Sampling, Distribution, Dispersal

A Comparison of Tick Collection Materials and Methods in Southeastern Virginia

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Abstract

In field studies of tick ecology, observed patterns may be biased by sampling methods. Results can vary by species, life stage, and habitat, and understanding these biases will improve comparisons of data across studies as well as assessment of human disease risk. A direct comparison of flagging versus dragging was conducted in southeastern Virginia. Transects were surveyed over a 6-wk period to identify differences in species and life stage collected, as well as differences between corduroy and denim material and inspection method for drags. Flagging collected more *Ixodes affinis* Neumann (Acari: Ixodidae) adults and *Amblyomma americanum* L. (Acari: Ixodidae) adults than dragging. Ground inspection was more efficient than tree inspection for collection of *I. affinis* adults, with no significant difference in inspection method for any other species or life stage. Corduroy was found to be more effective than denim in collecting nymphal *A. americanum*, although this may be an artifact of three large samples for corduroy collection of these ticks. There was no significant difference in *Ixodes scapularis* Say (Acari: Ixodidae) collection in any comparison. Dragging, tree inspection, and denim were not found to be more efficient in any scenario. This is the first comparison of flagging and dragging conducted in the southeastern United States. The community composition of ticks in this region greatly differs from regions where studies of these commonly used sampling techniques have been conducted. As the distributions of ticks continue to change over time, it will be important to evaluate best practices annually.

Key words: tick dragging, tick flagging, tick surveillance

As distributions of ticks and their associated pathogens change over time, human risk of exposure also changes (Eisen et al. 2016). Risk measurements vary with species and life stage, and each tick sampling method differs in the type of information it can provide. The recent recommendations for best practices from the United States Centers for Disease Control and Prevention (CDC) include the use of dragging for the collection of *Ixodes* species (CDC 2019) and either dragging or flagging for the collection of non-*Ixodes* hard ticks (CDC 2020). There is no discussion in these guides of the method for handling the drag or flag for inspection, but some researchers are known to hang the material from a nearby tree while others leave the material on the ground. Understanding these differences in collection techniques and how they influence observed patterns is critical both for understanding the risk of tick-borne pathogens within the sampling area as well as for understanding how to compare data from different studies.

Flagging and dragging are two commonly used methods to sample questing ticks (Sonenshine 1993). Flagging consists of attaching light-colored cloth, e.g., flannel, denim, or corduroy, to a dowel rod like a flag and sweeping it through vegetation and along the ground (Ginsberg and Ewing 1989). Dragging takes comparable material that is pulled or dragged behind the individual by a rope handle attached to a wooden base (Ginsberg and Ewing 1989, CDC 2019). Although both flagging and dragging methods have been demonstrated to be efficient in collecting questing ticks, it has been suggested that survey results may be affected by the sampling method selected (Sonenshine 1993, Schulze et al. 1997). Tick collection methods have demonstrated varying efficacy dependent on habitat characteristics, tick species and life stage, and questing behavior (Ginsberg and Ewing 1989, Petry et al. 2010, Dantas-Torres et al. 2013). Indeed, Sonenshine (1993) recommended flagging in areas with dense vegetation and dragging in more open habitat.

Researchers throughout the world have compared the efficacy of flagging versus dragging. Rulison et al. (2013) found that neither collection technique had a significant advantage for sampling nymphal *I. scapularis* in the eastern and central United States. In Italy, Dantas-Torres et al. (2013) found that collection success was dependent upon tick species, life stage, habitat, and time of year with flagging as generally the most efficient in collecting adults. The largest number of studies comparing the efficacy of various other tick sampling techniques have been conducted in the Midwest and northeastern regions of the United States (Ginsberg and Ewing 1989, Schulze et al. 1997, Petry et al. 2010, Rynkiewicz and Clay 2014). In New York, Ginsberg and Ewing (1989) compared a number of tick-sampling methods: a combination of flagging and dragging used interchangeably—this included ticks from the investigator's clothing at the time of collection, walking samples, dry ice-baited traps, and sampling from a host species, the white-footed mouse (*Peromyscus leucopus*). They reported comparable collections from dragging and walking surveys for *I. scapularis* adults and collected more *A. americanum* than *I. scapularis* using carbon dioxide traps including sites where *I. scapularis* predominated in flagging samples. Phenology of larval *I. scapularis* on *P. leucopus* differed from those collected through flagging, with a greater yield of host-sampled ticks earlier in their seasonal period. In New Jersey, Schulze et al. (1997) evaluated three common tick sampling methods: walking surveys, dragging, and dry ice-baited traps, and two experimental methods: pitfall traps and leaf litter samples. Schulze et al. (1997) found walking surveys to be more efficient for collecting *I. scapularis* adults than dragging; however, immature *I. scapularis* were rarely collected during walking surveys. All life stages of *A. americanum* were collected in greater quantities in drags over walking surveys and responded positively to CO₂, while pitfall traps and leaf litter samples yielded very few ticks. In Indiana, Rynkiewicz and Clay (2014) compared dragging, carbon dioxide-baited trapping, and rodent surveys, examining the relationship between tick abundance and microclimate conditions. *Amblyomma americanum* were collected by dragging and CO₂ trapping, *I. scapularis* were only found on rodents, and *Dermacentor variabilis* Say (Acari: Ixodidae) were collected using all three sampling techniques. Additionally, they found that more ticks were collected via rodent sampling and dragging when conditions were hotter and drier.

A direct comparison of flagging versus dragging has never been tested in the southeastern United States. The community composition of ticks in this region is different from other regions of the United States. In the northeast, *I. scapularis* dominates, while in the Midwest, *D. variabilis* is more prevalent. In the southeast, the predominant tick species is *A. americanum* (Merten and Durden 2000, Stromdahl and Hickling 2012). *Amblyomma maculatum* Koch (Acari: Ixodidae) is also found throughout the southeast but in lower number (Nadolny and Gaff 2018b). Additionally, *I. affinis*, a known sylvatic vector of *Borrelia burgdorferi*, the causative agent of Lyme disease, has established populations in southeastern Virginia, altering the overall tick community structure in the state (Nadolny et al. 2011). Few surveys have collected *I. affinis* through flagging or dragging, but those that have often found them in low densities (Clark 2004, Harrison et al. 2010, Nadolny et al. 2011, Nadolny and Gaff 2018a).

In this study, we compared the efficacy of flagging and dragging in wooded habitat at a wildlife preserve (PT2). We have sampled at PT2 since 2009 as part of a long-term field surveillance project in southeastern Virginia, and we regularly collect all tick species found in southeastern Virginia, i.e., *A. americanum*, *D. variabilis*,

I. scapularis, and *I. affinis* at PT2 except for *A. maculatum*. Previous sampling at this site indicates a well-established population of *A. americanum*. In this longitudinal study, transects were surveyed over a 6-wk period to identify differences in number of each species and life stage collected comparing flagging and dragging techniques as well as differences between corduroy and denim material and inspecting method for drags.

Methods

Drag and Flag Construction

In order to compare the efficiency of various tick collection techniques, two 'flags' and two 'drags' were constructed using wooden dowel rods and either white denim or white, fine wale corduroy. This experiment sought to compare flagging and dragging in practical tick collecting, and so while considerable effort was made to ensure equivalency of all samples, traditional sizes for each material were used, which meant a larger fabric area for the drags. However, we estimated that with the sweeping movement of flagging, approximately the same area was covered with both techniques.

To construct the flags, the fabric was cut to make a 500 × 500 cm flag with one edge folded over and sewn down its edge parallel to the fold. This provided a sleeve at the edge of flag for the 1.5 m wooden dowel rod to fit snugly into. The top of this sleeve was also sewed shut to prevent the flag from sliding down the dowel rod while in use. To prevent the flag from sliding up and off the rod while in use, a grommet was placed in the corner of the flag, and a screw eye ring was inserted into the dowel rod just below the flag and connected to the grommet by a small carabiner. The flag was then swept from side to side in front of the field technician while walking slowly along the transect.

To construct the drags, the fabric was cut to a 1 × 1 m drag with one edge folded over and sewn to create a sleeve through which a 1-m wooden dowel rod was inserted. For the drag, both ends of the sleeve remained opened so that the dowel rod would have some length outside the fabric on each end. On each end of the dowel, a screw eye ring was inserted. Then a string was tied through each ring so that the field worker could loop the string around the waist and pull the drag slowly along the transect.

Pilot Study

A pilot study was conducted at PT2 in 2015 to assess the association of plant communities with tick species. This work was done at a small spatial scale to compare ground cover to tick species. Each of the two transects sampled regularly as part of a long-term surveillance project was subdivided into 54 sections of 8 m each. For each grid cell, photographs were taken, and percent of ground cover was estimated using ImageJ (imagej.net). Regular flagging samples were taken weekly for 7 wk. One hundred and eighty-eight adult ticks were collected and identified using morphological keys (Sonenshine 1979, Kierans and Litwak 1989) for a total of 150 *A. americanum*, 38 *I. affinis*, and 2 *D. variabilis*. Herbaceous ground cover varied from 0 to 80%. While *A. americanum* were found in 44 of the 54 sections with little relationship to ground cover, *I. affinis* were found in only 20 sections. While some *I. affinis* were found in sections with minimal ground cover, they were more frequently found in sections with greater than 50% ground cover. This information was used to establish the transects for this study inasmuch as all transects were laid out to contain approximately equal portions with high levels of ground cover.

Transect Lay-out

Twelve 100 m transects (Fig. 1c) were established within the wild-life preserve (PT2) in Portsmouth, VA (Fig. 1b), chosen for its high biodiversity and known tick density. In order to fit all transects within the same approximate area of the preserve, transect lines used included single 200 m lines, two 100 m lines, or four 50 m lines without overlap, and each transect was marked with flags every 25 m. Both sampling techniques cover approximately 1 m in width resulting in 200 m² total area sampled for each transect. All transects were located within a mixed pine-hardwood habitat with closed canopy and nearly identical flora and fauna. Within the habitat, there is variation in the percent ground cover with highly uneven distribution of areas with high cover, e.g., areas with ferns or ivy. Using the information from the previously described pilot study, each transect was designed to include at least 10 m of high-density ground cover in an effort to ensure equal probability of collection of *Ixodes* spp. as determined in the pilot study. There was additional variation such as the density of short shrubs, but this was by design, as flagging in practice is used in more rugged environments while dragging in practice is used in more uniform, ground-level vegetation.

Sampling Protocol

The 12 transects provided three replicates of each of the four basic treatments: flagging with denim cloth, flagging with corduroy cloth, dragging with denim cloth, and dragging with corduroy cloth. Each transect was sampled during the initial set up on 14 May 2018, then twice each week for the next 5 wk weather permitting (May 22, 24 and June 1, 5, 7, 12, 14, 19, 21). This gave a total of 10 samples. All samples were collected between 10 a.m. and 3 p.m., and the order transects were sampled in and the individual assigned to each transect was randomized each day. As an additional factor, dragging collection was subdivided to test the effect of inspection method. Traditionally, some researchers will leave the drag on the ground while looking for ticks, but others will hang the drag from a nearby tree to inspect it. Ground and tree inspections of the drags were divided by day so that ground checks were used on all drag transects on Tuesdays and tree checks on Thursdays. All flagging collections were inspected with the flag on the ground.

All transects were walked at a slow, even pace. For flagging, field workers held the end of the dowel rod at approximately waist

level and swept it between 90 and 180 degrees along the ground in front of them so that the entire area of the transect was sampled. Field workers thoroughly inspected the flag for ticks every 25 m, as well as immediately removing any ticks noticed while flagging. For dragging, the string on the dowel rod was looped over the body and held around the waist so that the drag was just far enough behind the worker that it would not be kicked and so that the fabric remains flat on to the ground. The worker would then pull the drag like a sled to allow the fabric to ‘drag’ across the ground. If the fabric became displaced while dragging, it was immediately fixed before continuing. Both sides of the drag were inspected twice every 25 m, with the drag either remaining on the ground where it lay, or carried to the nearest tree to hang, no more than a couple meters away to limit the risk of ticks falling off the drag, before being checked. The side of the drag in contact with the ground was alternated between each inspection.

Tick Processing

All ticks collected at each transect were placed into a plastic vial with a small piece of crumpled mesh. Adult ticks were placed directly into the vial, while juvenile ticks were collected on blue painters’ tape before putting them in the vial. Each vial was also marked with the transect, date, time, cloud cover, initials of collector, and the temperature as reported for that location at the time of transect completion. Standard smartphone weather apps were used to report the temperature; to prevent variation from different apps, only one field worker would take temperature readings each day. Vials were returned to the laboratory where they were frozen in a –20°C freezer for at least 24 h before being identified by species and life stage using standard morphological keys (Sonenshine 1979, Kierans and Litwak 1989).

Statistical Analysis

To compare surveillance methods based on collection technique, inspection method, and type of material as fixed effects and transect as a random block effect, the significance of the difference between the mean number of ticks collected for each species and life stage was analyzed by one-way and two-way analysis of variances (ANOVAs) and Tukey’s multiple comparison using with R software (R Core Team 2019). $P \leq 0.05$ was considered statistically significant. For simplicity of implementation, replicates remained in their fixed positions, and so to ensure findings were not a result of

Fig. 1. (a) This study was conducted at a field site location in southeastern Virginia (marked by the star). (b) This field site (PT2) is a wildlife preserve that includes a number of walking trails around a man-made lake. Experimental transects for this project were established in the area near the Kids Trail, shown by the square. (c) The transects made use of the areas of closed canopy forest with varying ground cover along the Kids Trail and the nearby grounds accessible from the Kids Trail. CD1, CD2, and CD3 were the transects that were sampled with a corduroy drag. CF1, CF2, and CF3 were the transects that were sampled with a corduroy flag. DD1, DD2, and DD3 were the transects that were sampled with a denim drag. DF1, DF2, and DF3 were the transects that were sampled with a denim flag. All transects were 200 m.

pseudoreplication, transect was included as a block, and an ANOVA analysis was also done using the entire summer as a single sampling event for each method normalized by the number of collections for that sample. A final two-way ANOVA was done for dragging collection only to compare inspection method since flagging collection only used one inspection method.

Results

A total of 425 ticks (152 adults and 273 nymphs) were collected over the 2-mo sampling period. The four species collected included *A. americanum* (87 adults and 249 nymphs), *I. scapularis* (24 nymphs), *I. affinis* (61 adults), and *D. variabilis* (4 adults; Table 1). Overall abundance varied with respect to species, life stage, material, collection method, and inspection method. All flagging samples combined were more efficient in collecting *I. affinis* adults ($F = 8.695$, $P = 0.003957$) and *A. americanum* adults ($F = 6.774$, $P = 0.0106$) than all dragging samples combined. Flagging collections resulted in more ticks on average than dragging with the exception of week 23 (Fig. 4). In terms of inspection method, ground inspection, which included all flagging samples and half of the dragging samples, was more effective for *I. affinis* adults ($F = 15.07$, $P = 0.000184$; Fig. 2) than tree inspection, which was only used for dragging samples. Within the dragging samples, this difference remained

significant with more *I. affinis* for dragging samples with ground inspection ($F = 32.00$, $P = 8.346 \times 10^{-7}$). No significant differences were found for inspection method on number collected of any other species or life stage. Corduroy was found to be a more effective material in collecting *A. americanum* nymphs ($F = 7.735$, $P = 0.00645$; Fig. 3), but this may be an artifact of the three particularly large samples for corduroy collection of *A. americanum* nymphs (Fig. 3). No *Ixodes* spp. were collected when the combination of denim and tree inspection was used. There was no overall difference in the numbers of *I. scapularis* nymphs collected by dragging and flagging. In fact, there was no statistical difference in *I. scapularis* collection in any comparison. Dragging, tree inspection, and denim were not found to be more efficient in any scenario.

While significantly more *I. affinis* were collected through flagging than dragging when combined for both tree and ground inspection methods, inspecting drags on the ground improved the likelihood of finding adults to statistically equal to flagging (Fig. 2). Material was not found to significantly influence the number of this species collected in a sample. Only one of the 61 *I. affinis* was collected from a tree inspected corduroy drag (Table 1). Zero *I. affinis* were collected with the combination of denim and tree inspection.

More *A. americanum* adults were collected by flagging than dragging ($F = 6.774$, $P = 0.0106$), while collection method was not significant for *A. americanum* nymphs. Inspection method was not

Table 1. Number of visits and species per transect

Transect	Number of samples	Total <i>Ixodes scapularis</i> nymphs	Total <i>Ixodes affinis</i> adults	Total <i>Amblyomma americanum</i> nymphs	Total <i>Amblyomma americanum</i> adults	Total <i>Dermacentor variabilis</i> adults
CD1	10	3	8	8	4	0
CDG1	5	0	8	5	3	0
CDT1	5	3	0	3	1	0
CD2	10	4	2	53	4	0
CDG2	5	1	1	34	3	0
CDT2	5	3	1	19	1	0
CD3	10	0	0	18	3	0
CDG3	5	0	0	6	1	0
CDT3	5	0	0	12	2	0
CF1	10	2	2	76	18	0
CF2	10	8	16	12	6	0
CF3	10	0	3	2	2	0
DD1	10	0	3	12	4	0
DDG1	5	0	3	4	4	0
DDT1	5	0	0	8	0	0
DD2	10	2	2	11	5	1
DDG2	5	2	2	4	3	0
DDT2	5	0	0	7	2	1
DD3	10	0	3	11	8	0
DDG3	5	0	3	6	2	0
DDT3	5	0	0	5	6	0
DF1	10	1	11	26	21	0
DF2	10	3	8	16	12	3
DF3	10	1	3	4	0	0
Grand total	120	24	61	249	87	4

CD1, CD2, and CD3 are the transects that were sampled with a corduroy drag, which was inspected either on the ground (CDG1, CDG2, and CDG3) or hanging from a nearby tree (CDT1, CDT2, and CDT3). CF1, CF2, and CF3 are the transects that were sampled with a corduroy flag, which was only inspected on the ground. DD1, DD2, and DD3 are the transects that were sampled with a denim drag, which was inspected either on the ground (DDG1, DDG2, and DDG3) or hanging from a nearby tree (DDT1, DDT2, and DDT3). DF1, DF2, and DF3 are the transects that were sampled with a denim flag, which was only inspected on the ground. The unusually high number of *A. americanum* nymphs collected from CD2 reflects the frequent phenomena that ticks are not uniformly distributed even within small areas. Only one of the 61 *I. affinis* was collected from a tree inspected corduroy drag (CDT2).

Fig. 2. This box and whisker plot shows the number of *I. affinis* collected per sample by different combinations of collection and inspection method: dragging with ground inspection, dragging with tree inspection, and flagging with ground inspection in southeastern Virginia. Tree inspection was not used when flagging in accordance with common practice.

Fig. 3. This box and whiskers plot shows the number of *Amblyomma americanum* nymphs per sample compared by collection method and material: corduroy dragging, corduroy flagging, denim dragging, and denim flagging.

significant for *A. americanum* of either life stage. Corduroy collected more *A. americanum* nymphs than denim ($F = 7.735$, $P = 0.00645$; Fig. 3), but material was not significant for adult *A. americanum* collection. While the average number of *A. americanum* nymphs collected was approximately equal, corduroy does show an increase in the number of nymphs per sample, but this difference is driven by the large numbers collected from one transect. Corduroy had three outliers for nymphal *A. americanum* collection (Fig. 3). These larger numbers of *A. americanum* nymphs were not uniformly distributed among transects (Table 1). All other combinations of material, collection and inspection method were not significant for *A. americanum*.

There was no overall difference in the numbers of *I. scapularis* nymphs collected by dragging and flagging. In fact, there was no significant difference in *I. scapularis* collection in any comparison. Zero *I. scapularis* were collected when denim was combined with tree inspection. A total of four *D. variabilis* adults were collected over the 6-wk sampling period. Three of these ticks were collected using a denim flag and ground inspection, and one was collected using a denim drag and tree inspection. While there was some variation over the course of the study, the results were overall consistent across all weeks for all species and life stages (Fig. 4).

All of these trends remained true for the relationships when the average number of ticks collected per sample was used, but statistical significance was lost because of the reduction to three samples for each method. Statistical significance was maintained, and all trends were consistent when the inspection method was assessed for dragging samples only.

Discussion

This study demonstrates that flagging and dragging are not equal sampling methods for all tick species and life stages. Flagging was more effective in collecting *I. affinis* adults, *A. americanum* adults, and more ticks in general, while dragging did not collect more total ticks for any species or life stage. More *I. affinis* adults were collected through flagging or dragging when drags were inspected on the ground compared with dragging when drags were inspected in trees. Hanging drags in trees to look for and collect ticks after dragging is a common practice in other regions of the United States. Tick surveillance programs in these areas are predominantly concerned with collecting *I. scapularis* because it is the dominant tick species and because of its role in the transmission of *B. burgdorferi*. Inspection method is not discussed in the Center for Disease Control's protocols for surveillance of *I. scapularis* (CDC 2019) or in most studies (Ginsberg and Ewing 1989, Schulze et al. 1997, Dantas-Torres et al. 2013, Rulison et al. 2013, Rynkiewicz and Clay 2014), and this is likely because there is no difference in using tree over ground inspection in historic *I. scapularis* regions. However, inspection method could play a role in collection of *I. affinis* and possibly southern *I. scapularis*. Northern nymphal *I. scapularis* have demonstrated different questing behavior compared to their southern counterparts (Arsnoe et al. 2019). While Arsnoe et al. (2019) measured host-seeking in terms of nymphs being visible on and above the leaf litter, it is possible that their questing behavior may also differ in their attachment abilities and thus alter their likelihood to stay on a vertical flag versus one kept flat on the ground. Indeed, while the numbers collected in 2018 were too low for statistical results in this study, in our anecdotal observations, southern *I. scapularis* ticks are less active, often curling up and rolling off of the cloth

Fig. 4. Mean numbers of total ticks collected per week from 200 m transects in southeastern Virginia. Ticks collected by flagging (F) are shown in the lighter gray bars, and ticks collected by dragging (D) are shown in the dark gray bars. The error bars represent the standard error of the mean.

as soon as they are flagged. The community composition of ticks in southeastern Virginia differs from other regions in the United States (Wright et al. 2014). These ticks have different life histories and behaviors which may explain why tree inspection should not be a recommended inspection method for collecting ticks in areas in the southeast.

Corduroy was more effective for collecting *A. americanum* nymphs and more ticks overall, but there was no significant difference between materials for any other species or life stage. The high numbers of overall ticks collected by corduroy alone and corduroy paired with flagging were likely from the variation in the *A. americanum* nymph samples. Denim is particularly not recommended for dragging collection as evidenced by zero *Ixodes* spp. collected when researchers used trees to inspect the denim drags. This may be attributed to an inability of these species to hold on to a vertical smooth (denim) material versus one with ridges (corduroy). Dragging, tree inspection, and denim were never demonstrated to be more efficient in collecting any species or life stage of tick during the sampling period. In a future study, denim and corduroy should be compared with the CDC's recommended rubberized cotton flannel sheeting (CDC 2019), which was published after the completion of this study.

Sampling method seemed to be the most important for collection of *I. affinis*. While this tick is not known to bite humans, it is an important vector in the sylvatic cycle of *B. burgdorferi* in the Mid-Atlantic region (Harrison et al. 2010, Nadolny et al. 2011, Nadolny and Gaff 2018a). This tick species is undergoing a range expansion (Nadolny and Gaff 2018a), and thus surveillance in many new areas need to adapt collection methods to ensure detection of this species. These ticks do not stay on a drag long and are more likely to be lost, and so flagging may be a better means of surveying for this species than dragging. Based on the results of this study, for any future study looking for *I. affinis*, it is important to use ground inspection to keep losses of *I. affinis* adults at a minimum.

Amblyomma americanum adults were collected in higher numbers with flagging compared with dragging, while nymphs were collected in similar numbers between the two sampling methods. Material mattered for nymph collection, with corduroy collecting more, but was not important for sampling adult *A. americanum*. Corduroy collection of *A. americanum* nymphs was skewed by three outlier samples from two transects. It is possible that nymph differences in material were skewed by the tendency of immature *A. americanum* to cluster (Oliver 1989). Two out of the three large numbers of nymphs were collected from visits to the same transect, although all three of these sampling events used corduroy as the material. The samples of *A. americanum* nymphs were not uniform because ticks are not uniformly distributed in the landscape. Immature *A. americanum* ticks are often found in large numbers in areas that were likely where hosts rested and thus many engorged larvae could have fallen off in the prior year (Oliver 1989), and so additional studies need to be done to tease apart the influence of nonuniformity of distribution with the role that material plays in the number collected per sample. Finally, this study was ended prior to the start of *A. americanum* larvae season since the removal of larval clusters from corduroy would be very challenging. This is a reason that a smoother material, e.g., denim, is preferred in areas dominated by *A. americanum*.

Similar to the findings of Rulison et al. (2013), there were no consistent differences between flagging and dragging as sampling methods for nymphal *I. scapularis*. Additionally, there was no significant difference for any comparison between sampling techniques for *I. scapularis*. In this study, there were considerably fewer *I. scapularis* nymphs collected compared to other flagging and dragging studies (Ginsberg and Ewing 1989, Schulze et al. 1997, Rulison et al. 2013). This is likely due to a difference in questing behavior between northern and southern *I. scapularis* nymphs. Arsnoe et al. (2019) demonstrated that northern variants were eight times as likely to quest on or above the surface of the leaf litter as their southern counterparts. Due to this behavioral difference, we are less likely to encounter southern *I. scapularis* nymphs, which may have contributed to low collection numbers compared to surveys in other regions. Not surprisingly given the closed canopy wooded habitat, few *D. variabilis* adults were collected, and those few were only collected using denim, with most collected by flagging ($n = 3$) than dragging and tree inspection ($n = 1$). However, our sample size was too small to make any conclusions on the efficacy of these sampling methods for collection of *D. variabilis*.

The results of our survey may be representative of the tick community that is active during early summer in other areas of the southeast, particularly along the coastal plain. Dragging can be difficult in this region due to swamp or marsh habitat and a thick understory, even in closed canopy forests. Dragging is impeded in these areas with lots of vegetation due to an inability to make contact with the leaf litter or getting snagged on shrubs and brambles. Ecotones are also often found to be optimal habitat for ticks (Sonenshine 1993) but can be equally difficult to drag in this region due to dense vegetation. Flagging and dragging are not equal sampling methods with respect to species and life stage particularly for *I. affinis*. The appropriate sampling design for tick surveillance ultimately depends on the goals of researchers, the community composition of ticks, and habitat specific to their region. Variation in the efficacy of tick sampling methods is likely due to differences in species and life stage behavior. As ticks continue to move and expand their range, it will be important to evaluate best practices annually.

Data Availability Statement

Data from this study are available from the Dryad Digital Repository: doi:10.5061/dryad.00000002b (Espada 2020).

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