Abstract. With over 70 species established in New York State, mosquitoes are one of the most common pests in the northeast. As juveniles, mosquitoes live in small or temporary bodies of water. Once they are grown, they primarily consume nectar. Only during oviproduction will female mosquitoes ingest blood, making them ideal vectors for pathogens that cause diseases such as encephalitis and malaria. Mosquitoes may take blood from a number of different organisms, and the species they bite are important determinants of whether or not mosquitoes are a problem for humans. Mosquitoes survive in various types of habitats from cities to wetlands, and it is likely that animal species richness influences the spatial distribution of mosquito species across habitats. In sites around the Cary Institute campus, CDC Light Traps and containers of dry ice were used to collect adult mosquitoes in different habitats over 6 weeks. To assess host species diversity, point counts were completed and “hair snares” were placed near traps. We found 23 species of mosquitoes in communities ranging from 9 species at our most human-influenced site to 15 and 16 species at a wetland and forested field site. This paper examines how these mosquito communities vary with host composition and why this might be important for understanding disease risk in the Hudson Valley.

INTRODUCTION

Mosquitoes are found in nearly all habitats, from cities to wetlands to drier areas with little aquatic presence (Syed and Leal 2009). Their flexibility or specificity to certain sites has been noted and explored in past research experiments; mosquitoes of the genera Culex, for instance, can dominate in urban habitats due to their ability to reproduce in habitats that are ecologically damaged or ephemeral (Johnson et. al. 2008). Previous studies have also noted that understanding the diversity of mosquito species in different habitats can elucidate the role habitat may play on vector-borne disease transmission (Johnson et. al. 2008), or where these populations may grow and develop (LaDeau 2013), and could assist in the management of vector populations and their diseases. However, there is little understood about how mosquito hosts play a role in the vector’s distribution. Mosquito hosts range from birds (McLean et. al. 2006; Hamer et. al. 2009), mammals (Molaei et. al. 2006), amphibians, and reptiles (Cupp et. al. 2004). Some species, such as Culex pipiens, feed nearly exclusively on birds; the American robin and gray catbird have been noted as Culex favorites (McLean 2006; Molaei et. al. 2006; Savage et. al. 2007; Hamer et. al. 2009; Thiemann et. al. 2011). Others, like Culex salinarius, have been found to feed on both mammals and birds alike (Molaei et. al. 2006). These are examples of specialist and generalist feeders—specialists feed on specific taxa, while generalists seemingly have no preference.

Every species—mosquito and otherwise—have specific habitat preferences that fulfill their survival needs. For instance, if one habitat supports a large and healthy population of birds, it can be assumed that avian specialists will be the most common mosquitoes in the area. Mammal-feeders should follow mammal populations, and generalists have no specific preference. However, host species can vary from site to site, even if the habitats are fairly similar. Mosquito species whose ranges cover extensive areas would have to adapt to survive on what is available. A previous study found that while Culex pipiens samples taken from New York exhibited an almost solely avian preference, further south the species...
included mostly large mammals into their diet (Gingrich and Williams 2005; Savage et. al. 2007). When the habitat and biodiversity changed, so did C. pipiens’ feeding preference. Specialist feeders can therefore create disease reservoirs in different taxa, while more generalist species are likely to be bridge vectors (Gingrich and Williams 2005).

Understanding how to follow mosquito feeding preferences could help researchers identify current and possible disease reservoirs. If mosquitoes are truly spatially distributed based on host preference, then reservoirs can vary by environment. Pinpointing habitats where diseases may arise in certain taxa could not only benefit the host species in question, but the surrounding population, who may be at risk of contracting the disease as well.

In order to understand the relationships mosquito species have with their habitats, it is necessary to comprehend the basics of their life history. Females will acquire a bloodmeal before laying eggs, a dietary requirement for oviproduction (Wang et. al. 2011; LaDeau et. al. 2013). Mosquitoes require a source of standing water to lay their eggs and for their larvae to develop (Wang et. al. 2011). Thus, the availability of larval habitat should also play into the spatial distribution of mosquito species. The list of standing water sources is almost as vast as the number of mosquito species—leaf litter, tires, hoof prints, containers of any size or make, puddles, ponds, tree holes, and similar habitats (Norris 2004, Yasuoka & Levins 2007, Trevejo & Eidson 2008, Hollis 2011, Kirkhoff et. al. 2013, LaDeau et. al. 2013). Very often, specific mosquito species will have their own preference of standing water habitat; Ochlerotatus tristeriatus, for instance, prefers to lay its eggs in tree holes (Ellis et. al. 2006), while Aedes japonicus larvae are often found in artificial containers (Crans 2004). The preference can also depend on the surrounding environment; for example, Culex salinarius has a salt tolerance and can develop in coastal areas (Crans, Rutgers University). As mainly urban and suburban mosquitoes, Culex spp. seem to prefer to lay their eggs in artificial containers and vernal pools (Crans, Rutgers University; Farajollahi et. al. 2005, Molaei et. al. 2006). Some species oviposit on the surface of aquatic habitats, while others lay eggs on wet surfaces found near water (Silver 2008). Once fully developed, mosquitoes often do not travel very far from their source, though species that are more likely to lay eggs in temporary habitats like vernal pools may migrate up to 100km (Sellers 1980).

In more forested habitats, the types of standing water often associated with mosquito production are vernal pools. These temporary pools are larger than the average puddle, and persist anywhere from days to months. Vernal pools are also common in fragmented habitat, when land connectivity may be replaced by a multitude of vernal pools; in sites with a great number of vernal pools in one place there are more possible larval sites for mosquitoes (Colburn et. al. 2008, Liebowitz and Brooks 2008, Windmiller and Calhoun 2008). Indeed, studies surrounding mosquitoes and vernal pools have identified nearly 30 species of mosquitoes that rely on vernal pools (Colburn et. al. 2008). As humans continue to push deeper into forest habitats, this means that there are ample places for mosquitoes to lay eggs that are within flying distance of human residences.

Females may live and congregate in habitats with some aquatic ecological features, while drier areas may host mosquito species that live exclusively in tree holes. Around the Cary Institute, there are around 42 vernal pools, and several large wetland areas. Mosquitoes in the immediate area have substantial habitat to oviposit, and with changes in land use and climate this habitat may only increase. The management of mosquito species could benefit from the implications of this study by better pinpointing which species are more likely to appear in one habitat than another.

This study seeks to identify if mosquito diversity is based in host preference and larval habitat availability. Mosquitoes should be most abundant and diverse in a wetland habitat, which would be plentiful in host community and larval habitat. Drier or more residential habitats should have fewer possible larval habitats and should be more homogenous, which should result in fewer mosquito species.
living in the area. Specialists should be found in habitats where their hosts reside, and generalists should be found in every habitat regardless of host community composition or larval habitat availability. It is additionally possible that mosquito diversity is not affected by host preference or larval habitat availability.

**MATERIALS AND METHODS**

**Study Sites**

I selected three specific sites around the Cary Institute campus as representations of different habitats (Figure 1a-c). The selection of habitat would act as a proxy for juvenile aquatic habitat and potential host diversity. The first site was located near a residential property (Bacon Flats (BF)) that sits on the edge of forest and is more than 1 km from nearest pond. The second site, Fern Glen (FG), is a forested wetland habitat with a persistent pond with amphibians present. The third site is a forested field (Tire Field (TF)), which is dry habitat for observed small mammals and is mowed several times throughout the season. Additionally, there is a number of vernal pools in the forests bordering the site. TF was added to the experiment in midsummer, with collections beginning on July 8, 2014. This site was added to provide an additional level of site diversity.

**Mosquito Trapping**

I used CDC Light Traps to collect female mosquitoes. I removed the lights from the traps to avoid capturing any non-mosquito insects, and used dry ice as bait. As it evaporates, the dry ice emits CO₂, which attracts mosquitoes seeking a bloodmeal (Johnson et. al. 2008; Hamer et. al. 2009; Thiemann et. al. 2011; Becker et. al. 2014). Trapping was completed weekly, over the course of 7 weeks from June to August of summer 2014 on the Cary Institute grounds. Trapping was highly dependent on weather, and as a result some trapping days were missed due to severe storms.

Each site was assigned two CDC traps. The first trap was set on the edge of the habitat, while the second was placed in the interior of the site. The traps were separated by at least 25m to ensure that there was no overlap in trapping, and to account for mosquitoes that may prefer edge habitats to interior and vice versa. Traps were hung 1.5 meters off the ground for a period of 36 hours, twice per week. At the end of each trapping period, batteries would be replaced, collection containers would be gathered and new ones attached to the traps to begin another 36-hour trapping period.

In addition to the CDC traps, a handheld vacuum trap was utilized to collect mosquitoes resting on vegetation, which they are likely to do after consuming a bloodmeal (Cupp et. al. 2004). Vacuuming was conducted over all vegetation within a radius of 2m around each trap.

All mosquitoes collected in the field were brought back to the lab and subjected to freezing conditions for euthanization. Once they were immobilized, mosquitoes were relocated to smaller vials, organized by site and placed in the freezer for storage. Samples were identified to genus or species using the *Identification Guide to the Mosquitoes of Connecticut* (Andreadis et al. 2005).

**Larval Collections**

Ovitrapes were pint-sized black cups filled with leaf-infused water and lined with seed paper. Ovipositing females would lay their eggs either on the water surface (e.g. Culex) or along the side of the trap on the seed paper (e.g., Aedes), facilitating the collection of eggs and larvae for the study. I put out four ovitraps around each CDC trap: two ovitraps were placed 5m away from the trap, two were placed 10m away. Traps were set at tree bases, in bushes, tall grasses, and similar locations that were easily accessible to
ovipositing females. After placement, the traps were left out for one week. Ovitrapping occurred twice throughout the experiment (the weeks of June 26 and July 9).

Once ovitraps were collected, they were thoroughly assessed for larval and egg presence: seed paper was taken from each trap and set to dry for 36 hours before soaking, and water was poured onto light-colored dishes to identify hatched larvae. Any free-swimming larvae were collected and placed in site-specific containers filled with clean water. If they were too small to identify, they were allowed to grow for an additional 72 hours, or before pupation. All larvae were eventually euthanized with an ethanol solution, and identified to species or genus (Andreadis et al. 2005). Eggs that had not hatched were placed in clean water after the drying period and placed in an incubator. Any arthropods or non-mosquito larvae found within the ovitraps at the time of analysis were recorded.

In addition to ovitraps, standardized dips were conducted at FG, the only site with a constant water source. These samples were taken at four points around the FG pond, two dips per point. Any larvae found within the collection cup after dipping were put into vials and brought back to lab and identified. Dipping tests were conducted on June 18, July 12, July 29, and July 30.

**Host Diversity Assessments**

Twenty minutes per site was spent recording visual sightings of all potential hosts present, including amphibians, reptiles, and large mammals (squirrels, white-tailed deer, humans). Only potential hosts were recorded—organisms that were not available to bloodseeking mosquitoes (i.e. submerged salamanders) were not included in host assessments. Organisms that were not observed at a site were not counted in the index, even if they were known to inhabit the area.

To account for small mammals, I established “hair snares” at each trap site. These “snares” were 8” PVC pipes lined with duct tape and baited with peanut butter, and were 1” and 2” wide. I set up two of these snares (one of each width) a meter away from each CDC trap site. After one week out, snares were returned to the lab where the tape lining the pipe was removed and labeled. Fur samples lining the duct tape were analyzed under a dissection microscope for identification.

To analyze avian diversity, point counts were conducted on July 11 and August 1. Beginning at 5am, birdsong at each trap site was monitored and recorded on a digital device for 15 minutes. Every species heard and sighted was noted. Recordings were further analyzed to assess, in depth, which bird species were present the recording was made.

**Statistical Analysis**

Species richness is here defined as the number of species, and species diversity is the species richness and the evenness of their abundance, or the number of individuals of a species. The species richness of adult and larval mosquitoes, and host species was calculated by counting the number of individuals identified per trap site. The mean abundance of pest species was also calculated. We used the Shannon Diversity Index (H) to compare species diversity of mosquito and host communities at each site:

\[ H = -\sum p_i \ln p_i \]

Where \( p_i \) is the proportion of abundance of the species with the greatest richness within the dataset and \( \ln \) is the natural log. Host abundance values (\( p \)) were assigned by finding the overall richness of a site and then dividing the abundance of recorded species by the overall richness. The data is organized into matrices that recorded the frequency of a species’ sighting: when a species was caught on a specific trapping day, or if a species was sighted or heard in a point count or snare analysis, where 1=present and 0=absent. This matrix was a crucial tool that facilitated the calculation of the \( p \) value.
RESULTS

Mosquito Species Richness & Diversity

A total of 3,391 adult mosquitoes were captured and identified throughout the summer, along with 122 juveniles collected by ovitrapping and dipping. A total of 23 mosquito species were accounted for across all three sites (Figure 2a-c). Bacon Flats had the smallest number of mosquito species (n=9), while 15 and 16 species were sampled from Fern Glen and Tire Field, respectively. Mosquito species that either specialized in mammals or preferred to feed primarily on mammals made up 65.2% of the total species catalogued. More generalist species were found at Bacon Flats than at the other sites, including of 66.6% of the species identified from that site compared to 46.6% from Fern Glen and 43.8% from Tire Field.

Bacon Flats also had the lowest Shannon Diversity for mosquito species sampled (H=1.191). Fern Glen and Tire Field had diversity values very close to each other, with Tire Field emerging as the site with the greatest mosquito diversity (H=2.644) and Fern Glen following close behind (H=2.512).

Host Species Richness and Diversity

Host taxa richness varied across sites (Figure 3a-c). Birds were the most abundant host group found at each site, although the greatest number of avian species was observed at Tire Field (n=27), followed by Bacon Flats (n=21) and Fern Glen (n=14). Bacon Flats and Fern Glen were the only two sites where amphibians and reptiles were recorded, though Fern Glen’s was a little greater than Bacon Flat’s populations (n=2 and n=1, respectively). Mammals were most abundant at Bacon Flats (n=5), least abundant at Tire Field (n=1), and moderately abundant at Fern Glen (n=3).

Tire Field had the greatest overall host diversity (H=3.313), compared to Bacon Flats (H=3.1) and Fern Glen (H=2.828). Avian hosts were found to be the most diverse across sites (BF H=2.994; FG H=2.586; TF H=3.257). Bacon Flats had the greatest mammal diversity (H=1.428), followed by Fern Glen (H=1.041) and Tire Field (H=0). Both Bacon Flats and Tire Field had zero diversity values for amphibian and reptile species, making Fern Glen the leader in amphibian and reptile diversity (H=0.563).

Mosquito and Host Diversity

We found that there was no clear association between diversity values for hosts and mosquitoes (Figure 4).

Pest Mosquito Species Richness and Diversity

A pest species was defined as any mosquito species (both generalists and specialists) that fed on humans. 16 of the mosquito species (70% of the species observed in this study) have been known to feed on humans. The diversity value and mean abundance of just these pest species were calculated for each site, and then plotted against each other to pinpoint the site that would present the greatest threat to human health (Figure 5). Tire Field had the highest mean abundance and diversity values (μ=127.5, H=3.207). Fern Glen had a greater mean abundance of pest species than Bacon Flats (μ=45.875 compared to μ=43.5625, respectively), though Bacon Flats had a higher diversity value (H=3.1 compared to Fern Glen’s H=2.82).

DISCUSSION

After calculating diversity values for mosquito and host species at each site, we found no clear relationship between mosquito diversity and host diversity (Figure 4). However, several interesting patterns emerged from the data to present pictures of mosquito ecology in these habitats. First, two
species in particular, *Coquillettidia perturbans* and *Ochlerotatus trivittatus*, were found to have the highest abundance across sites (as adults). Since larval habitats, such as vernal pools and tree holes, were not found at every site, the high abundance of these species suggests that they have very successful and widespread dispersal. Though the data indicates that mosquito species are not tethered to their specific hosts, there was only one exception. *Culex territans*, which is a frog specialist (Savage et. al. 2007), was found exclusively at Fern Glen, and although it did not make up a large portion of the species trapped there, its presence at this particular site may suggest that, in certain cases where the hosts themselves are specialized to a particular kind of environment, some mosquitoes species will need to remain in one place.

At this study’s commencement, we predicted that FG would be the site of the greatest mosquito and host diversity, due to the constant water sources and the presence of more amphibians and reptiles than the other two sites. The data collected throughout the course of this study supports a very contrary finding. While it did not have the smallest diversity value for mosquito species, Fern Glen’s H value was not the largest value either. This wetland site also had the smallest diversity value for host species. As the only study site with a constant water source and a known population of amphibians and turtles, however, this low diversity value may be a somewhat inaccurate representation of the actual host species present.

For instance, salamanders were commonly seen sunbathing at the surface of the pond; however they were not counted in the Fern Glen diversity index because they were always fully submerged and therefore would not have been fed on by blood-seeking mosquitoes. Frogs, toads, and turtles were also known to inhabit Fern Glen, however their sightings were scarce and therefore we could not merely assume that they would be present at trapping. That does not mean that the few individuals identified during trapping were the only inhabitants of Fern Glen.

This issue of host presence was similar throughout all sites. While birds were seemingly the most common hosts found—indeed, Tire Field had the greatest overall host diversity value due to the fact that more bird species were identified there than at other sites—a number of different mammal and amphibian/reptile species also call these habitats home. For instance, one wood frog was found at Bacon Flats, and a leopard frog was found in an ovitrap placed in Tire Field. Furthermore, while mammals such as humans and chipmunks were some of the species most easily accounted for, my host counts were unable to account for larger mammals that could not fit in my “hair snares”. White-tailed deer, red foxes, and even black bears have all been known to lumber around the sites in this study. However, without any actual numeric data to confirm that these species visited the areas near my sites, it became difficult to actually count them as being present for the study.

As a result, the data I collected on host diversity is severely lacking. Relying solely on observational methods, and methods that provided a bias against large species, to account for the organisms found in these different habitats created an inaccurate portrait of the host diversity there. Future studies should perhaps invest in trap cameras to capture evidence of larger organisms (mesopredators, humans, etc.), or other methods that would present a more accurate representation of a site’s non-mosquito diversity.

As one of the most prevalent hosts in the immediate area, humans were readily considered in the diversity index, especially when cataloguing pest species of mosquito. Human-feeders were found at every site, with 65.2% of the mosquito species identified as mammal specialists or those that preferred to feed on mammals, and 70% of the species identified as known pests. However, no human specialists were found at any of the sites. This may be due to the fact that an urban habitat, which would have a large concentration of humans, was not surveyed for this study. As such, human specialists would not be expected to reside in the woods. Despite the lack of human specialists, several species known to be aggressive towards humans (Erwin et. al. 2002, Goddard and Harrison 2005, Scott et. al. 2014) were readily identified throughout the study: *Ochlerotatus triseriatus*, *Ochlerotatus trivittatus*, which were found at every site, and *Psorophora ciliata*, which was only captured at Tire Field. These mosquito
species have been known to aggressively bite and even pursue humans (Meece et. al. 2003, Barnard & Xue 2004, Stein et. al. 2013; University of Florida), and although all will feed from humans they will also readily feed on any warm-blooded organism.

However, pest species were most often found at Tire Field and not at the actual residential site, Bacon Flats. It is possible that the reasoning behind this is purely temporal—perhaps the most aggressive human feeders do not emerge during the time spent trapping in the summer. It is also possible that the richness of pests found at Tire Field compared to Bacon Flats could be due to the fact that Bacon Flats is a fairly homogenous habitat, so there may be less opportunity for rare or specialist species to settle there. Indeed, Bacon Flats had a higher concentration of generalists than any other site, with 66.6% of the species identified from that site falling within the generalist category. Tire Field is an expansive forested field with little human activity and is bordered by vernal pool-laden woods. It is possible that the diversity of the habitat itself has created a more welcoming environment for a larger variety of mosquito species.

In addition to mosquito species that posed as general beings of annoyance to human beings, 15 species were identified that are known vectors for various human pathogens, such as West Nile Virus (WNV) and encephalitis (Erwin et. al. 2002, Farajollahi et. al. 2005, Molaei et. al. 2006, Hamer et. al. 2009, Kilpatrick et. al. 2010). These species, however, were not found in high numbers, and the known WNV vectors (Culex spp.) were not found near human settlements. This could be due to the fact that Culex mosquitoes are mostly ornithophilic (Kilpatrick et. al. 2006, Molaei et. al. 2006, Savage et. al. 2007, Loss et. al. 2009), in which case their bloodseeking activities could be mainly focused on finding bird hosts. Additionally, it is possible that temporal conditions played a role in the low numbers of possible vector species being captured (Spielman 2001, Kunkel et. al. 2006, Kilpatrick et. al. 2008, Kilpatrick et. al. 2010).

There are a number of ways this study could have been improved, which all can be taken into consideration for any future studies. First, beginning trapping—both adult and larval—across all three sites at the same time would ensure that mosquito species emerging at different times in the season would not be missed. Host diversity counts should also begin at the same time as mosquito trapping to account for the same temporal shifts throughout the season. Trap cameras should be placed within all trap sites in order to account for larger mammals (including humans), in addition to the “hair snares” placed by the mosquito light traps. Methods into assessing amphibian and reptile diversity should be investigated, while it may also be helpful to refer to previous literature or diversity counts within the past decade.

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LITERATURE CITED


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APPENDIX

**FIGURE 1.** (a) Bacon Flats, a residential site with no consistent water source. (b) Fern Glen, a marshy wetland with a permanent pond and a hiking trail. (c) Tire Field, a forested field with no human disturbance.

**FIGURE 2.** A total of 23 mosquito species were accounted for across all three sites. Bacon Flats had the smallest number of mosquito species (n=9), while 15 and 16 species were sampled from Fern Glen and Tire Field, respectively.
FIGURE 3. Tire Field (c) had the greatest host richness compared to Bacon Flats (a) and Fern Glen (b), although most of this richness was composed of avian species rather than mammals or amphibians/reptiles.

FIGURE 4. There is no clear relationship between host diversity and mosquito diversity. Tire Field had the highest mosquito and host diversity. Fern Glen also had high mosquito diversity, but had the lowest host diversity value. Bacon Flats had the lowest mosquito diversity and a modest host diversity value.
**FIGURE 5.** Overall mosquito diversity across sites compared to the mean number of pest species captured at each location in order to assess pest species prevalence at sites with low mosquito diversity. Pest species of mosquito were most common at Tire Field than at any other site.