Assessing Surveillance, Density, and Pathogens in the Ticks of Shelter Island, New York

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By
Kate Elizabeth Thornburg
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ABSTRACT

Shelter Island, New York is a 32 km^2 island off Long Island that has many opportunities for outdoor recreation, and its large lot sizes preserve green spaces among housing. Located in Suffolk County, it has a high rate of tick-borne diseases, and habitat for several tick species that can transmit pathogens to humans. The island has a history of tick surveillance, a now defunct four-poster program, and performs regular white-tailed deer culling. Despite this, tick densities and pathogen prevalence are not well understood. A surveillance program was undertaken in the summer of 2020 to sample seven sites, 4 on Mashomack Preserve, and 3 in other areas of Shelter Island. Surveillance with drags cloths was performed once weekly for a period of eight weeks. Four medically important tick species were collected. *Ixodes scapularis* and *Amblyomma americanum* were the most abundant species. *Dermacentor variabilis* was also present, but rare. Additionally, a single specimen of *Haemaphysalis longicornis* was collected, marking the first confirmed record of this invasive species on Shelter Island. Density of nymphs was determined for the two most abundant species, and pathogen testing was conducted on a subset (10%) of tick samples.

A small number (7.41%) of tested *I. scapularis* were positive for *Anaplasma phagocytophilum* and *Babesia microti*, and 3.41% were positive for *Borrelia burgdorferi* and *Babesia. miyamotoi*. This result was lower than reported *B. burgdorferi* infection rates for *I. scapularis* collected from other locations in Suffolk County. No pathogens were detected in *A. americanum*, but over half (55.81%) were positive for a rickettsiae endosymbiont (*Rickettsia* spp.) that is not known to be pathogenic to humans and animals.

Study results and recommendations for future surveillance and public education on risk and tick bite prevention were developed and submitted to Shelter Island and Mashomack Preserve officials. The presence of pathogens and a new invasive tick species on the island indicate the importance of continued surveillance and testing.

Key words: Blacklegged tick, *Ixodes scapularis*, Lone star tick, *Amblyomma americanum*, drag sampling, Shelter Island, Borrelia burgdorferi, Babesia microti, Anaplasma phagocytophilum, Borrelia miyamotoi

BIOGRAPHICAL SKETCH

Kate Thornburg attended Mount Holyoke College, a small liberal arts college in Western Massachusetts where she majored in Biology. One of the seven sisters, Mount Holyoke is known for its classic architecture and traditions dating back hundreds of years. There her work in the Talcott greenhouse led her to develop an interest in ants. She traveled to Portal, Arizona, during an internship with the American Museum of Natural History, where she conducted research on the dynamic interactions of ant species. She wrote her thesis on supercoloniality and behavior in *Brachymyrmex depilis*, the same species that initially piqued her interest in the greenhouse. After graduating, she worked for two years rearing mosquitoes in a lab that focused on malaria and mosquito mating behavior. This allowed her to combine her interest in insects as well as human disease. It was during this time that she learned about the Vector Borne Disease Biology program with Cornell. She joined the program in 2019, and worked with ticks during her time there to expand her knowledge of disease vectors.

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Literature Review

The Growing Importance of Tick-Borne Disease

Tick-borne disease has a large and growing impact in the northeast region of the United States. Current trends show that ticks have expanded their range in the north, and in New York (Sonenshine, 2018). Tick bites can lead to serious disease in humans and animals, and perception of tick presence may even impact willingness to utilize outdoor spaces. This review examines tick-borne disease in Shelter Island, NY and the surrounding areas, their pathogens and tick sampling methods.

Shelter Island

Shelter Island is a small island nestled between the eastern north and south forks of Long Island in Suffolk County, New York. The town of Shelter Island encompasses the entirety of the 32.37 km^2 island, which is then divided up into smaller villages and neighborhoods. Shelter Island's population is around 2,000 people in the winter season but increases in the summer months up to 10,000 with vacation home residents on the island. A large portion of the island, about 8.25 km^2, makes up The Nature Conservancy's Mashomack Preserve, which remains undeveloped (Soren, 1978).

There is little change in elevation on Shelter Island, with the highest point being around 30m and the lowest at sea level (Soren, 1978). Shelter Island has a moderate temperate climate with highs of 29.5 C and lows of 12.5 C in the summer months (CLIMOD 2, 2020). It experiences frequent rainfall except in the summer months, with an average of 84.3 mm (CLIMOD 2, 2020). Relative humidity from the nearby town of Southold averaged 79.4% over the summer months during 2020 (Network for Environmental and Weather Applications 2021). The southern part of the island is home to a combination of freshwater and brackish wetlands, and tidal wetlands circle the island. The average precipitation per year is 1077 mm (Nelson, Pope, and Voorhis, 2014). A large portion of the island is covered in deciduous forest, at 47.2%. The next largest category includes roads and open areas such as lawns, consisting of 17.3% of the island. Two smaller but important categories of cover are pasture/hay and emergent herbaceous wetlands at 5.2% and 5.6% respectively. The category of pasture/hay includes large areas of mowed or long grass (Nelson, Pope, and Voorhis, 2014). The two largest groups of land use categories are low density residential and parks & open space, at 34.6 and 32.3 respectively. The next largest category is vacant, at 14.5%. All other categories make us less than 7% of land usage (Nelson, Pope, and Voorhis, 2014).

Mashomack Preserve

Acquired by The Nature Conservancy in 1979, Mashomack Preserve is a nature preserve with publicly accessible hiking and kayak trails (The Nature Conservancy, 2020). While it has multiple trails that are open for public hiking, beach access is not permitted, and a portion of the preserve known as the Katherine Ordway Wildlife Refuge is not accessible to the public (The Nature Conservancy, 2020). There are many service roads that transect the property that provide vehicle access to all sections, including the wildlife refuge. Mashomack is maintained, with frequent mowing on the trails, a rotational mowing regime in the meadows, and occasional removal of invasive and nuisance plant species (Novarro pers comm, 2020). Most parts of the preserve are accessed regularly by staff, either for maintenance or surveying (Novarro pers comm, 2020). The preserve is known for its habitat and species diversity, with secondary oak-hickory forests, freshwater and brackish wetlands and open meadows (The Nature Conservancy 2020). It has over 200 species of birds and is a frequent destination for visitors (The Nature Conservancy 2020). During the COVID-19 outbreak in 2020, the preserve experienced a 2-3x increase in visitors, and extended its hours of operation to allow continued usage by visitors (Novarro pers comm, 2020).

Many of the trails on the preserve double as service roads, and as a result most are wider than a normal walking trail. In addition, the substrate is usually a mixture of sand, gravel and rock, and dirt. Therefore, it is possible to avoid grass and other foliage when hiking on the trails, which can lower questing tick exposure. Mashomack warns visitors about tick risk in its online trail map and via trail signage (https://www.nature.org/content/dam/tnc/nature/en/documents/MashomackTrailMap_page11.pdf). Due to the ongoing COVID-19 outbreak, the visitor center was closed, so visitors to the preserve in Summer 2020 were not able to access any paper educational materials onsite.

Ticks on Shelter Island

Data from the Suffolk County Department of Health indicates that public health tick vectors including the blacklegged tick (*Ixodes scapularis*), American dog tick (*Dermacentor variabilis*), and lone star tick (*Amblyomma americanum*) are present in the county (Suffolk County Government, 2019). Only data for *I. scapularis* and *A. americanum* on Shelter Island are included in the report, and a comprehensive report that includes Shelter Island also listed only these two tick species (Suffolk County, 2019, Curtis et al., 2011). The American dog tick, *Dermacentor variabilis* has been recorded on the island during routine tick drags to monitor the efficacy of tick control methods (Payne, pers. comm).

Ixodes scapularis, known as the black legged tick, the primary vector for the Lyme disease pathogen, *Borrelia burgdorferi,* in the United States. It has spread throughout the Midwest and Northeast, and has continued to expand (Hamer, 2010). *Ixodes scapularis* feeds on a wide variety of hosts including humans, but larvae and nymphs in the northeast preferentially bite the white footed mouse, *Peromyscus leucopus*. Adults are commonly associated with a northeastern form of the white-tailed deer, *Odocoileus virginianus borealis*. (Hamer, 2010, Spielman et al., 1985). In the Southeastern United States, larvae and nymphs have been found to parasitize several species of lizards (Durden et al., 2002). It is possible that this has an impact on Lyme disease infection in the South, as lizards are not competent for *B. burgdorferi*. It is important to note that *I. scapularis* often fail to find alternate hosts when these lizards are removed from the environment, suggesting that host associations vary strongly between the Southeast and Northeast (Swei et al., 2011). Nymphs are active in early summer, from May to July, and the larvae from July to September (Spielman et al., 1985). Adults may feed during late summer until spring, remaining active throughout the winter when it is above freezing. *I. scapularis* is commonly found at forest edges, and quests in a passive manner compared to other tick species (Spielman et al., 1985).

A. americanum is a multi-host tick that occurs from Texas into the eastern United States and ranging as far north as Maine (Childs and Paddock, 2003). They are aggressive and non-specific biters, although it is indicated that this tick prefers larger mammals to smaller ones. *A. americanum* is commonly found in woodland environments, part-sun and shaded grassy areas and bushes (Childs and Paddock, 2003, Jackson et al., 1996). Adults and nymphs are active in early summer, from April to June, whereas larvae are active afterwards. All life stages are capable of overwintering. *Amblyomma americanum* is a competent vector for multiple human diseases, including Rocky Mountain spotted fever, (*Rickettsia rickettsii*) Human monocytic ehrlichiosis (*Ehrlichia chaffeensis* and *Ehrlichia ewingii*) and tularemia (*Francisella tularensis*) (Childs and Paddock, 2003).

D. variabilis is common throughout most of the United States east of the Rocky Mountains, and in parts of California and Oregon (Burgdorfer, 1969). The adults are common from May through July, while nymphs peak in July (Kollers et al., 2000). The larval peak is bimodal, rising in early spring and late summer (Burgdorfer, 1969, Kollers et al., 2000). *D. variabilis* can transmit the pathogens that cause Rocky Mountain spotted fever, and tularemia (Burgdorfer, 1969, Kollers et al., 2000). These ticks are commonly found on mice and other dominant small mammal species when immature, but they will readily feed on dogs, deer, and humans as adults (Kollers et al., 2000, Cohen et al., 2010, Burgdorfer, 1969). Where present, raccoons (*Procyon lotor*) are parasitized at a high rate (Cohen et al., 2010). *D. variabilis* is known to actively move towards human and animal-made trails (Burg, 2001).

It is likely that other tick species may be found on the island that are either unknown or not significant to human health. The Asian long horned tick (*Haemaphysalis longicornis*), may be present on the island. This is an invasive tick that originated in Asia and was discovered in the United States in New Jersey in 2017 (Beard, 2018). It has been collected from locations in 12 states including New York (Division of Vector-Borne Diseases, CDC, 2020, Beard 2018). *H. longicornis* is an important vector of both human and animal disease in China and Japan (Beard 2018). In addition, can reproduce by parthenogenesis, meaning that single ticks are able to establish a population (Beard, 2018). To date, male ticks have not been found in the United States (Division of Vector-Borne Diseases, CDC, 2020).

While it is not currently known to transmit human disease in the United States, it has been proven competent to transmit R. rickettsii under laboratory conditions (Beard, 2018, Stanley et al., 2020). In early 2020 a cattle pathogen associated with *H. longicornis*, *Theileria orientalis*, was detected in ticks in *H. longicornus* in Virginia (Thompson, 2020).). This marked the first time that a pathogen has been detected in this invasive tick in the United States (Thompson, 2020). *T. orientalis* causes the parasitic infection bovine theileriosis (Thompson, 2020). While primarily a veterinary concern, the detection of *Theileria* parasites in *H. longicornis* highlights the importance of continued surveillance of this non-native species (Thompson, 2020).

Tick Management on Shelter Island

Four posters are feeding stations that provide food bait to apply acaricides on treated rollers to white tailed deer, *O. v. borealis* (Pound et al., 2000). Deer are attracted to the feeding stations and use them frequently (Pound et al., 2000). Investigators in Rhode Island placed between 20-25 four posters over the course of the study and evaluated the reduction of *I. scapularis* (Miller et al., 2009). These authors noted a 43% decline in nymphs after 4 years and a 2 year lag in population increases after the removal of the four posters (Miller et al., 2009).There was no reported change in larval or adult *I. scapularis* (Miller et al., 2009). A similar study was conducted in Maryland where 25 amitraz treated four poster devices were placed in each of three sites (Carroll et al., 2002) Both *I. scapularis* and *A. americanum* populations were significantly reduced (Carroll et al., 2002). On Shelter Island, corn bait was used to attract deer and apply permethrin (Curtis et al., 2011). Sixty four-poster devices were deployed on Shelter Island at different sites (including Mashomack Preserve) starting in 2008 (Curtis et al., 2011). As of 2019, these four-poster devices were no longer due to NYS DEC regulations requiring all property owners within 228.6 m (750 feet) of the device give their written consent (New York State Department of Environmental Conservation, 2019). From 2008-2010 Curtis and others studied the impacts of these four-poster devices on deer and tick populations on Shelter Island (2011). Significant reductions in tick numbers were observed, notably numbers of *A.*

americanum nymphs which decreased by 84-85% at both the north and south sample sites. *I. scapularis* nymphs decreased by 41% at the north site, which was not significant, but 81% at the south site (Curtis et al., 2011).

The four-poster bait attracted other animals. Photos from camera traps deployed around the four posters showed deer and raccoons frequently coming into contact with the treated rollers (Curtis et al., 2011). Gray squirrels (*Sciurus carolinensis*) and birds also visited the four posters but rarely came into contact with the rollers (Curtis et al., 2011). Deer harvests were recorded during 2008 and 2009 at 245 and 423 animals, respectively, in the treatment area (Curtis et al., 2011). During the study four posters needed to be serviced weekly due to permethrin rollers drying out (Curtis et al., 2011). The authors cited concerns about the relatively high cost of the four posters as well as potential permethrin resistance in ticks because of exposure to treated deer (Curtis et al., 2011).

Currently the only island-wide tick reduction strategy on Shelter Island is deer culling. Going forward, four poster applications will be the responsibility of private landowners or Homeowners' Associations (Payne, pers comm, 2020). Deer management continues on the island, with both recreational hunting and town-sponsored management in place. However, much like the four-poster control program, the town requires individual property owner permissions for hunting on private property. This may lead to very high deer populations in certain parts of the island, which may have a localized effect on tick density. Another possibility is that due to patchy deer management, the deer population will remain high, and spread back into the managed areas during the off season.

The Town of Shelter Island Deer Management Plan notes that recreational hunting is only sufficient to keep the deer population stable (Shelter Island Police Department and Shelter

Island Deer & Tick Committee, 2019). A culling program implemented in 2018 almost doubled the amount of deer taken, and modeling indicated this led to a 10% decrease in the population later that year (Shelter Island Police Department and Shelter Island Deer & Tick Committee 2019). The management plan states that as recreational hunting is variable, culling efforts must be associated with hunting success (Shelter Island Deer & Tick Committee 2019). This plan seeks to expand both the hunting and culling programs throughout the island with the eventual goal of reducing deer populations to ≤80/km² by the year 2022 (Shelter Island Deer & Tick Committee 2019). A camera survey conducted in 2017 using the Branch Antlered Buck method indicated a density of 144 deer per km² (Jacobson et al., 1997, Payne, 2019).

Tick-Borne Illness in the Northeast

Anaplasmosis, caused by *Anaplasma phagocytophilum,* is distributed through the upper Midwest and northeast parts of the United States (CDC, 2018). Symptoms include fever, chills, tiredness, muscle aches and gastrointestinal distress (CDC, 2018). This disease is treated with the antibiotic doxycycline when anaplasmosis is suspected (CDC, 2018).

Babesiosis is an infection caused by the parasite *Babesia microti* (CDC, 2018). It is found in the northeast and upper Midwest (CDC, 2018). While most infections are caused by the bite of infected tick, the disease may also be transmitted through blood transfusions (CDC, 2018). Symptoms can include fever, chills, tiredness, joint pain, gastrointestinal distress and dark urine (CDC, 2018). People without a spleen, or impaired immune function are considered at higher risk for serious babesiosis (CDC, 2018). For those who are symptomatic, a combination of an antibiotic and antiparasitic-drugs are recommended (CDC, 2018).

Ehrlichiosis is commonly caused by *Ehrlichia ewingii, Ehrlichia murine-like* (EML) and *Ehrlichia chaffeensis*. The disease occurs in the southern United States, ranging from the coast to

Texas (CDC, 2018). Its distribution is association with the occurrence of *A. americanum* (CDC, 2018). EML has recently caused multiple infections in the Midwest and it is believed to be transmitted by *I. scapularis*, rather than *A. americanum* (CDC, 2018). Symptoms include fever, chills, headache, gastrointestinal distress, confusion, muscle pain, and conjunctivitis (CDC, 2018). Children may present with a rash (CDC, 2018). Ehrlichiosis presents in a similar manner to anaplasmosis, and should be treated rapidly with antibiotics like doxycycline when suspected (CDC, 2018).

Lyme disease is a bacterial infection caused by *Borrelia burgdorferi* sensu stricto and *B. mayonii* s.s. It is distributed through the upper Midwest and northeast, with a small pocket of cases in the Pacific Northwest (CDC, 2018). It occurs in two stages if not treated. The first is characterized by flu-like symptoms, swollen lymph nodes and the characteristic erythema migrans (EM) rash (CDC, 2018). The second stage may involve arthritis and join pain, neurological manifestations and cardiac involvement (CDC, 2018). Lyme disease can be treated with a number of antibiotics including doxycycline (CDC, 2018).

Another species of *Borrelia*, *Borrelia miyamotoi*, may cause similar symptoms, although it is less likely to produce a rash (CDC, 2019). *B. miyamotoi* is a relatively new infection, and little is known about it where it is found or how commonly it occurs (CDC, 2019). Like Lyme disease, it can be treated with doxycycline (CDC, 2019). In addition to *B. miyamotoi* infection, the EM rash may also be confused with that of Southern Tick-Associated Rash Illness (STARI), which can develop after the bite of *A. americanum* (CDC, 2019). Flu-like symptoms accompany this rash, but the long-term symptoms that can follow the EM rash are not known (CDC, 2019). Currently, the cause of STARI is not known (CDC, 2019).

Rocky Mountain Spotted Fever (RMSF) is an infection caused by *Rickettsia rickettsia* and can be found throughout most of the United States (CDC, 2018). Fever, chills, gastrointestinal illness, severe headache and neurological symptoms can be seen with the infection (CDC, 2018). A macropapular rash may appear, and petechial rash heralds severe disease (CDC, 2018). RMSF is a severe infection and should be treated with doxycycline immediately (CDC, 2018). RMSF progresses rapidly and can be fatal without intervention (CDC, 2018).

While not an infectious pathogen, the bite of *A. americanum* may cause an allergy to the carbohydrate galactose-α-1,3-galactose (known as "alpha-gal"), and is thought to be an important cause of this allergy in the United States (Steinke et al., 2015, Commons and Platts-Mills, 2013). This is mediated by the IgE antibody, and may cause anaphylaxis after consuming red meat (Commons and Platts-Mills, 2013). Other symptoms can include urticaria, angioedema, nausea and vomiting. Diagnosis of alpha-gal-meat allergy is often complicated by the 3-6 hour delayed reaction after meat ingestion (Commons and Platts-Mills, 2013). Currently it is believed that all life stages of *A. americanum* may trigger the development of an alpha-gal allergy (Steinke et al., 2015). Due to the atypical presentation of delayed anaphylaxis, and the lack of education for healthcare providers on alpha-gal meat allergy, patients who develop alpha-gal allergies often experience long delays in diagnosis, difficulty obtaining necessary diagnostic tests, and frequent visits to emergency services for anaphylaxis (Flaherty et al., 2017). There is still much research needed on alpha-gal to fully understand its causes and the role that *A. americanum* plays in transmission.

Co-infections in Ticks

Ticks in the northeast can be infected with multiple pathogens at once. Yuan et al (2020) found that in 11 counties in New York, 7.9% of *I. scapularis* collected were coinfected with *B. burgdorferi* and other pathogens. Half of those other co-infections were with *B. microti* (Yuan et al., 2020) A similar study from Suffolk County reported coinfections in 45% of *I. scapularis* adults and 11% of nymphs collected (Tokarz et al., 2017). Tokarz et al (2017) also reported similar infection rates with *B. microti* (2017). *Babesia microti* and *B. burgdorferi* share the white-footed mouse (*Peromyscus leucopus*) as a reservoir species and recent studies indicate not only that coinfection in *P. leucopus* increases *B. microti* parasitemia, but that, if the host species was coinfected, the proportion of infected nymphs increased (Dunn et al., 2014, Eisen and Eisen, 2018).

Another study conducted in Suffolk County included *A. americanum* and *D. variabilis* and those authors did not find evidence of coinfections in either tick species, while they did in *I. scapularis* (Sanchez-Vicente et al., 2019). When *A. americanum* populations in nine states were examined, coinfections were found in 4.3% of ticks collected (Mixson et al., 2006). Interestingly, that study reported higher *E. chaffeensis* prevalence on Shelter Island (27%) compared to the overall prevalence of 5% across the tick's range (Mixson et al., 2006).

Little evidence exists of coinfection in *D. variabilis*, with only a single paper reporting a coinfection of three rickettsial pathogens, *R. bellii, R. montanensis*, and *R. rickettsii* (Carmichael and Fuerst, 2009). It should be noted that *R. bellii* and *R. montanensis* are nonpathogenic in humans (Carmichael and Fuerst, 2009).

Risk of Tick-Borne Illness in Suffolk County and the United States

Of all the tick-borne illnesses in New York, Lyme disease is by far the most common and important to human health. Recent data for Suffolk County (2018) reported 476 laboratory-confirmed human cases of *Borrelia burgdorferi* (New York State Department of Health, 2018). In the neighboring counties of Nassau, Putnam and Westchester, 137, 319 and 312 cases were reported respectively. In total, New York reported 7,320 cases of Lyme disease in 2018. These cases are not laboratory confirmed, but rather based on a subsample, as is standard protocol for the state.

Most Lyme disease cases in the United States are reported from the Northeast region. Almost half of the 33,666 cases reported in 2018 were from New York, New Jersey, and Pennsylvania. When comparing three-year average incidence per 100,000 persons, New York has the lowest rate at three 14.5 cases. New Jersey reported 36.6 cases, and Pennsylvania 68.1. While Connecticut is not considered part of the same the same region as New York, New Jersey, and Pennsylvania by the CDC, it still provides a useful comparison at 36.2 cases (CDC, 2019). New York seems to have an artificially low rate, which is resolved when removing New York City from the comparison. When this is done, the rate of cases per 100,000 jumps to 58.5 (New York State Department of Health, 2018). When taking this into account along with Suffolk County's relatively high amount of Lyme compared to surrounding counties both in New York and Connecticut, Suffolk County and including Shelter Island are an important location to study ticks.

Other tick-borne illnesses are less common on Long Island, but with the exception of Rocky Mountain Spotted Fever (RMSF), New York continues to lead the region in incidence of all other tick-borne infections. In 2018, Suffolk County reported 36 cases of anaplasmosis, with the surrounding counties of Westchester reporting 20 cases, Nassau five, and 25 from Putnam (New York State Department of Health, 2018). The CDC does not report raw data but a map of infection indicates Suffolk County had between 6 to 20 cases per million (Biggs et al., 2016). In the same year, New York reported 696 cases of babesiosis, with 157 in Suffolk County (New York State Department of Health, 2018). Westchester reported 85, Nassau 9, and Putnam 28 (New York State Department of Health, 2018).

Suffolk County reported 44 cases of ehrlichiosis in 2018, with 9 in Westchester County, 3 in Nassau and 2 in Putnam (New York State Department of Health, 2018). CDC's infection incidence map indicates Suffolk County reports between 6 and 20 cases of *E. chaffeensis* ehrlichiosis per million (Biggs et al., 2016). Rocky Mountain Spotted Fever is much less common in the Northeastern states, but nine cases were reported in Suffolk County in 2018 (New York State Department of Health, 2018). Westchester had three cases, while Nassau had four and Putnam, two (New York State Department of Health, 2018). The CDC reports RMSF in a grouping of spotted fever rickettsiosis, a category that includes *Rickettsia parkeri* and other *Rickettsia* sp. that are not yet well characterized (Biggs et al., 2016). Suffolk County reports an incidence of >0 to ≤ 5 per million (Biggs et al., 2016).

New York State reports the most cases in the region for many tick borne infections except for and RMSF. Suffolk County also leads nearby counties in infection rate.

Tick Sampling Methods

A variety of methods may be used to sample *I. scapularis* and *A. americanum*, ranging in efficacy and the labor required. When comparing sampling methods and collection rates for *I. scapularis*, there was a significant difference between larvae and nymphs (Falco and Fish, 1992). Sampling using $C0_2$ traps was more effective when collecting larvae and using a drag cloth was more effective for nymph. Life stages collected from mice did not match dragging results. Variable results have been reported for the efficacy of dragging and walking surveys (Shulze et al., 1997). A two-factor increase in adult ticks was found when conducting walking surveys, although previous studies had found them to be comparable (Shulze et al., 1997). Only one of these studies evaluated *A. americanum*, but reported the same, while Shulze et al (1997) found twice as many adults when dragging compared to walking. *I. scapularis* larvae and nymphs were less frequently collected while dragging when compared to *A. americanum,* where all life stages were much more abundant (Shulze et al., 1997). Dry ice traps were also much more effective for *A. americanum* in all life stages than *I. scapularis.* This may be explained by differences in behavior. *A. americanum* will actively pursue a host, while *I. scapularis* tends to host seek by questing higher up in the vegetation. It is important to remember that larvae of *A. americanum* tend to occur in clusters of hundreds, which could easily skew any density estimates (Shulze et al., 1997).

Estimating Tick Abundance

Density provides a useful metric of tick abundance, such as DON (density of nymphs) or DOF (density of females) (Eisen et al., 2018). When combined with infection rates, these are known as DIN (density of infected nymphs) or DIF (density of infected females) (Eisen et al., 2018).

A later study found that mark-recapture is a suitable measure to estimate absolute tick populations for nymphs and adults (Daniels et al., 2000). Due to the small size of larvae, they were not used in mark-recapture studies, however, the authors suggested that removal studies would be suitable to estimate larval populations. As tick abundance, specifically nymphs, correlates with reported Lyme cases per year, an estimate of population size should prove a useful tool. Data from Westchester County, NY, which is around 200 km from Shelter Island, showed that infection rates of *B. burgdorfer*i varied much less per year than the nymphal abundance (Daniels et al., 2000). Additionally, data collected from their mark-recapture study suggested that adult *I. scapularis* populations are low, and relatively few nymphs survived to adulthood (Daniels et al., 2000). Consequently, it may be possible to sample only nymphal populations using mark-recapture methods to get a working estimate of absolute tick abundance. This would reduce the labor and time needed which may make this kind of sampling more feasible for smaller towns and communities (Daniels et al., 2000). The authors also noted that tick populations may beaffected at a very local level by environmental conditions, which would make extrapolating these tick population estimates difficult in areas with many different habitats (Daniels et al., 2000). It may be possible to integrate GIS data to determine how many locations would need to be sampled to create a robust estimate of tick population size.

Pathogen Testing and Calculating Tick-borne Disease Infection Rates

The CDC has produced a guide to surveillance for *I. scapularis,* as well as for the metastriate ticks which include *A. americanum, H. longicornis* and *D. variabilis* (CDC 2018, 2020). The guide provides recommendations on the number of ticks required for pathogen testing and calculating the infection rate varies depending on the infection prevalence in the target area. While a power of 80% is optimal, for many reasons that exact power may not be possible. Sample size calculators are available to determine power, such as EpiInfo, produced by the CDC. A rough sample size of 50 ticks is recommended (Division of Vector-Borne Diseases, CDC, 2020). Among the common metrics used is density of nymphs (DON). This is defined as the total number of nymphs collected per area sampled and is often scaled to $100m^2$ by multiplying the number of nymphs by $100m^2$ and dividing by the area sampled (CDC, 2018). Another metric is the density of infected nymphs (DIN), which is determined by multiplying the DON by infection prevalence (CDC, 2018). Prevalence is the percentage of ticks infected out of the total collected, and is sometimes known as the entomological risk index, or ERI (CDC, 2018).

Infection prevalence has recently been determined for *I. scapularis* on Shelter Island, with 66% *B. burgdorferi* in adults, and nymphs at 20% in 2018 (Suffolk County Government 2018). Nymphal *I. scapularis* were also infected with *B. microti* at a rate of 20% (Suffolk County Government 2018). All other pathogens were found in *I. scapularis* at <20% (Suffolk County Government, 2018). In *A. amblyomma, E. ewingii* was the most frequently detected, with an 8% infection rate (Suffolk County Government, 2018). All other pathogens were found to be below <5% (Suffolk County Government, 2018).

Biases in Sampling Methods

In one study, when flagging and dragging, more ticks were found on clothing than the drag cloth (Ginsberg and Ewing, 1988). Those authors also suggested that users may avoid dragging through thick bushes that could trap or tear the fabric, which may bias collection. They also recommended the walking survey method, where a person walks through the target habitat and checks themselves for ticks, as it represents the reality of a human contacting a tick compared to a piece of fabric (Eisen et al., 2018). Another important consideration is collector bias. To reduce this, surveys should be conducted by the same person (Ginsberg and Ewing, 1988). The environment and sampling method can also bring their own set of biases to tick collection as tick numbers vary depending on the collection method for each location sampled (Daniels et al., 2000). It is possible that denser vegetation is harder to drag through, whereas a smaller flag may be easier to use and contacts more of the vegetation. In addition, when dragging, the cloth should never reach above the highest shrub and will contact the ground frequently. Walking surveys and flagging will reach higher and contact the ground much less frequently (Schulze et al., 1997). This could bias collection if tick species prefer to quest from different heights or habitats. In addition to vegetation height and sampling method, the weather will also impact collections (Schulze et al., 1997) The highest success was observed after rain with high humidity once the sampling site had dried, while windy conditions reduced success (Schulze et al., 1997). Therefore, weather should be standardized during collections, or at least noted. An important factor to consider is the purpose of collection (Daniels et al., 2000). The authors noted that flagging or dragging would be preferable if the goal was to evaluate populations over time, as they can be standardized more easily. These methods are low cost, effective and require fewer resources than using dry ice traps or host trappings (Daniels et al., 2000).

A study on Fire Island, a long, narrow island off Long Island and about 115 km from Shelter Island, examined sampling methods (Ginsberg and Ewing, 1988). Habitats on Fire Island consist of beach grass, and low shrubs and trees (Ginsberg and Ewing, 1988). Walking surveys were found to be more accurate than flagging or dragging, which were equated here (Ginsberg and Ewing, 1988). However, ticks that prefer leaf litter would not be adequately sampled by this survey method. They also noted that *A. americanum* were overrepresented in dry ice traps when compared to dragging. Nymphal *A. americanum* outperformed *I. scapularis* in lab tests to determine mobility, which may explain their abundance in dry ice traps (Ginsberg and Ewing, 1988).

A study in New Hampshire categorized sites into high, medium, and low Lyme disease incidence (Walk et al., 2009). Ticks were found to be most abundant in coastal counties, as is seen in Massachusetts and Maine (Walk et al., 2009). Adult ticks were targeted as the authors believed them to best represent individuals that will pass on Lyme disease. *B. burgdorferi* was found be almost twice as common at sites designated as high incidence sites (Walk et al., 2009). This is similar to what has been reported from neighboring states. Contrary to a previous study by Falco et al (1999), Walk et al (2009) found a high correlation between Lyme disease and the entomological risk index. They believe that improved knowledge of Lyme disease over the past ten years may be responsible for this change (Walk et al., 2009). In addition, Falco et al (1999) reported erythema migrans (EM), which may not represent the true burden of Lyme disease. They found that nymphal, but not adult ticks were strongly associated with EM rash, and that nymphs should be the focus of surveillance and control (Falco et al., 1999). In addition, they note that while in other locations adult ticks may play important roles in transmission of Lyme disease, at least in southern New York, they play only a minimal role (Falco et al., 1999).

Shelter Island and the surrounding counties have high tick abundance and incidence of tick-borne disease. At least three medically important tick species are found on the island and the pathogens they carry pose risks to human health. While the four-poster program is no longer active on the island, a deer culling program has been implemented. There are a variety of tick sampling and surveillance methods available that would target the species present on Shelter Island. In addition, pathogen testing may help to determine the prevalence of infection and entomological risk.

CHAPTER 2

Assessing Surveillance, Density, and Pathogens in the Ticks of Shelter Island, New York

Introduction

Tick bites and tick-borne disease are an important concern in New York State where some regions experience the highest incidence of tick-borne infections in the United States (Campbell et al., 2015). Suffolk County, located on New York State's Long Island, is an area with high tick abundance and reported cases of tick borne human and animal infections. In 2018, New York reported 7320 cases of Lyme disease. In the same year, Suffolk County reported 476 laboratory confirmed cases, higher than the surrounding counties (NYSDOH, 2018). Primary tick-borne infections in Suffolk County include Lyme borreliosis, anaplasmosis, ehrlichiosis, and babesiosis (NYSDOH, 2018).

Shelter Island, a small island between the North and South Forks of Long Island, has not officially reported human tick-borne infections for residents and visitors from the island, but they may be high (Suffolk County Government, 2018). The black-legged tick, *Ixodes scapularis,* the Lonestar tick, *Amblyomma americanum,* and the American dog tick, *Dermacentor variabilis,* are present on the island (Suffolk County Government, 2018, Payne pers. comm., 2020). Tick exposure and potential for tick-borne diseases is a concern among Shelter Island residents. In addition to year-round permanent residents, who are knowledgeable about ticks and tick-borne disease, a large population of summer residents and tourists visit the island annually.

To address the risk of tick borne diseases, a Deer and Tick Committee was established to assist the community in addressing the high tick density on the island. A tick control study using the deployment of 4-poster devices across the island was established in 2008, with efficacy monitored through tick surveillance conducted once per year (Curtis et al., 2011, Pound et al., 2000). These devices consist of a bait station containing corn, and four acaricide applicators which consist of foam paint rollers impregnated with an acaricide (Pound et al., 2020). Deer must stick their heads into a small area to access the bait, which brings their necks into contact with the rollers (Pound et al., 2000). In 2019, the 4-poster program was discontinued after changing New York State DEC regulations (Payne pers. comm., 2020).

To continue assisting Shelter Island with their assessment of the risk for tick-borne diseases, I conducted a study in summer 2020 which included the Mashomack Nature Preserve. I evaluated tick diversity, abundance, and pathogen presence on the island. In addition, I performed analyses of historical data to determine tick abundance and temporal trends over multiple years. Of particular interest were changes in tick density since termination of the 4-poster program in 2019. Here, I present my results including high density areas on the island, relative species abundance, trends between the nature preserve and town, and risk to residents and tourists.

Study Area

Shelter Island, NY has a moderate climate with highs of 29.5 C° and lows of 12.5 C° during the summer months (CLIMOD 2, 2020). Rainfall is heavy except in the summer, with an average of 84.3 mm per month (CLIMOD 2, 2020). The average annual precipitation is 1,077 mm (Nelson, et al., 2014). Deciduous forest covers 47.2% of the island. The next largest land categories are roads and open grassy areas such as lawns, consisting of 17.3% of the island. The two largest land use categories are low density residential and parks/open spaces, at 34.6% and 32.3% of the total area respectively. The next largest category is vacant, at 14.5%. All other categories make up less than 7% of land usage (Nelson et al., 2014).

Seven areas were selected for monitoring, based on prior tick sampling information, habitat diversity, ease of transport, and access (Table 1, Fig 2).

Table 1. Sampling sites on Shelter Island, NY and their characteristics during the summer 2020 sampling period.

Site Label	Name	Location	Coordinates	Habitat
A	Triangle	Mashomack	41° 3'15.30"N 72°16'57.76"W	Full shade, leaf litter
B	Water Tower	Mashomack	41° 3'17.41"N 72°18'27.69"W	Full sun, grassy
C	Red Trail	Mashomack	41° 3'33.87"N 72°18'55.87"W	Sun and shade
D	Visitor Center	Mashomack	41° 3'28.14"N 72°19'17.59"W	Full shade
E	Quaker Cemetery	Town	41° 4'40.59"N 72°20'35.30"W	Full shade
F	Catholic Cemetery	Town	41° 5'18.49"N 72°20'18.53"W	Full shade
G	Williams	Town	41° 3'45.04"N 72° 20' 7.00"W	Filtered sun and shade

Site A (Triangle, Table 1, Fig. 2) was approximately 0.16 km from the blue trail, a 6.9 km dirt road that covers most of Mashomack Preserve, accessed by a private maintenance road. The site was mostly bare dirt with low growing plants and grasses. This remote site was not frequently used by preserve staff and was infrequently mowed. This has allowed for tall grass and plant growth on the road which was not seen at the other sites. Due to high amounts of catbrier (*Smilax spp*.), only the road was sampled to determine tick abundance and species. White-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), and Eastern chipmunks (*Tamias striatus*) were commonly observed during sampling events.

Site B (Water Tower, Table 1, Fig. 2) was off the main access road and included sections of the yellow trail, a short 1.45 km trail which consisted of a mixture of mowed grass and gravel. This site is a meadow and three lone conifers provide the only shade. The plant life consists of tall grass, bramble, catbrier and milkweed. There is no leaf litter. Deer, eastern wild turkeys (*Meleagris gallopavo*), passerine birds, and osprey (*Pandion haliaetus*) were commonly observed here. This site had a heavy deer presence, with trails, scat and beds seen at each visit.

Site C (Red Trail, Table 1, Fig. 2) was at a convergence of the red and yellow trails. Due to catbrier prevalence in the forest, only the trail was sampled. It consisted of leaf litter, dirt, and mowed grass. Chipmunks and passerine birds were commonly seen here. Deer were not observed during the survey period but deer trails were noted.

Site D (Visitor Center, Table 1, Fig. 2) was roughly 100 meters from the Mashomack Preserve Visitor Center on the red trail. All visitors must walk this dirt trail to gain further access to the preserve. This site contained heavy leaf litter mixed with patches of dirt. Roughly half of the ground was covered and dominated by plants in the blueberry family (Ericaceae), catbrier, and oaks (*Quercus* spp.). Deer, chipmunks, and passerine birds were commonly seen here. A HOBO data-logger was placed here to collect temperature and relative humidity data from 13 June 2020, to 9 August 2020.

Site E (Quaker Cemetery, Table 1, Fig. 2) was at a working organic farm and former slave plantation, Sylvester Manor. It was named for the historical Quaker Cemetery dating back to the 1700s, and it still hosts a Quaker meeting place. In addition, there were many walking trails that connected this area to the remainder of Sylvester Manor. It had heavy leaf litter and under 50% plant cover. It was dominated by catbrier, oaks, Japanese spikenard (*Aralia* spp.), and Ericaceous plants. Chipmunks, eastern gray squirrels (*Scirurus carolinensis*), and passerine birds were commonly seen here.

Site F (Catholic Cemetery, Table 1) was at the site of a current cemetery, Our Lady of the Isle. The ground cover was almost entirely leaf litter, with very little undergrowth. Most plants were catbrier, with some barberry (*Berberis* spp.), and American beech (*Fagus grandifolia*). There were no trails here, only a paved road that was used to access the cemetery. The drag area was in a wooded location not used by visitors. Chipmunks, gray squirrels, and passerine birds were frequently seen here.

Site G (Williams, Table 1, Fig. 2) was located on private property, an unmanaged wooded strip bordering many private houses, and it contained a section of grass >1.8-m high. It did not appear to be used by residents due to extreme overgrowth. Grapes (*Vitis* spp.), wineberry (*Rubus phoenicolasius*) catbrier, and barberry were the most common plant species. There was some bare ground and leaf litter, with patches of grass where the sun reached the ground. Deer were more visible at this site than any other sampling location, with deer sightings during every visit. In addition, deer sign, such as beds, scat, and bones were common here.

A HOBO Pro V2 data-logger (Onset Corp, Bourne, MA) was placed at site A to collect temperature and relative humidity data from 13 June 2020 to 9 August 2020. Additional weather data was accessed nearby at the Greenport Power House weather station; the closest weather station to Shelter Island (CLIMOD2, 2020).

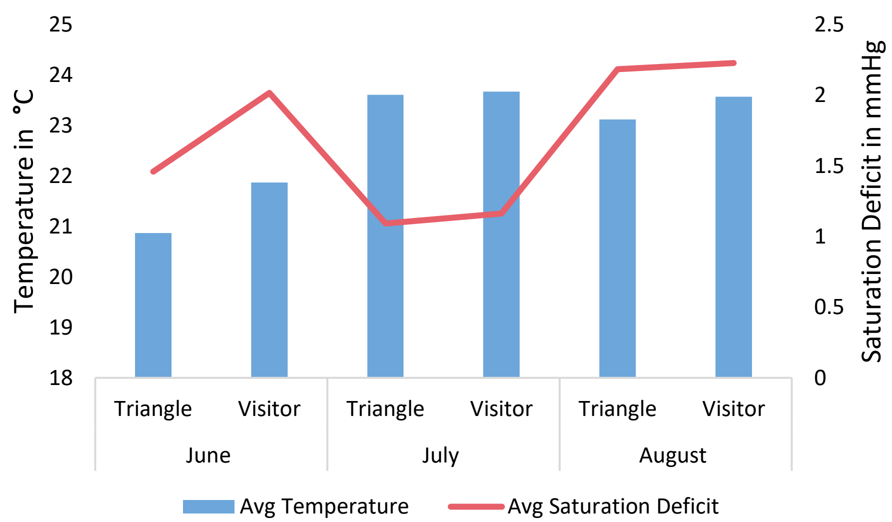

Fig. 1. Mean temperature (bars) and saturation deficit (line) over time from 2 sites on Shelter Island. Data were collected from 13 June – 9 August, 2020.

Fig. 2. Images of sampling sites on Shelter Island and Mashomack Preserve taken during summer 2020. Locations are labeled by site. Site F, the Catholic Cemetery is not shown.

Methods

Tick Collections

A 1-m^2 single-sided, corduroy drag cloth was used to collect ticks (Eisen et al., 2018). The cloth was sewn to a wooden dowel, and a nylon rope was attached to ease dragging. Two washers were sewn into the corners of the drag cloth to serve as weights. The drag cloth was pulled behind the collector as they walked at a slow pace, ensuring that it was in contact with the ground and any vegetation.

Transects of 100m were established in areas where catbrier prevented the use of grids. In other locations, blocks 30m on each side were established, and 3, 30m line transects were sampled within the block. (Fig. 3). Transects and grids on Mashomack Preserve were marked with flagging tape every 10m as a guideline. Starting on 26 July 2020, transects located off Mashomack Preserve were extended to 200m in length, and blocks to 50m on a side to increase tick collections. Both sides of the drag cloths were checked every 10m. Any ticks on the cloth were collected and placed in a glass vial either with 70% ethanol. A subset were collected live into a vial and frozen at -20°C for pathogen testing due to storage constraints. Larvae were collected with a garment lint roller and then frozen. Vials were labeled with the site, date, and time. The complete transect distance (100 or 200 m) was sampled each visit. The complete block (all 30m or 50m transects) was sampled during each sampling event. Transportation was limited due to the ongoing COVID-19 outbreak, restricting collections in other parts of Shelter Island.

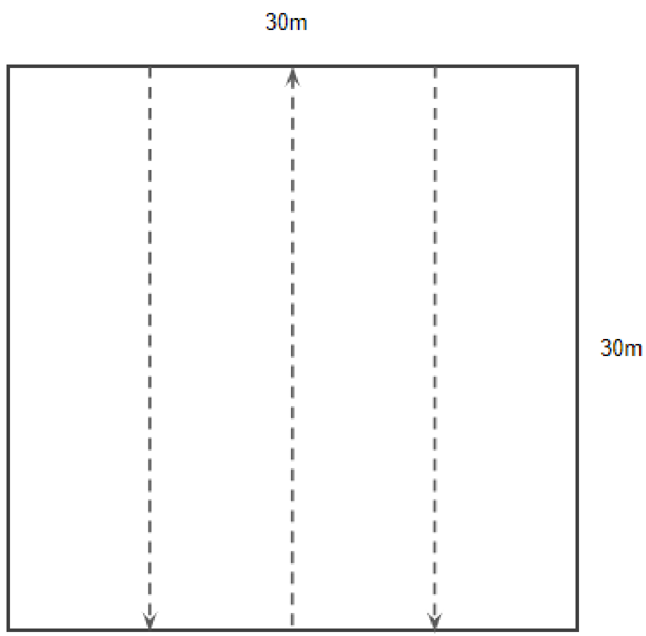

Fig. 3. A diagram of the sampling block layout with 3, 30m transects followed while dragging for ticks. Drags were checked every 10 meters.

Tick Identification

Ticks were identified to species by examining them under a dissecting microscope at 20x magnification and using published keys (Kierans and Litwak 1989, Kierans and Durden 1998, Dubie et al., 2017). The life stage, sex, and species of each tick was recorded. After identification, ticks were stored in a freezer at -20 °C until transport to Cornell University for pathogen testing.

Pathogen Testing

Ticks were placed in individual microcentrifuge tubes by species and life stage. Samples were homogenized and then tested for pathogens following the methods described in Goodman et al. (2020). Briefly, extraction was performed using an automated extraction machine (Thermo Fisher Scientific). The OpenArray Tick Nanochip workflow was performed and samples were tested for fourteen tick-borne pathogens as in Goodman et al (2020). Assays were done using custom printed plates for the target pathogens. Raw results are available in Appendix 1.

While overall infection rates for two tick species on Shelter Island were previously reported, it was not clear how prevalence varied across environments as these samples are collected from a single sample at one location (Romano, 2020, Suffolk County Government, 2019, Novarro, pers. comm). Therefore, I used a rough sample size to calculate infection rates (Division of Vector-Borne Diseases, CDC. Atlanta & Ft. Collins 2020). I calculated DON per $100m^2$ according to Eisen et al. (2018), where the number of ticks collected was multiplied by $100m^2$ and then divided by the area sampled. Density of infected nymphs (DIN) was calculated by multiplying the DON by the percent of infected ticks, and the density of infected adults was calculated by multiplying the density of adults (DOA) by the percent of infected ticks (CDC. 2020). The Pooled Infection Rate Add-In Microsoft Excel macro tool from the CDC was used (Division of Vector-Borne Diseases, CDC. Atlanta & Ft. Collins 2020). This model was used to calculate the point estimate, which was used to determine the DIN and DIA in cases where the infection rate was unknown. Samples sizes were too low to calculate confidence intervals.

Historical Data

To compare my results with prior trends, I analyzed historical tick collection data from Shelter Island. These data were from flagging collections conducted once annually in late June-early July, with occasional tick collections performed in the fall or early spring months. The data were collected by flagging for 30s 30 times at different sites annually. Flagging was also performed using this methods at the same sites in 2020, and were presented as year 2020 in the historical data.

Data Analysis

Tick data were analyzed for differences among location using Kruskal Willis and pairwise Wilcoxon tests in R Studio 2020, version 4.0.3 (RStudio Team, 2020) using the following package: ggplot2 (Wickham, 2016). The historical data were analyzed with the following packages: ggplot2 (Wickham, 2016), dplyr (Wickham, François, Henry and Müller, 2021), and reshape2 (Wickham, 2020). Correlation was calculated for historical data using the Spearman Correlation Coefficient. Figures were formatted with ggthemes (Arnold, 2021), gridExtra (Auguie, 2017) and ggpubr (Kassambara, 2020). Saturation deficit was analyzed using the formula from Randolph and Storey (1999).

Results

Weather. No significant differences in max/min temperatures and saturation deficit were detected between the two data logger locations. Variation in weather data collected is presented in Fig. 1. Saturation deficit was calculated using relative humidity and temperature. It is a measure of the drying power of atmosphere (Randolph and Storey, 1999).

Overall tick collections. A total of 862 non-larval ticks were collected in 2020; 588 were collected by dragging (Table 2). An additional 139 were collected with dragging over non-standardized distances to increase tick numbers for infection testing. All life stages *of A. americanum* were collected during the sampling period, while no adult *I. scapularis* were collected and only adult *D. variabilis* were collected. A single adult female *H. longicornis* was collected.

Table 2. Nymphs compared by sampling site and species. Tick counts are shown, with overall density at the sampling site per 100m^2 in parentheses. Distance sampled is shown in meters.

Location	Site	Total Distance Sampled (m²)	*A. americanum*	*I. scapularis*
Visitor Center	D	830	219 (22.12)	25 (2.30)
Red Trail	C	780	51 (6.38)	21 (2.63)
Tower	B	820	11 (1.23)	2 (0.25)
Triangle	A	790	28 (3.50)	9 (1.13)
Williams	G	950	34 (3.62)	9 (0.96)
Catholic Cemetery	F	940	6 (0.64)	6 (0.64)
Quaker Cemetery	E	940	19 (2.02)	18 (1.91)

Collections by species and site. A total of 2,014 *A. americanum* were collected (adult = 37, nymph = 368, larva = 1,609 and 3,998; Table 2). *I. scapularis* were also collected (adult = 100, nymph = 90, larva = 3,908; Table 2). Very few *D. variabilis* were observed (adult = 15, nymph = 0, larva = 0), and were limited to sites A, B, and G. The other 2 tick species were found at every location. The highest number of ticks were found at Site D (Visitor Center; *A. americanum* = 219, *I. scapularis* = 25). A maximum DON of 64.4 (*A. americanum*), and 6.7 (*I. scapularis*), and an average of 7.5 (*A. americanum*) and 1.63 (*I. scapularis*) were reported. The single *H. longicornis* was collected from Site G (Williams).

Pairwise comparisons of DON at sampling locations was conducted using the Wilcoxon rank sum method. The DON was greatest at the Visitor Center for *A. americanum* and was significantly different from all other locations (Fig. 4, Table 3). No differences in DON was detected by location for *I. scapularis* (Fig. 5). DON was compared by epiweek and no significant difference was found.

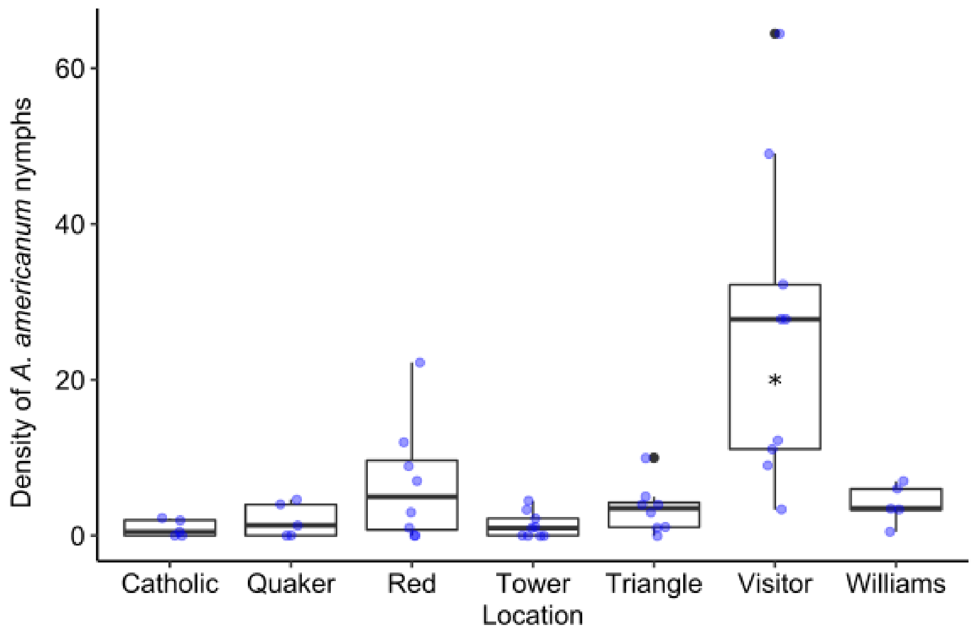

Fig. 4. Boxplot showing *A. americanum* nymphal density per 100m^2 (DON) at each sampling location during the 2020 sampling season. DON at the visitor site was significantly greater than all other locations (Kruskal-Wallis, P < 0.05).

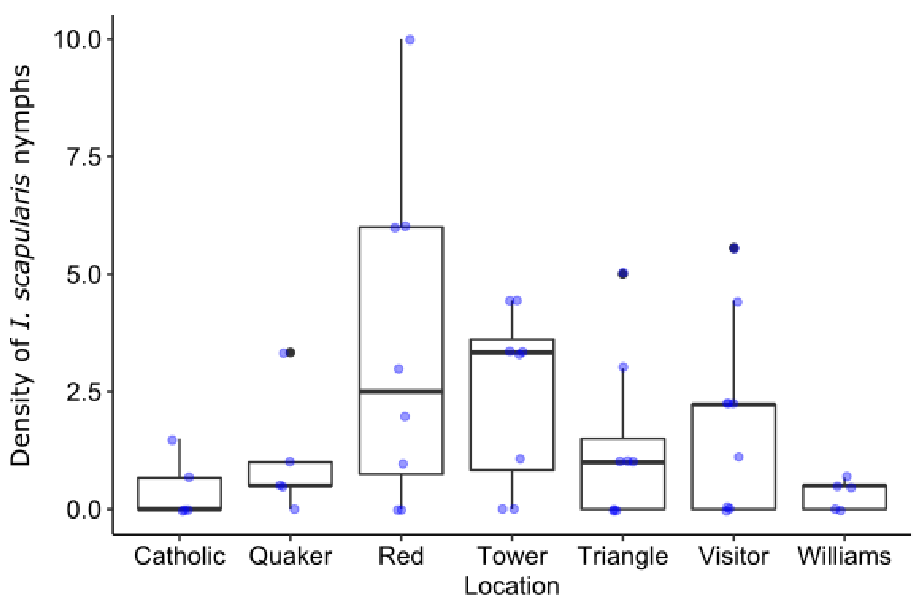

Fig. 5. Boxplot showing *I. scapularis* nymphal density data per 100m^2 (DON) the locations sampled in 2020. No locations were significantly different from each other (Kruskal-Wallis, P < 0.05).

Table 3. Pairwise comparison of average *A. americanum* DON/100 m². The column indicates the location where DON was recorded, and the row the location it is being compared to. Bold indicates a significant effect (Wilcoxon rank sum test, P <0.05).

	Catholic	Quaker	Red	Triangle	Tower	Visitor
Quaker	0.789	-	-	-	-	-
Red	0.277	0.502	-	-	-	-
Triangle	0.210	0.707	0.789		-	-
Tower	0.877	0.760	0.267	0.225	-	-
Visitor	**0.023**	**0.040**	**0.045**	**0.023**	**0.013**	**-**
Williams	0.132	0.476	0.883	0.789	0.132	0.047

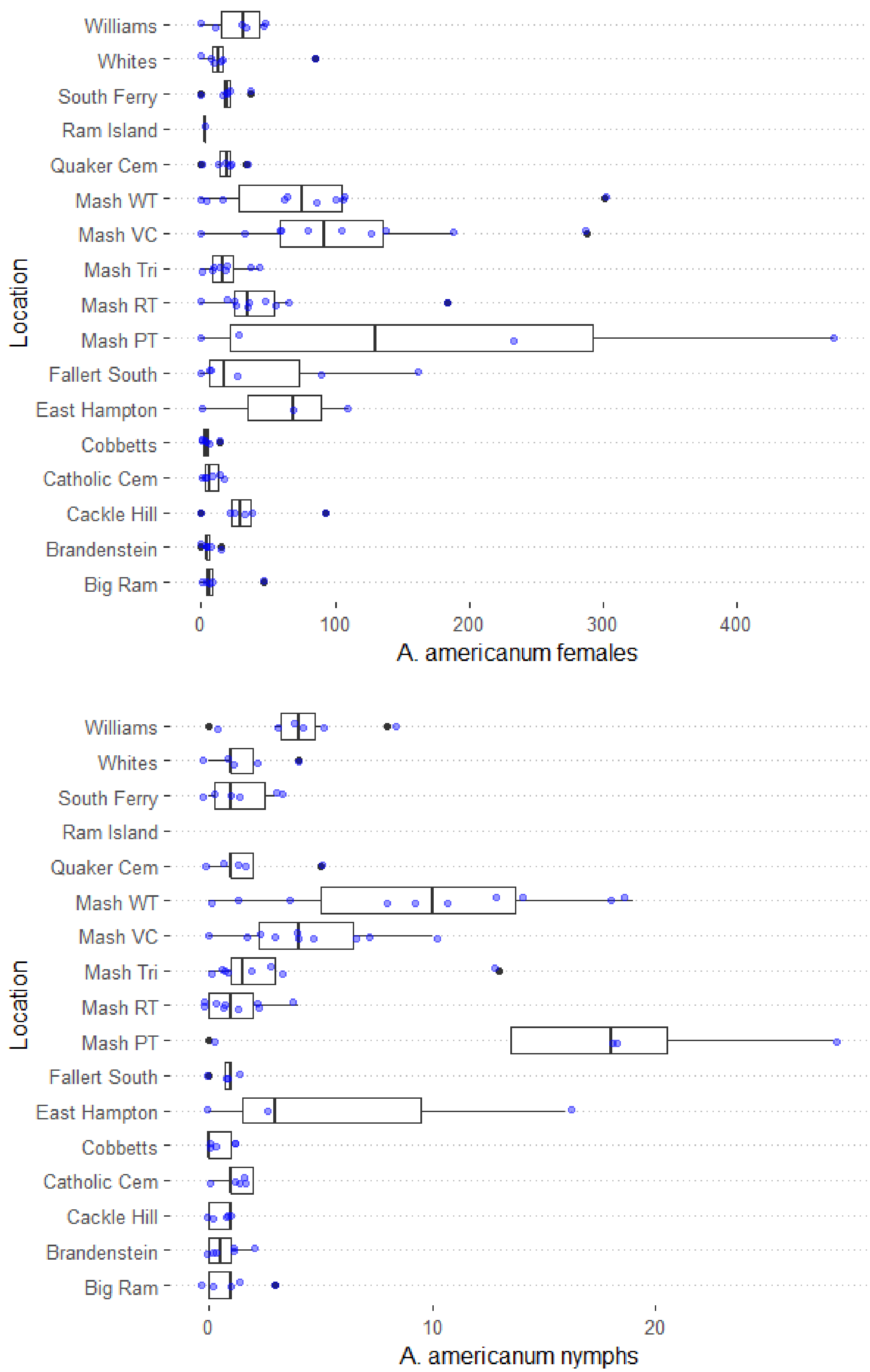

Fig. 6. Boxplot showing *A. americanum* adult female and nymphal counts at locations sampled from 2012 – 2020. No locations were significantly different from each other.

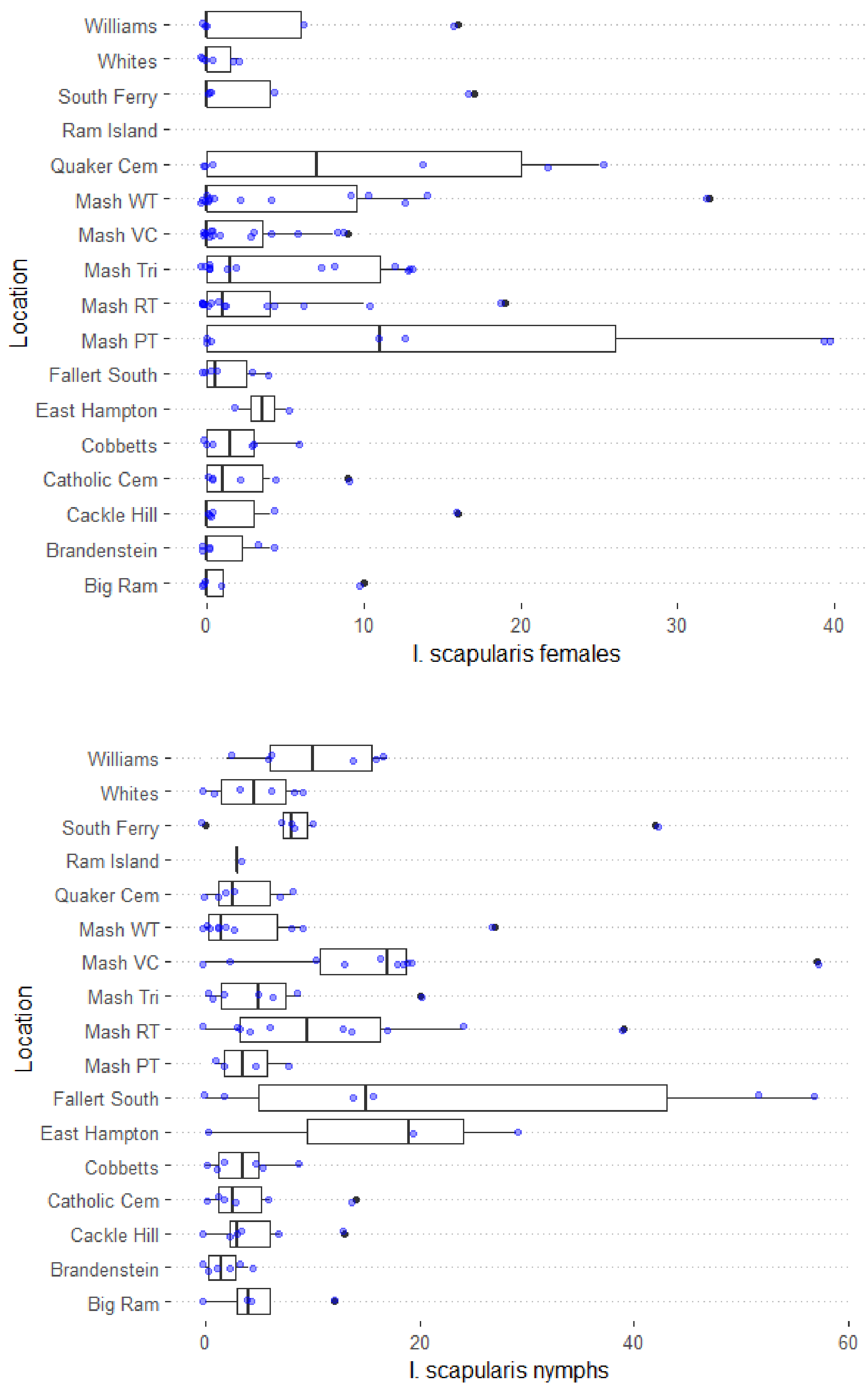

Fig. 7. Boxplot showing *I. scapularis* adult female and nymphal counts at locations sampled from 2012 – 2020. No locations were significantly different from each other.

Historical data. A Kruskal-Wallis rank sum analysis was run and determined that there was no significant difference between the locations sampled from 2012 – 2012 for any of the tick species or life stages (Figs. 6 & 7). When considering Mashomack Point (Mash PT) as a control location where no four poster was placed, the tick counts out the other locations were averaged and compared using Kruskal-Wallis rank sum analysis. *I. scapularis* nymphs were significantly difference in abundance on the point compared to the average of other locations ($P < 0.05$). This data should be considered with caveat that the control site did not have replicates.

Pathogen testing results. A total of 202 samples were tested including 27 *I. scapularis*, 172 *A. americanum,* and 3 *D. variabilis.* Two *I. scapularis* samples were positive for *A. phagocytophilum* and two for *B. microti*. A single *I. scapularis* sample was positive for *B. burgdorferi,* and a single sample tested positive for *B. miyamotoi.* Thirteen of the *I. scapularis* samples tested positive for *Rickettsia spp.*, a non-pathogenic endosymbiont. A large number (96) *A. americanum* samples tested positive for *Rickettsia spp*. *D. variabilis* did not test positive for pathogens or the rickettsial endosymbiont. While many of the samples were collected from the visitor center (112 *A. americanum*, 2 *I. scapularis*), all ticks positive for pathogens were collected from the Quaker cemetery, Catholic cemetery and triangle, low density areas (Table 2). All positive ticks were nymphs; however, no adult *I. scapularis* were collected. One sample was coinfected with *B. burgdorferi* and *B. microti* and was collected from the triangle site.

Table 4. Percent of tested ticks from Shelter Island, NY, infected with pathogens during summer 2020. Raw number of positive ticks are in parentheses.

	A. phagocytophilum	*B. microti*	*B. burgdorferi*	*B. miyamotoi*	*Rickettsia spp.*
A. americanum	0	0	0	0	55.81% (96)
I. scapularis	7.41% (2)	7.41% (2)	3.71% (1)	3.71% (1)	48.15% (13)

Table 5. Density per 100m^2 of infected *I. scapularis* nymphs (DIN) from Shelter Island, NY by pathogen during summer 2020.

	A. phagocytophilum	*B. microti*	*B. burgdorferi*	*B. miyamotoi*	*Rickettsia spp.*
I. scapularis	12.07	12.07	6.05	6.05	78.49

Discussion

Overall, my work demonstrated a range of tick abundance across locations sampled on Shelter Island. All tick species collected have been previously reported on the island. The density for *A. amblyomma* was higher than *I. scapularis* at all sites visited except for site E. The site with the highest overall DON per 100m2 was site D, which is located on Mashomack Preserve.

One limitation of this study was the number of locations surveyed. Historically, 10 locations were surveyed throughout the town, and 5 on the Preserve, one of which was the control site for the 4-Poster program. Due to the COVID-19 pandemic, transportation was limited to 4 non-control sites on the Preserve, and 3 in the Town of Shelter Island. The Town sites were selected for relative distance and accessibility. Broader sampling capacity would have led to more sampling sites and greater power to determine hotspots across the island.

The tick species with highest abundance on Shelter Island was *A. americanum*. There are few studies on abundance of *A. americanum* in New York. Ginsberg et al (1991) reported an increase in *A. americanum* in 1990 compared to previous sampling efforts. Recent studies have focused on pathogen detection and characterization. This represents a gap in the knowledge that may become problematic as more people are bitten by *A. americanum*.

I did not collect high numbers of *I. scapularis* in 2020, likely due to my sampling season not extending into the fall. It is also possible that the drought in the northeast impacted *I. scapularis* tick numbers on Shelter Island. While most of New York reported average precipitation, Shelter Island had between 5-10 cm less precipitation than normal between the months of June to August (NCEI, 2020). Tick surveillance reports from Maine also indicated a reduction in *I. scapularis* nymphs likely due to hot, dry weather during the summer (University of Maine, 2020). When examining collection data, 2,549 *I. scapularis* larvae were collected, compared to 816 *A. americanum* larvae. This suggests that the low *I. scapularis* collections were influenced by something other than population numbers.

Despite the large number of *A. americanum* collected compared to *I. scapularis*, only *I. scapularis* nymphs were positive for pathogens. As only three *D. variabilis* adults were tested, the fact they did not test positive for any pathogens is not surprising. The low infection rate of *B. burgdorferi* is unusual, as the island has a much high level historically (20%); however this may be due to the low amount of nymphs collected and tested (Suffolk County Government, 2018). The infection rate for *B. microti* was closer to historical data (20%) but still lower (Suffolk County Government, 2018).One coinfection was detected, *B. microti* and *B. burgderfori*. Both *A. americanum* and *I. scapularis* tested positive for a *Rickettsia* spp., a non-pathogenic endosymbiont that has been detected in *I. scapularis*. Non-pathogenic rickettsial endosymbionts are well-recorded in *I. scapularis*, but less is known about them in *A. americanum*. A recent analysis of the bacterial community of *A. americanum* revealed that rickettsial species are the second most common bacterial group detected (Maldonado-Ruiz et al., 2021).

As residents of Shelter Island know that ticks can transmit disease, it is likely they will make that connection with any tick bite. However, the alpha-gal allergy may become a concern with time for residents and tourists. The bite of *A. americanum* can cause an allergy to the carbohydrate galactose-α-1,3-galactose (alpha-gal), and is thought to be the most important cause of the allergy in the United States (Steinke et al., 2015, Commons and Platts-Mills, 2013). This is mediated by the IgE antibody, and can lead to anaphylaxis after consuming red meat (Commons and Platts-Mills, 2013. More education on alpha-gal and its association with *A. americanum* bites is warranted. This was particularly concerning due to the high abundance of *A. americanum* on Shelter Island and Mashomack Preserve when compared to *I. scapularis*. It is also important to remember that despite the lack of pathogen detection in 2020, *A. americanum* is reported to be positive for pathogens on the island, and the risk of tick-borne illness remains.

Reported seasonal peaks of nymphal and adult ticks of *A. americanum and I. scapularis* in the Northeastern United States vary widely in the literature. One study from Tennessee showed an adult peak in *A. americanum* during April through June, and no distinctive nymphal peak (Gerhardt et al., 1998.) However, another study in the same state showed an adult peak during May to August, and a nymphal peak May to September (Ludwig et al., 2016). This second study occurred in 2016, 20 years later than the first paper. It is possible that a change in the timing of the nymphal and adults peak occurred over time. Meanwhile, a separate study that modeled *A. americanum* over 15 years showed similar nymphal and adult peaks from March to June or July. It included sites from Maryland, Arkansas, Texas, and Georgia (Mount et al., 1993). In Mississippi, adult ticks peaked during May to June, and nymphal ticks in May to August (Jackson et al., 1996).

The Final Report of the Suffolk County Tick and Vector Borne Diseases Task Forces (https://www.suffolkcountyny.gov/LinkClick.aspx?fileticket=8EEMHropezs%3d&portalid=0) indicated that the adult and nymphal peak completely overlapped each other, peaking during the months of May to June (Suffolk County Department of Health Services, 2015). These data were consistent with what I collected during the summer 2020 sampling season and suggests that *A. americanum* peaks vary geographically across the United States.

The greatest number of ticks were collected at the Visitor Center site, indicating that most visitors to Mashomack Preserve are at risk of coming into contact with a tick. Visitors who do not reside or work at the Preserve, enter and exit through the Visitor Center. While this risk is concerning to visitors, it also may provide a significant educational opportunity. Due to the COVID-19 pandemic, the Visitor Center was closed in summer 2020. Under normal operations, the Visitor Center is staffed and provides educational materials and videos. The opportunity exists to educate visitors with flyers as they are about to enter the most high-risk area of the Preserve. It is important to note that the Visitor Center was not historically statistically different or one of the sites with the highest number of ticks. Whether this is emerging trend should be observed further.

Currently, ticks on the island are sampled once during the summer months, during the last week of June to the first week of July. A second period of sampling was historically conducted during the last week of October to the first week of November. Both sampling periods have been used since 2012, however until 2016 data was only collected on Mashomack Preserve. Due to the exclusion of *A. americanum* from the fall sampling, complete information was not collected, and historical data analysis for the fall season was lacking.

To create a streamlined sampling protocol, I recommend targeting the larval and adult peaks with dragging or flagging sampling for both *I. scapularis* and *A. americanum* rather than sampling only once per season. If further data is desired and the capability to expand sampling exists, then sampling effort could be increased. Locations that show historically high tick counts, or high density from my current (2020) sampling effort, such as the visitor center, should be prioritized, as well as sites that tested positive for pathogens (Table 4).

These collections should include approximately 8 to12 locations to monitor tick density, utilizing drag sampling once per month from the last week of May to the first week of June and maintained the sampling period from the last week of October first week of November. This approach would encompass the larval and adult peaks for *I. scapularis*, *A. americanum* and *D. variabilis* as reported in Suffolk County (Campbell et al., 2015). In the future, I recommend continually evaluating tick counts and DON at the sampling sites, as well as pathogen testing if possible. Sites where no ticks are collected over a period of time may be removed as necessary. This could be repeated yearly to ensure the inclusion of appropriate sites.

This option may not be appropriate for Mashomack Preserve because the current sampling sites cover most of the trails that visitors access, and choosing other sites that are accessible and visited by tourists may not be possible. However, when examining tick abundance data from the preserve, all sites have a high abundance and should continue to be surveyed in the future unless further data collection indicates a change. This would allow for flexibility in choosing other sites on the island excluding the Preserve. For example, four sites may be selected on the Preserve, and an additional 4 to 8 sites on the rest of the island.

Management Recommendations

Based on these results, I recommend that historic sampling protocols be updated to focus on capturing density based tick data. A more complete sampling protocol may indicate further *A. americanum* hotspots similar to the Visitor Center location. Infection data indicated that while *I. scapularis* nymphs were collected less frequently, they were the only pathogen-infected ticks, and therefore represented a larger risk for people and pets. Additionally, the infected ticks were found in areas that had low DON. This indicates that monitoring infection data will be important to evaluate potential risk in the future. DON may indicate bite risk, but because no *A. americanum* were infected, it does not give a true indication of infection risk. It will be important to convey this properly in any future educational materials, as visitors may falsely believe that a low density of ticks may indicate a low disease transmission risk. Despite this, the risk of the alpha-gal allergy remains and so the high density of *A. americanum* cannot be ignored. Additionally, it is possible that other pathogens may be introduced that *A. americanum* is competent for. Evaluating and continually updating the sampling protocol will be necessary in the future. Despite the extended length of historical data spanning over almost ten years, it shows little value in comparing density due to time based sampling method. Sampling should be combined with periodic infection testing at both hotspots and sites that may not have unusually high tick abundance but are otherwise well-trafficked to create a comprehensive picture of the infection risk on the island.

Outreach & Education

Shelter Island presents an interesting case for education and outreach. With the removal of the four-posters, there are no island-wide, chemical tick control measures in place. In their review on the prevention of tick-borne diseases Piesman and Eisen (2008), suggested that education is the most important factor when attempting to prevent tick borne illness. Other discussions of Lyme disease have highlighted the risk travelers face when they are not familiar with the local area or are uncertain how to access information regarding risk. Donohoe et al (2015) recommended that tick hotspots be clearly marked and disease risk identified. These educational measures could be implemented on the island by placing brochures and warning signs at the Visitor Center on Mashomack Preserve, as well as at trailheads. With the high abundance of *A. americanum* found on the island, it would be beneficial to include images of *A. americanum* and information about this tick, with the goal of increasing local knowledge about this tick species.

APPENDIX 1: Historical tick sampling comparisons

Fig. a1. *A. americanum* and *I. scapularis* nymphs and adults compared from 2016 – 2020 when sampling was conducted on Mashomack Point, where no four poster was located. Other locations are averaged. *A. americanum* nymphs are faceted due to a single collection of 473 nymphs in 2018 to present an accurate scale for other years.

APPENDIX 2: Tick sampling data

Table a1. Tick collection totals by life stage, site, and date for *A. americanum* (*A.a.*), *I. scapularis* (*I.i*) and *D. variabilis* (*D. v.*) collected in summer 2020 on Shelter Island, NY.

Date	Location	*A. a.* F	*A. a.* M	*A. a.* nymph	*A. a.* larvae	*I. s* F	*I. s.* M	*I. s.* nymph	*I. s.* larvae	*D. v.* F	*D. v.* M
7/9/2020	Catholic	0	0	2	0	0	0	3	0	0	0
7/16/2020	Catholic	0	0	3	0	0	0	0	0	0	0
7/26/2020	Catholic	0	0	1	0	0	0	0	8	0	0
8/1/2020	Catholic	0	0	0	0	0	0	2	153	0	0
8/9/2020	Catholic	0	0	0	271	0	0	1	12	0	0
7/9/2020	Quaker	1	0	0	0	0	0	3	0	0	0
7/16/2020	Quaker	0	0	7	0	0	0	3	399	0	0
7/26/2020	Quaker	0	0	8	0	0	0	5	223	0	0
8/1/2020	Quaker	0	0	4	0	0	0	5	229	0	0
8/9/2020	Quaker	0	0	0	485	0	0	2	43	0	0
6/22/2020	Red	3	1	7	0	0	0	3	0	0	0
6/26/2020	Red	0	0	20	0	0	0	3	0	0	0
6/29/2020	Red	1	0	8	0	0	0	3	0	0	0
7/6/2020	Red	2	1	12	0	0	0	6	0	0	0
7/14/2020	Red	0	0	3	0	0	0	4	176	0	0
7/22/2020	Red	0	0	1	0	0	0	1	110	0	0
7/30/2020	Red	0	0	0	1	0	0	1	6	0	0
8/6/2020	Red	0	0	0	0	0	0	0	0	0	0
6/13/2020	Tower	1	1	1	0	0	0	0	0	0	0
6/16/2020	Tower	0	0	3	0	0	0	0	0	0	0
6/24/2020	Tower	1	1	0	0	0	0	0	0	1	1
6/29/2020	Tower	0	0	0	0	0	0	0	0	0	0
7/6/2020	Tower	0	0	1	0	0	0	2	0	2	0
7/14/2020	Tower	0	0	4	0	0	0	0	0	2	2
7/22/2020	Tower	0	0	2	36	0	0	0	0	1	0

7/30/2020	Tower	0	0	0	0	0	0	0	0	0	0
8/6/2020	Tower	0	0	0	0	0	0	0	0	0	0
6/20/2020	Triangle	4	1	10	0	0	0	3	0	0	0
6/27/2020	Triangle	4	1	3	0	0	0	1	0	0	0
7/5/2020	Triangle	1	1	4	0	0	0	0	0	0	0
7/7/2020	Triangle	3	0	1	0	0	0	1	0	0	0
7/14/2020	Triangle	0	0	5	0	0	0	1	311	1	0
7/21/2020	Triangle	0	0	0	0	0	0	1	175	1	1
7/29/2020	Triangle	0	1	4	33	0	0	2	176	0	0
8/4/2020	Triangle	0	0	1	299	0	0	0	72	0	0
6/14/2020	Visitor	1	1	25	0	0	0	0	0	0	0
6/16/2020	Visitor	0	0	10	0	0	0	2	0	0	0
6/26/2020	Visitor	1	0	9	0	0	0	1	0	0	0
6/29/2020	Visitor	0	1	49	0	0	0	10	0	0	0
7/6/2020	Visitor	0	1	58	0	0	0	6	0	0	0
7/14/2020	Visitor	0	0	29	0	0	0	4	0	0	0
7/22/2020	Visitor	0	0	25	0	0	0	1	68	0	0
7/30/2020	Visitor	0	0	3	22	0	0	0	19	0	0
8/6/2020	Visitor	0	0	11	20	0	0	1	125	0	0
7/9/2020	Williams	0	0	7	0	0	0	3	133	0	1
7/16/2020	Williams	0	0	9	0	0	0	0	217	0	0
7/26/2020	Williams	1	2	7	0	0	0	2	486	0	0
8/1/2020	Williams	0	0	10	157	0	0	4	440	0	2
8/9/2020	Williams	0	0	1	285	0	0	0	327	0	0

APPENDIX 3: Tick infection testing results

Table a2. Tick pathogen testing results by species and life stage, and location collection from summer 2020 on Shelter Island, NY. PWV = Powassan virus, HRTV = Heartland virus, RSFG = Rickettsia spotted fever group, MH = *Mycoplasma haemocanis*, TO = *Theileria orientalis*, *RS* = *Rickettsia spp*, a rickettsial endosymbiont. Spp. = species. Visitor extra refers to extra dragging performed to collect ticks for testing.

Site	Spp.	Stage	MS2	*A. marginale*	*A. phagocytophilum*	*B. microti*	*Bartonella spp*	*B. burgdorferi*	*B. mayonii*	*B. miyamotoi*	HRTV	MH	PWV	RSFG	*RS*	SFTSV	TO
Triangle	*A.a.*	Nymph	15.412														
Triangle	*A.a.*	Nymph	14.671														
Triangle	*A.a.*	Nymph	14.503												10.3		
Triangle	*A.a.*	Nymph	14.777												9.7		
Triangle	*A.a.*	Male	15.498												8.2		
Triangle	*A.a.*	Nymph	15.323			10.5		15.9							16.7		
Triangle	*I.s.*	Nymph	15.694												18.9		
Manor	*A.a.*	Male	16.022														
Manor	*A.a.*	Nymph	14.779												12.0		
Manor	*A.a.*	Nymph	14.982														
Triangle Extra	*A.a.*	Female	15.81												8.5		
Triangle Extra	*D.v.*	Female	15.622														
Triangle Extra	*A.a.*	Nymph	14.985												18.7		
Triangle Extra	*A.a.*	Nymph	14.868												16.9		
Triangle Extra	*A.a.*	Nymph	15.646														
Manor	*I.s.*	Nymph	16.135												11.0		
Manor	*I.s.*	Nymph	15.356														
Red	*I.s.*	Nymph	15.21														
Red	*I.s.*	Nymph	15.022												14.0		
Visitor Extra	*A.a.*	Nymph	15.139														
Visitor Extra	*A.a.*	Nymph	14.864												11.4		
Visitor Extra	*A.a.*	Nymph	15.737												10.9		

Visitor Extra	*A.a.*	Nymph	15.601												9.5		
Visitor Extra	*A.a.*	Nymph	15.579												17.2		
Visitor Extra	*A.a.*	Nymph	14.993												17.1		
Visitor Extra	*I.s.*	Nymph	14.909												20.5		
Visitor	*A.a.*	Nymph	15.575														
Visitor	*A.a.*	Nymph	14.74												10.9		
Visitor	*A.a.*	Nymph	15.206												10.8		
Manor	*A.a.*	Male	14.912														
Manor	*A.a.*	Male	15.514												11.0		
Manor	*A.a.*	Male	15.117												12.8		
Manor	*I.s.*	Nymph	14.737														
Manor	*I.s.*	Nymph	14.804														
Manor	*A.a.*	Female	15.747														
Manor	*A.a.*	Female	16.146												8.8		
Manor	*A.a.*	Nymph	14.818												17.3		
Manor	*A.a.*	Nymph	15.199												11.8		
Catholic	*I.s.*	Nymph	14.786							16.0					17.7		
Catholic	*I.s.*	Nymph	14.811												16.5		
Quaker	*A.a.*	Nymph	14.733												13.1		
Quaker	*A.a.*	Nymph	14.978												18.0		
Quaker	*A.a.*	Nymph	14.961												13.3		
Quaker	*A.a.*	Nymph	14.791														
Quaker	*I.s.*	Nymph	14.963			20.3											
Quaker	*I.s.*	Nymph	14.536														
Quaker	*I.s.*	Nymph	14.776														
Quaker	*I.s.*	Nymph	15.053		19.0							18.9			19.9		
Quaker	*I.s.*	Nymph	14.144												19.7		
Williams	*D.v.*	Male	15.204														
Williams	*D.v.*	Male	15.069														
Williams	*A.a.*	Nymph	14.738														
Williams	*A.a.*	Nymph	14.262												14.5		

Williams	*A.a.*	Nymph	14.628														
Williams	*A.a.*	Nymph	14.947														
Williams	*A.a.*	Nymph	14.444														
Williams	*A.a.*	Nymph	14.715														
Williams	*A.a.*	Nymph	14.67												14.2		
Williams	*A.a.*	Nymph	14.497												12.9		
Williams	*A.a.*	Nymph	14.417												17.5		
Williams	*A.a.*	Nymph	14.953												11.5		
Williams	*Ixo*	Nymph	14.641														
Williams	*Ixo*	Nymph	14.264												18.9		
Williams	*I.s.*	Nymph	14.517												17.5		
Williams	*I.s.*	Nymph	14.536												18.4		
Triangle	*A.a.*	Nymph	14.742														
Visitor	*I.s.*	Nymph	14.163														
Visitor	*A.a.*	Nymph	13.995												11.5		
Visitor	*A.a.*	Nymph	14.349														
Visitor	*A.a.*	Nymph	14.74												12.9		
Visitor	*A.a.*	Nymph	14.384														
Visitor	*A.a.*	Nymph	14.449														
Visitor	*A.a.*	Nymph	14.046														
Visitor	*A.a.*	Nymph	14.198														
Visitor	*A.a.*	Nymph	14.016														
Visitor	*A.a.*	Nymph	14.376														
Visitor	*A.a.*	Nymph	14.083												10.6		
Visitor	*A.a.*	Nymph	14.616												13.4		
Visitor Extra	*A.a.*	Nymph	13.884														
Visitor Extra	*A.a.*	Nymph	14.096														
Visitor Extra	*A.a.*	Nymph	14.19														
Visitor Extra	*A.a.*	Nymph	14.417														
Visitor Extra	*A.a.*	Nymph	13.787												12.5		

Visitor Extra	*A.a.*	Nymph	14.446												13.1		
Visitor Extra	*A.a.*	Nymph	13.833														
Visitor Extra	*A.a.*	Male	14.173												11.1		
Point	*A.a.*	Female	14.769												11.0		
Point	*A.a.*	Nymph	14.478												14.2		
Point	*A.a.*	Nymph	14.5												15.7		
Point	*A.a.*	Nymph	14.431												16.9		
Point	*A.a.*	Nymph	14.755														
Point	*A.a.*	Nymph	14.896														
Point	*A.a.*	Nymph	14.784														
Point	*A.a.*	Male	14.756														
Point	*A.a.*	Male	14.165														
Point	*Amb*	Nymph	14.301														
Point	*A.a.*	Nymph	14.396												15.1		
Point	*A.a.*	Nymph	14.325												12.4		
Point	*A.a.*	Nymph	14.561												16.6		
Point	*A.a.*	Nymph	14.644												18.9		
Point	*A.a.*	Nymph	14.796												16.2		
Point	*A.a.*	Nymph	14.616												13.9		
Point	*A.a.*	Nymph	14.286														
Point	*A.a*	Nymph	14.234														
Point	*A.a.*	Nymph	14.535												17.4		
Point	*A.a.*	Nymph	14.525												16.6		
Point	*A.a.*	Nymph	14.555												14.6		
Point	*A.a.*	Nymph	14.62														
Point	*A.a.*	Nymph	14.212														
Point	*A.a.*	Nymph	14.735														
Point	*A.a.*	Nymph	14.136												15.2		
Point	*A.a.*	Male	14.715												12.4		
Point	*D.v.*		14.956														
Point	*D.v.*		14.872												19.9		
Point	*D.v.*		14.716														

Visitor Extra	*A.a.*	Nymph	14.526														
Visitor Extra	*A.a.*	Nymph	14.313														
Visitor Extra	*A.a.*	Nymph	13.874												20.1		
Visitor Extra	*A.a.*	Nymph	14.064												15.2		
Visitor Extra	*A.a.*	Nymph	13.779														
Visitor Extra	*A.a.*	Nymph	13.782												17.3		
Visitor Extra	*A.a.*	Nymph	13.835												19.8		
Visitor Extra	*A.a.*	Nymph	14.275														
Visitor Extra	*A.a.*	Nymph	13.857												16.4		
Visitor Extra	*A.a.*	Nymph	14.032														
Visitor Extra	*A.a.*	Nymph	13.815												15.2		
Visitor Extra	*A.a.*	Nymph	13.831												16.8		
Visitor Extra	*A.a.*	Nymph	14.242														
Visitor Extra	*A.a.*	Nymph	14.207														
Visitor Extra	*A.a.*	Nymph	14.289												11.4		
Visitor Extra	*A.a.*	Nymph	16.519												19.6		
Visitor Extra	*A.a.*	Nymph	14.056												18.0		
Visitor Extra	*A.a.*	Nymph	14.596												16.4		
Visitor Extra	*A.a.*	Nymph	14.254														
Visitor Extra	*A.a.*	Nymph	14.134														

Visitor Extra	*A.a.*	Nymph	14.139														
Visitor Extra	*A.a.*	Nymph	14.109														
Visitor Extra	*A.a.*	Nymph	14.491												16.5		
Visitor Extra	*A.a.*	Nymph	14.364												17.1		
Visitor Extra	*A.a.*	Nymph	14.219												21.3		
Visitor Extra	*A.a.*	Nymph	14.561												21.6		
Visitor Extra	*A.a.*	Nymph	14.207												15.9		
Visitor Extra	*A.a.*	Nymph	14.214												13.8		
Visitor Extra	*A.a.*	Nymph	14.336												17.9		
Visitor Extra	*A.a.*	Nymph	14.523												17.4		
Visitor Extra	*A.a.*	Nymph	14.575												15.4		
Visitor Extra	*A.a.*	Nymph	14.306														
Visitor Extra	*A.a.*	Nymph	14.375												14.1		
Visitor Extra	*A.a.*	Nymph	14.3												13.3		
Visitor Extra	*A.a.*	Nymph	14.119														
Visitor Extra	*A.a.*	Nymph	14.842												20.3		
Visitor Extra	*A.a.*	Nymph	15.231														
Visitor Extra	*A.a.*	Nymph	14.43														
Visitor Extra	*A.a.*	Nymph	14.684														
Visitor Extra	*A.a.*	Nymph	14.368														

Visitor Extra	*A.a.*	Nymph	14.717												14.7		
Visitor Extra	*A.a.*	Nymph	14.147												16.4		
Visitor Extra	*A.a.*	Nymph	14.218												15.8		
Visitor Extra	*A.a.*	Nymph	14.201														
Visitor Extra	*A.a.*	Nymph	14.375														
Visitor Extra	*A.a.*	Nymph	14.017														
Visitor Extra	*A.a.*	Nymph	14.1												20.2		
Visitor Extra	*A.a.*	Nymph	14.268														
Visitor Extra	*A.a.*	Nymph	14.23												15.4		
Visitor Extra	*A.a.*	Nymph	14.381												12.1		
Visitor Extra	*A.a.*	Nymph	14.3												19.8		
Visitor Extra	*A.a.*	Nymph	14.091														
Visitor Extra	*A.a.*	Nymph	14.063												17.9		
Visitor Extra	*A.a.*	Nymph	13.751												16.0		
Visitor Extra	*A.a.*	Nymph	14.393														
Visitor Extra	*A.a.*	Nymph	15.368												16.8		
Visitor Extra	*A.a.*	Nymph	15.154												16.0		
Visitor Extra	*A.a.*	Nymph	14.35												14.5		
Visitor Extra	*A.a.*	Nymph	14.511												18.5		
Visitor Extra	*A.a.*	Nymph	14.263												14.6		

Visitor Extra	*A.a.*	Nymph	14.86												12.8		
Visitor Extra	*A.a.*	Nymph	14.94												15.2		
Visitor Extra	*A.a.*	Nymph	14.659														
Visitor Extra	*A.a.*	Nymph	14.987														
Visitor Extra	*A.a.*	Nymph	14.137												17.9		
Visitor Extra	*A.a.*	Nymph	14.448														
Visitor Extra	*A.a.*	Nymph	14.525												16.8		
Visitor Extra	*A.a.*	Nymph	14.612														
Visitor Extra	*A.a.*	Nymph	14.908														
Visitor Extra	*A.a.*	Nymph	14.7												13.8		
Visitor Extra	*A.a.*	Nymph	14.413												16.7		
Visitor Extra	*A.a.*	Nymph	14.606														
Visitor Extra	*A.a.*	Nymph	14.912														
Visitor Extra	*A.a.*	Nymph	14.769														
Visitor Extra	*A.a.*	Nymph	14.561														
Visitor Extra	*A.a.*	Nymph	14.432														
Visitor Extra	*A.a.*	Nymph	14.773														
Visitor Extra	*A.a.*	Nymph	14.592												16.4		
Visitor Extra	*A.a.*	Nymph	14.761												17.2		
Visitor Extra	*I.s.*	Nymph	14.879														

Visitor Extra	*I.s.*	Nymph	14.476														
Visitor Extra	*I.s.*	Nymph	14.521														
Visitor Extra	*A.a.*	Male	14.793														
Quaker	*I.s.*	Nymph	14.579		18.9												
Quaker	*I.s.*	Nymph	14.063														
Catholic	*I.s.*	Nymph	14.737												13.7		
Williams	*A.a.*	Nymph	14.297												14.7		

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