlin, C, Stewart Jr., R. (2012). Evidence for Competition Between *Ixodes scapularis* and *Dermacentor albipictus* Feeding Concurrently on White-Tailed Deer. *Exp. Appl. Acarol*. 58: 301-314. doi: 10.1007/s10493-012-9574-5

Baggs, RB, Miller, SA. (1973). Nutritional Iron Deficiency as a Determinant of Host Resistance in the Rat. *The Journal of Nutrition.* 103(11): 1554-1560.

Barker, IK, McEwen, SA, Surgeoner G. A, Artsob H. (1988). *Borrelia burgdorferi*, the Agent of Lyme Disease, in Tick Vectors and Wildlife Reservoirs in Southern Ontario. *Ont. Dis. Surv*. Rep. 9: 151–154.

Bedford, N, Hoekstra, H. (2015). Peromyscus Mice as a Model for Studying Natural Variation. *eLife*. 4, e06813. https://doi.org/10.7554/eLife.06813

Binning SA, Barnes JI, Davies JN, Backwell PRY, Keogh JS, Roche DG. (2014). Ectoparasites modify escape behaviour, but not performance, in a coral reef fish. *Anim. Behav.* 93:1–7. doi: 10.1016/j.anbehav.2014.04.010

Binning, S, Shaw, A, Roche, D. (2017). Parasites and Host Performance: Incorporating Infection into Our Understanding of Animal Movement. *Integrative and Comparative Biology.* 57(2): 267-280. doi: 10.1093/icb/icx024

Bitam, I, Dittmar, K, Parola, P, Whiting, M, Raoult, D. (2010). Fleas and Flea-borne Diseases.

Elsevier. 14(8): e667-e676. doi: 10.1016/j.ijid.2009.11.011

Bobbie, C, Schmidt, E, Foley, J, Schulte-Hostedde, A. (2016). The Presence of Parasitic Mites on Small Mammals in Algonquin Provincial Park, Ontario, Canada. *Can. J. Zool.* 95:61-65. doi: 10.1139/cjz-2016-0085

Bordes, F, Morand, S. (2011). The Impact of Multiple Infections on Wild Animal Hosts: A Review. *Infect. Ecol. Epidemiol.* 1: 7346. doi: 10.3402/iee.v1i0.7346

Bordes, F, Guégan, JF, Morand, S. (2011). Microparasite Species Richness in Rodents is Higher at Lower Latitudes and is Associated with Reduced Litter Size. *Oikos*. 120(12): 1889-1896. doi: 10.1111/j.1600-0706.2011.19314.x

Brunner, J, Cheney, L, Keesing, F, Killilea, M, Logiudice, K, Previtali, A, Ostfeld, R. (2011). Molting Success of *Ixodes scapularis* Varies Among Individual Blood Meal Hosts and Species. *J. Med. Entomol.* 48(4): 860-866. doi: 10.1603/ME10256

Buchholz, M, Dick, C. (2017). Ecology of Rodent - Ectoparasite Associations in South-Central Kentucky. *Northeastern Naturalist*. 24(2): 97-109. doi: 10.1656/045.024.0201

Carbayo, J, Martin, K, Civantos, E. (2019). Habitat Type Influences Parasite Load in Algerian Psammodromus (*Psammodromus algirus*) Lizards. *Canadian Journal of Zoology*. 97(2): 172-180. doi: 10.1139/cjz-2018-0145

Careless, P. (2019, May 30). Phone interview.

Clark, K, Durden, L. (2002). Parasitic Arthropods of Small Mammals in Mississippi. *Journal of Mammalogy*83(4): 1039-1048.

Clow, K, Ogden, N, Lindsay, L. R, Michel, P, Pearl, D, Jardine, C. (2016). Distribution of Ticks and the Risk of Lyme Disease and Other Tick-Borne Pathogens of Public Health Significance in Ontario, Canada. *Vector-Borne and Zoonotic Diseases.* doi: 10.1089/vbz.2015.1890

Clow, K, Leighton, P, Ogden, N, Lindsay, L. R, Michel, P, Pearl, D, Jardine, C. (2017). Northward Range Expansion of *Ixodes scapularis* Evident Over a Short Timescale in Ontario, Canada. *PLoS ONE.* 12(12): e0189393. doi: 10.1371/journal.pone.0189393

Clow, K, Weese, JS, Rousseau, J, Jardine, CM. (2018). Micorbiota of Field-collected *Ixodes scapularis* and *Dermacentor variabilis* from Eastern and Southern Ontario, Canada. *Ticks and Tick-borne Diseases*. 9(2): 235-244. doi: 10.1016/j.ttbdis.2017.09.009

Cramer, M. (2014). Seeds of Doubt: Feeding Preferences of White-Footed Deer Mice

(*Peromyscus leucopus noveboracensis*) and Woodland Deer Mice (*Peromyscus maniculatus gracilis*) on Maple (genus *Acer*) Seeds. *Can. J. Zool.* 92: 771-776.

Crins, W, Gray, P, Uhlig, P, Wester, M. (2009). The Ecosystems of Ontario, Part 1: Ecozones and Ecoregions. *Ministry of Natural Resources – Science & Information Branch.* Technical Report SIB TER IMA TR-01.

Cummings, J: Vessey, S. (1994). Agricultural Influences on Movement Patterns of White-Footed Mice (*Peromyscus leucopus*). *Am. Midl. Nat*. 132: 209–218.

DeNatale, C, Burkot, T, Schneider, B, Zeidner, N. (2002). Novel Potential Reservoirs for Borrelia sp. and the Agent of Human Granulocytic Ehrlichiosis in Colorado. *Journal of Wildlife Diseases*. 38(2): 478-482. doi: 10.7589/0090-3558-38.2.478

Dlugosz, E, Downs, C, Khokhlova, I, Degen, A, Krasnov, BR. (2014). Ectoparasite Performance When Feeding on Reproducing Mammalian Females: An Unexpected Decrease When on Pregnant Hosts. *The Journal of Experimental Biology.* 217: 1058-1064. doi:10.1242/jeb.098376

Dougherty, E, Carlson, C, Bueno, V, Burgio, K, Cizauskas, C, Clements, C, Seidel, D, Harris, N. (2015). Paradigms for Parasite Conservation. *Conservation Biology*. 30(4): 724-733. doi: 10.1111/cobi.12634

Durden, L, Polur, R, Nims, T, Banks, C, Oliver J. (2004). Ectoparasites and Other Epifaunistic Arthropods of Sympatric Cotton Mice and Golden Mice: Comparisons and Implications for Vector-Borne Zoonotic Diseases. *J. Parasitol*. 90(6): 1293-1297

Esser, HJ, Foley, JE, Bongers, F, Herre, EA, Miller, MJ, Prins, HHT, Jansen, PA. (2016). Host Body size and the Diversity of Tick Assemblages on Neotropical Vertebrates. *Int. J. Parasitol: Parasites and Wildlife.* 5(3): 295-304. doi: 10.1016/j.ijppaw.2016.10.001

Estrada-Peña, A, de la Fuente, J. (2014). The Ecology of Ticks and Epidemiology of Tick-Borne Viral Diseases. *Antiviral Research.* 108: 104-128. doi: 10.1016/j.antiviral.2014.05.016

Falco, R, Fish, D. (1991). Horizontal Movement of Adult *Ixodes dammini* (Acari, Ixodidae) Attracted to CO2- Baited Traps. *J Med Entomol.* 28(5): 726–729.

Falls, JB. (1953). Activity and Local Distribution of Deer Mice in Relation to Certain Environmental Factors. Ph.D. Thesis, Department of Zoology, University of Toronto 221 pp. APM Reprint # 5527.

Falls, JB, Falls, EA, Fryxell, J. (2007). Fluctuations of Deer Mice in Ontario in Relation to Seed

Crops. *Ecological Monographs.* 77(1): 19-32

Ferreri, L, Giacobini, M, Bajardi, P, Bertolotti, L, Bolzoni, L, Tagliapietra, V, Rizzoli, A, Rosa, R. (2014). Pattern of Tick Aggregation on Mice: Larger Than Expected Distribution Tail Enhances the Spread of Tick-Borne Pathogens. *PLoS Computational Biology.* 10(11): 1-12. doi: e1003931.

Fitze, P, Tschirren, B, Richner, H. (2004). Life History and Fitness Consequences of Ectoparasites. *J. Anim. Ecol.* 73(2): 216-226. doi: 10.1111/j.0021-8790.2004.00799.x

Fryxell, J, Falls, JB, Falls, EA, Brooks, R. (1998). Long-Term Dynamics of Small-Mammal Populations in Ontario. *Ecology*. 79(1): 213-225

Gaitan, J, Millien, V. (2016). Stress Level, Parasite Load, and Movement Pattern in a Small Mammal Reservoir Host for Lyme Disease. *Can. J. Zoology*. 94: 565–573. doi: 10.1139/cjz-2015-0225

Galloway, T. (2012). Ectoparasites of Rabbits and Hares in Manitoba, Canada, with Observations on Age-Specific Dispersal in *Haemodipsus setoni* (Phthiraptera: Anoplura: Polyplacidae). *The Canadian Entomologist*. 144(3): 439-446. doi: 10.4039/tce.2012.44

Garcia-Elfring, A, Barrett, RDH, Combs, M, Davies, TJ, Munshi-South, J, Millien, V. (2017). Admixture on the Northern Front: Population Genomics of Range Expansion in the White-Footed Mouse (*Peromyscus leucopus*) and Secondary Contact with the Deer Mouse (*Peromyscus maniculatus*). *Heredity.* 119: 447-458.

Glicken, A, Schwab, R. (1980). Modes of Ectoparasite Reinfestations of Deer Mice (*Peromyscus maniculatus*). *J. Wildlife Diseases.* 16(4): 577-586. doi: 10.7589/0090-3558-16.4.577

Gómez-Rodríguez, R, Gutiérrez-Granados, G, Montiel-Parra, G, Rodríguez-Moreno, A, Sánchez,-Cordero, V. (2015). Diversity and Coexistence of Ectoparasites in Small Rodents in a Tropical Dry Forest. *Biotropica*. 47(4): 484-490. doi: 10.1111/btp.12229

Godinho, L, Cripps, J, Coulson, G, Lumsden, L. (2013). The Effect of Ectoparasites on the Grooming Behaviour of Gould's Wattled Bat (*Chalinolobus gouldii*): An Experimental Study. *Acta Chiropterologica.* 15(2): 463-472. doi: 10.3161/150811013X679080

Grant, S, Wiesinger, J, Beard, J, Carntorna, M. (2003). Iron-Deficient Mice Fail to Develop Autoimmune Encephalomyelitis. *J. Nutrit.* 133(8): 2635-2638. doi: 10.1093/jn/133.8.2635

Halsey, S, Miller, J. (2018). A Spatial Agent-Based Model of the Disease Vector *Ixodes*

scapularis to explore Host-Tick Associations. *Ecological Modelling*. 387: 96-106. doi: 10.1016/j.ecolmodel.2018.09.005

Halsey, S, Allan, B, Miller, J. (2018). The Role of *Ixodes scapularis*, *Borrelia burgdorferi* and Wildlife Hosts in Lyme Disease Prevalence: A Quantitative Review. *Tick and Tick-Borne Diseases*. 9: 1103-1114. doi: 10.1016/j.ttbdis.2018.04.006

Hamer, S, Hickling, G, Walker, E. Tsao, J. (2014). Increased Diversity of Zoonotic Pathogens and *Borrelia burgdorferi* Strains in Established Versus Incipient *Ixodes scapularis* Populations Across the Midwestern United States. *Infection, Genetics and Evolution.* 27: 531-542. doi: 10.1016/j.meegid.2014.06.003

Hersh, M, LaDeau, S, Previtali, M., Andrea, Ostfeld, R. (2014). When is a Parasite not a Parasite? Effects of Larval Tick Burdens on White-Footed Mouse Survival. *Ecology*. 95(5): 1360-1369

Hurtrez-Bousses, S, Perret, P, Renaud, F, Blondel, J. (1997). High Blowfly Parasitic Loads Affect Breeding Success in a Mediterranean Population of Blue Tits. *Oecologia* 112(4): 514–517. doi: 10.1007/s004420050339

Jones, H, Pekins, P, Kantar, L, Sidor, I, Ellingwood, D, Lichtenwalner, A, O'Neal, M. (2019). Mortality Assessment of Moose (*Alces* alces) Calves During Successive Years of Winter Tick (*Dermacentor albipictus*) Epizootics in New Hampshire and Maine (USA). *Canadian Journal of Zoology*. 97(1): 22-30. doi: 10.1139/cjz-2018-0140

Kada, S, McCoy, K, Boulinier, T. (2017). Impact of Life Stage-Dependent Dispersal on the Colonization Dynamics of Host Patches by Ticks and Tick-Borne Infectious Agents. *Parasites & Vectors*. 10(1): 375. doi: 10.1186/s13071-017-2261-y

Kaur, D, Jaiswal, K, Mishra, S. (2017). Effect of Tick Infestation on Parameters of Calves. *J. Entomol. Zool. Studies.* 5(4): 107-111

Keirans, J, Hutcheson, HJ, Durden, L, Klompen, JSH. (1996). *Ixodes* (Ixodes) *scapularis* (Acari: Ixodidae): Redescription of all Active Stages, Distribution, Hosts, Geographical Variation, and Medical and Veterinary Importance. *J. Med. Entomol.* 33(3): 297-318. doi: 10.1093/jmedent/33.3.297

Khatchikian, C, Prusinski, M, Stone, M, Backenson, PB, Wang, I, Foley, E, Seifert, S, Levy, M,

Brisson, D. (2015). Recent and Rapid Population Growth and Range Expansion of the Lyme Disease Tick Vector, *Ixodes scapularis*, in North America. *Evolution*. 69(7): 1678-1689. doi: 10.1111/evo.12690

Krasnov, BR, Khokholva, I, Shenbrot, G. (2002). The Effect of Host Density on Ectoparasite Distribution: An Example of a Rodent Parasitized by Fleas. *Ecology*. 83(1): 164-175.

Krasnov, BR, Stanko, M, Morand, S. (2007). Host Community Structure and Infestation by Ixodid Ticks: Repeatability, Dilution Effect and Ecological Specialization. *Oecologia*. 154(1): 185-194. doi: 10.1007/s00442-007-0824-x

Krasnov, BR, Matthee, S, Lareschi, M, Korallo-Vinarskaya, NP, Vinarksi, MV. (2009). Co-occurrence of Ectoparasites On Rodent Hosts: Null Model Analyses of Data from Three Continents. *Oikos*. 119(1): 120-128. doi: 10.1111/j.1600-0706.2009.17902.x

Krasnov, BR, Stanko, M, Lareschi, M, Khokhlova, I. (2020). Species Co-Occurrences in Ectoparasite Infracommunities: Accounting for Confounding Factors Associated with Space, Time, and Host Community Composition. *Ecological Entomology.* 1-14 doi: 10.1111/een.12900

Lareschi, M, Krasnov, BR. (2010). Determinants of Ectoparasite Assemblage Structure on Rodent Hosts from South American Marshlands: The Effect of Host Species, Locality and Season. *Medical and Veterinary Entomology*. 24(3): 284-292. doi: 10.1111/j.1365-2915.2010.00880.x

Leighton, P, Koffl, J, Pelcat, Y, Lindsay, LR, Ogden, N. (2012). Predicting the Speed of Tick Invasion: An Empirical Model of Range Expansion for the Lyme Disease Vector *Ixodes scapularis* in Canada. *J. App. Ecol.* 49(2): 457-464 doi: 10.1111/j.1365-2664.2012.02112.x

Leo, S, Millien, V. (2017). Microsatellite Markers Reveal Low Frequency of Natural Hybridization Between the White-Footed Mouse (*Peromyscus leucopus*) and Deer Mouse (*Peromyscus maniculatus*) in Southern Quebec, Canada. *Genome*. 60: 454-46

Lindsay, LR, Barker, IK, Surgeoner, GA, McEwen, SA, Elliott, LA, Kolar, J. (1991). Apparent Incompetence of Dermacentor Variabilis (Acari: Ixodidae) and Fleas (Insecta: Siphonaptera) as Vectors of Borrelia Burgdorferi in an *Ixodes dammini* Endemic Area of Ontario, Canada. *Journal of Medical Entomology.* 28(5): 750-753. doi: 10.1093/jmedent/28.5.750.

Lindsay, LR, Drebot, M, Weiss, E, Artsob, H. (2001). Hantavirus Pulmonary Syndrome in Manitoba. *Canadian Journal of Infectious Diseases*. 12(3): 169-173. doi: 10.1155/2001/425260

Long Point. (2019). *Ontario Parks*. Retrieved from https://www.ontarioparks.com/park/longpoint

Maaz, D, Krucken, J, Blumke, J, Richter, D, McKay-Demeler, J, Matuschka, F, Hartmann, S, von Samson-Himmelstjerna, G. (2018). Factors Associated with Diversity, Quantity and Zoonotic Potential of Ectoparasites on Urban Mice and Voles. *PLoS ONE.* 13(6): e0199385. doi: 10.1371/journal.pone.0199385

Mabbott, NA . (2018) The Influence of Parasite Infections on Host Immunity to Co-Infection With Other Pathogens. *Front Immunol.* 9: 2579. doi: 10.3389/fimmu.2018.02579

Mallory, M, Forbes, M, Galloway, T. (2006). Ectoparasites of Northern Fulmars *Fulmarus glacialis* (Procellariiformes: Procellariidae) from the Canadian Arctic. *Polar Biology.* 29: 353-357. doi: 10.1007/s00300-005-0063-8

McKenzie, K. E. (1998). Chapter 31 Largest Blood Meal. *In University of Florida Book of Insect Records* (pp. 76-79). Gainesville, FL: University of Florida, Department of Entomology & Nematology. Retrieved from: http://entnemdept.ufl.edu/walker/ufbir/files/pdf/UFBIR_Chapter31.pdf

Minigan, J, Hager, H, Peregrine, A, Newman, J. (2018). Current and Potential Future Distribution of the American Dog Tick (*Dermacentor variabilis*, Say) in North America. *Ticks and Tick-borne Diseases.* 9: 354-362. doi: 10.1016/j.ttbdis.2017.11.012

Mize, E, Tsao, J, Maurer, B. (2011). Habitat Correlates with the Spatial Distribution of Ectoparasites on *Peromyscus leucopus* in Southern Michigan. *Journal of Vector Ecology*. 36(2): 308-320. doi: 10.1111/j.1948-7134.2011.00171.x

Mlera, L, Meade-White, K, Dahlstrom, E, Baur, R, Kanakabandi, K, Virtaneva, K, Porcella, S, Bloom, M. (2018). *Peromyscus leucopus* Mouse Brain Transcriptome Response to Powassan Virus Infection. *Journal of Neurovirology*. 24(1): 75-87. doi: 10.1007/s13365-017-0596-y

Møller, AP. (1991). Parasite Load Reduces Song Output in a Passerine Bird. *Anim. Behav.* 41(4): 723-730. doi: 10.1016/S0003-3472(05)80909-1

Morshed, M, Scott, J, Fernando, K, Mann, R, Durden, L. (2003). Lyme Disease Spirochaete,

Borrelia burgdorferi Endemic at the Epicenter in Rondeau Provincial Park, Ontario. *J. Med. Entomol.* 40(1): 91-94. doi: 10.1603/0022-2585-40.1.91

Myers, P, Lundrigan, B, Hoffman, S, Poor Haraminac, A, Seto, S. (2009). Climate-induced Changes in the Small Mammal Communities of the Northern Great Lakes Region. *Global Change Biology*. 15: 1434-1454. doi: 10.1111/j.1365-2486.2009.01846.x

Nicolas, G, Bennoun, M, Porteu, A, Mativet, S, Beaumont, C, Grandchamp, B, Sirito, M, Sawadogo, M, Kahn, A, Vaulont, S. (2002). Severe Iron Deficiency Anemia in Transgenic Mice Expressing Lier Hepcidin. *PNAS*. 99(7): 4596-4601. doi: 10.1073□pnas.072632499

Nichols, J, Hines, J, Pollock, K. (1984). Effects of Permanent Trap Response in Capture Probability on Jolly-Seber Capture-Recapture Model Estimates. *JSTOR.* 48(1): 289-294. doi: 10.2307/3808491

Nunn, C, Altizer, S, Sechrest, W, Cunningham, A. (2005). Latitudinal Gradients of Parasite Species Richness in Primates. *Diversity and Distributions*. 11(3): 249-156. doi: 10.1111/j.1366-9516.2005.00160.x

Ogden, NH, Barker, IK, Beauchamp, G, Brazeau, S, Charron, DF, Maarouf, A, Morshed, MG, O'Callaghan, CJ, Thompson, RA, Waltner-Toews, D, Waltner-Toews, M, Lindsay, LR. (2006). Investigation of Ground Level and Remote-Sensed Data for Habitat Classification and Prediction of Survival of *Ixodes scapularis* in Habitats of Southeastern Canada. *J. Med. Entomol.* 43(2): 403-414. doi: 0022-2585/06/0403Ð0414$04.00/0

Ogden, N, Lindsay, LR, Leighton, P. (2013). Predicting the Rate of Invasion of the Agent of Lyme disease *Borrelia burgdorferi*. *J Appl Ecol*. 50: 510¬518. doi: 10.1111/1365¬2664.12050

Ogden, N, Barker, IK, Francis, CM, Heagy, A, Lindsay, LR, Hobson, KA. (2015). How Far North are Migrant Birds Transporting the Tick *Ixodes scapularis* in Canada? Insights from Stable Hydrogen Isotope Analyses of Feathers. *Ticks and Tick-borne Diseases.* 6:715-720. doi: 10.1016/j.ttbdis.2015.06.004

Ontario Biodiversity Council. (2015). State of Ontario's Biodiversity [web application]. Ontario Biodiversity Council, Peterborough, Ontario. Retrieved from http://ontariobiodiversitycouncil.ca/sobr

Ostfeld, R, Miller, M, Schnurr, J. (1993). Ear Tagging Increases Tick (*Ixodes Dammini*)

Infestation Rates of White-Footed Mice (*Peromyscus leucopus*). *J. Mamm.* 74(3): 651-655. doi: 10.2307/1382286

Ostfeld, R, Miller, M, Hazler, K. (1996). Causes and Consequences of Tick (*Ixodes scapularis*) Burdens on White-Footed Mice (*Peromyscus leucopus*). *Journ. Mamm.* 77(1): 166-273. doi: 10.2307/1382727

Pedersen, A, Greives, T. (2008). The Interaction of Parasites and Resources Cause Crashes in a Wild Mouse Population. *Journ. Anim. Ecol.* 77:370-37. doi: 10.1111/j.1365-2656.2007.01321.x

Pfäffle, M, Petney, T, Elgas, M, Skuballa, J, Taraschewski, H. (2009). Tick-Induced Blood Loss Leads to Regenerative Anaemia in the European Hedgehog (*Erinaceus europaeus*). *Parasitology*. 136: 443–452. doi:10.1017/S0031182009005514

Pishchany, G, Skaar, E. (2012). Taste for Blood: Hemoglobin as a Nutrient Source for Pathogens. *PLoS Pathogens.* 8(3): e1002535

Poulin, R. (1991). Group-living and the Richness of the Parasite Fauna in Canadian Freshwater Fishes. *Oecologia*. 86: 390-394.

Poulin R. (2010). Parasite manipulation of host behavior: an update and frequently asked questions. *Adv Study Behav.* 41:151–86. doi: 10.1016/S0065-3454(10)41005-0

Poulin, R, Guegan, J. (2000). Nestedness, Anti-Nestedness, and the Relationship Between Prevalence and Intensity in Ectoparasite Assemblages Of Marine Fish: A Spatial Model Of Species Coexistence. *International Journal for Parasitology.* 30(11): 1147-1152. doi: 10.1016/S0020-7519(00)00102-8

Preisser, W. (2019). Latitudinal Gradients of Parasite Richness: A Review and New Insights from Helminths of Cricetid Rodents. *Ecography.* 42(7): 1315-1330. doi: 10.1111/ecog.04254

Public Health Ontario. (2019). Ontario Lyme Disease Map 2019 Estimated Risk Areas. *Ontario Agency for Health Protection and Promotion*. Toronto, ON: Queen's Printer for Ontario.

Queen Elizabeth II Wildlands. (2019). *Ontario Parks*. Retrieved from https://www.ontarioparks.com/park/queenelizabeth2wildlands

Rasid, H, Baker, D, Kreutzwiser, R. (1992). Coping with Great Lakes Flood and Erosion Hazards: Long Point, Lake Erie, vs. Minnesota Point, Lake Superior. *Journal of Great Lakes Research*. 18(1): 29-42.

Reeves, W, Dowling, A, Dasch, G. (2006). Rickettsial Agents from Parasitic Dermanyssoidea (Acari: Mesostigmata). *Experimental and Applied Acarology*. 38: 181-188. doi: 10.1007/s10493-006-0007-1

Rohde, K, Heap, M. (1998). Latitudinal Differences in Species and Community Richness and in Community Structure of Metazoan Endo- and Ectoparasites of Marine Teleost Fish. *International Journal for Parasitology.* 28(3): 461-474. doi: 10.1016/s0020-7519(97)00209-9

Rosales, F, Jang, J, Pinero, D, Erikson, K, Beard, J, Ross, C. (1999). Iron Deficiency in Young Rats Alters the Distribution of Vitamin A Between Plasma and Liver and Between Hepatic Retinol and Retinyl Esters. *The Journal of Nutrition*. 129(6): 1223-1228.

Rynkiewicz, E, Hawlena, H, Durden, L, Hastriter, M, Demas, G, Clay, K. (2013). Associations Between Innate Immune Function and Ectoparasites in Wild Rodent Hosts. *Parasitol. Res.* 112: 1763-1770. doi: 10.1007/s00436-013-3335-1

Salkeld, DJ, Padgett, KA, Jones, JH. (2013). A Meta-Analysis Suggesting that the Relationship Between Biodiversity and Risk of Zoonotic Pathogen Transmission is Idiosyncratic. *Ecology Letters.* 16(5): 679-686. doi: 10.1111/ele.12101

Scholten, TH. (1977). Human Tick Infestations in Ontario: Findings at the Toronto Public Health Laboratory, 1967-1977. *Canadian Journal of Public Health*. 68(6): 494-496.

Schwanz, L, Voordouw, M, Brisson, D, Ostfeld, Richard. (2011). *Borrelia burgdorferi* Has Minimal Impact on the Lyme Disease Reservoir Host *Peromyscus leucopus*. *Vector-Borne and Zoonotic Diseases*. 11(2): 117-124. doi: 10.1089=vbz.2009.0215

Scott, J, Anderson, J, Durden, L. (2012). Widespread Dispersal of *Borrelia burgdorferi*-infected Ticks Collected from Songbirds Across Canada. *J. Parasitol.* 98(1): 49-59. doi: 10.1645/GE-2874.1

Shaw, M, Keesing, F, McGrail, R, Ostfeld, R. (2003). Factors Influencing the Distribution of Larval Blacklegged Ticks on Rodent Hosts. *Am. J. Trop. Med. Hyg.* 68(4): 447-452. doi: 10.4269/ajtmh.2003.68.447

Sonenshine, D. (2018). Range Expansion of Tick Disease Vectors in North America: Implications for Spread of Tick-Borne Disease. *Int. J. Environ. Res. Public Health.* 15: 478. doi:10.3390/ijerph15030478

Talagrand-Reboul, E, Boyer, P, Bergstrom, S, Vial, L, Boulanger, N. (2018). Relapsing Fevers:

Neglected Tick-Borne Diseases. *Frontiers in Cellular and Infection Microbiology.* 8:98. doi: 10.3389/fcimb.2018.00098

Thomas F, Poulin R, Brodeur J. (2010). Host manipulation by parasites: a multidimensional phenomenon. *Oikos*. 119:1217–23. doi: 10.1111/j.1600-0706.2009.18077.x

Thompson, PM, Corpe, HM, Reid, RJ. (1998). Prevalence and Intensity of the Ectoparasite *Echinophthirius horridus* on Harbour Seals (*Phoca vitulina*): Effects of Host Age and Inter-Annual Variability in Host Food Availability. *Parasitology*. 117(4): 393-403. doi: 10.1017/S0031182098003072

Thompson, SN, Kavaliers, M. (1994). Physiological Bases for Parasite-Induced Alterations of Host Behaviour. *Parasitology.* 109: S119-S138. doi: 10.1017/S0031182000085139

Toh, SQ, Glanfield, A, Gobert, G, Jones, M. (2010). Heme and Blood-Feeding Parasites: Friends or Foes? *Parasites & Vectors.* 3(108): 1-10. doi: 10.1186/1756-3305-3-108

Watson, TG, Anderson, RC. (1976). *Ixodes scapularis* Say on White-Tailed Deer (*Odocoileus virginianus*) from Long Point, Ontario. *J. Wild. Dis*. 12: 66–71.

Webber, Q, McGure, L, Smith, S, Willis, C. (2014). Host Behaviour, Age, and Sex Correlate with Ectoparasite Prevalence and Intensity In a Colonial Mammal, the Little Brown Bat. *Behaviour*. 152(1): 83-105. doi: 10.1163/1568539X-00003233

Weldon, L, Abolina, S, Lenzi, L, Bourne, C, Riley, E, Viney, M. (2015). The Gut Microbiota of Wild Mice. *PLoS One.* 10(8): e0134643. doi: 10.1371/journal.pone.0134643

Westblade, L, Simon, M, Mathison, B, Kirkman, L. (2017). *Babesia microti*: from Mice to Ticks to an Increasing Number of Highly Susceptible Humans. *Journal of Clinical Microbiology.* 55(10): 2903-2912. doi: 10.1128/JCM.00504-17

Wheeler, T, Threlfall, W. (1986). Observations on the Ectoparasites of some Newfoundland Passerines (Aves: Passeriformes). *Canadian Journal of Zoology*. 64(3): 630-636. doi: 10.1139/z86-093

Wilder, S, Meikle, D. (2004). Prevalence of Deer Ticks (*Ixodes scapularis*) on White-Footed Mice (*Peromyscus leucopus*) in Forest Fragments. *J. Mammal.* 85(5): 1015-1018. doi: 10.1644/020

Wilkinson, PR. (1967). The Distribution of *Dermacentor* Ticks in Canada in Relation to Bioclimatic zones. *Canadian Journal of Zoology*. 45(4): 517-537. doi: 10.1139/z67-066

Whitworth, TW, Bennett, GF. (1992). Pathogenicity of Larval Protocalliphora (Diptera:

Calliphoridae) Parasitizing Nestling Birds. *Can. J. Zool*. 70(11): 2184–2191. doi: 10.1139/z92-295

Wolf, M, Baltzi, G. (2001). Increased Prevalence of Bot Flies (C*uterebra fontinella*) on White-Footed Mice (*Peromyscus leucopus*) Near Forest Edges. *Can. J. Zool.* 79: 106-109. doi: 10.1139/cjz-79-1-106

Wolff, J. (1985). Maternal Aggression as a Deterrent to Infanticide in *Peromyscus leucopus* and *P. maniculatus*. *Anim. Behav*. 33: 117-123.

Wolff, J. (1994). Reproductive Success of Solitarily and Communally Nesting White-Footed Mice and Deer Mice. *Behav. Ecol.* 5(2): 206-209. doi: 1045-2249/94/$5.00

Wood, H, Dillon, L, Patel, S, Ralevski, F. (2016). Prevalence of *Rickettsia* species in *Dermacentor variabilis* ticks from Ontario, Canada. *Ticks and Tick-borne Diseases*. (7)5: 1044-1046. doi: 10.1016/j.ttbdis.2016.06.001

General Figures and Tables

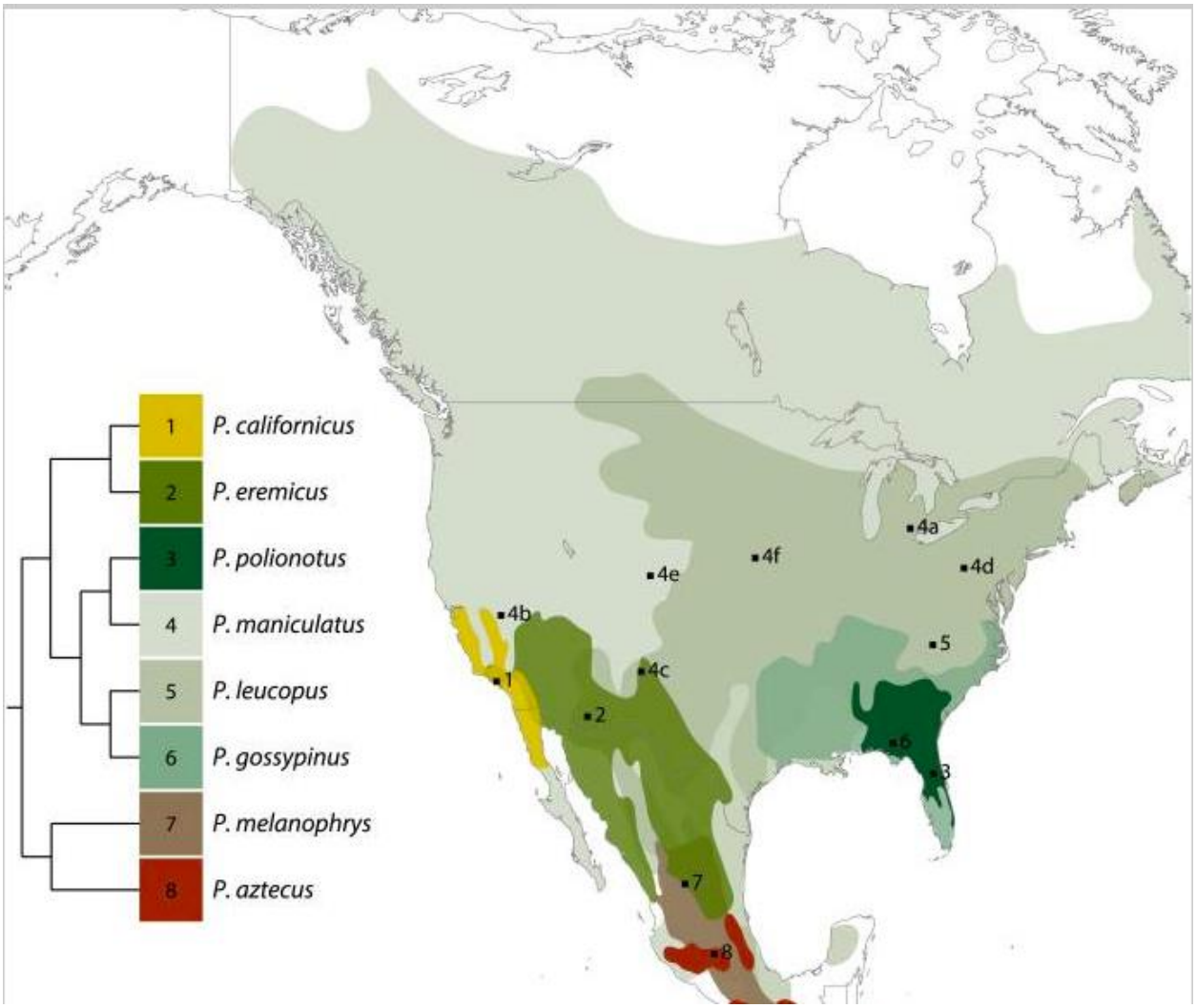

Figure 0.1. North American ranges of all *Peromyscus* species found in North America. The deer mouse (*Peromyscus maniculatus*) is the most abundant species of this genus, and as this figure shows, it also has the most extensive range (Bedford and Hoekstra, 2015).

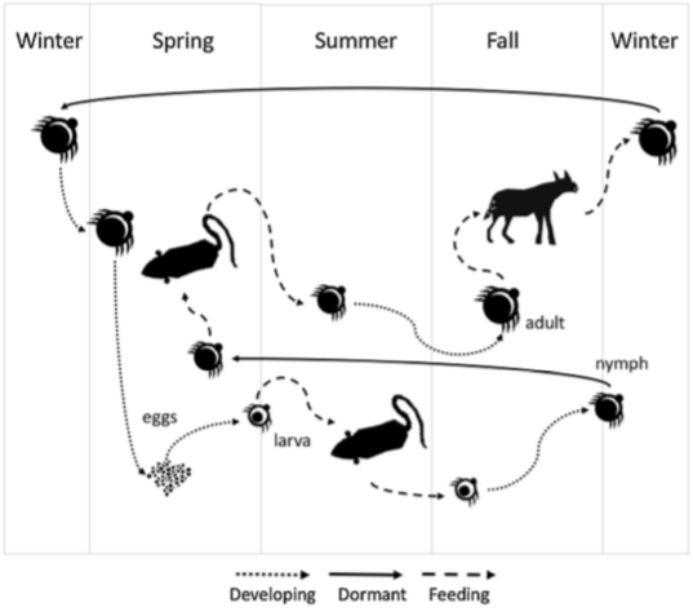

Figure 0.2. The life cycle of blacklegged ticks (*Ixodes scapularis*), illustrating the different developmental stages throughout seasons (Halsey & Miller, 2018).

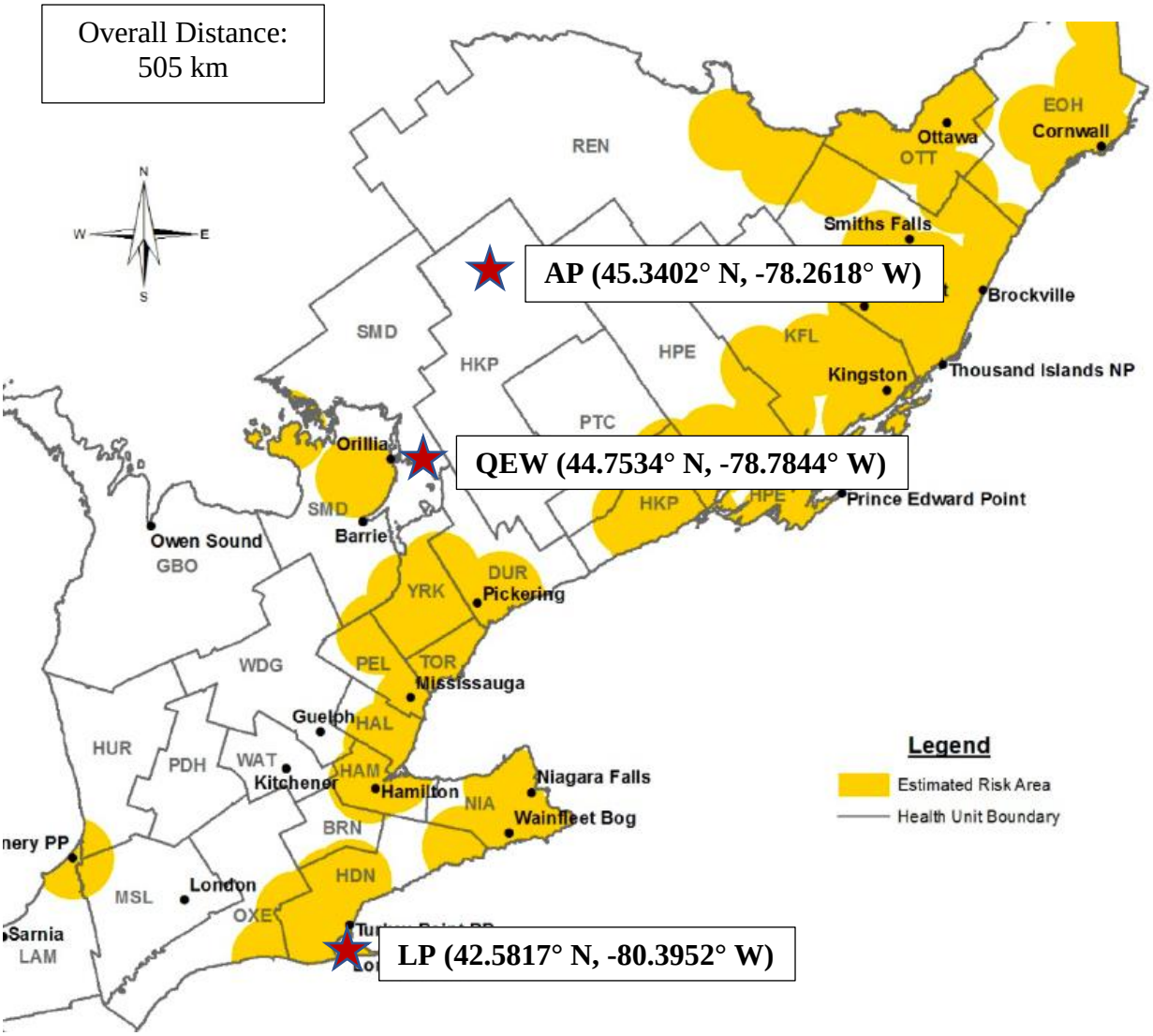

Figure 0.3. The estimated risk areas in Ontario, Canada where people are most likely to come in contact with blacklegged ticks, based on passive and active tick surveillance (Public Health Ontario, 2019). Red stars indicate sampling site locations.

Figure 0.4. The current distribution of suitable habitat for wood ticks in North America, highlighting the area of Ontario, Canada where the study is focused. The darker grey an area is, the better suited habitat that space is for wood ticks (Minigan *et al*., 2018).

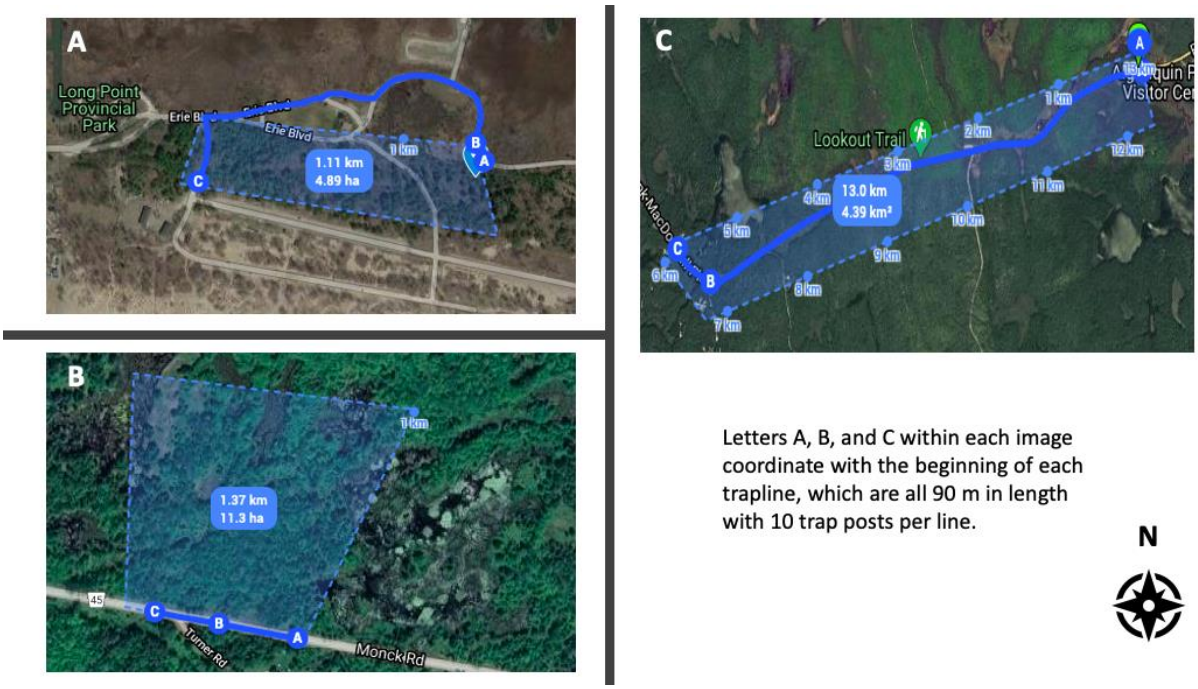

Figure 0.5. Land area of field sites where traplines were set and sampling occurred in Ontario, Canada. A) field Site at Long Point Provincial Park, B) field site at Queen Elizabeth II Wildlands Provincial Park, and C) field site at Algonquin Provincial Park (Google Maps, 2020).

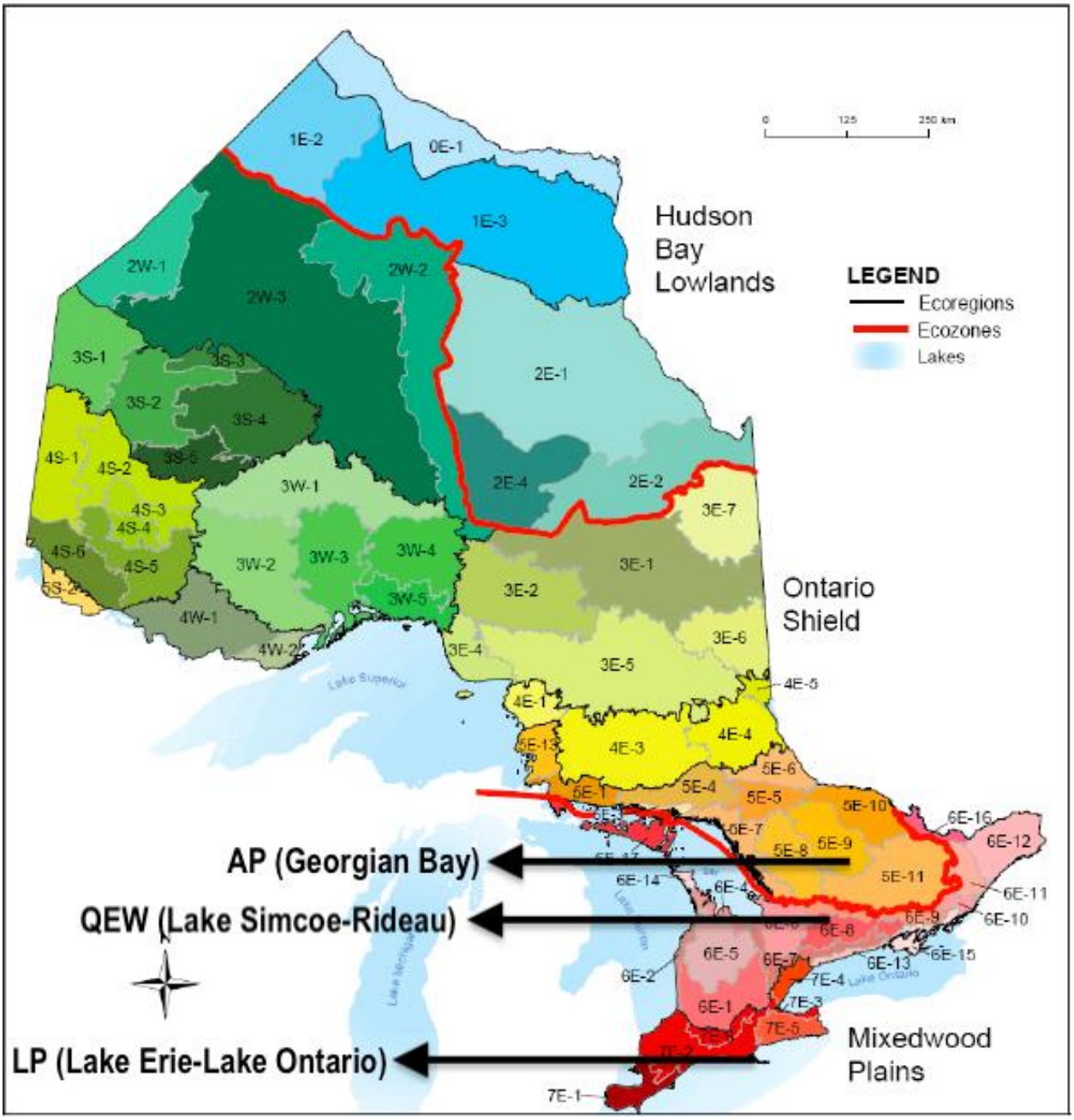

Figure 0.6. Map of the ecoregions (delineated by black lines) and ecozones (delineated by red lines) of Ontario, Canada, including the ecoregions and ecozones for each site, depicting the similarities and differences across locations (Crins *et al*., 2009). Black arrows indicate where sampling sites are and their respective ecoregions (in parentheses).

Chapter I:

Effects of Ixodid Tick Infestation on Hemoglobin Levels of *Peromyscus maniculatus*

Abstract:

Deer mice (*Peromyscus maniculatus*) are hosts to several ixodid tick species as well as the associated tick-borne pathogens they can spread. As tick expansion continues northwards in Ontario, Canada, naïve host populations of deer mice are likely to become infested by ticks and susceptible to the physiological effects that ticks can have on them via blood-feeding. Prevalence of hematophagous ticks, such as blacklegged ticks (*Ixodes scapularis*) and wood ticks (*Dermacentor variabilis*), can affect hemoglobin levels of the deer mice they infest. Hemoglobin levels were compared and analyzed in deer mice populations at three different sites with varying exposures to ticks. It was expected that hemoglobin levels would significantly differ depending on how long ticks have been established at the sites and that a mouse population that was naïve to ticks would have the highest hemoglobin levels. My results suggested that the abundance of blacklegged and wood ticks on individual mice had the most significant negative effect on the hosts' hemoglobin levels, but the interaction between these two species did not. Average hemoglobin levels between populations also varied, where there was a significant difference between the source population with the longest established tick populations and the source population where neither blacklegged nor wood ticks were prevalent, As the ticks' ranges expand and they become more abundant, it is important to understand how their prevalence and intensity can alter the physiology of their hosts, potentially affecting their own range expansion and the spread of the diseases they may carry.

Key Words: *Dermacentor variabilis*, hemoglobin, *Ixodes scapularis*, parasite-host interactions, *Peromyscus maniculatus*

Introduction

Hematophagous ectoparasites can negatively impact vertebrate hosts via blood-feeding (Bordes & Morand, 2011; Godinho *et al*., 2013) and the physiological effects of these parasites on hosts have been widely studied (Dryden *et al*., 1991; Carleton, 2008; Pfäffle *et al*., 2009; Hersh *et al*., 2014; Jones *et al*., 2019). Blood-feeding by ectoparasites is known to be detrimental to the physiology of their hosts and can even prove fatal in some cases. This includes reduced hemoglobin levels (Carleton, 2008), regenerative anemia (Pfaffle *et al*., 2009) and blood-loss related mortality (Jones *et al*., 2019). Blood-feeding is important for ectoparasites, such as hard-bodied ticks (Acari: Ixodidae), because they require blood meals to reach subsequent life stages (Arsnoe *et al*., 2015).

As the global climate warms and more habitat becomes suitable for ixodid tick species, the geographic ranges of these species expand into new areas (Leighton *et al*., 2012; Clow *et al*., 2016; 2017; Minigan *et al*., 2018) where they can potentially establish and infest host populations previously naïve to such interactions. Variation in parasite susceptibility between naturally exposed and naïve populations has mostly focused on internal parasites (Brockhurst *et al*., 2007; Hasu *et al*., 2009; Sheath *et al*., 2018). On an individual level, cattle calves (*Bos taurus*) infested with cattle ticks (*Rhipicephalus microplus*) have exhibited a significant reduction in hematological parameters, including hemoglobin when compared with non-infested calves (Kaur *et al*., 2017). However, little is known about the effect of tick infestations on naïve mammal host populations, despite the geographic expansion of these pathogen vectors (Rand et al., 1993; Larson et al., 2018; Sonenshine, 2018). In general, naïve hosts are especially vulnerable to initial parasitic interactions (Brockhurst *et al*., 2007). Contrastingly, hosts that have prior exposure to ectoparasites tend to be more resistant than hosts that have not been exposed to

them, due to their adaptive responses to promote stronger immune defences (Kennedy, 2010; Jones *et al*., 2015).

Local adaption has been studied between parasite and host, but there is a general gap in literature on the relationship between hematophagous parasites and the effects of their blood-feeding on their hosts (Kutzer & Armitage, 2016; Papkou *et al*., 2016). Several blacklegged ticks (*Ixodes scapularis*) can feed from white-footed mice at once or in succession due to exposed skin lesions that allow ticks to infest the same host despite a strong inflammatory response at the bite sites that can increase host resistance (Anderson *et al*., 2017). Yet, insufficient immune responses during developmental periods could result in mice being vulnerable to other bacterial infections (Rosales *et al*., 1999; Dlugosz *et al*., 2014).

Blacklegged ticks and wood ticks (*Dermacentor variabilis*) tend to feed on *Peromyscus* mice at immature stages (Ostfeld *et al*., 1996; Sonenshine, 2018), and have been documented sharing the same areas on a host (micro-habitat; Morshed *et al*., 2003; Shaw *et al*., 2003; Gómez-Rodríguez *et al.,* 2015). Although white-footed mice tend to be the preferred host of blacklegged ticks, and deer mice (*P. maniculatus*) have been found to have lower rates of tick infestations, both host species are equally competent vectors of tick-borne pathogens (*i.e., Borrelia burgdorferi* and *Anaplasma phagocytophilum*: Rand *et al*., 1993; Larson *et al*., 2018). However, since ticks feed on mice for a short period of time, it is difficult to measure long-term impacts that they may have on host fitness. There is evidence that immature blacklegged ticks may not affect mouse survival in established host populations (Hersh *et al*., 2014), although there are no comparative studies between experienced host populations that have cohabited with an established tick population versus inexperienced (naïve) ones.

Other ectoparasites that feed on *Peromyscus* mice for long periods of time (*i.e.* fleas, mites) are consistently affecting the vulnerability of their host's immune response (Mize *et al*., 2011), reducing hemoglobin levels due to constant blood loss (O'Brien *et al*., 2003), which could cause an insufficient production of hemoglobin, and therefore iron (Judy & Price, 1958; Andrews, 1997). Black rats (*Rattus rattus*) that were iron-deficient were found to be susceptible to infections of the pathogen *Salmonella typhimurium* (Baggs & Miller, 1973). It is possible that similar results may be found in other rodents. Investigating how ectoparasite feeding habits influence the hematology of deer mouse hosts – with and without the presence of ticks – will allow us to better understand how blacklegged and wood ticks may impact northern mouse populations as these ectoparasites move northward (Leighton *et al*., 2012).

The objective of this study was to determine if ticks have a significant effect on the hematology of deer mice by examining how tick prevalence and intensity influence the host's hemoglobin levels. Such data may provide insight into how ticks can affect the overall fitness of their hosts, and how these hosts adapt to novel parasites. I hypothesized that: 1) if mice are parasitized by a greater intensity of ticks, then they will have lower hemoglobin levels compared to mice infested with ticks at low intensities because more blood-feeding is occurring at one time and 2) deer mice that live in areas where ticks are prevalent should have lower hemoglobin levels than deer mice living in unestablished areas because they are being affected by blood-feeding. Individual mice that are infested with higher abundances of ticks are expected to have lower hemoglobin levels compared to a mouse with low tick abundance as more ticks are feeding from the same source at one time. Further, mice that inhabit areas where ticks are prevalent and at high intensities are expected to have lower hemoglobin levels than mice that are ecologically naïve to these parasites. Examining the differences between naïve and experienced mouse

populations as well as between individual mice can bridge the gap in our understanding of tick effects on a host and help make better predictions on how hematology can affect the ecology of *Peromyscus* mice.

Methods

All methods in this study were approved by the Animal Care Committee (ACC) at Laurentian University, file number 6017269.

Field Methods

Three sites in Ontario, Canada were visited for data and specimen collection from May-August 2019. The most southern site, Long Point Provincial Park (LP; 42.5817° N, -80.3952° W) is an area where tick populations have been long-established (Watson & Anderson, 1976; Lindsay *et al*., 1991). The other two sites, Queen Elizabeth II Wildlands Provincial Park (QEW; 44.7534° N, -78.7844° W) and Algonquin Provincial Park (AP; 45.3402° N, -78.2618° W) are areas with no established populations of blacklegged ticks, although QEW has known established wood tick populations (Minigan *et al.*, 2018; P. Careless, 2019, pers. comm.).

Neither blacklegged ticks nor wood ticks have colonized AP (Public Health Ontario, 2019). The sites chosen for this experiment convey the progression of the geographic expansion of blacklegged ticks and wood ticks in Ontario from both its southern and eastern populations (Hamer *et al.*, 2014; Clow *et al*., 2016; 2017; Minigan *et al*., 2018), and are areas within the geographical habitat range of deer mice (Bedford and Hoekstra, 2015). Traplines were placed in similar forest types across sites to maintain a consistent or similar habitat, although sites were from different ecozones (Crins *et al*., 2009).

At each site, three traplines at least 0.3 km apart from each other were set up with twenty Longworth traps (Penlon Ltd., Oxford, U.K) set per line. Traplines were 90 m long and had two Longworth traps 10 m apart from each other every 10 m (Fryxell *et al.,* 1998; Falls *et al*., 2007). Traps were baited with water-soaked sunflower seeds and were set half an hour before sunset (2000h-2100h). Deer mice are nocturnal, and so traps were checked half an hour before sunrise (0430h-0530h) after the mice had been active through the night (Clark & Durden, 2002). The traps were set five days each week. They were checked three days consecutively, with a one-day break, and then checked for two more consecutive days to reduce the amount of stress mice may have from repeated captivity or trap response behaviour (Nichols *et al*., 1984). To account for change in seasonality, and the difference in parasite assemblages that occurs within the spring-summer months, I alternated between the three sites in ascending latitude, spending one week at each site, three times, for a total of three weeks per site. By alternating sites within the season, I was able to account for the different assemblages that may be occurring within populations, as temperatures change and specific parasites occur in different quantities, therefore removing potential seasonality bias to my analysis for parasite community assemblages.

Mice caught in traps were removed from traps and weighed using a Pesola® scale (±0.1g). Age of mice was determined by body mass, where mice that were 15 g or less were considered juveniles, while mice greater than 15 g were considered adults (Banfield, 1974; Schmidt *et al*., 2019). Sex and reproductive status were determined visually. Enlarged testes in males and perforated vagina and presence of nipples in females indicated reproductive individuals (Gaitan & Millien, 2016). No pregnant females were included in this study. Age of ticks was determined by the number of legs the tick had, where larva have six legs, and nymphs have eight (Lindquist *et al*., 2016). Julian date was also recorded.

While mice were collected from the traps at each site, each mouse was examined for 60 seconds for sampling of ectoparasites (Patterson *et al*., 2013). Individual arthropod specimens were combed off the host using a louse comb sterilized with ethanol (Hawlena *et al*., 2006; Patterson *et al*., 2013) or plucked off, using sterilized tweezers (Bobbie *et al*., 2016). These specimens were then placed in a collection vial with 80% ethanol (Krogmann & Holstein, 2010). Tick identification was completed using guides (Lindquist *et al*., 2016; Dubie *et al*., 2017), and were later confirmed by Dr. Robbin Lindsay from the National Microbiology Laboratory (Winnipeg, Manitoba, Canada). Similarly, fleas were identified using an identification key (Holland, 1985), and later confirmed by Dr. Terry Galloway from the University of Manitoba. Two unique mite groups from the Trombiculidae family were identified by Dr. Heather Proctor (University of Alberta).

Deer mice were distinguished from white-footed mice by morphological characteristics. This included differences in ear length (mm; Kamler *et al*., 1998), tail bi-colouration, tail "penciling" (J. Bowman, 2019, pers. comm.), and degree of dorsoventral colouration (Buchholz & Dick, 2017). Captured mice were placed in a 50 mL Falcon conical centrifuge tube with a hole in the tip to allow for the mouse to breathe. By pushing gently above the tibia, the right hind leg of the mouse could be extended out of the tube and shaved using an electric trimmer (Parasuraman *et al*., 2010). Needles (20G) were used with a 1 mL syringe in accordance with Standard Operating Procedure for Laurentian University and general principles of laboratory blood collection (Parasuraman *et al*., 2010). Blood samples consisted of 0.007 mL of blood/g of the mouse's body mass taken from the saphenous vein in the shaved hind leg of the mouse via needled syringe (Randolph, 1980; National Research Council Institute for Laboratory Animal Research, 1996; PREDICT One Health Consortium, 2016). On site, the blood samples were

placed in a microcuvette specialized for the handheld hemoglobin analyzer to test for hemoglobin concentrations: HemoCue Hb 201+ analyzer (HemoCue AB, Ängelholm, Sweden; Tufts *et al*., 2013; Weldon *et al*., 2015). Results were given immediately by the analyzer and recorded.

It was necessary to shave the legs of the mice in order to acquire blood samples and so the shaved areas were also used as markers on the mice to indicate that they were already sampled to prevent sampling an individual more than once (Powell & Proulx, 2003). Although it is common for small mammals to be given ear tags for identification purposes in field studies (Harper & Austad, 2001; Hersh *et* al., 2014; Torre *et al*., 2016; Buchholz & Dick, 2017), Ostfeld et al. (1993) found that this method will increase tick infestations on the host's ears. It should be noted that the exposed shaved legs of the mice in this study were never observed to have ticks or bite marks in this specific area, implying shaving the legs of the mice did not result in the same bias as ear tags do in attracting ticks. Instead, because their right legs were shaved, it was clear which mice were recaptures when returning to each site every three weeks, as the fur on these mice had not fully regrown. These mice were documented as recaptures and were not repeatedly included in any statistical analysis as multiple blood samplings taken from one individual could provide lower hemoglobin levels over time (Weixelbaumer *et al*., 2010), biasing the study. The legs of these recaptures were re-shaved, however, so that upon returning to the site in the next cycle, they could be clearly identified again.

Statistical Methods

Statistical analyses were conducted in the R environment (R core Team 2019 [v3.6.1]) using base R and the package 'ggplot2' (Wickham, 2016 [v3.2.1]) for figures and linear models.

Prevalence and mean intensity of ectoparasites were completed using QPweb (Reiczigel *et al*., 2019), where prevalence refers to the percentage of mice in a population with one or more ectoparasites (Bush *et al*., 1997). For the purpose of this study, mean intensity refers to the mean number of ectoparasites found across mice in a population, whereas abundance refers to the number of ectoparasites found on individuals (Bush *et al*., 1997). Shapiro-Wilks tests showed that hemoglobin levels were distributed normally across individuals ($p = 0.09$).

To determine differences in hemoglobin levels among sites, a one-way ANOVA and Tukey's Honest Significance test were conducted, where hemoglobin levels were the response variable, and source population site (LP, QEW, AP) was the predictor. To account for possible variation across individuals in the ANOVA, a general linear model was performed first to see if there were any significant differences across host sex, host age, host body mass, and host population location (field site). In this model, only field site had a significant effect on hemoglobin levels (Table 1.1). Similar results were found in a general linear model with tick abundance (both blacklegged and wood ticks together) as the response variable (Table 1.2), suggesting that there is no difference in tick loads across host age, sex, or body mass; only field site. The effect of differences in tick prevalence on mouse population hemoglobin levels was measured by comparing mean hemoglobin levels between sites and across all individuals (LP+QEW+AP) when 1) all mice are included, 2) only mice infested with ticks are included, or 3) only mice not infested with ticks are included. Standard deviations of these means were also included. One-way ANOVAs were conducted for all individuals, and for each source population site comparing hemoglobin levels and tick prevalence (both blacklegged and wood tick together).

Julian date, population density per site (using Lincoln-Peterson index to determine population size via mark-recapture data; Grimm *et al*., 2014), and engorgement level of ticks (determined visually, and categorized as 0 = not engorged, 1 = moderately engorged, 2 = substantially engorged; Hofhuis *et al*., 2017) were variables initially considered. However, after performing Pearson correlation coefficient (r) tests, they were found to be heavily correlated with other variables – Julian date and session (r = 0.92), population density and location (r = -0.99), tick engorgement level and wood tick abundance (r = 0.90). The package 'tidyverse' (Wickham *et al*., 2019 [v1.3.0]) was used to test variance inflation factors (VIF) for independent variables. To avoid severe multicollinearity, the variables with the highest VIF values were removed from analyses (Julian date, tick engorgement, and population density; Harrison *et al*., 2018; Frost, 2019).

Across all individuals from all three sites, multiple general linear regressions were considered with hemoglobin levels as the response variable, comparing the effects of the predictor variables: number of wood ticks, number of blacklegged ticks, wood nymph presence (Y or N), presence of *Neotrombicula sp*. (Y or N) and presence of Trombiculidae *sp*. (Y or N) – separately and together (*Neotrombicula sp*. presence only, Trombiculidae *sp*. presence only, both *Neotrombicula sp.* and Trombiculidae *sp*. together), number of *O. leucopus*, sessions when each site was visited, age of mouse (adult or juvenile), sex of mouse (male or female), reproductive status of mouse (reproductive or non-reproductive), body mass of mouse, and source population (LP, QEW, AP). Sessions were divided by early (May-June), mid (June-July), and late (July-August) summer. Only two blacklegged nymphs were found, so only the presence of wood nymphs was measured.

Since the sample size for this study was small (n = 44), Akaike information criterion correction (AICc) tests were conducted via R package 'AICcmodavg' (Mazerolle, 2019 [v2.2-2]). AICc analyses were used to determine linear models that provided the strongest explanation for affecting hemoglobin levels when ΔAICc is < 3. Here all models within this threshold or 'top model set' were considered equally plausible (Harrison *et al*., 2018). Pearson's correlation formula was used to determine correlation coefficients between all predictor variables included in the top models and hemoglobin levels to determine effect sizes (Weber *et al*., 2019). VIF multicollinearity tests were conducted on all multiple linear regression models where all independent variable VIF values were < 5 (Harrison *et al*., 2018; Frost, 2019). Source population was removed as a predictor, since it had VIF > 5 (5.13) and was moderately correlated with blacklegged tick and wood tick abundances (r = -0.64 and -0.54, respectively). This was expected, as the populations are from field sites that were chosen based on their differences in tick prevalence and intensity. Wood nymphs had the highest VIF (3.65), because it was correlated with wood tick abundance (r = 0.65). All other variables had VIF < 3. Once the top model set was determined, multiple linear regression models were performed using the variables from these models.

Results

A sample of 44 individual deer mice was included in analyses among all sites. This included 16 adult female mice and 3 juvenile female mice, 20 adult male mice, and 5 juvenile male mice. Dr. Terry Galloway identified one flea species, which was found across sites: *Orchopeas leucopus.* Dr. Heather Proctor was able to identify two separate groups from the mite specimens collected. One group was identified to the genus *Neotrombicula.* The other group was

determined to be taxonomically distinct enough to be a different species from the *Neotrombicula* specimens but could not be identified beyond family; Trombiculidae. For the purpose of this study, the mite groups will be defined as '*Neotrombicula sp.*' and 'Trombiculidae *sp.*'. There were 111 wood ticks (16 of which were nymphs), 28 blacklegged ticks (2 of which were nymphs), and 15 *O. leucopus*. There were 15 instances of Trombiculidae *sp.* and 8 of *Neotrombicula sp.* on deer mice. The average population sizes for each site across sessions (early, mid, and late summer) were determined by the Lincoln-Peterson index: LP = 28 mice, QEW = 18 mice, AP = 11 mice. These results, along with the land area used for trap sites determined population density (Table 1.3). LP had the greatest intensity and prevalence of both focal tick species, while AP had the highest prevalence of *O. leucopus* and *Neotrombicula sp.*, and QEW had the highest prevalence of Trombiculidae *sp.* (Table 1.4).

Models with the lowest AICc scores determined which predictor variables may be affecting the response variable – deer mouse hemoglobin levels – most significantly. Across the top two models, the explanatory variables were the abundance of blacklegged ticks on an individual, the abundance of wood ticks on an individual, the interaction between these two species, and the prevalence of wood tick nymphs on an individual (Table 1.5). Multiple linear regressions were performed for the two models. Across the models, it was found that the abundance of blacklegged ticks and wood ticks significantly affected hemoglobin levels ($p < 0.05$), but their interaction did not ($p = 0.65$; Table 1.6; Figure 1.1). Pearson correlation coefficients between hemoglobin levels and tick species (both tick species together, blacklegged ticks, and wood ticks separately) were moderately and negatively correlated ($r = -0.49, -0.51, -0.5$, respectively; Table 1.7), suggesting moderate effect size (Weber *et al.*, 2019). Across the two models, the prevalence of wood nymphs was also found to significantly affect hemoglobin

levels, ($p = 0.01$ and $p = 0.03$; Table 1.6; Figure 1.2), however, the Pearson correlation value for wood nymphs was both negatively and weakly correlated to hemoglobin (-0.21; Table 1.7), suggesting small effect size (Weber *et al*., 2019). However, the estimates for wood nymph presence were positive (1.95, 1.85; Table 1.6), despite a negative relationship with hemoglobin levels (Figure 1.2). This could be due to collinearity between wood nymph presence and wood tick abundance. None of the top models included *Neotrombicula sp*. presence, Trombiculidae *sp* presence, *O. leucopus* abundance, field sessions, host age, host sex, host reproductive status, host body mass, or source population (LP, QEW, AP), suggesting that these variables had no significant effect on hemoglobin levels.

An ANOVA testing the differences in hemoglobin levels across sites showed that there was a significant difference between hemoglobin levels depending on the location where the deer mice were found ($p = 0.01$). A Tukey's Honest Significance test showed that AP hemoglobin levels were significantly higher than those at LP ($p\ adj = 0.01$), and the differences between LP and QEW hemoglobin levels and between QEW and AP were not significant ($p\ adj = 0.09$ and $p\ adj = 0.99$, respectively; Figure 1.3). These differences are further explained by the differences in hemoglobin levels due to tick prevalence. ANOVA results showed a significant difference when ticks were present versus absent on a host across sites ($p = 0.012$), although within sites, this difference was not significant: QEW ($p = 0.492$), and AP ($p = 0.814$; Table 1.8). Since LP mice were all infested, tick prevalence could not be compared, however, LP mice did have the lowest mean hemoglobin levels (Table 1.8).

Discussion

Tick Abundance

I found that blacklegged tick and wood tick abundances negatively affected hemoglobin levels in individual hosts. Most individual mice had hemoglobin levels much greater than 13.2 g/dL, which is the known average of lab-reared deer mice not infested with any ectoparasites (Wiedmeyer *et al*., 2014), suggesting this number is not necessarily accurate for wild-caught mice, so it is unknown if the reduction in hemoglobin levels for the deer mice in this study is adversely affecting their physiology. However, the prevalence and intensity of ticks has been shown to have a negative effect on hemoglobin levels in other mammals. Two studies found that cattle calves infested with ticks had substantially lower hemoglobin levels compared to their non-infested counterparts (Rahman *et al.*, 2009; Kaur *et al*., 2017). Similarly, a study on moose calves (*Alces alces*) that were moderately to severely infested with winter ticks (*Dermacentor albipictus*) had high mortality rates (Jones *et al*., 2019). Results from my study also show that tick prevalence and intensity affect hemoglobin levels in deer mice, which could be detrimental to their physiology. In European hedgehogs (*Erinaceus europaeus*), the blood-feeding activity of two tick species of the genus *Ixodes* (*I. ricinus* and *I. hexagonus*) resulted in lower hemoglobin levels and led to regenerative anemia in several individuals (Pfäffle *et al*., 2009). Gaitan & Millien (2016) found that white-footed mice infested with higher intensities of blacklegged ticks tended to have lower movement rates than mice hosting fewer ticks, suggesting that higher infestation rates incapacitate these mice in some way. This may be related to lower hemoglobin levels, which can reduce motor skills (Nicolas *et al*., 2002). These studies suggest that the negative impacts of blacklegged ticks and wood ticks could be detrimental, although not necessarily fatal. Hersh *et al*. (2014) found that larval tick burdens do not affect the survival of

Peromyscus hosts, and the majority of the ticks found in LP and QEW were found at the larval stage. Since larval ticks cannot transmit pathogens, but rather acquire them during this life stage (and then pass them on as nymphs; Huang *et al*., 2019), Hersh *et al*.'s (2014) study suggests that it is not the tick burden alone which is affecting their hosts.

Since blacklegged ticks were only found in LP, the mice in LP are the only ones whose hemoglobin levels are affected by these ticks. However, the prevalence and intensity of wood ticks at this site was much higher and so it could be that tick abundances and mouse population sources are confounding variables. Although one of the top AICc models suggested that the interaction between wood ticks and blacklegged ticks should also be a variable included in this relationship (Table 1.5), the interaction was not significant ($p = 0.65$; Table 1.6). This suggests that each species is affecting their hosts independently rather than jointly, where there are no confounding effects. Moreover, despite both species having a significant negative effect on hemoglobin levels mice, the abundances of blacklegged ticks and wood ticks varied greatly. Infections can play a role in the hematology of the host, reducing blood cell counts, which can result in anemia (Westblade *et al*., 2017). Some infections can affect a host's hematology, such as *Babesia* protozoans that invade mature red blood cells and directly affect hemoglobin concentrations (Borggraefe *et al*., 2006). Blacklegged ticks can transmit *Babesia* protozoans to their *Peromyscus* hosts, but wood ticks cannot (Westblade *et al*., 2017), so it may be possible that blacklegged ticks are indirectly affecting hemoglobin levels in hosts rather than directly by transmitting pathogens that affect the blood. A recent study found that *Babesia odocoilei* was identified in blacklegged ticks at Long Point (Milnes *et al*., 2019), so it is possible this may be occurring in the focal host species, but this variable was not examined in this analysis.

My results did not find any differences in hemoglobin levels across host's sex. This is supported by several studies, where there was no difference found in hemoglobin levels between male and female deer mice (Wiedmeyer *et al*., 2014; Eleftheriou *et al*., 2020). Similarly, deer mouse hemoglobin levels did not differ across age or body mass. To my knowledge, no studies on hemoglobin levels differing with age or body mass have been conducted in mice or small mammals, but in humans age does not appear to influence hemoglobin levels (Raisinghani *et al*., 2019). Similarly, hemoglobin levels in humans have not been found to be affected by differences in body mass index (Ghadiri-Anari *et al*., 2014), however, tick burdens in *Peromyscus* mice are known to be associated with both body mass and sex (Dallas *et al*., 2012).

Nymph Prevalence

Wood tick nymphs were shown to have a significant effect in the given models. Since nymph ticks are larger than larval ticks and can feed from hosts for longer periods of time (Estrada-Peña & de la Fuente, 2014; Kocan *et al*., 2015), they should consume more blood than their larval counterparts. Nymphs are also more likely to spread diseases, further affecting their host's physiology (Lindquist *et al*., 2016). The larger blood meals and potential effects nymphs have on their host's physiology could explain how the prevalence of wood tick nymphs negatively affects hemoglobin levels. Yet, the majority of nymphs in this study were of this species only so the effects of blacklegged nymphs are unknown. Although there are few studies comparing the differences in blood meal volume between ticks in general, Koch and Sauer (1984) found that adult female wood ticks ingested greater volumes compared to blacklegged ticks. Although there has not been any comparison between larval and nymph ticks of these species, wood ticks tend to be larger than blacklegged ticks when unfed and engorged (Lindquist

et al., 2016). It is thus possible that nymph wood ticks can acquire greater blood volumes than nymph blacklegged ticks. However, there was an imbalanced sample size between wood and blacklegged nymphs, so it could be simply that nymphs in general affect hemoglobin levels more than larval ticks in the study system.

Hemoglobin Variation Across Sites and Tick Prevalence

It was expected that the variation in tick species prevalence between deer mouse populations would affect hemoglobin levels, such that deer mice from a population where ticks have not established would have higher hemoglobin levels than mice from a population where ticks have established. Within sites, it was expected that there would be a difference in hemoglobin levels depending on the tick species intensities. I found that hemoglobin levels significantly differed across sites, but not within sites. In particular, hemoglobin levels differed significantly between LP, which had the highest tick intensity and greatest tick prevalence, and AP, which did not have an established wood tick or blacklegged tick population. Within sites, there was not a significant difference in hemoglobin levels (Table 1.3). However, the sample sizes across sites were imbalanced, and QEW in particular had a small sample size (n = 8), with only two infested hosts, while the one individual infested with a tick in AP, was not infested with a focal species. Due to the constraint in individual site sample size, results within each site may be misleading, and tick infestation effects could be hidden. Even so, my results suggest that in general, tick abundance can reduce a host's hemoglobin level and that tick intensity affects hematology. Within a population, however, hemoglobin levels are not significantly different between infested mice and non-infested mice. It is possible that by having prior exposure (or lacking exposure) to these ectoparasites in a population, the immune defences within the

population are similar (Nédélec *et al.*, 2016). This could result in non-significant differences in hemoglobin levels, regardless of whether or not ticks are prevalent on individual hosts in that population.

Limitations & Future Directions

One variable not accounted for that could have an effect on hemoglobin levels in deer mice was the water intake of individual mice. Water-deprived laboratory mice that were restricted water for over 24 h, were documented to have significant variation in hematocrit, depending on the extent of their deprivation (Bekkevold *et al*., 2013). Mice caught in traps in my study were not deprived of water, but it is possible that while trapped (or pre-trapping) individual mice may have been dehydrated for several hours. Other variables that were not included in this study include the microbiota of the host, which can affect hematology and can differ significantly across sites (Weldon *et al*., 2015). Additional differences in sites must also be considered. Smaller land areas will have smaller mouse (and therefore smaller tick) populations. Therefore, negative impacts caused by tick infestations may hold a greater significance to the overall population in smaller populations, which have less genetic diversity and higher rates of disease transmission (King & Lively, 2012). This could be another reason why hemoglobin levels in LP were lower than the other two sites. Since LP is a much smaller site, having a land area of only 1.50 km^2, the mouse population is not only smaller but denser than QEW or AP. The population density for LP is also inflated in this study, due to flooding in the area, which caused the mice to be more concentrated in dry areas where traps were placed (Wijnhoven *et al*., 2005). Such environmental factors were not applicable in QEW or AP, which are already larger habitats. Sample size imbalance could also play a role, as only seven deer mice from QEW were

included in analyses. These mice also had low intensities of wood ticks, although some mice caught during this study in this area were found with high wood tick infestations, but hemoglobin samples were not taken so these individuals were not included in any analysis. Future research should focus on other factors that may play a role in parasite-host relationships, such as other co-infections and the microbiota, which can affect host physiology and hematology. To gain a better understanding of how naïve host populations may respond to the introduction of tick species to their ecosystem, experiments where these hosts are infected with ticks should be considered in a lab setting.

Conclusion

Overall, hemoglobin levels differed at different degrees of tick abundance across individuals and prevalence across populations, although the most significant difference was between a population with a high infestation rate and a population with little to no infestation, suggesting that low infestation rates do not impact deer mice detrimentally on a hematological level. Tick abundance appeared to contribute most to hemoglobin differences on an individual level. When examining the effects of both tick species together, blacklegged ticks only, and wood ticks only, hemoglobin levels declined significantly when there were greater intensities of ticks on an individual. Despite blacklegged ticks being found at lower intensities than wood ticks, both species significantly affected hemoglobin levels. This may be due to the bacteria blacklegged ticks can carry or a difference in the host's resistance to blacklegged ticks relative to wood ticks, though further studies must be conducted to determine this. These results may suggest that deer mouse populations that will soon be introduced to blacklegged ticks may see their hemoglobin levels decrease more substantially compared to when wood ticks are introduced

to a new mouse population. The susceptibility to tick infestations and their effects has the potential to alter ecosystem processes that can ultimately affect other host species as zoonotic pathogens are transmitted from mice to ticks to other host species at higher trophic levels, including humans. Future studies should consider variables that were not measured in this project, including internal parasites and bacteria that the host may be infected with, as well as their gut microbiota, which can influence the overall biology of the host, including hemoglobin concentrations. More research on the interactions between co-occurring species should be done, considering the impacts of invasive introductions to naïve populations, as well as the co-evolution and local adaptations of parasites and hosts. This, along with experimental studies exposing naïve hosts to new parasites will help increase our understanding of host-parasite interactions.

Literature Cited

Andrews, N. (1997). Iron Deficiency: Lessons from Anemic Mice. *Yale J. of Biol. Med.* 70: 219-226.

Anderson, J, Moore, I, Nagata, B, Ribeiro, J, Valenzuela, J, Sonenshine, D. (2017). Ticks, *Ixodes scapularis*, Feed Repeatedly on White-Footed Mice Despite Strong Inflammatory Response: An Expanding Paradigm for Understanding Tick-Host Interactions. *Frontiers in. Immunology*.8(1784): 1-15. doi: 10.3389/fimmu.2017.01784

Arsnoe, I, Hickling, G, Ginsberg, H, McElreath, R, Tsao, J. (2015). Different Populations of Blacklegged Tick Nymphs Exhibit Differences in Questing Behavior That Have Implications for Human Lyme Disease Risk. *PLoS ONE*. 10(5): e0127450. doi:10.1371/journal.pone.0127450

Baggs, RB, Miller, SA. (1973). Nutritional Iron Deficiency as a Determinant of Host Resistance in the Rat. *The Journal of Nutrition.* 103(11): 1554-1560.

Banfield, AW. (1974). The Mammals of Canada. 1st ed. Toronto and Buffalo: University of Toronto Press.

Bedford, N, Hoekstra, H. (2015). Peromyscus Mice as a Model for Studying Natural Variation. *eLife*. 4, e06813. https://doi.org/10.7554/eLife.06813

Bekkevold, CM, Robertson, KL, Reinhard, MK, Battles, AH, Rowland, NE. (2013). Dehydration Parameters and Standards for Laboratory Mice. *Journal of the American Association for Laboratory Animal Science.* 52(3): 233-239.

Bobbie, C, Schmidt, E, Foley, J, Schulte-Hostedde, A. (2016). The Presence of Parasitic Mites on Small Mammals in Algonquin Provincial Park, Ontario, Canada. *Can. J. Zool.* 95:61-65. doi: 10.1139/cjz-2016-0085

Bordes, F, Blumstein, D, Morand, S. (2007). Rodent Sociality and Parasite Diversity. *Biol. Lett*. 3: 692-694. doi:10.1098/rsbl.2007.0393

Borggraefe, I, Yuan, J, Telford, SR, Menon, S, Hunter, R, Shah, S, Spielman, A, Gelfand, J, Wortis, H, Vannier, E. (2006). *Babesia microti* Primarily Invades Mature Erythrocytes in Mice. *Infection and Immunity*. 74: 3204–3212. doi: 10.1128/IAI.01560-05

Brockhurst, M, Buckling, A, Poullain, V, Hochberg, M. (2007). The Impact of Migration from Parasite-Free Patches on Antagonistic Host-Parasite Coevolution. *Evolution*. 61: 1238. doi:10.1111/j.1558-5646.2007.00087.x

Buchholz, M, Dick, C. (2017). Ecology of Rodent - Ectoparasite Associations in South-Central Kentucky. *Northeastern Naturalist*. 24(2): 97-109. doi: 10.1656/045.024.0201

Bush, AO, Lafferty, KD, Lotz, JM, Shostak, AW. (1997). Parasitology Meets Ecology on its Own Terms: Margolis *et al*. Revisited. *The Journal of Parasitology.* 83(4): 575-583. doi: 10.2307/3284227

Careless, P. (2019, May 30). Phone interview.

Carleton, R. (2008). Ectoparasites Affect Hemoglobin and Percentages of Immature Erythrocytes but Not Hematocrit in Nestling Eastern Bluebirds. *Wilson J. Ornithology.* 120(3): 565-568. doi: 10.1676/07-158.1

Clark, K, Durden, L. (2002). Parasitic Arthropods of Small Mammals in Mississippi. *Journal of Mammalogy*83(4): 1039-1048.

Clow, K, Ogden, N, Lindsay, L. R, Michel, P, Pearl, D, Jardine, C. (2016). Distribution of Ticks and the Risk of Lyme Disease and Other Tick-Borne Pathogens of Public Health Significance in Ontario, Canada. *Vector-Borne and Zoonotic Diseases.* doi: 10.1089/vbz.2015.1890

Clow, K, Leighton, P, Ogden, N, Lindsay, L. R, Michel, P, Pearl, D, Jardine, C. (2017). Northward Range Expansion of *Ixodes scapularis* Evident Over a Short Timescale in Ontario, Canada. *PLoS ONE.* 12(12): e0189393. doi: 10.1371/journal.pone.0189393

Dallas, TA, Foré, SA, Kim, H. (2012). Modeling the influence of *Peromyscus leucopus* body mass, sex, and habitat on immature *Dermacentor variabilis* burden. *Journal of Vector Ecology*. 37(2): 338-341.

Dryden, MW, Gaafar, SM. (1991). Blood Consumption by the Cat Flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). *J. Med. Entomology.* 28: 394–400.

Dubie, T, Grantham, R, Coburn, L, Noden, B. (2017). Pictorial Key for Identification of Immature Stages of Common Ixodid Ticks Found in Pastures in Oklahoma. *Southwestern Entomologist.* 42(1): 1-14.

Dlugosz, E, Downs, C, Khokhlova, I, Degen, A, Krasnov, BR. (2014). Ectoparasite Performance When Feeding on Reproducing Mammalian Females: An Unexpected Decrease When on Pregnant Hosts. *The Journal of Experimental Biology.* 217: 1058-1064. doi:10.1242/jeb.098376

Eleftheriou, A, Luis, AD. (2020). Leukocyte Evaluation of the Free-Ranging Deermouse

(*Peromyscus maniculatus*) from Montana, USA. *Journal of Wildlife Diseases.* 56(3). doi: 10.7589/2019-04-104

Estrada-Peña, A, de la Fuente, J. (2014). The Ecology of Ticks and Epidemiology of Tick-Borne Viral Diseases. *Antiviral Research.* 108: 104-128. doi: 10.1016/j.antiviral.2014.05.016

Falls, JB, Falls, EA, Fryxell, J. (2007). Fluctuations of Deer Mice in Ontario in Relation to Seed Crops. *Ecological Monographs.* 77(1): 19-32

Frost, J. (2019). Regression Analysis (1st ed.). Statistics by Jim. [self-published]. Retrieved from www.statisticsbyjim.com

Fryxell, J, Falls, JB, Falls, EA, Brooks, R. (1998). Long-Term Dynamics of Small-Mammal Populations in Ontario. *Ecology*. 79(1): 213-225

Gaitan, J, Millien, V. (2016). Stress level, Parasite Load, and Movement Pattern in a Small Mammal Reservoir Host for Lyme Disease. *Can. J. Zoology*. 94: 565–573. doi: 10.1139/cjz-2015-0225

Ghadiri-Anari, A, Nazemian, N, Vahedian-Ardakani, H. (2014). Association of Body Mass Index with Hemoglobin Concentration and Iron Parameters in Iranian Population. *ISRN Hematology*. doi: 10.1155/2014/525312

Godinho, L, Cripps, J, Coulson, G, Lumsden, L. (2013). The Effect of Ectoparasites on the Grooming Behaviour of Gould's Wattled Bat (*Chalinolobus gouldii*): An Experimental Study. *Acta Chiropterologica.* 15(2): 463-472. doi: 10.3161/150811013X679080

Grimm, A, Gruber, B, Henle, K. (2014). Reliability of Different Mark-Recapture Methods for Population Size Estimation Tested against Reference Population Sizes Constructed from Field Data. *PLoS ONE.* 9(6): e98840. doi: 10.1371/journal.pone.0098840

Halsey, S, Allan, B, Miller, J. (2018). The Role of *Ixodes scapularis*, *Borrelia burgdorferi* and Wildlife Hosts in Lyme Disease Prevalence: A Quantitative Review. *Tick and Tick-Borne Diseases*. 9: 1103-1114. doi: 10.1016/j.ttbdis.2018.04.006

Harrison, X, Donaldson, L, Correa-Cano, ME, Evans, J, Fisher, DN, Goodwin, CED, Robinson, BS, Hodgson, DJ, Inger, R. (2018). A Brief Introduction to Mixed Effects Modelling and Multi-Model Inference in Ecology. *PeerJ.* 6: e4794. doi: 10.7717/peerj.4794

Harper, J, Austad, S. (2001). Effect of Capture and Season on Fecal Glucocorticoid Levels in Deer Mice (*Peromyscus maniculatus*) and Red-Backed Voles (*Clethrionomys gapperi*). *Gen. Comp. Endocrin.* 123(3): 337-344. doi: 10.1006/gcen.2001.7682

Hamer, S, Hickling, G, Walker, E. Tsao, J. (2014). Increased Diversity of Zoonotic Pathogens and *Borrelia burgdorferi* Strains in Established Versus Incipient *Ixodes scapularis* Populations Across the Midwestern United States. *Infection, Genetics and Evolution.* 27: 531-542. doi: 10.1016/j.meegid.2014.06.003

Hasu, T, Benesh, DP, Valtonen, ET. (2009). Differences in Parasite Susceptibility and Costs of Resistance Between Naturally Exposed and Unexposed Host Populations. *J. Evol. Biol.* 22: 699-707. doi:10.1111/j.1420-9101.2009.01704.x

Hawlena, H, Krasnov, BR, Abramsky, Z, Khokhlova, IS, Saltz, D, Kam, M, Tamir, A, Degen, A. (2006). Flea Infestation and Energy Requirements of Rodent Hosts: Are There General Rules? *Functional Ecology.* 20: 1028-1036.

Hersh, M, LaDeau, S, Previtali, M., Andrea, Ostfeld, R. (2014). When is a Parasite not a Parasite? Effects of Larval Tick Burdens on White-Footed Mouse Survival. *Ecology*. 95(5): 1360-1369

Hofhuis, A, van de Kassteele, J, Sprong, H, van den Wijngaard, C, Harms, M, Fonville, M, Docters van Leeuwen, A, Simoes, M, van Pelt, W. (2017). Predicting the Risk of Lyme borreliosis After a Tick Bite, Using a Structural Equation Model. *PLoS One*. 12(7): e0181807. doi: 10.1371/journal.pone.0181807

Holland, George. (1985). The Fleas of Canada, Alaska and Greenland (Siphonaptera). *Memoirs of the Entomol. Soc. Can.* 117: 3-632.

Huang, C, Kay, S, Davis, S, Tufts, D, Gaffett, K, Tefft, B. (2019). High Burdens of *Ixodes scapularis* larval ticks on white-tailed deer may limit Lyme Disease Risk in a Low Biodiversity Setting. *Ticks Tick Borne Dis.* 10(2): 258-268. doi:10.1016/j.ttbdis.2018.10.013.

Jones, C, Brunner, J, Scoles, G, Owen, J. (2015). Factors Affecting Larval Tick Feeding Success: Host, Density and Time. *Parasites & Vectors*. 8: 340. doi: 10.1186/s13071-015-0955-6

Jones, H, Pekins, P, Kantar, L, Sidor, I, Ellingwood, D, Lichtenwalner, A, O'Neal, M. (2019). Mortality Assessment of Moose (*Alces* alces) Calves During Successive Years of Winter Tick (*Dermacentor albipictus*) Epizootics in New Hampshire and Maine (USA). *Canadian Journal of Zoology*. 97(1): 22-30. doi: 10.1139/cjz-2018-0140

Judy, H, Price, N. (1958). Hemoglobin Level and Red Blood Cell Count Findings in Normal

Women. *JAMA*. 167(5): 563-566. doi:10.1001/jama.1958.02990220033010

Kamler, JF, Pennock, DS, Welch, C, Pierotti, RJ. (1998). Variation in Morphological Characteristics of the White-footed Mouse (*Peromyscus leucopus*) and the Deer Mouse (*P. maniculatus*) under Allotopic and Syntopic Conditions. *The American Midland Naturalist*. 140(1): 170-179. doi: 10.1674/0003-0031(1998)140[0170:VIMCOT]2.0.CO;2

Kaur, D, Jaiswal, K, Mishra, S. (2017). Effect of Tick Infestation on Parameters of Calves. *J. Entomology. Zool. Studies.* 5(4): 107-111

Keirans, J, Hutcheson, HJ, Durden, L, Klompen, JSH. (1996). *Ixodes* (Ixodes) *scapularis* (Acari: Ixodidae): Redescription of all Active Stages, Distribution, Hosts, Geographical Variation, and Medical and Veterinary Importance. *J. Med. Entomol.* 33(3): 297-318. doi: 10.1093/jmedent/33.3.297

Kennedy, MA. (2010). A Brief Review of the Basics of Immunology: The Innate and Adaptive Response. *Vet. Clin. North Am. Small Anim. Pract.* 40(3): 369-379. doi: 10.1016/j.cvsm.2010.01.003.

King, KC, Lively, CM. (2012). Does Genetic Diversity Limit Disease Spread in Natural Host Populations? *Heredity (Edinb)*. 109(4): 199-203. doi: 10.1038/hdy.2012.33

Kocan, K, de la Fuente, J, Coburn, L. (2015). Insights into the Development of *Ixodes scapularis*: A Resource for Research on a Medically Important Tick Species. *Parasites & Vectors*. 8: 592. doi: 10.1186/s13071-015-1185-7

Koch, H, Sauer, J. (1984(. Quantity of Blood Ingested by Four Species of Hard Ticks (Acari: Ixodidae) Fed on Domestic Dogs. *Ann. Entomol. Soc. Am.* 77:142-146. doi: 10.1093/aesa/77.2.142

Krogmann, L, Holstein, J. (2010). "Chapter 18: Preserving and Specimen Handling: Insects and Other Invertebrates". In Eymann, Jutta, Degreef, Jerome, Hauser, Christoph, Monje, Juan Carlos, Samyn, Yves, VandenSpiegel, Didier. (Eds), *Manual on Field Recording Techniques and Protocols for All Taxa Biodiversity Inventories*. (463-481). ABC Taxa

Kutter, MA, Armitage, SA. Maximising Fitness in the Face of Parasites: A Review of Host Tolerance. (2016). *Zoology.* 119(4): 281-289. doi: 10.1016/j.zool.2016.05.011

Larson, SR, Lee, X, Paskewitz, SM. (2018). Prevalence of Tick-borne Pathogens in Two Species of *Peromyscus* Mice Common in Northwestern Wisconsin. *Journal of Medical Entomology.* 55(4): 1002-1010. doi: 10.1093/jme/tjy027

Lindsay, LR, Barker, IK, Surgeoner, GA, McEwen, SA, Elliott, LA, Kolar, J. (1991). Apparent Incompetence of Dermacentor Variabilis (Acari: Ixodidae) and Fleas (Insecta: Siphonaptera) as Vectors of Borrelia Burgdorferi in an *Ixodes dammini* Endemic Area of Ontario, Canada. *Journal of Medical Entomology.* 28(5): 750-753. doi: 10.1093/jmedent/28.5.750.

Lindquist, E, Galloway, T, Artsob, H, Lindsay, LR, Drebot, M, Wood, H, Robbins, R. (2016). *A Handbook to the Ticks of Canada (Ixodida: Ixodidae, Argasidae)*. Biological Survey of Canada.

Mazerolle, M. (2019). AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c). R package version 2.2-2. https://cran.r-project.org/package=AICcmodavg.

McKenzie, K. E. (1998). Chapter 31 Largest Blood Meal. *In University of Florida Book of Insect Records* (pp. 76-79). Gainesville, FL: University of Florida, Department of Entomology & Nematology. Retrieved from: http://entnemdept.ufl.edu/walker/ufbir/files/pdf/UFBIR_Chapter31.pdf

Milnes, E, Thronton, G, Levéillé, A, Delnatte, P, Barta, J, Smith, D, Nemeth, N. (2019). *Babesia odocoilei* and Zoonotic Pathogens Identified from *Ixodes scapularis* Ticks in Southern Ontario, Canada. *Ticks and Tick-Borne Diseases.* 10: 670-676. doi: 10.1016/j.ttbdis.2019.02.016

Minigan, J, Hager, H, Peregrine, A, Newman, J. (2018). Current and Potential Future Distribution of the American Dog Tick (*Dermacentor variabilis*, Say) in North America. *Ticks and Tick-borne Diseases.* 9: 354-362. doi: 10.1016/j.ttbdis.2017.11.012

Mize, E, Tsao, J, Maurer, B. (2011). Habitat Correlates with the Spatial Distribution of Ectoparasites on *Peromyscus leucopus* in Southern Michigan. *Journal of Vector Ecology*. 36(2): 308-320. doi: 10.1111/j.1948-7134.2011.00171.x

Morshed, M, Scott, J, Fernando, K, Mann, R, Durden, L. (2003). Lyme Disease Spirochaete, *Borrelia burgdorferi* Endemic at the Epicenter in Rondeau Provincial Park, Ontario. *J. Med. Entomol.* 40(1): 91-94. doi: 10.1603/0022-2585-40.1.91

National Research Council (US) Institute for Laboratory Animal Research (1996). Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US). doi: 10.17226/5140

Nédélec, Y, Sanz, J, Baharian, G, Szpiech, Z, Pacis, A, Dumaine, A, Grenier, J, Freiman, A,

Sams, A, Hebert, S, Sabourin, A, Luca, F, Blekhman, R, Hernandez, R, Pique-Regi, R, Tung, J, Yotova, V, Barreiro, L. (2016). Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens. *Cell*. 167(3): 657-669. doi: 10.1016/j.cell.2016.09.025

Nichols, J, Hines, J, Pollock, K. (1984). Effects of Permanent Trap Response in Capture Probability on Jolly-Seber Capture-Recapture Model Estimates. *JSTOR.* 48(1): 289-294. doi: 10.2307/3808491

Nicolas, G, Bennoun, M, Porteu, A, Mativet, S, Beaumont, C, Grandchamp, B, Sirito, M, Sawadogo, M, Kahn, A, Vaulont, S. (2002). Severe Iron Deficiency Anemia in Transgenic Mice Expressing Lier Hepcidin. *PNAS*. 99(7): 4596-4601. doi: 10.1073□pnas.072632499

O'Brien, E, Morrison, B, Johnson, LS. (2003). Assessing the Effects of Haematophagous Ectoparasites on the Health of Nestling Birds: Haematocrit vs. Haemoglobin levels in House Wrens Parasitized by Blow Fly Larvae. *Journ. Avian Biol.* 32(1): 73-76. doi: 10.1034/j.1600-048X.2001.320110.x

Ostfeld, R, Miller, M, Schnurr, J. (1993). Ear Tagging Increases Tick (*Ixodes Dammini*) Infestation Rates of White-Footed Mice (*Peromyscus leucopus*). *J. Mamm.* 74(3): 651-655. doi: 10.2307/1382286

Ostfeld, R, Miller, M, Hazler, K. (1996). Causes and Consequences of Tick (*Ixodes scapularis*) Burdens on White-Footed Mice (*Peromyscus leucopus*). *Journ. Mamm.* 77(1): 166-273. doi: 10.2307/1382727

Papkou, A, Gokhale, CS, Traulsen, A, Schulenburg, H. (2016). Host-parasite Coevolution: Why Changing Population Size Matters. *Zoology*. 119(4): 330—338. doi: 10.1016/j.zool.2016.02.001

Parasuraman, S, Raveendran, R, Kesavan, R. (2010). Blood Sample Collection in Small Laboratory Animals. *J. Pharmacology. Pharmacotherapy.* 1(2): 87-93. doi: 10.4103/0976-500X.72350

Park, H, Hong, S, Kim, K, Cho, S, Lee, W, Kim, Y, Lee, S, Park, YK. (2015). Characterizations of Individual Mouse Red Blood Cells Parasitized by *Babesia microti* using 3-D Holographic Microscopy. *Sci. Rep.* 5: 10827. doi: 10.1038/srep10827

Patterson, JE, Neuhaus, P, Kutz, SJ, Ruckstuhl, KE., (2013). Parasite Removal Improves Reproductive Success of Female North American Red Squirrels (*Tamiasciurus hudsonicus*). *PLoS One*. 8(2): e55779. doi: 10.1371/journal.pone.0055779

Pfäffle, M, Petney, T, Elgas, M, Skuballa, J, Taraschewski, H. (2009). Tick-Induced Blood Loss Leads to Regenerative Anaemia in the European Hedgehog (*Erinaceus europaeus*). *Parasitology*. 136: 443–452. doi:10.1017/S0031182009005514

Powell, R, Proulx, G. (2003). Trapping and Marking Terrestrial Mammals for Research: Integrating Ethics, Performance Criteria, Techniques, and Common Sense. *ILAR Journal*. 44(4): 259-276.

PREDICT One Health Consortium. (2016). PREDICT Operating Procedures: Rodent Sampling Methods.

Public Health Ontario. (2019). Ontario Lyme Disease Map 2019 Estimated Risk Areas. *Ontario Agency for Health Protection and Promotion*. Toronto, ON: Queen's Printer for Ontario.

Rahman, M. M, Mostofa, M, Jahan, MS, Kamal, MAHM. (2009). Comparative efficacy of Neem leaves and Ivermectin (Ivomec®) Against Ectoparasites in Calves. *J. Bangladesh Agril. Univ*. 7(1): 73-78. doi: 10.22004/ag.econ.208340

Raisinghani, N, Kumar, S, Acharya, S, Gadegone, A, Pai, V. Does Aging Have an Impact on Hemoglobin? Study in Elderly Population at Rural Teaching Hospital. *J. Family Med. Prim. Care*. 8(10): 3345-3349. doi: 10.4103/jfmpc.jfmpc_668_19

Rand, P, Lacombe, E, Smith, R, Rich, S, Kilpatrick, W, Dragoni, C, Caporale, D. (1993). Competence of *Peromyscus maniculatus* (Rodentia: Cricetidae) as a Reservoir Host for *Borrelia burgdorferi* (Spirochaetares: Spirochaetaceae) in the Wild. *Journal of Medical Entomology*. 30(3): 614-618. doi: 10.1093/jmedent/30.3.614

Randolph, JC. (1980). Daily Energy Metabolism of Two Rodents (*Peromyscus leucopus* and *Tamias striatus*) in Their Natural Environment. *Physiol Zool* 53: 70-81.

Reiczigel, J, Marozzi, M, Fabian, I, Rozsa, L. (2019). Biostatistics for Parasitologists – A Primer to Quantitative Parasitology. *Trends in Parasitology*. 35(4): 277-281.

Rosales, F, Jang, J, Pinero, D, Erikson, K, Beard, J, Ross, C. (1999). Iron Deficiency in Young Rats Alters the Distribution of Vitamin A Between Plasma and Liver and Between Hepatic Retinol and Retinyl Esters. *The Journal of Nutrition*. 129(6): 1223-1228.

Rynkiewicz, E, Hawlena, H, Durden, L, Hastriter, M, Demas, G, Clay, K. (2013). Associations

Between Innate Immune Function and Ectoparasites in Wild Rodent Hosts. *Parasitol. Res.* 112: 1763-1770. doi: 10.1007/s00436-013-3335-1

Schmidt, E, Mykytczuk, N, Schulte-Hostedde, A. (2019). Effects of the Captive and Wild Environment on Diversity of the Gut Microbiome of Deer Mice (*Peromyscus maniculatus*). *ISME Journal*. 13: 1293-1305. doi: 10.1038/s41396-019-0345-8

Shaw, M, Keesing, F, McGrail, R, Ostfeld, R. (2003). Factors Influencing the Distribution of Larval Blacklegged Ticks on Rodent Hosts. *Am. J. Trop. Med. Hyg.* 68(4): 447-452. doi: 10.4269/ajtmh.2003.68.447

Sheath, D, Dick, J, Dickey, J, Guo, Z, Andreou, D, Britton, JR. (2018). Winning the Arms Race: Host–Parasite Shared Evolutionary History Reduces Infection Risks in Fish Final Hosts. *Biology Letter*. 14(7): 20180363. doi: 10.1098/rsbl.2018.0363

Sonenshine, D. (2018). Range Expansion of Tick Disease Vectors in North America: Implications for Spread of Tick-Borne Disease. *Int. J. Environ. Res. Public Health.* 15: 478. doi:10.3390/ijerph15030478

Torre, I, Freixas, L, Arrizabalaga, A, Diaz, M. (2016). The Efficiency of Two Widely used Commercial Live-traps to Develop Monitorting Protocols for Small Mammal Biodiversity. *Ecological Indicators*. 66: 481-487. doi: 10.1016/j.ecolind.2016.02.017

Tufts, D, Revsbech, I, Cheviron, Z, Weber, R, Fago, A, Storz, J. (2013). Phenotypic Plasticity in Blood-Oxygen Transport in Highland and Lowland Deer Mice. *J. Exper. Biol.* 216: 1167-1173. doi: 10.1242/jeb.079848

Watson, TG, Anderson, RC. (1976). *Ixodes scapularis* Say on White-tailed Deer (*Odocoileus virginianus*) from Long Point, Ontario. *J. Wildl. Dis.* 12: 66-71.

Weber, M, Stevens, R, Diniz-Filho, JAF, Grelle, CEV. (2016). Is There a Correlation Between Abundance and Environmental Suitability Derived from Ecological Niche Modelling? A Meta-Analysis. *Ecography*. 40(7): 817-828. doi: 10.5061/dryad.g2fd2

Weixelbaumer, K, Raeven, P, Redl, H, van Griensven, M, Bahrami, S, Osuchowski, M. (2010). Repetitive Low-Volume Blood Sampling Method as a Feasible Monitoring Tool in a Mouse Model of Sepsis. *SHOCK*. 34(4): 420-426. doi: 10.1097/SHK.0b013e3181dc0918

Weldon, L, Abolina, S, Lenzi, L, Bourne, C, Riley, E, Viney, M. (2015). The Gut Microbiota of Wild Mice. *PLoS One.* 10(8): e0134643. doi: 10.1371/journal.pone.0134643

Westblade, L, Simon, M, Mathison, B, Kirkman, L. (2017). *Babesia microti*: from Mice to Ticks

to an Increasing Number of Highly Susceptible Humans. *Journal of Clinical Microbiology*. 55(10): 2903-2912. doi: 10.1128/JCM.00504-17

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Wickham, H, Averick, M, Bryan, J, Chang, W, D'Agostino McGowan, L, Francois, R, Grolemund, G, Hayes, A, Henry, L, Hester, J, Kuhn, M, Pedersen, TL, Miller, E, Bache, SM, Muller, K, Ooms, J, Robinson, D, Seidel, DP, Spinu, V, Takahashi, K, Vaughan, D, Wilke, C, Woo, K, Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*. 4(43): 1686. https://doi.org/10.21105/joss.01686

Wiedmeyer, C, Crossland, J, Veres, M, Dewey, M, Felder, M, Barlow, S, Vrana, P, Szalai, G. (2014). Hematologic and Serum Biochemical Values of 4 Species of Peromyscus Mice and Their Hybrids. J. Am. Assoc. Lab. Anim. Sci. 53(4): 336-343.

Wijnhoven, S, van der Velde, G, Leuven, R, Smits, A. (2005). Flooding Ecology of Voles, Mice, and Shrews: The Importance of Geomorphological and Vegetational Heterogeneity in River Floodplains. *Acta Theriologica*. 50: 453-472. doi: 10.1007/BF03192639

Figures and Tables

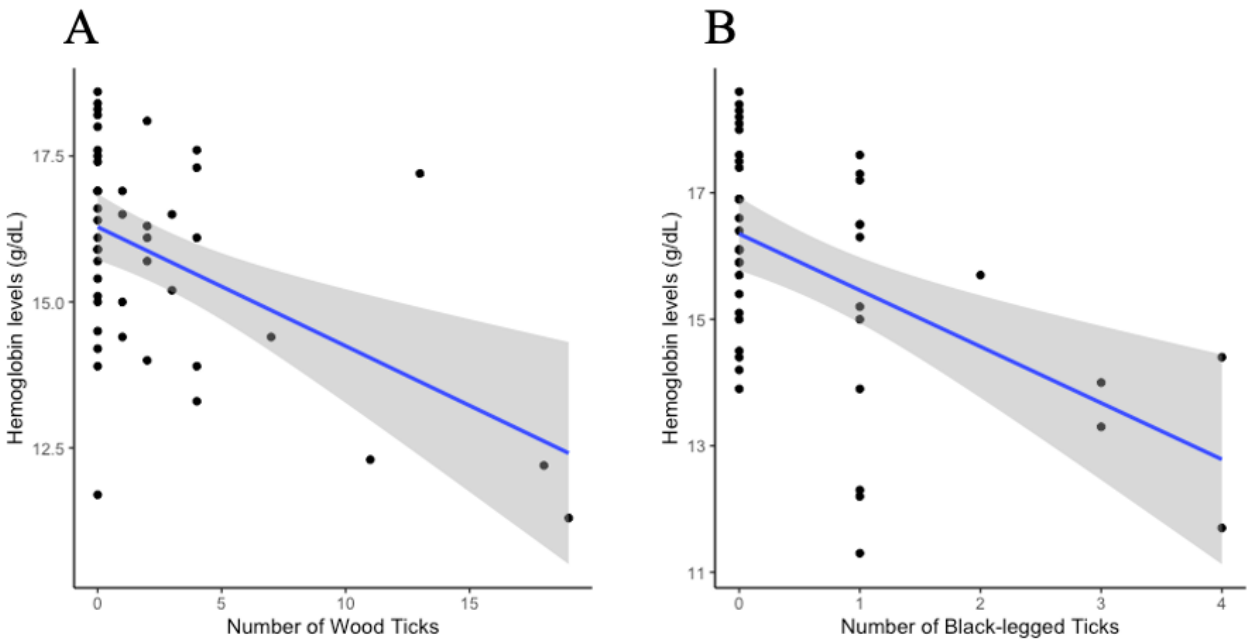

Figure 1.1. General linear regression results depicting the effect of tick abundance on hemoglobin levels in deer mice (*Peromyscus maniculatus*; n = 44). Each point indicates an individual host. Shaded area indicates the 95% confidence interval. A) Wood tick abundance is significantly affecting hemoglobin levels (p-value < 0.01) as is B) blacklegged tick abundance (p-value < 0.01;Table 1.5).

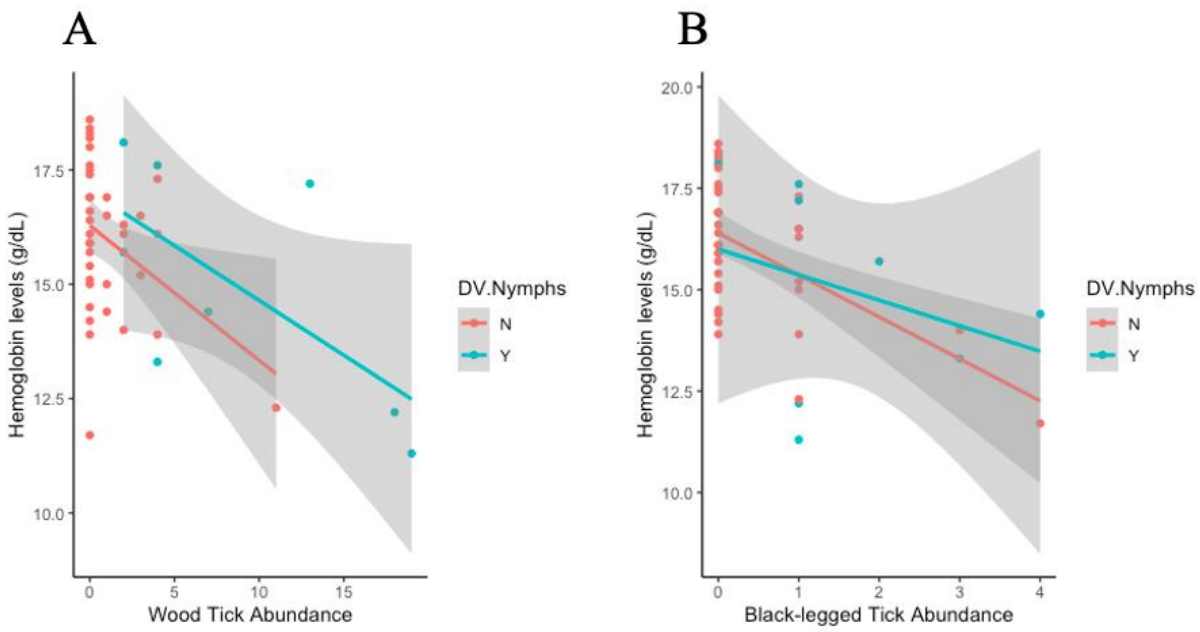

Figure 1.2. General linear regression results depicting the effect of tick abundance on hemoglobin levels in deer mice (*Peromyscus maniculatus*; n = 44) when wood (*D. variabilis*) nymphs are present on the host. Blue points indicate that wood nymphs were prevalent on that individual host. Shaded area indicates the 95% confidence interval. A) Wood tick abundance was significantly affecting hemoglobin levels (p-value < 0.01) as was B) blacklegged tick (*I. scapularis*) abundance (p-value < 0.01), but their interaction was not (p-value = 0.65). The presence of wood nymphs (DV Nymphs) was significant (p < 0.05; Table 1.5).

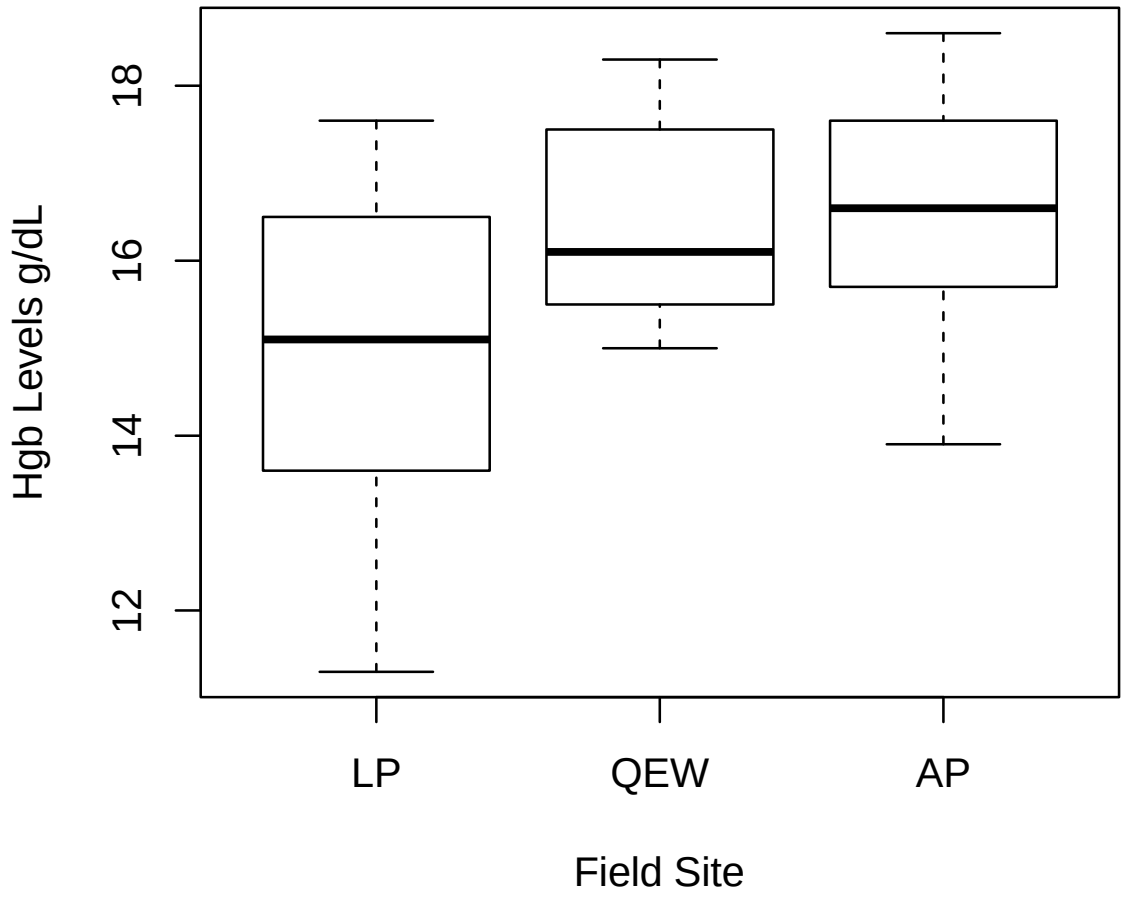

Figure 1.3. Hemoglobin levels across each site (LP: n = 20. QEW: n = 7, AP: n = 17). ANOVA results show a significant difference across sites (p = 0.01). Based on a Tukey's Honest Significance test the comparative differences between sites are LP-QEW (p adj = 0.09), LP-AP (p adj = 0.01), and QEW-AP (p adj = 0.99). LP = Long Point Provincial Park, QEW = Queen Elizabeth II Wildlands Provincial Park, AP = Algonquin Provincial Park.

Table 1.1. Results for general linear model examining the effects of deer mice (*Peromyscus maniculatus*) sex, age, body mass, and population (field site) on hemoglobin levels. This model had a residual standard error of 1.748 on 39 degrees of freedom. Multiple R-squared: 0.1973, Adjusted R-squared: 0.1149. Bolded values indicate variables where $p < 0.05$.

Variable	Estimate	Std. Error	t - value	Pr(>|t|)
Intercept	14.86	1.30	11.44	$4.93e^{-14}$
Host sex	-0.35	0.54	-0.65	0.52
Host age	0.06	0.84	0.07	0.94
Host body mass	-0.01	0.06	-0.07	0.94
Field site	0.59	0.20	2.92	**0.006**

Table 1.2. Results for general linear model examining the effects of deer mice (*Peromyscus maniculatus*) sex, age, body mass, and population (field site) on general tick abundance (both blacklegged and wood ticks together). This model had a residual standard error of 4.331 on 39 degrees of freedom. Multiple R-squared: 0.6151, Adjusted R-squared: 0.5756. Bolded values indicate variables where $p < 0.05$.

Variable	Estimate	Std. Error	t - value	Pr(>|t|)
Intercept	11.32	3.22	3.52	0.001
Host sex	0.92	1.34	0.68	0.50
Host age	3.46	2.07	1.67	0.10
Host body mass	-0.06	0.14	-0.40	0.69
Field site	-3.80	0.51	-7.60	**$3.28e^{-9}$**

Table 1.3. Study sites, geographic coordinates, land area of parks and field sites, as well as average population size across (measured across sessions, determined by Lincoln-Peterson index) to determine population densities.

Sites	Latitude and Longitude	Total Land Area of Provincial Park	Land Area of Field Site	Average Population Size	Population Density per Field Site
Long Point Provincial Park (LP)	42.5817° N, -80.3952° W	1.50 km^2	0.31 km^2	28 mice	90 mice/ km^2
Queen Elizabeth II Wildlands Provincial Park (QEW)	44.7534° N, -78.7844° W	335.05 km^2	0.47 km^2	18 mice	38 mice/ km^2
Algonquin Provincial Park (AP)	45.3402° N, -78.2618° W	7723 km^2	4.39 km^2	11 mice	2.5 mice/ km^2

Table 1.4. Prevalence and mean intensity (average abundance) of ectoparasites found on deer mice (*Peromyscus maniculatus)* across all sites and per site. Numbers in parentheses indicate the sample size of deer mouse hosts for each row. Since mite groups were only measured as present (1) or absent (0) for all analysis, their intensities are unknown. LP = Long Point Provincial Park, QEW = Queen Elizabeth II Wildlands Provincial Park, AP = Algonquin Provincial Park.

	I. scapularis	*D. variabilis*	*O. leucopus*	Trombiculidae *sp.*	*Neotrombicula sp.*
All Sites (44)					
Prevalence	37.8%	48.9%	20.0%	33.3%	17.8%
Mean intensity	0.63 +/- 1.06	2.45 +/- 4.53	0.34 +/- 0.89	N/A	N/A
LP (20)					
Prevalence	85.0%	95.0%	10.0%	0.0%	5.0%
Mean intensity	1.40 +/- 1.19	5.10 +/- 5.67	0.15 +/- 0.49	N/A	N/A
QEW (7)					
Prevalence	0.0%	28.6%	12.5%	85.7%	0.0%
Mean intensity	N/A	0.86 +/- 1.57	0.71 +/- 1.89	N/A	N/A
AP (17)					
Prevalence	0.0%	0.0%	35.3%	47.1	41.2%
Mean intensity	N/A	N/A	0.41 +/- 0.62	N/A	N/A

Table 1.5. AICc models with lowest scores (Δ AICc < 3) indicating what variables are affecting hemoglobin levels in deer mice (*Peromyscus maniculatus*). IS = blacklegged ticks (*Ixodes scapularis*) abundance, DV = wood ticks (*Dermacentor variabilis*) abundance, and DV Nymphs = wood tick nymph presence.

Model	AICc	Δ AICc	AICc Wt	Cum. Wt	LL
DV + IS + DV Nymphs	161.97	0.00	0.29	0.29	-75.20
DV + IS + DV:IS + DV Nymphs	164.43	2.46	0.09	0.59	-75.08

Table 1.6. Top two models based on AICc scores for what variables affect hemoglobin levels in deer mice (*Peromyscus maniculatus*). Bolded rows indicate variables where $p < 0.05$. AICc scores for each model are in parentheses. IS = blacklegged ticks (*Ixodes scapularis*) abundance, DV = wood ticks (*Dermacentor variabilis*) abundance, and DV Nymphs = wood nymph presence.

Model	Estimate	Std. Error	t - value	Pr(>|t|)	R^2
DV + IS + DV Nymphs (161.97)	Intercept: 16.60 DV: -0.25 IS: -0.88 DV Nymphs: 1.95	Intercept: 0.26 DV: 0.06 IS: 0.23 DV Nymphs: 0.77	Intercept: 63.87 DV: -4.01 IS: -3.90 DV Nymphs: 2.55	Intercept: $< 2e^{-16}$ **DV: 0.0003** **IS: 0.0004** **DV Nymphs: 0.01**	Multiple: 0.471 Adjusted: 0.431
DV + IS + DV:IS + DV Nymphs (164.43)	Intercept: 16.63 DV: -0.28 IS: -0.96 DV:IS: 0.04 DV Nymphs: 1.85	Intercept: 0.27 DV: 0.09 IS: 0.29 DV:IS: 0.08 DV Nymphs: 0.80	Intercept: 61.40 DV: -2.97 IS: -3.33 DV:IS: 0.46 DV Nymphs: 2.30	Intercept: $< 2e^{-16}$ **DV: 0.005** **IS: 0.002** DV:IS: 0.65 **DV Nymphs: 0.03**	Multiple: 0.474 Adjusted: 0.420

Table 1.7. Pearson correlation coefficient values between predictor variables and hemoglobin levels in deer mice (*Peromyscus maniculatus*). The variable 'Tick abundance' includes the abundance of both tick species together, in order to compare correlation variation when blacklegged ticks (*Ixodes* scapularis; IS) and wood ticks (*Dermacentor variabilis*; DV) are interacting together.

Predictor variable	Pearson correlation coefficient (r)
Tick abundance	-0.49
IS abundance	-0.51
DV abundance	-0.50
DV Nymph presence	-0.21

Table 1.8. Mean hemoglobin levels from each site including all deer mice (*Peromyscus maniculatus*) hosts, only hosts infested with ticks, and only hosts not infested with ticks. P values given are one-way ANOVA results between hemoglobin levels of deer mice infested with ticks versus mice without ticks. Bolded values indicate variables where $p < 0.05$. LP = Long Point Provincial Park, QEW = Queen Elizabeth II Wildlands Provincial Park, AP = Algonquin Provincial Park.

Sites	Mean Hgb levels (g/dL)	Mean Hgb levels with tick infested mice only (g/dL)	Mean Hgb levels with non-infested mice only (g/dL)	Pr(>F)
All Sites	15.78 +/-1.86	15.17 +/-2.00	16.46 +/-1.46	**0.012**
LP	14.89 +/-1.97	14.89 +/-1.97	N/A	N/A
QEW	16.49 +/- 1.33	17.1 +/-1.41	16.24 +/-1.38	0.492
AP	16.55 +/-1.47	16.90*	16.53 +/-1.52	0.814

* only one tick present at this site; not a focal species

Chapter II:

Ectoparasite community assemblages found on *Peromyscus maniculatus* across varying degrees of ixodid tick exposure

Abstract:

Ectoparasites are fundamental to ecosystems, playing a key role in trophic regulation. Fleas, mites, and ticks are common hematophagous ectoparasites that infest shared mammalian hosts. One common host in Ontario, Canada, is the deer mouse (*Peromyscus maniculatus*). As the climate warms and the geographic ranges of blacklegged ticks (*Ixodes scapularis*) and wood ticks (*Dermacentor variabilis*) expand, their introduction to new ecosystems may alter current ectoparasite communities. At three different sites where exposure to ticks varied (both in terms of tick diversity and abundance), ectoparasite community structures found on deer mouse hosts were compared and analyzed, focusing on species co-occurrences and habitat partitioning on the host. I predicted that when varying tick species were prevalent, ticks would dominate the micro-habitat niches often inhabited by other parasites, thereby significantly altering parasite community structure. My results suggest that blacklegged ticks and wood ticks could have a positive association with each other, but a negative or random association with other ectoparasite species, even when they do not occupy the same micro-habitat niche. Sampling site played a significant role in community assemblages as well, possibly due to the differences in tick exposure. As the ticks' ranges expand and they become more abundant, it is important to understand how their prevalence can potentially alter the dynamics in an ectoparasite community, affecting the transmission of pathogens that may spread within an ecosystem, from one host to another.

Key Words: Community ecology, fleas, mites, parasite-host interactions, *Peromyscus maniculatus*, ticks

Introduction

Parasite species rarely occupy a host in isolation (Hellard *et al*., 2015). Rather, assemblages of parasites infest an individual host (Bordes *et al*., 2007; Kappeler *et al*., 2015). Parasites experience intraspecific and interspecific relationships and directly or indirectly compete with each other (Bordes & Morand, 2011). Interspecific interactions may be synergistic, where one parasite species facilitates the other, or antagonistic, where one species suppresses the other (Fenton *et al*., 2010; Hellard *et al*., 2015). For instance, hard ticks (Acari: Ixodidae) can promote infestation rates of other tick species (Lutermann *et al*., 2015), and parasitic mites (Acari: Mesostigmata) have been found to act as biological controls to mosquito infestations (Atwa *et al*., 2017). Fleas (Insecta: Siphonaptera) and mites have also been found to experience antagonistic relationships with ticks that they share a host with (Krasnov *et al*., 2010b; Lutermann *et al*., 2015; Hoffman *et* al., 2016).

Not all co-occurring species may have a clearly defined synergistic or antagonistic relationship, however. In one study analyzing the concurrent feeding of two tick species (blacklegged tick, *Ixodes scapularis* and winter tick, *Dermacentor albipictus*) on white-tailed deer (*Odocoileus virginianus*), blacklegged ticks were more abundant and prevalent in general, although their prevalence did not alter the distribution of winter ticks (Baer-Lehman *et al*., 2012). Blacklegged ticks can be the dominant species on a host when present, although other ectoparasites may be more competitive as blacklegged ticks tend to have lower success rates of attaching to a host (Baer-Lehman *et al*., 2012). Since it is rare for only one ectoparasitic species to be infesting a host (Bordes & Morand, 2011), and there is a possibility of interspecific competition occurring between ectoparasites (Baer-Lehman *et al*., 2012), co-occurrences can

affect parasite species diversity on a host, which in turn can cause cumulative effects of parasitism on the ecology and evolution of the host (Bordes & Morand, 2011).

Differences in ecology between parasites may affect interactions between parasitic species sharing a host. Fleas migrate between their host and the host's nest, which can affect their interspecific relationships with other ectoparasites as they are not consistently sharing the host (Krasnov *et al*., 2010a). Energy requirements for immune responses to parasites can also differ depending on the host species. In one study where two gerbil species (*Gerbillus andersoni* and *Gerbillus dasyurus*) were exposed to fleas, one species was significantly affected by parasitizing fleas more so than the other, suggesting that even closely related organisms may not have similar immune responses to certain parasites (Hawlena *et al*., 2006).

Host associations within an ecosystem and seasonality differences in macro-habitats (*i.e.*, habitats with complex flora and fauna; landscape ecosystems) may also influence variation in host preferences by parasites (Bouchard *et al*., 2011). This may result in differences in parasite loads between conspecific hosts from different habitats. For instance, Halsey and Miller (2018) explored host-tick associations between blacklegged ticks and two of their hosts – white-footed mice (*Peromyscus leucopus*) and white-tailed deer – over time. Their results suggest that when parasites and hosts co-evolve, the hosts are able to adapt a more effective grooming technique. At the same time, an adaptive behavioural strategy for ectoparasites to combat host grooming is to feed on areas where it is difficult to groom (Fracasso *et al*., 2019). Many ectoparasites can feed from several different micro-habitat niches on their hosts (Balashov, 1972), however, depending on the parasite's size and mobility, as well as the host's defense mechanisms, parasites tend to prefer specific parts of the host's body and will partition the micro-habitat with their competitors (Poulin, 2011).

The purpose of this study is to compare the community assemblage of ectoparasites on deer mice from three localities across Ontario, examining the differences between communities with and without ticks. Specifically, I compared ectoparasite attachment sites on hosts, species distributions between sampling landscape sites, and co-occurrences of ectoparasites with varying tick prevalence. I predicted that when ticks were occurring on a host, these ectoparasites would dominate the micro-habitat niches often inhabited by other parasites. There has been evidence that blacklegged ticks can be the more abundant species on a host relative to another tick species, but the impact of tick prevalence in general on the co-occurrence patterns of other ectoparasites is currently unknown (Baer-Lehman *et al*., 2012). I predicted that when ticks are occurring on an individual host, there would be a negative co-occurrence with other ectoparasites, particularly in the preferred areas on the mouse's body where several species are known to congregate (*i.e.* the ears, where ticks and mites are commonly found; Ostfeld *et al*., 1993; Baer-Lehman *et al*., 2012; Bobbie *et al*., 2016) due to interspecific competition. Knowing how the ectoparasite community changes when ticks are a part of the community is important for understanding how the mouse host will be influenced by an encounter with a newly introduced parasite that can affect parasite diversity as well as the host's ecology and evolution (Bordes & Morand, 2011).

Methods

All methods in this study were approved by the Animal Care Committee (ACC) at Laurentian University, file number 6017269.

Field Methods

Three sites were visited for data and specimen collection from May-August 2019. The sites chosen for this experiment took into consideration the current and predicted ranges of blacklegged ticks and wood ticks in Ontario, Canada (Hamer *et al.*, 2014; Clow *et al*., 2016; 2017; Minigan *et al*., 2018), and were areas within the geographical habitat range of deer mice (Bedford and Hoekstra, 2015). These sites were Long Point Provincial Park (LP; 42.5817° N, -80.3952° W), Queen Elizabeth II Wildlands Provincial Park (QEW; 44.7534° N, -78.7844° W) and Algonquin Provincial Park (AP; 45.3402° N, -78.2618° W). At LP, populations of blacklegged ticks and wood ticks are well established (Watson & Anderson, 1976; Lindsay *et al*., 1991). Only wood ticks are known to be established at QEW (Minigan *et al.*, 2018; P. Careless, 2019, pers. comm.), and neither blacklegged ticks or wood ticks have established in or colonized AP (Hamer *et al.*, 2014; Clow *et al*., 2016; 2017; Minigan *et al*., 2018; Public Health Ontario, 2019).

Traplines were set up at QEW and LP, modelled after the long-term traplines used in AP (Fryxell *et al.* 1998; Falls *et al*., 2007). To replicate these lines, three traplines at least 0.3 km apart from each other were set at the field sites, with twenty Longworth traps placed (Penlon Ltd., Oxford, U.K) per line. Each trapline was 90 m in length and had two Longworth traps 10 m apart from each other every 10 m (Fryxell *et al.*, 1998; Falls *et al*., 2007). Traps were placed in similar forest types across sites to maintain a consistent or similar habitat, although sites were from different ecozones (Crins *et al*., 2009). Three of the long-term traplines at AP were exclusively used for this project during this time as well. Traps were baited with water-soaked sunflower seeds and were set half an hour before sunset (2000h-2100h) and checked half an hour before sunrise (0430h-0530h). The traps were checked five days each week; three days

consecutively, with a one-day break, and two more consecutive days to reduce the amount of stress in the mice from repeated captivity or trap response behaviour (Nichols *et al*., 1984). To account for change in seasonality, and the difference in parasite assemblages that occurs within the spring-summer months, I alternated between these three sites in ascending latitude, spending one week at each site, three times, for a total of three weeks per site.

Deer mice were distinguished from white-footed mice by morphological characteristics: differences in ear length (in mm; Kamler et al., 1998), tail bi-colouration, tail "penciling" (J. Bowman, 2019, pers. comm.), and degree of dorsoventral colouration (Buchholz & Dick, 2017). Deer mice were collected from traps at each site, where the right leg of each mouse was shaved to denote individuals as recaptures. Recaptures were not included in any statistical analysis to avoid bias. Individual mice were examined for 60 seconds for sampling of ectoparasites (Patterson *et al*., 2013). Individual arthropod specimens were combed off the host using an ethanol sterilized louse comb (Hawlena *et al*., 2006; Patterson *et al*., 2013) or plucked off using sterilized tweezers (Bobbie *et al*., 2016) and placed in a collection vial, preserved in 80% ethanol (Krogmann & Holstein, 2010). During this time, the position on the mouse (ears, head, middle, back, and extremities) where parasites were found was recorded. Extremities included forelimbs, hindlimbs, genitals, and tail. Tick identifications were done by hand using several guides (Lindquist *et al*., 2016; Dubie *et al*., 2017), and were later confirmed by Dr. Robbin Lindsay from the National Microbiology Laboratory (Winnipeg, Manitoba, Canada). Fleas were identified using an identification key (Holland, 1985), and later confirmed by Dr. Terry Galloway (University of Manitoba). Dr. Heather Proctor from the University of Alberta was able to identify two taxonomically unique mite groups from the Trombiculidae family, one of which she identified to the genus; *Neotrombicula*.

Age of mice was determined by body mass; mice that were 15 g or less were considered juveniles (Banfield, 1974; Schmidt *et al*., 2019). Sex and reproductive status (non-reproducvtive/reproductive) were determined visually. Enlarged testes in males and perforated vagina and presence of nipples in females indicated reproductive individuals (Gaitan & Millien, 2016). No pregnant females were included in this study. Other external factors were also documented, such as field sites where samples were collected, and the temporal session when the samples were collected. This was divided by early (May-June), mid (June-July), and late (July-August) summer of 2019.

Statistical Methods

Measurements of prevalence and intensity of ectoparasites were completed using QPweb (Reiczigel *et al*., 2019). For the purpose of this study, mean intensity refers to the mean number of ectoparasites found on mice across the population, prevalence refers to the percentage of hosts that have the specified parasite infesting them in a population, and abundance refers to the number of ectoparasites found on individuals (Bush *et al*., 1997). Statistical analyses were conducted in the R environment (R core Team 2019 [v3.6.1]). For all statistical analyses, the ectoparasite species and groups included were: *I. scapularis, D. variabilis, O. leucopus, Neotrombicula sp*., and Trombiculidae *sp*. Although in AP, the squirrel tick (*Ixodes angustus*) was also present, there was only one instance of this species present on a single host (n = 1). Thus, this species and its host were not included in any ordination or co-occurrence analyses.

Constrained and unconstrained ordination methods were used to determine relationships between ectoparasites using the 'vegan' package (Oksanen *et al.*, 2019 [v3.6.1]). Specifically, correspondence analyses (CA) were performed using presence-absence data of ectoparasites on

hosts. An unconstrained CA was conducted comparing the ectoparasite species and where they were found on individual mice micro-habitat sites (head, ears, middle, back, extremities, and unknown). Species and site scores for each axis were recorded where site scores were weighted averages of species scores.

A canonical correspondence analysis (CCA) was also conducted, where the analysis was constrained by environmental factors: host body mass, sex, age, reproductive status, field site, and session. Eigenvalues were extracted from these data (both constrained and unconstrained). A permutation test for constrained correspondence analysis (permutations = 9999) was performed to test the specified environmental factors for significance to ectoparasite species distributions (Oksanen, 2019). This was done using a significance test for each variable as a 'marginal' term in the model such that separate significance tests for each variable would be performed, rather than assessing environmental constraints sequentially, where the order of terms can influence significance (Legendre *et al*., 2011). A similar test was conducted to determine significance by CCA axes as well, where permutations = 9999 (Legendre *et al*., 2011; Oksanen, 2019). A non-metric multidimensional scaling (NMDS) model was also performed on parasite loads across individual hosts as well to compare dissimilarity of ectoparasite communities between field site locations (LP, QEW, AP). NDMS is a dissimilarity index where ordination spacing represents distance in a dissimilarity index (Oksanen, 2019). For this analysis, each host was considered a unique site to determine if there were similar species found co-occurring across sampling field sites.

A species pair-wise co-occurrence analysis was also conducted using the package 'cooccur' (Griffith *et al*., 2016 [v3.1]) to determine if co-occurrences between species were positive, negative, or random across micro-habitats (individual hosts) based on a statistical

probabilistic co-occurrence model. Here, each mouse was considered a site, where there was no expectation that parasites should be found at the same macro-habitat location (field site). This R implementation is metric-free, distribution-free, and randomization-free (Griffith *et al*., 2016) and has a similar methodology as a matrix-level approach (Arita, 2016). In this package, the algorithm calculates the observed and expected frequencies of co-occurrences between each pair of species based on presence/absence data distributed among a set of sampling sites (*i.e*., deer mouse hosts; Veech, 2013; Griffith *et al*., 2016). The analysis reports significant positive or negative co-occurrences as well as random co-occurrences based on differences between observed and calculated expected co-occurrences across sites (Griffith *et al*., 2016; Adair *et al*., 2018). Expected co-occurrence (Q_{exp}) is measured, such that:

$$Q_{exp} = \Sigma(P_j \times j)$$

where P_j is the probability of co-occurrence at exactly j sites, and j is the number of co-occurrence sites (Veech, 2013). When co-occurrences are extremely low or extremely high relative to the expected co-occurrence, these associations are considered to be negatively or positively co-occurring, respectively (Veech, 2013; Griffith *et al*., 2016). When each host is regarded as a micro-habitat, this analysis does not take into consideration the population source (macro-habitat; LP, QEW, AP) that the host is from. Further analyses were conducted within each population source as well to constrain the results by field site for comparison.

Results

A sample of 70 individual deer mice hosts across field sites were included in analyses. This included 23 female mice and 47 male mice (Table 2.1). Dr. Terry Galloway identified one flea species from the collected samples across sites: *Orchopeas leucopus*. Dr. Heather Proctor

was able to identify two taxonomically separate groups from the mite specimens collected. One group she identified as the genus *Neotrombicula.* The other group could not be identified beyond family; Trombiculidae. For the purpose of this study, the mite groups will be defined as '*Neotrombicula sp*.' and 'Trombiculidae *sp*.', respectively. Across hosts, 162 wood ticks and 39 blacklegged ticks were collected, none of which were adults, along with 22 *O. leucopus*. There were 26 instances of Trombiculidae *sp*. and 10 of *Neotrombicula sp*. present on deer mice (Table 2.2). It should be noted that each field site had a different assemblage of parasites. Trombiculidae *sp.* was not present at LP, *Neotrombicula sp.* and blacklegged ticks were not present at QEW, and neither blacklegged ticks nor wood ticks were present at AP. *O. leucopus* was present at all sites (Table 2.2).

An unconstrained correspondence analysis plot of ectoparasite prevalence based on their location on deer mice showed that both tick species and mite groups aggregated at the ears of their hosts, while *O. leucopus* tended to be found across extremities and back (Figure 2.1). Eigenvalues in the unconstrained model (CA) indicate that axis CA1 explained approximately 74% of the variation within the dataset, with *O. leucopus* being highly differentiated from the other species, having the lowest value and only negative axis CA1 value (-2.240). *O. leucopus'* preferred micro-habitats (back and extremities) also had the most differentiated site scores on axis CA1 (-2.338 and -1.953, respectively; Table 2.3). A NMDS model was performed to determine the species distributions of ectoparasite groups or species across field sites. Blacklegged ticks were only prevalent in LP, although LP presented a somewhat similar species distribution with QEW (Figure 2.2; see the overlapping ellipses) as wood ticks were prevalent at both sites (Figure 2.2; Table 2.2). However, there was greater overlap between the parasite distribution of QEW and AP (Figure 2.2), suggesting a larger overlap in ectoparasite

communities, as both sites have similar prevalence of *O. leucopus* and Trombiculidae *sp*. (Table 2.2). The greatest disparity in species distributions was between LP and AP (Table 2.2).

When comparing eigenvalues of constrained and unconstrained models, the unconstrained model had a larger proportion rank (CA: 0.624, CCA: 0.376) and inertia (CA: 1.259, CCA: 0.759), however, the axis CCA1 in the constrained model explains the greatest proportion of variance overall in the constrained model (0.333; Table 2.4). On this axis, field site had the highest biplot score for constraining variables (0.991) and both tick species had the lowest species scores (*I. scapularis*: -0.608, *D. variabilis*: -0.400; Table 2.5). A permutation test examining the importance of constraining environmental variables assessed that field site significantly affected the ectoparasite communities across hosts ($p = 0.0001$; Table 2.6). A permutation test determined that axis CCA1 was the most significant axis in this model as well ($p = 0.0001$; Table 2.6).

My analysis of relationships among ectoparasites detected 10 pairwise associations of statistically significant species co-occurrences when considering hosts as unique habitats/sites. The only positively co-occurring pair was blacklegged ticks and wood ticks (Figure 2.3), which were expected to occur across only 13.7 sites, but were found together at 22 (Table 2.7). Both tick species had negative associations with the mite groups and *O. leucopus* (Figure 2.3), where the expected co-occurrences were much lower than the observed co-occurrences (Table 2.7). The co-occurrences between the mite groups and *O. leucopus*, however, appeared to be random (Figure 2.3), such that species pairs were not observed more or less than expected by random chance. All co-occurrence analyses constrained by field sites determined that the co-occurrences between all ectoparasites were random, suggesting none of these associations between ectoparasites were positively or negatively co-occurring at LP, QEW, or AP (Table 2.8).

Discussion

Ectoparasite Distributions Across Field Sites

The purpose of this study was to compare community assemblage of ectoparasites on deer mice from populations with varying tick exposure. I hypothesized that when ticks were infesting a host, they would dominate the micro-habitat so that few or no other ectoparasite species (fleas and mites) would be sharing the host, especially in preferred attachment sites (*i.e.* ears). Although studies have found that host parameters such as body mass and sex affect parasite community assemblages (Gaitan & Millien, 2016; Sponchiado *et al*., 2016), this was not the case in my study. However, field site played an important role in the observed structure. The three field sites that were assessed are geographically different, occurring at different latitudes and in different ecozones (Crins *et al*., 2009). LP is the most southern site and was the only field site where blacklegged ticks were prevalent. Although wood ticks were prevalent at LP and QEW, there were greater ectoparasite community similarities between QEW and AP. At these two sites, *O. leucopus* and Trombiculidae *sp*. were more prominent than the other ectoparasite species. The intensity of wood ticks in QEW was low and did not seem to affect the occurrence of other ectoparasites found at this site. There are several possible reasons why this may be when comparing QEW and LP, where wood ticks were present: 1) the occurrence of blacklegged ticks affects the prevalence of other ectoparasites more than wood ticks, 2) the intensity of wood ticks affects the infestation rates of co-occurring ectoparasites more so than its presence on a host does, 3) the field site location is affecting the species distribution in a way that was not measured (*i.e.* abiotic factors such as weather or temperature, differences in flora or fauna between habitats). Field site had the highest biplot score for constraining variables, significantly affecting the ectoparasite communities across hosts, and it is possible that the abiotic environmental

factors of these sites are influencing ectoparasite communities (Gómez-Rodríguez *et al.*, 2015). Thus, there may be environmental characteristics not considered in this study that are influencing occurrences of different ectoparasites.

Ectoparasite Distributions Across Hosts

The correspondence analysis plot illustrates that both tick species and both mite groups prefer the ears as their attachment site, although wood ticks can also be found on the head and middle of their host's body (Figure 2.1). Fleas, on the other hand, were never found on the head of any of the deer mice, suggesting some exclusion or difference in preference in attachment or feeding sites. Siphonaptera (flea) species differ from Acari species (mites and ticks) in that they invest more time associating with their host, alternating between the host's body and the host's nest (Krasnov *et al*., 2010a). While all three groups are obligate parasites, fleas do not attach to one site on the host to feed nor do they move on to another host as ticks do (Krasnov *et al*., 2010a), which can affect associations seen in this study.

Despite fleas moving freely across their hosts, the micro-habitat partitioning of mites and ticks infesting deer mice in this analysis was similar to the body partitioning results found in a study on the painted spiny pocket mouse (*Liomys pictus*) in Mexico (Gómez-Rodríguez *et al.*, 2015). Although the painted spiny pocket mouse lives in an area with generally higher biodiversity than the deer mice in this study, both host species were observed to have ectoparasite communities with mutually exclusive areas on the body occupied by certain ectoparasite groups. On painted spiny pocket mice, hard tick species, including *Ixodes* sp. were found mainly on the head (neck, cheek pouch, and snout), and while some mite species were found exclusively on the ears of their hosts, others were found across the body, from the dorsal

posterior to the ears (Gómez-Rodríguez *et al.,* 2015). The mite species in this thesis were also unknown; yet, two distinct groups were defined: *Neotrombicula sp*. was found in LP and AP and Trombiculidae *sp*. in AP and QEW. *Neotrombicula sp*. is known to infest their host's ears in AP (Bobbie *et al*., 2016), and were found to be prevalent at this field site. Although also prevalent in LP, they were only found on two individual hosts, while tick prevalence was extremely high at this site. Thus, it may be possible that mutual exclusion of micro-habitats is occurring here, where the tick species are outcompeting the mites that prefer the same attachment sites. It is also possible that at this site, *Neotrombicula sp*. associates with a different rodent host and so their prevalence was not as high in this area. Without knowing the actual species of mite, however, it is difficult to discern its ecology.

Ectoparasite Co-occurrences

Results differed in co-occurrences when considering hosts as unique micro-habitats versus hosts constrained by their macro-habitats (field site/population source). When hosts were considered individual micro-habitats blacklegged ticks and wood ticks were positively co-occurring, and both tick species were negatively co-occurring with Trombiculidae *sp*. and fleas. Wood ticks were also negatively co-ocurring with *Neotrombicula sp*., whereas blacklegged ticks and *Neotrombicula sp*. were randomly co-occurring. All other co-occurrences were random (Figure 2.3).

It is common for different tick species to feed from the same host (Baer-Lehman *et al*., 2012; Gómez-Rodríguez *et al.,* 2015; Cayol *et al*., 2018). In some cases, the incidence rate of a tick species will significantly increase when their host is co-infested by other tick species (Biguezoton *et al*., 2016). There are several cases where an ectoparasite species infesting a

mammalian host will have increased intensities when there are co-infestations by other taxa on the host, often due to how these interactions compromise the host's immune response (Matthee & Krasnov, 2009; Krasnov *et al*., 2010b; Lutermann *et al*., 2015). It is also possible for one tick species to facilitate another tick species in a parasite community, however; it is typical for these interspecific relationships to be antagonistic (Matthee & Krasnov, 2009; Lutermann *et al*., 2015). A study on eastern rock elephant shrew (*Elephantulus myurus*) ectoparasite communities reported that when experimentally reducing abundances of several tick and flea species from hosts, most inter-taxon relationships of ectoparasites were antagonistic, although some tick species interactions resulted in facilitation of one another (Lutermann *et al*., 2015). Much like Lutermann *et al*.'s (2015) results, the positive co-ocurrence between blacklegged ticks and wood ticks suggests that the competition for attachment sites or blood-feeding resources does not dictate the broad co-occurrence patterns between these species.

While mite and flea co-occurrences were random, there were marked negative co-occurrences found between blacklegged ticks and Trombiculidae *sp*., and *O. leucopus* as well as between wood ticks and Trombiculidae *sp*., *Neotrombicula sp*., and *O. leucopus*. An experimental study that removed a dominant hard tick species from hosts found that the removal of this ectoparasite increased the abundances of co-infesting chiggers and lice (Hoffman *et al*., 2016). Similarly, in my study, when blacklegged ticks and wood ticks were not present on a host, the prevalence of *O. leucopus* and mites was higher in deer mice. These results are also similar to Lutermann *et al*.'s (2015) findings, where fleas were more prevalent when ticks were removed and vice versa. This may be due to direct competition for resources. Although fleas and mites parasitize *Peromyscus* mice for long periods of time and are consistently impacting their host's immune response (Mize *et al*., 2011), ticks require larger blood meals for shorter periods of time

(McKenzie, 1998), and since they require greater volumes of a shared resource, they may be outcompeting other blood-feeding ectoparasites. Unfortunately, since surveying only occurred over a short field season between May and August of one year – and the hosts' nests were not examined – sampling effort was limited, so there could be hidden effects impacting both the host and parasite fitness that could be noted over a longer time period (Krasnov & Matthee, 2010).

When micro-habitats were constrained by their population sources, all co-occurrences between ectoparasites were considered random. This result suggests that there are no associations between any of the ectoparasites, though the unbalanced and small sample sizes for each population source could be affecting the results, as expected pair-wise co-occurrences are the product of the two species' probability of occurrence multiplied by the number of sampling sites (Veech, 2013; Griffith *et al*., 2018). As mentioned previously, sampling effort could also be affecting these results. Although it is possible for co-occurrences between species to be random, it seems unlikely that all species in this study are randomly co-occurring, since many of these ectoparasites favoured the same micro-habitat niches (specifically the ears). In order to further test co-occurrences between these species, an experimental study in which a parasite is removed from the micro-habitat and the parasite community is re-examined (Lutermann *et al*., 2015) should be conducted.

Conclusion

This study focused on parasite community assemblage processes at the host individual level. However, both population and (macro) community dynamics can affect an individual's parasite load and must also be considered (Gómez-Rodríguez *et al.*, 2015; Hellard *et al*., 2015). Specifically, interactions at one ecological level can cascade up to other levels, affecting

intraspecific and interspecific relationships between organisms (Hellard *et al*., 2015). It is especially important to consider these other interactions, as several pathogenic parasites are expanding their geographic ranges northwards (Gasami *et al*., 2018), which may alter current associations between species. The results of my study suggest that the prevalence of blacklegged ticks and wood ticks could affect community assemblages. As these species expand their ranges (Leighton *et al*., 2012; Gasami *et* al., 2018; Minigan *et al*., 2018), it is possible that they will outcompete other ectoparasites in their micro-habitat.

There is evidence that tick-borne diseases do expand with the increased distribution of *Ixodes* ticks and it is likely that this trend will continue (Ostfeld & Brunner, 2015). Many of the ectoparasites currently found on deer mice (*Peromyscus maniculatus*) are vectors for several pathogens that can be transmitted to humans (Lindsay *et al*., 2001; DeNatale *et al*., 2002; Westblade *et al*., 2017; Mlera *et al*., 2018; Talagrand-Reboul *et al*., 2018), indicating the epidemiological importance of these hosts. Since the blacklegged tick and wood tick are vectors of pathogens that can be transmitted to humans (blacklegged tick: *Borrelia burgdorferi;* Sonenshine, 2018; wood tick: *Rickettsia rickettsii;* Durden *et al*., 2004) and these tick species are expanding their geographical ranges throughout Ontario (Leighton *et al*., 2012; Clow *et al*., 2016; 2017; Minigan *et al*., 2018), it is an important next step to see how they are associating with other ectoparasites that may also transmit zoonotic pathogens.

By understanding the differences in community assemblages between host populations where ticks are present or absent, we can better predict how ectoparasite communities may change when these tick species do eventually expand northwards into new populations. Furthermore, more research is needed to better understand how their presence may affect

parasite-parasite interactions as well as the transmission of zoonotic pathogens that may be able to spread between parasites and hosts.

Literature Cited

Adair, KL, Wilson, M, Bost, A, Douglas, AE. (2018). Microbial Community Assembly in Wild Populations of the Fruit Fly *Drosophila melanogaster*. *ISME Journal.* 12(4): 959-972. doi: 10.1038/s41396-017-0020-x

Anderson, J; Moore, I; Nagata, B; Ribeiro, J; Valenzuela, J; Sonenshine, D. (2017). Ticks, *Ixodes scapularis*, Feed Repeatedly on White-Footed Mice despite Strong Inflammatory Response: An Expanding Paradigm for Understanding Tick-Host Interactions. *Frontiers in. Immunology.*8(1784): 1-15. doi: 10.3389/fimmu.2017.01784

Arita, HT. (2016). Species Co-occurrence Analysis: Pairwise Versus Matrix-Level Approaches. *Global Ecology and Biogeography*. 25: 1397-1400. doi: 10.1111/geb.12418

Atwa, AA; Bilgrami, A; Al-Saggaf, AIM. (2017). Host-parasite Interaction and Impact of Mite Infection on Mosquito Population. *Revista Brasileira de Entomologia*. 61(2): 101-106. doi: 10.1016/j.rbe.2017.03.005

Baer-Lehman, M; Light, T; Fuller, N; Barry-Landis, K; Kindlin, C; Stewart Jr., R. (2012). Evidence for Competition Between *Ixodes scapularis* and *Dermacentor albipictus* Feeding Concurrently on White-Tailed Deer. *Exp. Appl. Acarol*. 58: 301-314. doi: 10.1007/s10493-012-9574-5

Balashov, Y.S. (1972). Bloodsucking Ticks (Ixodoidea)-Vectors of Disease in Man and Animals. Misc. Publ. Entomol. Soc. Am. 8, 376.

Banfield, AW. (1974). The Mammals of Canada. 1st ed. Toronto and Buffalo: University of Toronto Press.

Bedford, N; Hoekstra, H. (2015). Peromyscus Mice as a Model for Studying Natural Variation. *eLife*. 4, e06813. https://doi.org/10.7554/eLife.06813

Biguezoton, A; Adehan, S; Adakal, H; Zoungrana, S; Farougou, S; Chevillon, C. (2016). Community Structure, Seasonal Variations and Interactions Between Native and Invasive Cattle Tick Species in Benin and Burkina Faso. *Parasites & Vectors.* 9(43): 1-16. doi: 10.1186/s13071-016-1305-z

Bobbie, C; Schmidt, E; Foley, J; Schulte-Hostedde, A. (2016). The Presence of Parasitic Mites on Small Mammals in Algonquin Provincial Park, Ontario, Canada. *Can. J. Zool.* 95:61-65. doi: 10.1139/cjz-2016-0085

Bordes, F; Blumstein, D; Morand, S. (2007). Rodent Sociality and Parasite Diversity. *Biol. Lett*.

3: 692-694. doi:10.1098/rsbl.2007.0393

Bordes, F; Morand, S. (2011). The Impact of Multiple Infections on Wild Animal Hosts: A Review. *Infect. Ecol. Epidemiol.* 1: 7346. doi: 10.3402/iee.v1i0.7346

Bouchard, C; Beauchamp, G; Nguon, S; Trudel, L; Milord, F; Lindsay, L. Robbin; Belanger, D; Ogden, Nicholas. (2011). Associations Between *Ixodes scapularis* Ticks and Small Mammal Hosts in a Newly Endemic Zone in Southeastern Canada: Implications for *Borrelia burgdorferi* Transmission. *Ticks and Tick-Borne Diseases.* 2: 183-190. doi:10.1016/j.ttbdis.2011.03.005

Bowman, J. (2019, May 3). Personal interview.

Buchholz, M, Dick, C. (2017). Ecology of Rodent - Ectoparasite Associations in South-Central Kentucky. *Northeastern Naturalist*. 24(2): 97-109. doi: 10.1656/045.024.0201

Bush, AO, Lafferty, KD, Lotz, JM, Shostak, AW. (1997). Parasitology Meets Ecology on Its Own Terms: Margolis *et al*. Revisited. *The Journal of Parasitology.* 83(4): 575-583. doi: 10.2307/3284227

Careless, P. (2019, May 30). Phone interview.

Cayol, C; Jääskeläinen, A; Koskela, E; Kyröläinen, S; Mappes, T; Siukkola, A; Kallioi, ER. (2018). Sympatric Ixodes-tick Species: Pattern of Distribution and Pathogen Transmission Within Wild Rodent Populations. *Scientific Reports*. 8(1666): 1-10. doi:10.1038/s41598-018-35031-0

Clow, K; Ogden, N; Lindsay, L. R; Michel, P; Pearl, D; Jardine, C. (2016). Distribution of Ticks and the Risk of Lyme Disease and Other Tick-Borne Pathogens of Public Health Significance in Ontario, Canada. *Vector-Borne and Zoonotic Diseases.* doi: 10.1089/vbz.2015.1890

Clow, K; Leighton, P; Ogden, N, Lindsay, L. R; Michel, P; Pearl, D; Jardine, C. (2017). Northward Range Expansion of *Ixodes scapularis* Evident Over a Short Timescale in Ontario, Canada. *PLoS ONE.* 12(12): e0189393. doi: 10.1371/journal.pone.0189393

Crins, W; Gray, P; Uhlig, P; Wester, M. (2009). The Ecosystems of Ontario, Part 1: Ecozones and Ecoregions. *Ministry of Natural Resources – Science & Information Branch.* Technical Report SIB TER IMA TR-01.

DeNatale, C; Burkot, T; Schneider, B; Zeidner, N. (2002). Novel Potential Reservoirs for

Borrelia sp. and the Agent of Human Granulocytic Ehrlichiosis in Colorado. *Journal of Wildlife Diseases*. 38(2): 478-482. doi: 10.7589/0090-3558-38.2.478

Dubie, T; Grantham, R; Coburn, L; Noden, B. (2017). Pictorial Key for Identification of Immature Stages of Common Ixodid Ticks Found in Pastures in Oklahoma. *Southwestern Entomologist.* 42(1): 1-14.

Durden, L; Polur, R; Nims, T; Banks, C; Oliver J. (2004). Ectoparasites and Other Epifaunistic Arthropods of Sympatric Cotton Mice and Golden Mice: Comparisons and Implications for Vector-Borne Zoonotic Diseases. *J. Parasitol*. 90(6): 1293-1297

Falls, JB; Falls, EA; Fryxell, J. (2007). Fluctuations of Deer Mice in Ontario in Relation to Seed Crops. *Ecological Monographs.* 77(1): 19-32

Fenton, A; Viney, M; Lello, J. (2010). Detecting Interspecific Macroparasite Interactions from Ecological Data: Patterns and Process. *Ecology Letters.* 13(5): 606-615. doi: 10.1111/j.1461-0248.2010.01458.x.

Fracasso, G; Matthysen, E; Dhondt, A; Heylen, D. (2019). Experimental Study of Micro-Habitat Selection by Ixodid Ticks Feeding on Avian Hosts. *International Journal for Parasitology.* 49(13-14): 1005-1014. doi: 10.1016/j.ijpara.2019.09.003

Fryxell, J; Falls, JB; Falls, EA; Brooks, R. (1998). Long-Term Dynamics of Small-Mammal Populations in Ontario. *Ecology*. 79(1): 213-225

Gaitan, J; Millien, V. (2016). Stress level, Parasite Load, and Movement Pattern in a Small Mammal Reservoir Host for Lyme Disease. *Can. J. Zoology*. 94: 565–573. doi: 10.1139/cjz-2015-0225

Gasami, S; Bouchard, C; Ogden, NH; Adam-Poupart, A; Pelcat, Y; Rees, EE; Milord, F; Leighton, P; Lindsay, RL; Koffi, JK; Thivierge, K. (2018). Evidence for Increasing Densities and Geographic Ranges of Tick Species of Public Health Significance Other than *Ixodes scapularis* in Quebec, Canada. *PLoS ONE.* 13(8): e0201924. doi: 10.1371/journal.pone.0201924

Godinho, L; Cripps, J; Coulson, G; Lumsden, L. (2013). The Effect of Ectoparasites on the Grooming Behaviour of Gould's Wattled Bat (*Chalinolobus gouldii*): An Experimental Study. *Acta Chiropterologica.* 15(2): 463-472. doi: 10.3161/150811013X679080

Gómez-Rodríguez, R; Gutiérrez-Granados, G; Montiel-Parra, G; Rodríguez-Moreno, A;

Sánchez,-Cordero, V. (2015). Diversity and Coexistence of Ectoparasites in Small Rodents in a Tropical Dry Forest. *Biotropica*. 47(4): 484-490. doi: 10.1111/btp.12229

Griffith, D; Veech, J; Marsh, C. (2016). cooccur: Probabilistic Species Co-Occurrence Analysis in R. Journal of Statistical Software, 69(2), 1-17. doi:10.18637/jss.v069.c02

Halsey, S; Miller, J. (2018). A Spatial Agent-Based Model of the Disease Vector *Ixodes scapularis* to explore Host-Tick Associations. *Ecological Modelling*. 387: 96-106. doi: 10.1016/j.ecolmodel.2018.09.005

Hamer, S; Hickling, G; Walker, E. Tsao, J. (2014). Increased Diversity of Zoonotic Pathogens and *Borrelia burgdorferi* Strains in Established Versus Incipient *Ixodes scapularis* Populations Across the Midwestern United States. *Infection, Genetics and Evolution.* 27: 531-542. doi: 10.1016/j.meegid.2014.06.003

Hawlena, H; Krasnov, BR; Abramsky, Z; Khokhlova, I. S; Saltz, D; Kam, M; Tamir, A; Degen, A. (2006). Flea Infestation and Energy Requirements of Rodent Hosts: Are There General Rules? *Functional Ecology.* 20: 1028-1036.

Hazler, K; Ostfeld, R. (1995). Larval Density and Feeding Success of *Ixodes scapularis* on Two Species of *Peromyscus*. *Journal of Parasitology*. 81(6): 870-875. doi: 10.2307/3284032

Hellard, E; Fouchet, D; Vavre, F; Pontier, D. (2015). Parasite-Parasite Interactions in the Wild: How to Detect Them? *Trends in Parasitology*. 31(12): 640-652. doi: 10.1016/j.pt.2015.07.005

Hoffman, S; Horak, I; Bennet, N; Lutermann, H. (2016). Evidence for Interspecific Interactions in an Ectoparasite Infracommunity of a Wild Mammal. *Parasites & Vectors*. 9(58): 1-11. doi: 10.1186/s13071-016-1342-7

Holland, George. (1985). The Fleas of Canada, Alaska and Greenland (Siphonaptera). *Memoirs of the Entomol*. Soc. Can. 117: 3-632.

Kamler, JF, Pennock, DS, Welch, C, Pierotti, RJ. (1998). Variation in Morphological Characteristics of the White-footed Mouse (*Peromyscus leucopus*) and the Deer Mouse (*P. maniculatus*) under Allotopic and Syntopic Conditions. *The American Midland Naturalist*. 140(1): 170-179. doi: 10.1674/0003-0031(1998)140[0170:VIMCOT]2.0.CO;2

Kappeler, P; Cremer, S; Nunn, C. (2015). Sociality and Health: Impacts of Sociality on Disease Susceptibility and Transmission in Animal and Human Societies. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 370(1669): 20140116. doi: 10.1098/rstb.2014.0116

Krasnov, BR; Shenbrot, G; Khokhlova, I; Poulin, R. (2006). Is Abundance a Species Attribute? An Example with Haematophagous Ectoparasites. *Oecologia*. 150: 132-140. doi: 10.1007/s00442-006-0498-9

Krasnov, BR, Matthee, S. (2010). Spatial Variation in Gender-Biased Parasitism: Host-related, Parasite-Related and Environment-Related Effects. *Parasitology*. 137(10): 1527–1536. doi: 10.1017/S0031182010000454

Krasnov, BR, Matthee, S; Lareschi, M; Korallo-Vinarskaya, NP; Vinarski, MV. (2010a). Co-occurrence of Ectoparasites on Rodent Hosts: Null Model Analyses of Data from Three Continents. *Oikos*. 119(1): 120-128. doi: 10.1111/j.1600-0706.2009.17902.x

Krasnov, BR; Stanko, M; Morand, S. (2010b). Competition, Facilitation or Mediation via Host? Patterns of Infestation of Small European Mammals by Two Taxa of Haematophagous Arthropods. *Ecol Entomol*. 35(1): 37–44. doi: 10.1111/j.1365-2311.2009.01153.x

Krogmann, L; Holstein, J. (2010). "Chapter 18: Preserving and Specimen Handling: Insects and Other Invertebrates". In Eymann, Jutta; Degreef, Jerome; Hauser, Christoph; Monje, Juan Carlos; Samyn, Yves; VandenSpiegel, Didier. (Eds), *Manual on Field Recording Techniques and Protocols for All Taxa Biodiversity Inventories*. (463-481). ABC Taxa

Legendre, P; Oksanen, J; ter Braak, CJF. (2011). Testing the Significance of Canonical Axes in Redundancy Analysis. *Methods in Ecology and Evolution*. 2: 269-277.

Leighton, P; Koffl, J; Pelcat, Y; Lindsay, LR; Ogden, N. (2012). Predicting the Speed of Tick Invasion: An Empirical Model of Range Expansion for the Lyme Disease Vector *Ixodes scapularis* in Canada. *J. App. Ecol.* 49(2): 457-464 doi: 10.1111/j.1365-2664.2012.02112.x

Lindsay, LR; Drebot, M; Weiss, E; Artsob, H. (2001). Hantavirus Pulmonary Syndrome in Manitoba. *Canadian Journal of Infectious Diseases*. 12(3): 169-173. doi: 10.1155/2001/425260

Lindquist, E; Galloway, T; Artsob, H; Lindsay, LR; Drebot, M; Wood, H; Robbins, R. (2016). *A Handbook to the Ticks of Canada (Ixodida: Ixodidae, Argasidae)*. Biological Survey of Canada.

Lutermann, H; Fagir, DM; Bennett, NC. (2016). Complex Interactions Within the Ectoparasite Community of the Eastern Rock Sengi (*Elephantulus myurus*). *Int J Parasitol: Parasite Wildlife*. 4(1): 148–158. doi: 10.1016/j.ijppaw.2015.02.001

Matthee, S; Krasnov, BR. (2009). Searching for the Generality in the Patterns of Parasite Abundance and Distribution: Ectoparasites of a South African Rodent, *Rhabdomys pumilio*. *Int J Parasitol*. 39(7): 781–788. 42. doi:10.1016/j.ijpara.2008.12.003

Minigan, J; Hager, H; Peregrine, A; Newman, J. (2018). Current and Potential Future Distribution of the American Dog Tick (*Dermacentor variabilis*, Say) in North America. *Ticks and Tick-borne Diseases.* 9: 354-362. doi: 10.1016/j.ttbdis.2017.11.012

Mlera, L; Meade-White, K; Dahlstrom, E; Baur, R; Kanakabandi, K; Virtaneva, K; Porcella, S; Bloom, M. (2018). *Peromyscus leucopus* Mouse Brain Transcriptome Response to Powassan Virus Infection. *Journal of Neurovirology*. 24(1): 75-87. doi: 10.1007/s13365-017-0596-y

Nichols, J; Hines, J; Pollock, K. (1984). Effects of Permanent Trap Response in Capture Probability on Jolly-Seber Capture-Recapture Model Estimates. *JSTOR.* 48(1): 289-294. doi: 10.2307/3808491

Oksanen, J. (2019). Vegan: An Introduction to ordination. http://cran.r-project.org/web/packages/vegan/vignettes/intro-vegan.pdf.

Oksanen, J; Blanchet, G; Friendly, M; Kindt, R; Legendre, P; McGlinn, D; Minchin, P; O'Hara, RB; Simpson, G; Solymos, P; Stevens, MH; Szoecs, E; Wagner, H. (2019). Vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Ostfeld, R; Miller, M; Schnurr, J. (1993). Ear Tagging Increases Tick (*Ixodes Dammini*) Infestation Rates of White-Footed Mice (*Peromyscus leucopus*). *J. Mamm.* 74(3): 651-655. doi: 10.2307/1382286

Ostfeld, R; Brunner, J. (2015). Climate Change and *Ixodes* tick-borne diseases of Humans. *Phil Trans. R. Soc. B.* 370(1665): 1-11. doi: 10.1098/rstb.2014.0051

Patterson, JE; Neuhaus, P; Kutz, SJ; Ruckstuhl, KE., (2013). Parasite Removal Improves Reproductive Success of Female North American Red Squirrels (*Tamiasciurus hudsonicus*). *PLoS One*. 8(2): e55779. doi: 10.1371/journal.pone.0055779

Poulin, R. (2011). Evolutionary Ecology of Parasites. Princeton University Press, Princeton.

Reiczigel, J; Marozzi, M; Fabian, I; Rozsa, L. (2019). Biostatistics for Parasitologists – A Primer to Quantitative Parasitology. *Trends in Parasitology*. 35(4): 277-281.

Rynkiewicz, E; Hawlena, H; Durden, L; Hastriter, M; Demas, G; Clay, K. (2013). Associations

Between Innate Immune Function and Ectoparasites in Wild Rodent Hosts. *Parasitol. Res.* 112: 1763-1770. doi: 10.1007/s00436-013-3335-1

Schmidt, E; Mykytczuk, N; Schulte-Hostedde, A. (2019). Effects of the Captive and Wild Environment on Diversity of the Gut Microbiome of Deer Mice (Peromyscus maniculatus). *The ISME Journal*. 13: 1293-1305. doi: 10.1038/s41396-019-0345-8

Sonenshine, D. (2018). Range Expansion of Tick Disease Vectors in North America: Implications for Spread of Tick-Borne Disease. *Int. J. Environ. Res. Public Health.* 15: 478. doi:10.3390/ijerph15030478

Sponchiado, J; Melo, GL; Martins, TF; Krawczak, FS; Jacinavicius, FC; Labruna, MB; Barros-Battesti, DM; Cáceres, NC. (2017). Ectoparasites of Small-mammals: Determinants of Community Structure in South American Savannah. *Parasitology*. 144(4): 475-483. doi:10.1017/S0031182016001906

Talagrand-Reboul, E; Boyer, P; Bergstrom, S; Vial, L; Boulanger, N. (2018). Relapsing Fevers: Neglected Tick-Borne Diseases. *Frontiers in Cellular and Infection Microbiology.* 8:98. doi: 10.3389/fcimb.2018.00098

Veech, JA. (2013). A Probabilistic Model for Analysing Species Co-occurrences. *Global Ecology and Biogeography*. 22(2): 252-260. doi: 10.1111/j.1466-8238.2012.00789.x

Watson, TG; Anderson, RC. (1976). *Ixodes scapularis* Say on White-tailed Deer (*Odocoileus virginianus*) from Long Point, Ontario. *J. Wildl. Dis.* 12: 66-71.

Westblade, L; Simon, M; Mathison, B; Kirkman, L. (2017). *Babesia microti*: from Mice to Ticks to an Increasing Number of Highly Susceptible Humans. *Journal of Clinical Microbiology.* 55(10): 2903-2912. doi: 10.1128/JCM.00504-17

Figures and Tables

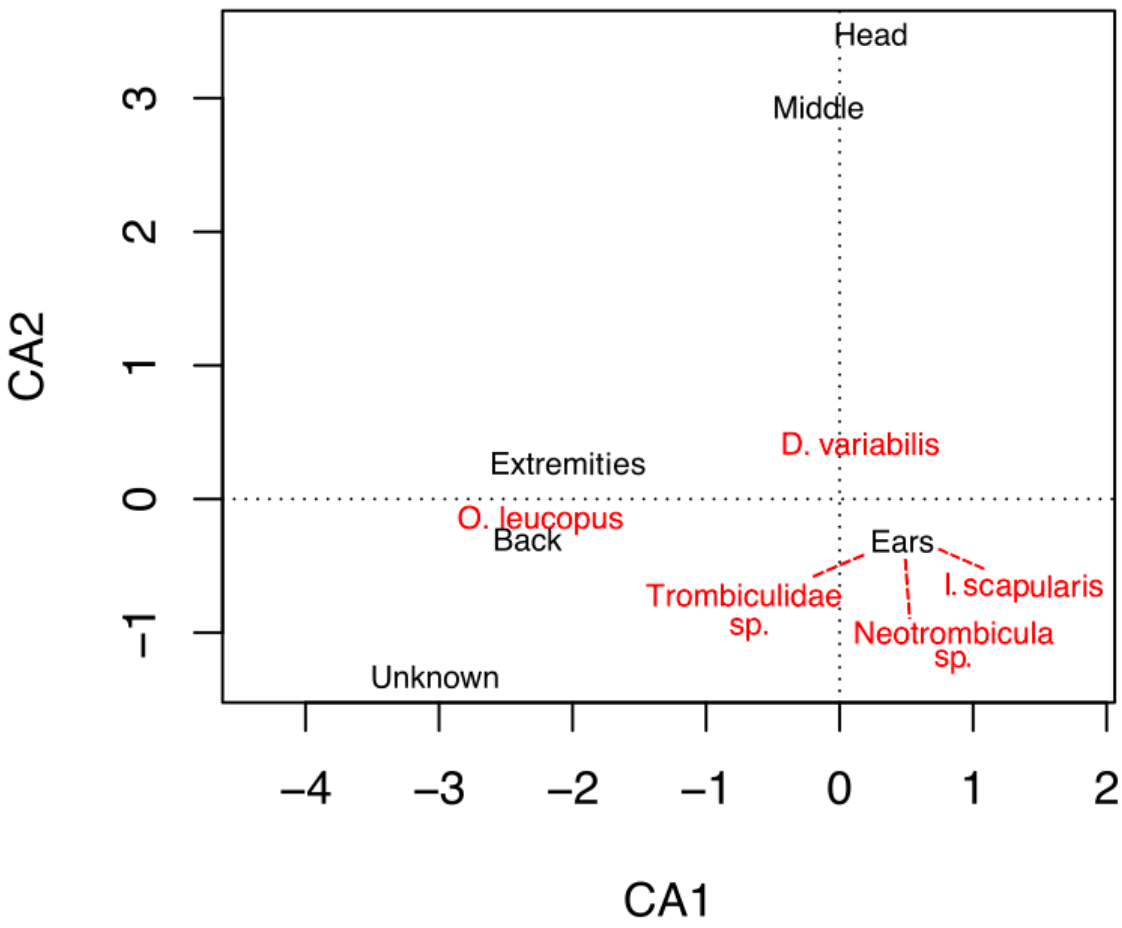

Figure 2.1. Correspondence analysis plot of ectoparasite (red text) prevalence to body location (black text) on deer mice (*Peromyscus maniculatus*; n = 70). Dotted red lines indicate that these groups were overlapping on the specified body location.

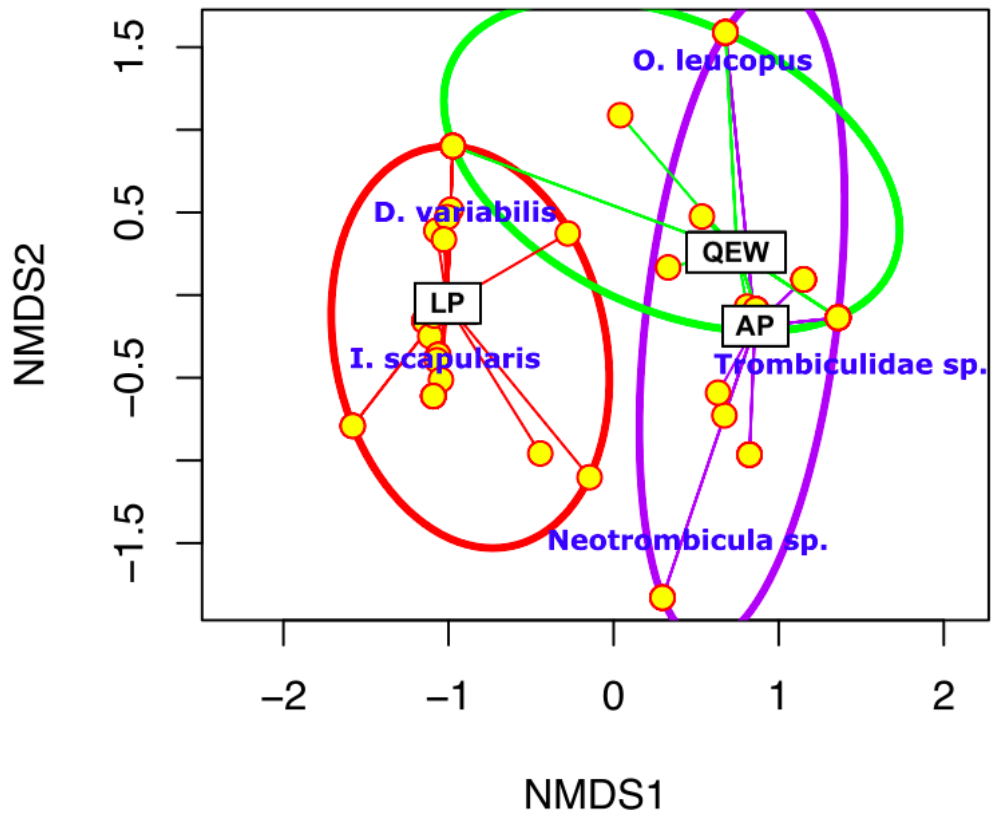

Figure 2.2. Non-metric multidimensional scaling plot of individual deer mice (*Peromyscus maniculatus*; n = 70) and the ectoparasites they host across field sites. Each yellow dot represents a single host (micro-habitat site). The coloured lines extending out from the site names are measurements of dissimilarity, whereby longer lines indicate greater dissimilarity. Red ellipse represents the distribution of Long Point (LP) individuals (hosts and parasites), green ellipse represents Queen Elizabeth II Wildlands (QEW), and purple ellipse represents Algonquin (AP) individuals.

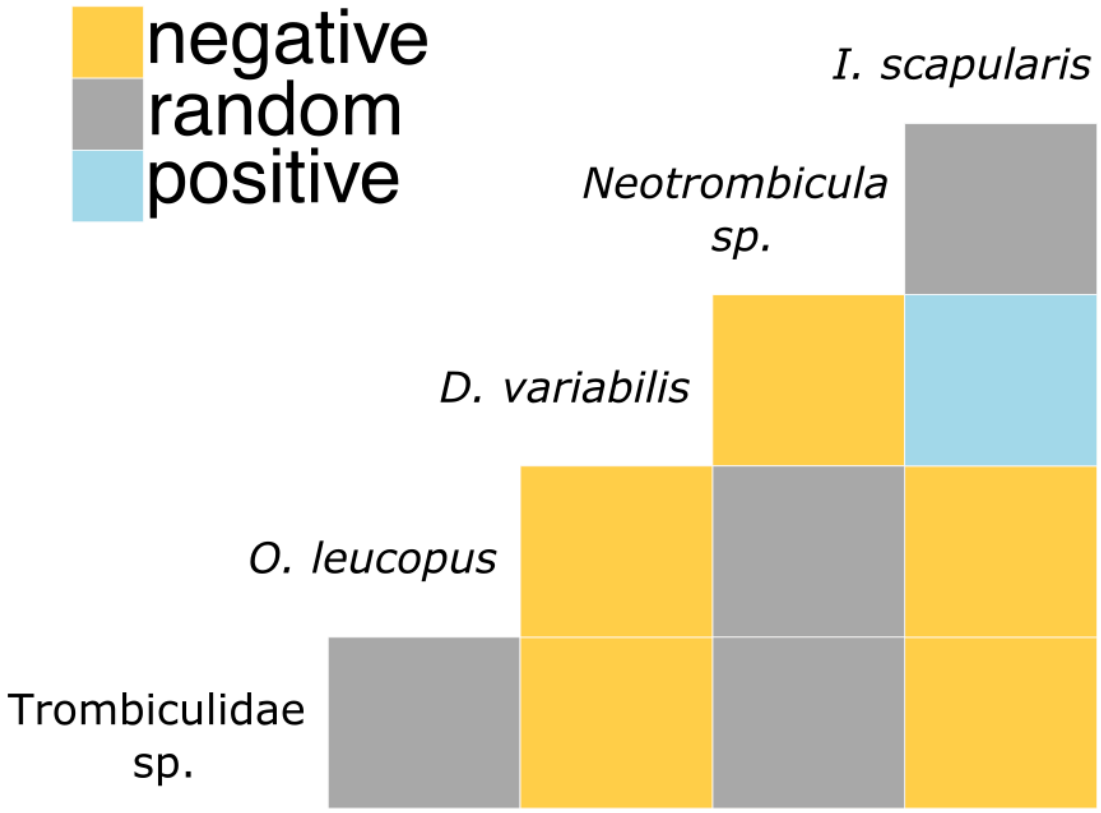

Figure 2.3. Heat map depicting the positive, negative, and random species associations determined by the probabilistic co-occurrence model (Table 2.7) for ectoparasites found across individual deer mice (*Peromyscus maniculatus*) hosts (n = 70). Species names are positioned to indicate the columns and rows that represent their pairwise relationships with other species.

Table 2.1. Host parameters for deer mice (*Peromyscus maniculatus*) depicting distributions of average body mass (with standard deviation), sex, age, and reproductive status across all sites and within sites as well as temporal sessions. Numbers in parentheses indicate the sample size of hosts for each indicated category. NR = non reproductive and R = reproductive. LP = Long Point Provincial Park, QEW = Queen Elizabeth II Wildlands Provincial Park, AP = Algonquin Provincial Park.

	All Sites (70)	LP (31)	QEW (20)	AP (19)
Average body mass (g)	19.67 +/- 5.28	20.21 +/- 6.67	18.73 +/- 3.85	19.79 +/- 3.95
Host sex				
	Male (47)	Male (21)	Male (13)	Male (13)
	Female (23)	Female (10)	Female (7)	Female (6)
Host age				
	Adult (59)	Adult (26)	Adult (15)	Adult (18)
	Juvenile (11)	Juvenile (5)	Juvenile (5)	Juvenile (1)
Host reproductive status				
	NR (37)	NR (22)	NR (11)	NR (4)
	R (33)	R (9)	R (9)	R (15)
Session				
	Early (10)	Early (5)	Early (3)	Early (2)
	Mid (25)	Mid (12)	Mid (8)	Mid (5)
	Late (35)	Late (14)	Late (9)	Late (12)

Table 2.2. Prevalence and mean intensity of ectoparasites found on deer mice (*Peromyscus maniculatus*) across all sites and per site. Numbers in parentheses indicate the number of hosts (first column) and parasites for each indicated column. It should be noted that both mite groups had prevalence measured only; intensities unknown. LP = Long Point Provincial Park, QEW = Queen Elizabeth II Wildlands Provincial Park, AP = Algonquin Provincial Park.

	I. scapularis	*D. variabilis*	*O. leucopus*	Trombiculidae *sp.*	*Neotrombicula sp.*
All Sites (70)	(39)	(162)	(22)	(26)	(10)
Prevalence	54.3%	55.6%	30.5%	37.1%	14.3%
Mean intensity	0.56 +/- 0.97	2.33 +/- 3.94	0.31 +/- 0.77	N/A	N/A
LP (31)	(39)	(139)	(2)	(0)	(2)
Prevalence	77.4%	93.5%	3.2%	0.0%	6.5%
Mean intensity	1.25 +/- 1.12	4.52 +/- 4.86	0.06 +/- 0.36	N/A	N/A
QEW (20)	(0)	(23)	(10)	(14)	(0)
Prevalence	0.0%	55.0%	25.0%	70.0	0.0%
Mean intensity	N/A	1.15 +/- 2.08	0.50 +/- 1.19	N/A	N/A
AP (19)	(0)	(0)	(10)	(12)	(8)
Prevalence	0.0%	0.0%	42.9%	63.1	42.1%
Mean intensity	N/A	N/A	0.53 +/- 0.61	N/A	N/A

Table 2.3. Species and site scores for unconstrained correspondence analysis (Figure 2.1) of ectoparasite prevalence to micro-habitat sites on deer mice (*Peromyscus maniculatus*) hosts (n = 70). Eigenvalues and proportions explained for both CA axes are also included. Inertia = 0.859 (scaled chi-square).

	CA1	CA2
Eigenvalues	0.739	0.119
Proportion explained	0.861	0.139
Species scores		
D. variabilis	0.148	0.413
I. scapularis	0.469	-0.316
O. leucopus	-2.240	-0.159
Trombiculidae *sp.*	0.469	-0.316
Neotrombicula sp.	0.469	-0.316
Site scores		
Head	0.299	3.767
Ears	0.469	-0.316
Middle	-0.158	2.930
Back	-2.338	-0.304
Extremities	-1.953	0.267
Unknown	-3.030	-1.331

Table 2.4. Correspondence analysis inertia (scaled chi-square), proportion ranks, and eigenvalues for both unconstrained and constrained axes for parasite loads found on individual deer mice (*Peromyscus maniculatus*) hosts (n = 70). Proportion explained for eigenvalue contribution to scaled chi-square is in parentheses. Constrained environmental factors include host body mass, sex, age, reproductive status, field site, and session. Mite groups only provided prevalence data, while both tick species and *O. leucopus* provided intensity values.

	Constrained (CCA)	Unconstrained (CA)
Inertia	0.759	1.259
Proportion Rank	0.376	0.624
Axis 1 Eigenvalues	0.672 (0.333)	0.457 (0.227)
Axis 2 Eigenvalues	0.070 (0.035)	0.450 (0.223)
Axis 3 Eigenvalues	0.015 (0.007)	0.268 (0.133)
Axis 4 Eigenvalues	0.002 (0.001)	0.084 (0.040)

Table 2.5. Species scores and biplot scores for constraining variables in constrained (CCA) and unconstrained (CA) correspondence analyses for ectoparasite communities across individual deer mice (*Peromyscus maniculatus*) hosts (n = 70). Since CA is unconstrained, all biplot scores for constraining variables are equal to zero. HRS = host reproductive status.

	CCA1	CCA2	CCA3	CCA4	CA1	CA2
Species scores						
Trombiculidae *sp*.	1.518	-0.248	-0.091	-0.077	-1.613	0.147
Neotrombicula sp.	1.744	-0.706	0.255	0.119	1.019	-2.839
O. leucopus	1.385	0.673	-0.068	0.044	1.300	1.270
I. scapularis	-0.608	-0.191	-0.239	0.035	0.195	-0.127
D. variabilis	-0.400	0.039	0.069	-0.009	-0.018	0.009
Biplot scores						
Host body mass	-0.106	-0.234	-0.124	0.510	0	0
Host sex	-0.072	-0.290	0.144	-0.810	0	0
Host age	-0.070	-0.482	0.293	0.329	0	0
HRS	0.287	-0.678	-0.499	0.374	0	0
Site	0.991	0.010	-0.002	0.042	0	0
Session	-0.060	-0.571	0.728	0.014	0	0

Table 2.6. Permutation test results for constrained correspondence analysis (permutations = 9999). The test was performed to assess the specified environmental factors for significance to ectoparasite communities across deer mice (*Peromyscus maniculatus*) hosts (n = 70). Bolded terms indicate significant alpha values (< 0.05). Permutation test results for CCA axes significance is also included here (permutations = 9999). Bolded terms indicate significant alpha values (< 0.05). HRS = Host reproductive status.

	Df	Chi Square	F	Pr(>F)
Environmental Factors				
Host body mass	1	0.007	0.343	0.840
Host sex	1	0.010	0.524	0.699
Host age	1	0.013	0.641	0.618
HRS	1	0.035	1.773	0.117
Field site	1	0.660	33.029	**0.0001**
Session	1	0.033	1.644	0.155
Residual	63	1.259	N/A	N/A
Axis Significance				
CCA1	1	0.672	34.691	**0.0001**
CCA2	1	0.070	3.615	0.333
CCA3	1	0.015	0.774	0.991
CCA4	1	0.002	0.080	1.000
Residual	65	1.259	N/A	N/A

Table 2.7. Summary of probabilistic co-occurrence model of pair-wise co-occurrences for ectoparasites found across individual deer mice (*Peromyscus maniculatus*) hosts (n = 70) that were each considered a site (micro-habitat) in this analysis.

Species 1	Species 2	Number of micro-habitats that have species 1	Number of micro-habitats that have species 2	Observed number of micro-habitats having both species	Expected number of micro-habitats having both species	Probability of micro-habitats having both species	Probability of co-occurrence freq. < observed # of co-occurrence micro-habitats if both species were distributed randomly	Probability of co-occurrence freq. > observed frequency
Neotrombicula sp.	Trombiculidae *sp.*	27	10	5	3.9	0.055	0.875	0.321
Neotrombicula sp.	*O. leucopus*	27	15	7	5.8	0.083	0.847	0.331
Neotrombicula sp.	*I. scapularis*	27	24	0	9.3	0.132	0.000	1.000
Neotrombicula sp.	*D. variabilis*	27	40	8	15.4	0.220	0.001	1.000
Trombiculidae *sp.*	*O. leucopus*	10	15	2	2.1	0.031	0.636	0.686
Trombiculidae *sp.*	*I. scapularis*	10	24	1	3.4	0.049	0.077	0.990
Trombiculidae *sp.*	*D. variabilis*	10	40	2	5.7	0.082	0.013	0.999
O. leucopus	*I. scapularis*	15	24	1	5.1	0.073	0.009	0.999
O. leucopus	*D. variabilis*	15	40	4	8.6	0.122	0.008	0.999
I. scapularis	*D. variabilis*	24	40	22	13.7	0.196	1.000	0.000

Table 2.8. Summary of probabilistic co-occurrence model of pair-wise co-occurrences for ectoparasites found across individual deer mice (*Peromyscus maniculatus*) hosts (n = 70) that were each considered a site (micro-habitat) in this analysis, constrained by field site (macro-habitat). Numbers in parentheses indicate the sample size of hosts for each site. QEW = Queen Elizabeth II Wildlands.

Species 1	Species 2	Number of micro-habitats that have species 1	Number of micro-habitats that have species 2	Observed number of micro-habitats having both species	Expected number of micro-habitats having both species	Probability of micro-habitats having both species	Probability of co-occurrence freq. < observed # of co-occurrence micro-habitats if both species randomly distributed	Probability of co-occurrence freq. > observed frequency
Long Point (31)								
Neotrombicula sp.	*O. leucopus*	2	1	0	0.1	0.002	0.936	1.000
Neotrombicula sp.	*I. scapularis*	2	24	1	1.5	0.050	0.407	0.955
Neotrombicula sp.	*D. variabilis*	2	29	2	1.9	0.060	1.000	0.873
O. leucopus	*I. scapularis*	1	24	1	0.8	0.025	1.000	0.774
O. leucopus	*D. variabilis*	1	29	1	0/9	0.030	1.000	0.936
I. scapularis	*D. variabilis*	24	29	22	22.5	0.724	0.594	1.000
QEW (20)								
Trombiculidae *sp.*	*O. leucopus*	15	5	2	3.8	0.188	0.073	0.995
Trombiculidae *sp.*	*D. variabilis*	15	11	8	8.2	0.413	0.605	0.779
O. leucopus	*D. variabilis*	5	11	3	2.8	0.138	0.779	0.605
Algonquin (19)								
Trombiculidae *sp.*	*Neotrombicula sp.*	12	8	5	5.1	0.266	0.663	0.703
Trombiculidae *sp.*	*O. leucopus*	12	9	5	5.7	0.299	0.430	0.870
Neotrombicula sp.	*O. leucopus*	8	9	2	3.8	0.199	0.115	0.985

General Discussion

There is a clear difference in the hematology and community assemblage of deer mice when tick exposure varies, particularly when both blacklegged ticks and wood ticks are prevalent. The prevalence of both blacklegged ticks and wood ticks has been demonstrated to affect their host's hematology and ectoparasite community structures. Deer mice that were infested with blacklegged ticks had hemoglobin levels significantly lower than mice that were not infested. In most cases, blacklegged ticks were found on mice with wood ticks. Mice with higher intensities of wood ticks also had significantly lower hemoglobin levels, specifically in LP. LP had the highest tick intensities and was the only site where blacklegged ticks were prevalent. Without confounding effects, these two species affected their host's hematology separately, as their interaction was found to be nonsignificant, although the results in Chapter 2 suggest these species do often occur together. Wood tick nymphs, which are larger than larval ticks (Lindquist *et al*., 2016), were also shown to have a negative effect on hemoglobin levels.

Low hemoglobin levels can result in iron deficiency and anemia (Andrews, 1997; O'Brien *et al*., 2003). In lab studies focused on rodents, there is evidence that iron deficiency causes fatigue, trouble focusing, reduced immune response, and loss of motor skills (Rosales *et al*., 1999; Nicolas *et al*., 2002; Hentze *et al*., 2004; Camaschella, 2015). This could in turn affect the range expansion of the infested host (Binning *et al*., 2017), potentially suppressing the expansion of pathogens they carry to new populations by migrating less (Huang *et al*., 2019) or intensifying the spread of pathogens within their current one by reducing genetic diversity (King & Lively, 2012). Since other variables, such as the host's microbiota, endoparasite loads, and diseases were not included in any analysis, there are still many questions that must be answered

to fully understand the deer mouse's hematology, although the prevalence of the two tick species appears to affect hemoglobin levels.

These two species of ticks also appeared to affect the ectoparasite community assemblages of their hosts. It is possible that there is a positive co-occurrence association between the two tick species. Similar to the hematology results, blacklegged ticks and wood ticks were often found together and did not seem to deter each other. Again, LP was the only site examined that had both tick species, where most of the hosts had tick infestations, but few had fleas or mites prevalent. In contrast, the greatest prevalence of mites and fleas was seen in AP - where neither tick species has currently established. In the unconstrained analysis, it was found that while blacklegged ticks and wood ticks were positively co-occurring with each other, these ticks negatively co-occurred with fleas and mites (except blacklegged ticks and *Neotrombicula sp*., which were random), whereas fleas and mites were randomly co-occurring. The absence of co-occurrence between ticks and mites could be due to interspecific competition, as they all preferred the same attachment site/microhabitat niche (ears). However, fleas were also negatively co-occurring with ticks, despite partitioning the habitat, possibly due to differences in their ecology and host reliance (short term versus long term infestations).

Field sampling site also played a significant role in species distributions in my system. When micro-habitats were segregated by their macro-habitats, all co-occurrences between ectoparasites were deemed random. Although the total number of ectoparasite species found across sites was not different, there was a difference in tick species prevalent at each site. Host populations at lower latitudes have been documented to have higher parasite species diversity (Preisser, 2019; Bordes *et al*., 2011). Although, the latitudinal range in this study was small, each site was in a different ecozone (Crins *et al*., 2009). Differences in habitat types can have

significant effects on parasite loads (Carbayo *et al*., 2019) as well as differences in host population sizes (Papkou *et al*., 2016) so these variables should be considered in further studies. Similar to the host's hematology, an individual's microbiota, endoparasites, and pathogens can also affect their parasite community assemblage (Budischak *et al*., 2012) and should be examined as well.

The results of my thesis suggest that further consideration must be given on how the ever-increasing prevalence of these tick species will affect ecosystems that will soon come into contact with them. QEW is expected to have blacklegged ticks invade in the upcoming years (Leighton *et al*., 2012; Ogden *et al*. 2013; Clow *et al.,* 2016; 2017), and soon after AP will be invaded by both tick species (Leighton *et al*., 2012; Ogden *et al*. 2013; Minigan *et al*., 2018). As naïve hosts come into contact with these tick species, it is important to understand how tick prevalence will affect the host's physiology via hematology as well as their ectoparasite communities. Deer mice in particular, are vectors to numerous pathogens, passed on to numerous other species via ticks and other ectoparasites (Lindsay *et al*., 2001; DeNatale *et al*., 2002; Westblade *et al*., 2017; Mlera *et al*., 2018; Talagrand-Reboul *et al*., 2018). If prevalence or intensity of ticks reduces the prevalence of other ectoparasites found on a host or within a host's population, this will in turn reduce parasite species richness, thereby reducing biodiversity. Although it has been suggested that higher host biodiversity can dilute the spread of tick-borne diseases (Ogden & Tsao, 2009; Dantas-Torres, 2015), parasite diversity may also act as a control for the spread of zoonotic pathogens (Johnson *et al*., 2013). This is important to consider, as tick-borne viruses are arguably of the greatest medical concern in North America, particularly those transmitted by the blacklegged tick (Kocan *et al*., 2015; Kulkarni *et al*., 2015; Eisen & Eisen, 2018). It has been documented that tick burdens do not affect the survival of *Peromyscus* hosts

(Hersh *et al.,* 2014), and that these hosts may have higher innate immune functions that allow them to enhance their tolerance for high tick burdens compared to other rodents (such as *Microtus* spp.; Rynkiewicz *et al*., 2013). Since the effects of low hemoglobin levels via parasite loads have not been previously tested on wild *Peromyscus*, it is not known how this deficiency may alter their physiology or immunology.

Further studies on interactions between these tick species and *Peromyscus* hosts should be conducted, particularly between experienced hosts and naïve hosts that may respond differently to these parasitic interactions, physically and immunologically. Since these tick species are expanding their ranges into areas where naïve hosts inhabit (Bedford and Hoekstra, 2015; Clow *et al*., 2016; 2017; Minigan *et al*., 2018), future research should focus on how these interactions may differ. Understanding these dynamics can allow us to better predict how new host populations will react to ixodid ticks. Other parasite interactions should also be considered, including associations occurring between flea, mite, and lice species, as well as other tick species currently prevalent in these areas and those tick species that are expected to expand their ranges into these regions in the future. Recognizing these parasite-parasite relationships will allow for greater insight into changes in species richness and biodiversity in a micro-habitat as well as provide information on the transmission of diseases that can spread from host to parasite (and vice versa) or even possibly indirectly from one parasite to another.

Literature Cited

Andrews, N. (1997). Iron Deficiency: Lessons from Anemic Mice. *Yale J. of Biol. Med.* 70: 219-226.

Bedford, N; Hoekstra, H. (2015). Peromyscus Mice as a Model for Studying Natural Variation. *eLife*. 4, e06813. https://doi.org/10.7554/eLife.06813

Binning, S; Shaw, A; Roche, D. (2017). Parasites and Host Performance: Incorporating Infection into Our Understanding of Animal Movement. *Integrative and Comparative Biology.* 57(2): 267-280. doi: 10.1093/icb/icx024

Bordes, F; Guégan, JF; Morand, S. (2011). Microparasite Species Richness in Rodents is Higher at Lower Latitudes and is Associated with Reduced Litter Size. *Oikos*. 120(12): 1889-1896. doi: 10.1111/j.1600-0706.2011.19314.x

Bowman, J. (2019, May 3). Personal interview.

Buchholz, M, Dick, C. (2017). Ecology of Rodent - Ectoparasite Associations in South-Central Kentucky. *Northeastern Naturalist*. 24(2): 97-109. doi: 10.1656/045.024.0201

Bush, AO, Lafferty, KD, Lotz, JM, Shostak, AW. (1997). Parasitology Meets Ecology on Its Own Terms: Margolis *et al*. Revisited. *The Journal of Parasitology*. 83(4): 575-583. doi: 10.2307/3284227

Budischak, S; Jolles, A; Ezenwa, V. (2012). Direct and Indirect Costs of Co-Infection in the Wild: Linking Gastrointestinal Parasite Communities, Host Hematology, and Immune Function. *International Journal for Parasitology: Parasites and Wildlife*. 1: 2-12. doi: 10.1016/j.ijppaw.2012.10.001

Camaschella, C. (2015). Iron-Deficiency Anemia. *N. Engl. J. Med.* 372: 1832-1843. doi: 10.1056/NEJMra1401038

Carbayo, J; Martin, K; Civantos, E. (2019). Habitat Type Influences Parasite Load in Algerian Psammodromus (*Psammodromus algirus*) Lizards. *Canadian Journal of Zoology*. 97(2): 172-180. doi: 10.1139/cjz-2018-0145

Clow, K; Ogden, N; Lindsay, L. R; Michel, P; Pearl, D; Jardine, C. (2016). Distribution of Ticks and the Risk of Lyme Disease and Other Tick-Borne Pathogens of Public Health Significance in Ontario, Canada. *Vector-Borne and Zoonotic Diseases.* doi: 10.1089/vbz.2015.1890

Clow, K; Leighton, P; Ogden, N, Lindsay, L. R; Michel, P; Pearl, D; Jardine, C. (2017).

Northward Range Expansion of *Ixodes scapularis* Evident Over a Short Timescale in Ontario, Canada. *PLoS ONE.* 12(12): e0189393. doi: 10.1371/journal.pone.0189393

Dantas-Torres, F. (2015). Climate Change, Biodiversity, Ticks and Tick-Borne Diseases: The Butterfly Effect. *International Journal for Parasitology: Parasites and Wildlife*. 4(3): 452-461. doi: 10.1016/j.ijppaw.2015.07.001

DeNatale, C; Burkot, T; Schneider, B; Zeidner, N. (2002). Novel Potential Reservoirs for Borrelia sp. and the Agent of Human Granulocytic Ehrlichiosis in Colorado. *Journal of Wildlife Diseases*. 38(2): 478-482. doi: 10.7589/0090-3558-38.2.478

Eisen, RJ; Eisen, L. (2018). The Blacklegged Tick, *Ixodes scapularis*: An Increasing Public Health Concern. *Trends Parasitol.* 34(4): 295-309. doi: 10.1016/j.pt.2017.12.006

Hentze, M; Muckenthaler, M; Andrews, N. (2004). Balancing Acts: Molecular Control of Mammalian Iron Metabolism. *Cell*. 117(3): 285-297. doi: 10.1016/S0092-8674(04)00343-5

Hersh, M; LaDeau, S; Previtali, M., Andrea; Ostfeld, R. (2014). When is a Parasite not a Parasite? Effects of Larval Tick Burdens on White-Footed Mouse Survival. *Ecology*. 95(5): 1360-1369

Huang, C; Kay, S; Davis, S; Tufts, D; Gaffett, K; Tefft, B. (2019). High Burdens of *Ixodes scapularis* larval ticks on white-tailed deer may limit Lyme Disease Risk in a Low Biodiversity Setting. *Ticks Tick Borne Dis.* 10(2): 258-268. doi:10.1016/j.ttbdis.2018.10.013.

Johnson, PTJ; Preston, DL; Hoverman, JT; LaFonte, BE. (2013). Host and Parasite Diversity Jointly Control Disease Risk in Complex Communities. *PNAS.* 110(42): 16916-16921. doi: 10.1073/pnas.1310557110

King, KC; Lively, CM. (2012). Does Genetic Diversity Limit Disease Spread in Natural Host Populations? *Heredity (Edinb)*. 109(4): 199-203. doi: 10.1038/hdy.2012.33

Kocan, K; de la Fuente, J; Coburn, L. (2015). Insights into the Development of *Ixodes scapularis*: A Resource for Research on a Medically Important Tick Species. *Parasites & Vectors*. 8: 592. doi: 10.1186/s13071-015-1185-7

Kulkarni, M; Berrang-Ford, L; Buck, P; Drebot, M; Lindsay, LR; Ogden, N. (2015). Major Emerging Vector-Borne Zoonotic Diseases of Public Health Importance in Canada. *Emerging Microbes & Infections.* 4(1): 1-7. doi: 10.1038/emi.2015.33

Leighton, P; Koffl, J; Pelcat, Y; Lindsay, LR; Ogden, N. (2012). Predicting the Speed of Tick Invasion: An Empirical Model of Range Expansion for the Lyme Disease Vector *Ixodes scapularis* in Canada. *J. App. Ecol.* 49(2): 457-464 doi: 10.1111/j.1365-2664.2012.02112.x

Lindquist, E; Galloway, T; Artsob, H; Lindsay, LR; Drebot, M; Wood, H; Robbins, R. (2016). *A Handbook to the Ticks of Canada (Ixodida: Ixodidae, Argasidae)*. Biological Survey of Canada.

Lindsay, LR; Drebot, M; Weiss, E; Artsob, H. (2001). Hantavirus Pulmonary Syndrome in Manitoba. *Canadian Journal of Infectious Diseases*. 12(3): 169-173. doi: 10.1155/2001/425260

Minigan, J; Hager, H; Peregrine, A; Newman, J. (2018). Current and potential future distribution of the American dog tick (*Dermacentor variabilis*, Say) in North America. *Ticks and Tick-borne Diseases.* 9: 354-362. doi: 10.1016/j.ttbdis.2017.11.012

Mlera, L; Meade-White, K; Dahlstrom, E; Baur, R; Kanakabandi, K; Virtaneva, K; Porcella, S; Bloom, M. (2018). *Peromyscus leucopus* Mouse Brain Transcriptome Response to Powassan Virus Infection. *Journal of Neurovirology*. 24(1): 75-87. doi: 10.1007/s13365-017-0596-y

Nicolas, G; Bennoun, M; Porteu, A; Mativet, S; Beaumont, C; Grandchamp, B; Sirito, M; Sawadogo, M; Kahn, A; Vaulont, S. (2002). Severe Iron Deficiency Anemia in Transgenic Mice Expressing Lier Hepcidin. *PNAS*. 99(7): 4596-4601. doi: 10.1073□pnas.072632499

O'Brien, E; Morrison, B; Johnson, LS. (2003). Assessing the Effects of Haematophagous Ectoparasites on the Health of Nestling Birds: Haematocrit vs. Haemoglobin levels in House Wrens Parasitized by Blow Fly Larvae. *Journ. Avian Biol.* 32(1): 73-76. doi: 10.1034/j.1600-048X.2001.320110.x

Ogden, NH; Barker, IK; Beauchamp, G; Brazeau, S; Charron, DF; Maarouf, A; Morshed, MG; O'Callaghan, CJ; Thompson, RA; Waltner-Toews, D; Waltner-Toews, M; Lindsay, LR. (2006). Investigation of Ground Level and Remote-Sensed Data for Habitat Classification and Prediction of Survival of *Ixodes scapularis* in Habitats of Southeastern Canada. *J. Med. Entomol*. 43(2): 403-414. doi: 0022-2585/06/0403Ð0414$04.00/0

Ogden, NH; Tsao, JI. (2009). Biodiversity and Lyme Disease: Dilution or Amplification?

Epidemics. 1(3): 196-206. doi: 10.1016/j.epidem.2009.06.002

Ogden, N; Lindsay, LR; Leighton, P. (2013). Predicting the Rate of Invasion of the Agent of Lyme disease *Borrelia burgdorferi*. *J Appl Ecol*. 50: 510¬518. doi: 10.1111/1365¬2664.12050

Papkou, A; Gokhale, C; Traulsen, A; Schulenburg, H. (2016). Host-parasite Coevolution: Why Changing Population Size Matters. *Zoology*. 119(4): 330-338. doi: 10.1016/j.zool.2016.02.001

Preisser, W. (2019). Latitudinal Gradients of Parasite Richness: A Review and New Insights from Helminths of Cricetid Rodents. *Ecography*. 42(7): 1315-1330. doi: 10.1111/ecog.04254

Rosales, F; Jang, J; Pinero, D; Erikson, K; Beard, J; Ross, C. (1999). Iron Deficiency in Young Rats Alters the Distribution of Vitamin A Between Plasma and Liver and Between Hepatic Retinol and Retinyl Esters. *The Journal of Nutrition*. 129(6): 1223-1228.

Rynkiewicz, E; Hawlena, H; Durden, L; Hastriter, M; Demas, G; Clay, K. (2013). Associations Between Innate Immune Function and Ectoparasites in Wild Rodent Hosts. *Parasitol. Res.* 112: 1763-1770. doi: 10.1007/s00436-013-3335-1

Talagrand-Reboul, E; Boyer, P; Bergstrom, S; Vial, L; Boulanger, N. (2018). Relapsing Fevers: Neglected Tick-Borne Diseases. *Frontiers in Cellular and Infection Microbiology.* 8:98. doi: 10.3389/fcimb.2018.00098

Westblade, L; Simon, M; Mathison, B; Kirkman, L. (2017). *Babesia microti*: from Mice to Ticks to an Increasing Number of Highly Susceptible Humans. *Journal of Clinical Microbiology.* 55(10): 2903-2912. doi: 10.1128/JCM.00504-17