Effects of Infection with Brainworm Parasite (*Parelaphostrongylus tenuis*) on the Climbing Behaviour in a Host Gastropod Species (*Deroceras laeve*)

by

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A thesis submitted to the Department of Biological Sciences, University of Manitoba,

in partial fulfilment of the requirements for the course
BIOL 4100 (Honours Thesis)

for the degree of
Bachelor of Science (Honours)

Abstract

Brainworm parasite (*Parelaphostrongylus tenuis*) is a nematode parasite which typically infects white-tailed deer (WTD – *Odocoileus virginianus*) as a definitive host, where it often causes no severe harm. It develops through larval stages in terrestrial gastropods and is eventually ingested by the WTD to complete its life cycle. Brainworm can infect other ungulates accidentally, most commonly moose (*Alces alces*), where it causes serious neurological disease and often death. Here, I investigate the potential role of parasite-modified behaviour in influencing the ingestion of the gastropod hosts by the ungulate host. I hypothesize that when infected with *P. tenuis* larvae, a gastropod host, the meadow slug (*Deroceras laeve*) will display altered vertical climbing behaviour, predicting an increase in vertical climbing activity. Vertical climbing behaviour was assessed amongst 15 infected and 45 uninfected slug hosts at four different times throughout the day, on two separate days. I found a significant effect of the interaction between the weight of slugs, their infection status, and the time of day on vertical climbing behaviour. This is novel evidence to support the parasite-modified behaviour hypothesis in *P. tenuis* and suggests that climbing behaviours may play a role in the transmission of brainworm. Further research would be beneficial, as replicates in this study were low, and the presence of coinfection with a secondary parasite in some samples may have provided a confounding effect.

Acknowledgements

I would like to wholeheartedly thank my supervisor, Dr. Jillian Detwiler, for her continuous support and guidance throughout the creation of this thesis. Your expertise and encouragement were invaluable. Additionally, I would like to thank Dr. John Markham and Dr. Byron Van Nest for their feedback and advice in the analysis and review of this project. I would also like to thank Sidney Mann for introducing me to the field sites and collection and laboratory techniques required for this project, along with all the other members of the Detwiler lab who assisted in the data collection: Trina White, Steven Ajibi, and Joshita Sehgal. I would also like to thank the Markham lab for the use of their Arduino processor and sensors. I would lastly like to thank my family and friends who supported me through this process.

Again, thank you all for your support and encouragement, this project would not have been possible without you.

Finally, I would like to acknowledge that this project was conducted on Treaty No. 1 Territory, the original lands of Anishinaabeg, Ininiwak, Anisininewuk, Dakota Oyate and Dene peoples, and on the National Homeland of the Red River Métis.

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Introduction

Parelaphostrongylus tenuis, commonly known as the brainworm or meningeal worm, is a protostrongylid nematode with a parasitic lifestyle involving at least two hosts to complete its development (Lankester and Anderson 1968). The adult parasite is most commonly found in *Odocoileus virginianus*, the white-tailed deer (WTD) (Anderson 1963). In this host species, the larvae migrate along the spinal column to the brain, where they mature in the subdural space surrounding the brain (Anderson 1964; Slomke et al. 1995). While this parasite is typically not seriously harmful to WTD, it often infects other wild ungulates, such as moose (*Alces alces*) or reindeer (*Rangifer tarandus*), and is also known to infect domesticated species such as the llama (*Lama glama*) and its relatives (Anderson 1964; Upshall et al. 1987; Rickard et al. 1994; Pidwerbesky et al. 2023). In these other hosts, the parasite can cause serious neurological pathology, leading to paralysis or death in some cases (Anderson 1964). In moose, *P. tenuis* larvae remain in the spinal cord and midbrain, where they cause extensive damage to the nervous tissue (Anderson 1964). Moose are at especially high risk where they overlap with WTD populations, as the prevalence of brainworm infection in WTD can be high (e.g. western Manitoba, 41-55%; Pidwerbesky et al. 2023).

The first larval stage (L1) of *P. tenuis* is excreted in the feces of WTD (McCoy and Nudds 1997). Once in the environment, L1 larvae need to infect an intermediate gastropod host through either contact and penetration, or ingestion by the gastropod (Lankester 2001). In this host, the parasite develops through two larval stages, from the L1 to the second (L2) and third larval stage (L3) (Anderson 1963; Maze and Johnstone 1986). After developing within the gastropod host, the L3 larvae are now infective to their next host and can be transmitted back to WTD, most likely through accidental ingestion of the infected gastropods by WTD during their foraging (Maze and Johnstone 1986). The adult parasite sexually reproduces within the ungulate definitive host, and eggs are laid either directly into the bloodstream or in the fibrotic dura tissues, where the larvae then hatch and enter the bloodstream (Anderson 1963). Once in circulation, the eggs, or larvae, emerge in the alveoli of the lungs (Anderson 1963). Here, any unhatched eggs will hatch, and the L1 larvae travel up along the mucous lining of the lungs into the mouth, where they are finally ingested into the digestive tract to be excreted, completing the lifecycle (Anderson 1963). The larval stages of *P. tenuis* are known as dorsal-spined larvae (DSL), for a characteristic spine protruding from the dorsal side of the caudal end of the parasite (Anderson 1963). The parasite has been documented to use at least 18 different species of gastropod hosts, including primarily terrestrial snails and slugs, but also some freshwater snails (Anderson 1963; Lankester and Anderson 1968; Maze and Johnstone 1986). One of the more common gastropod hosts used by *P. tenuis* is the meadow slug (*Deroceras laeve*), which is frequently used when studying the interactions of this parasite with its hosts (Maze and Johnstone 1986; McCoy and Nudds 2000).

The slug *D. laeve* is a widespread species with a global distribution and the ability to inhabit a broad range of environmental conditions (Anderson 2005). These slugs are native to North America but have been documented across Europe and Asia, including much more tropical climates in countries such as India and Sri Lanka (Gupta et al. 2023; Thilakarathne et al. 2024). *Deroceras laeve* are generalist consumers and will typically spend their time in the low grass or leaf litter of forested habitats (Lankester and Anderson 1968; Fox and Landis 1973; Gupta et al. 2023). They have relatively broad environmental requirements, with a range of temperatures at which they can survive (Getz 1959; Thompson et al. 2006). *Deroceras laeve* show no preference for temperatures between 14°C and 26°C and survived freezing at -8°C for at least five hours (Getz 1959). While they do not have specific moisture requirements, they are not especially resistant to desiccation and prefer damp habitats and substrates (Thompson et al. 2006). They will, however, remain active in the shade once the vegetation has dried out (Lankester and Anderson 1968). Despite this lack of specificity, their activities and behaviour are still driven by the need to remain in a suitable environment on the forest floor (Getz 1959).

As mentioned above, *D. laeve* is an intermediate host for *P. tenuis*, meaning that it is infected by the L1 larvae from the feces of WTD (Lankester and Anderson 1968). Although they are a commonly known host for *P. tenuis*, the most common mode of transmission between the two hosts is still unclear (McCoy and Nudds 2000). One hypothesis is that infected slugs are accidentally ingested by WTD as they graze on leaf litter in the forest (McCoy and Nudds 2000). However, the prevalence of *P. tenuis* in gastropods collected from the forest floor is generally much lower when compared to WTD. In one study area, only between 2.8% and 6.3% of gastropods were infected, whereas WTD had a prevalence of over 60% (Lankester and Anderson 1968). An alternative hypothesis is that WTD become infected when they consume parasitized gastropods that have climbed onto grasses and shrubs (McCoy and Nudds 2000).

Parasite-modified behaviour is a phenomenon that has been observed in a number of other parasites (Poulin 1994; Cézilly et al. 2010). Cases of behavioural modification are most common in parasite species where the hosts of the parasite are trophically linked (Cézilly et al. 2010). This phenomenon is hypothesized to occur because the altered behaviour of the infected host increases the likelihood of ingestion by the definitive host (Poulin 1994; Cézilly et al. 2010). There are several ways in which parasite-modified behaviour by *P. tenuis* could manifest. One hypothesis is that *P. tenuis* alters the behaviour of its gastropod hosts by increasing their vertical climbing (McCoy and Nudds 2000). Since WTD prefer to browse in low shrubs and leaves, this would increase the likelihood of their ingestion by WTD, and therein the transmission of *P. tenuis* to WTD (McCaffery et al. 1974; McCoy and Nudds 2000). WTD foraging also follows a circadian rhythm, with an upstart of activity briefly before dawn, less intensive activity during the day, and several hours of foraging after sunset in the summer time (Montgomery 1963). A similar pattern in the timing of climbing has also been found in *Mesodon sayanus*, one of the gastropod host species of *P. tenuis* (McCoy and Nudds 2000). However, there was no difference in climbing between uninfected and infected snails, so to date, there is no support for the hypothesis that brainworm infection modifies the climbing behaviour of gastropod hosts (McCoy and Nudds, 2000).

My objective for this study was to investigate the effect of the infection of brainworm on the climbing behaviour of one of its gastropod hosts, the meadow slug *D. laeve*. This gastropod species was chosen for this study as it is considered one of the main gastropod hosts for *P. tenuis* transmission, yet the effects of parasitism on its climbing behaviour are unknown (Anderson 1963; McCoy and Nudds 2000). In addition, this species does not normally display climbing behaviours, so the effects of parasitism may be easier to distinguish from a more arboreal gastropod such as *M. sayanus* (McCoy and Nudds 1997, 2000). I hypothesized that infection by *P. tenuis* larvae alters the climbing behaviour of the slugs, predicting that infected slugs will climb higher than uninfected slugs following the circadian browsing patterns of WTD. Investigation into the effects of larval *P. tenuis* on its intermediate hosts is key to understanding how the parasite is transmitted and to better estimate the risk of infection to ungulate hosts.

Materials and Methods

Obtaining Adult Slugs for Parasite Exposure

Deroceras laeve were collected from two locations (49°50'52.8"N, 97°14'50.4"W and 49°50'56.4"N, 97°14'54.8"W) within Assiniboine Forest in Winnipeg, Manitoba, Canada. At each location, slugs were collected using 30 0.25 m^2 cardboard sheets 0.25 m^2 in size, sprayed with water and laid directly on the forest floor. Cardboard traps were laid purposefully in areas of the forest that appeared the most desirable to the slugs, such as damp but not flooded ground and sufficient leaf litter or substrate to provide the slugs with access to the cardboard sheets. This approach was used to collect as many slugs as possible with the given sampling effort. The collection trips began on June 5th, 2024, and proceeded throughout the summer until October 10, 2024. Collection trips happened once weekly until the end of August and then once every two weeks during September and October.

A collection trip involved setting traps in the evening just before sunset and collecting them the following morning at dawn. In the field, slugs were removed from the top and bottom of the cardboards and then stored in small plastic containers (7.5cm in diameter, 4.5cm deep). Upon returning to the university, slugs were maintained in the laboratory in larger plastic containers (10cm wide, 15cm long, and 10cm deep) consisting of five or six individuals each. Containers had approximately one to two cm of wet vermiculite on the bottom with a piece of soaked paper towel above and were covered in perforated plastic wrap sealed with a rubber band. Slugs were fed thin slices of carrot along with Fluker's® High-Calcium Cricket Diet as a calcium supplement. Containers were cleaned twice per week, which involved changing the paper towel and replacing the slugs' food.

Obtaining DSL Nematodes for Gastropod Exposures

WTD feces were collected from a forested area within Assiniboine Park (49°52'10.8"N, 97°14'04.2"W). Feces were collected through visual searches of areas known to be frequented by local WTD. Fecal sample collection was biased towards fresher samples, those darker and more moist in appearance, as these are more likely to contain *P. tenuis* larvae (Peterson et al. 1996)**.** A pair of researchers searched for typically two hours and stored fecal samples individually in resealable plastic bags. Upon returning to the laboratory, samples were frozen at -20°C until sedimentations were conducted.

To extract brainworm larvae from the feces, I followed the Baermann technique from Forrester and Lankester (1997), which was further modified here. Fecal samples were weighed and then enclosed in a mesh envelope in a single layer to prevent DSL from falling off one pellet onto another pellet. Given the variable size and shape of the fecal samples, some samples were divided into two mesh envelopes. Envelopes containing fecal samples were submerged in the beakers containing approximately 200mL of distilled water for one hour. Samples that could not lie flat in the beakers due to their larger size and/or shape were submerged in water for 90 minutes and were 'turned over' after 45 minutes to allow DSL to slough off both sides of the sample. Following their submersion fecal samples were removed from the water and disposed of. After allowing the water an additional five minutes to settle, the water from each beaker was poured into a larger beaker to pool the larvae. Once full, every 10 - 15 minutes the top two-thirds of the water from the pool was poured off into a secondary beaker to ensure that no DSL were accidentally discarded. Once the water from all the beakers had been added to the pool, five 1mL aliquots were examined for any live, motile DSL, and the number of DSL visible in each was counted to estimate the concentration of DSL. Additionally, five DSL from the pool were preserved in 100% ethanol for later molecular identification. Six sedimentations were performed on September 10, 17, and 24 and October 1, 8, and 22, 2024. For the first four sedimentations, I used between 31 and 34 fecal samples collected between July 10, 2024 and September 2, 2024. For the final two sedimentations, I used a similar number of samples collected between October 3 and 21, 2024. Slugs were either exposed to DSL or sham-exposed on the same day that sedimentation was conducted. This timing was used because extracted larvae had stopped moving and were presumed dead after I attempted to store them in a refrigerator overnight (approximately 18h). In all exposures, each container of slugs was randomly assigned a treatment of exposed or sham-exposed. Slugs were all fasted for 24 hours before treatment, as slugs may potentially ingest DSL as well (Jenkins et al. 2006).

Exposures were conducted using two different procedures, the first was based on an existing protocol used in the Detwiler laboratory, which was then modified to increase the encounter rate of the slugs with the parasite in an attempt to improve the likelihood of infection following exposure. The first exposure took place in a plastic ice cube tray, each well (2.5cm x 2.5cm x 2.5cm) filled with 1mL of either distilled water (sham-exposed) or sedimented solution with DSL (exposed). One slug was placed in each well and a piece of window screen was placed over the wells to prevent the slugs from escaping over the course of the three-hour exposure. After this first exposure, it was decided that this size of well was too large, as the slugs were often not in contact with the water or DSL solution. Subsequently, I developed the modified procedure for the additional three exposures, which used a silicone ice cube tray with wells (1.5cm x 1.5cm x 1.0cm). As before, one slug was placed in each well with 1mL of either the DSL solution or distilled water. Given the apparent desire of the slugs to climb out of the water to breathe, and to increase my exposure success due to lower-than-expected DSL concentrations, 0.5mL of water was pipetted out of each well prior to adding the slug. This reduced the volume of water without reducing the number of DSL present. Doing so allowed the slugs to remain at the bottom of the wells in contact with our treatment water and keep their breathing pore out of the water, allowing them to breathe. Any water removed from the wells was examined for DSL, and none were ever found. A 40-micron mesh was used to cover the tray to allow air to circulate but prevent the slugs from escaping. Every 15 minutes, the slugs were checked to ensure they remained in contact with the bottom of their well, and if they had climbed out of the water, they were tapped gently back into the water using a scoopula. Following exposure, the slugs were rinsed with distilled water to remove any unpenetrated DSL to ensure a synchronized development of the parasite (Jenkins et al. 2006). Slugs were then placed into their containers and returned to the animal holding facility, where they were maintained for 28 days to allow the DSL to develop from the L1 to the L3 stage in a room with a mean temperature of 22°C, mean relative humidity of 30% and a 12 hr light:dark cycle (Anderson 1963).

Behavioural Trials

My experimental design was modified from a similar experiment conducted by McCoy and Nudds (2000). Glass vases (28 cm in height x 9.5 cm in diameter), known hereafter as arenas, were used to measure the slugs' climbing behaviours. Arenas were lined on the bottom with a wet paper towel and covered in perforated plastic wrap to maintain moisture levels during the experiment. Randomly, three arenas each day were assigned an Adafruit DHT11 sensor, inserted and suspended through the covering, to measure the temperature and relative humidity of the arena's atmosphere. This data was relayed through an Arduino Uno® processing board to a small LCD display, where it was noted and recorded at each time of measurement. Slugs were weighed to the nearest milligram to obtain a body mass measurement at the time of the trials. Size is an indicator of age and body condition, and so mass was recorded to control for the difference in climbing height due to these factors (Cordoba et al. 2011). Each arena was randomly assigned a slug, with only one slug per arena to ensure their behaviours were independent. Slugs were added no later than 07:00 to provide a 30-minute acclimatization period. Measurements were then taken four times throughout the day, at 07:30, 11:30, 15:30, and 19:30. At these times, the height of each slug from the bottom of its arena was measured in centimetres using a ruler held up to the side of the arena. Height was measured at the point halfway between the highest and the lowest points of the slug. Following the final measurement, slugs were removed and stored in individual containers, and the arenas were cleaned and rinsed in preparation for the next set of trials. Climbing was assessed twice for each slug, 28 and 30 days post-exposure, to account for variation in climbing behaviour between days. Four sets of behavioural trials were conducted, one for each of the exposure groups.

Slug Dissection

Following the behavioural trials, all slugs (sham-exposed and exposed) that survived were dissected to confirm their infection status. Given that some slugs may not have become infected following exposure, slugs could be assigned to three categories: sham-exposed, exposed-uninfected, and exposed-infected. Any parasites discovered during these dissections were digitally imaged and then preserved in 100% ethanol for later molecular identification.

Statistical Analysis

Given that some exposed slugs did not have any DSL following dissection, I ran a linear model to determine if the height climbed differed between sham-exposed and exposed-uninfected based on weight, time of day, and infection status. An ANCOVA test comparing the two groups showed no difference, so I combined those two groups into one, referred to hereafter as 'uninfected'.

A general linear mixed model was used to assess the effect of infection status and the time of day on the height climbed by the slugs. The weight of each slug was included as a covariate in the model, and a nested random effect was included to account for slug identity and the repeated measure of climbing on two days. A variable inflation factor test was conducted to test for multicollinearity amongst the factors. Assumptions of normality and homogeneity of variance amongst the data were evaluated by visual inspection of plots of the residuals from a linear model. A log transformation was applied to the data to ensure that the data met these assumptions. A set of possible models was ranked using

Akaike Information Criterion corrected for small sample sizes ($\Delta\mathrm{AIC}_c$). The best ranking model had the lowest $\Delta\mathrm{AIC}_c$, and was chosen for use in my analysis (Anderson and Burnham, 2002).

Results

In Assiniboine Forest, slug counts were highest at the end of the spring (June 5 and 18, 2024), then dropped to very low levels throughout the summer (June 25 to September 3, 2024). My collection counts rose again in the fall (September 19 to October 10, 2024). I collected, on average, 19.2 ± 5.95 slugs per collection trip; the trend in collection count over the sampling season can be seen in Figure 1.

Two of the attempted sedimentations, conducted on September 17 and 24, 2024, resulted in insufficient concentrations of DSL for exposure, with only 10 and 13.7 DSL/mL, respectively. The other four sedimentations resulted in concentrations of 34.8, 61, 21.4, and 67.5 DSL/mL, respectively (mean ± SE: 46.2 ± 10.9 DSL/mL). My four rounds of exposures resulted in 51 exposed slugs and 49 sham-exposed slugs. Throughout the 28-day development period, only 66.7% of exposed slugs and 69.1% of sham-exposed slugs survived. Slug mortality also occurred between the days of the behavioural experiments (1 exposed, 1 sham-exposed). Following my four trials, I had complete data for 30 slugs of each treatment. However, an additional four slugs (2 sham-exposed and 2 exposed) died before I was able to dissect them and confirm their infection status.

Dissection showed that 15 of the exposed slugs were infected with putative *P. tenuis* larvae (Table 1). The identification is considered putative until DNA sequencing is performed as *P. tenuis* is difficult to morphologically distinguish from *P. andersoni*, and are known to occur in the same area and even co-infect WTD with *P. tenuis* (Lankester and Hauta 1989; Pidwerbesky et al. 2023). Initial visual identification indicated that these nematodes were in the L2 stage of development based on the presence of the shed cuticle of the first stage still encasing the body (Figure 2A), which is invariably found in this stage of larval development (Anderson 1963). The intensity of infection in these 15 infected slugs ranged from 1 to 9 parasites, with a mean of 4.6 ± 0.7 per individual (Table 1). Further, 29 slugs were found to be infected with an unknown adult nematode parasite. Again, this parasite was not positively identified, but both gravid adult specimens as well as newly hatched larvae were found within the slugs of both my infected and uninfected groups (Table 1, Figure 2B). Infection intensity for this parasite was not well recorded, as often there were too many young larvae to count within the slug.

After accounting for deaths and a lack of infection following exposure, 15 slugs were in the infected treatment group, and 45 slugs were in the uninfected control group. The best model included a significant three-way interaction amongst slug weight, infection status, and time of day ($\chi^2 = 20.35$, $P < 0.001$), as well as a two-way interaction between slug weight and time of day ($\chi^2 = 8.85$, $P = 0.031$). Vertical height climbed was significantly different based on two of the fixed effects as a result of this, slug weight ($\chi^2 = 17.56$, $P < 0.001$) and infection status ($\chi^2 = 6.18$, $P = 0.012$), with larger slugs climbing higher than smaller slugs, and infected slugs climbing higher than uninfected slugs (Table 2, Figure 3). Time of day was not significant as a main effect ($\chi^2 = 2.31$, $P = 0.509$) but was a component of both significant interaction terms of the model (Table 2). Conditional r^2 for the model was 65%, with the marginal r^2 at 24%, indicating a relatively large amount of the variation in the model being explained by the random effects (41%).

Temperature and relative humidity data recorded from the DHT sensors showed no substantial variation between any of the trials, with temperatures ranging from 21°C to 24°C with a mean and standard error of 22.8 ± 0.09°C and relative humidity constant at 95% across all trials.

Discussion

Given that the prevalence of infection in naturally infected slugs is low, obtaining infected slugs for laboratory experiments is essential. The results of my field collections suggest that in Assiniboine Forest, it is best to collect slugs from nature in early to mid June, as the highest numbers of slugs were found during these collection trips. Slug counts rose from mid-September to early October, suggesting that this is another period in which many field-collected slugs can be obtained. This increase in the Fall was expected based on sampling from the previous year (Mann, unpublished).

Sedimentations suggested that fecal samples collected from July to October were likely to have concentrations of DSL below 100 DSL/mL. Previous studies also found that peak larval shedding occured between February and May, and began to decrease into June and July (Slomke et al. 1995; Peterson et al. 1996). In addition, this study also further developed an exposure protocol aimed at increasing the number of slugs that become infected following exposure. Past studies reported that exposing gastropods to 500 – 1000 DSL led to a 100% infection rate in their hosts (Kutz et al. 2000; Jenkins et al. 2006). In these latter cases, DSL were obtained from animals kept in captivity, where it is easier to obtain infected feces in a timely manner. Given that our concentrations were drastically lower, this study also further developed an exposure protocol aimed at increasing the encounter rate between the DSL and slugs to increase the number of infected slugs when lower concentrations of DSL are used. In the smaller trays, approximately two-thirds of slugs exposed to over 60 DSL/mL were found to be infected with putative *P. tenuis* larvae, whereas none of those exposed to the 21.4 DSL/mL were infected. Thus, my results provide guidelines for how to time field collections of gastropods and feces and how to compensate for low DSL concentrations.

The results of my behavioural trials support the hypothesis that infection with brainworm alters the vertical climbing behaviour of *D. laeve* slugs. I showed that time of day, size of slug, and infection status of the slug all interact to affect the vertical climbing behaviour of *D. laeve*, indicating that the effect of infection in the slug is dependent on the weight of the slug and the time of day. This contradicts the findings of the previous work done with brainworm, where it was found that infection status had no effect on the climbing behaviour of another gastropod host, *Mesodon sayanus* (McCoy and Nudds 2000). The lack of an effect of infection in the latter was likely because *M. sayanus* is considered to be an “arboreal” species, tending to naturally spend more time off the forest floor than other gastropod host species of *P. tenuis*, including *D. laeve* (McCoy and Nudds 1997). Thus, the strength of the effect of the parasite on climbing would have to be potentially greater to be observed. In contrast, the ground-dwelling tendency of *D. laeve* is better suited to find evidence of induced climbing behaviours. This is novel evidence for this hypothesis, suggesting that climbing may play a role in transmission as larger, infected individuals are more likely to climb than smaller, uninfected individuals. My results indicated that the time of day alone did not significantly affect the height climbed by individuals, but rather the effect was dependent on the weight of the slug. As slugs increased in size, they tended to climb higher during each time point. Further, there was an increase in height climbed as slugs increased in size for all the time points except the early morning. This is also in disagreement with a past study on the climbing behaviour of various host gastropod species, which found that time of day alone influenced the climbing behaviours of host species (McCoy and Nudds, 1997).

Some of the variation in climbing among infected slugs could have been due to variation in the development of the DSL. Only some of the infected slugs were found to be infected with what appeared to be, based on their morphology, L3 larvae. The intermediate larval development of *P. tenuis* to the L3 stage has been documented to be completed within 27 days when in other terrestrial snail host species (*Zonitoides arboreus* and *Discus cronkhitei*) maintained at a temperature of at least 18°C (Anderson, 1963). While 18°C is listed as the minimum temperature, storage of these hosts for larval development occurred outdoors during May and June in southern Ontario, so daytime temperatures may have been much higher and promoted faster development than in my study (Anderson, 1963). The slugs in my study were maintained at temperatures fluctuating between 21°C and 24°C, suggesting more time is required either at these temperatures or in this host species for L3 larvae to develop. Only L3 larvae are infective to the ungulate hosts, so it remains unclear whether climbing is advantageous for the parasite, as presumably they would not be able to develop after being consumed by the definitive host (Anderson 1963).

A potentially confounding effect on gastropod climbing in my study could be the influence of infection or co-infection with the unknown adult nematode. Given the small sample sizes for uninfected and infected slugs with DSL, I did not further divide the dataset into more groups. The identity of the adult nematode remains unknown, though its morphology, location within the slugs, and other characteristics suggest it may be *Cosmocercoides dukae*, a nematode parasite found naturally and primarily in *D. laeve* (Vanderburgh and Anderson 1987). As with the DSL from my dissections, visual identification is insufficient to define this parasite, so molecular identification would need to be done before further analysis. This parasite is transmitted between slugs in a number of ways, but given that it is slug-slug transmission, climbing does not seem to be an advantage to this parasite's transmission (Anderson, 1960). Further, infection intensity and age of host can negatively affect host survival, with younger (smaller) hosts with higher intensities of parasites having higher mortality than older hosts. In our study, this may explain the effect of slug weight on climbing, with smaller individuals being more affected by infection. Field data suggests that singly infected and co-infected slugs with this adult nematode are most likely to be obtained later in the fall, from late September through October. Additional collections during this period can be conducted to increase the sample size to compare how co-infection affects slug climbing.

It is also unclear if this unknown adult nematode influenced the mortality of the slugs, as the bodies decay quickly following death, making it difficult to ascertain. Further, this non-DSL parasite may have influenced the success rate of the exposures as well as the development to the L3 stage. Parasite competition is known to reduce fitness and delay development, and pre-infection with a parasite can also inhibit that host's ability to be infected with another parasite (Jourdane et al. 1990; Fredensborg and Poulin 2005; Tang et al. 2009). It is difficult to determine if this affected the success of exposure since approximately half of the successfully exposed *D. laeve* specimens were found to be co-infected with this unknown nematode.

The effect of infection was clear despite concerns of a small sample size of infected slugs. *Mesodon sayanus* is more amenable to studies of parasite-modified behaviour, as they are known to have a longer life span in the laboratory compared to *D. laeve* (McCoy and Nudds, 1997; McCoy and Nudds, 2000). The rate at which *D. laeve* die in the laboratory can make it challenging to have a sufficient number of survivors for adequate sample sizes (McCoy and Nudds, 1997; McCoy and Nudds, 2000). An increased sample size would increase the confidence in these results and the ability to further assess other factors, such as coinfection. Continuation of this project would be beneficial in further assessing this hypothesis. Repetition to increase sample size, as well as the collection of specimens in the early season to avoid pre-infection with secondary parasites, are important for future work here.

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Tables

Table 1. Infection status of slugs from vertical climbing behavioural trials with nematodes, given as a percentage with the actual number in brackets, and mean intensity of infection with brainworm (*Parelaphostrongylus tenuis*) larvae (DSL), given as the mean and standard error.

Treatment Group	Infection Status	Sham-exposed	Parasite-exposed	
		% (total)	% (total)	Mean ± SE
DSL uninfected	Uninfected	73.3 (22)	13.3 (4)	0
	Non-DSL Nematode	26.7 (8)	36.7 (11)	0
DSL infected	DSL Nematode	0 (0)	23.3 (7)	4.57 ± 1.11
	Coinfection[a]	0 (0)	26.7 (8)	5.13 ± 0.95
	Total	(30)	(30)	4.63 ± 0.72

[a] Coinfected with non-DSL nematode putatively identified as an adult nematode and the DSL of putative brainworm, *Parelaphostrongylus tenuis*.

Table 2. Analysis of deviance table for the best model explaining the vertical climbing behaviour in *Deroceras laeve* slugs infected with brainworm (*Parelaphostrongylus tenuis*) larvae (DSL).

Source	χ^2	df	P-value
Weight[a]	17.563	1	**< 0.001**
Infection Status[b]	6.187	1	**0.013**
Time of Day[c]	2.314	3	0.510
Weight * Time of Day	8.854	3	**0.031**
Weight * Infection Status * Time of Day	20.353	4	**< 0.001**

[a] Weight refers to mass of the slug in grams.

[b] Infection Status is the presence or absence of *P. tenuis* larvae following exposure to DSL.

[c] Time of Day includes one of four times within a 12h period in which climbing was assessed.

Figures

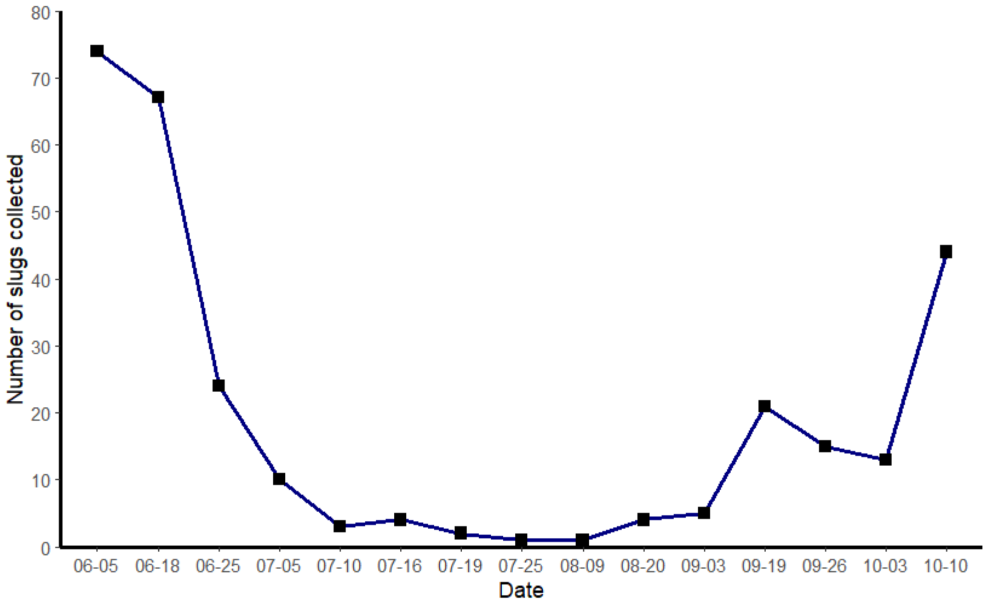

Figure 1. The trend of slug collection counts over 18 weeks from the late spring to the fall of 2024 in Assiniboine Forest in Winnipeg, Manitoba. Each point represents a sampling trip, where 60 cardboard traps were used to collect slugs overnight. The first collection occurred on June 5th and the last on October 10th.

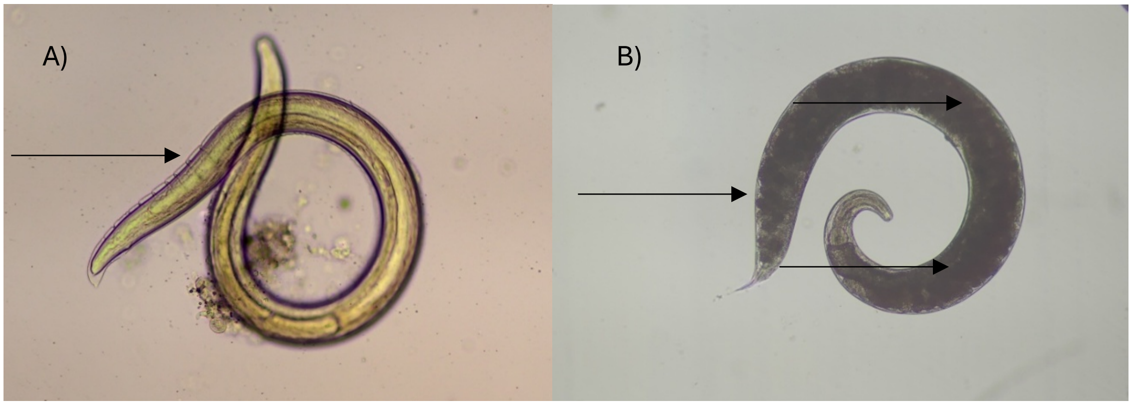

Figure 2. Microscope images of nematode parasites recovered from field-collected *Deroceras laeve* specimens experimentally exposed to putative *Parelaphostrongylus tenuis* stage 1 (L1) larvae. Fig. 2A depicts a suspected L2 *P. tenuis* larva, as indicated by the lingering shed cuticle of the L1. Fig. 2B depicts a suspected gravid female *Cosmocercoides dukae*, with eggs indicated by the arrows.

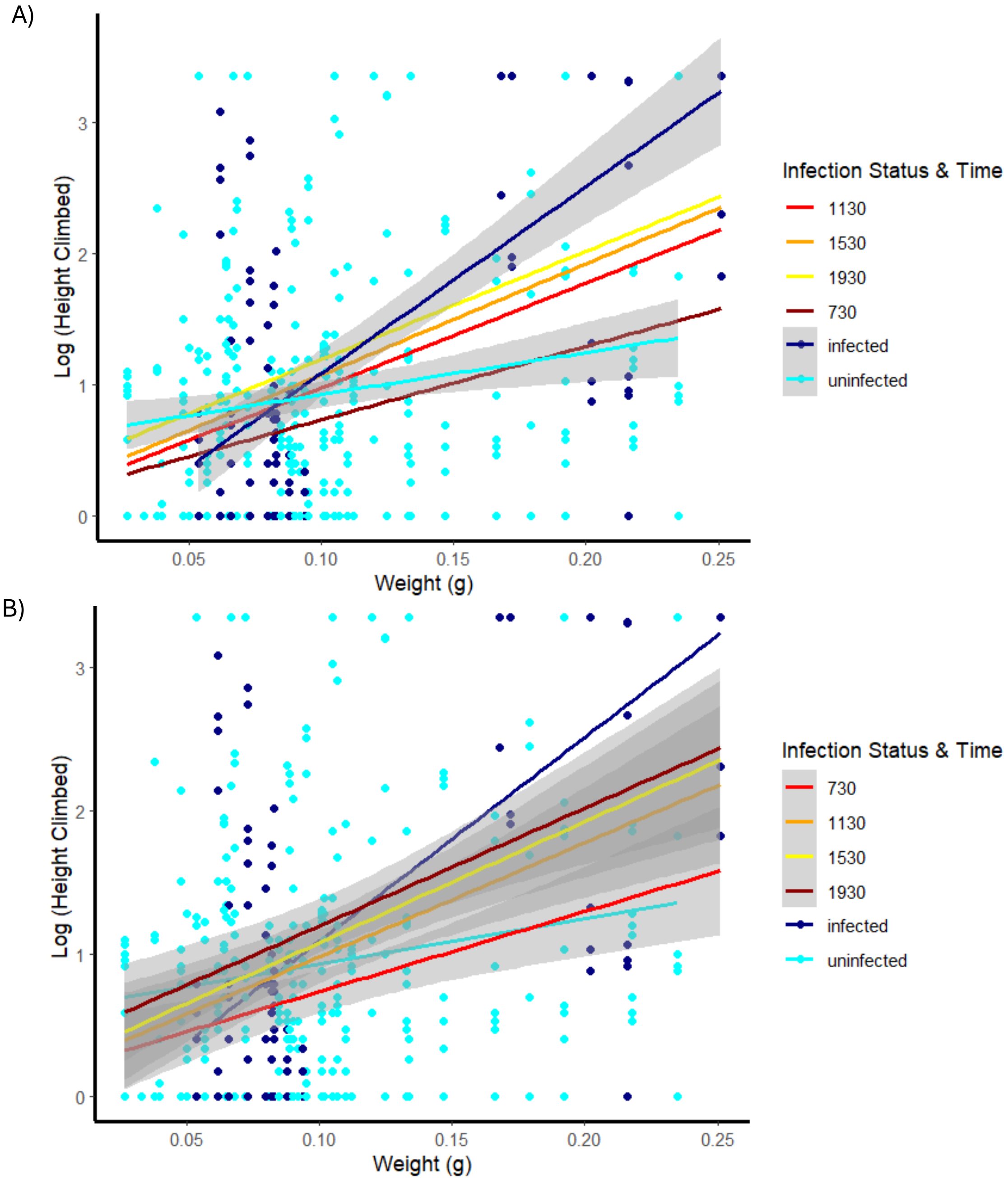

Figure 3. Relationship of log-transformed height climbed to the weight of a meadow slug (*Deroceras laeve*) depending on the time of day and infection with brainworm (*Parelaphostrongylus tenuis*) larvae. Fig. 3A displays 95% confidence intervals for the infected versus uninfected groups, and Fig. 3B displays 95% confidence intervals for the various times of the day.