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Contrasting Tick Species Behaviors: A Course-Based Undergraduate Research Experience (CURE)

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Abstract

Tick-borne diseases are on the rise throughout the world, and there is a need to better understand tick behavior in order to identify potential new interventions. Ticks have a complex life history and can survive months off-host. There is a lack of large-scale data on off-host tick behavior, which leaves a gap in understanding of tick biology outside of tick-host interactions. Introducing undergraduate students to authentic research early in their studies can help prepare them for independent inquiry in upper-level classes. To address the student needs and fill gaps in tick research, students in introductory biology courses recorded observations of ticks in sealed terraria each week for one semester. Students recorded 11,905 observations of two species of nymphal ticks over 10 weeks. The results showed that *Amblyomma americanum* nymphs were observed more frequently and quested higher than *Ixodes scapularis* nymphs.

Keywords: tick behavior, vector biology, crowdsourcing, undergraduate research

1 Introduction

Despite the documented rise in tick-borne infectious disease in the United States [2], knowledge regarding the efficacy of ticks as disease vectors and differences between tick species is limited by the challenges of conducting controlled tick behavioral research. Each tick species varies in its vector competence, the physiological capacity to act as a viable host for a given pathogen and its ability to transmit that pathogen to either a human or animal host that can then become infected. The blacklegged tick *Ixodes scapularis*, for example, is a competent vector for the pathogen *Borrelia burgdorferi*, the causative agent of Lyme disease. Vector competence of the blacklegged tick is only one piece of the puzzle in evaluating human risk of exposure to the Lyme disease pathogen. Density of nymphs and density of infected nymphs are important ecological parameters to consider [11, 12], but even beyond these measures, tick behavioral characteristics can further affect the likelihood that ticks will transmit a pathogen. In North America, blacklegged ticks are a major concern in the northeast and midwest regions, but different species pose risks elsewhere in the country. The lone star tick *Amblyomma maculatum* is a widespread, abundant tick in the southeast and south central states. Lone star ticks can act as vectors for several pathogens, including *Ehrlichia chaffeensis* and *Rickettsia amblyommatis*, which can cause ehrlichiosis and spotted fever, respectively. Lone star tick bites are also implicated in the development of alpha-gal syndrome, a delayed meat allergy that can lead to life-threatening anaphylaxis in sensitive individuals. A potential key to prevention of tick-borne diseases is better understanding of vector behavior [5], particularly with respect to questing. Questing is a host-seeking behavior, during which ticks will cling to vegetation or other vertical supports and extend their forelegs with the goal of encountering a suitable host. Comparative studies have demonstrated differences in questing behaviors among blacklegged tick populations and provide evidence for the hypothesis that behavior is an important additional factor to consider in overall transmission risk [1]. For example, ticks that quest higher on vegetation or those that emerge from leaf litter more frequently have a greater chance of encountering potential human hosts [1]. Questing fatigue might be a potential limiting factor that can affect the level of activity that ticks sustain over time. Studies examining questing behavior in controlled settings range in duration from several days to multiple weeks [6, 13, 14, 15]. However, observation of questing activity or questing heights have not yet been explored beyond several days for the lone star tick nor for blacklegged ticks in controlled indoor en-

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vironments. Longer duration studies that are more comparable to the active season for a species or life stage can provide an opportunity to observe any apparent questing fatigue that might occur in the ticks. Previous efforts to examine tick questing behavior have often suffered from limited observations and low statistical power and have suggested themselves to approaches employing large numbers of independent observers [7]. Such studies tend to rely on a limited number of trained observers, which introduces the question of whether some differences can be attributed to observer biases related to recognition of individual ticks, detection of ticks on different substrates, and observer fatigue. Nevertheless, improving on the current approaches to behavioral studies can be critical to expand our understanding of the effects of tick behavior on transmission risks to aid in prevention efforts, such as vegetation control and public education.

At Old Dominion University (ODU) as at most universities, there are several departmental and university-level undergraduate research initiatives; however, the large majority are targeted at upper-level students, and most involve "super-mentoring" arrangements where a very limited number of students are involved in intensive mentoring environments resembling graduate-level work. Few opportunities exist for large-scale introduction to hands-on experiential research experiences at the introductory levels, and in our experience, the student seeking an authentic research experience at the upper level often has a limited understanding of what research entails and inadequate technical and critical thinking skills for successful entry into super-mentoring environments. Some initiatives have arisen over the last several years at ODU toward Course-based Undergraduate Research Experiences (CUREs).

In 2018, members of the Department of Biological Sciences at ODU piloted a large-scale CURE targeted to students in 100-level introductory biology courses, both for biology majors and non-majors. This program, entitled Reinventing Undergraduate Laboratory Experiences (RULE), was intended to develop institutional knowledge on implementation of an authentic CURE into existing course curricula with a goal of reaching large numbers of students. Our group also had an explicit concurrent goal of employing large numbers of student observers to generate usable scientific data in support of ongoing tick ecology research by the Tick Research Team at ODU. Crowdsourcing and citizen science have been used successfully in tick research to update tick records in the United States [8, 10] and beyond [9], but this project is a novel use of student crowdsourcing to examine behavior and detectability of ticks.

Here we present the findings of the Fall 2018 year of the 10-week RULE project. The goal of the research in this study was to quantify the differences in questing behavior of *A. americanum* and *I. scapularis* nymphs in questing height, frequency, and longevity. Our null hypothesis was that there would be no differences between species. Alternately, we hypothesized that *A. americanum* would be more active, and therefore would be observed more frequently than *I. scapularis*. Further, this greater activity might lead to a tendency for *A. americanum* to quest higher because of their active pursuit (hunting) versus sit-and-wait (ambushing) behavior [16]. By including repeated measures from many independent observers, we could assess variation between observers. Finally, we wanted to determine whether either species showed any overall decline in questing activity throughout the course of the experiment, which could indicate the onset of questing fatigue.

2 Materials and Methods

Six sealed terraria were constructed for tick observation (Figure 1). Each terrarium had approximately one inch of soil and a few leaves covering approximately 4 g of 1–2 mm diameter acrylamide/potassium acrylate copolymer, crosslinked insect feeding crystals (Tasty Worms Nutrition, Inc., Longwood, Florida, U.S.A.) soaked in 250 ml of water for moisture retention. Dowel rods were placed as a potential substrate for climbing with colored marks at every centimeter. Pathogen-free *I. scapularis* and *A. americanum* nymphs were purchased from Oklahoma State University colonies. Three terraria were dedicated to each species, with approximately 25 nymphs of the given species per tank. Terraria were sealed with clear, silicone caulk after ticks were introduced. Velcro straps added pressure to the seal to reinforce containment. A hole (approximately 7.5 cm in diameter) cut into the plexiglass lid allowed for ventilation. The hole was secure with a double layer of mesh to prevent nymphs from escaping. A heating pad and light were set up for each tank. The lights were on a 12-hour light/dark timer. Finally, each tank was placed on a bench in the teaching lab with a perimeter of double-sided tape as an extra precaution against nymphal escape.

2.1 Student instructions

Instructors explained basics of tick biology and the tick research program at ODU during the students' first lab session. The purpose of the experiment and data collection procedure was also explained. Instructors introduced students to the initial hypothesis that some tick species may quest higher than others, but also offered the opportunity for students to form new hypotheses based on what they observed for the duration of the course. Paper data sheets were printed for each class to record details such as date, time, room number, and terrarium ID (see Supplemental Materials). A section was included for binary choices regarding whether condensation was present, the lights were on or off, and if fungal growth was visible. Space was included for up to 10 observations per tank to detail species observed and questing height. If additional space was needed, students could write in on the back of the page. Students were asked to submit data sheets even if no ticks were observed that day to record zero observations.

The observations took place during Fall 2018 semester in three adjacent classrooms (labeled Rooms 1, 2, 3), with one *I. scapularis* and one *A. americanum* terrarium in each teaching lab. Two courses participated in this project including introductory biology for majors (226 students in 12 lab sections) and general biology for non-majors (113 students in 5 lab sections). During the semester from September through December 2018, the students documented what they observed in the tick terraria for five to ten minutes of every weekly lab session. For each session, every student individually completed a data sheet. The observations were an ungraded exercise, and there was no personal information other than the student's name on the paper sheets. At the end of the semester, students learned basic data analysis and wrote a paper detailing their results and conclusions. In addition to the student learning objectives, the cumulative data that the students collected were used to assess tick behavior for significant patterns. All data were entered into a database by members of the ODU Tick Research Team. Raw data available for download from `https://github.com/hollygaff/tick_observations`.

2.2 Statistical methods

The total number of observations was recorded based on the number of students who submitted reports. Tick counts were a subset of the total observations where information about each visible nymph was recorded. Course sections had variable numbers of students enrolled and different attendance rates over the semester. Data were normalized to account for student participation and to obtain a mean number of nymphs of each species per section, per week, since students in the same course section were observing the same ticks. The denominator for the mean was obtained by using individual student IDs to calculate the number of reports submitted for each of 154 section and week combinations. Normalized counts by species were compared using the "chisq.test" function in R 4.2.2, with Monte Carlo simulation (2000 replications by default) to determine if we could reject the null hypothesis, that there were no differences in expected proportions between species. Significant differences in nymphal counts were examined between sections, room numbers, and weeks. Finally, a randomized complete block design was used to evaluate the effect of species and room on mean questing heights for each terraria, based on the observation that *I. scapularis* appeared to quest at lower heights than *A. maculatum*.

Figure 1: Example terrarium with dowel rods marked by centimeter.

3 Results

A total of 13,063 tick observations were counted over the course of the semester across all classrooms and meeting times. Of this total, 11,905 observations had information about nymphs and 1158 recorded no observed ticks on a given day by a specific observer. Data were collected for 10 weeks total. Observations by date, classroom, and meeting time revealed variation in individual student observations of the same ticks at the same time. Some students consistently recorded higher tick counts compared to their peers within the same section. After normalizing the data, students were significantly more likely to observe *A. americanum* than *I. scapularis*, $\chi^2(\mathrm{N} = 154) = 15752$, $p = 0.001$ (Fig. 2).

Students in rooms 1 and 2 recorded greater variation across *A. americanum* counts than *I. scapularis*. In Room 3, variation in nymphal counts was similar between species. Differences in observations by room were significant for both species, *A. americanum*, $\chi^2(\mathrm{NA}, \mathrm{N} = 154) = 286.62$, $p = 0.001$; *I. scapularis*, $\chi^2(\mathrm{N} = 154) = 254.89$, $p = 0.002$. When we considered data by class section rather than room, only *I. scapularis* observations were significantly different between sections, $\chi^2(\mathrm{N} = 154) = 2478.3$, $p = 0.043$, whereas no significant differences were detected between sections for observations of *A. americanum*, $\chi^2(\mathrm{N} = 154) = 2820.4$, $p = 0.182$. No significant differences were observed by week.

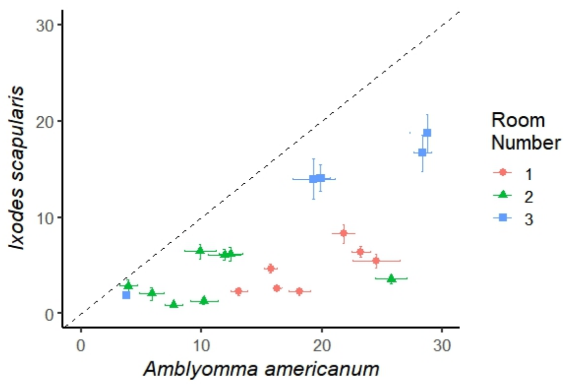

Figure 2: Average number of observations of each species per week. The dashed line indicates an expected proportion of observations per species if the number of observations were equal between them. To account for differences in student participation, data were normalized for effort by obtaining a nymph count for each species divided by the number of student reports, using unique student initials to account for individual observations, rather than using total nymph counts per section or week. *Amblyomma americanum* were more likely to be observed than *I. scapularis* across all sections. Weekly observations are pooled by course section.

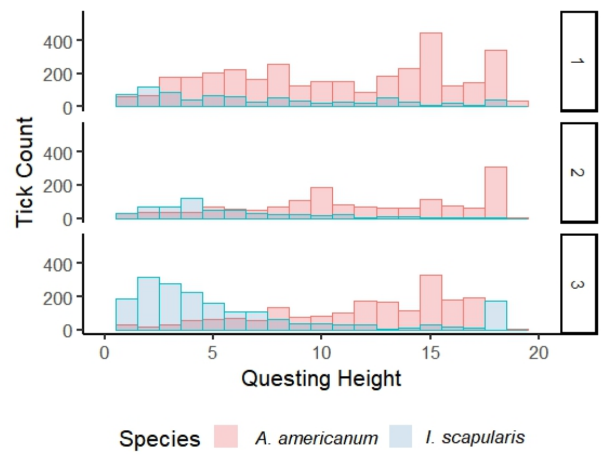

Figure 3: Total number of ticks reported by questing heights of both species by room (treatment blocks 1, 2, and 3). *Amblyomma americanum* were overall more likely to be observed on the ceiling, whereas *I. scapularis* were often observed near the soil surface.

On average, classes submitted reports for about seven weeks each. All data sheets included lines to record both species. Therefore, true zeroes could be reported when any number of ticks were observed for one species, and zero were observed for the other species on the same day. Distinguishing between missing data and zero observations was not possible if reports were not submitted to record the zero observations. In these cases, a missing student report would not be added into the denominator, so these would have all been treated like “NA” rather than zero.

Clear differences in questing heights were observed by species, $F(1,) = 3838.4$, $p < 2 \times 10^{-16}$, as well as by room $F(2,) = 207.7$, $p < 2 \times 10^{-16}$ (Fig. 3). Observers noted that most of the *A. americanum* nymphs counted were on the glass walls or the plexiglass ceiling rather than the dowel rods. Some observers counted ticks that were visible on the soil or leaves, but not clearly questing. Typically, higher questing heights were associated with *A. americanum* and nymphs observed closer to the soil level were more likely to be *I. scapularis*.

Attempts to control condensation by altering the volume of moisture in the soil and polyacrylamide crystals were not successful. Previous trials run without moisture crystals ended up in rapid desiccation of the tank substrate and subsequent death of nymphs before the semester concluded.

4 Discussion

We were able to demonstrate that citizen science, in this case classroom crowdsourcing, was a reasonable way to collect behavioral data with many replicates over a substantial period of time. Placing tanks in the classroom increased the number of observations and reduced the effort on the part of individual students to collect data compared to outdoor tick gardens. These data also help us to understand how individual variation in observations might influence research outcomes. Understanding variation between observers can help inform experimental design in experiments such as tick gardens, which take place outdoors or in more difficult study environments.

The strongest and most consistent trends were the differences between species, both in total observations and questing heights. These differences are likely explained by differences in species behaviors. *A. americanum* are hunters, whereas *I. scapularis* wait for a host to pass by and ambush the host, grasping on fur, feathers, or skin to hitch a ride [16]. The Oklahoma colony *I. scapularis* nymphs are more likely similar to the southern *I. scapularis* populations, which show less aggressive questing behavior compared to northern populations. In this case, sub-species level characteristics may also play a role in the differences observed between species in our study. In the southeastern United States (U.S.), *A. americanum* occur abundantly and contribute to the relatively high prevalence of ehrlichiosis caused by the pathogen *E. chaffeensis* compared to other regions in the U.S. *A. americanum* can also harbor bacteria and viruses that cause human disease of emerging concern, such as spotted fever rickettsiosis [3], tularemia *Francisella tularensis*, Heartland virus disease and Bourbon virus disease [4]. Although these ticks are known as aggressive hunters, we are able to demonstrate here that student observations can help quantify distinct differences between species, which may also be useful in comparing populations or activity under altered environmental conditions. In addition, we observed no apparent decline in their questing activity over the course of 10-weeks, suggesting the ability to sustain high levels of question activity over a long period of time. The CURE framework can facilitate further research on questing duration of unfed nymphs, to identify the physiological limits of *A. maculatum* questing duration under a range of conditions. Such data could be useful in developing a dynamic energy budget that, combined with density and ecological data collected from the field, can help determine temporal risk of exposure in different locales throughout the active season.

We suspect that some of the difference seen between species could be attributed to the relative ease with which students could distinguish *A. americanum* from soil particles or mold growth. More students reported difficulty seeing *I. scapularis* nymphs, especially if they were low on the glass walls of the tank or on dowel rods later in the semester as fungal bodies developed. This likely explains some of the significant differences in observations between sections, which were taught by different instructors, as some instructors reported asking students to be more thorough in counting the nymphs that were more difficult to see. Such discrepancies in species identification would be less likely to influence future studies comparing populations of the same species, or tick species with similar visibility.

Measurements for questing heights were complicated by students occasionally choosing different starting points from which to measure height. The initial instructions directed students to measure from the top of the soil level to the bottom of the tank ceiling. However, some measurements exceeded the maximum height (20 cm), and therefore were excluded. We also found that some students reported measurements in inches rather than centimeters, or did not indicate units, in which case we made inferences based on observations from the same class period.

Many of the challenges we faced in this process were a result of errors made in consistency in training across course sections and error in student observations. Students did not receive grades for their submissions, so some students consistently opted out or submitted results that weren't consistent with other observations from their classmates. Collecting and compiling data was a long and labor-intensive process. Missing data sheets complicated efforts to handle zero data in our results. Finally, heavy condensation and fungal growth in the tanks made observations difficult on most days.

Overall, we found that this large-scale CURE was successful in providing information that can translate to field studies. We confirmed that behavioral differences, namely higher activity levels, can be quantified with minimally-trained observers. Activity levels in ticks, particularly *I. scapularis* nymphs, is an important parameter for assessing human risk of exposure to tick-borne pathogens. This type of study would be especially valuable for species such as the Gulf Coast tick (*A. maculatum*) for which behavior of immature ticks is poorly understood, in large part because free-living immature ticks are rarely collected in the field. Similar study designs would also be useful for testing a variety of substrates and other variables to evaluate questing responses in ticks.

Checklist for future large-scale CURE projects would include: 1) a solid data management plan and proper training of instructors, 2) buy-in from the department or key collaborators and stakeholders who can monitor student attitudes as well as research outcomes, and 3) a plan to convey to students that the scientific process is dynamic. Students should understand through this process that experiments may not work smoothly, collecting data can be repetitive, challenges arise, and outcomes may be unexpected.

Author Contributions

Sara Benham and Shovan Dutta helped with investigation, data curation, data analysis, and writing. Rohan Madamsetti helped with investigation, editing, and data analysis. Clayton Wright helped with conceptualization and investigation. Alena Anderson helped with data curation and validation. David Gauthier helped to conceptualize the CURE and with writing. Holly Gaff helped to conceptualize the CURE, with writing, and provided overall supervision.

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