THE EFFECT OF FOREST FRAGMENTATION ON LYME DISEASE RISK

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Abstract. Unknown to the United States three decades ago, Lyme disease is now the most prevalent vector-borne disease in the country. Caused by the bacterium *Borrelia burgdorferi*, Lyme disease is typically transmitted to humans by the bite of a blacklegged tick (*Ixodes scapularis*) in the nymph stage. For this reason the density of infected nymphs is considered the most precise ecological indicator of Lyme disease risk. The white-footed mouse (*Peromyscus leucopus*) is the most competent reservoir for the transmission of Lyme disease to blacklegged ticks, and has been shown to reach extraordinary densities in small woodlots (<1-2 ha) created by forest fragmentation. Thus we hypothesized that Lyme disease risk would be inversely related to the area of forest patches. Forest fragments, ranging from 0.7 ha – 7.6 ha, were chosen using GIS maps of the forested cover of Dutchess County, New York, as were three continuously forested areas. Density estimates were taken by drag sampling, and infection prevalence was assessed by dissecting ticks and determining the presence or absence of *Borrelia burgdorferi* spirochetes. Analysis of forest fragments demonstrated a strong, inverse relationship with patch size for blacklegged nymphal tick density ($R^2 = 0.51$, $P = 0.02$, $N = 14$), infection prevalence ($R^2 = 0.43$, $P = 0.011$, $N = 14$), and their product, the density of infected nymphs ($R^2 = 0.65$, $P = 0.01$, $N = 14$). Larval density, on the other hand, showed no relationship with patch area ($R^2 = 0.01$, $P = 0.78$, $N = 14$). Multiple linear regression of the combined influence of patch area and density of larvae, for fragments only, found the density of infected nymphs to be a negative linear function of patch area ($P = 0.018$), but not significantly related to the density of larvae ($P = 0.099$) (model $R^2 = 0.52$). When forest fragments were combined with three continuous sites, the effect of patch area was dampened, seemingly because high larval densities in the continuous sites appeared to drive very high nymph densities. Nevertheless, infection prevalence of those nymphs was quite low compared to the small fragments, and nymph infection prevalence was, in fact, a negative exponential function of forest area for all sites ($R^2 = 0.57$, $P < 0.0005$, $N = 17$). These results suggest that Lyme disease risk is inversely related to forest patch area, with small patches (<1-2 ha) having higher Lyme disease risk due to higher densities of infected nymphs. Efforts to manage the spread of Lyme disease should be directed towards the prevention of the fragmentation of forests into patches of less than 1-2 ha, given that these patches appear particularly prone to high densities of infected nymphal-blacklegged ticks.

INTRODUCTION

Human activities in the northeastern United States have resulted in the fragmentation of what was once a predominantly forested landscape. Forest fragmentation reduces the total area of habitat and average patch size, and increases the ratio of edge to interior and the mean distance between patches (Murcia 1995). One key consequence of forest fragmentation is a reduction of species diversity in remnant forest patches, a pattern that has been demonstrated for both birds (Blake and Karr 1987) and mammals (Rosenblatt et al. 1999). However, certain species, typically habitat generalists or species characterized by high population densities and small home range sizes, appear to thrive in highly fragmented landscapes. One such organism, the white-footed mouse (*Peromyscus leucopus*), has been shown to attain unusually high densities in small forest fragments, possibly resulting from restricted rates of emigration due to patch isolation, a decrease in abundance of both predators and competitors, and an increase in edge habitat (Nupp and Swihart 1998). Densities of *P. leucopus* tend to be inversely correlated with forest patch area (Nupp and Swihart 1996, Krohne and Hoch 1999).

We predicted that the reduced species diversity and increased abundance of *P. leucopus* that accompany forest fragmentation would increase risk of human exposure to Lyme disease. Lyme disease is caused by the bacterium, *Borrelia burgdorferi*, which, in eastern and central North America, is transmitted by the bite of an infected blacklegged tick, *Ixodes scapularis*. Larval ticks typically hatch uninfected with *B. burgdorferi*, and attempt a blood meal from any of dozens of species of mammals, birds, and lizards. Larvae that feed from white-footed mice are highly likely to become infected, but those that feed from most other mammals, birds, and reptiles are unlikely to become infected (Anderson and Magnarelli 1993, Lane et al. 1991, Ostfeld and Keesing 2000a). Larvae acquiring a *B. burgdorferi* infection molt into infected nymphs, which may then transmit the infection to humans, causing Lyme disease. The majority of Lyme disease cases are thought to be transmitted by nymphal ticks, owing to their small size and summer peak in activity, which coincides with human recreational use of the outdoors (Falco and Fish 1989; Barbour and Fish 1993). Adult ticks also may be infected, but are larger, easier to detect, and active in late fall and early spring (Lane et al. 1991).

Lyme disease risk is related to both the proportion of nymphs that are infected (or the nymphal infection prevalence: NIP) and the density of infected nymphs (DIN) (Ostfeld and Keesing 2000b, Ostfeld et al. in press). NIP is a function of the distribution of larval meals among the community of vertebrate hosts, and is expected to increase with: (1) increasing absolute density of white-footed mice; and (2) decreasing species diversity in the host community (Ostfeld and Keesing 2000a,b, Schmidt and Ostfeld 2001). On the other hand, causes of variation in DIN are more complex. DIN is the product of NIP and the total density of nymphs (DON). Several factors can influence DON, including the abundance of larvae and survival of those larvae to the nymphal stage. Larval density, in turn, is determined largely by the distribution of white-tailed deer (Wilson et al. 1985), since this species is the primary host for adult blacklegged ticks and the site of mating and larval production (Ostfeld et al. 1996).

Although mouse populations are expected to increase with decreasing forest patch area (Nupp and Swihart 1996, Krohne and Hoch 1999), fragmented areas may be less frequently visited by white-tailed deer (Nixon et al., 1991). Therefore, small patches can be expected to support lower densities of larval ticks but high availability of mouse hosts, which should boost both larval feeding success and probability of acquiring an infection. The net effect of forest patch size on the density and infection prevalence of nymphal ticks may be hard to predict.

Given the presumptive effects of forest fragmentation on vertebrate hosts for ticks and the effects of the vertebrate community on the density and infection prevalence of ticks, we generated the following general hypotheses: (1) NIP is negatively correlated with forest patch area; and (2) DON is negatively correlated with forest patch area and positively correlated with the density of larval ticks. Therefore, (3) DIN is negatively correlated with forest patch area and positively correlated with the density of larval ticks.

METHODS

We established a field-sampling program to determine the distribution of ticks among forest patches of different areas and the prevalence of *Borrelia burgdorferi* infection within ticks within Dutchess County, southeastern New York (41°50' N, 73°45' W). Based on previous investigations that indicated that white-footed mouse densities increased dramatically in small (<2 ha) forest patches compared to larger (>2 ha) patches (Nupp and Swihart 1996, Krohne and Hoch 1999), we selected 14 forest fragments from 0.7 to 7.6 ha. We included three larger, continuously forested sites of 458, 988, and 1,825 ha in area, in order to extend our comparison of highly fragmented to less fragmented landscapes.

All forest patches were selected to be a minimum of 250m from the next nearest forested site. All sites were dominated by sugar maple (*Acer saccharum*) or red maple (*A. rubra*), because maple-dominated forest has been shown to support the highest densities of blacklegged ticks in Dutchess County sites (Ostfeld 1997).

Oak-dominated sites were avoided since considerable variation among years can exist in this habitat due to masting (Ostfeld et al., 1996). Study sites were chosen using GIS maps of Dutchess County, which were prepared from 1994-5 USDA black and white aerial photos by the Dutchess County Environmental Management Council (Millbrook, NY). Their classification into land cover types was based on the LUNR Classification Manual from Cornell University's Institute for Resource Information Systems (Ithaca, NY). Sites were ground-truthed to confirm size and tree species composition.

Nymphal ticks were collected from all sites during the peak in nymphal activity (10 June – 15 July, 2000), and larval ticks were collected from all sites during the peak in larval activity (August, 2000). Although the nymphal cohort in 2000 arose from larvae present in 1999, we assumed that larvae are in approximately steady state from year to year, and that the 2000 larval cohort would represent average abundance through time. At each site we established a set of parallel line transects totaling 400m. Transects were at least 10m apart and varied from 50 to 100m in length. We used a standard drag-sampling technique (e.g., Falco and Fish, 1992) to determine the density of ticks among patches of different area and to collect ticks for determination of spirochete prevalence. The drag-sampling method has been demonstrated to be both reliable and efficient in censusing *I. scapularis* (Falco and Fish, 1992), and to minimize biases among habitat types in sampling effectiveness (Ostfeld et al., 1995). Thus, a 1-m^2 drag cloth was dragged the length of each transect, held as close to the ground as possible. We stopped to examine the cloth every 20m, and all ticks were removed with fine forceps and either maintained alive until dissection, or preserved in 70% ethanol for later identification.

At least 20 nymphal ticks were examined per site for the presence of *Borrelia burgdorferi* using immunofluorescence microscopy. Ticks were washed once in 70% ethanol and twice in deionized water, placed in an Eppendorf tube, and ground in phosphate buffered saline (PBS). Three 5-mL aliquots of tick suspension were placed in separate wells in multiwell slides, air-dried, and fixed in cold acetone for 10 min. Fluorescent rabbit anti-*Borrelia* conjugate was added to wells and incubated for 45 minutes at 37^{o}C. Slides were then washed in PBS, dried, and placed in fluorescent-antibody mounting medium. We examined the slides under an Olympus BH-2 binocular microscope. If spirochetes were not detected immediately, the three wells per individual were examined systematically. Each individual was categorized as positive or negative for *B. burgdorferi.*

Our general approach was to use simple regression analyses to test whether the density of nymphs (DON), the nymph infection prevalence (NIP), and the density of infected nymphs (DIN) were a significant function of patch area. Because nymph parameters may be influenced by larval abundance via demographic forcing, we also used multiple regression analyses to test for the effects of both density of larvae (DOL) and patch area (independent variables) on DON, NIP, and DIN (dependent variables). To assess the effects of patch size per se, we initially analyzed the 14 fragments separately. We followed this with analyses that included the three continuous areas. The areas of the 14 forest fragments were analyzed untransformed, but for the analysis of all 17 sites we used log (area), to correct for the strongly leptokurtic distribution.

RESULTS

Analyses of Forest Fragments

Nymph density, nymph infection prevalence, and their product, the density of infected nymphs, were all found to be a negative function of patch area. A simple regression found DON to be a significant negative exponential function of patch area (R^2 = 0.51, P = 0.02, N = 14; Fig. 1; exponential model R^2 33% better fit than linear model R^2). Nymph density averaged 0.1/m^2 in the five smallest fragments (<1.2 ha), and declined to an average of 0.03/m^2 in larger fragments. NIP was a significant negative linear function of patch area (R^2 = 0.43, P = 0.011, N = 14; Fig. 2). The

percentage of infected nymphs decreased from an average of 70% in the smallest fragments, to an average of 48% in larger fragments. DIN was significantly related to patch area by a negative exponential function (R^2 = 0.65, P = 0.01, N = 14; Fig 3; exponential model R^2 50% better fit than linear model R^2). The five smallest fragments supported an average of 0.07 infected nymphs/m^2, whereas the larger fragments contained an average of 0.01 infected nymphs/m^2. A simple regression indicated that DOL was not significantly related to patch area (R^2 = 0.01, P = 0.78, N = 14; Fig. 4).

Both DOL and patch area appeared to influence the nymphal population. Multiple linear regression of the combined influence of patch area and DOL found the density of nymphs to be a positive linear function of the DOL (P = 0.047) and a negative linear function of patch area (P = 0.029) (model R^2 = 0.53). Multiple linear regression of the combined influence of patch area and DOL on DIN was qualitatively similar. DIN was a negative linear function of patch area (P = 0.018), but was not significantly related to the DOL (P = 0.099) (model R^2 = 0.52).

Analyses of Forest Fragments plus Continuous Forest Sites

Inclusion of the continuous forest sites revealed somewhat different determinants of Lyme disease risk than did analyses of the fragments only. Multiple linear regression of the combined influence of patch area and DOL for all sites indicated that the DON was positively related to DOL (P = 0.001), but not significantly related to patch area (P= 0.40; model R^2 = 0.88; Fig. 5).

A simple regression found NIP to be a significant negative exponential function of forest area (R^2 = 0.57, P < 0.0005, N = 17; Fig. 6; exponential model R^2 16% better fit than linear model R^2). Multiple linear regression of the combined influence of patch area and DOL on NIP indicated a negative linear correlation with patch area (P = 0.01), but no significant correlation with DOL (P = 0.63) (model R^2 = 0.49). The percent of nymphs infected decreased from an average of 70% in the smallest fragments, to an average of 32% in the continuous sites. Multiple linear regressions across all sites found DIN to be a positive linear function of DOL (P = 0.001), but not significantly related to patch area (P = 0.34) (model R^2 = 0.88; Fig. 7).

DISCUSSION

We found that, in highly fragmented landscapes, the density of nymphs, nymph infection prevalence, and their product, the density of infected nymphs, were inversely correlated with forest patch area. These metrics of the nymphal tick population are ecological indicators of Lyme disease risk. The elevated risk of exposure to Lyme disease in the smallest patches appears to result from two interrelated phenomena. First, the loss of biodiversity that accompanies forest fragmentation (Blake and Karr 1987, Rosenblatt et al. 1999) reduces the abundance of tick hosts that are poor reservoirs for Lyme disease bacteria (Ostfeld and Keesing 2000a,b). Low biodiversity decreases the fraction of tick meals that are taken from poor reservoirs and increases NIP (Ostfeld and Keesing 2000a). Second, because the vertebrate species lost from the smallest forest fragments tend to be predators on and competitors with white-footed mice (Rosenblatt et al. 1999, Schmidt and Ostfeld 2001), regulation of *P. leucopus* may be weaker in smaller patches, resulting in high absolute density of mice observed in several studies (Nupp and Swihart 1996, 1998, Krohne and Hoch 1999, Rosenblatt et al. 1999). Such an increase in both the relative and absolute density of white-footed mice, which are the principal Lyme disease reservoir, would appear to increase both NIP and DON, and consequently, their product, DIN (Ostfeld and Keesing 2000b).

When three continuously forested areas were included in the analyses, the pattern changed somewhat. Although NIP continued to show a strong, inverse correlation with forest area, the inverse relationship between DON and forest area was destroyed, largely because one of the continuous sites supported enormously high nymphal abundance. Abundance of nymphs should be a function of the abundance of the prior year's larvae, a process we call demographic forcing, and the survival of the larval cohort to the nymphal

stage. We postulate that the continuously forested areas were more heavily used than were the fragments by white-tailed deer (Nixon et al. 1991). Because white-tailed deer are the primary host for adult ticks and are a critical determinant of the distribution of larval ticks (Wilson et al. 1985, Telford et al. 1992), attraction of deer might have resulted in high numbers of larval ticks in continuous sites. In the absence of a survival bias due to patch size, high larval densities, in turn, would cause high nymphal densities to occur in these sites. Indeed, we found a strong positive correlation among sites between DOL and DON. Thus, for analyses of the 14 fragments only, both density and infection prevalence of nymphs decreased with increasing patch area, whereas when the three continuous sites were included, only NIP remained a decreasing function of forest area.

Thus it seems that in a fragmented forest landscape, the principle determinant of Lyme disease risk is patch area, which is inversely related to the density of infected nymphal *I. scapularis*, while in a continuously forested landscape, the density of larval ticks plays a key role. Given the trend demonstrated by white-footed mice with respect to forest area in a fragmented landscape, it seems highly likely that this host for juvenile ticks is the cause of the inverse relationship between Lyme disease risk and forest fragment size. On the other hand, much evidence indicates that the abundance of larval ticks is associated with deer abundance. Little can be done to alter the relationship between the density of larvae and the density of infected nymphs in continuously forested areas, as efforts to control the deer population in such areas have little effect on *Ixodes* tick population dynamics (Wilson et al., 1988, Van Buskirk and Ostfeld 1995). However, much can be done to halt forest fragmentation and the increase in Lyme disease risk that it appears to cause. Efforts to manage the spread of Lyme disease should be directed towards preventing fragmentation of the deciduous forests of the northeastern United States, particularly in areas of high Lyme disease incidence. The creation of forest fragments of less than 1-2 ha should especially be avoided, given that these patches are particularly prone to high white-footed mouse densities, and thus higher densities of infected nymphal-blacklegged ticks.

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APPENDIX

Thirty-one field sites were sampled either for the density of nymphs and nymph infection prevalence, or just for nymph infection prevalence. Fourteen of the thirty-one field sites were not analyzed in this study, for various *a priori* reasons, which were: insufficient maple dominance, insufficient *Ixodes scapularis* population to provide reasonable sample size, and/or infeasibility of drag sampling technique. The seventeen sites that were included in our analyses, the fourteen fragments and three continuous forests, were the only sites that met all criteria and were thus included in this investigation.

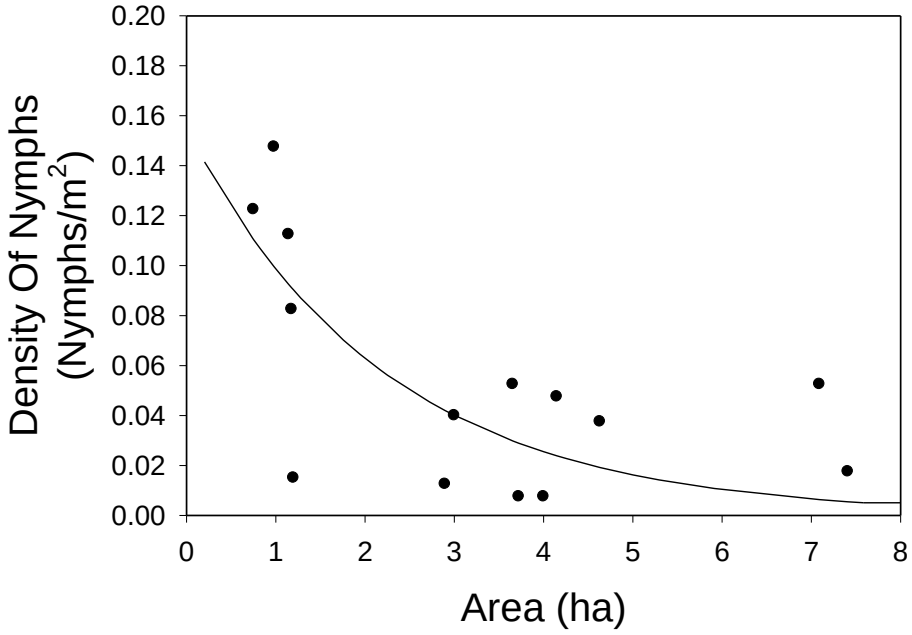

FIGURE 1. Density of nymphal *I. scapularis* versus the area of forest fragments in a highly fragmented landscape. $R^2 = 0.51$, $P < 0.02$, $N = 14$; exponential model R^2 33% better model than linear model R^2.

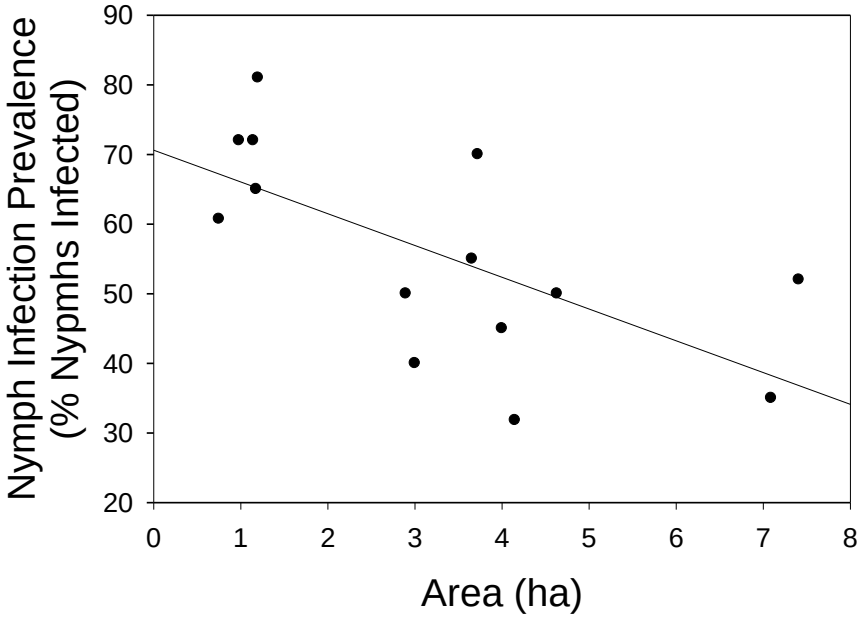

FIGURE. 2. The percentage of nymphal *I. scapularis* infected with Lyme Disease versus the area of forest fragments in a highly fragmented landscape. $R^2 = 0.43$, $P < 0.01$, $N = 14$.

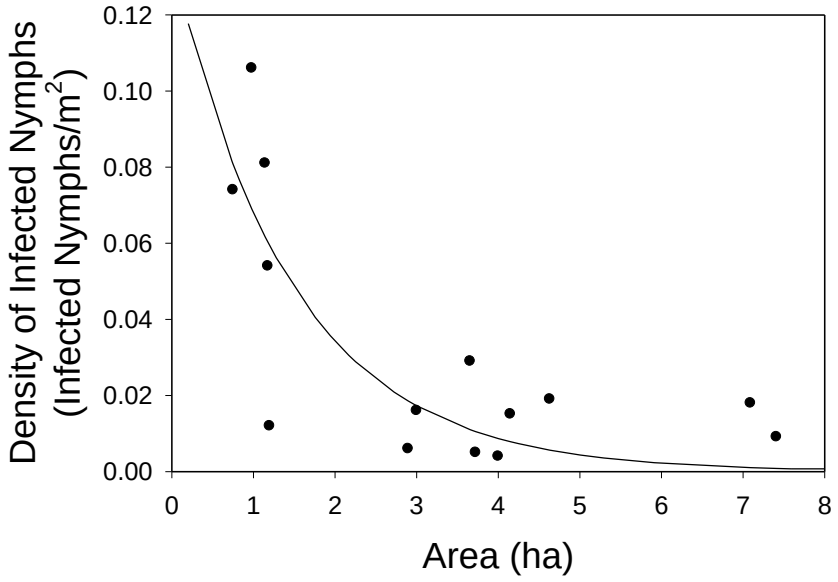

FIGURE. 3. The density of nymphal *I. scapularis* infected with Lyme Disease versus the area of forest fragments in a highly fragmented landscape. The density of infected nymphs is considered the best ecological indicator of Lyme Disease risk. $R^2 = 0.65$, $P < 0.01$, $N = 14$; exponential model R^2 50% better model than linear model R^2.

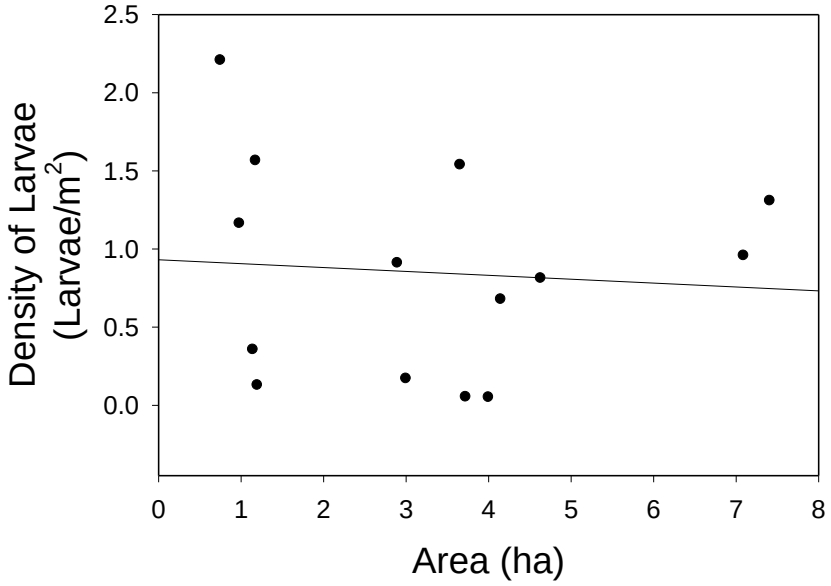

FIGURE 4. Density of larvae versus the area of forest fragments in a highly fragmented landscape. $R^2 = 0.01$, $P = 0.78$, $N = 14$.

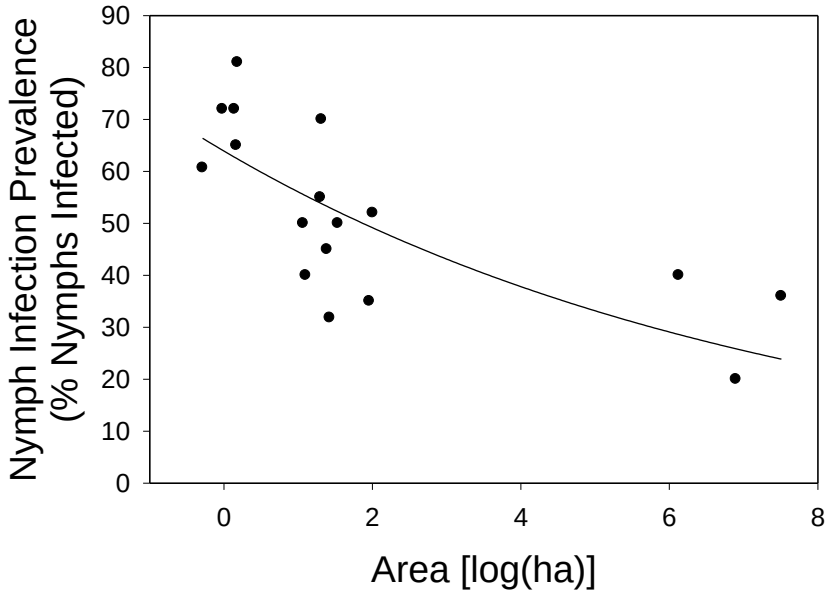

FIGURE 5. Density of nymphal *I. scapularis* versus forest area in both a highly fragmented and relatively unfragmented landscape.

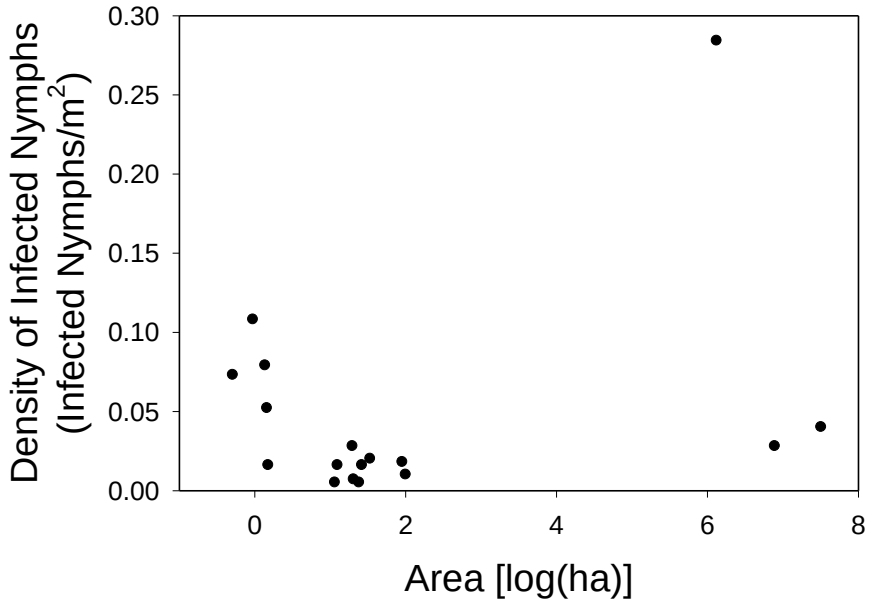

FIGURE 6. The percentage of nymphal *I. scapularis* infected with Lyme disease versus forest area in both a highly fragmented and relatively unfragmented landscape. $R^2 = 0.57$, $P < 0.0005$, $N = 17$; exponential model R^2 16% better model than linear model R^2.

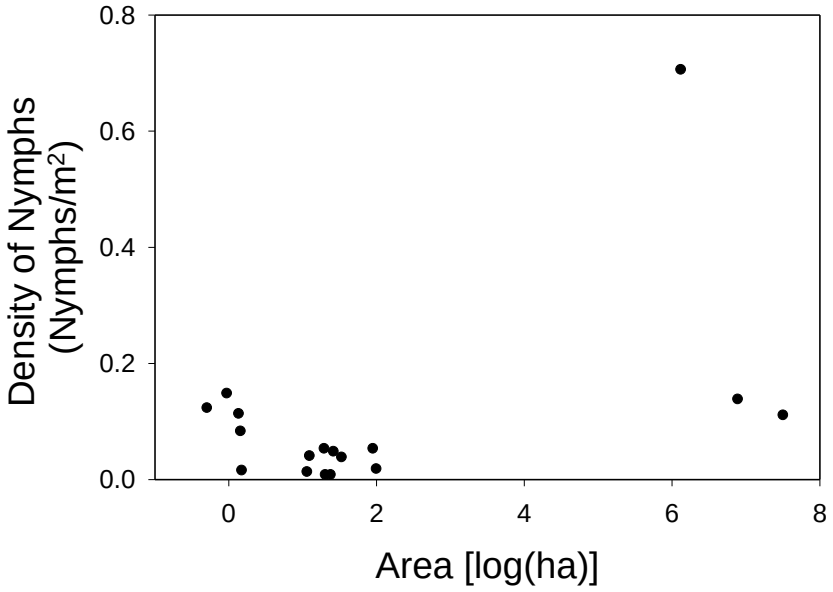

FIGURE 7. The density of nymphal *I. scapularis* infected with Lyme disease versus forest area in both a highly fragmented and relatively unfragmented landscape.