Influence of weather, environment, and infection on gastropod intermediate hosts of parelaphostrongylid nematodes

by

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Abstract

Temporal and spatial variation in the transmission of parelaphostrongylid nematodes like brainworm (*Parelaphostrongylus tenuis*) and muscle worm (*Parelaphostrongylus andersoni*) is regulated by the environment and their hosts. White-tailed deer (*Odocoileus virginianus*) are the primary ungulate host that deposit first-stage larvae into the environment, while gastropods (slugs and snails) are required by these parasites for development to the third stage that is infective to ungulates. Gastropod distributions are known to vary temporally and in response to environmental variation, but their habitat selection preferences have yet to be adequately characterized. Furthermore, the habitat preferences of infected gastropods remain unknown, as observed prevalence in naturally infected hosts is low. The first chapter used transect sampling to investigate the effect of temporal variation and several environmental factors on gastropod presence and abundance in an area where moose (*Alces alces*) are recovering after population decline. Site and month influenced gastropod species composition, driven by three host species (*Deroceras laeve, Succinea ovalis, Zonitoides arboreus*). Across species, statistical models explained gastropod presence better than abundance. Presence was positively correlated with soil pH and humidity and varied among three study sites. Gastropod presence was most likely at a site where deer are currently absent, indicating that transmission risk would increase if deer were to begin occupying it. The second chapter concerned gastropod microhabitat preferences, as well as vertical climbing behaviour, within an urban park and forest inhabited by deer infected with parelaphostrongylids. From June-October, gastropod presence increased and was associated with higher humidity. Abundance also increased over time, and was associated with higher humidity, along with lower air temperature and soil moisture. Infected gastropods were found, but none exhibited vertical climbing behaviour. The results of this thesis bring further insight into

parelaphostrongylid transmission by identifying factors that influence the habitat selection of gastropods, which play a significant role in the life cycles of these parasites. Population declines in wild North American ungulates of conservation concern may be caused in part by the pathology and mortality resulting from infection with parelaphostrongylid nematodes. Therefore, understanding transmission patterns would enable conservation biologists to effectively intervene and reduce transmission to declining ungulate populations, such as moose and caribou (*Rangifer tarandus*).

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Introduction

The survival and transmission of a parasitic organism are determined by its ability to infect a host, avoid being removed by the host or inhibited by the host's immune system, and ensure that the subsequent stage within the life cycle or offspring can exit the current host and enter the environment or infect another host (Goater *et al*., 2014). Transmission is further regulated by the frequency of encounters between parasites and hosts, as well as the ability of parasites to establish infections in hosts, also referred to as compatibility. In other words, for the successful transmission and continuation of a parasitic life cycle to occur, the parasite must encounter compatible hosts (Combes, 2004). For parasites with complex life cycles, which involve the use of more than one host for the maturation of successive developmental stages, life cycle completion is further complicated by the need to successfully encounter and infect compatible hosts multiple times (Combes, 2004). Although encounter rate and compatibility exert significant influences on parasite transmission, their effects have yet to be characterized for many parasites (Buhnerkempe *et al*., 2015).

Just as encounter rate and compatibility modify parasite transmission, abiotic and biotic conditions influence the rate at which parasites encounter compatible hosts (Sousa, 1990; Combes, 2004). Two species of nematode, *Protostrongylus stilesi* and *Protostrongylus rushi*, which parasitize bighorn sheep (*Ovis canadensis*), demonstrate the influences of both types of conditions. One route of infection for both nematode species is the consumption of gastropods (slugs and snails) containing larvae by bighorn sheep. Generally, to prevent dessication, gastropods preferentially occupy habitats with increased moisture levels (Rogerson *et al*., 2008). Due to this preference, the risk of transmission of *P. stilesi* and *P. rushi* to bighorn sheep is greatest near water sources, as sheep are more likely to encounter and consume gastropods

containing *P. stilesi* and *P. rushi* larvae in these riparian habitats. As for biotic influences on transmission, in addition to ingestion of infected gastropods, both *P. stilesi* and *P. rushi* can be transmitted transplacentally from infected ewes to their fetal lambs (Rogerson *et al*., 2008).

In the context of wildlife conservation, information regarding abiotic and biotic influences on transmission can be used to determine the most effective methods for reducing transmission to host species of concern (Sousa, 1990; Garwood *et al*., 2023). For example, as described previously, riparian habitats facilitate transmission of the lungworms *P. stilesi* and *P. rushi* to bighorn sheep. Decommissioning water sources within bighorn sheep range is a potential intervention that could reduce transmission by altering gastropod habitat (Rogerson *et al*., 2008). Biotic influences on transmission are also important to consider when determining management strategies. Although the antihelminth treatment Ivermectin can be used to clear infections in non-fetal bighorn sheep, it is not effective at killing *P. stilesi* and *P. rushi* infecting fetal lambs. Therefore, drug treatment is not the only intervention that should be used in this system (Rogerson *et al*., 2008).

It has been established that biotic and abiotic influences on parasite transmission should be considered when creating interventions meant to reduce infections in hosts of conservation concern (Rogerson *et al*., 2008). However, these influences may vary at different temporal and spatial scales. If it is unknown when and where hosts of concern are being infected, as well as which factors facilitate infection and how, then interventions that effectively reduce transmission cannot be designed (Buhnerkempe *et al*., 2015). Without intervention, unregulated parasite transmission can have significant negative consequences for host populations, especially if infection causes pathology or mortality, such as the neurological symptoms that the nematode

Parelaphostrongylus tenuis (brainworm) causes in several ungulate species including moose (*Alces alces*) (Anderson, 1972).

The negative consequences of brainworm transmission are demonstrated by cause-specific mortality surveys such as one conducted by Carstensen *et al*. (n.d.) in Minnesota, which implicated *P. tenuis* in the deaths of a significant proportion of both collared and uncollared moose. By extension, this negative effect at the population level can lead to change at the community or ecosystem level, with local extinction of certain host populations and subsequent reduced biodiversity being the most extreme outcomes (Dawe and Boutin, 2016). White-tailed deer (*Odocoileus virginianus*) (subsequently referred to as deer) are the usual host of brainworm, in which infections cause little to no pathology (Anderson, 1963). In addition to moose (Anderson, 1965a), brainworm also infects other wild ungulates of conservation concern, including caribou (*Rangifer tarandus*), and elk (*Cervus canadensis*) (Anderson *et al*., 1966; Behrend and Witter, 1968). Domestic species such as sheep (*Ovis aries*) and llamas (*Lama glama*) can also become infected (Lankester, 2002). Additionally, there have been incidences of non-native ungulates kept in North American captivity for conservation purposes being infected with brainworm (Rowley *et al*., 1987). Brainworm infections in non-deer hosts are of concern to conservation biologists because they lead to symptoms like blindness, paraplegia, walking in circles, and possible mortality (Anderson, 1964). For North American moose, brainworm infections are an additional pressure on populations already exhibiting decline due to factors like human harvest, climate change, predation, and other parasites like liver fluke (*Fascioloides magna*) and winter tick (*Dermacentor albipictus*) (Murray *et al*., 2006; Manitoba Fish and Wildlife, 2020). Due to the differential impact of brainworm infection on deer and other ungulate hosts, it has been hypothesized that in addition to milder winters and habitat disturbance by

humans, brainworm may facilitate the expansion of deer via the removal of potential competitors like moose (Schmitz and Nudds, 1994). Consequently, the ungulate species composition within these communities may be altered (Anderson, 1965a; Ditmer *et al*., 2020; McGraw *et al*., 2021).

Along the spectrum of knowledge regarding parasite transmission, brainworm and its transmission to various species of ungulates lies somewhere in the middle. Many surveys have assessed the transmission of this parasite, but an effective intervention has yet to come (Lankester, 2018; Garwood *et al*., 2023). Conservation initiatives trying to reduce transmission to vulnerable non-deer species regard the presence of infected deer as the most significant biotic influence on brainworm transmission (Oliveira-Santos *et al*., 2021). Prevalence in deer is typically high (e.g. 82%) (Slomke *et al*., 1995), which could mean high potential for transmission to non-deer hosts, as deer shed brainworm larvae that penetrate gastropods and mature to the stage that is infective to ungulates (Lankester and Anderson, 1968; Slomke *et al*., 1995). Like the previously described *P. stilesi* and *P. rushi*, susceptible ungulates are infected with brainworm when they consume an infected gastropod (Lankester and Anderson, 1968; Rogerson *et al*., 2008). This has led to interventions such as establishing and keeping deer population densities below thresholds, which have had limited success at reducing brainworm prevalence or transmission. For example, in northeastern Minnesota, infection prevalence in co-occurring deer remained high and local moose populations experienced significant decline, even though deer population size is below the threshold recommended by the brainworm literature (Lankester, 2018; Ditmer *et al*., 2020; McGraw *et al*., 2021). Consequently, factors other than deer density should be considered when determining how to reduce transmission (Behrend and Witter, 1968; Lankester and Peterson, 1996; Severud *et al*., 2023).

In addition to deer infection prevalence and population density, another biotic factor that affects the transmission of brainworm is the rate at which deer shed brainworm larvae into the environment via their feces (Slomke *et al*., 1995). Regardless of age, Slomke *et al*. (1995) observed that deer shed the most larvae during spring. The likelihood of these larvae encountering suitable gastropod hosts is another biotic factor to consider. While brainworm larvae can infect several gastropod species, some are more suitable than others. For example, it has been suggested that the thin mucus secreted by the slug *Deroceras laeve* makes it easier for larvae to penetrate and begin developing, leading to increased prevalence and intensity in these individuals (Lankester, 2018). Conversely, infection experiments have shown that brainworm larvae take longer to mature in another slug species, *Deroceras reticulatum*, which could delay transmission to ungulates (Lankester and Anderson, 1968). In addition to biotic processes modifying transmission, there are several abiotic factors that also influence the continuation of brainworm's life cycle. Larvae shed by deer must survive in the environment until they encounter a gastropod belonging to a compatible host species as the gastropod crawls over deer feces or the adjacent soil (Lankester and Anderson, 1968; Duffy *et al*., 1999). Before this occurs, the larvae are dependent on favourable climatic conditions. While they can tolerate freezing temperatures, repeated drying and temperature shifts significantly reduce their longevity, and even if they can survive to encounter a gastropod host, they may not have sufficient energy to penetrate it or establish infection after penetration (Shostak and Samuel, 1984). Moist conditions and cool temperatures are optimal for brainworm larvae to persist in the environment (Lankester and Anderson, 1968; Shostak and Samuel, 1984). Even after successfully infecting a gastropod, brainworm larvae are still regulated by weather, as their development is influenced primarily by

temperature and humidity, with warm, humid conditions shortening development time, while development is halted during winter (Anderson, 1963; Lankester and Anderson, 1968).

The studies that have characterized factors influencing the life cycle of brainworm demonstrate that there are other potential ways to intervene and reduce transmission, and that the focus of intervention design should not be restricted to deer (Behrend and Witter, 1968). Since brainworm uses a complex life cycle, gastropods are another group of hosts that interventions could be directed at. One potential benefit of targeting gastropods is that it is a more direct way to reduce transmission to ungulate hosts of concern, since the consumption of infected gastropods is what leads to infection (Anderson, 1965b; Ditmer *et al*., 2020; Severud *et al*., 2023). However, this route is not without its problems. The most pressing issue is that gastropod surveys rarely find infected gastropods, from 0% (Severud *et al*., 2023) to around 2.7% prevalence (Platt, 1989). This low prevalence could potentially be explained by the rate at which immature brainworm larvae on or around deer feces encounter gastropods belonging to compatible host species. While Boag (1983) observed that terrestrial snails (*Euconulus fulvus*, *Nesovitrea occidentalis*, *Zonitoides arboreus*, *Vitrina alaskana*, *Discus whitneyi*, *Vertigo gouldi*) may be attracted to weathered bighorn sheep feces, Garvon and Bird (2005) found the terrestrial snail *Anguispira alternata* was attracted to fresh white-tailed deer feces. This difference may be due to the two studies using different gastropod species, along with feces from different ungulate species (Boag, 1983; Garvon and Bird, 2005). Others have advanced hypotheses suggesting that the actual gastropod prevalence may be higher than what was estimated because the typical methods used to sample gastropods may exclude infected individuals from collection (McCoy and Nudds, 1997; 2000). Regardless of cause, this lack of knowledge concerning the distribution and habitat preferences of infected gastropods presents a problem for those trying to reduce

brainworm transmission. As stated previously, if the timing and location of encounters between infected gastropods and susceptible ungulates of conservation concern is unknown, then interventions meant to reduce those encounters will not be effective (Garwood *et al*., 2023).

Although past surveys have returned few or no infected gastropods, they have provided insight into the habitat preferences of gastropods in general (Platt, 1989; Severud *et al*., 2023). For example, the presence and abundance of gastropods vary temporally and are influenced by several abiotic factors (Lankester and Anderson, 1968). Generally, gastropods have demonstrated a preference for habitats with a daily average humidity of at least 76.0%, with cool temperatures no higher than approximately 21.4°C, and increased rainfall (Maskey *et al*., 2015). Increased gastropod abundance has also been found in areas with mixed or upland conifer habitat types (Kearney and Gilbert, 1978; Vanderwaal *et al*., 2015), along with habitats where the ground is densely covered in leaf litter or low vegetation (Maze and Johnstone, 1986). Soil chemistry represents another set of abiotic factors that can influence gastropod habitat selection, in which neutral soils, with increased calcium and moisture have been correlated with higher gastropod abundance (Maze and Johnstone, 1986; Vanderwaal *et al*., 2015). Regarding time, gastropod response has been less consistent, with abundance varying by species (Lankester and Peterson, 1996), but there have been general associations between increased gastropod abundance and late summer or fall (Rowley *et al*., 1987; Pidwerbesky, 2022). While these observations are important to inform the creation of interventions, they are typically quite general and broad scale. More specific findings regarding the fine scale habitat conditions that gastropods are influenced by is necessary to more precisely identify where gastropods are more likely to occur, or in higher abundance, and by extension, where ungulates of concern are more likely to encounter and

consume them, potentially being infected with brainworm as a result (Kearney and Gilbert, 1978; Garwood *et al*., 2023).

In addition to the general patterns of gastropod presence and abundance, the issue of low gastropod infection prevalence is also important to investigate further, as infected gastropods are the specific factor of concern in the reduction of brainworm transmission. Low gastropod prevalence obscures the context in which transmission occurs at this stage of brainworm's life cycle, as some have argued that gastropod prevalence is too low for the high prevalence observed in deer to occur via the random ingestion of infected gastropods during foraging (McCoy and Nudds, 2000). Others suggest that deer might elect to eat gastropods, possibly as a calcium resource, as gastropods are an important calcium resource for other vertebrates like birds (Mand *et al*., 2000), and deer have been observed to prey on bird nestlings for the calcium in their bones (Pietz and Granfors, 2000). Another hypothesis advanced to explain the disparate prevalences between deer and gastropod hosts is referred to as the climbing hypothesis. Proponents claim that infection with brainworm causes gastropods to climb vegetation to the height at which ungulates, particularly deer, forage. If true, this would constitute behavioural manipulation of a host by a parasite to encourage transmission to the next host. In addition to potentially identifying another biotic mechanism that influences brainworm transmission, the climbing hypothesis also calls into question the accuracy of the standard gastropod sampling method, cardboard trapping. If the climbing hypothesis is supported, then the low prevalence observed using cardboard traps, which only sample ground-dwelling gastropods, does not reflect actual prevalence. It would also mean that this method is biased against infected gastropods whose behaviour is being altered by the parasite to climb vegetation and instead, visual searches of vegetation and soil should be employed to overcome this bias, as both climbing and ground-dwelling gastropods can be

collected with this method. Both in the field and the lab, investigations of the climbing hypothesis have been limited, despite the significant implications it would have for brainworm research and conservation (McCoy and Nudds, 1997; 2000; Cyr, 2015).

Chapter 1 concerns how gastropod intermediate host presence and abundance vary in moose habitat. Understanding the habitat preferences of gastropods is an important part of determining where and when brainworm hosts of conservation concern encounter gastropods and potentially become infected (Pidwerbesky, 2022). Temporal variation, weather, habitat type, soil chemistry, and ground cover were investigated as potential influences (Maze and Johnstone, 1986; Hawkins *et al.,* 1998; Maskey *et al*., 2015; Vanderwaal *et al*., 2015). Characterizing conditions that lead to increased gastropod presence and abundance could help wildlife managers identify areas where moose are more at risk of becoming infected with brainworm, and by extension, areas where land management to reduce gastropod abundance and moose risk can be applied (Vanderwaal *et al*., 2015; Severud *et al*., 2023).

Chapter 2 also concerns variation in gastropod presence and abundance in response to temporal variation and environmental factors. It is a follow-up to Chapter 1, which focused on general gastropod habitat preference. The goal of this chapter was to specifically investigate the habitat preferences of infected gastropods by sampling in homogenous habitat where infected deer are known to occur. Temporal variation and the same environmental characteristics were sampled, but rainfall was an additional factor investigated for it's influence on gastropods (Maskey *et al*., 2015). Furthermore, sampling occurred more frequently and was conducted later into the year. Sampling in known brainworm range increases the likelihood of collecting infected gastropods, further clarifying how deer become infected and spread brainworm, and again, how aberrant host infection risk can be predicted and areas for management can be identified

(Lankester and Peterson, 1996). Additionally, this chapter concerns the effect of brainworm infection on gastropod behaviour, which may explain the low gastropod prevalence observed in this and the previous chapter, as well as other brainworm surveys (Platt, 1989; Severud *et al*., 2023). Assessments of gastropod behaviour were conducted in the field, using timed visual searches of vegetation and litter. This represents additional necessary investigation into the climbing hypothesis and will indicate how the infective stage of brainworm within gastropods encounters ungulate hosts (McCoy and Nudds, 2000; Cyr, 2015).

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Chapter 1: Humidity and soil pH predict presence of terrestrial gastropods that transmit brainworm (*Parelaphostrongylus tenuis*) to moose (*Alces alces*) in protected habitat

Abstract

The nematode *Parelaphostrongylus tenuis* (brainworm) may be contributing to declines in moose (*Alces alces*) populations across North America. Although general trends in gastropod distributions driven by temporal and environmental variation have been observed, specific predictions concerning where the increased likelihood of gastropod presence or higher abundance have yet to be made. To investigate the response of gastropod presence and abundance to temporal variation and select abiotic characteristics, I sampled in eastern Manitoba where restrictions on moose hunting have been imposed due to concerns about the population size. Using linear transects consisting of cardboard traps, three sites were sampled for gastropods monthly from June-August 2022. Additionally, microhabitat was assessed at a subset of the traps. From all cardboards, 254 gastropods were collected. Nine species were observed, including *Deroceras laeve*, *Discus whitneyi*, *Succinea ovalis*, *Zonitoides arboreus*, *Deroceras reticulatum*, and *Cochlicopa lubrica*, which are compatible brainworm hosts. Multivariate analysis demonstrated that overall gastropod species composition varied by site and over time, with the two factors having an interactive effect. From a subset of the cardboards, generalized linear modelling indicated that gastropod presence varied significantly among the sites and was positively associated with increased humidity and soil pH. Additionally, humidity and soil pH had a marginally significant positive effect on gastropod abundance. This study suggests that gastropod community composition, presence, and abundance vary over space and time. Furthermore, areas not inhabited by deer that seemingly have low risk of infection may be sites

where risk is greatest if infected deer were to invade the area due to favourable gastropod microhabitat conditions causing high gastropod diversity, presence, and abundance.

Introduction

Declines in North American moose (*Alces alces*) populations began with historic human industrialization and persist due to past and current habitat loss, working synergistically with other pressures (Anderson, 1972; Manitoba Fish and Wildlife, n.d.). The extirpation or extinction of North American moose will have significant consequences for the environment and co-occurring humans due to their ecological, industrial, and cultural importance (Dawe and Boutin, 2016; Severud *et al*., 2023). Negative influences on moose populations include human harvest, climate change, and parasites such as brainworm (*Parelaphostrongylus tenuis*), liver fluke (*Fascioloides magna*), and winter tick (*Dermacentor albipictus*) (Murray *et al*., 2006). The nematode brainworm may be a cause of moose population decline because it has been identified as a direct and indirect cause of moose mortality. For example, in northeastern Minnesota, *P. tenuis* was detected in 23% of GPS-collared moose and 42% of uncollared moose reported as sick or dead (Carstensen *et al*., n.d.). To reduce brainworm infections in moose and ameliorate this potential threat to population stability, the temporal and spatial patterns controlling transmission of this parasite should be investigated (Pidwerbesky, 2022; Garwood *et al*., 2023).

To become infected with brainworm, moose and other ungulates ingest gastropods (slugs or snails) infected with the third-stage larvae of the parasite. Infected white-tailed deer (*Odocoileus virginianus*) maintain the presence of brainworm because their feces are the source of immature first-stage larvae that infect gastropods (Lankester and Anderson, 1968; Duffy *et al*., 1999). Due to the significance of deer in the life cycle of brainworm, wildlife managers have limited deer population densities or encouraged their hunting to reduce brainworm infections in

moose. This approach is based on the hypothesis that moose infection risk is directly indicated by the population density of sympatric deer (Lankester, 2018). However, surveys have demonstrated that brainworm transmission to moose occurs even when co-occurring deer are at or below prescribed population levels (Ditmer *et al*., 2020). Therefore, brainworm transmission may be influenced by other factors that should also be considered when determining how to reduce infections in moose. These factors include temporal, climatic, and environmental variation because they regulate the life cycle of brainworm (Behrend and Witter, 1968). For example, while fawns shed the most first-stage larvae in general, spring is when deer of all age classes shed the most larvae (Slomke *et al*., 1995). Furthermore, these first-stage larvae are more likely to survive and infect a gastropod in cool, moist climatic conditions as their survival and infectivity are significantly reduced by being dried, or frozen and thawed (Shostak and Samuel, 1984). The presence and abundance of gastropod hosts can also change temporally and in response to their environment (Pidwerbesky, 2022). This variation is important for transmission, since first-stage larvae shed by deer must penetrate and infect a gastropod as it crawls on or around deer feces to mature into the third stage that can infect deer and moose (Anderson, 1963; Duffy *et al*., 1999). Because brainworm requires gastropods to complete their life cycle, it has been suggested that gastropods should be investigated as another indicator of moose infection risk, as well as another target for interrupting brainworm transmission (Vanderwaal *et al*., 2015).

Using gastropod host presence or abundance as an indicator of moose infection risk requires knowing what influences gastropod distribution patterns. By extension, the presence of conditions that are positively correlated with the increased likelihood of gastropod presence or higher abundance can serve as an identifier for where moose are most at risk of infection and where interventions to reduce infection risk can be employed (Ditmer *et al*., 2020). Previous

gastropod surveys have demonstrated that monthly variation, habitat type, climate, ground cover, and soil chemistry influence gastropod distributions (Maze and Johnstone, 1986; Pidwerbesky, 2022). These factors can have an influence on a larger spatial scale, such as climatic conditions and habitat type (Hawkins *et al*., 1998; Vanderwaal *et al*., 2015), or on finer scale microhabitat traits, such as ground cover and soil chemistry (Platt, 1989). Past surveys have typically occurred at the habitat level, from which predictions concerning moose risk can be made, although they may lack the specificity to be effective (Pidwerbesky, 2022). Additionally, these surveys only sample a few of the variables correlated with changes to gastropod distribution at a time. The simultaneous sampling of as many of these influential variables as possible is needed to determine whether they differ in their magnitude of influence and their subsequent utility as indicators of moose risk (Wareborn, 1970; Garwood *et al*., 2023).

In addition to temporal change, relative humidity, air temperature, and habitat type are abiotic variables known to have a general influence on gastropods at a broad scale (Lankester and Anderson, 1968; Kearney and Gilbert, 1978; Hawkins *et al*., 1998; Cyr, 2015). The relationship between seasonal progression and gastropod abundance has been well documented, with the overall conclusion being that peak abundance occurs in late summer or fall (Pidwerbesky, 2022). However, some studies have observed differing abundances over time at the species level, as well as between slugs and snails in general (Lankester and Anderson, 1968; Cyr *et al*., 2014). As for the influence of climate on gastropods, habitats with increased humidity are preferred to avoid lethal dessication (Kearney and Gilbert, 1978). For example, one survey found that the average relative humidity during days when gastropods were collected was significantly higher than that of days where no gastropods were collected (Cyr, 2015). Temperature also influences gastropod distributions, with cool or moderate temperatures leading

to increased gastropod abundance, most likely also due to the need to avoid dessication (Hawkins *et al*., 1998). The habitat composition of a given area can also influence it's usage by gastropods. For example, Kearney and Gilbert (1978) observed a positive relationship between mixed forests and gastropod abundance, while Vanderwaal *et al*. (2015) found that habitats composed of upland coniferous forest had increased gastropod abundances.

Fine-scale microhabitat characteristics may indicate what makes the previously described habitat-level characteristics attractive to gastropods (Kearney and Gilbert, 1978). For example, habitat type can indicate the kind of forest found within a certain area (Vanderwaal *et al*., 2015). The leaf litter produced by different forests has different chemical properties, and by extension, will differentially affect the chemistry of the underlying soil (Wareborn, 1970). The microhabitat characteristics considered in this study include ground cover type, as well as soil moisture content, pH, and calcium concentration (Platt, 1989; Maskey *et al*., 2015). The type and proportion of ground cover in an environment can influence whether gastropods will inhabit it. At the fine-scale, certain ground cover types create more favourable conditions for gastropods. While bare rock or soil leaves gastropods vulnerable to dessication, cover types like leaf litter or low vegetation are thought to preserve moisture and prevent heating, which facilitates increased gastropod abundance (Kearney and Gilbert, 1978; Maze and Johnstone, 1986). For example, Maze and Johnstone (1986) collected the most gastropods from an area thick with low vegetation. As for soil chemistry, gastropods typically prefer areas with soil of a higher moisture content, neutral pH (around 7.0), and with a higher concentration of calcium (Maze and Johnstone, 1986), although other surveys have observed a lack of influence on gastropod abundance by soil pH and calcium (Platt, 1989).

It is important to identify conditions that increase the likelihood of gastropod presence, increase abundance, or alter species composition as these conditions can be used to identify sites where moose may be more at risk of ingesting gastropods and becoming infected with brainworm (Ditmer *et al*., 2020). By reducing one of the pressures that may be negatively affecting moose population stability, North American moose populations may be conserved and sustained into the future, fulfilling both human and ecological purposes (Dawe and Boutin, 2016; Severud *et al*., 2023). The objective of this study was to survey gastropod habitat and microhabitat to determine how species composition, presence, and abundance vary temporally and in response to environment. I hypothesized that temporal variation, climate, habitat type, ground cover type, and soil chemistry would affect gastropod presence and abundance. I predicted that increased gastropod presence and abundance would be observed in late summer (August), and in habitats that are cool in temperature (approximately 15°C) and of increased humidity (at least 80%), containing upland coniferous forest, and leaf litter or low vegetation as ground cover, along with moist soil that is neutral and calcium rich.

Methods

Linear Transect Sampling.

Within the northern half of Game Hunting Area 26 of Manitoba, Canada, six potential sampling sites were randomly selected using ArcGIS 3.1.2. (ESRI, 2023) in areas where moose and deer or their tracks were observed during an aerial survey (Dupont, unpublished). Subsequently, three sites were chosen for their feasibility of access for sampling by foot as well as the co-occurrence of moose and deer (Site A), or the occurrence of moose alone (Sites B, C) (Fig. 1.1). Habitat composition of the sites was determined using the summarize within feature in ArcGIS (ESRI, 2023). Broadleaf forest was the predominant habitat type in site A, with the rest

of the area being divided between other habitat types making up no more than 10% of the area. Site B was primarily composed of broadleaf (23%) and mixed wood (29%) forest types, along with some coniferous forest (17%) and barren or exposed land (16%). Site C was split between shrubland (36%), coniferous forest (29%), and exposed or barren land (25%). Presence of water at the sites was negligible, as the habitat composition at all sites was </= 1% water (Agriculture and Agri-Food Canada, 2024).

Each site was sampled once monthly from June-August 2022 using two linear transects composed of 68 0.25 m^2 cardboard squares spaced 10 m apart (Cyr *et al*., 2014; Pidwerbesky, 2022). Transects belonging to the same site were laid at least 100 m apart (Vanderwaal *et al*., 2015; Pidwerbesky, 2022). Transects were laid after sunrise, in which the underside of each cardboard was moistened with water before being laid on the ground and secured using a wire flag stuck through the center of cardboard. After layout, cardboards were left for about 24 hours before being retrieved. During retrieval, the underside of each cardboard was examined for the presence of gastropods, with any present being collected (Boag, 1982). Ten cardboards within each transect were selected for additional microhabitat sampling using a random number generator. At these cardboards, the proportion of four ground cover types (rock, leaf litter, vegetation, soil) were visually estimated, and a soil core approximately 10 cm deep was taken after the top layer of litter had been moved away. To compensate for the high amount of randomly selected cardboards where no gastropods were found, an additional 3-5 cardboards were also selected and sampled in the same way to better assess gastropod microhabitat preferences. At the start and end of transect retrieval, air temperature and relative humidity were measured to determine the average climatic conditions of the transect using a 3000 weather meter (Kestrel Instruments).

Lab Processing.

Collected gastropods were identified to species using dichotomous keys (Burch, 1962; Getz *et al*., 2017) before being dissected to assess infection status (Anderson, 1963). Any nematodes found were preserved in 100% EtOH (Pidwerbesky, 2022). To estimate moisture content, soil samples were spread out in 14 cm diameter Petri dishes and dried in an incubator for 24 hours at 100°C. The proportion of moisture present in a soil sample was determined using the weight of the sample before and after drying (United States Forest Service, 1954). Soil pH was assessed using a Professional Groline portable Soil pH Meter (Hanna Instruments) for most samples or an accumet XL50 meter with an accumet accucap combination pH electrode (Thermo Fisher Scientific) set at a default temperature of 23.8°C (represents the average temperature recorded by the Hanna Instruments probe). Following the manufacturer's protocols, 10 mL of dried soil was added to a 50 mL centrifuge tube, along with 25 mL dH_2O. The tube was shaken by hand for 30 seconds, after which the suspension was kept motionless for five minutes. Just before measurement, the tube was shaken briefly by hand again. For soil calcium measurement, 1 g of dried soil was added to a 50 mL tube, then 20 mL of 1 M ammonium acetate (approx. pH = 4.8). The tube was agitated on a reciprocating shaker for 1 hour at 250 rpm, then filtered using Whatman grade 6 filter paper. The calcium concentration of the filtrate was measured using a LAQUAtwin-Ca-11 water quality meter (Horiba) that had been calibrated at custom points (130 mg/L and 1800 mg/L calcium) using 135 mg/L and 1800 mg/L 1M ammonium acetate buffers, as directed by the manufacturer.

Statistical Analysis.

To identify geographic and temporal trends in gastropod community species composition, species abundances at all cardboards where gastropods were found were fourth root transformed

(Clarke and Gorley, 2015), from which a resemblance matrix using the Bray-Curtis similarity index was generated (Boyce and Ellison, 2001; Chao *et al*., 2005). Principal coordinate analysis (PCO) was conducted on the resemblance matrix, along with permutational multivariate analysis of variance using 9999 permutations (PERMANOVA) (Palily and Shankar, 2016). Multivariate analyses were conducted in PRIMER (v7 and PERMANOVA+) (PRIMER-E Limited, 2015).

To determine if gastropod presence or abundance were influenced by microhabitat, only data collected from the 10-15 randomly selected cardboards from each transect were included in analysis. Any cardboards with missing data, such as those where a soil sample could not be taken due to the presence of rock or water, were also excluded. To assess the influence of temporal and environmental factors on gastropod presence, I used logistic regression. The response variable was the presence of absence of gastropods at a cardboard. Explanatory continuous variables were average humidity and air temperature based on measurements taken at the first and last cardboard of each transect, soil moisture, soil pH, soil calcium, and the proportions of the four ground cover types were standardized before analysis (Dytham, 2011). Multicollinearity between the independent variables was checked for using variance inflation factors (VIFs), with a value >5 indicating multicollinearity (Zuur *et al*., 2007). Ground cover proportion variables were found to be perfectly correlated, so all ground cover proportions except vegetation, the most abundant ground cover proportion, were excluded (Palily and Shankar, 2016). Initially, a random effect term specifying that transect was nested within site had been included in the model, but models including this term did not converge, so the random effect was dropped from subsequent analysis. The full model containing a term for each independent variable was evaluated against subsets of it in which at least one term had been dropped. Interaction terms were not considered, which was consistent with similar analyses conducted by Vanderwaal *et al*. (2015). Model

evaluation was based on change in corrected Akaike information criterion (ΔAIC_c) (Burnham and Anderson, 2002). The fit of the best model was assessed using McFadden's pseudo-R^2, for which a pseudo-R^2 of 0.2-0.4 indicated that the model fit the data well (Louviere *et al*., 2000).

The influence of temporal and environmental variation on gastropod abundance was assessed using zero-inflated modelling to account for the many cardboards where no gastropods were found (Zuur *et al*., 2009; Hall, 2000). The same continuous predictor variables were standardized before analysis (Dytham, 2011), and VIFs were also used to assess multicollinearity (Zuur *et al*., 2007). The ground cover proportion variables were correlated, so the same process of ground cover variable exclusion and re-evaluation of VIFs was done as described above. Similarly, only the proportion of vegetation as ground cover was included. Subsets of the full model were compared against each other and the full model with best models being considered within 2 for ΔAIC_c being used to select the best model (Burnham and Anderson, 2002). Models were run with a Poisson distribution or two different negative binomial distributions that accounted for overdispersion within the count data (Zuur *et al*., 2009; Brooks *et al*., 2017). Models were run with a random effect term in which transect was nested within site, to account for the probable similarity between observations from cardboards from the same transects at the same site (Dytham, 2011), but interaction terms were not included, like methods used by Vanderwaal *et al*. (2015). The best models using each distribution type were also compared using ΔAIC_c and the fit of the best model out of the three distributions was assessed using R^2 (Burnham and Anderson, 2002; Ludecke *et al*., 2021). Generalized linear modelling was conducted using R version 4.2.1 (R Core Team, 2022) using the packages "lmtest" (Zeileis and Hothorn, 2002), "car" (Fox and Weisberg, 2019), "tidyverse" (Wickham *et al*., 2019), "ggpubr" (Kassambara, 2020), "rcompanion" (Mangiafico, 2022), "MuMIn" (Barton, 2023), "dplyr"

(Wickham *et al*., 2023), "AICcmodavg" (Mazerolle, 2020), "performance" (Ludecke *et al*., 2021), "glmmTMB" (Brooks *et al*., 2017), and "emmeans" (Lenth, 2022).

Results

Gastropods were present at 14.8% (181/1224) of all cardboards laid in GHA 26, from which 254 individual gastropods consisting of nine species were collected (Fig. 1.2). Six of these species are known hosts of brainworm: *Deroceras laeve*, *Discus whitneyi*, *Succinea ovalis*, *Zonitoides arboreus*, *Deroceras reticulatum*, and *Cochlicopa lubrica* (Pidwerbesky, 2022). The four non-intermediate host species were *Euconulus fulvus*, *Vitrina* spp., and *Catinella* spp. (Pidwerbesky, 2022) No gastropods were infected with L3s of brainworm. However, nematodes were found within two *D. whitneyi* snails, but were not putatively identified as brainworm due to the lack of the dorsal spine characteristic of *P. tenuis* and related species, along with the presence of ovoviviparous larvae within them (Anderson, 1963). Therefore, brainworm prevalence was 0%. From the cardboards randomly selected for additional soil and ground cover sampling, 133 gastropods were collected, comprising seven species: *D. laeve*, *D. whitneyi*, *S. ovalis*, and *Z. arboreus*, which can host brainworm, along with *E. fulvus*, *Vitrina* spp., and *Catinella* spp., which are not compatible hosts (Pidwerbesky, 2022).

The first axis of PCO analysis of gastropod species abundances at all cardboards explained 58.2% of the variation, with samples forming three major groups, with some indication of clustering by site and month (Fig. 1.3). The largest cluster, driven by the abundance of *D. laeve*, contains samples from all sites and months. The next largest group clustered along the vectors of *Z. arboreus* and other gastropod species, contains samples from all months at sites B and C, but only samples from site A recorded in July. The last group, samples where *S. ovalis* was most abundant, included samples from site A taken during June and July, and samples from

site B collected in August. This grouping reflects results of the PERMANOVA, in which gastropod species abundances were significantly affected by site (pseudo-$F_{2,172}$ = 9.96, P = 0.0001) and month (pseudo-$F_{2,172}$ = 10.02, P = 0.0001), as well as the interaction between the two factors (pseudo-$F_{2,172}$ = 6.49, P = 0.0001).

The best logistic regression model for gastropod presence included terms for average humidity, soil pH, and site (Table 1.1). Gastropod presence was more likely as average humidity increased from 65-100% (estimate = 0.41, χ^2 = 5.96, *P* = 0.015, Fig. 1.4a), and as soil pH increased from approximately 4.0-7.0 (estimate = 0.35, χ^2 = 4.89, *P* = 0.027 Fig. 1.4b). Generally, gastropod presence varied significantly among sites (χ^2 = 9.56, *P* = 0.0084), with presence being most likely at site C (estimate = 1.03) (Fig. 1.4c). However, this model did not fit the data well, as McFadden's pseudo-R^2 was 0.04.

For influences on gastropod abundance, the best zero-inflated model utilized a Poisson distribution and included terms for average humidity, soil pH, and the proportion of vegetation as ground cover. None of these variables had a significant effect on gastropod abundance (*P* > 0.05), although the effects of average humidity (χ^2 = 2.86, *P* = 0.091, Fig. 1.5a) and soil pH (χ^2 = 3.12, *P* = 0.077, Fig. 1.5b) approached significance (Table 1.2). Marginal and conditional R^2 for this model was 0.068 and 0.10, respectively, indicating poor fit.

Discussion

The lack of gastropods infected with parelaphostrongylids indicates that transmission risk in northern GHA 26 was low. The absence of infected gastropods at the three sites could be due to biological factors like the low number or complete absence of infected deer shedding first-stage larvae into the environment (Lankester and Anderson, 1968). Additionally, survival of first-

stage larvae may have been reduced due to unfavourable weather conditions, which would limit encounters between gastropods and larvae (Shostak and Samuel, 1984; Lankester, 2018). For example, Shostak and Samuel found that snails exposed to first-stage *P. tenuis* larvae kept at 8°C had greater infection intensities compared to those exposed to larvae that had been frozen and thawed. Although the effect of temperatures above 8°C was not investigated, their results and mine suggest that average air temperatures observed in GHA 26 (13.5-23.4°C) may have been too high for first-stage larvae to survive and successfully infect gastropods (Shostak and Samuel, 1984).

Within moose range, gastropod species composition had a complex association with spatial and temporal variation. Three brainworm host species, *D. laeve*, *S. ovalis*, and *Z. arboreus*, drove the clustering of samples in the PCO analysis, as they were the most abundant gastropod species (Pidwerbesky, 2022). These species also likely caused gastropod community composition to differ between the three sites and from June-August, as indicated by the PERMANOVA. Observed species abundances were relatively consistent with observations made by other gastropod surveys. *D. laeve* had the greatest abundance at all sites and throughout June-August, although abundance was slightly lower in June. *Z. arboreus* was collected from all sites, but only during June and July, while *S. ovalis* was present at sites A and B, and observed from June-August, but was most abundant during June and July. Lankester and Peterson (1996) also observed differing temporal abundances in these three brainworm host species while sampling gastropods in an area of Minnesota occupied by moose and deer. They observed *D. laeve* more consistently over June and July than in August, and although *Z. arboreus* was collected from June-August, abundance in June and July was greatest relative to August. As for *S. ovalis*, this species was collected from June-August, but was also most abundant during the first two months

of this interval. Differences between my results and theirs may have been due to the increased frequency of their gastropod sampling, in which they sampled weekly or biweekly. Furthermore, they sampled in more homogenous habitat, while habitat composition was more distinct across my three study sites (Lankester and Peterson, 1996).

From July-August 2022 in GHA 26, increased average humidity and soil pH indicated greater likelihood of gastropod presence, reinforcing my predictions and what other North American surveys of gastropods conducted moose habitat have observed. For example, a survey in Minnesota that sampled gastropods from June-November 2013 and from May-August 2014 using visual searches of vegetation found that on average, gastropods were collected on days when humidity was >/= 80% (Cyr, 2015). On average, site B had the highest soil pH (5.76) and was the site from which the greatest overall number of gastropods were collected. Similarly, other studies have found that gastropods typically prefer habitats with neutral soil (Maze and Johnstone, 1986), or at the very least, have noted that gastropods are averse to substrates that are too acidic (Wareborn, 1970; Vanderwaal *et al*., 2015). The increased likelihood of gastropod presence at site C may be because it possessed the highest proportion of coniferous forest, as areas containing a mix of coniferous and other forest types have also been found to host the most gastropods (Kearney and Gilbert, 1978; Nankervis *et al*., 2000; Cyr *et al*., 2014). Although this site had the lowest average humidity (83.23%) and soil pH (5.01), these values were still within the range found to be preferred by gastropods. The preference for this site by gastropods is likely due it's habitat type having a canopy that better preserves moisture compared to open habitat types where gastropod abundance is typically lower (Kearney and Gilbert, 1978; Nankervis *et al*., 2000, Vanderwaal *et al.*, 2015).

Gastropod presence did not vary temporally, which may have been due to seasonal abundance differing according to species (Lankester and Anderson, 1968; Kearney and Gilbert, 1978; Lankester and Peterson, 1996). At my study sites, *D. laeve* was most abundant during July and August, while observations of *S. ovalis* peaked in June and July, and *Z. arboreus* was absent during August. The near-significant influence of soil pH in addition to the lack of influence by soil moisture and calcium is an interesting result that could be explained by the differential importance of certain soil chemistry variables for different gastropod species. In other words, while each of these variables are important for gastropods in general, they may differ in their relative importance to certain gastropod species. For example, a given species could be regulated by soil moisture, while another is regulated by calcium (Wareborn, 1970). Soil pH may be the most significant soil chemistry variable for some species collected in this survey, such as *Z. arboreus* and *D. whitneyi*, as Maze and Johnstone (1986) found their abundance was greatest in an area where soil pH was 7.3, relative to other sites where soil pH ranged from 4.3-5.5.

The lack of influence on gastropod abundance by temporal variation or the environmental variables is inconsistent with the results of studies which I had used to formulate my predictions. However, the results of some other surveys may explain this lack of influence. Temporal variation might not have had a significant effect because species can differ in their seasonal abundance patterns (Kearney and Gilbert, 1978; Nankervis *et al*., 2000; Cyr *et al*., 2014). For example, while sampling gastropods over three intervals from May-September, Kearney and Gilbert (1978) found that *Z. arboreus* and *D. whitneyi* were most abundant during July, while *D. laeve* was most abundant in the August-September sampling interval. As for the lack of influence by soil chemistry, species-driven differences in habitat preference may also explain why this set of variables did not affect abundance (Wareborn, 1970). For example, Wareborn (1970) observed

that while most gastropod species, including *E. fulvus* and *C. lubrica*, preferred habitats with greater amounts of calcium and a soil pH >/= 5.6. However, a few other species like *Carychium tridentatum* and *Vitrea crystallina* were found more often in habitats with less calcium, while *Aegopinella nitidula* occurred most often where substrates had a pH of 4.6-5.5 (Wareborn, 1970). To date, the soil chemistry preferences of the three most abundant species I observed (*D. laeve, S. ovalis*, *Z. arboreus*) have yet to be characterized. Additionally, Platt (1989) observed differing gastropod abundances and species compositions at sites with similar soil calcium and pH measurements, this suggests that habitat type was more influential than soil chemistry. For example, Nicolai *et al*. (2019) observed that certain species preferred different habitat types. This latter survey is particularly relevant as it is one of the few gastropod surveys also conducted in Manitoba, in which species I had also collected, including *D. laeve* and *Z. arboreus*, were also observed. However, this study took place in a different habitat type uninhabited by moose (Nicolai *et al*., 2019).

Conclusions

Conditions that increase the likelihood of gastropod presence or facilitate increased abundance can be used to estimate parelaphostrongylid transmission risk (Ditmer *et al*., 2020). For example, based on results concerning gastropod presence, risk of brainworm transmission to moose inhabiting site A is relatively low, as deer are present, but it is unknown whether brainworm also occurs in this area, as the nematodes infecting snails collected there were not *P. tenuis*. This site had the highest average humidity, but this variable did not influence gastropod presence as much as the effect of sampling site. Considering both biotic and abiotic conditions, site B poses slightly more risk to moose, as this site had the greatest overall gastropod abundance, and the greatest average soil pH. As for site C, despite gastropod presence being

significantly more likely at this site, risk for the local moose population is also low, as this site had the fewest gastropods overall, along with the lowest average humidity and most acidic soil. Future research concerning the role of gastropods in brainworm transmission should make use of previous surveys and conduct a metanalysis of gastropod abundance patterns to characterize the temporal variation and habitat preferences of gastropod species known to host brainworm, and to evaluate whether these patterns are also subject to regional variation. Additionally, the specific microhabitat preferences of important host species of brainworm, like *D. laeve* and *Z. arboreus* should be characterized in the laboratory (Wareborn, 1970).

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Tables

Table 1.1 Results, fit, and terms included in the best three logistic regression models of gastropod presence (significance = $P < 0.05$, bolded).

Model	Variables	Degrees of freedom	χ^2	*P*	McFadden's pseudo-R^2	ΔAIC_c	Post-hoc comparison
1	Humidity	1	5.96	**0.015**	0.044	-	
	Soil pH	1	4.89	**0.027**			
	Site	2	9.56	**0.0084**			C>A, C>B
2	Humidity	1	7.00	**0.0081**	0.050	0.51	
	Soil pH	1	5.47	**0.019**			
	Prop. vegetation	1	1.60	0.21			
	Site	2	7.43	**0.024**			C>B
3	Soil calcium	1	1.30	0.25	0.049	0.81	
	Humidity	1	6.37	**0.012**			
	Soil pH	1	6.15	**0.013**			
	Site	2	9.95	**0.0069**			C>A, C>B

Table 1.2 Top zero-inflated models using a Poisson distribution for gastropod abundance at the randomly selected subset of cardboards (Near-significance = 0.05 > $P < 0.1$, bolded).

Model	Variables	Degrees of freedom	χ^2	P	Marginal R^2	Conditional R^2	ΔAIC_c
1	Humidity	1	2.86	**0.091**	0.068	0.10	-
	Soil pH	1	3.12	**0.077**			
	Prop. vegetation	1	2.44	0.12			
2	Humidity	1	3.05	**0.081**	0.079	0.11	0.31
	Soil pH	1	2.47	0.12			
	Soil moisture	1	2.08	0.15			
	Prop. vegetation	1	2.77	**0.096**			
3	Humidity	1	2.10	0.15	0.056	0.099	0.34
	Soil pH	1	2.21	0.14			

Figures

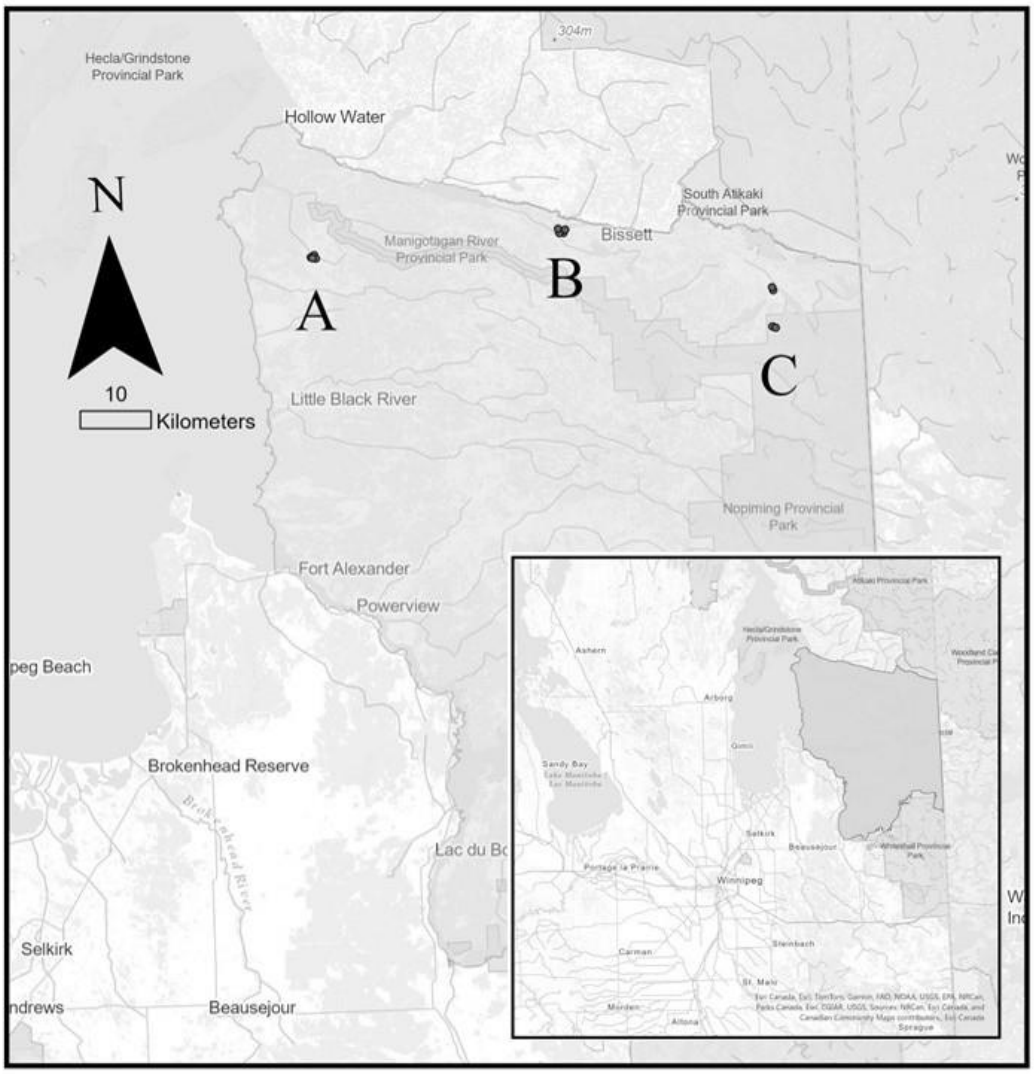


Figure 1.1 The three sites (A, B, C) at which gastropod species composition, presence, and abundance were sampled in moose habitat in southeastern Manitoba (Canada) from June-August 2022. Each site was sampled once per month using two linear transects (depicted with solid dots) approximately 680 m long (Figure generated using ArcGIS pro 3.1.2 (ESRI, 2023) and data from the Government of Manitoba).

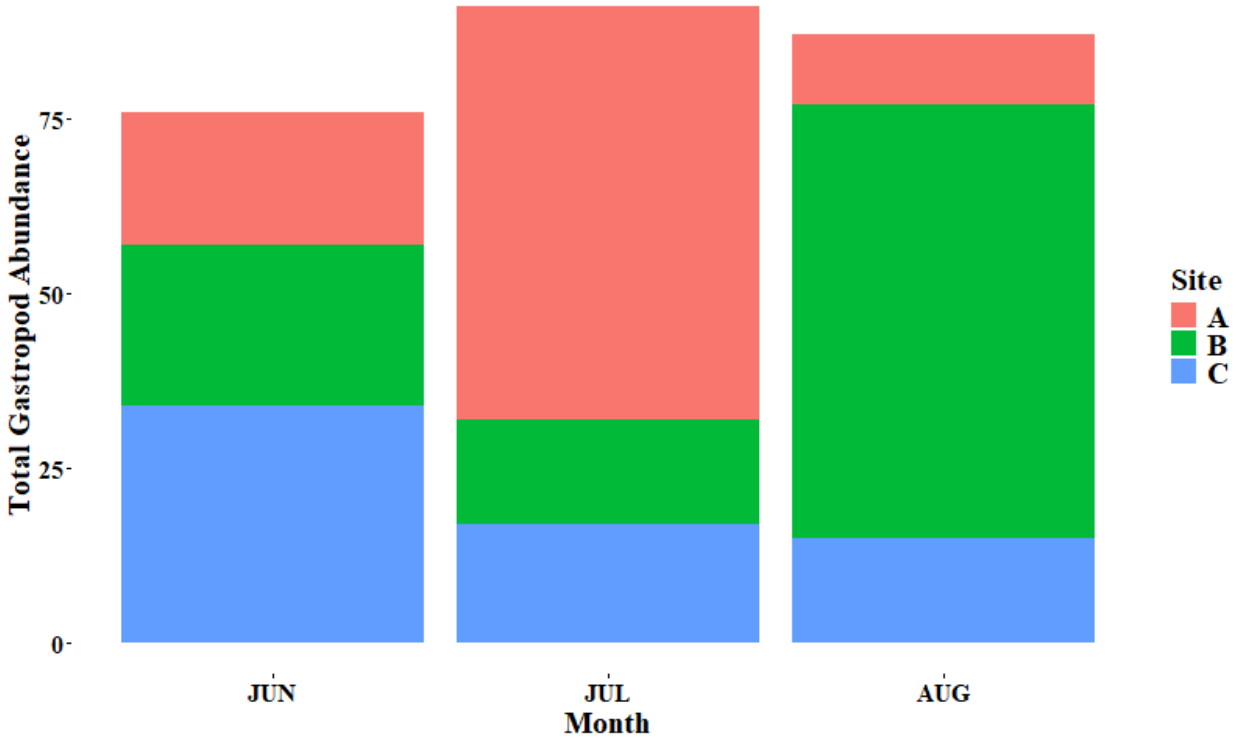


Figure 1.2 Overall gastropod abundance (individual counts) observed at the three sites in Game Hunting Area 26 from June-October 2022.

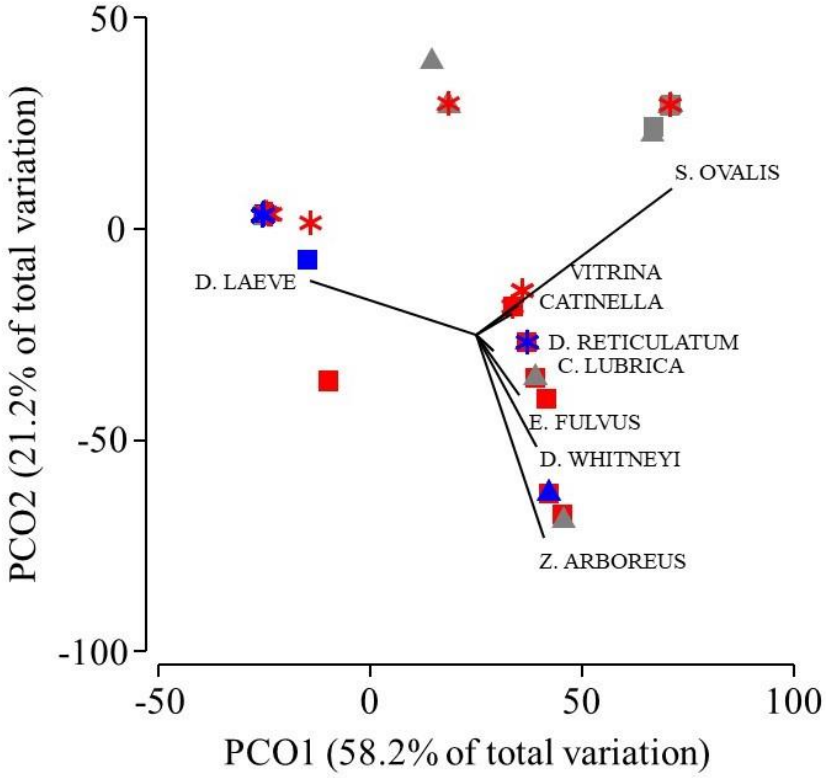
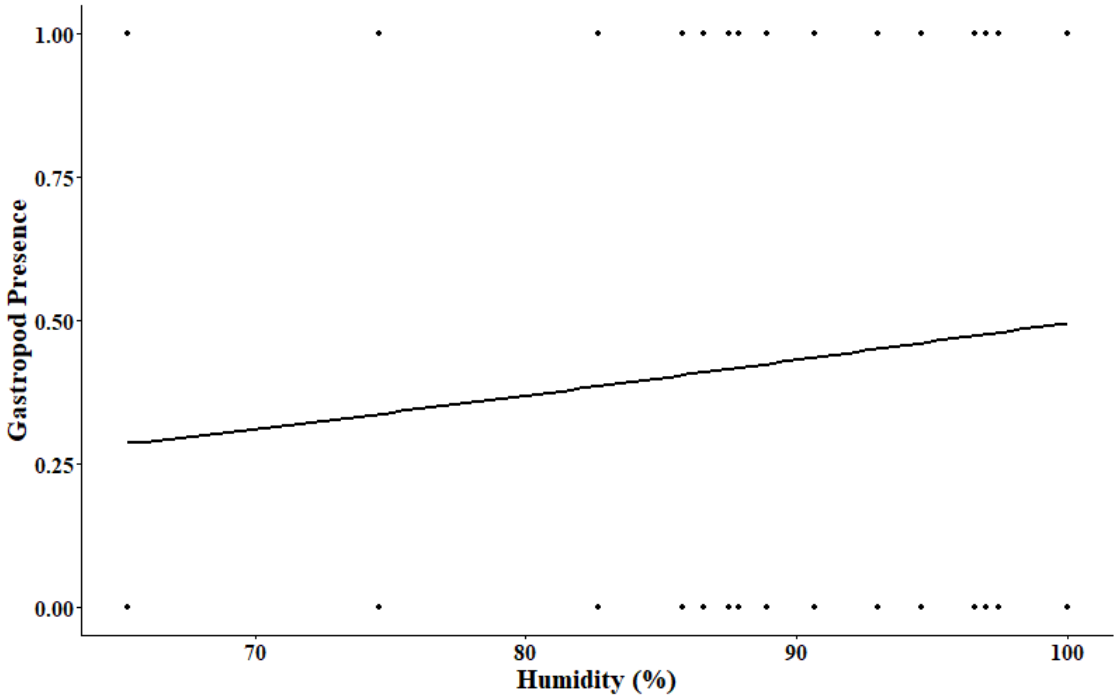
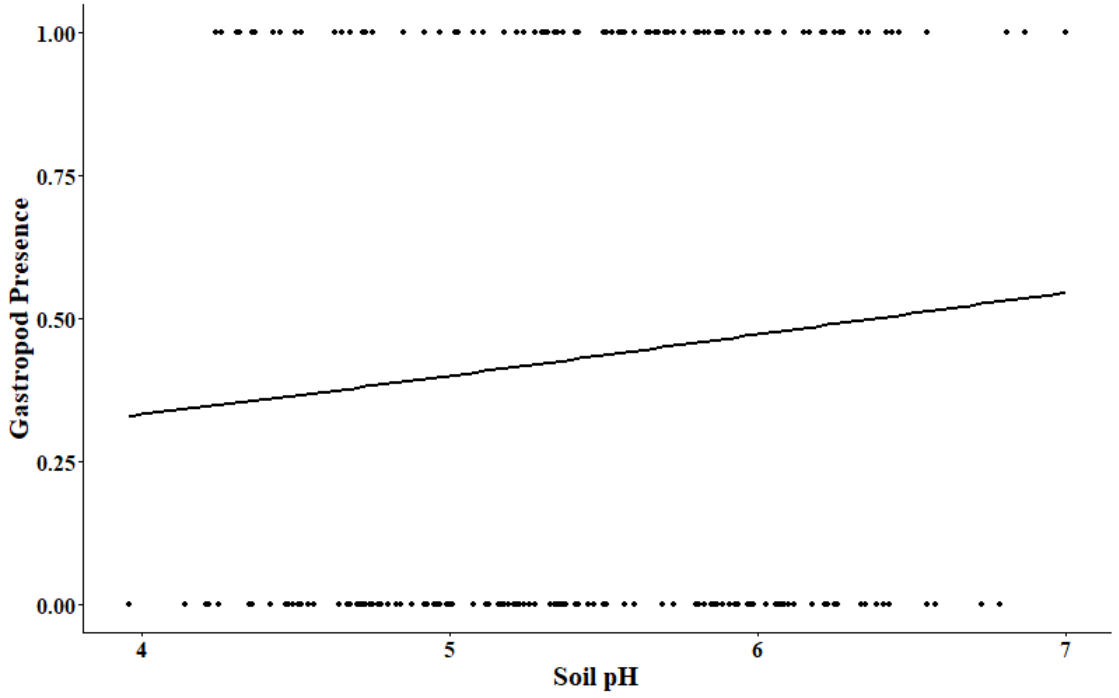


Figure. 1.3 Principal coordinates analysis plot of gastropod species composition at all cardboards (Sites are represented by colour (grey = site A, red = site B, blue = site C), months are represented by shape (square = June, triangle = July, star = August)).

A.


1.00
0.75
0.50
0.25
0.00
Gastropod Presence
70
80
90
100
Humidity (%)

B.


1.00
0.75
0.50
0.25
0.00
Gastropod Presence
4
5
6
7
Soil pH

C.

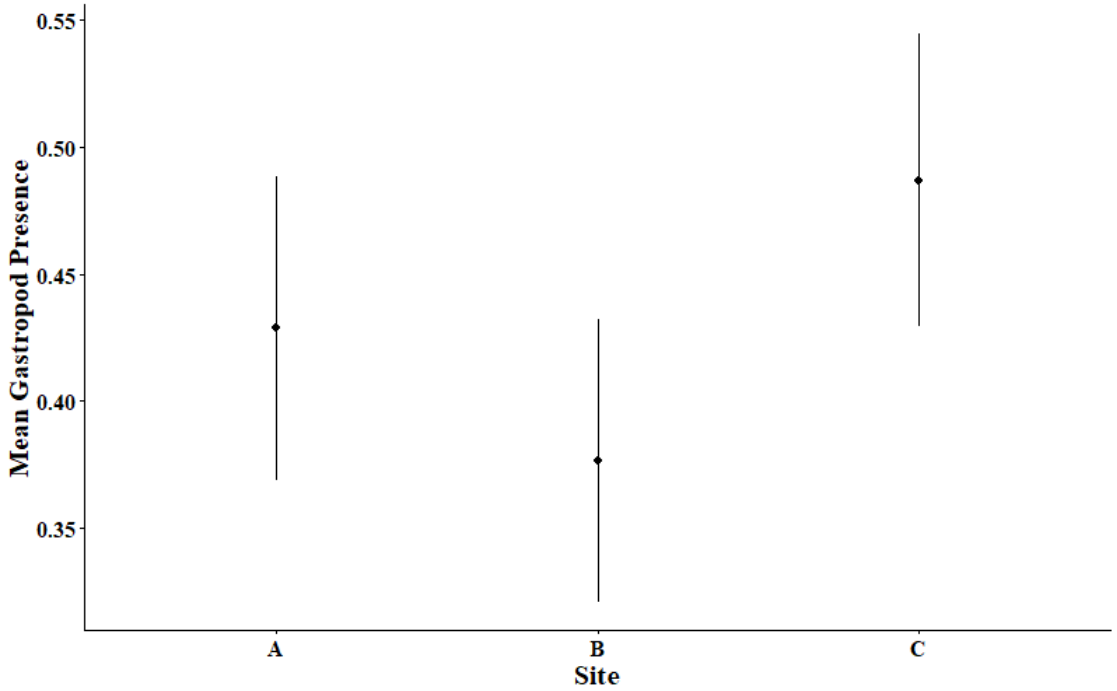


Figure 1.4 Positive relationships between the likelihood of gastropod presence and (A) average relative humidity and (B) soil pH within GHA 26, along with variation in gastropod presence by (C) site (mean ± SE).

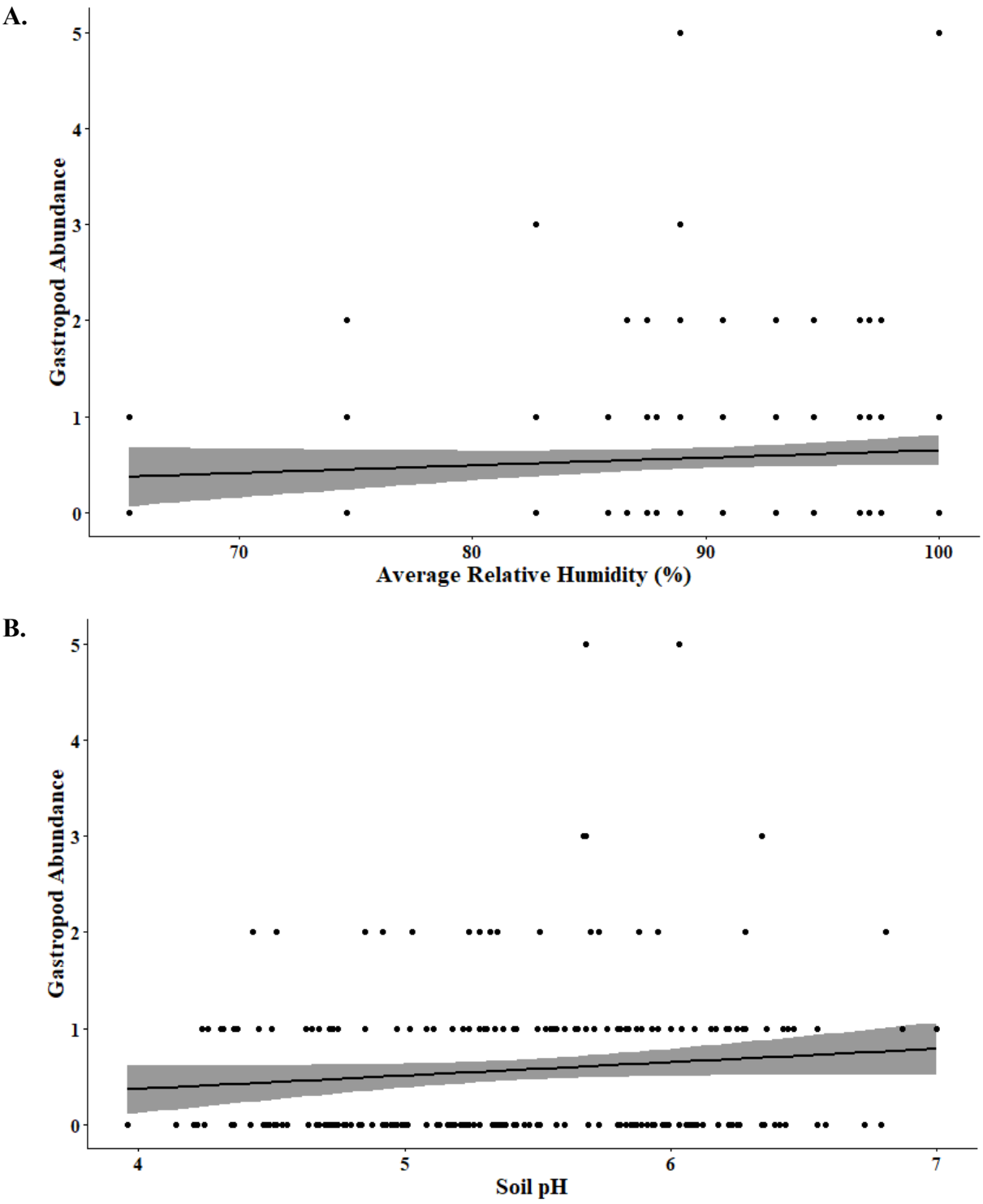


Figure 1.5 Near significant correlation of gastropod abundance (individual counts) with (A) average relative humidity ($P = 0.091$) and (B) soil pH ($P = 0.077$) within GHA 26 (shading represents 95% confidence interval).

Chapter 2: Terrestrial gastropod habitat selection: Temporal variation, weather, and microhabitat are more important than infection with parelaphostrongylid parasites

Abstract

Terrestrial gastropods are required hosts of parelaphostrongylid nematodes like brainworm (*Parelaphostrongylus tenuis*) and muscle worm (*Parelaphostrongylus andersoni*). To predict and reduce the transmission rates of these parasites to subsequent hosts like white-tailed deer (*Odocoileus virginianus*), factors that influence gastropod habitat selection should be identified. Gastropod distributions are influenced by temporal and environmental variation. Previous investigations of general gastropod response have found positive associations between the late summer and fall months, as well as habitats with cool, humid climates and moist, neutral soils. However, reliable predictions of where and when presence and abundance would be greatest still cannot be made. Furthermore, the influence of other factors on gastropod habitat selection, such as infection status, have yet to be determined. Gastropods and their habitat conditions were sampled from June-October 2023 at six sites within an urban park and forest in Winnipeg, Manitoba, using linear cardboard transects. In addition, visual searches of vegetation and leaf litter were conducted alongside the transects to assess whether parelaphostrongylid infection altered gastropod climbing behaviour. Cardboard transect surveys returned 1131 gastropods, of 10 distinct species. The slug *Deroceras laeve*, a significant parelaphostrongylid host, dominated the sample (957/1131). Multivariate analysis identified spatial and temporal changes in gastropod species composition, while generalized linear modelling indicated that gastropod presence increased over time and in response to higher humidity. Gastropod abundance was also positively influenced by these factors, along with lower temperatures and soil moisture content. The results of this study indicate that temporal variation, weather

conditions, and soil moisture could be used to predict when and where gastropod presence and abundance would be greatest, and by extension, where risk of parelaphostrongylid transmission is highest. Additionally, seven gastropods were found climbing, all of which were *D. laeve* except for a single *Vitrina* spp. Visual searches and cardboard traps did not differ in the proportion of infected gastropods returned, indicating that infection likely does not induce gastropods to climb vertically.

Introduction

Several nematode species within the genus *Parelaphostrongylus*, including brainworm (*Parelaphostrongylus tenuis*) and muscle worm (*Parelaphostrongylus andersoni*), must infect terrestrial gastropods for development to the third larval stage and transmission to subsequent hosts, primarily white-tailed deer (*Odocoileus virginianus*) (Ballantyne and Samuel, 1984). Deer become infected when they ingest a gastropod containing larvae of the parasite (Ballantyne and Samuel, 1984). In addition to deer, these nematodes can also infect other wild ungulates, which is cause for concern in wildlife management, because infection in these non-deer hosts may cause pathology and mortality and contribute to overall population decline (Pidwerbesky *et al*., 2023). Therefore, it is important to investigate how transmission rates might vary temporally and spatially, as deer expand their ranges northward, bringing their parasites with them (Anderson, 1972). Geographically, *P. tenuis* occurs in eastern North America, while *P. andersoni* may be more widely distributed, although further investigation is needed (Ballantyne and Samuel, 1984; Pidwerbesky *et al*., 2023). Pathology caused by brainworm infection in non-deer hosts has been the most well-characterized. In these hosts, infection leads to symptoms such as blindness, circling, and paraplegia (Anderson, 1964; 1965), which implicate this parasite in ungulate population decline, especially in moose (Murray *et al*., 2006; Carstensen *et al*., n.d.). Less is

known regarding the pathology and subsequent population-level impacts of infection by muscle worm (Pidwerbesky *et al*., 2023).

First stage larvae of these parelaphostrongylids infect gastropods like the slug *Deroceras laeve* through contact as this host crawls on or around the feces of deer (Duffy *et al*., 1999; Pidwerbesky *et al*., 2023). Infection risk for ungulate hosts of concern has been associated with areas in which conditions for increased gastropod abundance are favourable (Lankester and Anderson, 1968; Pidwerbesky *et al*., 2023). Therefore, altering landscape in areas with microhabitat conditions that increase the likelihood of gastropod presence or lead to increased gastropod abundance is a potential management strategy. This approach could reduce transmission to non-deer ungulate populations exhibiting decline, as reduced gastropod presence and abundance make ungulate ingestion of gastropods less likely (Ditmer *et al*., 2020).

Regardless of infection, the presence and abundance of gastropod hosts have been shown to vary temporally (Lankester and Peterson, 1996) and in response to several abiotic variables such as weather or climate (Hawkins *et al*., 1998; Cyr, 2015; Lankester, 2018), ground cover composition, and soil chemistry (Maze and Johnstone, 1986). For example, Pidwerbesky (2022) observed greater gastropod density in August and September relative to June and July. Moreover, the probability of gastropod presence has been positively correlated with humidity (Cyr, 2015), and ground temperatures around 15°C (Hawkins *et al*., 1998). Additionally, habitats with increased rainfall have been indirectly associated with increased gastropod abundance, as areas that receive more rainfall have higher rates of brainworm transmission (Jacques, *et al*., 2015; Maskey *et al*., 2015). In addition to temporal variation and weather, ground cover composition can also influence overall gastropod presence and abundance, with dense, shade-giving cover such as leaf litter or low vegetation being preferred by gastropods (Maze and Johnstone, 1986).

Soil chemistry is another determinant of gastropod habitat suitability, with the greatest average number of gastropods being found in soils with a pH of at least 5.6 (Wareborn, 1970) and moist, calcium-rich soils being preferred (Maze and Johnstone, 1986).

Previous studies of gastropod distributions have typically been applied at the habitat level, assessing general habitat composition and climate over large distances (≥ 150 km) (Maskey *et al*., 2015; Vanderwaal *et al*., 2015). This approach may reduce the ability of conservation initiatives to specifically predict where gastropod abundance and subsequent transmission risk might be greatest. Instead, finer-scale sampling of distinct microhabitat conditions immediately experienced by gastropods is needed (Kearney and Gilbert, 1978; Pidwerbesky, 2022). For example, although they illustrate how precipitation facilitates transmission, the previously mentioned studies that have investigated this variable typically use data from weather stations, which do not account for the significant variation in rainfall that can occur between 250000 m^2 blocks (Jensen and Pedersen, 2005). Furthermore, different habitat types possess distinct abiotic characteristics. For example, the tree species composition of certain habitat types leads to different soil chemistries, as trees produce leaf litter with distinct properties that uniquely affect the chemistry of the underlying soil as the litter degrades (Wareborn, 1970). Controlling for the effect of habitat type by sampling in similar habitats may lead to a better understanding of gastropod response to temporal variation, along with finer-scale characteristics such as ground cover and soil chemistry (Kearney and Gilbert, 1978; Maskey *et al*., 2015).

Additionally, gastropod activity and habitat selection may be influenced by other factors, such as brainworm infection itself, but this influence has not been adequately studied in natural or laboratory settings (McCoy and Nudds, 1997; 2000). One laboratory study compared the vertical climbing behaviour of *Appalachina sayana* (syn. with *Mesodon sayanus*) snails

experimentally infected with brainworm with that of uninfected snails, finding that the behaviour of the two groups did not differ significantly (McCoy and Nudds, 2000). These findings concern one gastropod species out of the almost 20 that brainworm is known to infect (McCoy and Nudds, 1997). Assessments of climbing behaviour in nature are also limited. A study conducted in Minnesota found climbing gastropods, although none were infected (Cyr, 2015). At present, these results suggest that parelaphostrongylid infection may not alter gastropod behaviour. However, it is unknown whether parelaphostrongylids alter gastropod behaviour in other species or in other parts of their ranges to influence transmission. Therefore, more investigation is needed in other regions inhabited by these parelaphostrongylids, and more gastropod host species need to be assessed.

Low brainworm prevalence in gastropods (<0.1%) (Lankester, 2018) has prevented the characterization of the habitat preferences of infected gastropods, as well as the conditions that lead to increased transmission (Pidwerbesky, 2022). Sampling gastropods in areas inhabited by deer with confirmed brainworm infections is a potential solution, as this would increase the likelihood of collecting infected gastropods (Lankester and Peterson, 1996). If enough infected gastropods are sampled, then their habitat preferences could be characterized, allowing for the identification of areas known as "hotspots of infection." These hotspots would harbour more infected gastropods and would be an ideal place to apply interventions to reduce gastropod abundance so transmission to aberrant hosts may be reduced (Maze and Johnstone, 1986; Ditmer *et al*., 2020; Pidwerbesky, 2022). The issue of low gastropod prevalence has also caused some to question how the life cycle of brainworm is maintained, as prevalence in deer is often significantly higher (84%) (Slomke *et al*., 1995) than that of gastropods (McCoy and Nudds, 2000). The hypothesis advanced to explain this disparity in prevalence proposes that infection

alters gastropod behaviour, making them climb vegetation to where they are more likely to be consumed by foraging ungulates (McCoy and Nudds, 2000). According to this hypothesis, the typical low gastropod prevalence observed by most surveys is due to the sampling method used, cardboard trapping (Boag, 1982), as this method only allows for the collection of gastropods moving across the substrate and excludes gastropods that have burrowed into the soil or climbed vegetation (Rogerson *et al*., 2008). Therefore, the actual infection prevalence in gastropods may be much higher than what is observed and sufficient for the maintenance of high prevalence in deer, but it has yet to be reliably assessed. Visual searches of vegetation or soil may return a more accurate estimate of prevalence because both climbing and non-climbing gastropods are collected (McCoy and Nudds, 1997). However, this method has yet to yield infection prevalences significantly higher than those returned by cardboard trapping (Platt, 1989; Cyr, 2015).

The first objective of this study was to assess gastropod habitat preferences in areas of similar habitat type to assess the influence of temporal variation and select abiotic conditions independent of habitat type, and within confirmed parelaphostrongylid range, to increase the probability of collecting infected gastropods. I hypothesized that gastropod community species composition, presence, and abundance would vary temporally and be influenced by climatic conditions, ground cover composition, and soil chemistry. I predicted that gastropod presence would be most likely, and abundance would be greatest, during later summer and fall (August-September), in cool, humid weather with more rainfall, in areas where leaf litter or short vegetation was the predominant ground cover type, and where soil is neutral and contains a higher proportion of moisture and calcium. The second objective of this study was to use cardboard traps and visual searches to determine whether parelaphostrongylid infection altered

gastropod climbing behaviour in nature. The two gastropod collection methods were also compared to determine whether they differed in their estimates of infection prevalence. I hypothesized that parelaphostrongylid infection would alter gastropod behaviour, predicting that infection would make gastropods climb vertically, and that visual searches would return more infected gastropods than cardboard trapping.

Methods

Transect Sampling of Gastropod Habitat.

Six sites within Winnipeg, Manitoba, Canada were selected for sampling, with three sites in an urban park, Assiniboine Park Conservancy (APC), and three in an urban forest, Assiniboine Forest (AF) (Fig. 2.1). Sites were selected based on the proportion of broadleaf forest present (>45%), usage by deer known to be infected with brainworm (Detwiler, unpublished), and the reduced likelihood of being disturbed by human visitors to APC and AF. Each site was sampled nine times approximately every two weeks from June-October 2023. The habitat types of the sites included varying proportions of broadleaf forest, shrubland, developed land, and grassland. Within APC, site 1 was 65% broadleaf forest, 25% shrubland, and 10% grassland, site 2 was 46% broadleaf forest and 54% developed land, while site 3 was 45% broadleaf and 55% developed land. Within AF, site 4 was 100% broadleaf forest, site 5 was 68% broadleaf forest and 32% shrubland, and site 6 was also 100% broadleaf forest (ESRI, 2023; Agriculture and Agri-Food Canada, 2024).

Sampling involved laying three transects consisting of 10 0.25 m^2 cardboard squares spaced five meters apart. The three transects were laid such that they were parallel to each other and separated by five meters (Boag, 1982). At each site, the cardboard transects were laid out in the

evening, with their undersides moistened with water, while their topsides were covered in plastic sheeting to prevent moisture loss (Cyr *et al*., 2014; Maskey *et al*., 2015). After approximately 12 hours, cardboards were retrieved and their undersides were examined for gastropods, with any present being collected (Boag, 1982; Cyr *et al*., 2014). Five cardboards at each site were randomly selected for additional gastropod habitat sampling, where a soil sample was taken, and the ground beneath the cardboard was visually examined and the proportions of four ground cover types (rock, soil, vegetation, leaf litter) were recorded (Maze and Johnstone, 1986). Measurements of weather were taken at each site during cardboard retrieval, in which a 3000 Weather Meter (Kestrel Instruments) was used to record the temperature and relative humidity of the site before and after cardboard retrieval, from which the average temperature and relative humidity were calculated. A HOBO Data Logging Rain Gauge (Onset Computer Corporation) was also installed at each site according to the manufacturer's manual to estimate total overnight precipitation at the sites during sampling.

In-Field Assessments of Behaviour.

Approximately one meter away from the randomly selected cardboards mentioned previously, a 0.25 m^2 quadrat was laid, and vegetation up to a height of 183 cm, as well as the leaf litter or top layer of soil within were visually searched for gastropods over 10 minutes. Gastropods found during searches were also collected, and the location in which they were found was noted, along with the height off the ground they were found at (Cyr, 2015; Severud *et al*., 2023).

Lab Processing.

Gastropods were identified to species using dichotomous keys (Burch, 1962; Getz *et al*., 2017) and dissected to assess infection status. Parelaphostrongylid larvae were identified based on morphological characteristics such as the presence of a dorsal spine (Ballantyne and Samuel, 1984). They and other helminths found were digitally imaged before being preserved in 100% ethanol and stored at -80°C for subsequent DNA barcoding (Pidwerbesky, 2022). Soil samples were weighed, dried for 24 hours at 100°C, and weighed again to determine moisture content (United States Forest Service, 1954). After suspension in distilled water, the pH of the dried soil samples was measured using a Professional Groline Portable Soil pH Meter (Hanna Instruments), as well as an Accumet XL50 meter with an Accumet Accucap combination pH electrode (Thermo Fisher Scientific), in accordance with protocols provided by the manufacturers. Calcium concentrations were measured using a LAQUAtwin-Ca-11 water quality meter (Horiba) calibrated using custom points (130 mg/L and 1800 mg/L calcium) with 135 mg/L and 1800 mg/L 1M ammonium acetate buffers. As directed by the manufacturer, dried soil was mixed with 1M ammonium acetate buffer (approx. pH = 4.8), which after filtration, was used to determine calcium concentration.

Statistical Analysis.

Multivariate analysis was used to determine how gastropod species compositions changed over time or differed between the sites in PRIMER (v7 and PERMANOVA+) (PRIMER-E Limited, 2015). Species abundances at all cardboards as well as exclusively the randomly selected cardboards were analyzed. Any cardboard at which no gastropods were found was excluded from analysis. To account for the overrepresentation of the meadow slug *Deroceras laeve*, species abundances at each cardboard were fourth root transformed (Clarke and Gorley, 2015), before resemblance matrices using Bray-Curtis similarity index were generated

(Boyce and Ellison, 2001; Chao *et al*., 2005). Principal coordinates analysis (PCO) was used to visualize dissimilarity between the sites by month, along with which gastropod species were driving that dissimilarity. Permutational multivariate analysis of variance (PERMANOVA: 9999 permutations) was used to assess whether gastropod species composition differed significantly between the sites and over time (Clarke and Gorley, 2015; Palily and Shankar, 2016). Environmental data (average humidity, average temperature, proportion of leaf litter as ground cover, soil moisture, pH, and calcium) from the randomly selected cardboards at each site was analyzed in a similar manner, although these variables were normalized, and the resemblance matrix generated was based on Euclidean distance. Overnight rainfall and proportion of rock and soil as ground cover had to be dropped due to the high amount of zero values recorded (Clarke and Gorley, 2015; Palily and Shankar, 2016). The proportion of vegetation was also excluded, as it was correlated with the proportion of leaf litter (Palily and Shankar, 2016). Correlations between patterns in gastropod species composition at randomly selected cardboard and the environmental measurements were assessed using the RELATE function in PRIMER (Clarke and Gorley, 2015; PRIMER-E Limited, 2015).

In the subsequent analyses, all cardboards were analyzed to determine if gastropod presence and abundance varied temporally or were influenced by average temperature, average humidity, and rainfall. Gastropod presence and abundance at the randomly selected cardboards were also modelled against those variables, but the proportions of the four ground cover types (rock, soil, vegetation, leaf litter), as well as soil moisture, pH, and calcium were included as additional predictor variables. Gastropod presence was analyzed using logistic regression, beginning with continuous variable standardization and checks for correlation using a variance inflation factor (VIF) >5 as an indicator of multicollinearity (Dytham, 2011; Zuur *et al*., 2007).

For analysis of randomly selected cardboards, the ground cover proportions exhibited multicollinearity, so the least represented proportion variables were dropped (Zuur *et al*., 2007; Palily and Shankar, 2016). Leaf litter was the most common ground cover proportion and only ground cover variable that was not excluded from analysis. Site was included as a random effect term to account for the increased probability that cardboards from the same site would be more similar (Dytham, 2011). Variations of the full model that included different numbers and combinations of variables were compared based on change in corrected Akaike information criterion (ΔAIC_c) (Burnham and Anderson, 2002). McFadden's pseudo-R^2 was calculated for the best model to evaluate how much variation within the response variable was explained, in which a value of 0.2-0.4 was considered ideal (Louviere *et al*., 2000).

Due to the high proportion of cardboards where zero gastropods were found, temporal and environmental influences on gastropod abundance were analyzed using zero-inflated modelling (Martin *et al*., 2005; Zuur *et al*., 2009). The same predictor variables were standardized and again assessed for multicollinearity using VIFs (Zuur *et al*., 2007; Dytham 2011). Again, ground cover proportion variables had to be dropped so only leaf litter remained (Palily and Shankar, 2016) and subsets of the full model were compared against each other based on ΔAIC_c value no greater than 2 (Burnham and Anderson, 2002) with the marginal and conditional R^2 values of the best model being calculated to determine the degree of model fit (Ludecke *et al*., 2021). The models were fitted with a Poisson distribution, along with two negative binomial distributions that account for either positive linear or quadratic relationships between the variance and the mean (Brooks *et al*., 2017). The best models using each distribution were then compared using ΔAIC_c (Burnham and Anderson, 2002). A paired t-test indicated that soil pH measurements differed significantly according to pH probe, with measurements from the

Professional Groline Portable Soil pH Meter (Hanna Instruments) being on average 0.392 greater than those taken using the Accumet XL50 (Thermo Fisher Scientific) ($t = -13.00$, $df = 14$, $P < 0.0001$). Measurements recorded using the Accumet XL 50 (Thermo Fisher Scientific) probe were transformed via the addition 0.392 before being used in all analyses (Dytham, 2011).

Several analyses were conducted to compare gastropod samples collected using visual searches with samples from the adjacent randomly selected cardboards. A Wilcoxon signed rank test was used to determine if one method led to the collection of more gastropods regardless of infection status, as a Shapiro-Wilk test revealed that the abundance data did not fit the assumptions of normality ($W = 0.72$, $P < 0.0001$). A Fisher's exact test was conducted to determine if the two methods differed in the number of infected and uninfected gastropods they returned, and a chi-square test was used to evaluate whether one of the methods returned the same numbers of gastropods belonging to species known to host brainworm and species known to be unsuitable brainworm hosts (Dytham, 2011). Excluding multivariate analysis, all analyses were conducted in R version 4.2.1 (R Core Team, 2022) using the packages "dplyr" (Wickham *et al*., 2023), "tidyverse" (Wickham *et al*., 2019), "car" (Fox and Weisberg, 2019), "MuMIn" (Barton, 2023), "rcompanion" (Mangiafico, 2022), "ggpubr" (Kassambara, 2020), "glmmTMB" (Brooks *et al*., 2017), "AICcmodavg" (Mazerolle, 2020), "lmtest" (Zeileis and Hothorn, 2002), "lme4" (Bates *et al*., 2015), and "performance" (Ludecke *et al*., 2021).

Results

A total of 1131 gastropods were collected from 25.4% (411/1620) of the cardboard traps (Fig. 2.2). Overall gastropod collection consisted of 10 species, six which are known to host brainworm: *D. laeve*, *Zonitoides arboreus*, *Discus whitneyi*, *Deroceras reticulatum*, *Succinea ovalis*, *Cochlicopa lubrica*, and four species that do not host brainworm: *Euconulus fulvus*,

Vitrina spp., and two species of the genus *Vallonia* (referred to as *Vallonia* spp. 1 and *Vallonia* spp. 2) (Pidwerbesky, 2022). Thirteen gastropods collected from all cardboards were infected with at least one nematode, however, two individuals could be eliminated as potential hosts of parelaphostrongylids based on morphology. Therefore, 0.97% (11/1131) of gastropods were infected with nematodes identified as parelaphostrongylid dorsal-spined larvae (Ballantyne and Samuel, 1984). This group of infected individuals was composed of four forest disc snails (*D. whitneyi*) and seven meadow slugs (*D. laeve*). Overall prevalence in *D. laeve* was 0.73% (7/957) and 57.14% (4/7) in *D. whitneyi.* From the randomly selected cardboards, 192 gastropods were collected, of eight species, with *D. whitneyi* and *C. lubrica* being absent from this subset. One of these gastropods was found on a cardboard that had been randomly selected, a *D. laeve* slug collected during late September from site 4, leading to an infection prevalence of 0.52% (1/192) in this subset of gastropods. All infected gastropods were collected from AF during August and September.

For the gastropod species abundances recorded at all cardboards, PCO analysis accounted for 48.8% of the variation and indicated four distinct groups (Fig. 2.3a). One group of samples consisted of gastropods collected from the urban forest sites (4-6) during June/July-October, dominated by the abundance of *D. laeve*. Another group represented gastropods collected from the same sites from July-October but was primarily composed of *Vitrina* spp. instead of *D. laeve*. The other two groups consisted of samples where *D. laeve*, *D. reticulatum*, *Z. arboreus*, and other snail species were observed. However, they grouped according to site, in which one group was composed of samples from all sites, while the other was exclusively composed of samples from the urban forest (sites 4-6). This clustering is reflected by the PERMANOVA results, in which gastropod community species composition differed between the sites (PERMANOVA,

pseudo-$F_{5,387}$ = 18.28, P = 0.0001) and by month (pseudo-$F_{4,387}$ = 2.79, P = 0.014), with an interaction between the two factors (pseudo-$F_{14,387}$ = 2.02, P = 0.0060). For gastropods collected from randomly selected cardboards, PCO analysis of gastropod species abundances indicated that 58.1% of variation was explained and demonstrated similar grouping as the PCO run with all cardboards, in which four distinct groups formed, mostly driven by the abundances of *D. laeve* and *Vitrina* spp. Again, the major group within this plot contained samples from July-October and sites 4-6, although the other three groups contained fewer samples from smaller spatial and temporal ranges. Additionally, one of these three smaller groups contained only samples from sites 1-3 (Fig. 2.3b). Site was the only factor to cause community species composition to differ significantly (PERMANOVA, pseudo-$F_{5,52}$ = 11.04, P = 0.0001). The significance of site is likely indicated by the separation of sites 1-3 and sites 4-6 in the PCO plot. An additional 33.26% of variation in the environmental measurements taken at the randomly selected cardboards was accounted for by PCO analysis. The samples appeared to form one broad grouping (Fig. 2.3c), which is consistent with PERMANOVA results that indicated the environmental variables differed by both site (pseudo-$F_{5,52}$ = 2.63, P = 0.0002) and month (pseudo-$F_{3,52}$ = 6.52, P = 0.0001). Spatial and temporal patterns within the gastropod species abundance and environmental data at the randomly selected cardboards were not correlated, although this is demonstrated in the PCO plot, in which the length of the gastropod species vectors indicate that they likely did not influence the groupings of the environmental samples (RELATE, rho = -0.024, $P > 0.05$).

The best logistic regression model to determine the effect of climatic and temporal variation on gastropod presence at all cardboards had near optimal fit to the data (McFadden's pseudo-R^2 = 0.17) and indicated that the likelihood of presence increased with average humidity

($\chi^2 = 24.87$, $P < 0.0001$) (Fig. 2.4a) and from June-October ($\chi^2 = 173.89$, $P < 0.0001$) (Fig. 2.4b) (Table 2.1). As for the effect of the same predictor variables, along with ground cover proportion and soil chemistry on gastropod presence at the randomly selected cardboards, the best logistic regression model fit the data similarly well (McFadden's pseudo-$R^2 = 0.16$). This model indicated that the probability of gastropod presence increased over the nine sampling trips from June-October ($\chi^2 = 28.85$, $P < 0.0001$) (Fig. 2.5) (Table 2.2).

Concerning gastropod abundance at all cardboards, evaluation of ΔAIC_c revealed that the best zero-inflated model distribution was a negative binomial in which the variance and mean had a positive quadratic relationship (Brooks *et al*., 2017). Additionally, this model also explained the most variation in the data out of the three options (conditional $R^2 = 0.53$). Again, average humidity had a positive effect ($\chi^2 = 9.41$, $P = 0.0022$) (Fig. 2.6a), while average temperature had an inverse relationship with gastropod abundance ($\chi^2 = 184.26$, $P < 0.0001$) (Fig. 2.6b) (Table 2.3). For zero-inflated modelling of abundance in response to habitat and microhabitat at randomly selected cardboards, the best zero-inflated model utilized a negative binomial distribution in which the variance and mean increased linearly, despite having the worst fit to the data (conditional $R^2 = 0.56$) (Brooks *et al*., 2017). This model indicated that gastropod abundance increased significantly over time ($\chi^2 = 43.30$, $P < 0.0001$) (Fig. 2.7a), and that soil moisture had a significant negative effect ($\chi^2 = 4.61$, $P = 0.032$) (Fig. 2.7b) (Table 2.4).

Visual searches of vegetation and substrate yielded 210 individual gastropods of 10 species, including known brainworm hosts *C. lubrica*, *D. laeve*, *D. whitneyi*, *S. ovalis*, and *Z. arboreus*, along with non-hosts *E. fulvus*, *Strobilops labyrinthica*, *Vallonia* spp. 1 and 2, and *Vitrina* spp. (Pidwerbesky, 2022). One gastropod within this group, a *D. whitneyi* snail collected from site 5 in the urban forest during mid-October, was infected with a nematode putatively

identified as parelaphostrongylid dorsal-spined larvae, yielding an infection prevalence of 0.5% (1/210) (Anderson, 1963). Seven gastropods were found climbing vegetation, one *Vitrina* spp. and six *D. laeve*, none of which were infected. One *D. laeve* that was found climbing had dried before dissection so infection status could not be reliably assessed. Therefore, this individual was excluded from the sample. The use of visual searches did not lead to the collection of more infected gastropods (Fisher's Exact Test, $P = 1.00$), or the collection of more gastropods in general (Wilcoxon Signed Rank Test, $V = 2361$, $P = 0.46$). However, gastropod samples collected using cardboard trapping contained higher proportions of gastropods belonging to known brainworm host species (Chi-square Test, $\chi^2 = 33.0$, $df = 1$, $P < 0.0001$).

Discussion

In the urban park and forest, gastropod species composition reflected the differing spatial and temporal abundance patterns of a few highly abundant gastropod species. *D. laeve*, a significant host of both muscle and brainworm (Pidwerbesky *et al*., 2023), was abundant at all urban forest sites during every month. Another brainworm host species, *Z. arboreus*, was found at all urban forest sites along with a single urban park site (site 1), and clustered with other host species that were less abundant, such as *D. reticulatum*, which was only found at the urban park (sites 1-3). Similar temporal abundance patterns were observed during a study conducted in Minnesota, particularly for *D. laeve* and *Z. arboreus* (Lankester and Peterson, 1996). However, they observed a decline in the abundances of these two species before they increased again from September-October. This conflicting observation could be a result of environmental differences, as they sampled in natural habitat, or differences in sampling frequency, as they sampled weekly from June-September (Lankester and Peterson, 1996). Like *D. laeve*, *Vitrina* spp., a species which does not host brainworm, was also abundant in the urban forest (sites 4-6), but from July-

October. The temporal abundance of this species matches what was observed by the previously mentioned Minnesota survey of gastropods in a natural space (Lankester and Peterson, 1996). The influence of site on species composition has also been observed by Platt (1989), in which *D. laeve* and *Z. arboreus* were observed at all three sites sampled, while *D. whitneyi* occurred at two of the sites and *Cochlicopa* spp. was found at just one of the sites.

The differences between the analysis of species composition at all cardboards and randomly selected cardboards is likely due to the comparatively small size of the randomly selected subset. Analysis excluded cardboard samples where no gastropods were found. The overall dataset included 411 samples, while the subset contained only 69. This reduction in samples also lead to the exclusion of the abundances of two gastropod species: *D. whitneyi* and *C. lubrica*, both hosts of brainworm (Pidwerbesky, 2022). In the PCO analysis, this caused the samples from sites 1-3 to form a distinct group, likely causing site to be the only significant factor in the PERMANOVA. This reduction in the dataset may also explain why temporal and spatial variations in gastropod species composition at the randomly selected cardboards were not correlated with that of the environmental measurements taken at those same cardboards.

This study shows that temporal variation, average air temperature and humidity, and soil moisture significantly influence gastropod presence and abundance. The higher average humidity within the urban forest sites is likely what facilitates occupancy by greater numbers of gastropods, as this variable was consistently identified as a positive influence on both gastropod presence and abundance. Sites with higher humidity also had a higher proportion of broadleaf forest and an increased amount of canopy cover provided by this habitat type. This association is reinforced by results observed in areas where the predominant habitat type was upland coniferous forest (Vanderwaal *et al*., 2015). Gastropod preference for forested habitat types may

be due to the shade provided by the forest cover, which helps to maintain higher humidity (Vanderwaal *et al*., 2015).

The increased likelihood of gastropod presence and increased abundance from summer to fall is consistent with what was predicted and what has been observed by other surveys (Rowley *et al*., 1987; Pidwerbesky, 2022). However, others have demonstrated that temporal abundance can differ according to species (Lankester and Peterson, 1996). For example, the slug *D. laeve* exhibited two peaks in abundance, the first during June and early July, and the second from late August to October. In contrast, another slug species, *D. reticulatum*, had a more unimodal abundance pattern, beginning in June, peaking in August, and dropping off in October (Lankester and Peterson, 1996). The same is true for the results concerning the effect of humidity and temperature, as studies conducted by Cyr (2015) and Vanderwaal *et al*. (2015) in Minnesota both found that humid, cool conditions were more suitable for gastropods. However, Rowley *et al*. (1987) observed a positive relationship between abundance and temperature while sampling gastropods every two weeks over one year. The conflicting results of this study may be due to their use of climate data from a weather station, the longer sampling interval, and latitudinal differences, as this study was conducted in Virginia, U.S.A. (Rowley *et al*., 1987). Contrary to my prediction, precipitation did not influence gastropod presence or abundance. However, this is likely due to the very infrequent precipitation that occurred at the sites during sampling, in which rainfall was recorded twice at 2/3 urban park sites (sites 1 and 2), once at a site in the urban park and a site in the forest (sites 3 and 6), and never at 2/3 urban forest sites (sites 4 and 5). This is notable because the straight-line distance between sites 1-3 and 4-6 was less than 3 km (ESRI, 2023). Also contrary to my predictions, soil moisture negatively affected gastropod presence and abundance. However, other surveys suggest that gastropod preference for soil moisture may have

an upper limit. Despite soil moisture being considered a significant resource for gastropods (Wareborn, 1970), the site where Maze and Johnstone (1986) observed the highest gastropod abundances was described as "well drained," while the site with the least gastropods was significantly moister. The lack of influence by any soil chemistry variables also conflicted with what was predicted (Maze and Johnstone, 1986; Vanderwaal *et al*., 2015). This may be a result of gastropod species differing in their soil chemistry preferences, which has been observed through lab experiments with other gastropod species but has yet to be investigated in species that host brainworm (Wareborn, 1970).

Parelaphostrongylid infection prevalence in the urban park and forest was low, which is consistent with that of other gastropod surveys 0-2.7% (Platt, 1989; Severud *et al*., 2023), but prevents the specific analysis of the habitat preferences of infected gastropods (Maze and Johnstone, 1986; Pidwerbesky, 2022). However, habitat preferences of infected and uninfected gastropods did not appear to differ, as all but two infected gastropods were collected from the urban forest sites with the highest proportions of broadleaf forest (site 4, 100% broadleaf forest; site 5, 68% broadleaf and 32% shrubland) (ESRI, 2023; Agriculture and Agri-Food Canada, 2024). Gastropod collection results from the cardboard transect surveys indicate that the presence and population density of infected deer are not the only factors that determine transmission rates and infection risk in aberrant hosts like moose (Severud *et al*., 2023). Despite the presence of infected deer, parelaphostrongylid transmission is reduced in the urban park, as the abiotic conditions there are unfavourable for gastropods, leading to reduced abundance and the absence of infected individuals. Conditions that negatively affect gastropods have a similar influence on the survival of first stage parelaphostrongylid larvae in the environment, which contributes to reduced gastropod infection prevalence (Vanderwaal *et al*., 2015). These represent

additional results suggesting that temporal variation, environmental conditions, and their effects on gastropod presence and abundance should be considered and managed by conservation initiatives aiming to reduce transmission to aberrant hosts in natural spaces (Behrend and Witter, 1968; Ditmer *et al*., 2020; McGraw *et al*., 2021).

No infected gastropods were found climbing, suggesting that parelaphostrongylid infection did not alter gastropod behaviour. This result differed from my prediction but was consistent with both field surveys and lab experiments (McCoy and Nudds, 2000; Cyr, 2015). Additionally, the one infected gastropod that was collected during the visual searches, a *D. whitneyi* snail, was collected from the substrate, indicating that the climbing behaviour of infected and uninfected gastropods is likely similar, which is consistent with the behavioural trials conducted by McCoy and Nudds (2000) using gastropods experimentally infected with brainworm. However, it is important to note that gastropod climbing behaviour can follow a circadian rhythm (McCoy and Nudds, 2000), and visual searches for my study were only conducted during the three to five hours following dawn. Other gastropod surveys that used both cardboard traps and visual searches have also observed that the two methods return similar infection prevalences (Platt, 1989), but that cardboard trapping typically returns more gastropods for less sampling effort relative to visual searches (Rogerson *et al*., 2008; Cyr, 2015). No difference was found between gastropod abundances or infection prevalences collected using visual searches and the subset of cardboards. Increasing sample size by sampling more cardboard traps and conducting more visual searches might have yielded higher infection prevalences and revealed potential differences between samples returned by the two methods, but it is important to consider the number of gastropods found at all cardboards. In approximately the same amount of time it took to collect 210 gastropods using visual searches (5 per site per trip), the cardboard trap surveys

returned 1131 gastropods (30 per site per trip). This difference in sample size suggests that cardboard trapping does not underestimate parelaphostrongylid prevalence, as infected gastropods are not climbing up from where sampling occurred. Additionally, cardboard trapping may increase the likelihood of collecting infected gastropods, as my results indicated that more individuals belonging to brainworm host species were found using this method.

The lack of infected gastropods found climbing vertically in my and other studies suggests that deer and other ungulates probably eat most infected gastropods from the ground (Cyr, 2015). Although some suggest that gastropod prevalence is too low for deer to consume infected gastropods by chance (McCoy and Nudds, 2000), others have pointed out that based on the foraging activity of deer, the observed gastropod prevalence is sufficient to maintain high prevalence in deer without manipulations to increase transmission by the parasite (Lankester and Peterson, 1996). Instead of investigating the effect of infection on climbing behaviour, future studies should investigate whether brainworm or other parelaphostrongylids cause gastropods to seek out habitats with increased temperature and humidity, as these conditions shorten the time it takes brainworm to develop to the third larval stage (Anderson, 1963; Lankester and Anderson, 1968).

Although one purpose of investigating parelaphostrongylid transmission is the conservation of declining aberrant host populations such as moose, the presence of moose or other aberrant hosts is not necessary for the characterization of the habitat preferences of gastropod hosts (Platt, 1989). Conservation strategies that reduce gastropod presence and abundance would likely reduce parelaphostrongylid infections in both aberrant hosts as well as deer. This reduction of transmission to deer would also be beneficial to aberrant hosts, as there would be less deer

maintaining the parelaphostrongylid life cycle and shedding larvae that could infect gastropods (Anderson, 1972; Garwood *et al*., 2023).

Conclusions

It is important to characterize the response of gastropod hosts to temporal and environmental variation because it can lead to the identification of factors that should be considered by wildlife managers trying to reduce parelaphostrongylid transmission to aberrant hosts of conservation concern (Pidwerbesky, 2022; Garwood *et al*., 2023). Gastropod presence and abundance were found to vary temporally and in response to reduced soil moisture and increased humidity. Furthermore, determining gastropod response to parelaphostrongylid infection is also significant because it further clarifies where in the environment infected gastropods occur, and by extension, how aberrant hosts of concern are becoming infected (McCoy and Nudds, 2000). Field assessments have revealed that regardless of infection status, gastropods seldom engage in climbing behaviour, indicating that ungulates likely become infected with parelaphostrongylids as they forage along the ground (Lankester and Peterson, 1996).

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Tables

Table 2.1 Variables included in and results of the best three logistic regression models used to analyze gastropod presence at all cardboards (significance = $P < 0.05$, bolded).

Model	Variables	Degrees of freedom	χ^2	*P*	McFadden's pseudo-R^2	ΔAIC_c
1	Humidity	1	24.87	**<0.0001**	0.17	-
	Sampling trip	1	173.89	**<0.0001**		
2	Humidity	1	22.99	**<0.0001**	0.18	0.27
	Temperature	1	1.72	0.19		
	Sampling trip	1	70.25	**<0.0001**		
3	Humidity	1	23.51	**<0.0001**	0.17	1.79
	Rainfall	1	0.23	0.63		
	Sampling trip	1	168.15	**<0.0001**		

Table 2.2 Top three logistic regression models used in the analysis of gastropod presence at the subset of cardboards that were randomly selected for microhabitat sampling (significance = $P < 0.05$, bolded).

Model	Variables	Degrees of freedom	χ^2	P	McFadden's pseudo-R^2	ΔAIC_c
1	Soil moisture	1	2.83	0.092	0.16	-
	Sampling trip	1	28.85	**<0.0001**		
2	Leaf litter	1	1.98	0.16	0.16	0.10
	Soil moisture	1	3.01	0.083		
	Sampling trip	1	27.92	**<0.0001**		
3	Soil pH	1	1.37	0.24	0.17	0.83
	Leaf litter	1	2.38	0.12		
	Soil moisture	1	3.08	0.079		
	Sampling trip	1	27.48	**<0.0001**		

Table 2.3 Best three zero-inflated regression models of overall gastropod abundance using a negative binomial distribution (significance = $P < 0.05$, bolded).

Model	Variables	Degrees of freedom	χ^2	*P*	Marginal R^2	Conditional R^2	ΔAIC_c
1	Humidity	1	9.41	**0.0022**	0.087	0.53	-
	Temperature	1	184.26	**<0.0001**			
2	Humidity	1	9.29	**0.0023**	0.088	0.53	1.85
	Rainfall	1	0.17	0.68			
	Temperature	1	179.83	**<0.0001**			

Table 2.4 Results of best models analyzing gastropod abundance at the randomly selected cardboards using a negative binomial distribution (significance = $P < 0.05$, bolded).

Model	Variables	Degrees of Freedom	χ^2	*P*	Marginal R^2	Conditional R^2	ΔAIC_c
1	Leaf litter	1	2.77	0.096	0.13	0.56	-
	Soil moisture	1	4.61	**0.032**			
	Sampling trip	1	43.30	**<0.0001**			
2	Leaf litter	1	3.34	0.068	0.14	0.58	0.28
	Soil moisture	1	2.97	0.085			
	Temperature	1	1.85	0.17			
	Sampling trip	1	9.63	**0.0019**			
3	Soil calcium	1	3.06	0.080	0.16	0.60	0.33
	Humidity	1	4.19	**0.041**			
	Leaf litter	1	2.73	0.098			
	Temperature	1	6.13	**0.013**			
	Sampling trip	1	3.51	0.061			

Figures

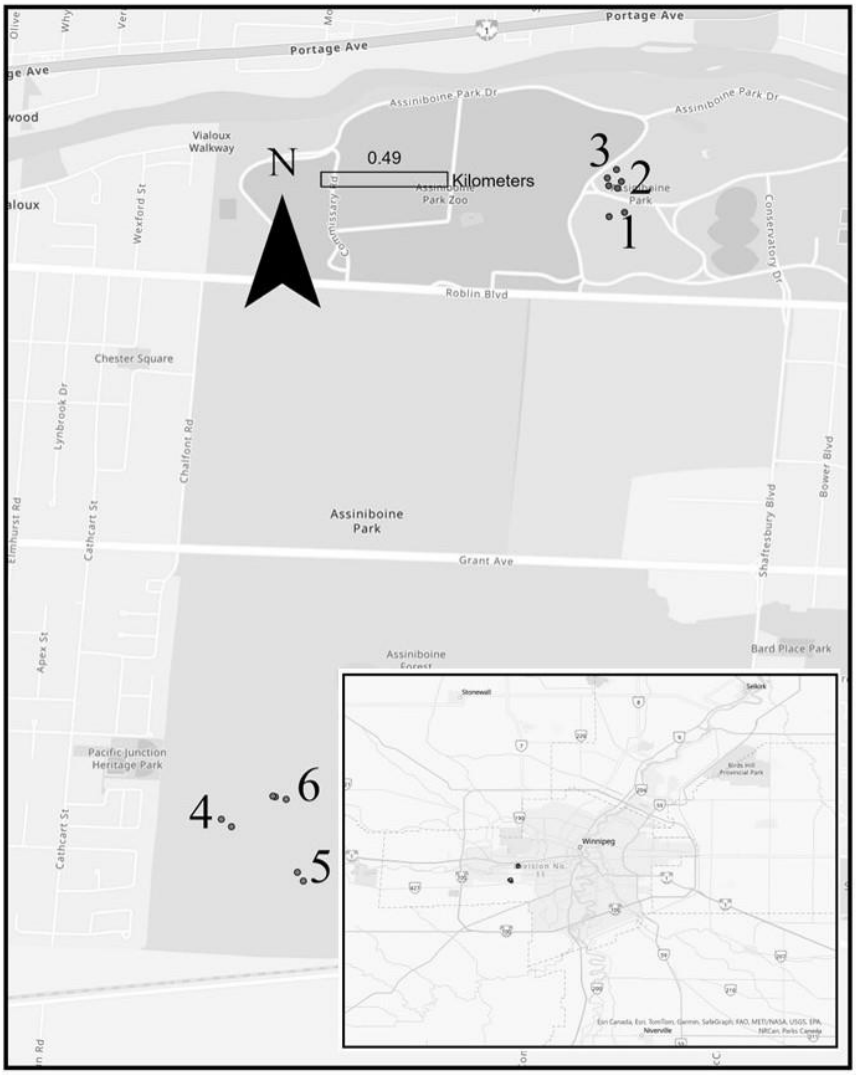


Figure 2.1 The six sites (1-6) where gastropod species composition, presence, and abundance were sampled in urban park (sites 1-3) and forest (sites 4-6) from June-October 2023 using bimonthly cardboard transect surveys (three adjacent transects approx. 50 m x 50 m) and visual searches in Winnipeg, Manitoba, Canada (solid dots depict start and end points of transects laid) (figure generated using ArcGIS pro 3.1.2 (ESRI, 2023)).

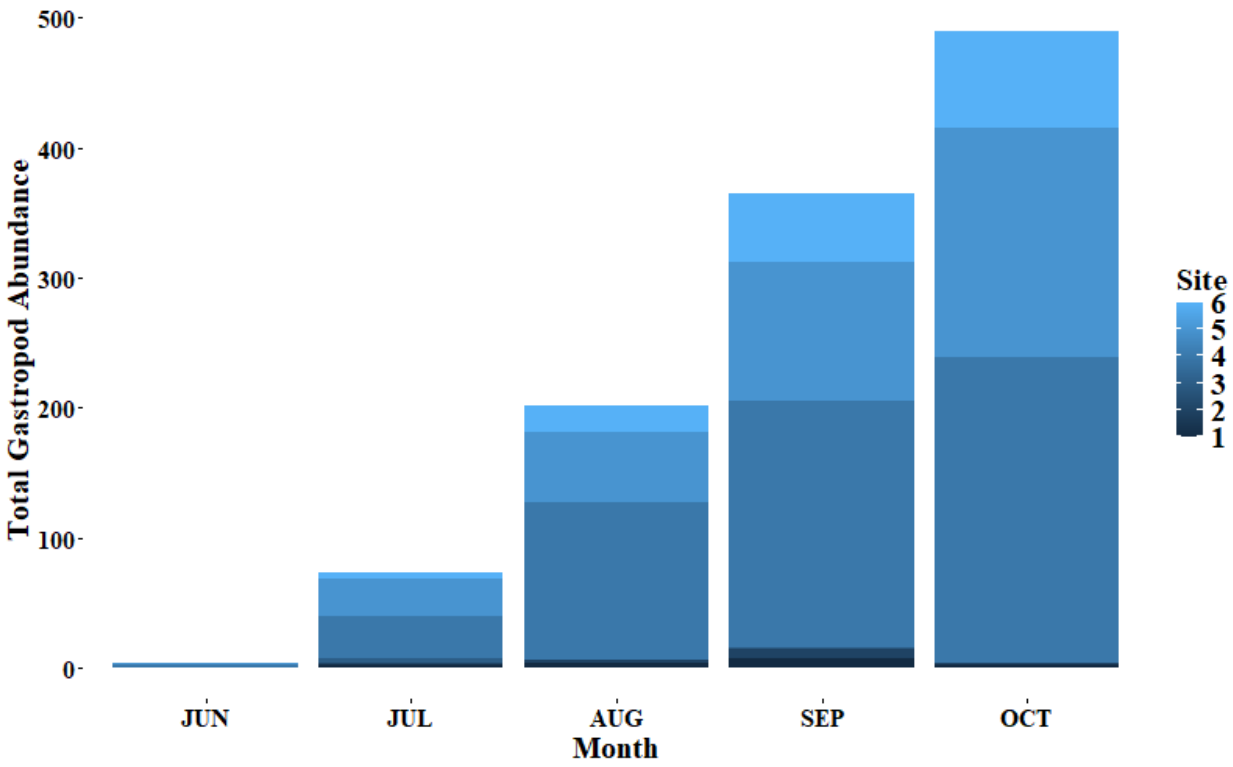
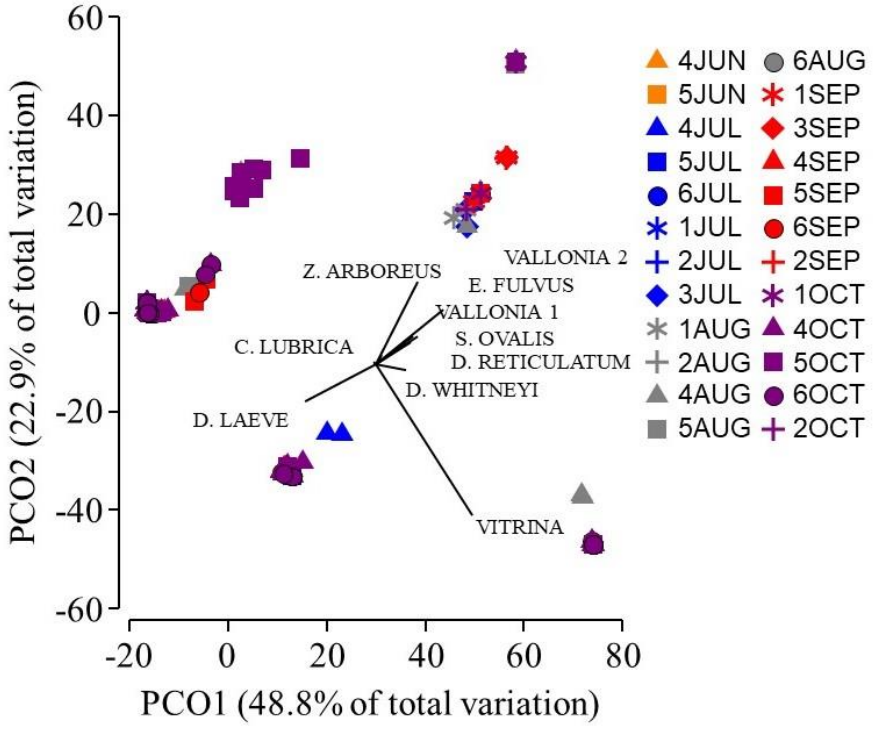
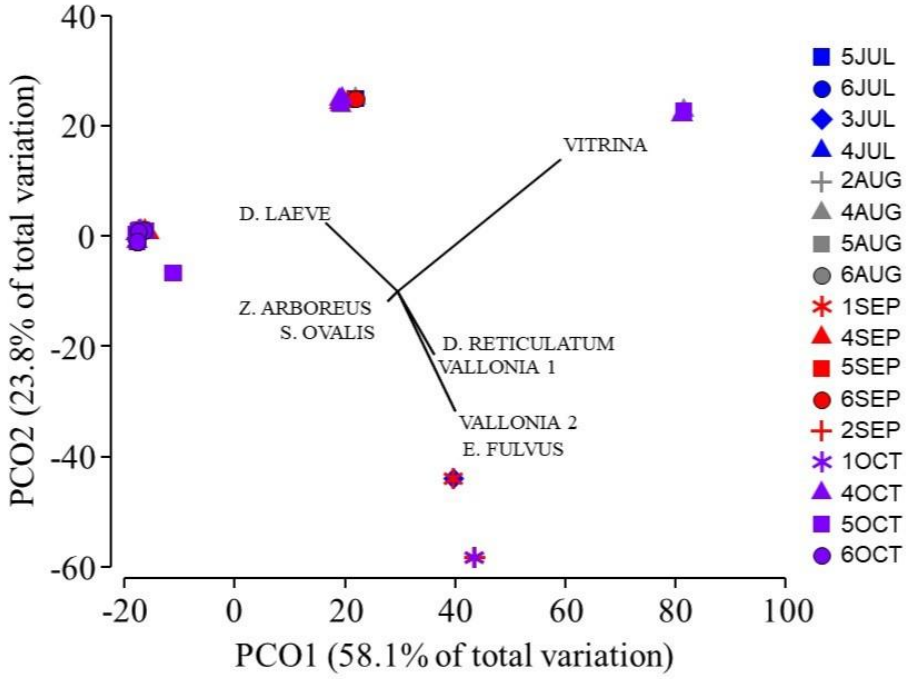


Figure 2.2 Total gastropods collected from all cardboards over time (June-October 2023) at the six sites within urban park (sites 1-3) and forest (sites 4-6).

A.


PCO2 (22.9% of total variation)
PCO1 (48.8% of total variation)
Z. ARBOREUS
VALLONIA 2
E. FULVUS
VALLONIA 1
S. OVALIS
C. LUBRICA
D. RETICULATUM
D. WHITNEYI
D. LAEVE
VITRINA
4JUN
5JUN
4JUL
5JUL
6JUL
1JUL
2JUL
3JUL
1AUG
2AUG
4AUG
5AUG
6AUG
1SEP
3SEP
4SEP
5SEP
6SEP
2SEP
1OCT
4OCT
5OCT
6OCT
2OCT

B.


PCO2 (23.8% of total variation)
PCO1 (58.1% of total variation)
VITRINA
D. LAEVE
Z. ARBOREUS
S. OVALIS
D. RETICULATUM
VALLONIA 1
VALLONIA 2
E. FULVUS
5JUL
6JUL
3JUL
4JUL
2AUG
4AUG
5AUG
6AUG
1SEP
4SEP
5SEP
6SEP
2SEP
1OCT
4OCT
5OCT
6OCT

C.

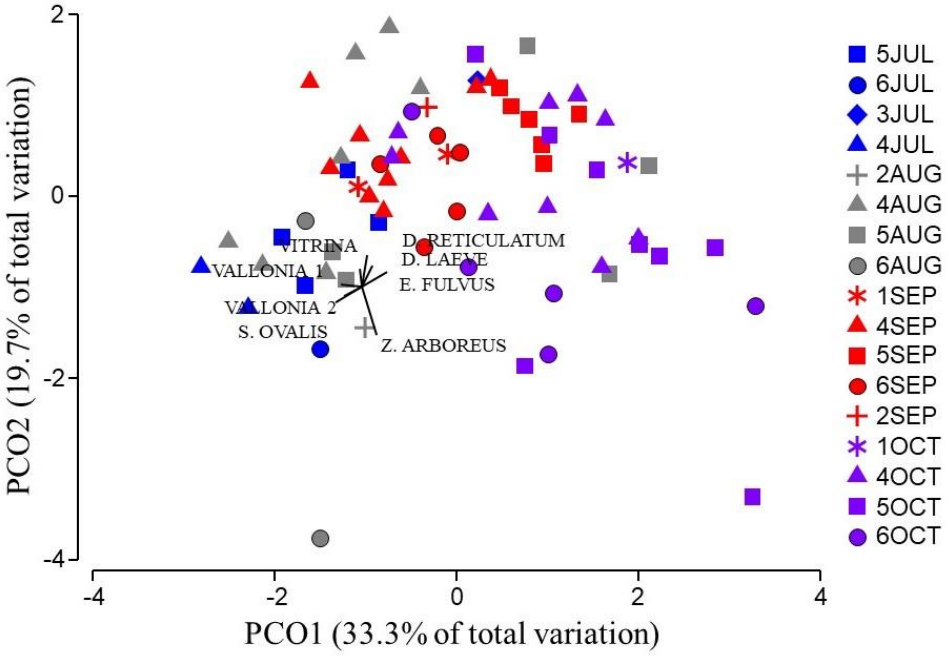


Figure 2.3 Visual representation of PCO analysis of gastropod species composition at (A) all cardboards and (B) randomly selected cardboards, along with (C) weather and microhabitat measurements observed at randomly selected cardboards (Figure legends indicate site (1-6) and sampling month (June-October)).

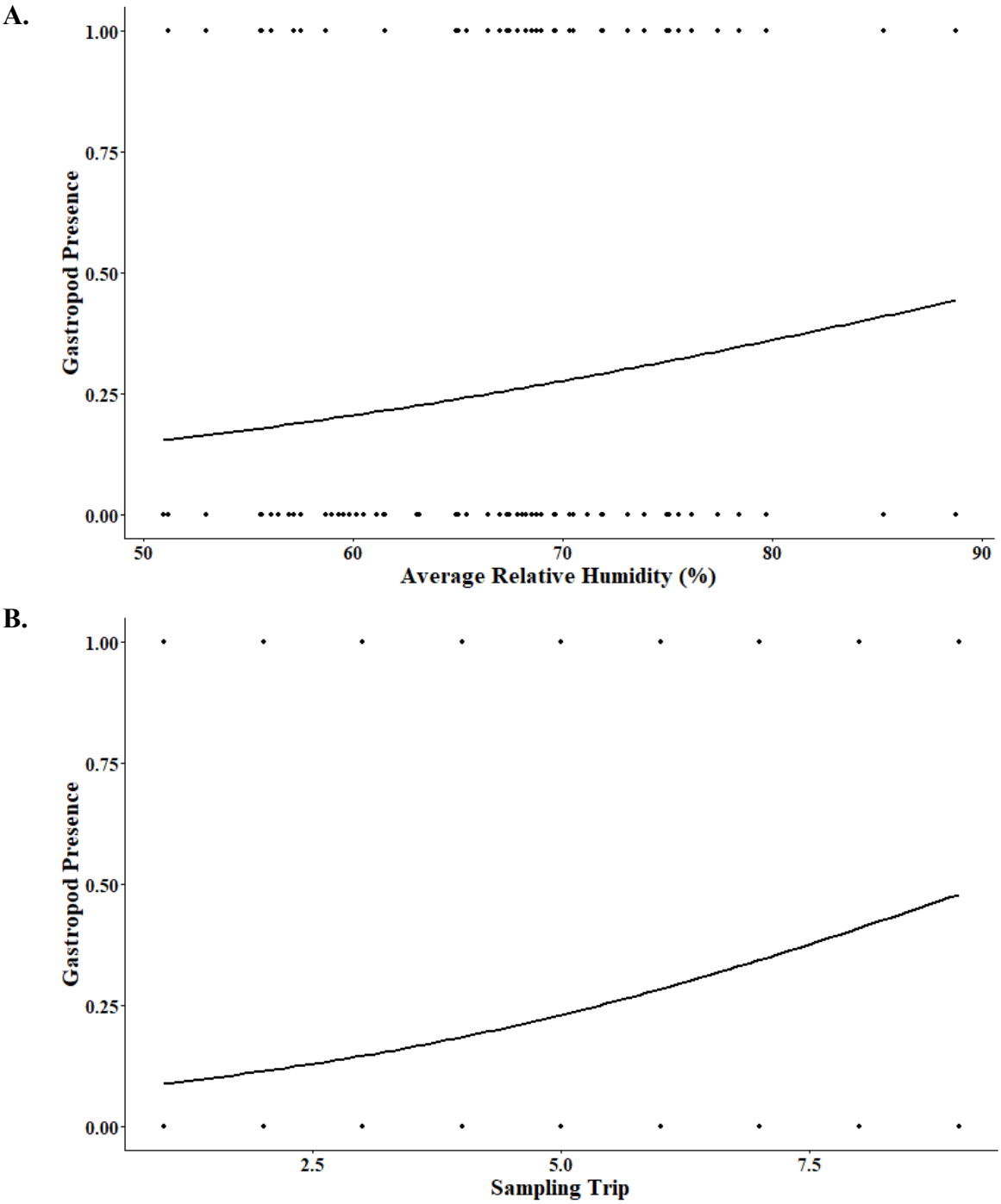


Figure 2.4 Regression curves indicating the positive relationship gastropod presence and (A) average humidity as well as (B) changes in gastropod presence over time at all cardboards sampled.

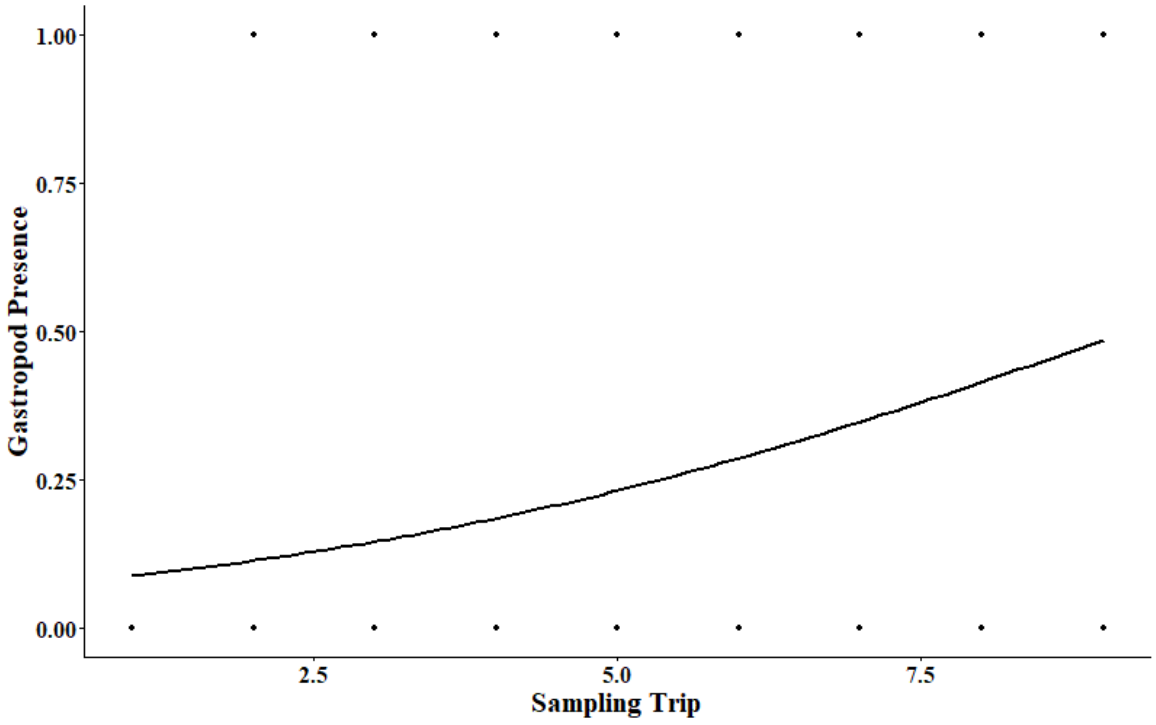


Figure 2.5 Increased likelihood of gastropod presence over time at randomly selected cardboards.

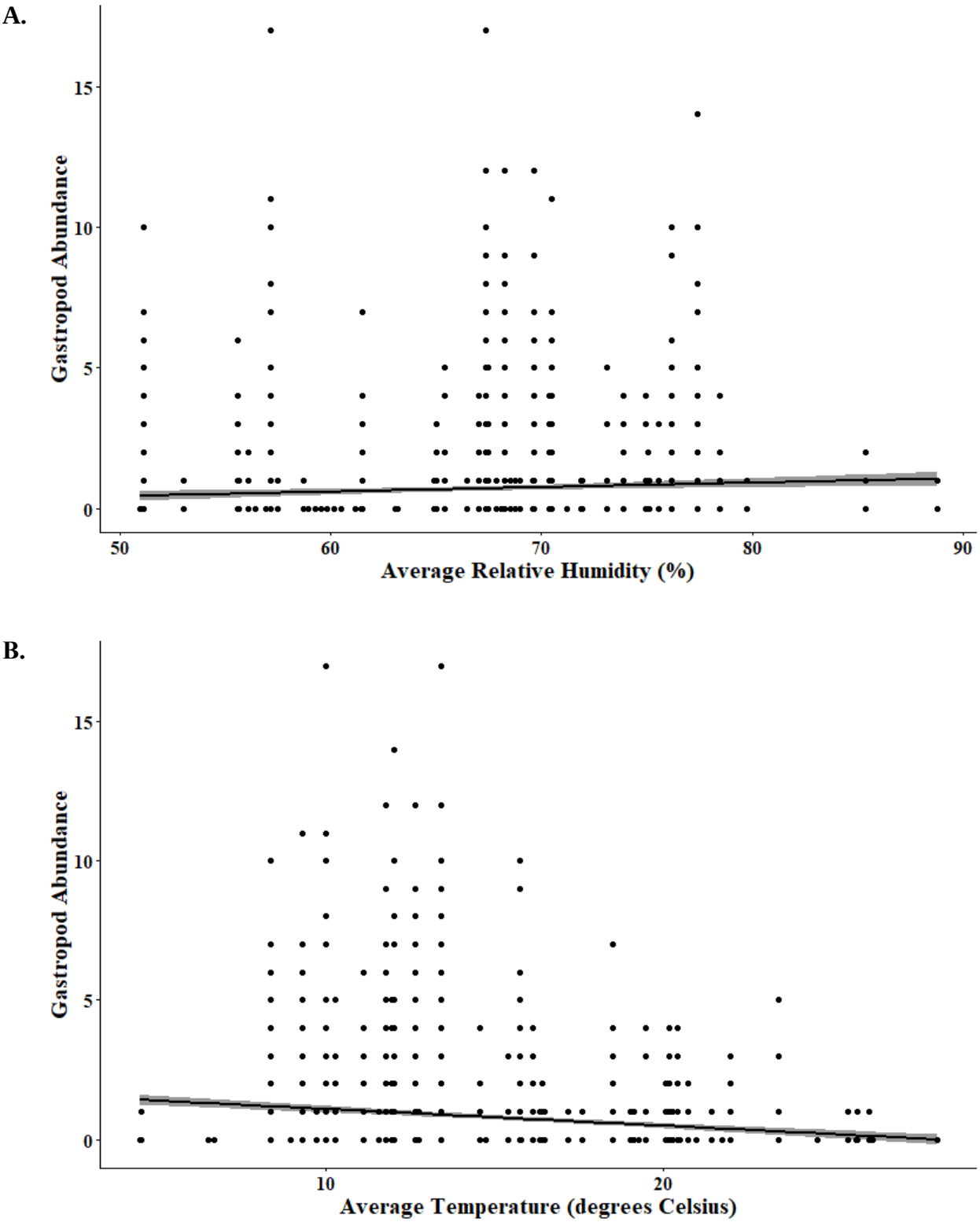
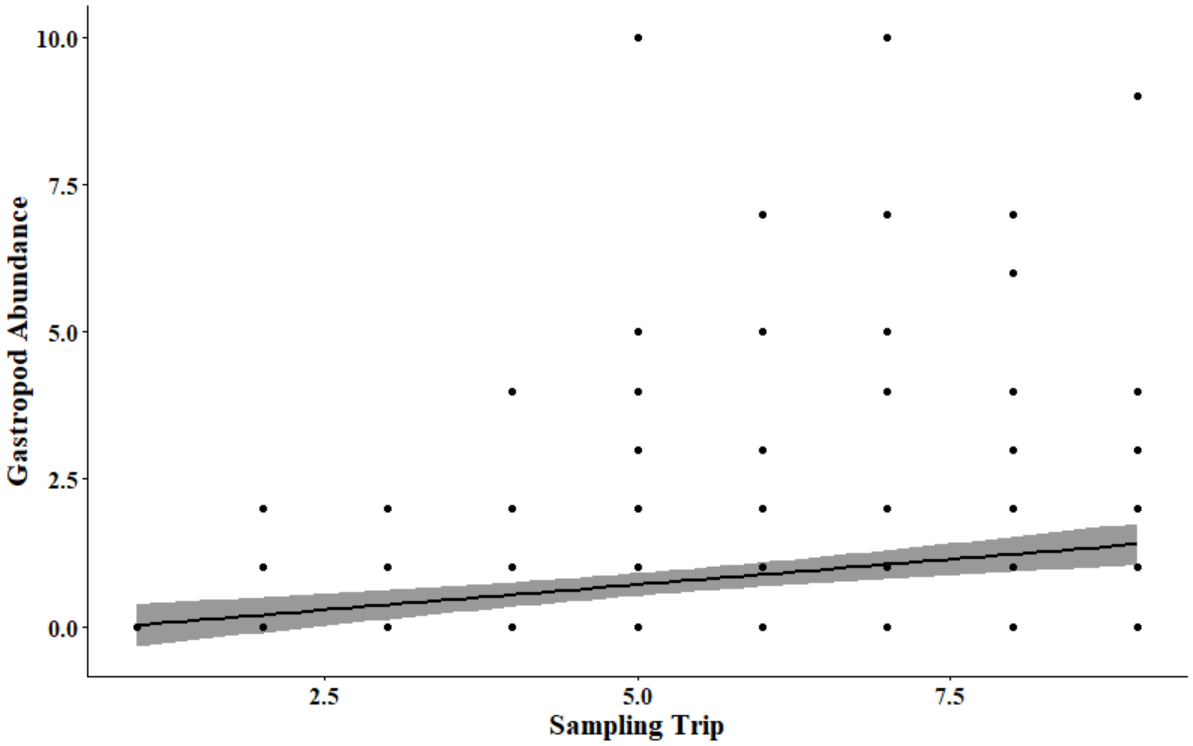


Figure 2.6 Effect of (A) average humidity and (B) temperature on gastropod abundance at all cardboards (shading depicts 95% confidence interval).

A.


Gastropod Abundance
10.0
7.5
5.0
2.5
0.0
2.5
5.0
7.5
Sampling Trip

B.

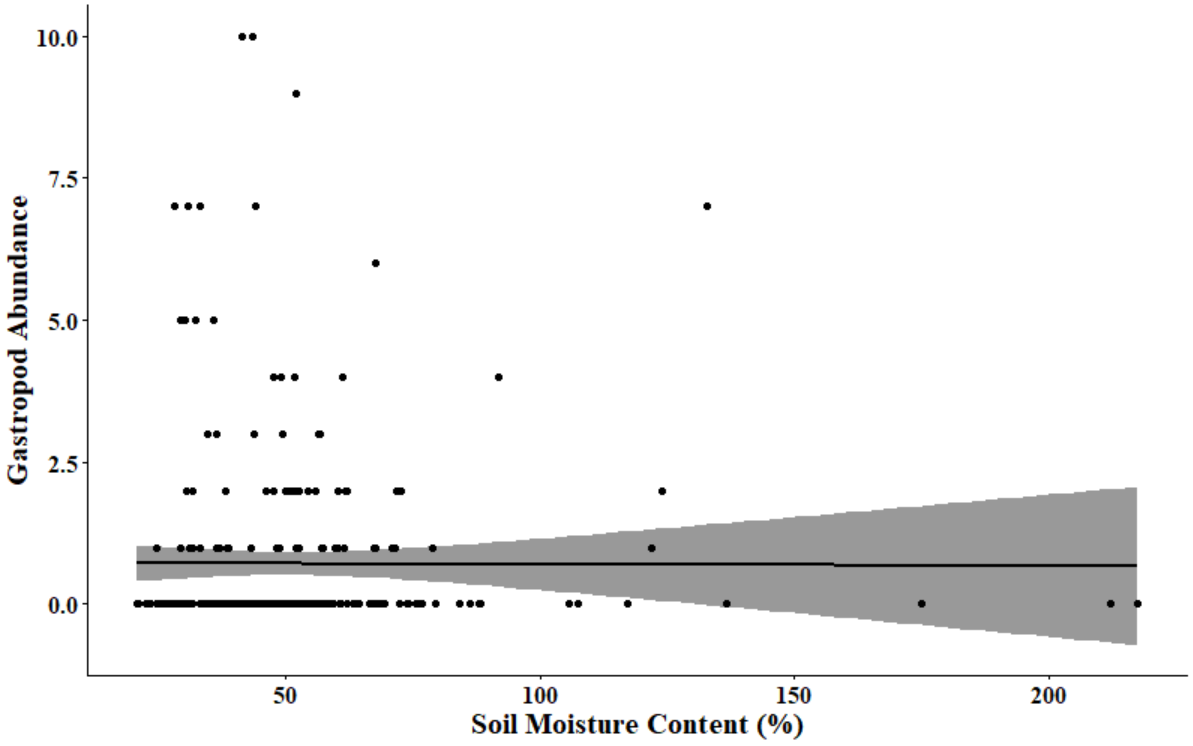

Gastropod Abundance
10.0
7.5
5.0
2.5
0.0
50
100
150
200
Soil Moisture Content (%)

Figure 2.7 (A) Temporal variation in gastropod abundance, as well as the negative association between abundance and (B) soil moisture content at randomly selected cardboards (shading depicts 95% confidence interval).

Thesis Conclusion

Biotic and abiotic influences on the gastropod hosts of parelaphostrongylid nematodes were assessed within different habitat types in southeastern Manitoba. It was important to survey gastropods in Game Hunting Area 26 (GHA 26) (Chapter 1) because there is concern for the resident moose (*Alces alces*) population, as it underwent a recent decline from which recovery has been slow. Restrictions on moose hunting have been imposed, but white-tailed deer (*Odocoileus virginianus*) also occur in this area and could be spreading parelaphostrongylids to moose. Sampling in the urban park and forest in Winnipeg (Chapter 2) allowed for more frequent assessment of gastropods over a longer period, which led to better temporal resolution concerning gastropod habitat preferences and behaviour. Additionally, these urban spaces were inhabited by deer confirmed to be hosts of parelaphostrongylids.

In GHA 26, gastropod species composition, presence, and abundance in response to temporal and environmental variation were investigated to identify abiotic factors that significantly influenced their distributions and the subsequent transmission of brainworm (*Parelaphostrongylus tenuis*) to moose. Infection prevalence within the collected gastropods was 0% (0/254), suggesting that from June-August, encounters between moose and infected gastropods are unlikely to occur. Gastropod species composition varied over time and between the three study sites, and the interaction between these two factors also had a significant effect. The interaction may be explained by the differing temporal and spatial patterns observed in the three most abundant species that are known brainworm hosts: *Deroceras laeve*, *Zonitoides arboreus*, and *Succinea ovalis*. Overall gastropod abundance was greatest at site B and during the month of July.

Humidity and soil pH were identified as significant influences on gastropod presence within GHA 26, with presence being most likely as humidity increased from 60-100% and as soil pH increased from approximately 4.25-7.00. Additionally, the likelihood of presence differed significantly among the sites, with the greatest probability occurring at the site C, which had the most coniferous forest making up it's habitat composition. Ranges of pH and humidity observed at each of the sites overlapped, although on average site A was the most humid and site B had the most alkaline soil. Further investigation is needed to identify the mechanisms underlying gastropod preference for certain habitat types, possibly by sampling gastropods in areas where abiotic conditions have already characterized. This approach would be unlike my study, in which sites were chosen randomly without prior knowledge of the habitat conditions. Regarding abiotic influences on gastropod abundance in GHA 26, neither temporal variation nor any of the environmental variables assessed had a significant effect. At most, the influences of humidity and soil pH were almost significant. This lack of influence has been observed in several other studies and may have been due to different gastropod species having conflicting habitat preferences. These results suggest that it may be beneficial to bring significant host species of brainworm, such as *D. laeve* and *Z. arboreus*, into the laboratory for the assessment of their microhabitat preferences in a controlled setting.

In the second chapter, temporal variation and several environmental characteristics were again investigated to identify abiotic influences on the species composition, presence, and abundance of gastropods. Sampling occurred at sites with significant proportions of broadleaf forest within an urban park (Assiniboine Park) and forest (Assiniboine Forest) to control for the effect of habitat type. This area was also selected because previous fecal sampling within the park indicated that deer inhabiting the area were infected with parelaphostrongylids. Sampling

where infected deer occurred was intended to increase the likelihood of collecting infected gastropods, as randomly selecting sites in GHA 26 had led to the collection of no infected gastropods. Furthermore, despite previous aerial surveys confirming that deer occurred at site A in GHA 26, and attempts to lay transects along deer paths, deer and their fecal pellets were rarely observed. Future studies should choose sites based on direct observation of deer usage. Rainfall was an additional variable that I wanted to investigate as a potential abiotic influence on gastropods. A rain gauge was installed at each site, and allowed for the collection of precipitation measurements at a finer spatial resolution than what would be given by data from a weather station. Although the infrequency of precipitation prevented it's inclusion in multivariate analysis and obscured it's effect on gastropod presence and abundance, neighboring sites within 3 km received differing levels of precipitation over the same time periods. This fine-scale difference would not be present in weather station data. Therefore, I recommend that future studies looking to determine the effect of rainfall on gastropod distributions should avoid the use of broad-scale weather station data, due to the high degree of spatial variability in precipitation.

More infected gastropods were collected from the urban park and forest than GHA 26. A total of 0.97% (11/1131) of all gastropods were infected with nematodes preliminarily identified as belonging to the genus *Parelaphostrongylus*. However, further molecular characterization is needed to determine the species of these nematodes, as they cannot be distinguished from each other by morphology alone. Gastropod sampling in urban park and forest was conducted for two months longer than what was done in GHA 26, from June-October instead of June-August. The shorter sampling interval for GHA 26 was due to the expenses and logistical challenges that come with maintaining a team of field personnel as the university term begins. However, future studies should sample gastropods during September and October, as this is when gastropod

abundance peaked during sampling of urban park and forest. Additionally, the most infected gastropods were collected during September. However, for both GHA 26 and the urban park and forest, patterns in gastropod species varied significantly over time and between sites, as well as in response to the interaction between time and site. There was also an interactive effect between site and sampling month. A subset of gastropod species composition samples differed only by site but attempts to relate their temporal and spatial patterns with those of corresponding abiotic measurements indicated no correlation. The differing gastropod species compositions between the sites was likely due to gastropod species having distinct temporal and spatial abundance patterns.

Like with GHA 26, gastropod presence in the urban park and forest was significantly affected by humidity, with presence being more likely as humidity increased from 50-90%. The likelihood of gastropod presence also increased significantly from June-October. Significant temporal variation was also observed in gastropod abundance, again with abundance increasing over time. Humidity also had a positive effect. Temperature and soil moisture content were additional abiotic characteristics that influenced abundance, although their effect was negative. Gastropods were less abundant as temperatures exceeded approximately 20°C and as soil moisture content increased above roughly 75%. These findings are like what was observed in GHA 26, in which humidity also had a positive influence. Additionally, while sampling in GHA 26 and in the urban park and forest both demonstrated the influence of soil chemistry on gastropods, they differed in that soil pH positively affected presence in GHA 26, while soil moisture negatively affected abundance in the urban park and forest. At the very least, the negative effect of temperature may have been observed in GHA 26 if sampling in this area could have taken place over a longer period. Taken together, these results indicate that in addition to

climatic or weather conditions, soil moisture and temporal variation should be considered when trying to determine the likelihood that ungulates will encounter and consume gastropods.

In addition to my study, only one other survey has concurrently used cardboard trapping and visual searches to determine if they yield differing gastropod prevalences. I and the other survey found that neither method had a significant effect. At most, cardboard trapping yielded significantly more gastropods of species that are compatible hosts of brainworm. Additionally, other surveys have observed the greatest prevalences at sites where general gastropod abundance is highest. Based on my limited data, infection with parelaphostrongylid nematodes does not seem to be a biotic influence on gastropod habitat selection or overall transmission. Furthermore, cardboard trapping remains a reliable way to assess gastropod infection prevalence and encounters between ungulates and gastropods probably occurs on the ground, where most gastropods, regardless of infection status, prefer to dwell. The survival of infected and uninfected gastropods is constrained by the need to avoid predators and prevent drying out. Vertical climbing would increase the likelihood of both outcomes. Furthermore, parelaphostrongylid larvae are unable to encounter subsequent ungulate hosts and continue their life cycle if their gastropod host desiccates or is consumed by anything other than a deer.

Low gastropod infection prevalence obtained by field results suggests that the biology of these parasites should be investigated in the laboratory. This setting allows for the obtainment of higher proportions of infected gastropods through controlled exposures than would be collected from a natural setting. Experimental infections of gastropods have illuminated the host range of brainworm and indicated the likely route of infection and development of larvae within gastropods, as well as the effect of climate on development rate. Lab experiments on the environmental tolerance of first-stage brainworm and *Parelaphostrongylus odocoilei* larvae have

identified conditions that favour brainworm larvae survival in the environment and subsequent ability to infect gastropods. Behavioural experiments using ungulate feces in various conditions led to the observation of gastropod attraction to fresh and weathered feces, which has implications for the rate of encounters between gastropod hosts and first-stage larvae. Additionally, while they did not indicate that infection alters climbing behaviour, previous lab experiments have shown that climbing behaviour can differ by gastropod species and circadian rhythm. These lab experiments with brainworm have yielded important findings regarding the rate at which brainworm encounters compatible hosts, as well as how encounter rate and compatibility are influenced by various biotic and abiotic characteristics. However, certain aspects of brainworm's biology have yet to be characterized. For example, the response to infection remains unknown for many gastropod host species. Furthermore, the ability for third stage muscle (*Parelaphostrongylus andersoni*) or brainworm larvae to emerge from gastropods has yet to be assessed, even though this phenomenon has been documented in gastropods infected with *P. odocoilei*.

The results of this thesis build on those of previous studies and bring further insight into how the transmission of brainworm and other parelaphostrongylid nematodes is influenced by biotic and abiotic factors. Gastropod distributions were significantly influenced by temporal variation, climatic and weather conditions, habitat type, and soil moisture content. Landscape-altering interventions, such as the opening of forest canopy or burns, could be conducted in areas with abiotic conditions known to increase gastropod presence or abundance. These practices would alter the abiotic conditions of the area, and may reduce gastropod presence and abundance, and by extension, the likelihood that co-occurring ungulates would encounter and consume gastropods, potentially becoming infected with parelaphostrongylids.

Appendix

R Code

```
#CHAPTER ONE

#Packages and setwd ####

library(dplyr)

library(tidyverse)

library(car)

library(MuMIn)

library(rcompanion)

library(ggpubr)

library(lmtest)

library(glmmTMB)

library(AICcmodavg)

library(performance)

library(emmeans)

#CH. 1 MICROHABITAT WORK ####

ngha26_data <- read.csv("NGHA26_verif.csv")

#DF PREP - Variables renamed
```

```
ngha26_data <- ngha26_data %>%
  rename(locat = "LOCATION",
         site = "SITE",
         transect = "TRANSECT",
         sitetrans = "SITETRANS",
         cb = "CARDBOARD",
         trip = "TRIP",
         humid = "HUMIDITY_AVG",
         air_temp = "AIR_TEMP_AVG",
         veg = "VEGETATION",
         lit = "LITTER",
         soil = "SOIL",
         rock = "ROCK",
         cover = "COVER",
         moisture = "SOIL_MOISTURE",
         lab_ph = "LAB_PH",
         lab_temp = "LAB_TEMP",
         calcium = "CALCIUM",
```

```
        gast_abun = "GASTROPODS")

#NA values dropped to create new df

ngha26_net <- drop_na(ngha26_data, calcium)

ngha26_net <- drop_na(ngha26_net, moisture)

#Gastropod counts made into pres/abs variable

ngha26_net <- ngha26_net %>%

  mutate(gast_pres = case_when(gast_abun > 0 ~ "1",

                               gast_abun == 0 ~ "0"))

#Recoding discrete/categorical variables

ngha26_net$site <- as.factor(ngha26_net$site)

ngha26_net$transect <- as.factor(ngha26_net$transect)

ngha26_net$sitetrans <- as.factor(ngha26_net$sitetrans)

ngha26_net$cover <- as.factor(ngha26_net$cover)

ngha26_net$gast_pres <- as.numeric(ngha26_net$gast_pres)

#Standardizing the continuous variables

ngha26_net <- ngha26_net %>%

  mutate(s.humid = scale(humid),

         s.air_temp = scale(air_temp),
```

```
        s.veg = scale(veg),
        s.lit = scale(lit),
        s.soil = scale(soil),
        s.rock = scale(rock),
        s.moisture = scale(moisture),
        s.lab_ph = scale(lab_ph),
        s.calcium = scale (calcium))
#------LOGISTIC REGRESSION - first checking for multicollinearity ####
net_logregvifs <- glm(gast_pres ~ site + trip + s.humid + s.air_temp + s.veg + s.lit
                + s.soil + s.rock + s.moisture +
                  s.lab_ph + s.calcium, data = ngha26_net)
vif(net_logregvifs)
alias(net_logregvifs)
net_lrvifs_lit <- lm(gast_pres ~ site + trip + s.humid + s.air_temp + s.veg
                + s.moisture +
                  s.lab_ph + s.calcium, data = ngha26_net)
vif(net_lrvifs_lit)
#Model formulas and results ####
```

```
netlr_all1 <- glm(gast_pres ~ site + trip + s.humid + s.air_temp +
                    s.veg + s.moisture + s.lab_ph + s.calcium,
          family = binomial(link = 'logit'), data = ngha26_net, na.action = na.fail)
modelset_netlr <- dredge(netlr_all1, beta = c("none"))
(netlr_mods <- get.models(modelset_netlr, subset = delta<4))
get.models(modelset_netlr, subset = 1) [1]  #Best model
get.models(modelset_netlr, subset = 2) [1]  #Second best model
get.models(modelset_netlr, subset = 3) [1] #Third best model
#Top 3 LR model results ####
netlr_top <- glm(gast_pres ~ s.humid + s.lab_ph + site + 1,
           family = binomial(link = "logit"), data = ngha26_net, na.action = na.fail)
summary(netlr_top)
Anova(netlr_top, type = 3)
nagelkerke(netlr_top)
#Tukey's Test of Model
marginal = emmeans(netlr_top, ~ site)
pairs(marginal, adjust="tukey")
netlr_top2 <- glm(gast_pres ~ s.humid + s.lab_ph + s.veg + site + 1,
```

```
            family = binomial(link = "logit"), data = ngha26_net, na.action = na.fail)

summary(netlr_top2)

Anova(netlr_top2, type = 3)

nagelkerke(netlr_top2)

marginal = emmeans(netlr_top2, ~ site)

pairs(marginal, adjust="tukey")

netlr_top3 <- glm(gast_pres ~ s.calcium + s.humid + s.lab_ph + site + 1,

             family = binomial(link = "logit"), data = ngha26_net, na.action = na.fail)

summary(netlr_top3)

Anova(netlr_top3, type = 3)

nagelkerke(netlr_top3)

marginal = emmeans(netlr_top3, ~ site)

pairs(marginal, adjust="tukey")

#Comparing the top models

net26lrmods <- list(netlr_top, netlr_top2, netlr_top3)

aictab(net26lrmods)

#------ZERO INFLATED MODELLING - again starting with check of VIFs ####

net_zivifs <- lm(gast_abun ~ site + trip + s.humid + s.air_temp + s.veg + s.lit
```

```
            + s.soil + s.rock + s.moisture + s.lab_ph + s.calcium, data = ngha26_net)

vif(net_zivifs)

alias(net_zivifs)

net_zivi_lit <- lm(gast_abun ~ site + trip + s.humid + s.air_temp + s.veg

              + s.moisture + s.lab_ph + s.calcium, data = ngha26_net)

vif(net_zivi_lit)

#Model formulas ####

#Running each model with a poisson and negative binomial distribution

netzi_allp1 <- glmmTMB(gast_abun ~ (1|sitetrans) + site + trip + s.humid + s.air_temp +

                    s.veg + s.moisture + s.lab_ph + s.calcium, zi = ~1,

                  family = poisson, data = ngha26_net, na.action = na.fail)

netzi_allnb1 <- glmmTMB(gast_abun ~ (1|sitetrans) + site + trip + s.humid + s.air_temp +

                     s.veg + s.moisture + s.lab_ph + s.calcium, zi = ~1,

                   family = nbinom1, data = ngha26_net, na.action = na.fail)

netzi_allnb2 <- glmmTMB(gast_abun ~ (1|sitetrans) + site + trip + s.humid + s.air_temp +

                     s.veg + s.moisture + s.lab_ph + s.calcium, zi = ~1,

                   family = nbinom2, data = ngha26_net, na.action = na.fail)

#Top Poisson models ####
```

```
modelset_netzip <- dredge(netzi_allp1, beta = c("none"))

(zipset_d4 <- get.models(modelset_netzip, subset = delta<4))

get.models(modelset_netzip, subset = 1) [1]

get.models(modelset_netzip, subset = 2) [1]

get.models(modelset_netzip, subset = 3) [1]

#Top nbinom1 models ####

modelset_netzinb1 <- dredge(netzi_allnb1, beta = c("none"))

(zinb1set_d4 <- get.models(modelset_netzinb1, subset = delta<4))

get.models(modelset_netzinb1, subset = 1) [1]

get.models(modelset_netzinb1, subset = 2) [1]

get.models(modelset_netzinb1, subset = 3) [1]

#Top nbinom2 models ####

modelset_netzinb2 <- dredge(netzi_allnb2, beta = c("none"))

(zinb2set_d4 <- get.models(modelset_netzinb2, subset = delta<4))

get.models(modelset_netzinb2, subset = 1) [1]

get.models(modelset_netzinb2, subset = 2) [1]

get.models(modelset_netzinb2, subset = 3) [1]

#Best 3 Poisson model results ####
```

```
netzip_top <- glmmTMB(gast_abun ~ s.humid + s.lab_ph + s.veg + (1 | sitetrans),
                      zi = ~1, data = ngha26_net, na.action = na.fail, family = poisson)
summary(netzip_top)
Anova(netzip_top, type = 3)
r2(netzip_top)
plot(residuals(netzip_top), fitted(netzip_top))

netzip_top2 <- glmmTMB(gast_abun ~ s.humid + s.lab_ph + s.moisture + s.veg + (1 | sitetrans),
                      zi = ~1, data = ngha26_net, na.action = na.fail, family = poisson)
summary(netzip_top2)
Anova(netzip_top2, type = 3)
r2(netzip_top2)

netzip_top3 <- glmmTMB(gast_abun ~ s.humid + s.lab_ph + (1 | sitetrans),
                       zi = ~1, data = ngha26_net, na.action = na.fail, family = poisson)
summary(netzip_top3)
Anova(netzip_top3, type = 3)
r2(netzip_top3)
```

```
net26zimods <- list(netzip_top, netzip_top2, netzip_top3)

aictab(net26zimods)

#Results of top nbinom1 model ####

netzinb1_top <- glmmTMB(gast_abun ~ s.humid + s.lab_ph + s.veg + (1 | sitetrans),

                zi = ~1, data = ngha26_net, na.action = na.fail, family = nbinom1)

summary(netzinb1_top)

Anova(netzinb1_top, type = 3)

r2(netzinb1_top)

#Results of best model with nbinom2 distribution ####

netzinb2_top <- glmmTMB(gast_abun ~ s.lab_ph + (1 | sitetrans),

                zi = ~1, data = ngha26_net, na.action = na.fail, family = nbinom2)

summary(netzinb2_top)

Anova(netzinb2_top, type = 3)

r2(netzinb2_top)

#Comparing top models to see which distribution is best ####

ngha26netzimods <- list(netzip_top, netzinb1_top, netzinb2_top)

aictab(ngha26netzimods)
```

```
#CHAPTER TWO ####

#setwd and load packages ###

library(dplyr)

library(tidyverse)

library(car)

library(MuMIn)

library(rcompanion)

library(ggpubr)

library(glmmTMB)

library(AICcmodavg)

library(lmtest)

library(lme4)

library(performance)

#CH. 2 MICROHABITAT WORK ###

gha38data <- read.csv("GHA38data.csv")

gha38data <- gha38data %>%

  rename(locat = "LOCATION",

         site = "SITE",
```

transect = "TRANSECT",

cb = "CARDBOARD",

trip = "TRIP",

humid = "AVG_HUMID",

temp = "AVG_TEMP",

rain = "SUM_RAIN",

rock = "ROCK",

soil = "SOIL",

lit = "LITTER",

veg = "VEGETATION",

cover = "COVER",

moisture = "SOIL_MOISTURE",

ph = "SOIL_PH",

adj_ph = "ADJ_PH",

ca = "CALCIUM",

slugs = "SLUGS",

snails = "SNAILS",

gast_abun = "GASTROPODS")

```
gha38data <- gha38data %>%
  mutate(gast_pres = case_when(gast_abun > 0 ~ "1",
                               gast_abun == 0 ~ "0"))
#ANALYSIS WITH RANDOMLY SELECTED CARDBOARDS FIRST
#df creation
gha38_net <- drop_na(gha38data, ca)
#recoding
gha38_net$site <- as.factor(gha38_net$site)
gha38_net$transect <- as.factor(gha38_net$transect)
gha38_net$gast_pres <- as.numeric(gha38_net$gast_pres)
#scaling
gha38_net <- gha38_net %>%
  mutate(s.humid = scale(humid),
         s.temp = scale(temp),
         s.rain = scale(rain),
         s.rock = scale(rock),
         s.soil = scale(soil),
         s.lit = scale(lit),
```

```
        s.veg = scale(veg),
        s.moisture = scale(moisture),
        s.ph = scale(ph),
        s.adjph = scale(adj_ph),
        s.ca = scale (ca))
#------LOGISTIC REGRESSION ####
#Checking for multicollinearity
net38lrvifs <- lm(gast_pres ~ site + trip + s.humid + s.temp + s.rain + s.veg + s.lit
              + s.soil + s.moisture + s.adjph + s.ca, data = gha38_net)
vif(net38lrvifs)
alias(net38lrvifs)
net38lrvifs_veg <- lm(gast_pres ~ site + trip + s.humid + s.temp + s.rain + s.lit
                  + s.moisture + s.adjph + s.ca, data = gha38_net)
vif(net38lrvifs_veg)
#Best 3 Model formulas ####
net38lradj <- glmer(gast_pres ~ (1|site) + trip + s.humid + s.temp + s.rain + s.lit +
                    s.moisture + s.adjph + s.ca,
                  family = binomial(link = 'logit'), data = gha38_net, na.action = na.fail)
```

```
modelset_net38lradj <- dredge(net38lradj, beta = c("none"))

(netlr38setadj <- get.models(modelset_net38lradj, subset = delta<4))

get.models(modelset_net38lradj, subset = 1) [1]

get.models(modelset_net38lradj, subset = 2) [1]

get.models(modelset_net38lradj, subset = 3) [1]

#Formulas + Results of the top 3 LR models ####

net38lradj_top <- glmer(gast_pres ~ s.moisture + trip + (1 | site),

                        family = binomial(link = "logit"), data = gha38_net,

                        na.action = na.fail)

summary(net38lradj_top)

Anova(net38lradj_top, type = 3)

nulllr38net <- glmer(gast_pres ~ 1 + (1|site), data = gha38_net, family = binomial)

nagelkerke(net38lradj_top, nulllr38net)



net38lradj_top2 <- glmer(gast_pres ~ s.lit + s.moisture + trip + (1 | site),

                         family = binomial(link = "logit"), data = gha38_net,

                         na.action = na.fail)

summary(net38lradj_top2)
```

```
Anova(net38lradj_top2, type = 3)

nagelkerke(net38lradj_top2, nulllr38net)



net38lradj_top3 <- glmer(gast_pres ~ s.adjph + s.lit + s.moisture + trip + (1 | site),

                         family = binomial(link = "logit"), data = gha38_net,

                         na.action = na.fail)

summary(net38lradj_top3)

Anova(net38lradj_top3, type = 3)

nagelkerke(net38lradj_top3, nulllr38net)



rslradjmods <- list(net38lradj_top, net38lradj_top2, net38lradj_top3)

aictab(rslradjmods)

#------ZERO-INFLATED MODELLING ####

#VIF checking

net38zivifs <- lm(gast_abun ~ site + trip + s.humid + s.temp + s.rain + s.veg + s.lit

                  + s.soil + s.moisture +

                    s.adjph + s.ca, data = gha38_net)

vif(net38lrvifs)
```

```
alias(net38lrvifs)


net38zivifs_soil <- lm(gast_abun ~ site + trip + s.humid + s.temp + s.rain + s.lit
                       + s.moisture + s.adjph + s.ca, data = gha38_net)
vif(net38zivifs_soil)
#Model formulas ####
adj38zip_full <- glmmTMB(gast_abun ~ (1|site) + trip + s.humid + s.temp + s.rain + s.lit +
                           s.moisture + s.adjph + s.ca, zi= ~1, data = gha38_net,
                         family = poisson, na.action = na.fail)
adj38zinb1_full <- glmmTMB(gast_abun ~ (1|site) + trip + s.humid + s.temp + s.rain + s.lit +
                             s.moisture + s.adjph + s.ca, zi= ~1, data = gha38_net,
                           family = nbinom1, na.action = na.fail)
adj38zinb2_full <- glmmTMB(gast_abun ~ (1|site) + trip + s.humid + s.temp + s.rain + s.lit +
                             s.moisture + s.adjph + s.ca, zi= ~1, data = gha38_net,
                           family = nbinom2, na.action = na.fail)


#Top models for each distribution ####
modelset_adj38zip <- dredge(adj38zip_full, beta = c("none"))
```

```
(adjzi38set <- get.models(modelset_adj38zip, subset = delta<4))

get.models(modelset_adj38zip, subset = 1) [1]


modelset_adj38zinb <- dredge(adj38zinb1_full, beta = c("none"))

(adjzinb38set <- get.models(modelset_adj38zinb, subset = delta<4))

get.models(modelset_adj38zinb, subset = 1) [1]


modelset_adj38zinb2 <- dredge(adj38zinb2_full, beta = c("none"))

(adjzinb38set <- get.models(modelset_adj38zinb2, subset = delta<4))

get.models(modelset_adj38zinb2, subset = 1) [1]


#Comparing the best models using each distribution ####

adj38zip_top <- glmmTMB(gast_abun ~ s.adjph + s.ca + s.humid + s.lit + s.temp + (1|site),

                        zi= ~1, data = gha38_net, family = poisson, na.action = na.fail)

adj38zinb_top <- glmmTMB(gast_abun ~ s.lit + s.moisture + trip + (1 | site),

                         zi= ~1, data = gha38_net, family = nbinom1, na.action = na.fail)

adj38zinb2_top <- glmmTMB(gast_abun ~ s.ca + s.humid + s.lit + s.temp + trip + (1 | site),

                          zi= ~1, data = gha38_net, family = nbinom2, na.action = na.fail)
```

```
adj38zinfmods <- list(adj38zip_top, adj38zinb_top, adj38zinb2_top)

aictab(adj38zinfmods)



#Top three nbinom1 models ####

summary(adj38zinb_top)

Anova(adj38zinb_top, type = 3)

r2(adj38zinb_top)

plot(residuals(adj38zinb_top), fitted(adj38zinb_top))



#second best model

get.models(modelset_adj38zinb, subset = 2) [1]

adj38zinb_top2 <- glmmTMB(gast_abun ~ s.lit + s.moisture + s.temp + trip + (1 | site),

                          zi= ~1, data = gha38_net, family = nbinom1, na.action = na.fail)

summary(adj38zinb_top2)

Anova(adj38zinb_top2, type = 3)

r2(adj38zinb_top2)



get.models(modelset_adj38zinb, subset = 3) [1]
```

```
adj38zinb_top3 <- glmmTMB(gast_abun ~ s.ca + s.humid + s.lit + s.temp + trip + (1 | site),
                          zi= ~1, data = gha38_net, family = nbinom1, na.action = na.fail)
summary(adj38zinb_top3)
Anova(adj38zinb_top3, type = 3)
r2(adj38zinb_top3)


adjzimods <- list(adj38zinb_top, adj38zinb_top2, adj38zinb_top3)
aictab(adjzimods)


#ANALYSIS WITH ALL CARDBOARDS ####
gha38_all <- gha38data %>%
  mutate(s.temp = scale(temp),
         s.humid = scale(humid),
         s.rain = scale(rain))
gha38_all$site <- as.factor(gha38_all$site)
gha38_all$transect <- as.factor(gha38_all$transect)
gha38_all$gast_pres <- as.numeric(gha38_all$gast_pres)
#------LOGISTIC REGRESSION ####
```

```
all38lrvifs <- lm(gast_pres ~ site + trip + s.humid + s.temp + s.rain, data = gha38_all)

vif(all38lrvifs)

#Model setup and results ####

all38lrfull <- glmer(gast_pres ~ (1|site) + trip + s.humid + s.temp + s.rain,

                     family = binomial(link = 'logit'), data = gha38_all, na.action = na.fail)

modelset_all38lr <- dredge(all38lrfull, beta = c("none"))

(alllr38set <- get.models(modelset_all38lr, subset = delta<4))

get.models(modelset_all38lr, subset = 1) [1]

all38lr_top <- glmer(gast_pres ~ s.humid + trip + (1 | site),

                     family = binomial(link = "logit"), data = gha38_all, na.action = na.fail)

summary(all38lr_top)

Anova(all38lr_top, type = 3)

nulllr38all <- glmer(gast_pres ~ 1 + (1|site), data = gha38_all, family = binomial)

nagelkerke(all38lr_top, nulllr38all)



get.models(modelset_all38lr, subset = 2) [1]

all38lr_top2 <- glmer(gast_pres ~ s.humid + s.temp + trip + (1 | site),

                      family = binomial(link = "logit"), data = gha38_all, na.action = na.fail)
```

```
summary(all38lr_top2)

Anova(all38lr_top2, type = 3)

nagelkerke(all38lr_top2, nulllr38all)



get.models(modelset_all38lr, subset = 3) [1]

all38lr_top3 <- glmer(gast_pres ~ s.humid + s.rain + trip + (1 | site),

                      family = binomial(link = "logit"), data = gha38_all, na.action = na.fail)

summary(all38lr_top3)

Anova(all38lr_top3, type = 3)

nagelkerke(all38lr_top3, nulllr38all)



alllrmods <- list(all38lr_top, all38lr_top2, all38lr_top3)

aictab(alllrmods)



#------ZERO-INFLATED MODELLING ####

all38zivifs <- lm(gast_abun ~ site + trip + s.humid + s.temp + s.rain, data = gha38_all)

vif(all38zivifs)

#Models ####
```

```
all38zip <- glmmTMB(gast_abun ~ (1|site) + s.humid + s.temp + s.rain, zi = ~1,
                    family = poisson, data = gha38_all, na.action = na.fail)
all38zinb1 <- glmmTMB(gast_abun ~ (1|site) + s.humid + s.temp + s.rain, zi = ~1,
                      family = nbinom1, data = gha38_all, na.action = na.fail)
all38zinb2 <- glmmTMB(gast_abun ~ (1|site) + s.humid + s.temp + s.rain, zi = ~1,
                      family = nbinom2, data = gha38_all, na.action = na.fail)
#Poisson distribution ####
modelset_all38zip <- dredge(all38zip, beta = c("none"))
(allzi38set <- get.models(modelset_all38zip, subset = delta<4))
get.models(modelset_all38zip, subset = 1) [1]


all38zip_top <- glmmTMB(gast_abun ~ s.humid + s.temp + (1 | site), zi = ~1,
                        family = poisson, data = gha38_all, na.action = na.fail)
summary(all38zip_top)
Anova(all38zip_top, type = 3)
r2(all38zip_top)


#nbinom1 distribution ####
```

```
modelset_all38zinb1 <- dredge(all38zinb1, beta = c("none"))

(allzinb138set <- get.models(modelset_all38zinb1, subset = delta<4))

get.models(modelset_all38zinb1, subset = 1) [1]



all38zinb1_top <- glmmTMB(gast_abun ~ s.humid + s.temp + (1 | site), zi = ~1,

                family = nbinom1, data = gha38_all, na.action = na.fail)

summary(all38zinb1_top)

Anova(all38zinb1_top, type = 3)

r2(all38zinb1_top)



#Top 3 nbinom2 distribution models and results ####

modelset_all38zinb2 <- dredge(all38zinb2, beta = c("none"))

(allzinb238set <- get.models(modelset_all38zinb2, subset = delta<4))

get.models(modelset_all38zinb2, subset = 1) [1]



all38zinb2_top <- glmmTMB(gast_abun ~ s.humid + s.temp + (1 | site), zi = ~1,

                family = nbinom2, data = gha38_all, na.action = na.fail)

summary(all38zinb2_top)
```

```
Anova(all38zinb2_top, type = 3)

r2(all38zinb2_top)

plot(residuals(all38zinb2_top), fitted(all38zinb2_top))



get.models(modelset_all38zinb2, subset = 2) [1]



all38zinb2_top2 <- glmmTMB(gast_abun ~ s.humid + s.rain + s.temp + (1 | site), zi = ~1,

                family = nbinom2, data = gha38_all, na.action = na.fail)

summary(all38zinb2_top2)

Anova(all38zinb2_top2, type = 3)

r2(all38zinb2_top2)



get.models(modelset_all38zinb2, subset = 3) [1]



all38zinb2_top3 <- glmmTMB(gast_abun ~ s.temp + (1 | site), zi = ~1,

                family = nbinom2, data = gha38_all, na.action = na.fail)

summary(all38zinb2_top3)

Anova(all38zinb2_top3, type = 3)
```

```
r2(all38zinb2_top3)


allzimods <- list(all38zinb2_top, all38zinb2_top2, all38zinb2_top3)

aictab(allzimods)


#Model comparison by distribution ####

all38zimods <- list(all38zip_top, all38zinb1_top, all38zinb2_top)

aictab(all38zimods)


#PRELIMINARY PH PROBE COMPARISON WITH TEMPERATURE ####

pH_comp <- read.csv("ph_comp.csv")


pH_comp <- pH_comp %>%

  rename(sample = "Sample",

         acc1 = "accumet1",

         acc2 = "accumet2",

         han = "hanna",

         acc1r = "accumet1_tenth",
```

```
        acc2r = "accumet2_tenth",
        hanr = "hanna_tenth")
pH_comp <- pH_comp %>%
  mutate(tempdiff = acc2 - acc1,
        metdiff1 = acc1 - han,
        metdiff2 = acc2 - han,
        tempdiffr = acc2r - acc1r,
        metdiff1r = acc1r - hanr,
        metdiff2r = acc2r - hanr)
#Normailty test on the differences
shapiro.test(pH_comp$tempdiff)
shapiro.test(pH_comp$metdiff1)
shapiro.test(pH_comp$metdiff2)
shapiro.test(pH_comp$tempdiffr)
shapiro.test(pH_comp$metdiff1r)
shapiro.test(pH_comp$metdiff2r)
t.test(Pair(acc2, acc1)~1, data=pH_comp)
t.test(Pair(acc1, han)~1, data=pH_comp)
```

```
t.test(Pair(acc2, han)~1, data=pH_comp)

wilcox.test(Pair(acc1, han)~1, data=pH_comp)

tidymetdiff1 <- read.csv("tidymetdiff1.csv")

wilcox.test(tidymetdiff1$pH ~ tidymetdiff1$Meter, paired = TRUE)



#------SAMPLING METHOD COMPARISON ####

CB_vs_search <- read.csv("CB_vs_search.csv")

gast_species <- read.csv("Gast_species.csv")

#Tidying CB_vs_search ####

CB_vs_search <- CB_vs_search %>%

  rename(location = "CB.LOCATION",

         site = "CB.SITE",

         transect = "CB.TRANSECT",

         cb = "CB.CARDBOARD",

         trip = "CB.TRIP",

         date = "CB.DATE",

         cb_slugs = "CB.SLUGS",

         cb_snails = "CB.SNAILS",
```

```
        cb_total = "CB.TOTAL",
        cb_inf = "CB.INFECTED",
        vs_slugs = "SEARCH.SLUGS",
        vs_snails = "SEARCH.SNAILS",
        vs_total = "SEARCH.TOTAL",
        vs_inf = "SEARCH.INFECTED",
        vs_climb = "SEARCH.CLIMBING")
#Column drop
CB_vs_search$SEARCH.LOCATION <- NULL
CB_vs_search$SEARCH.SITE <- NULL
CB_vs_search$SEARCH.TRANSECT <- NULL
CB_vs_search$SEARCH.CARDBOARD <- NULL
CB_vs_search$SEARCH.TRIP <- NULL
CB_vs_search$SEARCH.DATE <- NULL
CB_vs_search$VEGETATION.HEIGHT <- NULL
CB_vs_search$LOCATION <- NULL
CB_vs_search$HEIGHT <- NULL
#Tidying gast_species ####
```

```
gast_species <- gast_species %>%
  rename(method = "SAMPLE",
         species = "SPECIES.ID",
         prev = "NEMATODE.ID")
#Dropping entries with NA values under NEMATODE.ID
gast_species <- drop_na(gast_species, prev)
#Column drop
gast_species$SNAIL.ID <- NULL
gast_species$WIDTH..mm. <- NULL
#Creation of column assigning host status to each gastropod - IH or NIH
gast_species$status <- as.factor(ifelse(gast_species$species == "Deroceras laeve", 'IH',
                                ifelse(gast_species$species == "Deroceras reticulatum", 'IH',
                                  ifelse(gast_species$species == "Zonitoides arboreus", 'IH',
                                    ifelse(gast_species$species == "Discus whitneyi", 'IH',
                                      ifelse(gast_species$species == "Cochlicopa lubrica", 'IH',
                                        ifelse(gast_species$species == "Succinea ovalis", 'IH',
                                               'NIH')))))))
#Attempt to make contingency table
```

```
spp_table <- table(gast_species$'method', gast_species$status)

view(spp_table)

prev_table <- table(gast_species$'method', gast_species$prev)

view(prev_table)

#Effect of method on IH and NIH yield ####

sp_contin <- read.csv("sp_contin.csv")

chisq.test(sp_contin[-1], correct = TRUE)

#Effect of method on prevalence ####

prev_contin <- read.csv("prev_contin.csv")

fisher.test(prev_contin[-1])

#Effect of method on abundance ####

CB_vs_search <- CB_vs_search %>%

  mutate(count_diff = cb_total - vs_total)

shapiro.test(CB_vs_search$count_diff)

wilcox.test(CB_vs_search$cb_total, CB_vs_search$vs_total, paired = TRUE)
```