Virginia Outdoors Foundation Publication

Insect Biodiversity of the Preserve at Bull Run Mountains

Fellowship Report 1

April 2021

Meredith Hart and Summers Cleary
with Joe Villari

Virginia Outdoors Foundation

Acknowledgements and thanks

We would like to thank all of our VOF volunteers who contributed to this project, especially those that made such an intensive field work season possible.

Kevin Denham, a dedicated VOF Volunteer, acted as Meredith's dependable and constant field assistant. Brody Receveur, an undergraduate statistics student at George Mason University, kindly helped with data analyses.

We appreciate the Virginia Department of Conservation and Recreation's Division of Natural Heritage (DCR-DNH) for all of their previous work done within VOF's Bull Run Mountains Natural Area Preserve (BRMNAP) and for openly sharing their technical insight, guidance, and expertise in relation to this project.

Copperfox Distillery (based in Sperryville, Virginia) selflessly came to our rescue with a notable donation of ethanol preservative. This study project kicked-off just as a global supply shortage and production bottleneck of disinfecting agents was caused by the Covid-19 pandemic. Without Copperfox's generous contribution, our specimens would not have been able to be permanently preserved for further scientific study – and for that we will remain eternally grateful.

We are eternally grateful for the research that has guided our previous entomological understanding of the Bull Run Mountains. The significant entomological work completed within the Bull Run Mountains by Dr. Arthur Evans, Dr. Oliver Flint, and Dr. Dave Smith is especially worth mentioning.

Finally, we would like to thank all of those who engage with us online, come to our public events, and are taking the time to read this report. We appreciate each of you for opening your hearts and minds to our local six-legged kinfolk.

Meet the Authors

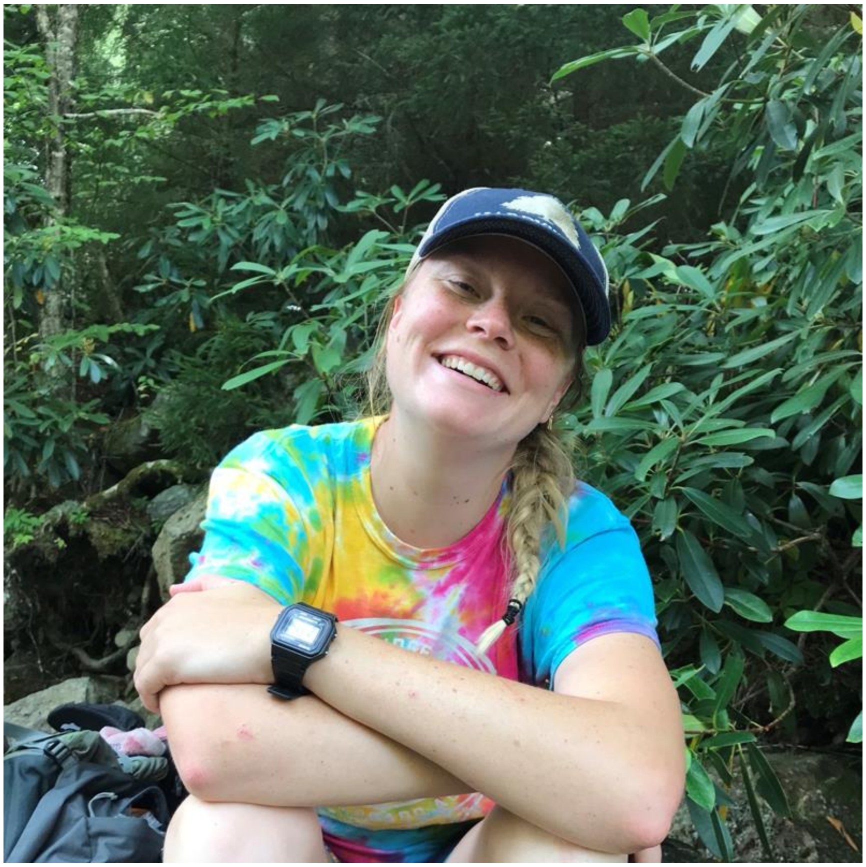
Meredith Hart

Meredith Hart is VOF's inaugural Natural Science Fellow. Currently in her junior year at George Mason University, Meredith is pursuing a B.S. in environmental science with a concentration in wildlife studies and a minor in global health.

Meredith's time at the Preserve was filled with a triumphant field season and a meticulous study of her very favorite thing … insects! Her interest in insects was piqued when she met a scholar from the National Institute of Health who told her about a cockroach that produces a type of milk for its young. Meredith's curiosity and unrivaled enthusiasm for entomology has led her to the Preserve, where she has spent the summer of 2020 collecting and analyzing the insect biodiversity of the Preserve.

Meredith brings fresh perspective and much needed passion for an often-overlooked class of the animal kingdom.

Meet the Authors



Summers Cleary

Summers' work on Bull Run Mountains Natural Area Preserve is varied, keeping her out in the field some days for field mapping, trail maintenance projects, and talking to preserve visitors, or in the office creating maps, doing geospatial analyses, and assisting with management initiatives.

Summers earned her bachelor's in geography and master's in geospatial analysis from the University of Mary Washington with an emphasis on human-environment interactions and biogeography. Her work in natural resource management began in graduate school as a field technician with the Virginia Department of Conservation and Recreation. She has worked and lived in New River Gorge National River in West Virginia, but lately has ambled slightly back east to work with VOF, applying her love for exploring and analyzing the outdoors on the daily.

When not working, Summers can be found painting and drawing features of the natural world, as well as running around the woods in her Rappahannock County home.

Meet the Authors

Joe Villari

Joe manages VOF's Bull Run Mountains Natural Area Preserve in Northern Virginia, which is VOF's largest and mostly visited reserve. His focus is on implementing science-based management practices that balance the conservation needs of the property, while maximizing its scientific and educational potential.

Originally from Prince George's County, Maryland, Joe grew up in the rural landscape of Fauquier County and considers the small towns of Paris and Marshall home. Fascinated with animals at an early age, he would go on to earn his bachelor's in conservation biology and master's in environmental science and policy from George Mason University.

Before coming to VOF, Joe worked for the Smithsonian Institution's National Museum of Natural History, where he received a Peer Recognition Award for his contributions.

Joe is a passionate advocate for habitat conservation, field, and specimen-based scientific research, and making science more accessible to the general public.

Contents

Section One: Introduction

The Virginia Outdoors Foundation's Bull Run Mountains Natural Area Preserve (BRMNAP) is a ~2,500 acre living laboratory and open-air museum that sits in the backyard of our nation's capital (see ***Figure 1***). As a state-designated Natural Area Preserve, its stewardship is dedicated to maximizing the scientific and educational potential of its natural and cultural resources, while ensuring the most pristine natural habitat possible.

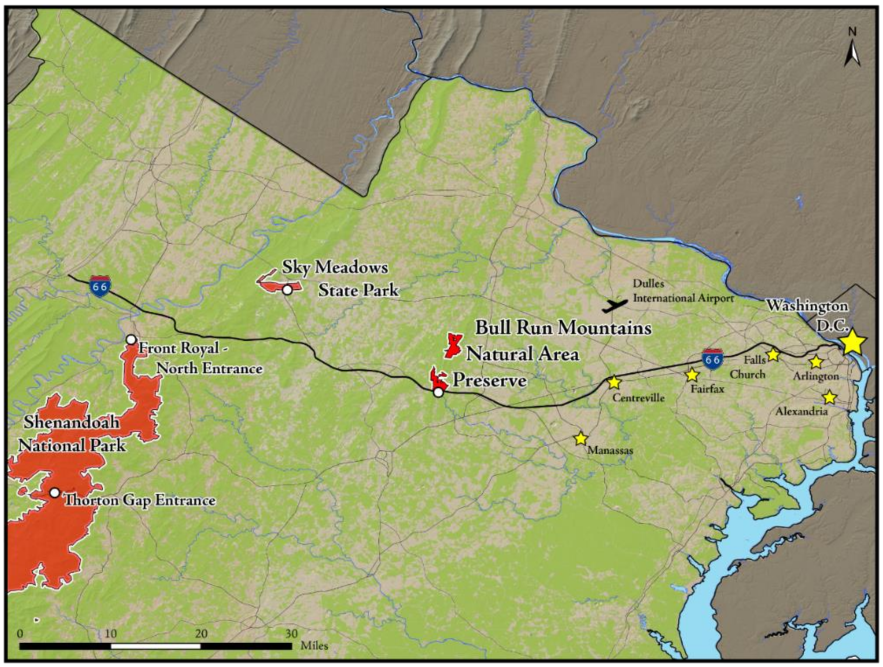


Figure 1*: Location of VOF's Bull Run Mountains Natural Area Preserve relative to Washington D.C. and other notable landmark features in the surrounding area.*

To best meet these objectives, The Preserve is divided into three sections and management units (see ***Figure 2***).

Inaugural Fellowship Program

VOF's Fellowship Program was developed to provide early career professionals with the opportunity to gain experience co-developing and executing a research project in their chosen interest area. While providing these key early career opportunities, this program also helps BRMNAP fill areas of specialized expertise and build internal capacity in the arenas of scientific and historical research, program development, multimedia, and/or other special projects that otherwise may not be possible with our small team of full-time staff.

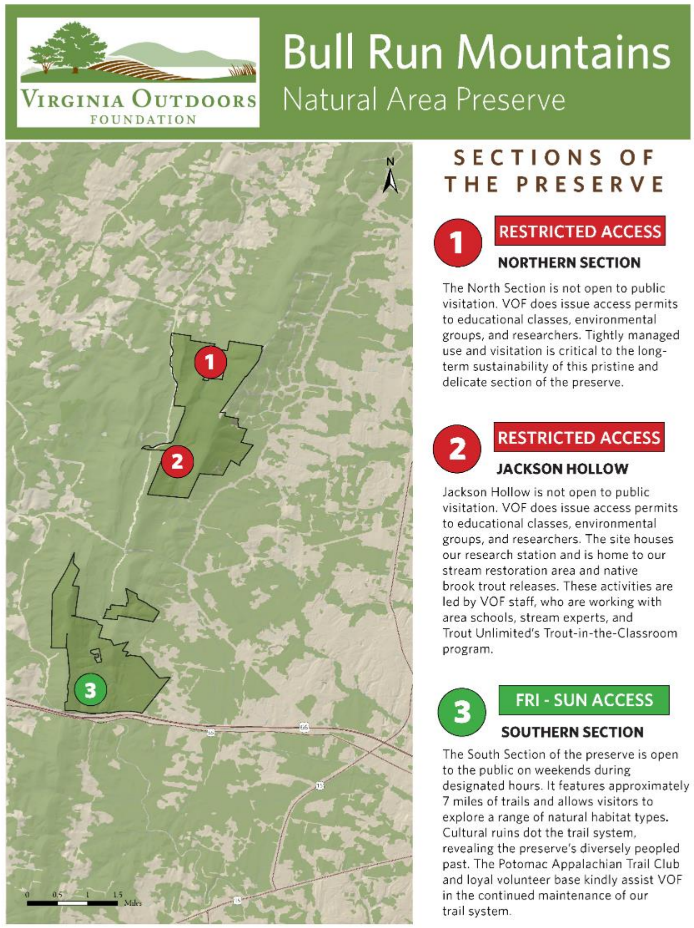


***Figure 2**: The Preserve's three management sections*

2020 Natural Science Fellowship

Meredith Hart served as Virginia Outdoors Foundation's (VOF's) inaugural Natural Science Fellow. Her research project aims to provide the Preserve's Managers and the general public with a better foundational awareness of the insect biodiversity that is protected by VOF's Preserve at Bull Run Mountains. Meredith completed this fellowship in the allotted 360 hours, with the support of VOF Preserve staff and volunteers. In 360 hours, our team went from project design and development, to fieldwork, to taxonomic identification, to the preparation of a photographic guide to the specimens collected, as well as the completion of several public outreach and engagement initiatives.

This report contains the fellowship products of Meredith's work. The physical specimens captured during these eight weeks of insect collecting are preserved in ethanol and fully publicly accessible to any researcher interested in building upon this project.

VOF Research Publications and Community Reports: A note to the reader

In addition to the Fellowship Program, this VOF Fellowship Report also formally introduces another new concept, the “Virginia Outdoors Foundation Publication”. This publication series will provide us with the forum to immediately communicate project deliverables in the necessary technical detail, but also within a manner that strives to be approachable and accessible to the communities that we serve. This first Virginia Outdoors Foundation Publication is the Fellowship Report focused on our inaugural 2020 Natural Science Fellowship Project.

This report divides the deliverables of this four-month research project into five main sections. While this guide can be consumed in its entirety, each section is designed to be understood and accessible as a standalone document.

Though these reports aim to disseminate relevant information related to our work in a timely fashion, further analyses of data will be performed to answer targeted research questions. The outcomes of those analyses will be submitted to appropriate peer-reviewed scholarly journals for wider specialized publication. For those reasons, data may not always be included in full. These reports instead act as an initial platform of a given project that will hopefully continue to be built upon over time.

Impacts of the 2020 Natural Science Fellowship

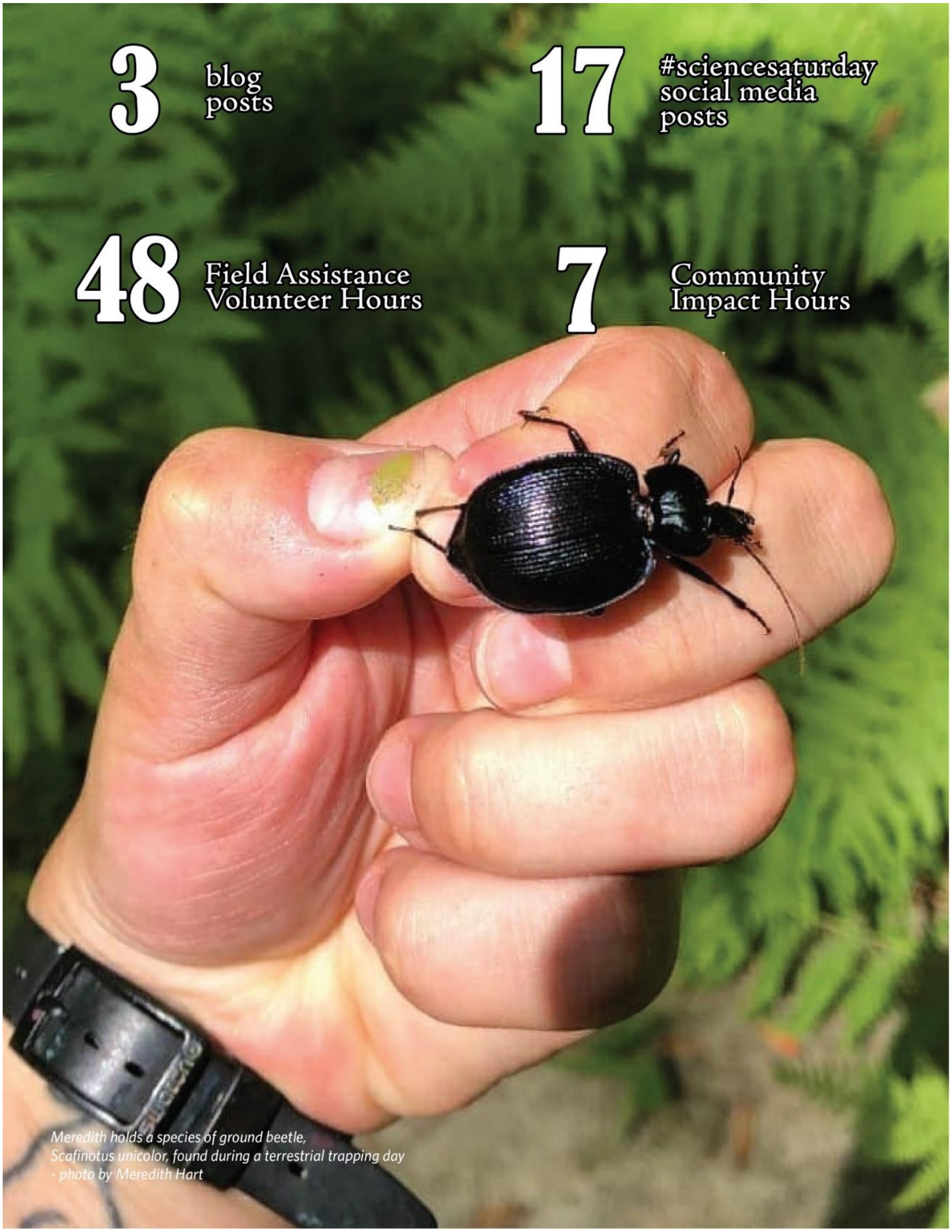
This project resulted in a number of outcomes that contribute to the scientific and educational value of the Preserve. These metrics will surely only amplify as time enables us with opportunities to continue to build upon this foundation.

In addition to the scientific and management value, Meredith engaged with the Preserve community in a number of ways. A summary of scientific and engagement related impacts are quantified on the following two pages.

In addition to what is contained within this report, there were regular social media and blog posts that invited our followers to join Meredith in her journey as the Natural Science Fellow – and learn some new things about insects along the way! A number of volunteer Community Scientists assisted Meredith in the field during her intense field collecting efforts, and Meredith even led multiple entomologically focused guided hikes for both kids and adults (which are captured in the following two pages under “Community Impact Hours”).

Scientific Impacts to Date

Public Engagement Impacts to Date



Meredith holds a species of ground beetle, Scafinotus unicolor, found during a terrestrial trapping day - photo by Meredith Hart

Section Two: Insect Biodiversity of the Preserve at Bull Run Mountains

1. Introduction

Global insect biodiversity demands further study and possible intervention, as insect taxa are in decline across many regions of the planet (Montgomery et al. 2020). Because of its permanently preserved status (Leahy & Erdle 2004), its uncommon element occurrences and closely monitored management practices (Fleming 1999,Fleming 2015), and its 10 distinct plant communities (Fleming 2002, Fleming 2016, Fleming, 2020), BRMNAP serves as a unique site to observe and study insect biodiversity.

Prior research on insect biodiversity within the Bull Run Mountains has been relatively extensive, if targeted.

The most recent endeavor was the Department of Conservation and Recreation – Department of Natural Heritage's (DCR-DNH) inventory on adult dragonflies and damselflies (Odonata) (Hobson 2019).

Dr. Dave Smith and Dr. Oliver Flint have, over the past nine years, collected and studied sawfly (Hymenoptera) and caddisfly (Trichoptera) specimens throughout the Bull Run Mountains (Smith 2006, Smith 2013, Flint 2014, Flint 2017). With thirteen trapping sites being utilized over a nine-year period to target select stream-dependent species, among many of their successes were the documentation of 223 of the 345 sawfly species that have been recorded in Virginia (around 65% of the state's known sawfly diversity).

Dr. Arthur Evans and colleagues have heavily contributed to the understanding of beetle diversity within the mountains, describing the natural history, distribution, defining characteristics, and taxonomic status of over 10 species (Evans 2009a-d, Evans 2011, Evans 2012, Evans and Steury 2012). The wealth of past information on insect biodiversity at the Preserve provides a necessary springboard for this more broadly-focused fellowship project.

BRMNAP serves as a biodiversity hotspot within Northern Virginia and continued research will only elevate its cumulative scientific importance. Although this study presents only one season's worth of data, and therefore has its limitations in extrapolation, it will serve as an important time-stamped "snapshot" into insect biodiversity. As such, this data may become essential to establishing future patterns of change in community structure and biodiversity.

A fragmented habitat is one with areas of divergence from an organism's preferred habitat. These can occur naturally (grassland to forest, cenotes, etc.) or as a result of human disturbance (such as urbanization). Insect behavior within forests is often linked directly to fragmentation and border presence; a significant number of insects prefer ovipositing on the fragmented edges, the edges displaying greater levels of communal variability (Ewers & Didham 2006). With this in mind, penetration of external forces into fragmented ecosystems can increase variability by creating more edge habitat, while negatively impacting overall diversity and natural compositions for that habitat.

Species with a higher trophic level and larger body sizes are at the most risk for extinction when their habitat fragments, while smaller species with increased adaptability generally survive the temporal gap. BRMNAP is ~2,500 acres currently separated into three non-contiguous sections. Because Preserve stewardship ends at certain boundary lines, depending on the land usage and preservation status on properties along these Preserve boundaries, the ecological disparities across the Bull Run Mountains could be a result of fragmentation similar to the effects seen on protected land vs unprotected land that borders it. Especially for colony species (such as ants) with displayed territoriality and multiple species per one forested area, this could be an interesting way to monitor species diversity across areas, and see how human activity fits in with fragmentation activities to contribute to habitat loss and decreases of dominant species.

“Biodiversity monitoring” refers to observing species variety in ecosystems and using it to track variations in ecosystem communities over time (Duro et al. 2007). At the present time, most biodiversity studies rely heavily on the presence of arthropod, specifically insect, communities (Hallmann et al. 2017). Beetles are the most widely used invertebrate as an indicator of biodiversity in the northern hemisphere (Silva et al. 2017). However, ants are the most favorable species for conducting biodiversity analysis, as their high diversity and biomass, easy collectability, short generation times, and sensitivity to environmental change highlight them as the ideal taxa for ecological indication and conservation projects (Peck et al. 1998). Ants are an ecological indicator in tropical ecosystems, given their prevalence and quick response to changes, and are used commonly as a way to evaluate whether or not an environment will recover from damage (Dahlgren et al. 2012). Though ants are not the only species used to evaluate biodiversity, other species have been utilized in establishing whether or not insect communities have an established effect on the plant communities present (Silva et al. 2017). A study done in Northern Australian territory found positive correlations between ant communities and other invertebrate taxa, as well as plant species. This is potentially due to ants’ different interactions with various beneficial communities, and their abilities to regulate a microclimate. In the future, these will be excellent avenues to expand the Preserve’s research endeavors. However, we began an inquiry into the insect biodiversity of the Preserve in general to set the stage for diving into more nuanced research topics.

While certain species can be used to indicate an environments robustness, this is not a forever-measurement of how well that environment will be able to adapt to changes, that range from climate change, to natural disasters, to increased urbanization. One currently studied change in northern temperate ecosystems is the effect of habitat fragmentation on smaller fauna (insect communities) (Schlaepfer 2018). Insect behavior changes along the edges of these fragments; increased transience between species and more oviposition occurs along these areas than in more dense parts of the forest (Schlaepfer 2018). Through biodiversity monitoring, different species are collected and evaluated against one another in order to find a correlation. Because of ants' role as a bioindicator and ecological indicator species, there is a positive correlation between ant diversity and surrounding species diversity (Schlaepfer 2018). An unexplored aspect of this, is how separate ant communities (and the territoriality between them) impacts the flora and fauna present in their separate "areas", and whether or not this can be considered a level of micro-fragmentation present on the species level.

Although ants and beetles make for useful spotlights when discussing the principals of insect biodiversity, a focus on the complete diversity is critical to monitoring and conservation. Not only to better document and track species survival rates during an ongoing insect apocalypse (Sánchez-Bayo and Wyckhuys 2019), but because several disparate species are often important indicators of environmental health for differing habitats. Many aquatic larvae (Odonata, Plecoptera, etc) are indicators of water quality (Cadmus et al. 2020). Through continual study and analysis into the total composition of insects on the preserve, a better understanding of insect populations, the plant communities they depend upon, and aquatic health may begin to emerge.

2. Materials & Methods

Over the course of June-September 2020, the insect biodiversity of Bull Run Mountains was investigated by our Fellowship team. Our Natural Science Fellow was allocated 360 paid hours that would include project design and development, fieldwork, specimen sorting and taxonomic identification, the preparation of a photographic guide, a series of blog posts, and weekly #sciencefellowshipsaturday posts on @bullrunmountains social media account.

Our focus was to identify and analyze insect biodiversity across the Preserve, specifically within the North Section and Jackson Hollow Section. Although we took a few exploratory surveys within our South Section, it was excluded from this initial full-scale study for the sake of time.

Five different insect collection types were used over the course of seven weeks, late May to late July. Aerial, aquatic, and terrestrial environments were sampled, and each collection method was set up and collected weekly. Collection types included: pitfall traps, three different aquatic collection methods, and sweep net collection methods. Specimens were preserved in ethanol, identified down to family level using a macroscope and relevant reference literature, then catalogued and analyzed to calculate biodiversity metrics.

3.1 Materials

Materials organized by collection type:

Pitfall traps

39 red solo cups (per week) transferred to vials and jars once in the lab

1 trowel to dig holes for placement

Approximately 16 liters of water spread between traps, each with a drop of Dawn dish soap added to the trap's liquid "catch'. Please note that this was employed instead of ethanol, as it effectively deterred the tampering of traps by black bears (*Ursus americanus*) and also acted as an overnight field preservative agent.

The ingredients in Dawn dish soap: Alcohol Denatured, C10-16 Alkyl dimethylamine Oxide, Colorants, Fragrances, Methylisothiazolinone, PEI-14 PEG-24/PPG-16 Copolymer, Phenoxyethanol, PPG-26, Sodium Chloride, Sodium Hydroxide, Sodium Laureth Sulfate, Sodium Lauryl Sulfate, Water, C9-11 Pareth-8 ("Dawn Ultra Dishwashing Liquid Dish Soap, Original, 8 Fl Oz - SmartLabel^{TM}" n.d.).

Sweep net collection method

1 sweep net, 8 glass jars (per week), 64oz of ethanol.

Aquatic collection methods

1 pool net (hard), 1 pool net (soft), 1 pond net, 8 glass jars (per week), 64oz of ethanol.

3.2 Methods

Three different collection methods are used to assess insect biodiversity across the North and Jackson Hollow Sections of the Preserve, once a week over an eight-week period from June to August. Pitfall traps, used to assess terrestrial arthropod diversity, are distributed in the two sections of the Preserve at 13 individual sites, with three pitfall traps per site. Sweep net collection methods are conducted at four individual sites throughout the two sections. Aquatic collection methods are conducted at eight individual sites along Hungry Run and Catharpin Creek in the North Section and Jackson Hollow Section, respectively. See ***Figure 3*** for locations of each collection site throughout the corresponding sections of the Preserve. From each collection site, specimens collected are identified to family level and stored for permanent preservation.

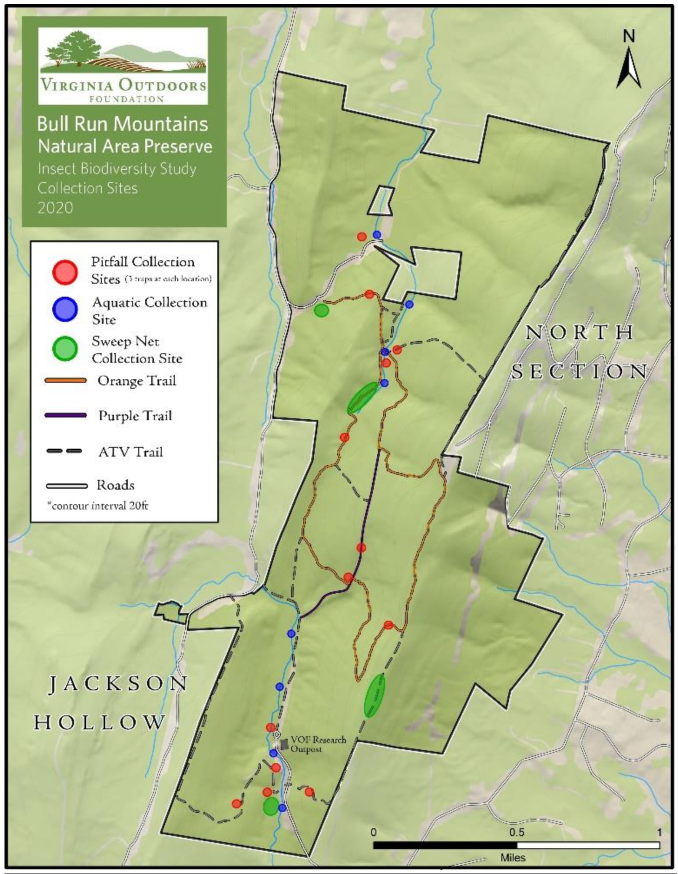


***Figure 3**: Pitfall, Aquatic, and Sweeping Collection sites across the North and Jackson Hollow Sections of BRMNAP.*

<u>*Pitfall traps*</u>

Pitfall traps are placed once a week in 13 separate locations across the North Section and Jackson Hollow of BRMNAP (see ***Figure 2***). Three individual pitfall traps are placed at each trap location marked in ***Figure 4***, totaling to 39 individual traps. At each site, the three traps are placed within a 10-meter radius, but never placed within the exact same spot within this area to account for territoriality of insect colonies and other factors that may induce the same species to encounter the same paths. If a territorial, social species with a specific pathway encounters a trap multiple times, it could impact the biodiversity conclusions, as the species richness will be skewed in the direction of that species.

To place the traps, an 8 inch hole is dug with a trowel, the pitfall traps (red solo cup) were placed inside, and the edges of the hole were filled in with dirt, creating a smooth surface, and then the cups were filled with the dawn dish soap solution. The water was prepared with Dawn Dish Soap and acted as a killing agent for the invertebrates that fell into the cup. Traps were left out for a 24-hour period.

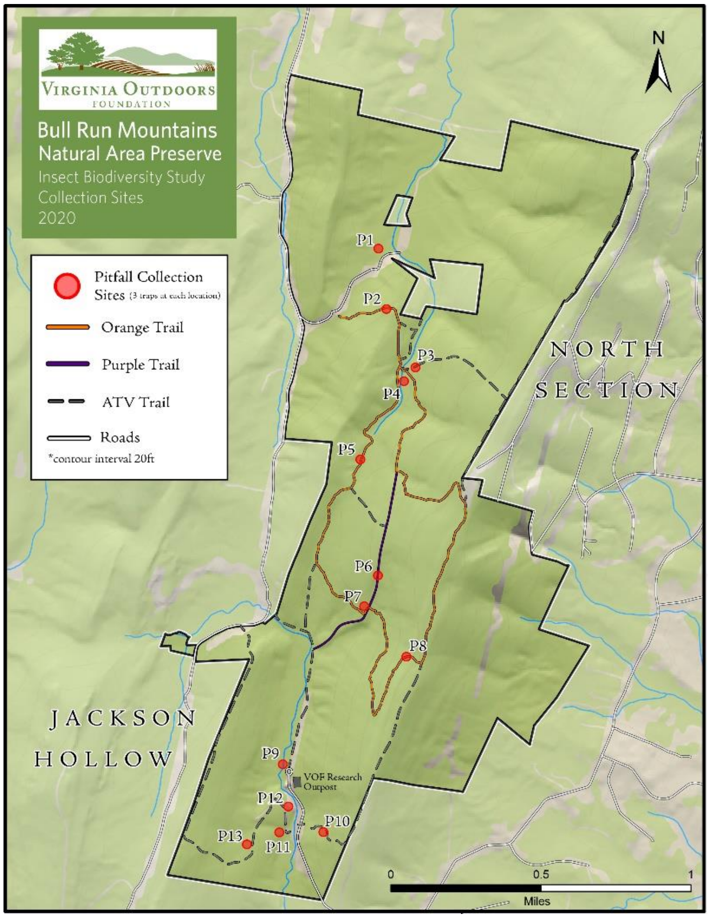


__Figure 4__: Pitfall traps across the North and Jackson Hollow Sections of BRMNAP. Three individual pitfall traps are placed at each site shown in the map.

Aquatic Collection Methods

Aquatic collecting is done once a week for an 8-hour period at eight individual sites across the sections of Catharpin Creek in Jackson Hollow and Hungry Run in the North Section (see ***Figure 5***). Three different collection types are used to collect a representative diversity of aquatic species at each of the eight sites.

A pond net is used for skimming the surface of the water to capture those invertebrates residing on the surface. Generally, five passes (or samples) with the pond net across the surface are conducted at each of the eight sites.

A hard pool net is used to capture and sample invertebrates that burrow into the sediment at the bottom of both creeks. Collection with this net involves digging into the sediment at the bottom of the creek and sifting through it, to reveal invertebrates. The sediment is sampled five times per site.

A third net, a small and soft pool net, functions as a kick net and is used to catch insects floating downstream at each of the eight sites. This net is placed at the bottom of the creek, facing upstream and weighted down with rocks for a total of 30 minutes. In the last five minutes, the researcher moves upstream to disturb the sediment (kicking & shuffling sediment with boots) in order to dislodge specimens.

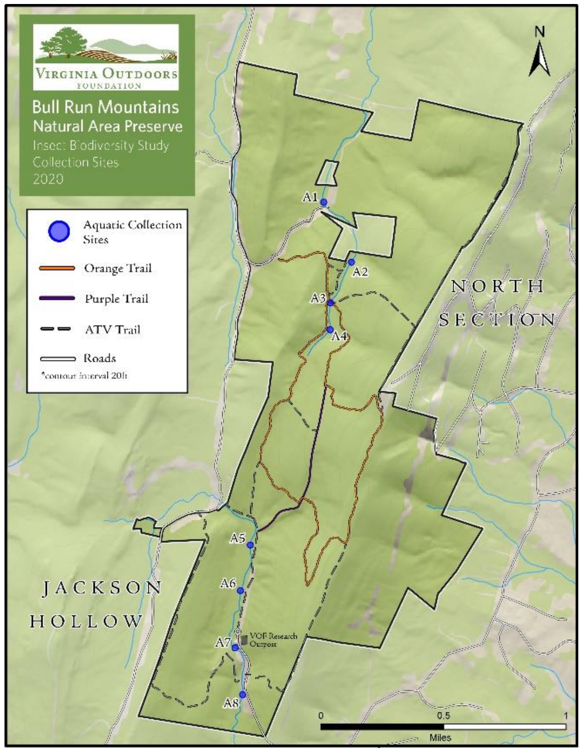


Figure 5: Aquatic collecting sites placed on Hungry Run in the North Section (A1 – A4) and Catharpin Creek in Jackson Hollow (A5 – A8).

Sweep Net Collection Method

Sweep net collecting is performed once a week at four different collection sites across the North Section and Jackson Hollow (see ***Figure 6***). A sweep net is swept across areas with high grasses and dense herbaceous layers for 10 minutes at each site. Multiple passes with the sweep net are conducted at each site for the entire 10 minutes. Insects within the grasses and herbaceous layers are captured within the net, then collected and placed into jars with 1oz of ethanol for preservation for remainder of field day.

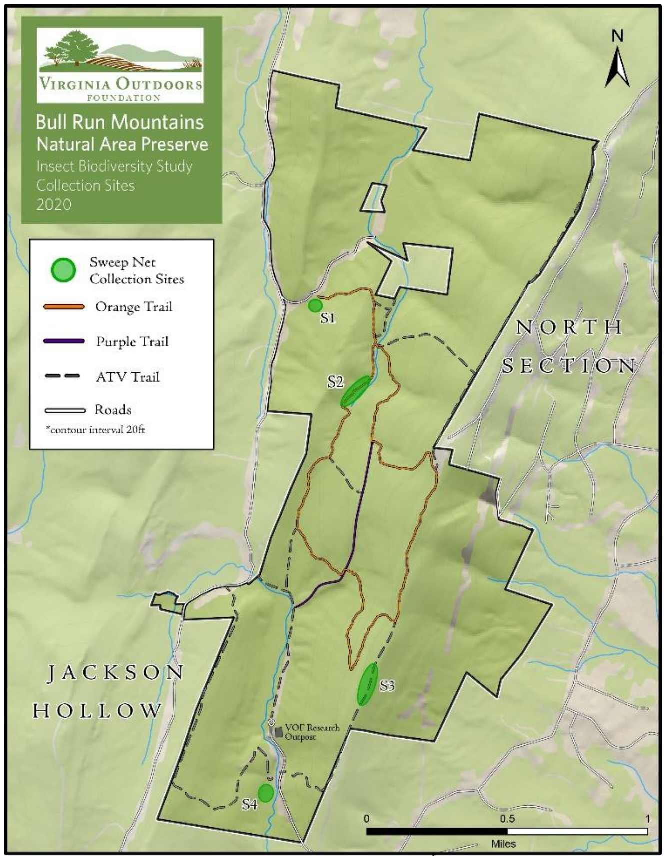


Figure 6: Sweep net collection sites in the North and Jackson Hollow sections of BRMNAP.

Preservation and Identification

As with sweep net protocols, specimens from each collecting method are euthanized in a small jar containing 1oz of ethanol, in which they are preserved for the remainder of the field session. Once in the lab specimens from each field jar are rinsed to remove excess debris and soil, then sorted into small vials according to collection type and location, and labeled with date, collection type, and location (e.g. 6-24-2020, Aquatic, A2). Each vial contains ethanol to ensure proper, long-term preservation.

Specimens in each vial are then analyzed under a macroscope and identified down to family level. An entire list of specimens collected is housed internally and available upon request. While working through the identification process, photographs are taken of each specimen for the creation of a photographic guide. This photographic guide serves as a useful tool for further inquiry into, and education on, the insect biodiversity of BRMNAP.

3. Analysis

Once identified down to family level, all data is catalogued into an Excel file and analyzed with the alpha diversity statistic.

Alpha diversity is the average species diversity in an area. Alpha diversity applied to this study shows the relative diversity at every individual pitfall, aquatic, and sweep net collection site. This measure of diversity, taken at a local scale, is important for future comparative density work in the BRMNAP to calculate the relative increase or decrease of local species diversity over time. Documentation of the trend of species diversity can identify patterns and indicators to observed trends, such as the presence of environmental stressors or overall ecosystem health decline.

Each individual collecting site is analyzed using alpha diversity and presented in graph format. See Appendix A to see all figures representing alpha diversity for each collection type.

Further specialized analyses will be performed by Preserve staff to answer questions pertinent to submission to peer-reviewed scholarly journals.

4. Discussion

When analyzing Alpha Diversity graphs of BRMNAP's insect populations over an eight-week collecting period, an important aspect of the project to consider is that the pitfall traps were placed in the same or similar locations every week. Traps were placed within a 10-meter radius of where they were the previous week. This slight weekly adjustment was in place to reduce the impact of social foraging insect behavior, while still reliably sampling the same 10-meter area. It is possible that abiotic and biotic factors were influencing their results (Ishaya et al. 2018). One biotic factor that routinely impacted these terrestrial trap sites was bear activity (and/or other mammal disturbances). On five occasions it was discovered that 1+ trap out of each 3 had been dug up and mangled in a particular area, and specifically the diversity for trap site P5 may have been impacted by this activity, as the animals had a higher activity in this region (see ***Figure 2***). Although ethanol and isopropyl alcohol have been defined as the most effective killing agents for different insect traps (Szinwelski et al. 2012), due to the issues we experienced with bear tampering, the dawn dish soap solution was substituted, and the problem decreased.

Alpha diversity among each different site is not impacted by diversity of the others and is one of the reasons that a gamma diversity analysis was overlooked. The gamma diversity statistic is a holistic approach that would have been comparing three different habitats. Thus, the populations within them may have displayed significant diversity according to the test, which would have impacted overall conclusions about the biodiversity of BRMNAP (Bynum n.d.).

From reviewing the alpha diversity statistics, we can ascertain that pitfall traps carry the greatest number of specimens from each family but are similar to sweep net collections in the total number of insect families present themselves. This means that the grassland living hemipterans most commonly captured in sweep net collection methods are able to live together in larger numbers than ground-living arthropods, with the exception of ants, which are social insects. Tallgrass prairie is one of the most endangered ecosystems in the United States, with much of it at risk due to farming, and so these microhabitats that can still support insect life in droves are crucially important in understanding how these insect communities live together (Hamilton 2005).

Aquatic insect alpha diversity was consistent over the summer, with increases in rainfall typically bearing the most correlation to spikes in diversity among different sites (Gurr et al. 2012), although this may be more detrimental than beneficial. For many of the insects observed, their predatory status is reliant on being able to hide from prey before they ambush, and a heavy rainfall on shallower areas (particularly in Catharpin Creek in the North Section) could uproot ecosystems, since it impacts the predator-prey dynamic.

5.1 Aquatic Data

Each aquatic collecting site offered different levels of total specimens collected. Subsequent graphs illustrate this disparity between each site (see Appendix A ***Figures A1 – A8***). Alpha diversity tests were performed on each collection site, accumulating all species present over the summer in order to gauge the diversity of each area, and the comparisons between different areas. This test is important in understanding biodiversity of aquatic species, as many of these species are ecological indicators, and their future presence or absence will be indicative of changes in water quality.

Over the course of the summer, the number of specimens collected varied with both rain flow and season. Jackson Hollow was observed to be the most specious. This could be due to Catharpin Creek in Jackson Hollow being deeper, and the mixed sediment allowing predators such as Plecoptera and Odonata ample feeding grounds. Softer sediment typically harbors predators more easily than rocks/pebbles, as these opportunistic feeders need to both disguise themselves and ambush prey quickly. Caddisfly larvae were more prominent in North Section collection sites on Hungry Run, once again due to habitat preferences. Generalists between the two macro-locations (North Section and Jackson Hollow) were Hemiptera, in the families Gerridae and Veliidae. These predatory insects are predators and scavengers, and some species (in Veliidae) are social hunters, which explains their large numbers across both areas.

Aquatic data is primarily important in monitoring water quality, and as Catharpin creek lies almost completely within the boundary of BRMNAP, it is likely that the chemical composition will remain the same (Hunter, MacDonald, and Carter 2010, Cadmus et al. 2020). This data will be valuable to BRMNAP in areas of development within the preserve, such as the trout-release project within Catharpin Creek.

5.2 Pitfall Data

Pitfall trap data displayed less overall specimen presence per species for the summer, but an overall greater diversity than in Aquatic habitats (see Appendix A ***Figures A9 - A21***). This is due to the variety of microhabitats sampled via pitfall traps across BRMNAP.

Over the course of the summer, the largest pitfall trap concerns revolved around 1) capturing vertebrates, and 2) bear and raccoon impact. The presence of bears and raccoons on BRMNAP bore a significant concern for the wellbeing of traps. Halfway through the trapping season ethanol was replaced with the dawn dish soap solution, which made preserving specimens a little more difficult, but decreased the occurrence of traps being dug out of the ground. All traps removed by foraging animals were recovered, and no plastic was left in the trapping sites. Over the course of eight weeks, only two vertebrates fell into the pitfall traps.

Dominant families represented in specimens collected included ecological indicators formicidae and oliopones (harvestmen). Because there were so many different species, observing trends is difficult without further analysis. Theories for an explanation of the differences in species between traps include elevation, habitat specialists, and the community dynamics between different species (EG: the presence of parasitoid wasps in correlation with their prey specific species).

Terrestrial data largely indicates the presence of numerous microhabitats among the different areas of BRMNAP. Microhabitats are buffers after deforestation events, influential in determining response by animal indicator groups, and increase diversity in terrestrial areas (Bogoni et al. 2013, Mehrabi et al. 2014, Torossian, Kordas, and Helmuth 2016). Future research with this dataset will allow researchers to compare test results to the different ecological areas of The Preserve, and monitor changes in diversity.

5.3 Sweep Net Data

Sweep net data was comprised almost entirely of Orthoptera and Hemiptera (see Appendix A ***Figures A22 – A25***). Many of these species are grass-specialists, with camouflage enabling them to live and hunt in the grassy and open areas of BRMNAP. The largest difference between insect presence on the North Section and Jackson Hollow came in the form of predatory species. Mantids were far more common in Jackson Hollow, perhaps due to its dense forest covering around the meadow (see ***Figure 4***, collection site S4), allowing juvenile mantids to seek arboreal cover once they grow older.

The insect presence in the North Section was incredibly sparse for predator species. The most common species were Orthoptera, specifically different kinds of Katydid. When identifying the insects found, one challenge was that many species were still juvenile, and either (in the case of mantids) lacking identifiable characteristics in terms of patterning, or completely different to the adult forms (all Pentatomidae nymphs).

In the beginning of the summer, calligrapher flies were a dominant species among sweep net specimens, but as the summer continued, they only made up a small percentage of the final volume. After heavy rainfall (and during rainfall of any kind) less specimens were captured, but the volume of specimens between sites did not typically change depending on the time of day.

Future uses for this data include an analysis of the graminoid composition as herbivory alters composition (Hartley, Gardner, and Mitchell 2003). Another area of concern for BRMNAP's natural plant communities and the insect populations that depend on them, is the influence of Joint-head grass (*Arthraxon hispidus*) and Japanese stiltgrass (*Microstegium spp.*) - common Virginia invasives dependent on elevation. Spraying and pulling are the best methods to be employed for control of Japanese stilt gras, many different insect species both feed upon and parasitize the joint head grass, so control efforts could be examined through targeted biological agents with that grass species.

5. Conclusion

6.1 Determining insect biodiversity of BRMNAP

Pitfall traps display the most consistent diversity compared to aquatic and sweep net collections. This could be due to the higher number of trap sites and, subsequently, the number of specimens, but also to the fact that there is typically more habitat variation among terrestrial arthropods due to microhabitat differentiation (Nittérus and Gunnarsson 2006).

There were the greatest spikes in habitat-based biodiversity among sweep net specimens. Of the 4 collection sites available for sweep net collecting, 1 was a high altitude location with blueberry bushes, 1 was a low elevation location with ferns, and 2 were (on opposite ends of the preserve) areas of similar elevation with tall grasses. Tall grasses were obviously the preferred habitat for sweep net species, and of the mutual specimens between locations there were only Diptera, Odonata, and some Coleoptera overlap, as the specialized plant Hemiptera (and Orthoptera) were confined to those more open areas. Aquatic collecting revealed greater diversity in two locations: 1) the preferred habitat of the families Gerridae/Veliidae, are deep areas with much overhang shade, and a small current. This is due to their predatory nature, and social hunting behaviors. Odonata and plecopteran species were more commonly found in shallow areas with a loose pebble cover, which allows these ambush predators to hide from their potential prey. Caddisfly larvae (Trichoptera) were non-discriminatory, as the family in question determines habitat on a species level, and thus both free floating and hidden forms of larvae were identified (Urbanič, Toman, and Krušnik 2005).

6.2 Areas for Improvement

Some flaws in the experimental design and methodologies were countered early on in the project- when ethanol was used to bait pitfall traps, they were subsequently disturbed or destroyed by black bears (*Ursus americanus*). To avoid further losses to bear activity (two weeks into the project), ethanol was swapped for a dish soap solution as the invertebrate killing agent and preservative. While pitfall traps were dug up at less frequent intervals using this dish soap solution, bear activity still continued to contribute to an overall loss of pitfall data.

Another area for improvement could be that this project was limited in "human power": increasing the biodiversity measurement team by even one person has a drastic significance on the amount of data collected (as displayed in aquatic collecting with the additional volunteer). Since biodiversity calculations rely on the accuracy and detail of widespread, equidistant collection sites, adding another person to assist with field work could have both enabled this project to increase in size, and accuracy.

6.3 Future plans

The specimens from this study are now part of VOF's research holdings and will be available to future researchers. A priority should be identifying these collections to finer taxonomic scale, as identification was only able to be completed down to the family level during this study (due to time restrictions).

Select specimen by-catch is also being prepared to be utilized in educational and science accessibility programming with K-12 students, university students, and for better training our many dedicated community/citizen scientists.

The data from this study is currently being utilized in research that should result in publications being submitted to peer-reviewed journals by VOF staff, fellows, and research associates. The data will also help the Virginia Outdoors Foundation make better informed land management decisions.

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Section Three: Appendices to the Technical Report

Appendix A

Alpha Diversity Graphs

Aquatic Collection Sites - Alpha Diversity Graphs

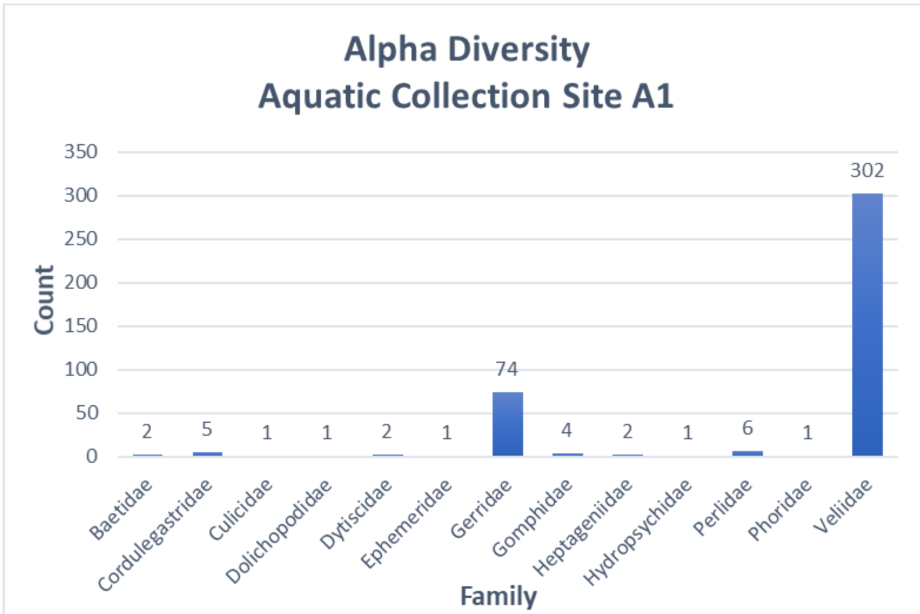

Figure A1: *Alpha Diversity of aquatic collection site A1 on Hungry Run in the North Section. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

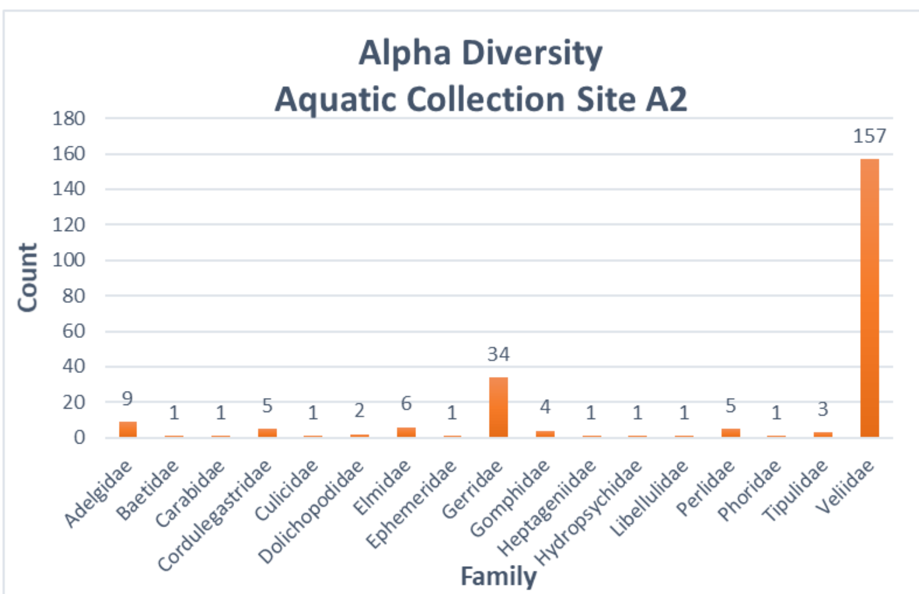

Figure A2: *Alpha Diversity of aquatic collection site A2 on Hungry Run in the North Section. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

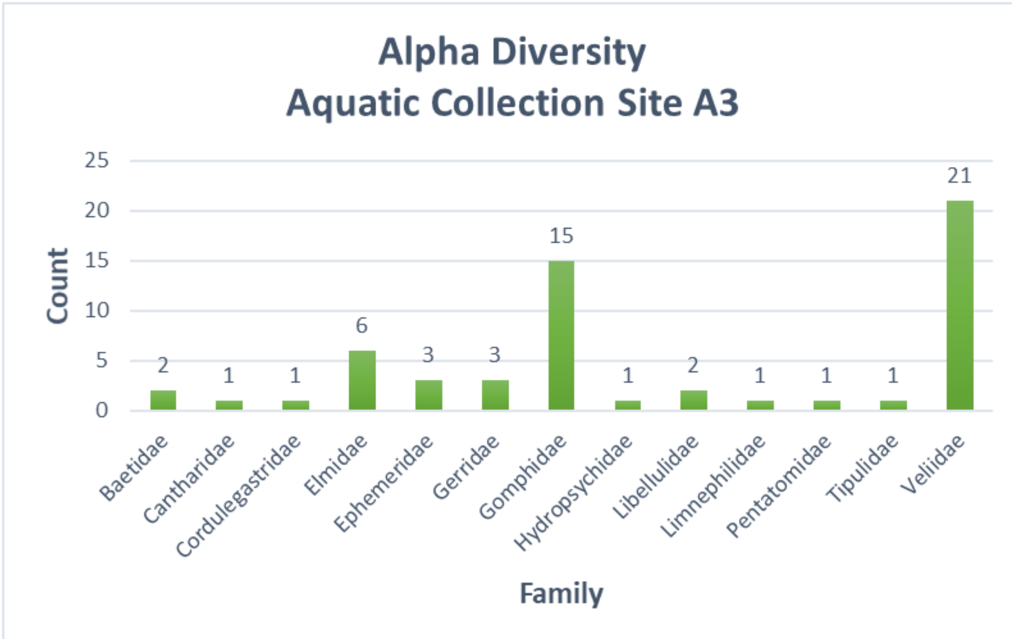

Figure A3: *Alpha Diversity of aquatic collection site A3 on Hungry Run in the North Section. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

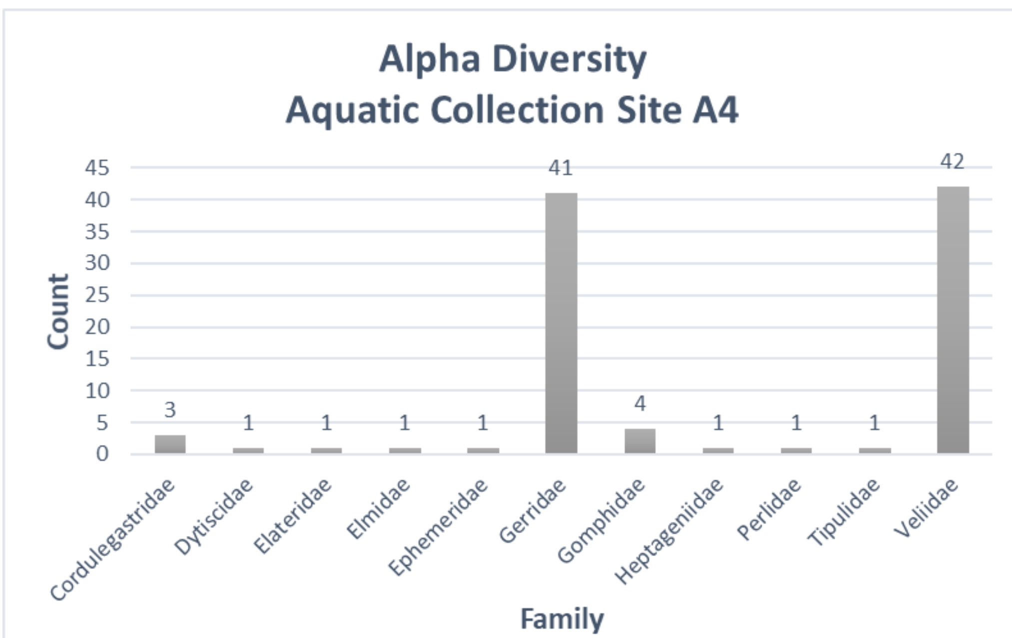

Figure A4: *Alpha Diversity of aquatic collection site A4 on Hungry Run in the North Section. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

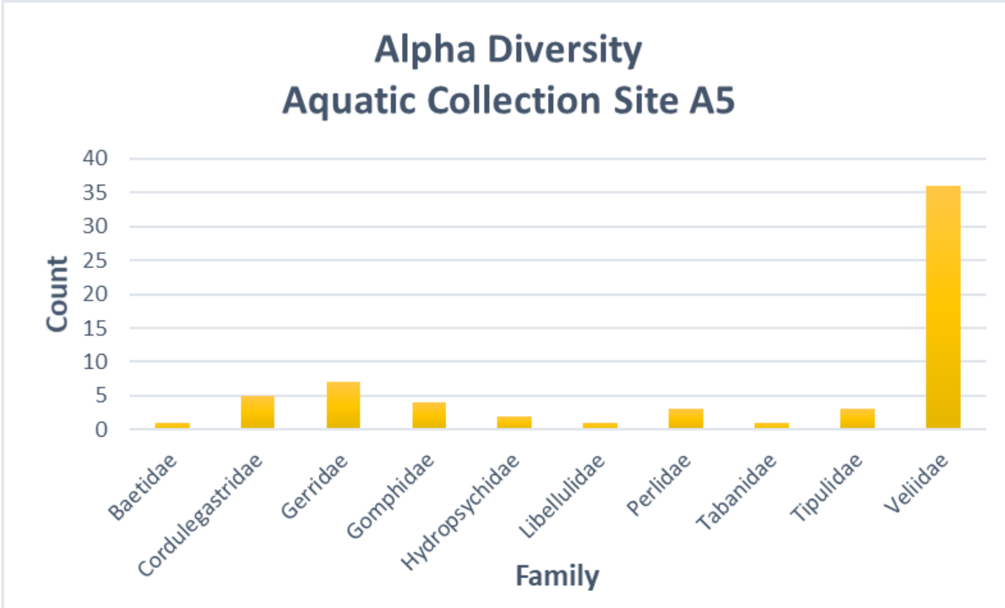

Figure A5: *Alpha Diversity of aquatic collection site A5 on Catharpin Creek in Jackson Hollow. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

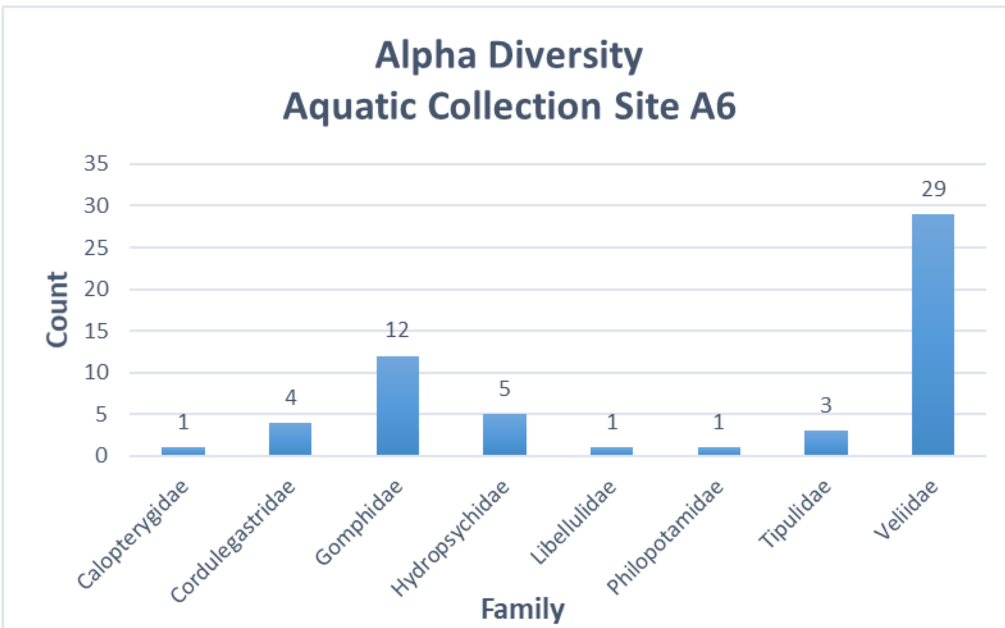

Figure A6: *Alpha Diversity of aquatic collection site A6 on Catharpin Creek in Jackson Hollow. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

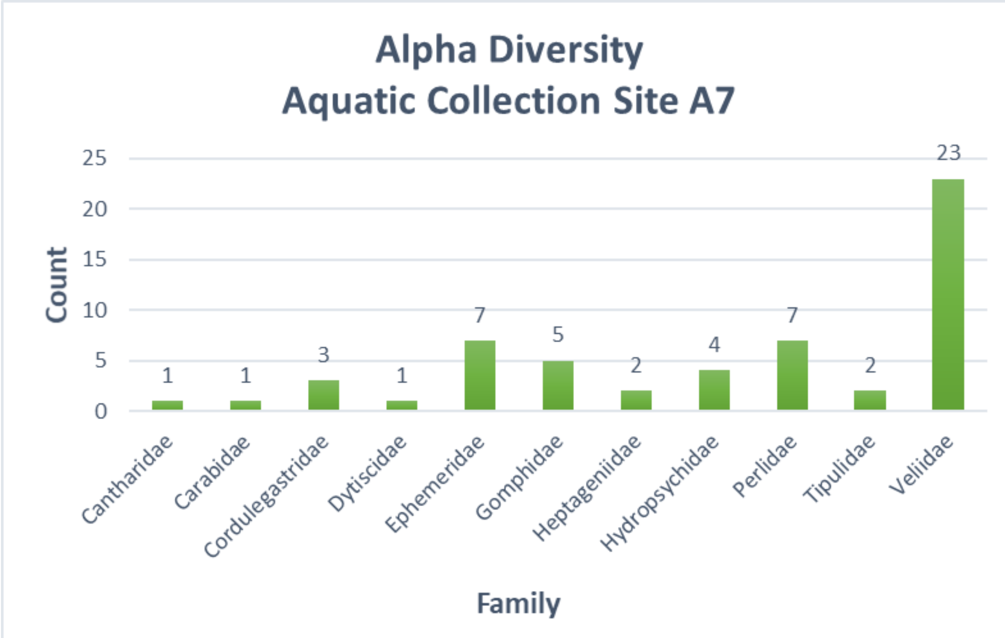

Figure A7*: Alpha Diversity of aquatic collection site A7 on Catharpin Creek in Jackson Hollow. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

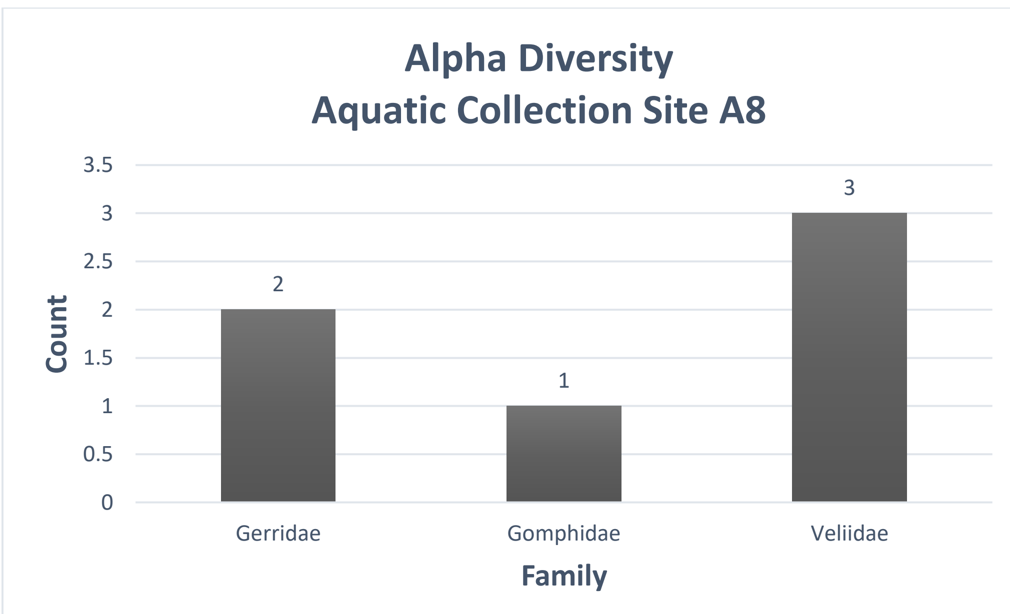

Figure A8*: Alpha Diversity of aquatic collection site A8 on Catharpin Creek in Jackson Hollow. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

Pitfall Trap Sites – Alpha Diversity Graphs

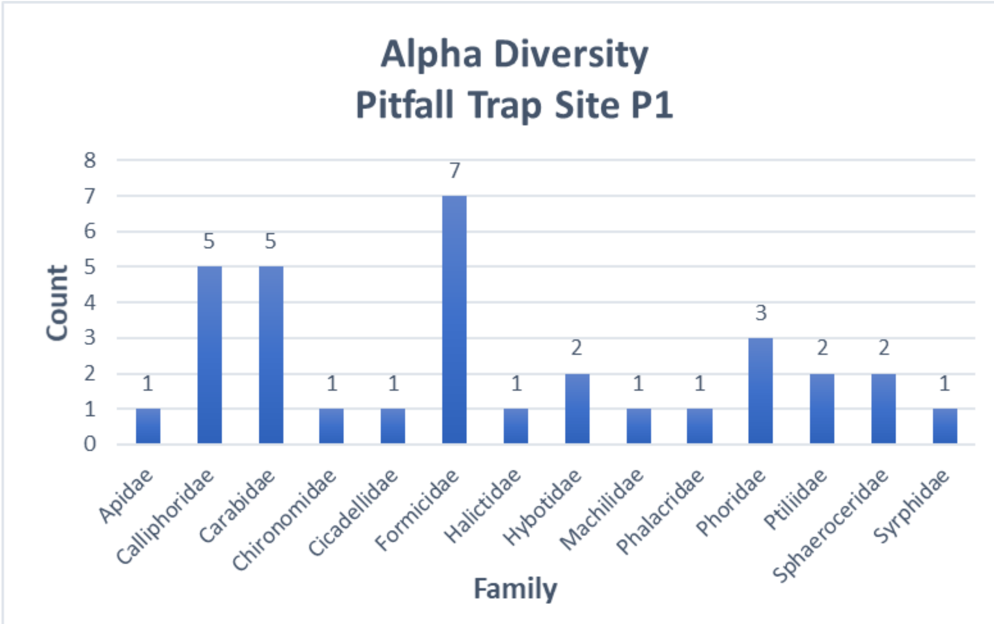

***Figure A9**: Alpha Diversity of Pitfall Trap P1 in the North Section. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

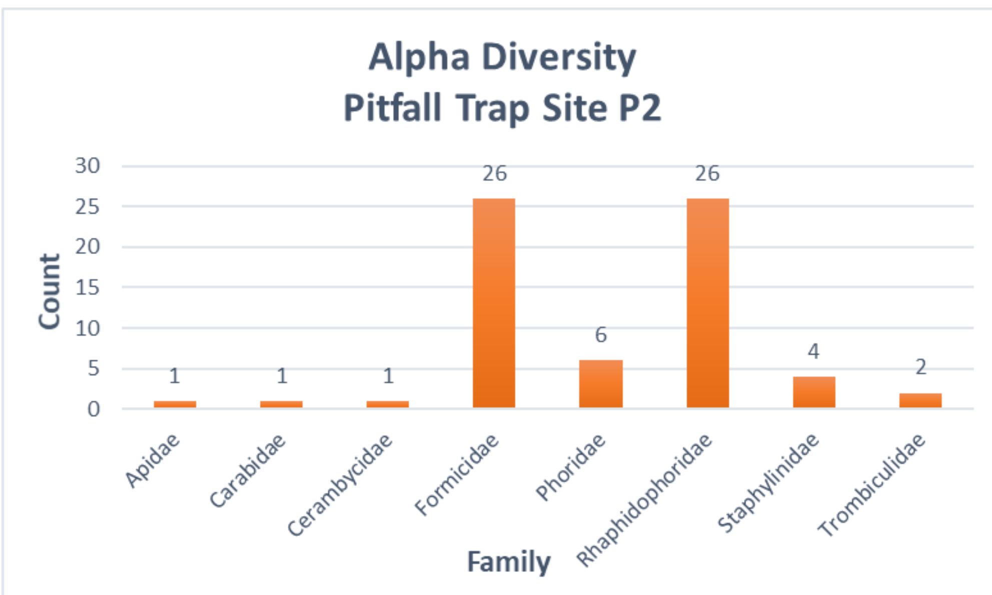

***Figure A10**: Alpha Diversity of Pitfall Trap P2 in the North Section. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

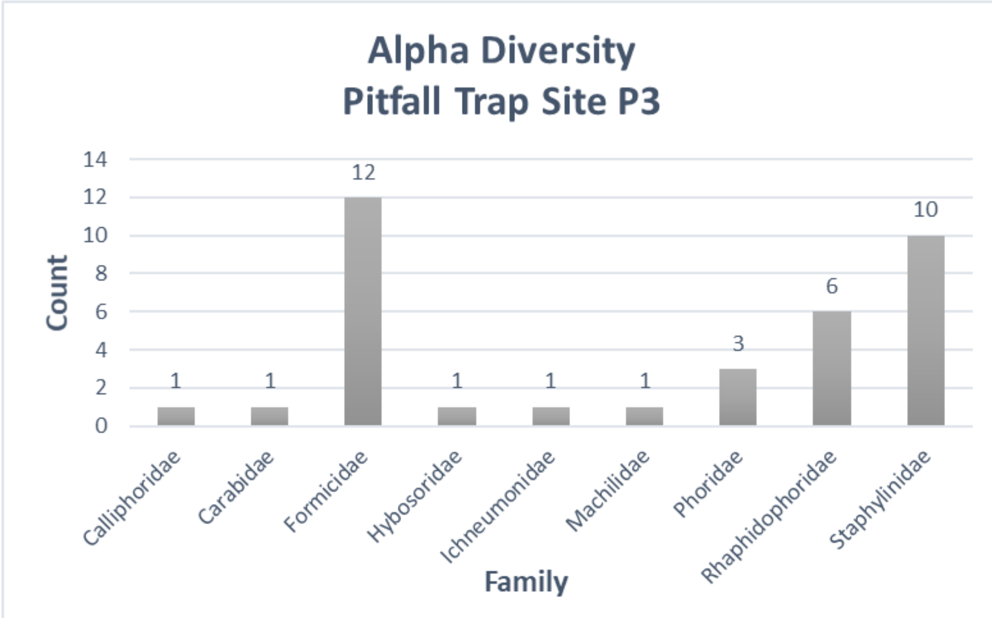

Figure A11: *Alpha Diversity of Pitfall Trap P3 in the North Section. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

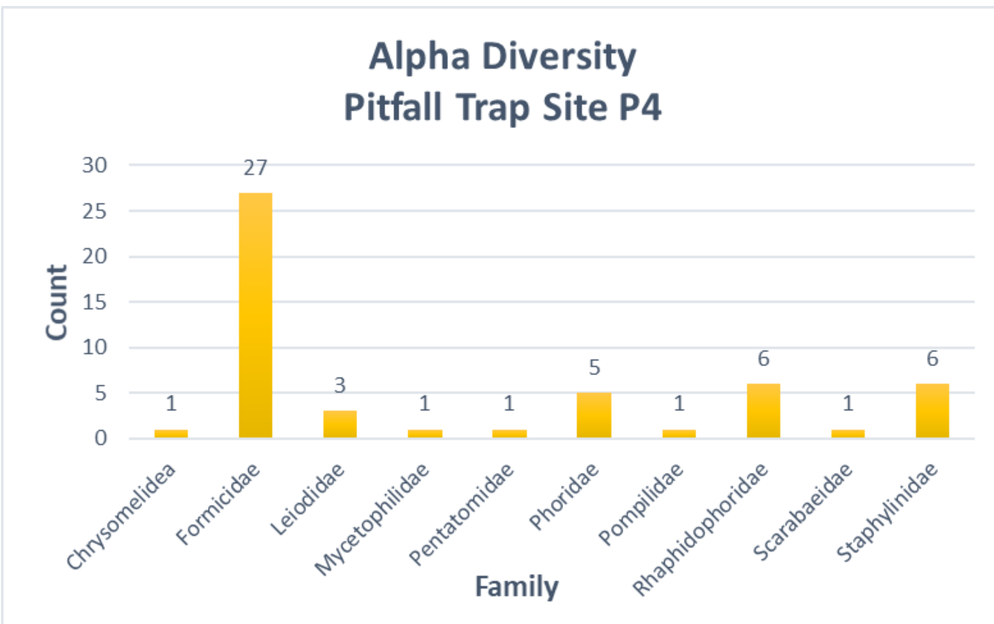

Figure A12: *Alpha Diversity of Pitfall Trap P4 in the North Section. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

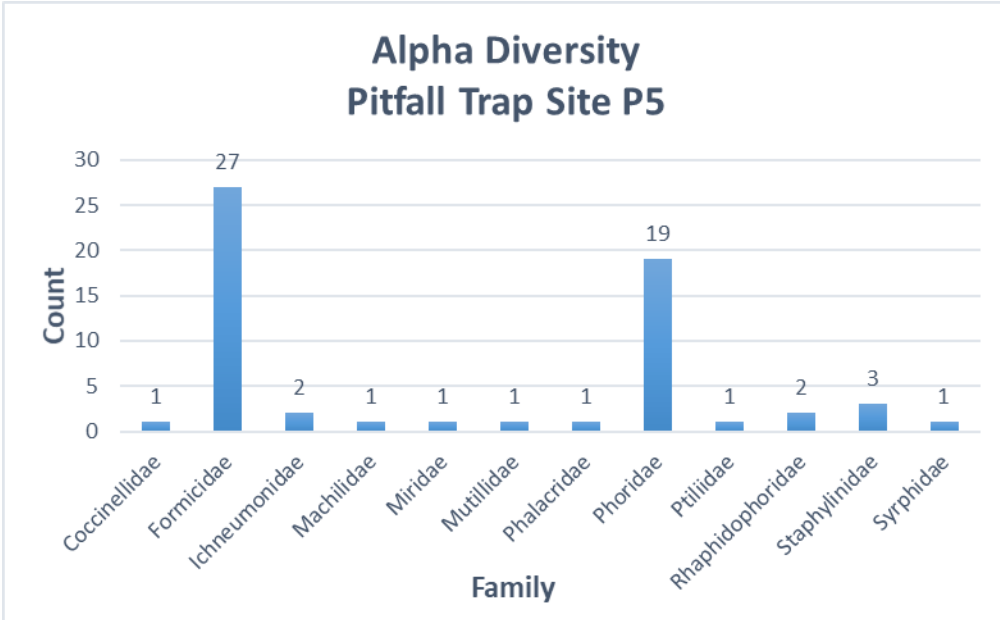

Figure A13*: Alpha Diversity of Pitfall Trap P5 in the North Section. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

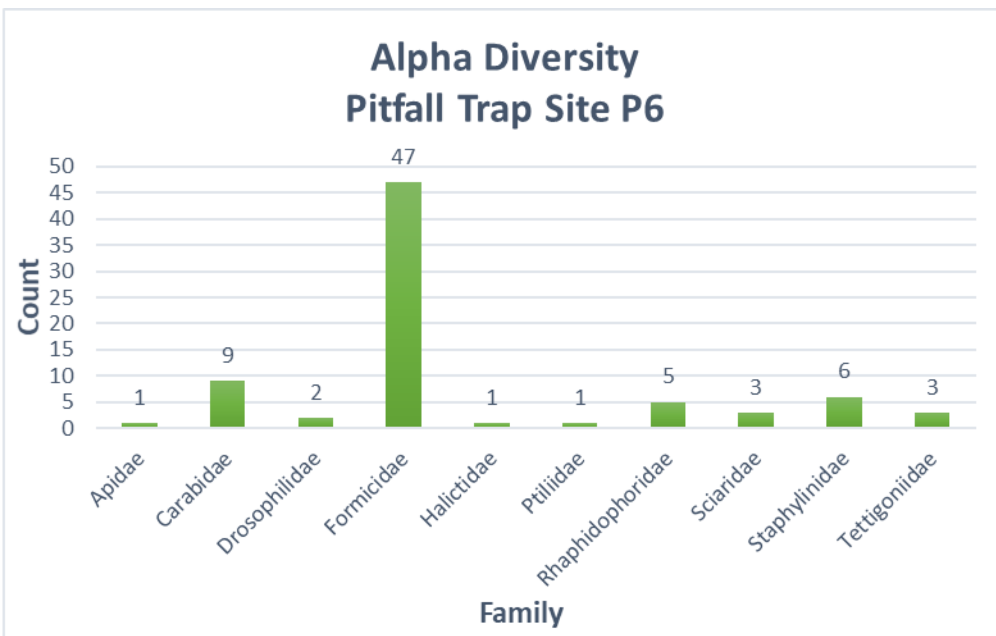

Figure A14*: Alpha Diversity of Pitfall Trap P6 in Jackson Hollow. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

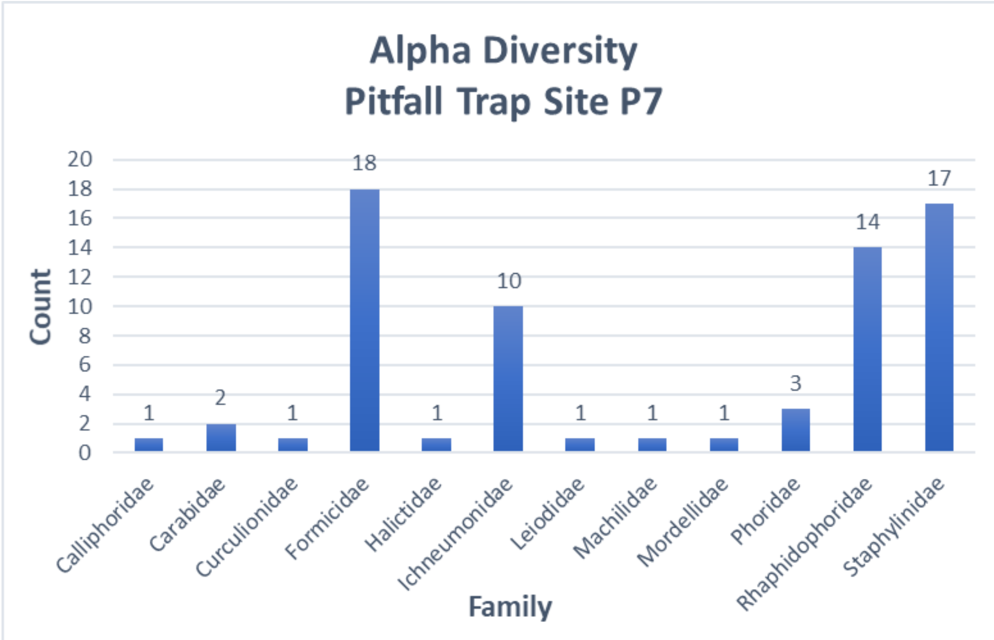

Figure A15: *Alpha Diversity of Pitfall Trap P7 in Jackson Hollow. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

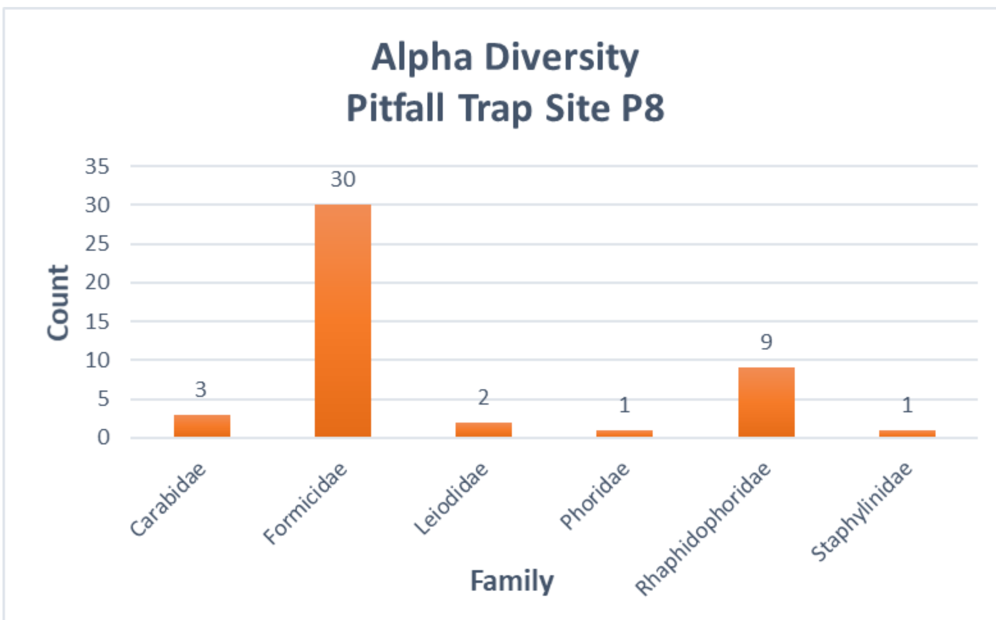

Figure A16: *Alpha Diversity of Pitfall Trap P8 in Jackson Hollow. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

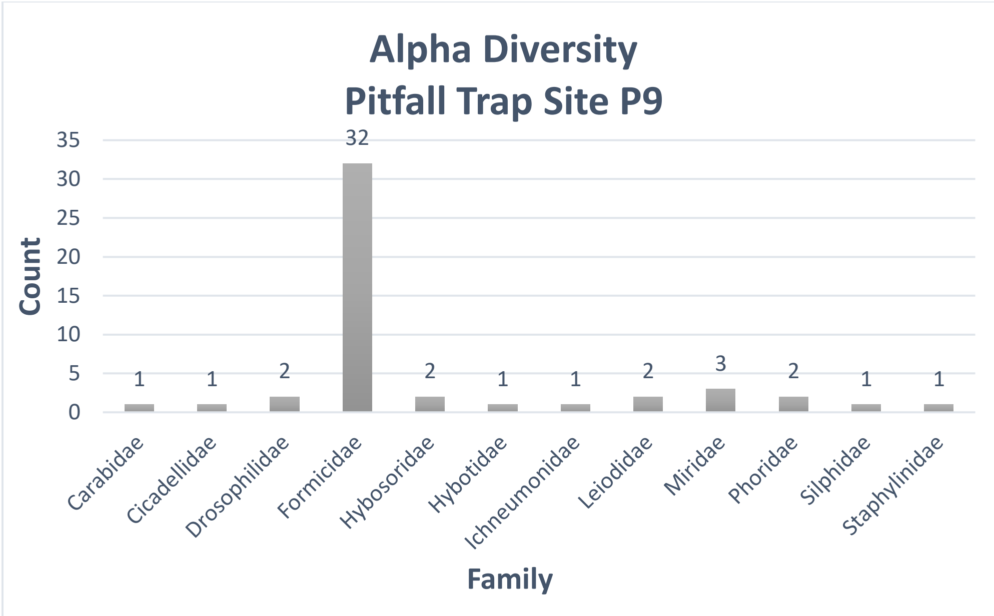

Figure A17: *Alpha Diversity of Pitfall Trap P9 in Jackson Hollow. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

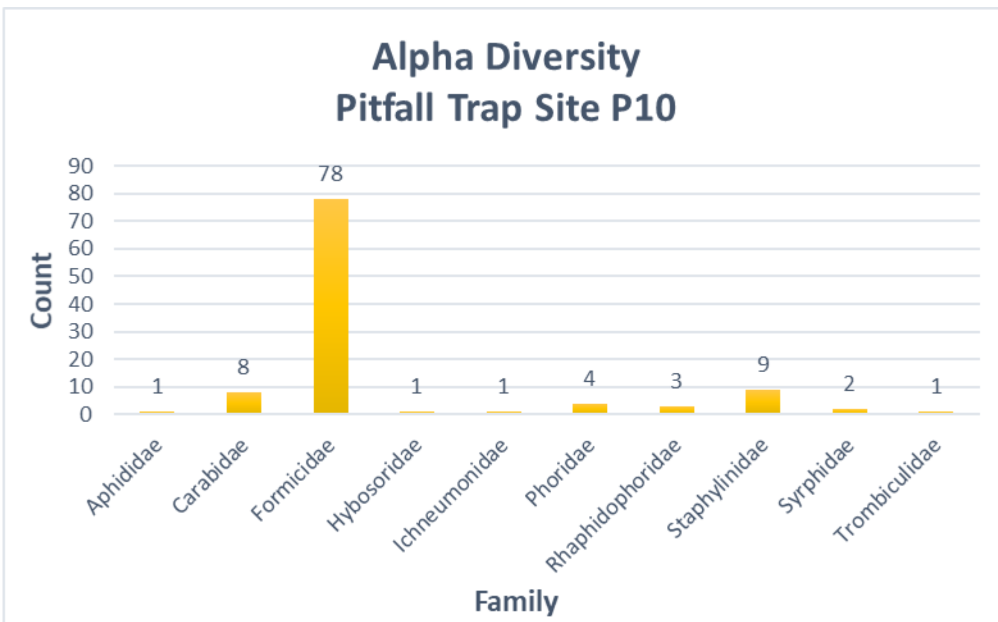

Figure A18: *Alpha Diversity of Pitfall Trap P10 in Jackson Hollow. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

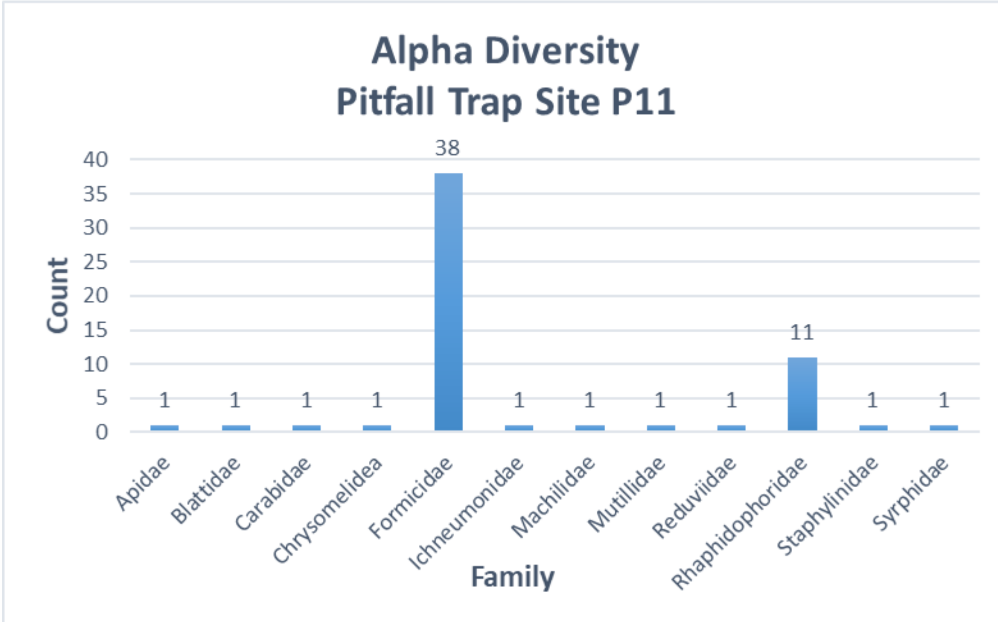

Figure A19: *Alpha Diversity of Pitfall Trap P11 in Jackson Hollow. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

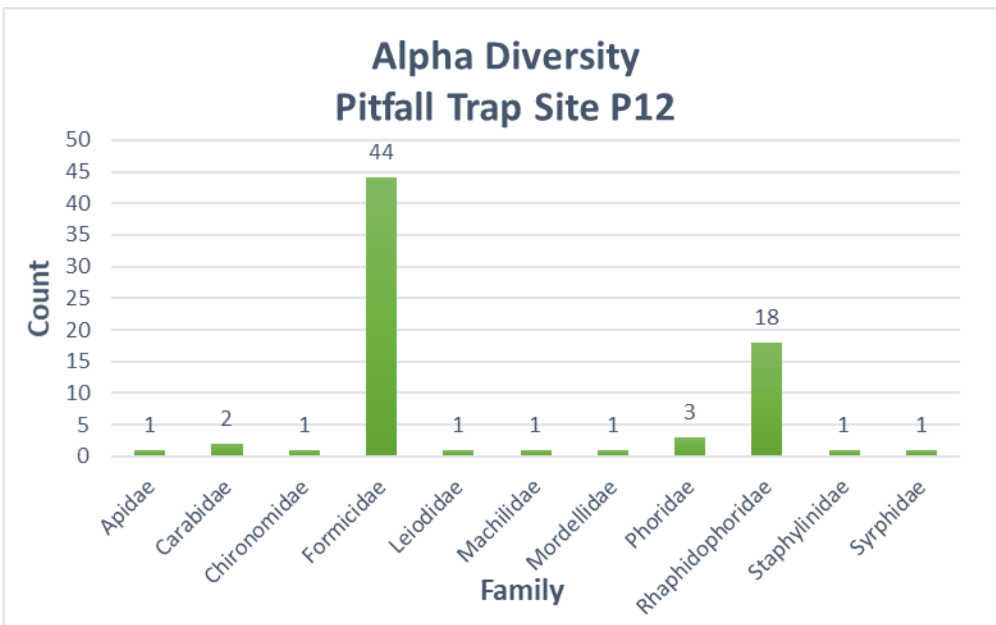

Figure A20: *Alpha Diversity of Pitfall Trap P12 in Jackson Hollow. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

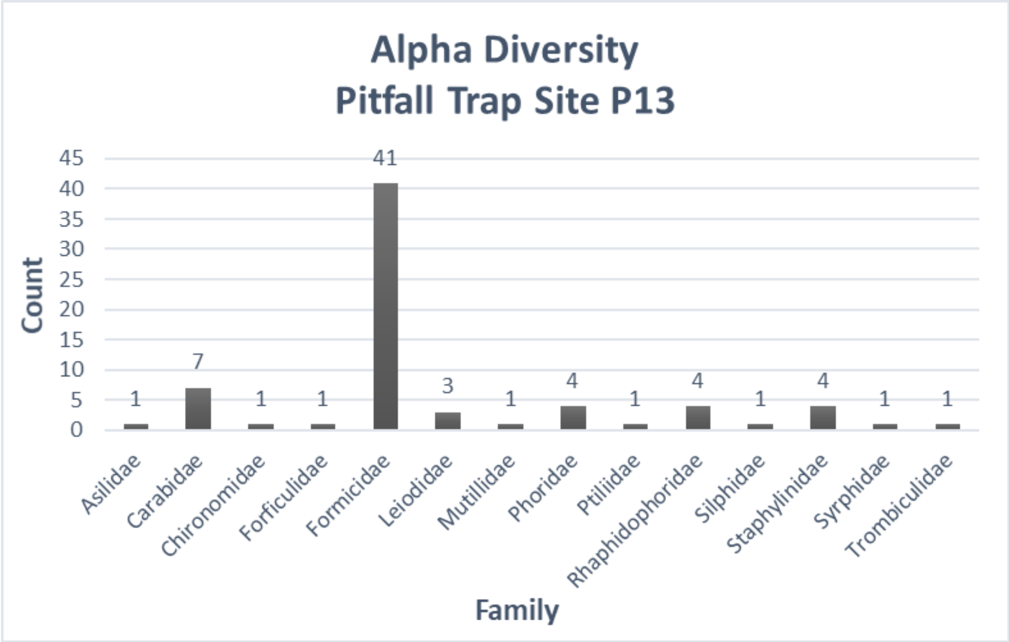

***Figure A21**: Alpha Diversity of Pitfall Trap P13 in Jackson Hollow. The graph shows total counts of specimens collected at this trap site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

Sweep Net Collection Sites – Alpha Diversity Graphs

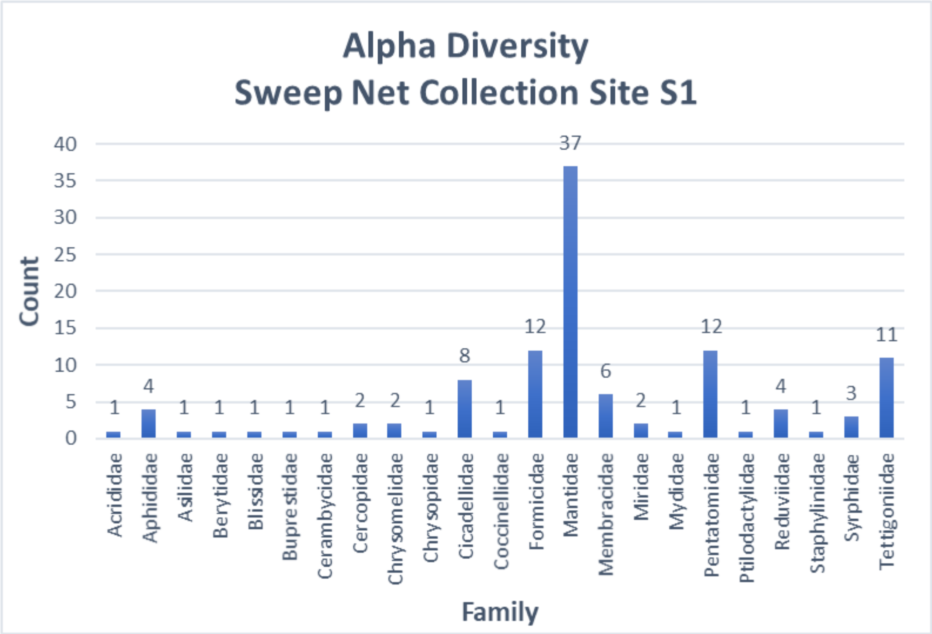

***Figure A22**: Alpha Diversity of Sweep Net Collection Site S1 in the North Section. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

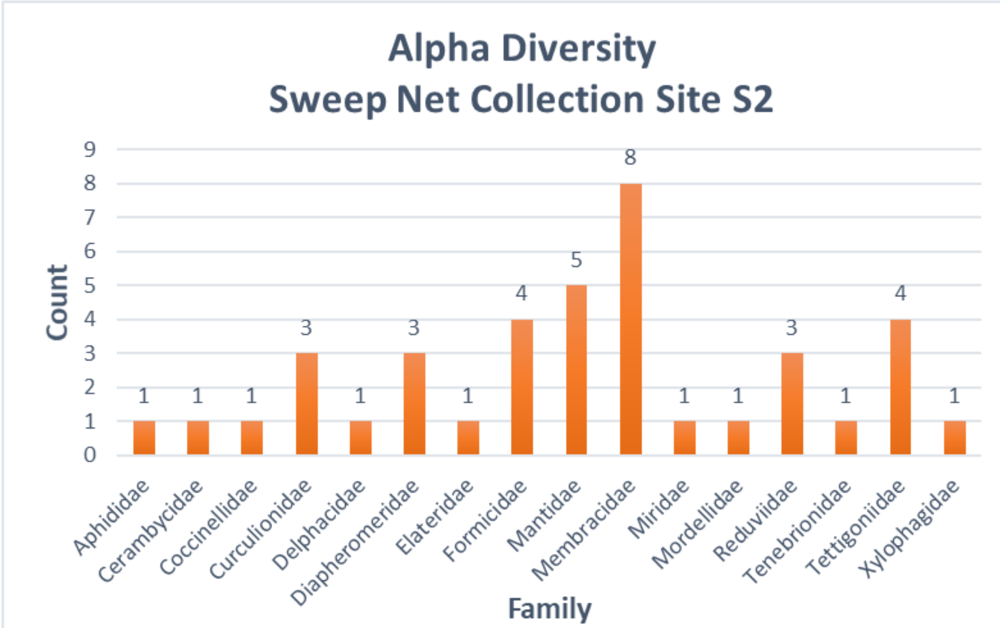

Figure A23*: Alpha Diversity of Sweep Net Collection Site S2 in the North Section. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

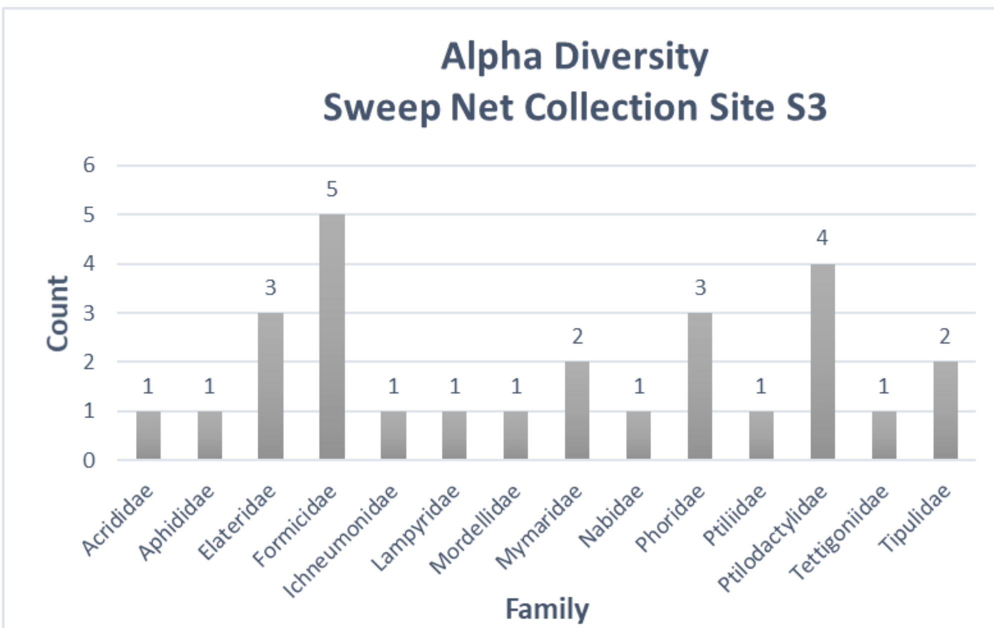

Figure A24*: Alpha Diversity of Sweep Net Collection Site S3 in Jackson Hollow. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

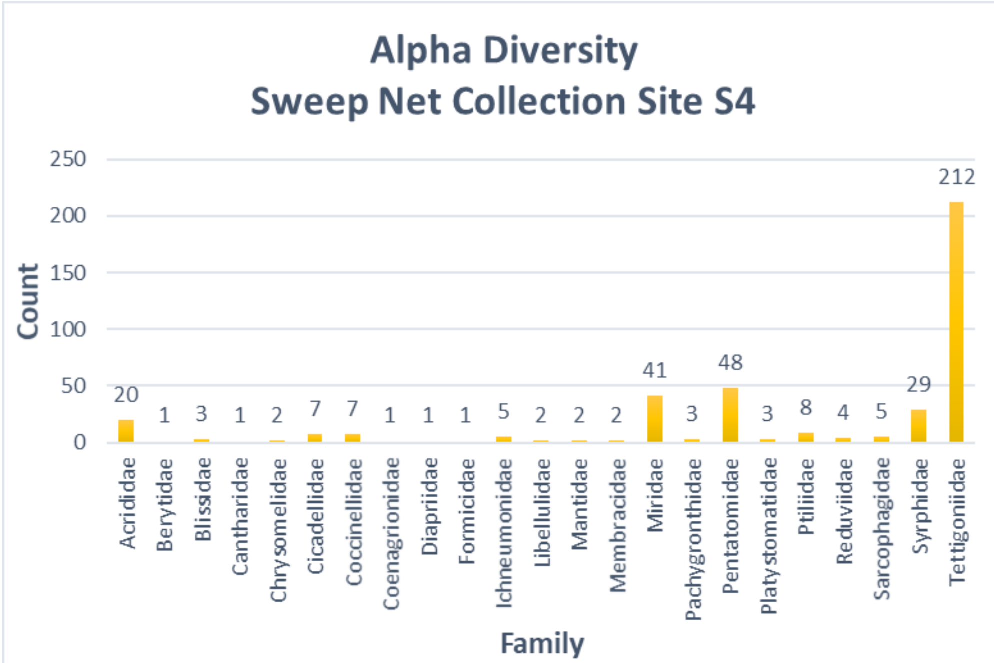

Figure A25*: Alpha Diversity of Sweep Net Collection Site S4 in Jackson Hollow. The graph shows total counts of specimens collected at this collection site throughout the entire eight weeks. Specimen counts are separated according to family identification.*

Section Four: Pictorial Examples of Taxa Gathered by Different Survey Methods

Pitfall specimens

Hymenoptera

BEES, WASPS, ANTS, AND SAWFLIES

Ichneumonidae (parasitoid wasps)
Habitat: leaf litter, ground layer
Identifying characteristics: tiny, antlike abdomen raised above thorax, slender waist and antennae 1x as long as body
Order: Hymenoptera
Family: Ichneumonidae
Seasonal Activity: spring/summer/fall

Halictidae (sweat bees)
Habitat: nests in bare soil
Identifying characteristics: shiny, two pairs of wings, large compound eyes, short antennae
Order: Hymenoptera
Family: Halictidae
Seasonal Activity: primarily active in summer, before dusk
Details: ground nesters that are eusocial

Pitfall specimens

Coleoptera

Beetles

Nitidulidae (sap beetles)
Habitat: feeds on flowers, sap, and decaying materials
Identifying characteristics: 0.9 - 15mm in length, tarsal segments are 5-5-5
Order: Coleoptera
Family: Nitidulidae
Seasonal Activity: present in higher concentrations in summer
Details: found commonly in deciduous forests, are good indicators or good soil quality

Carabidae (ground beetles)
Habitat: under stones, logs, debris
Identifying characteristics: filiform antennae, grooved elytra, large hind trochanters
Order: Coleoptera
Family: Carabidae
Seasonal Activity: night feeders, more active in summer

Pitfall specimens

Coleoptera

BEETLES

Odonteus (scarab beetles)
Habitat:
Identifying characteristics: clubbed antennae, oval shape
Order: Coleoptera
Family: Geotrupidae
Seasonal Activity: species dependent, each provide ecological functions in connection with seasonal change

Histeridae (scarab beetles)
Habitat:
Identifying characteristics: hard, rounded elytra, thick fossorial legs, clubbed antennae, oval shape
Order: Coleoptera
Family: Histeridae
Seasonal Activity: species dependent, each provide ecological functions in connection with seasonal change

Pitfall specimens

Coleoptera

BEETLES

Staphylinidae (rove beetles)
Habitat: soil
Identifying characteristics: 7 - 8 exposed abdominal segments, short ekytra
Order: Coleoptera
Family: Staphylinidae
Seasonal Activity: year-round activity
Details: indicators of good soil quality

Pitfall specimens

Diptera

True Flies

Hybotidae (dance flies)
Habitat: woodland edges
Identifying characteristics: large compound eyes, antennae distal to mouthparts, legs extend past abdomen
Order: Diptera
Family: Hybotidae
Seasonal Activity: warm days during the summer
Details: predatory behavior on the bark of trees

Diptera (flies)
Habitat: aerial in temperate environments
Identifying characteristics: two wings, halteres, tapered abdomen
Order: Diptera
Family: Unidentified
Seasonal Activity: during the warmest parts of the day in summer

Pitfall specimens

Diptera

True flies

Phoridae (scuttle flies)
Habitat: near decaying vegetation
Identifying characteristics: small, scurrying, humped back
Order: Diptera
Family: Phoridae
Seasonal Activity: most active in late summer/early fall

Dictyoptera

Termites, cockroaches, mantises

Blattodea (cockroaches and termites)
Habitat: beneath stones and in damp areas
Identifying characteristics: 3 cm, dark brown-red, adults have long, yellow wings
Order: Dictyoptera
Family: Blattodea
Seasonal Activity: primarily at night

Pitfall specimens

Archeognatha

Bristletails

Machilidae (bristletails)
Habitat: upper soil layers
Identifying characteristics:
3 long ceric, eyes on top of head and close together, wingless with humped thorax
Order: Archeognatha
Family: Machilidae
Seasonal Activity: year-round activity
Details: these archaic insects are indicators of good soil quality

Pitfall specimens

HEMIPTERA

True bugs

Reduviidae (assassin bugs)
Habitat: North, Central, and South America; wide species variety and multiple habitats
Identifying characteristics: tip of proboscis fits into a groove in the prosternum, long neck, bright coloration
Order: Hemiptera
Family: Reduviidae
Seasonal Activity: most active in daylight in the summertime

AQUATIC SPECIMENS

Hemiptera

TRUE BUGS

Gerridae (water striders)
Habitat: freshwater
Identifying characteristics: tibias longer than femur
Order: Hemiptera
Family: Gerridae
Seasonal Activity: active March - September
Details: social when young, predators

Gerridae (water striders)
Habitat: freshwater streams and creeks
Identifying characteristics: tibias longer than femur, abdomen widens as it approaches thorax
Order: Hemiptera
Family: Gerridae
Seasonal Activity: active March - September
Details: social when young, predators

AQUATIC SPECIMENS

HEMIPTERA

TRUE BUGS

Gerridae (water striders)
Habitat: freshwater streams and creeks
Identifying characteristics: tibias longer than femur, abdomen wider towards base and pointed
Order: Hemiptera
Family: Gerridae
Seasonal Activity: active March - September

Veliidae (riffle bugs)
Habitat: freshwater streams and creeks
Identifying characteristics: tibias do not extend past abdomen
Order: Hemiptera
Family: Veliidae
Seasonal Activity: active March - September
Details: social predators, gather near stream banks

AQUATIC SPECIMENS

EPHEMEROPTERA

MAYFLIES

Heptageniidae (mayflies)
Habitat: freshwater streams, under rocks
Identifying characteristics: flat, clear head
Order: Ephemeroptera
Family: Heptageniidae

Ephemeridae (mayflies)
Habitat: freshwater ecosystems, aerial once adult
Identifying characteristics: tusk-like projections from center of head
Order: Ephemeroptera
Family: Ephemeridae
Seasonal Activity: Active in the spring/summer/fall

AQUATIC SPECIMENS

COLEOPTERA

BEETLES

Dytiscidae (predaceous diving beetles)
Habitat: freshwater streams
Identifying characteristics: rounded elytra, large eyes, tiny, filiform antennae
Order: Coleoptera
Family: Dytiscidae

Elmidae (riffle beetles)
Habitat: freshwater creeks, in leaf litter/debris
Identifying characteristics: rounded elytra, pockmarked, long trochanter
Order: Coleoptera
Family: Elmidae
Seasonal Activity: Most active in summer/fall when there is larger drift density

AQUATIC SPECIMENS

ODONATA

DRAGONFLIES AND DAMSELFLIES

Gomphidae (club-tailed dragonflies)
Habitat: freshwater streams, sediment
Identifying characteristics: clubbed antennae, large mandibles on labium
Order: Odonota
Family: Gomphidae
Seasonal Activity: larvae active in summer, molt in summer into adults

AQUATIC SPECIMENS

Neuroptera

LACEWINGS, MANTIDFLIES, ANTLIONS

Chrysopidae (lacewing)
Habitat: freshwater streams and aerial, larvae live in creek sediment
Identifying characteristics: long, tusk-like mandibles
Order: Neuroptera
Family: N/A at this stage of development
Seasonal Activity: larvae active in summer, once molted they only live for a few days
Details: found underneath rocks

SWEEP NET SPECIMENS

COLEOPTERA

BEETLES

Curculionidae (weevils)
Habitat: near/on their feeder plants
Identifying characteristics: extended rostrum, curved, large femur
Order: Coleoptera
Family: Curculionidae

Coccinellidae (ladybugs)
Habitat: grasslands/forests/cities/rivers
Identifying characteristics: circular, spotted
Order: Coleoptera
Family: Coccinellidae

Sweep net specimens

COLEOPTERA

BEETLES

Chrysomelidae (leaf beetles)
Habitat: leaf litter
Identifying characteristics: relatively small, no visible neck, antennae dorsal to eyes
Order: Coleoptera
Family: Chrysomelidae
Seasonal Activity: adults become active in early spring

Mordellidae (tumbling flower beetles)
Habitat: fields, grasses
Identifying characteristics: extended elytra
Order: Coleoptera
Family: Mordellidae

Sweep net specimens

COLEOPTERA

BEETLES

Coccinellidae (ladybugs)
Habitat: fields, plants
Identifying characteristics: heart shaped thorax, mirrored spots on abdomen, clubbed antennae
Order: Coleoptera
Family: Coccinellidae

Chrysomelidae (leaf beetles)
Habitat: fields, grasses
Identifying characteristics: filiform antennae, rectangular elytra
Order: Coleoptera
Family: Chrysomelidae

Sweep net specimens

Hemiptera

TRUE BUGS

Pentatomidae (shield or stink bugs)
Habitat: grasslands
Identifying characteristics: gold edged scutellum, antennae bicolor and segmented
Order: Hemiptera
Family: Pentatomidae
Seasonal Activity: March through September

Pentatomidae (shield or stink bugs)
Habitat: grasslands
Identifying characteristics:
red and black coloring, same antennal patterns as adult (bicolor and segmented)
Order: Hemiptera
Family: Pentatomidae

Sweep net specimens

Hemiptera

LEAFHOPPERS, TREEHOPPERS, AND PLANT HOPPERS

Cicadellidae (leafhoppers)
Habitat: grasslands and fields
Identifying characteristics: pale green, scutellum pronounced, large compound eyes
Order: Hemiptera
Family: Cicadellidae
Details: ethanol preservation has stripped the color from this specimen

Cicadellidae (leafhoppers)
Habitat: grasslands
Identifying characteristics: vivid green and black striping
Order: Hemiptera
Family: Cicadellidae
Seasonal Activity: active in spring and summer

Sweep net specimens

Hemiptera

LEAFHOPPERS, TREEHOPPERS, AND PLANT HOPPERS

Membracidae (treehoppers)
Habitat: forests & grasslands on plants
Identifying characteristics: forward facing pronotum (horn)
Order: Hemiptera
Family: Membracidae
Seasonal Activity: active in summer

Miridae (plant or grass bugs)
Habitat: grasslands and fields
Identifying characteristics: long antennae and legs, bright green and black coloring, 1cm long
Order: Hemiptera
Family: Miridae
Seasonal Activity: most active in late summer
Details: ethanol preservation has stripped the color from this specimen

Sweep net specimens

Hemiptera

Plant bugs, Assassin bugs, stinkbugs

Miridae (plant or grass bugs)
Habitat: grasslands and fields
Identifying characteristics: long antennae and legs, bright green and black coloring, 1cm long
Order: Hemiptera
Family: Miridae
Seasonal Activity: most active in late summer
Details: ethanol preservation has stripped the color from this specimen