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THE EFFECTS OF THE HEMLOCK WOOLLY ADELGID ON ABUNDANCE AND NYMPHAL INFECTION PREVALENCE OF BLACK-LEGGED TICKS IN MAINE

by

Spencer Christian DeBrock

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Zoology)

The Honors College

University of Maine

May, 2018

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ABSTRACT

The black-legged tick (*Ixodes scapularis*) has recently made a tremendous impact in Maine due to its role as a vector for the bacterial pathogen *Borrelia burgdorferi*, the causative agent of Lyme disease. A lesser known, but equally concerning, invasive insect is the hemlock woolly adelgid (HWA; *Adelges tsugae*), a sap-sucking scale that is primarily responsible for the ongoing widespread decline of eastern hemlock in the northeast. Maine is currently experiencing a co-invasion of these species, and this study tests the hypothesis that the phenomenon of hemlock loss may facilitate the invasion of the black-legged tick by a combination of indirect effects. By killing eastern hemlock trees, the HWA alters forest structure (e.g., letting more light through the canopy) and changes the species composition of plant and wildlife communities, including important hosts of the black-legged tick. My study simulates the consequences of HWA infestation by comparing tick abundance and nymphal infection prevalence (NIP) in hemlock and hardwood stands in southern Maine and the Bangor area. I hypothesized that the HWA is indirectly increasing both tick abundance and Lyme disease risk in Maine by creating ecological conditions that alter abundance of deer and provide a more suitable microhabitat for the tick. I also predicted that NIP would differ between the two treatments, with ticks collected in deciduous stands having higher infection prevalence. My results showed no significant difference in either tick abundance or NIP between the two treatments. Additionally, I tested one mechanism that could explain these patterns by conducting deer scat surveys using standardized transects. There was no significant difference in deer scat counts between the treatments. Conclusions from this work could

inform park managers and Maine citizens about the likelihood of Lyme infection or tick bites in certain areas of forests or parks.

DEDICATION

I dedicate this work to my college experience including all the incredible people I met and the times that I had as well as to my emotional, spiritual, personal, and academic growth throughout.

ACKNOWLEDGEMENTS

Thank you to Lucy Guarnieri, Emma Lee, and Becca Gallandt for participating in data collection and Sara McBride and Christine Conte for making comments on my thesis throughout. Thank you to the Center for Undergraduate Research for funding this project. I also want to thank Ann Bryant for her wonderful instruction during pathogen testing for this part of my study could not have been done without her. Finally, a very special thanks goes to my advisor Allison Gardner, who was so patient, kind, and helpful throughout this entire project. She taught me so much and I am eternally grateful.

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INTRODUCTION

Biological invasions and facilitation

Biological invasions are a major driver of biodiversity loss, a key issue affecting all major biomes (Dias, 2017). The success of invasions is driven by factors associated with the introduced species themselves (e.g., their ability to outcompete native species), characteristics of the invaded habitats (e.g., presence of predators of the invader), and attributes of the process by which the introduction occurred, such as the initial number of introduced individuals (Duncan et al., 2003; Peoples & Goforth, 2017; Thuiller et al., 2010). Establishment of alien species is favored at locations where there are fewer predators, more available resources and absence of competitors (Duncan et al., 2003). Four transition phases are recognized in the process of invasion by an exotic organism: transport, release, establishment, and proliferation (Duncan et al., 2003). Invasive species cost our economies billions of dollars each year. These costs arise from economic loss in agriculture, forestry, energy, and negative effects on ecosystem services, as well as the cost of control and eradication efforts of the alien species (Dias, 2017). Further, non-native species can affect the quality of habitats available to native organisms and, in turn, the ecosystem services they provide or regulate (Guttierrez, 2017). Biodiversity or loss thereof, can provide ecosystem services that affect human health, especially in the emergence and transmission of vector borne disease (Sandifer et al., 2015). Most arguments for this point use the premise that loss of biodiversity can alter the abundance of competent and incompetent reservoir hosts or the vector itself, a premise known as "the dilution effect" (Sandifer et al., 2015)

Indeed, the establishment and proliferation of invasive species can be promoted by other species, even other alien species. This is known as biological facilitation, which is defined as any direct or indirect positive interaction between two species that promotes their coexistence and the diversity of one or both species (McIntire and Fajardo, 2014; Ruiter and Gaedke, 2016). Examples of direct facilitation include symbiotic effects between plants and mycorrhizal fungi, or between plants and pollinators; both examples have strong effects on plant species diversity and community organization (Thébault and Fontaine, 2010; Van der Heijden et al., 1998). Facilitation can also result from indirect effects. For example, ecosystem engineers such as beavers, earthworms, or eastern hemlock trees create or preserve habitats for other species, thereby encouraging their coexistence with those other species and increasing diversity in the ecosystem (Jones et al., 1994; Dangerfield et al., 1998; Wright et al., 2002; Eisenhauer, 2010). Biological facilitation has been reported to occur between two invasive arthropods. In one study, an invasive ant *Solenopsis invicta* had been known to receive important carbohydrate resources from the invasive mealybug *Antonina graminis* utilizing grasses (Helms & Vinson, 2003). The authors wondered if the mealybug was benefiting from the ant and found that mealybug occurrence increased significantly with increased proximity to *S. invicta* mounds (Helms & Vinson, 2003). They admit the nature of the facilitation of the mealybug by the ant is unknown however they list several hypotheses. Among these are: ants may protect the mealybugs from predators, they might actively transport first instar mealybugs, or, by removing the carbohydrate (honeydew), the ants aid in mealybug disease prevention.

Black-legged Tick

The black-legged tick (*Ixodes scapularis*) is an invasive arthropod in Maine. It quests for bloodmeals on forests floors, most notably feeding on white footed mice (*Peromyscus leucopus*) and white- tailed deer (*Odocoileus virginianus*) (Anderson 1988). While ticks may be a food source for other arthropods such as spider, ants, and beetles, as well as for grooming hosts, (Samish & Rehacek, 1999), they are also a well-known nuisance to humans as they are the main vectors of several tick-borne pathogens. The greatest threat being the etiological agent of Lyme disease (*Borrelia burgdorferi*), the most frequently reported vector-borne disease in the United States (CDC 2016). *Borrelia burgdorferi* cells are motile spirochetes which are neither gram-negative nor gram-positive (Barbour and Hayes 1986; Shapiro and Gerber 2000). Lyme disease is characterized by a “bull’s eye” rash at the site of infection, flu-like symptoms, and joint pain (CDC, 2016).

Recently, a combination of the tick’s expanding range and human modification of the landscape has contributed greatly to increased human-tick contact and potential for disease transmission (Ostfeld et al. 1995). Additionally, human land use over the past 60 years has transformed the northeastern United States from a primarily agricultural area to a heavily residential, urban environment (Goltz, 2012). Forests are starting to regenerate as agriculture has declined in New England, however now there are forests in varying stages of growth near and on residential lots. Forest edges, also a result of land use change, can further provide ideal habitats for *Ixodes scapularis* and its hosts thus increasing human-tick interaction (Lane et al. 1991; CDC 2016). Additionally, in the

Northeast, human-tick contact is maximized because increased summer human outdoor activity coincides with the peak of nymphal questing times (CDC 2016).

As with other ixodid ticks, *I. scapularis* is most vulnerable to water imbalance or desiccation when engaging in questing behavior, which involves the tick leaving the shelter of the leaf litter for areas with higher temperatures and lower humidity (Goltz, 2012). Questing behavior, which is utilized by most ticks, consists of the tick climbing vegetation and raising the first pair of legs to attach to passing hosts (Goltz 2012). Because *I. scapularis* spends about 98% of its life cycle off host, seasonal changes in temperature and humidity will greatly affect population growth and the spread of black-legged ticks (Needham and Teel 1991, Fish 1993, Bertrand and Wilson 1996). If temperatures are too high or humidity too low, they may desiccate due to their large surface area to volume ratio (Goltz, 2012). In fact, it has been found that abundance of larval and nymphal tick populations are greatest in forested habitats dominated by maple and oak during the summer months due to the presence of leaf litter from these trees (Ostfeld et al. 1995). Conversely, reduced light penetration restricts understory growth which could result in less suitable questing habitat, while soil properties could be too acidic or dry for the tick or be ideal for predators (Zhioua et al. 1999, Guerra et al. 2002).

The black-legged tick life cycle has been heavily studied in the northern U.S. and takes two years to complete (Eisen and Lane 2002). *Ixodes scapularis* has several developmental stages which consist of egg, larva, nymph, and adult, and in each stage the tick feeds on different hosts (Goltz 2012; Oliver 1989). After attaching to a host, the tick takes a blood meal and becomes engorged. Eggs are deposited in the early spring by females. After hatching, the larvae quest for a blood meal, typically small mammals and birds, in the summer (Goltz 2012; CDC 2016). Larval *I. scapularis* are reported to disperse only 2-3 meters from their egg mass (Stafford 1992). These larvae then overwinter, molt into nymphs, and become active in the spring and quest for hosts (e.g., small mammals, foxes, deer, humans) throughout the summer (Goltz 2012; CDC 2016). Once the tick has had a blood meal, it could potentially have *B. burgdorferi* in its gut, making it epidemiologically relevant. This is the time when black-legged ticks pose the greatest threat to humans because, not only are they vectors for diseases, but they are also very small and easily go unnoticed (CDC, 2016). After feeding on a second host, they drop from the host and molt into adults which are then active in the fall through early spring (Goltz 2012; Oliver 1989). Larvae and nymphs have an extremely broad host range, feeding on many different mammalian and avian species in the northeastern U.S. regions. The most important hosts being the white-footed mouse and eastern chipmunk (*Tamias striatus*), both being reservoir hosts for *B. burgdorferi,* and the white- tailed deer (Anderson 1988, 1989).

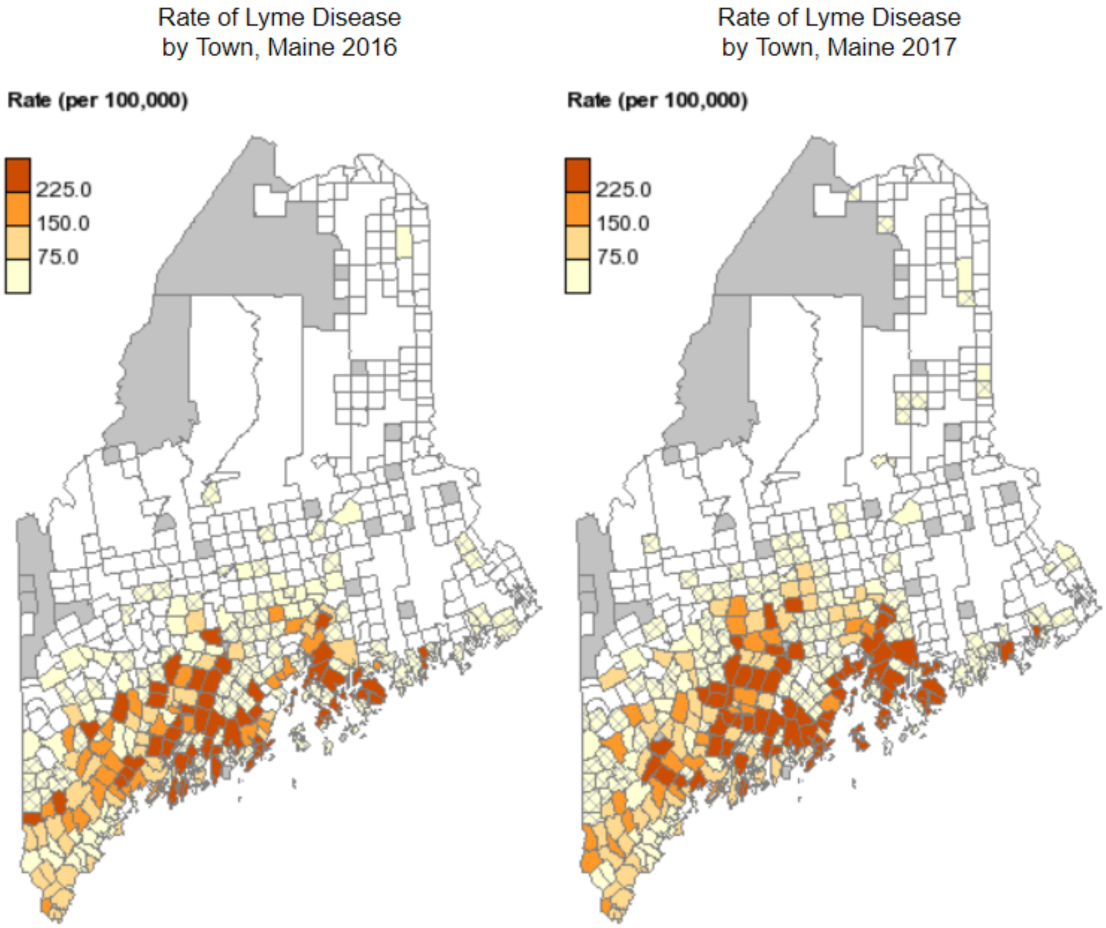

Figure 1: Change in Lyme disease by town in the state of Maine from 2016- 2017. Notice incidence is greatest along the coast and has been moving inland. Since, in the northeast, Lyme is only spread by the black-legged tick, this map can be used as a proxy for the tick's range in Maine.

Hemlock Woolly Adelgid

Another significant arthropod that has recently colonized Maine, and is primarily responsible for the loss of eastern hemlock throughout the Northeast, is a scale insect, the hemlock woolly adelgid (HWA; *Adelges tsugae*). The HWA is a small (~1.5-mm), aphidlike insect that was introduced into the eastern U.S. from Japan during the early 1950s (Orwig, 2011; Degrassi, 2018; McAvoy, 2017). Since its introduction, HWA has spread to 18 states from Georgia to southern Maine, threatening eastern hemlock trees (*Tsuga canadensis*). HWA has a complex, polymorphic life cycle, with two parthenogenetic generations each year in the eastern United States (Orwig, 2011, McAvoy, 2017). The HWA settles on the underside of young hemlock twigs and feeds on ray parenchyma cells via a long stylet, causing needle loss, bud mortality, and branch and tree mortality within 4–15+ years in the northern United States. This insect is invasive and is like a terrible disease to hemlock trees. The adelgid can infest one tree, kill the tree, and spread in a short time (Orwig, 2011). This is because HWA eggs and crawlers can disperse rapidly via wind, birds, deer, and human activities such as logging and transfer of infested nursery plants and therefore have the potential to devastate whole stands of hemlock trees. Rates of HWA spread have been estimated to be 8–20 km per year (Orwig, 2011). Once HWA is introduced into a forest, the damage is chronic. Hemlock trees cannot sprout or refoliate after needle loss, and therefore hemlock can be eradicated from a site once infested (Orwig, 2011; Degrassi, 2018). Eastern hemlock has no known resistance to HWA, and so continued infestation threatens a range- wide decline or elimination of this ecologically, culturally, and economically important tree species (Orwig, 2011).

The eastern hemlocks themselves are ecosystem engineers creating important structural diversity and habitat for a wide variety of wildlife species and modulating core ecosystem processes such as energy, nutrient flows, and water balance (Orwig, 2011). However, when the HWA kills the hemlock, the tree is no longer available to contribute its crucial ecosystem functions and, as a result, a dramatically new ecosystem is created (Ellison et al, 2005). In forest settings, hemlock decline and mortality often lead to a complete change in forest cover from a cool, dark, conifer-dominated forest to a warmer, sunlit mix of deciduous species, specifically Red maple (*Acer rubrum*), birch (*Betula spp.*) and Oak (*Quercus spp.*) (Orwig, 2011; Ellison et al., 2005; Orwig et al., 2002;

Tingley et al., 2002; Spaulding & Rieske, 2012; Orwig, 2007). Thus, infestation by HWA leads to thinning canopies and eventual tree death, leading to microenvironmental changes such as increased light, soil surface temperatures and moisture, and humidity changes due to leaf litter accumulation (Ellison et al, 2005; Orwig et al, 2002; Spaulding & Rieske, 2012; Orwig, 2007). Increased light in these new hardwood stands allows growth of plant species along the forest floor that would be ideal for questing ticks (Orwig et al, 2002). With hardwoods also comes accumulation of leaf litter and with that, shelter from the increased temperature due to light penetration and optimal humidity conditions so the tick avoids desiccation (Orwig 2011).

Importantly, the hemlocks' deep crowns and associated understory conditions provide important habitat for numerous wildlife species, from vertebrates to insects (Orwig, 2011; Ellison et al, 2005; Tingley et al, 2002; Spaulding & Rieske, 2012). One animal strongly associated with hemlock is the white- tailed deer, which congregates under these evergreens in winter for food and cover (Orwig, 2011, 2007). It is possible that hemlock death draws in more deer, because they would prefer to browse the early-successional deciduous trees (e.g., maple, birch, oak) that utilize the space previously occupied by the hemlock for food (Orwig et al, 2002; Tingley et al, 2002; Spaulding & Rieske, 2012). This movement of deer of will facilitate movement of the black-legged tick such that if more deer are in these hardwood forests, I predict more ticks will be as well. One of the most important hosts of the black-legged tick is the white footed mouse, since it is such a competent reservoir host (Anderson, 1988, 1983; Goltz, 2012, Fino, 2017) Although ubiquitous in the deciduous and mixed deciduous-coniferous forests of the northeastern United States (Baker, 1968), the white-footed mouse has been found to reach higher densities in habitats with a structurally complex understory and dense shrubby undergrowth (Anderson et al., 2003, Drickamer, 1990). Such habitats would likely be hardwood habitats due the increased light penetration allowing for a complex understory. The mouse also appears to favor older deciduous forest patches over coniferous patches (Schmid-Holmes and Drickamer, 2001; Schnurr et al., 2004). Because mice prefer the deciduous forests and the dense vegetation provided on the forest floor following hemlock disappearance, more ticks should be found in these hardwood forests.

This study investigates the phenomenon of hemlock loss due to HWA and its impacts on ticks. It is known from previous literature that ticks tend to be found in deciduous stands. Since hemlock death due to HWA creates deciduous forest, which may attract more small mammals and deer, it would stand to reason that black-legged ticks will spread to these forests. Tick abundance should then theoretically increase since more space will be available to occupy and more ticks would create an increased risk of Lyme disease. My study simulates the consequences of HWA infestation by comparing tick abundance and nymphal infection prevalence (NIP) in hemlock and hardwood stands in southern Maine and the Orono area. To determine NIP, the ticks were tested for bacterial pathogen, in order to characterize differences in Lyme disease prevalence between the two treatments. Finally, white- tailed deer because present ticks not only with a blood meal but also an excellent opportunity for dispersal and movement between patches and hosts. Therefore, I investigated deer activity to provide ecological context to the tick abundance data I would collect. Altogether, this study tests three hypotheses. First, I hypothesize that by creating a more suitable microhabitat (e.g., altering soil, temperature, and leaf litter composition) for the black-legged tick, hemlock loss by HWA infestations will lead to increased tick abundance. Second, I hypothesize that these same factors will lead to greater NIP in deciduous stands compared to hemlock stands due to increased tick longevity and likelihood to encounter blood- meal hosts. Finally, I predict deer scat, and thus deer activity, will be greater in hardwood stands because these stands contain foraging material preferable to deer.

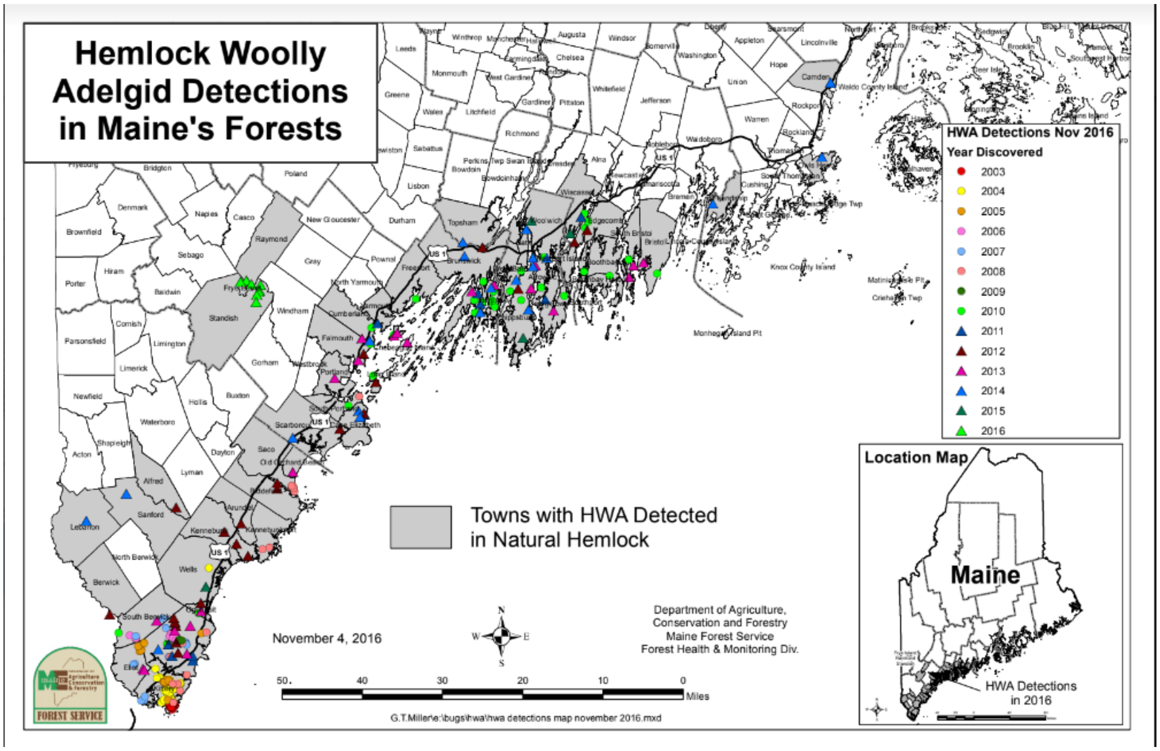

Figure 2: Range map of HWA in Maine as of 2016. Note the similarities between this map and the figure 1. The HWA is most numerous along the coast, much like Lyme disease incidence

METHODS

Tick Collecting

To test the hypothesis that tick abundance is greater in hardwood stands than in hemlock stands, HWA infestation was simulated by dragging for ticks in stands of two treatment types, hemlock stands and deciduous stands. Infestation had to be simulated due to the currently limited range of HWA in Maine. “Hemlock stands” simulate the forest before HWA infestation and the “deciduous stands” simulate the forest after HWA infestation. As such, the deciduous stands were chosen based on species composition of the stand. Specifically, stands containing maple and birch were selected as they are genera likely to succeed hemlock upon its death (Orwig et al., 2002; Spaulding & Rieske, 2012). Ticks were collected once a month from May through August and across a spatial gradient in Maine with sites along the Maine coastlines (Figure 3). A total of six sites (i.e., general areas containing both hemlock and hardwood stands) and 16 stands (i.e., areas within sites that contain the hemlock and hardwood within which dragging took place) were used in this study. The six sites included the DeMeritt Forest Trail System in Orono and Old Town, the Helen Gates lot in Gray, the Gillet family trust in Phippsburg, Indian Rest forest in Harpswell, Sewall Woods in Bath, and Well National Estuary Reserve in Wells (Figure 3). Only one site (Gillet Family trust in Gray, ME) was in an area currently infested with HWA.

Dragging lasted for 60 minutes at each stand which negated the need to have a standardized area for dragging; tick density was calculated per hour instead of per unit area. Abundance of both nymphs and adults were totaled for each stand in each site and this was used in statistical analysis. Ticks were collected by drag cloth sampling with several people dragging in each stand to divide the 60 minutes of dragging between them. Drag cloths are $1m^2$ corduroy cloths pulled over a pole, like a flag (Falco and Fish, 1992). The cloth is then dragged along the ground and questing ticks attach to it. Captured ticks were picked off the cloth with tweezers and put into vials of alcohol labeled with the site name, treatment type, and date. The ticks were later separated by life stage (larvae, nymph, adult), species (*Ixodes scapularis* or *Dermacentor variabilis*), and treatment, then counted. These counts were recorded on separate spread sheets for each site with columns to record collector name, time spent dragging, treatment type the tick(s) was acquired from, life stage, the number of specimens captured, and the number of the vial in which they were placed.

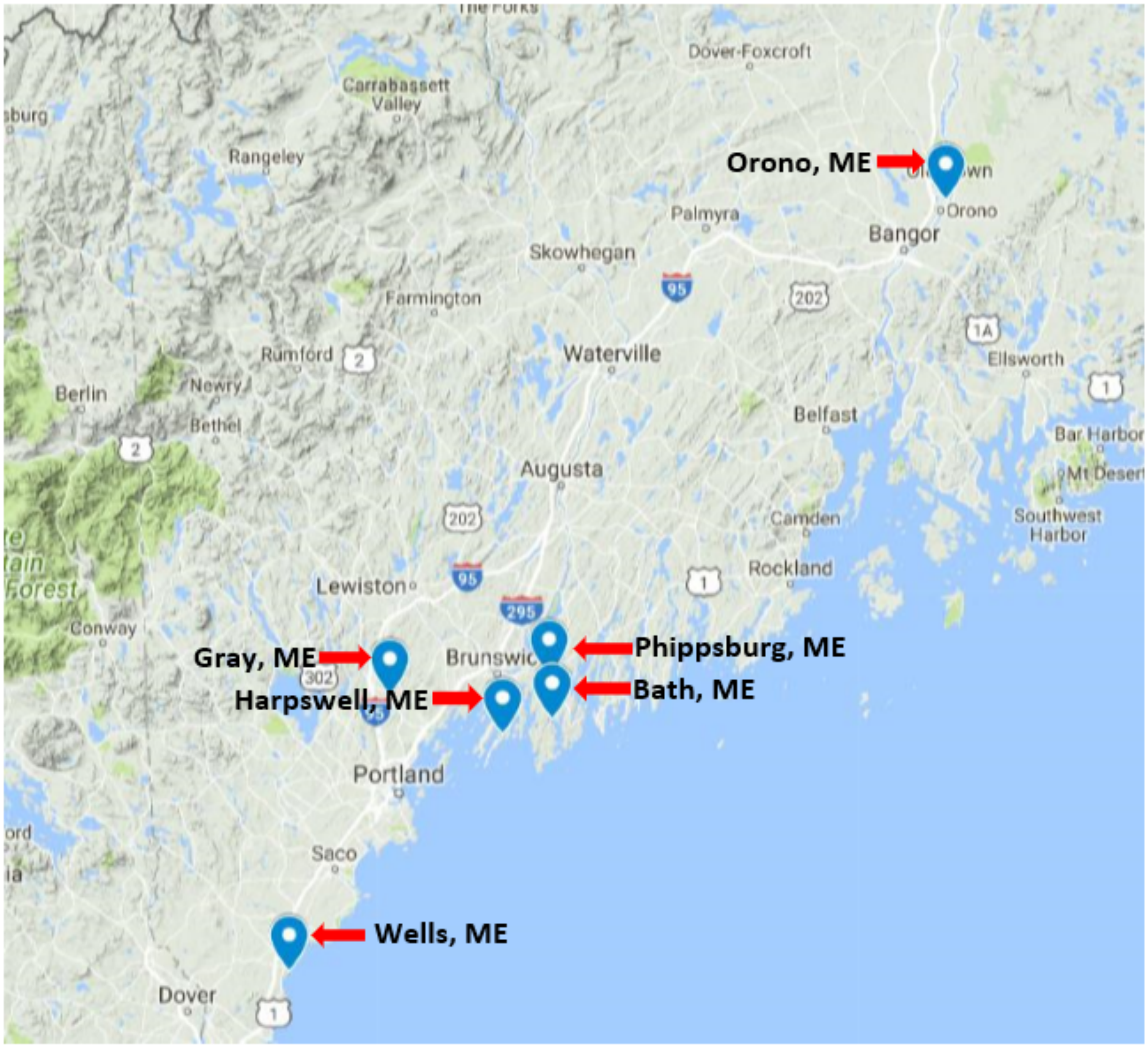

Figure 3: Map of the six sites used in my study. There were two stands within each of the four sites around Brunswick, ME however at DeMeritt Forest Trail System in Orono there were six stands to increase power. There were four stands in Wells National Estuary Reserve, however by dragging for 30 minutes in each stand I was able to combine the two hemlock stands and two hardwood stands thereby producing two stands (one of each treatment) in which I dragged a total of 60 minutes in each. These were utilized throughout the study.

Pathogen Testing

To test the hypothesis that there would be greater infection prevalence in hardwood stands than in hemlock stands, DNA was extracted from nymphal ticks using a Qiagen DNeasy Blood and Tissue Test Kit. A total of 50 ticks, all from Wells National Estuary Reserve, were tested, 26 from hemlock stands and 24 from deciduous stands.

Further, ticks from each month were tested, 18 from June split evenly between the two treatments, 16 from July, and 16 from August. Only nymphal ticks were tested due to the greater numbers they were found in and their epidemiological significance. Ticks from my site in Wells, ME were used due to the greater risk of Lyme disease in areas along the southern coast (CDC, 2016). To extract the DNA, the ticks were subjected to a protease K digestion overnight. DNA was extracted using the directions from the Qiagen kit and using several buffers to elute the DNA. Amplification of DNA was done using a nested Polymerase Chain Reaction (PCR) protocol targeting the 16s ribosomal species-specific gene (Ann Bryant and Griffin Dill, personal communication). Nested PCR was necessary to provide enough DNA to be visible in gel electrophoresis. Primers for this procedure targeted the *fla* gene (Brown et al. 2015) of *B. burgdorferi* and include, LD1 (5'-ATGCACACTTGGTGTTAACTA-3') and LD2 (5'-GACTTATCACCGGCAGTCTTA-3'). The nested PCR primers were TEC1 (5'-CTGGGGAGTATGCTCGCAAGA-3') and LD2. These were combined with PCR SuperMix 1x (Invitrogen 10572-014). PCR was performed accordingly: 95C for two min and then 35 cycles of 94C for 30 sec, 55C for 30 sec, and 72C for one min, concluding with a final 10 min extension at 72C. The products were run on a 2% agarose gel and compared to a positive control from a previous project, *Borrelia burgdorferi* DNA (ATCC 35210D-5 Strain 31, genomic DNA) diluted 1:10,000 in nuclease free water, and a small molecular weight ladder. The proportion of positive ticks for each treatment was recorded.

Deer Scat Surveys

In order to provide ecological context and a potential causal mechanism to explain the other data I collected, deer scat surveys were performed in each stand. Throughout

August, upon the last visit to each stand, I conducted deer scat surveys using a simple, standardized method. Using a 100m measuring tape, I used three belt transects all emanating from a central point so as to make a ray formation (adapted from Mayle et al, 2000). All deer scat, both on the transect line and within one meter on either side of it, were counted. A scat pile was defined by six or more scat pellets in one pile (Mayle et al, 2000). I recorded scat counts from each of the three transects created in each stand and then totaled the scat counts for each stand.

Statistical Analysis

A two-way ANOVA performed in R was used to analyze linear models generated based on several parameters, for nymphal and adult abundance separately. The fixed effects in the model were treatment (i.e., hemlock vs. deciduous stand) and month (i.e., June, July, or August), and site was included as a random effect. A normality test was also run to ensure the data were normally distributed. Initially, the data were not normally distributed, but this criterion was satisfied when the data were log transformed. To analyze the deer scat results, a paired T test was performed using Microsoft Excel. Data from two hemlock stands, both from Wells National Estuary Reserve, had to be dropped from analysis since there was no deciduous data from this site to pair it with in the T- test due to field site constraints. While there was a path in these deciduous stands and pockets of open area among the brush within which to drag for ticks, the brush itself was too dense and thorny to see deer scat on the ground or even move through. Further, there were no breaks in the brush large enough to accommodate three, 100m transects. Additionally, two stands in DeMeritt woods were not utilized because they were too geographically distant to be consider “paired” in this analysis. A chi squared test was run in both excel and R to evaluate the pathogen testing results and compare NIP between the treatments.

RESULTS

Adult abundance between the treatments types was marginally significant (p=.0987), while no significant difference in nymph abundance could be found between the hemlock and hardwood stands (p= .3461) (Figure 4). Fifty nymphal ticks, 26 from hemlock stands and 24 from deciduous stands, were tested for *B. burgdorferi* DNA. Of these ticks, nine ticks from the hemlock stands and eight from the hardwood stands, were pathogen positive. A chi squared test showed the difference in infection prevalence between treatments to be non- significant (p=1). In fact, the proportion of positive ticks was 33.3% in deciduous and 34.6% in hemlock stands, practically equal had the same number of ticks been used for each treatment during pathogen testing (Figure 5). Deer scat surveys were conducted using a standardized belt transect method. Between all deciduous stands together, 17 scat piles were counted, and between all hemlock stands 22 scat piles were counted (Figure 6). The difference in scat counts between the two treatments was found to be non- significant (p=.391) following a paired T- test.

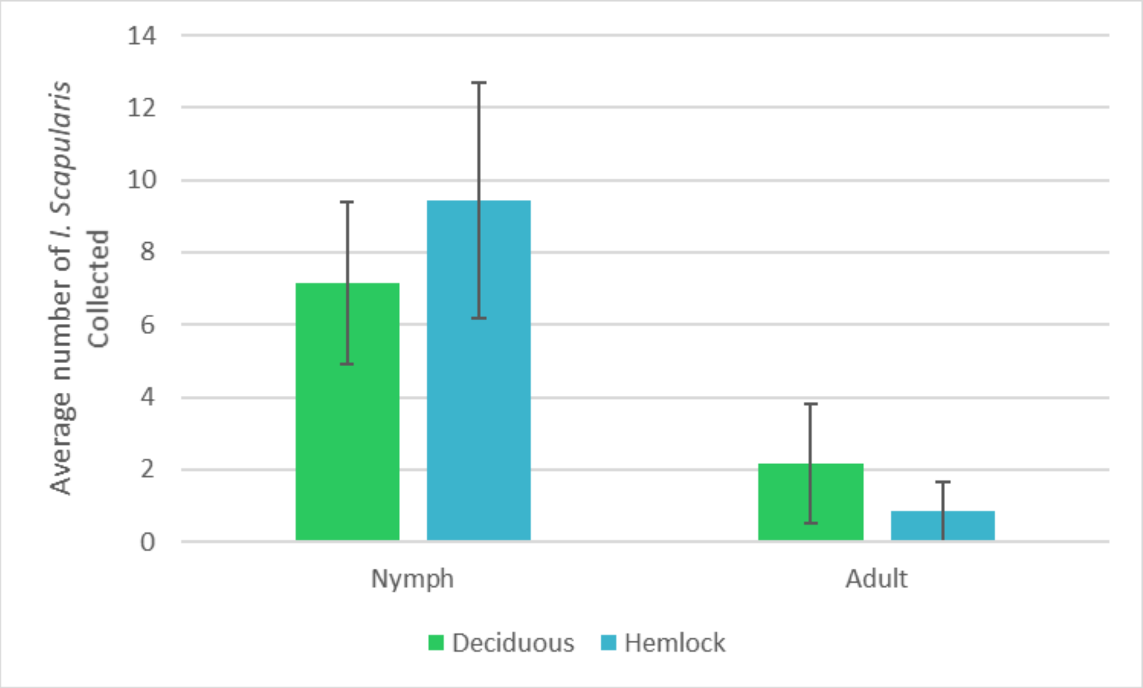

Figure 4: Average number of ticks of each epidemiologically significant life stage, nymph and adult, collected in each treatment type, hemlock and hardwood. Averages were calculated using 3 months of data, June – August, from each stand. The difference in adult abundance between the treatments was marginally significant while the difference in nymph abundance was non- significant.

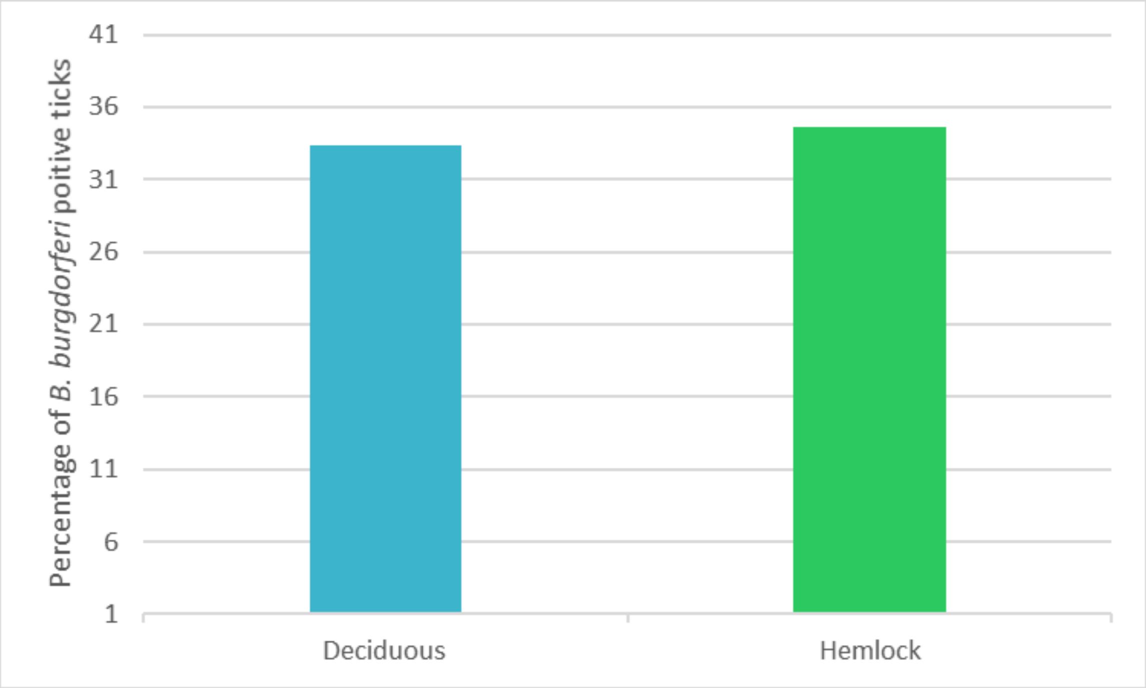

Figure 5: Proportion of ticks positive for B. burgdorferi DNA in each treatment type according to pathogen testing results. Note the percentages are equal and only skewed because there was one more tick tested from hemlock stands than from deciduous stands. There was no significant difference in infection prevalence between the two treatments.

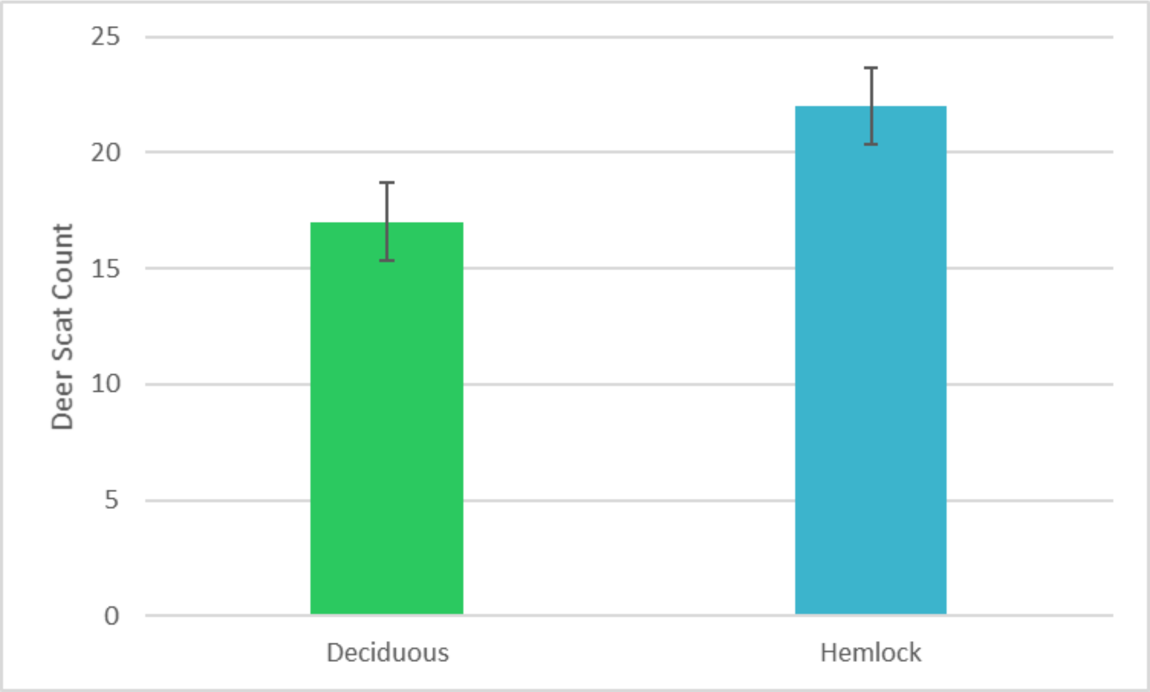

Figure 6: Total count of deer scat piles in each treatment. Deer scat results were non- significant between the two stand types.

DISCUSSION

Currently, there is much debate over what drives tick ecology and ecologists cannot agree on the primary influence on tick survival: host community composition, abiotic factors, or both. My research is a novel study of the relationship between the hemlock loss due to the HWA and black-legged tick abundance and NIP. This relationship has not been investigated before and was performed to ascertain if an invasive species was hindering or possibly even aiding another invasive species. Because several prior studies show that hardwood trees will take over forest stands following hemlock death, and that black-legged ticks primarily occur in deciduous forest habitats in New England (Carey et al., 1980; Ellison et al., 2005; Orwig et al., 2002), I hypothesized that tick abundance and NIP would be greater in hardwood stands. However, to the contrary, I found there was no significant difference in nymphal tick abundance between the hemlock and deciduous stands while there was a marginally significant difference in adult abundance between the treatment types. Furthermore, the treatments had no noticeable difference in NIP between them. These results go against not only common doctrine that ticks are more numerous in deciduous forests and leaf litter, but also against what most literature states. For example, in a study conducted in Maine by the vector biology lab at the Maine Medical Center, tick abundance was found to be negatively associated with the presence of eastern hemlock saplings compared to other shrub- layer species (Lubelczyk et al., 2004) Further, less ticks were found in forests with a mix of coniferous and deciduous litter than in forests composed entirely of hardwoods. My results are corroborated by another study that found adult abundance to be greater in deciduous forests (maple forests) than in coniferous stands (white pine habitat) (Lindsay et al., 1999).

The loss of a foundation species can have numerous consequences on an ecosystem and typically, we think of these consequences as negative, but they can have positive effects as well. For instance, presence of hemlock appears to result in low levels of arthropod species richness studies show hemlock removal by HWA alters composition and diversity of beetles and spiders and increases species richness of ants and other arthropods (Sackett et al., 2011, Rohr et al., 2009). When it comes to the black-legged tick, I propose there are two mechanisms by which loss of hemlock could positively affect tick abundance and pathogen prevalence, 1) by influencing abiotic factors creating more suitable conditions for tick survival and 2) by impacting host communities and their habitat. In the first mechanism, loss of hemlock has the potential to create more appropriate habitat and microclimate conditions for ticks due to successional tree species (Spaulding and Rieske, 2012). To explain the second mechanism requires an explanation of the ecology and preferred habitat of the tick's primary hosts. The following paragraphs elaborate on these two mechanisms.

Throughout my study, tick numbers were low, especially in August, however this has to do with the seasonality of the tick and nothing I could control for. Immature ticks are highly susceptible to desiccation and are at an increased risk of this fate while questing out of the humid, sheltering leaf litter (Needham and Teel 1986; Yoder and Spielman 1992); summer months only exacerbates this risk. Sites with increased light penetration, such as hardwood stands, are correlated with a decreased probability of tick-host interactions (Fino, 2017). This decreased interaction is likely due to the increased temperature from the sunlight and a higher risk of desiccation, therefore ticks would exhibit less questing behavior. Additionally, studies have found that increased temperatures and decreased relative humidity negatively affects development, oviposition, and hatching success, as well as overall survival of ticks (Bertrand and Wilson, 1996). Such a significant threat from the animal's microclimate would lead to fewer ticks questing on hot summer days, such as those days I was dragging, since they spend more time in the leaf litter to stay cool and in appropriate humidity. Furthermore, there is a significant amount of evidence that soil characteristics can impact ticks. From knowing that ticks are sensitive to temperature and relative humidity, one can already get a good idea of how soil can be detrimental or beneficial to ticks. Soils do not just effect ticks directly however, since soils with increased acidity and a high proportion of clay retain a greater amount of moisture. These conditions can enhance the growth of fungi and entomophagous nematodes that negatively affect tick populations (Zhioua et al., 1999, Guerra et al., 2002). Soil also influence plant community composition in forest stands which can indirectly affect ticks by providing shade, high quality questing position, and increasing the chance of encountering a host by attracting small mammals (Curtis 1959, Guerra et al., 2002). It has also been shown that oak species prefer sandier soils (Zhioua et al., 1999), which is important because oak species typically replace hemlock in early successional forests (Orwig et al., 2002), and how sandier soils affect the black-legged tick is unknown.

Although environmental conditions may significantly influence the maintenance of reproducing populations, factors such as host density and host species composition might have a greater influence on tick populations (Brownstein et al. 2003). In the North and East, the primary hosts for *I. scapularis* larvae and nymphs are white-footed mice eastern chipmunks (Anderson 1988), and gray squirrels (*Sciurus carolinensis*) (Lane et al. 1991). Adults prefer larger hosts such as white- tailed deer (Anderson, 1988). To provide ecological context to the tick abundance and NIP data, deer scat surveys were conducted which allowed me to assess deer activity in the stands in which I dragged. I had hypothesized that more deer scat would be found in deciduous stands however the data contradict this prediction and there was no significant difference in deer scat between hardwood and hemlock stands. The limitations of my study, discussed later, could be a reason for this nonsignificant result, for example the size of my hemlock stands could have influenced these results

The white-footed mouse has been found to reach higher densities in old growth deciduous forests where habitats contain structurally complex understory, dense undergrowth, and mast, such as acorns or nuts, produced by hardwood trees (Drickamer, 1990; Anderson et al., 2003). If more mice are found these deciduous stands more ticks should be as well since organisms should follow their food source. However, the idea that mice are more often found in hardwood stands is refuted in a study that showed significantly more mice captured and significantly more *B. burgdorferi* isolations collected in hemlock habitat than from deciduous habitats (Lord et al. 1992). Hemlock habitat is sparse but increases winter survival of mice, according to the authors, and thus possibly results in increased infection rates in mice; if more mice are infected more ticks in these hemlock stands will be too. Ungulate browsing and grazing can also have an adverse effect on small mammal populations. By decreasing plant species diversity, removing ground cover, and simplifying vegetative structure, ungulate browsing results in reduced protection and food supplies while increasing intraspecific competition in mice (Flowerdew and Ellwood, 2001; Côté et al., 2004). Taken altogether these effects of ungulate browsing on small mammals may lead to reduced tick- host interactions. The effects of HWA infestation on small mammal communities has indeed been studied. The study, conducted in 2018, had 4 treatment types, including hemlock and hardwood stands, and populations of common species were estimated with mark-recapture analysis (Degrassi, 2018). *Peromyscus spp*. were not affected by treatment in either year of the study and overall the author concluded that there is little evidence of a major shift in small mammal community structure in response to HWA invasion (Degrassi, 2018). Small mammals are major hosts of nymphal and larval ticks and there was no difference in relative small mammal abundance between sites in the aforementioned study thus proposing one explanation for why tick abundance did not differ between the two treatments in my study.

Similar mechanisms could have influenced infection prevalence in the two types of stands in my study. Insight from current literature shows that small mammal community structure did not change during HWA infestation of forests (Degrassi, 2018). If relative abundance of mice or other hosts are not changing as a result of hemlock death, infection prevalence should remain the same between the two stands since host movement is the same between stands. Another explanation could have to do with energy allocation of white footed mice or other host species. White footed mice prefer deciduous habitat for the food and shelter offered by these stands (Anderson et al., 2003). In these stands with greater food availability relative to coniferous stands, mice may be able to allocate more energy to grooming (parasite resistance) because food is relatively easy to come by (Wilder & Meikle, 2004). Deer scat results showed that there was no difference in deer activity between the two treatments, yet another possible explanation for the non-significant NIP result. The environmental factors discussed earlier that influence tick abundance and survival could have effects on host communities and therefore indirect effects on NIP. For example, hardwood stands allow increased light penetration which increases the temperature in the stand (Ellison et al., 2005) and so the ticks may quest less, negatively influencing NIP. However increased light also creates more understory growth (Ellison et al., 2005; Orwig et al., 2002) and suitable questing habitat. It could also create ideal habitat for important hosts of the tick (Anderson et al., 2003, 2008) thereby theoretically increasing tick- host interactions and influencing NIP. Conversely, hemlock stands allow less light through the canopy resulting in cooler temperatures (Orwig et al., 2002; Spaulding and Rieske, 2012) and possibly increased questing behavior. The shade created by hemlock trees, however, may also result in less understory growth (Ellison et al., 2005) and ideal habitat for small mammals leading to less tick- host interactions, negatively influencing NIP.

A major limitation to my study was low replication since I only had 8 hemlock and 8 hardwood stands. The marginally significant result suggests that my data may not have had enough power to yield a significant result. Power could have come from having many more replicates of each treatment (more field sites) or dragging for longer to collect more ticks. Further, this study was limited to one field season, and multiple field seasons would increase the power of statistical tests. This limitation is true of my deer scat survey data as well since I only collected data on deer scat in one month and so only had data from one visit for each of my sites. Finally, the study would have benefited from analysis of more ticks for pathogen prevalence since most NIP assays consist of more than 100 ticks (Brown et al, 2015; Aliota et al, 2014).

Another limitation of this study was tree species composition of available patches and patch isolation which was something I could not control. Most stands used in this study, especially the "hemlock" stands were mixed forest stands containing both hardwoods and confers. Further, several of my hemlock stands were very small in comparison to my deciduous stands. Although I dragged for ticks over a set period of time, there are only so many ticks that can be found in a stand. In small stands, ticks will not be as numerous as large stands, since there is less occupiable space. Furthermore, both small and large hemlock stands in my study were surrounded by deciduous forest. Therefore, ticks were probably in equal numbers in hemlock stands due to proximity to the deciduous stands. Movement between deciduous patches in my study would necessitate movement through hemlock patches and so tick moving between patches may be captured in hemlock stands by coincidence. Additionally, movement of deer and white-footed mice between deciduous patches further minimizes the difference in abundance between the treatment types since it would result in mice moving through the hemlock patches. If mice are moving between stands so are ticks, and so the prevalence of infected nymphs would be similar between the hemlock and hardwood stands as well. My deer scat results could be explained by this mechanism as mentioned earlier. It could be that deer were simply passing through the hemlock patches when they defecated. The size of the deciduous stands, and the possibility that deer are browsing on hardwood trees, would mean deer are naturally spending more time in these stands. If deer are spending a significant amount of time in these stands, they will also defecate in these stands. Perhaps if the stands were more isolated from each other or of equal size, deer scat, and activity, would significantly differ between the two treatments.

It should also be noted that drag cloth sampling has inherent flaws and could be a possible reason for low numbers of captured tick. Flaws of the drag cloth method include immature *I. scapularis* being unable to attach or remain on the drag cloths; they may attach and get knocked off during a single drag (Goltz, 2012). Vegetation may also block or prevent contact with the cloth, and immature *I. scapularis* may not quest high enough in vegetation to make contact with the cloth as they may be under the leaf litter or not actively questing in the first place (Goltz, 2012).

Future research may look into soil analysis since this is one aspect of my project that I did not complete. Upon my last trip to every stand I collected a soil sample in a small urine cup to later assess the type of soil, water concentration, heavy metal composition, etc. Therefore, one direction for future research is understanding the effects of hemlock loss on soil characteristics, and in turn how any soil alterations may affect tick abundance. Additional future directions could also investigate small mammal movements to confirm the effects of the HWA on small mammal communities seen in Degrasse (2018). This would include trapping in the two treatment types for small mammals, particularly preferred hosts of the black-legged tick such as white footed mice, eastern chipmunks, and gray squirrels (Anderson 1988; Lane et al. 1991). These small mammals could be ear tagged and removed of ticks. Such data could inform this study of host abundance and movement between stands. The number of ticks could be sorted into separate life stages and counted and the nymphs subject to pathogen testing. Future research could include a retrospective study investigating whether or not the phenomenon of hemlock loss is correlated with increased Lyme incidence, or the number of new cases per year, in northeastern states by utilizing public data from the CDC. Additionally, it would be beneficial in future work to not simulate this relationship but instead directly measure the effects of HWA by dragging in multiple afflicted sites and multiple non-afflicted sites.

Interestingly, a new problem is arising. Over the last 20 years, populations of another invasive insect from Japan, the elongate hemlock scale (*Fiorinia externa*), have increased dramatically, are distributed across more than 14 states, and often cooccur with HWA (Orwig, 2011). It is uncertain what impact two interacting invasive pests will have on hemlock ecosystems, however it is thought that the *F. externa* only has a minor effect on hemlock forests (Orwig, 2011; Preisser et al, 2008). With both of these pests killing hemlock trees, reversal of this ecosystem disaster will be difficult. Further, evidence shows the elongate hemlock scale is able to persist in cold environments (-15°C) meaning its spread north is almost inevitable (Preisser et al, 2008). Perhaps this will hold future consequences for the black-legged tick.

Overall, my findings imply that park managers should be aware that the presence of this invasive scale might be creating more suitable tick habitat and they should act appropriately. People utilizing infested parks or forests recreationally should be aware a possible increased risk of tick exposure. These findings could help determine local risk of exposure to this vector and to the disease. They also contest previous findings that black-legged ticks prefer deciduous brush over other habitat types. Finally, my study underscores the intricacies of ecological phenomena and how these complicate our understanding of disease ecology as well as control and prevention of vector- borne diseases.

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AUTHOR'S BIOGRAPHY

Spencer DeBrock was born in Kalamazoo, Michigan but spent most of his life in Naperville, Illinois. For that time, it seemed certain he would go to University of Illinois like so many other in his family. However, when his family moved to Newtown, Connecticut, he fell in love with the University of Maine. Throughout his time here Spencer involved himself in numerous ways: he is a member of Alpha Lambda Delta freshman honors society, president of the biology club, and a member of the blade society. He has been a tutor for the tutor program, a Maine Learning Assistant (MLA) for intro chem for seven semesters and intro bio for two semesters. Additionally, he has worked in several labs around campus, held an executive position in his fraternity Alpha Tau Omega, and even finds time to play intramural floor hockey. He has been able to do all this while also maintaining the high standard of academic excellence that he sets for himself, something he is especially proud of. After graduation, Spencer will be pursuing his Ph.D in biomedical sciences (epidemiology) at Texas A&M University.