# Moth Diversity at Big Oaks National Wildlife Refuge

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#### Introduction

Big Oaks National Wildlife Refuge is located in southeastern IN. It was once a munitions testing facility called Jefferson Proving Ground, where the U.S. Army conducted research and operations, including testing of various types of ammunitions (Jefferson Proving Ground 2016). Jefferson Proving Ground closed in 1995, and management of the land was handed over to the U.S. Fish and Wildlife Service; in 2000, the land was formally converted into a national wildlife refuge (Jefferson Proving Ground 2016).

Given its history, many areas of the refuge remain closed due to the presence of unexploded (and potentially live) ordnance. These closed areas have remained untouched by humans, allowing many unique habitats to flourish. The refuge encompasses over 50,000 acres of land, and the refuge contains one of the largest forested areas in the Midwest (About the Refuge 2016; Meretsky et al. 2006). Other areas of the refuge are frequently burned to maintain lush native tallgrass prairies. These habitats support a variety of state threatened and endangered species, including Henslow's Sparrow, Cerulean Warbler, river otters, and crawfish frogs (Wildlife and Habitat 2013). Surveys have been conducted on these taxa groups, as well as for butterflies during their annual 4th of July Butterfly Count. However, no surveys thus far have been conducted for moths.

One particular group of moths, the subfamily Arctiinae (or tiger moths), has been shown to be an indicator of overall moth species richness and may be a potential indicator taxon for eastern forest health (Summerville et al. 2004). Tiger moths have also been the focus of numerous studies regarding predator-prey relationships (especially those involving bats) as well as chemical ecology and sequestration (Boppré 1990; Corcoran et al. 2011).

This capstone project seeks to provide the first survey of moths on the refuge, and it has a number of objectives. The first is to compare the overall moth community structure between mature oak forests and native tallgrass prairies. The second is to compare overall tiger moth diversity and abundance across the forest and grassland habitat types. The third is to create a moth species checklist for Big Oaks NWR similar to the one that they have for butterflies. And the fourth is to create a display box for the refuge showcasing species found on the property.

We hypothesize that habitat type shapes the overall moth community structure due to differences in vegetation in those habitats. We therefore predict that moth forest communities will differ from moth grassland communities, and that communities of a particular habitat type will be similar to one another. Within the tiger moth subfamily Arctiinae, we predict that moths in the tribe Lithosiini (lichen moths) will be more abundant in forested habitats given the prevalence of lichen there, whereas moths in the tribe Arctiini (general tiger moths) will be more abundant in grassland habitats, given that most of these species feed on low-growing forbs and grasses as larvae.

### **Materials and Methods**

Surveys

A total of eight sites were surveyed at Big Oaks NWR, four of which were forested and four of which contained grasslands (Figure 1). A total of four blacklight traps were used (one per site), thus surveying took place over two nights. Three of the four traps were ordered from

BioQuip, while the fourth was homemade. To account for the differences in traps, the homemade trap was used in both habitat types (three times in forest sites and three times in grassland sites); the homemade trap was never used more than once at a particular site. Surveying was conducted during the nights of June 21–22 and 24–25, July 19–20 and 20–21, and August 14–15 and 15–16 of 2017. Traps ran approximately from 18:00 to 8:30.

Sites were selected based on habitat quality and accessibility; quality was based on the absence of small trees in grasslands and the absence of invasive species in both habitat types. Given the history of the refuge, finding good grassland sites proved challenging, as most are located in closed sections of the refuge. Also, during the summer of 2017, the grassland sites in the southern portion of the refuge were closed to enable a cleanup crew to dispose of unexploded ordnance.

Surveying was conducted during the week of the new moon in June and July in order to coincide with peak moth activity. However, in August, surveying was done on the week of the third quarter, as the start of fall semester classes fell on the week of the new moon. In July and August, surveying was done consecutively over two nights, but in June, severe weather prevented consecutive surveying; sites were surveyed two nights apart.

### Specimen Preparation

Vouchers from each site and from each month were field-pinned for further identification. A total of 4,795 moth specimens were collected; this total reflects only "macro" moths and a select group of large "micro" moths that could easily be pinned. This total contained 417 species, 328 of which were macro moths. All 328 macro moths were identified to family, and 323 of them were identified to genus/species level. For the purposes of this study, only macro moths were used. However, for the creation of the checklist, any micro moths that could be easily identified to species were also included in the checklist.

### Data Analysis

A multivariate analysis was conducted using R (version 3.5.0 for Windows). A Jaccard index was used to compare overall similarity/dissimilarity between the forest and grassland habitat types. This index was used at both the family level and the species level. A cluster analysis was also conducted for both the family and species levels as another way to look at the data. Finally, a Similarity Percentage (SIMPER) analysis was conducted to compare tiger moth community structure and abundance across the two habitat types.

#### **Results**

Figure 2 shows the results of the Jaccard's measure of dissimilarity for the family level. At this level, there appears to be no meaningful clustering of the data, suggesting that the sites surveyed bear no similarity to each other. The cluster dendrogram produced for the family level (Figure 3) also shows that the moth communities at these sites are not similar to each other.

Figure 4 shows the results of the Jaccard's measure of dissimilarity for the species level. Here, the moth forest communities cluster together, indicating similarity. However, the grassland communities are still quite scattered. The species cluster dendrogram (Figure 5) indicates that forest communities are similar to one another and are dissimilar from grassland communities. Even within the habitat clusters, sites surveyed in a given month are more similar to one another than they are to sites surveyed in a different month.

Tables 1–3 show the results of the SIMPER analysis for the tiger moths. For each month, the number and type of species that contributed most to the similarity/dissimilarity between habitats varied considerably (shown in the third column in the tables). June was the month with the most significant results, showing that moths in both the Lithosiini and Arctiini tribes were more abundant in forests. For July, only one lithosiine species was shown to be significantly more abundant in forests, while three other species (one lithosiine and two arctiines) had p-values that were greater than but close to 0.05. Here, the lithosiine species was more abundant in forests, while the two arctiine species were split between the habitat types. Finally, in August, only one species had a p-value close to 0.05, and it belonged to the Arctiini tribe; it was more abundant in grasslands.

### **Discussion**

At the family level, moth communities were not distinct between the forest and grassland habitat types. This is likely due to the fact that each moth family contains a diversity of species that occur in both habitats.

At the species level, forest communities were similar to one another and were distinct from grassland communities, which was expected; however, moth grassland communities were not similar to each other. This could have been due to the fact that the grassland sites surveyed may not have been uniform; as stated previously, the availability of grassland sites was reduced by the presence of the clean-up crew in the southern third of the refuge, so the sites chosen were not always consistent (some were close to forests, others contained some wet areas). Another reason for this unexpected trend could have been due to the fact that the grassland sites surveyed were at different points of succession. Two of the sites, 31 and 52, were burned in 2016, while site 54 was burned in 2015 and site 57 was burned in 2015 or earlier. This could have resulted in slight differences in vegetation present, resulting in different moth communities at these sites.

For the tiger moths, the species present varied by month; this is expected due to the different flight periods and life cycles of the species. Overall, moths in the tribe Lithosiini were found to be most abundant in forests, which was expected. However, moths in the tribe Arctiini were also more abundant in forested habitats, with only a few species more abundant in grasslands. This could be due to the fact that Arctiini is a very diverse tribe of moths with many species; some species could be more at home in forests. Another reason could be that many species in this tribe are generalists. *Hyphantria cunea*, for example, has been recorded on over 600 species of plants, so species in this tribe could be equally at home in both habitats (Warren 1970).

Understanding moth community structure, as well as tiger moth diversity and abundance, on Big Oaks NWR will be of value to future researchers, especially those who perform further surveys on the property. The checklist and display box created will also be of value to future educational and research programs on Big Oaks NWR.

### Acknowledgements

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## **Figures**

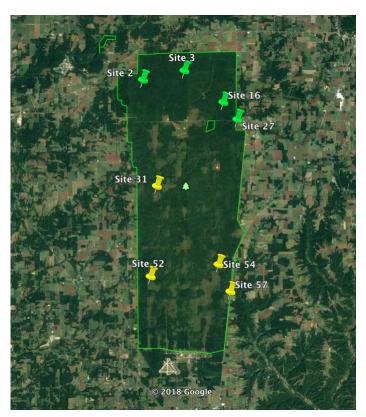


Figure 1. Map of Big Oaks NWR with chosen sites. Green pins represent forest sites, while yellow pins represent grassland sites

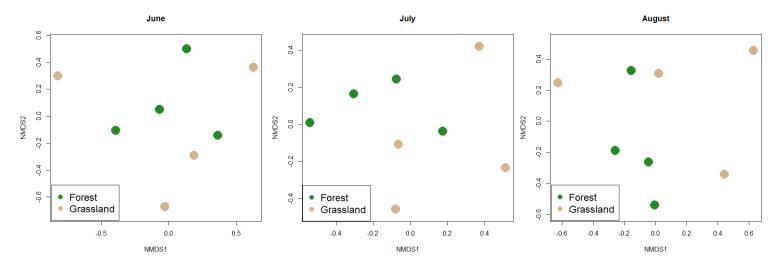


Figure 2. Results from NMDS performed using the Jaccard's measure of dissimilarity for family level for each month surveyed.

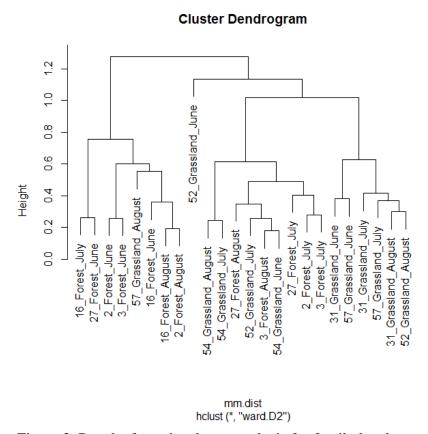


Figure 3. Results from the cluster analysis for family level.

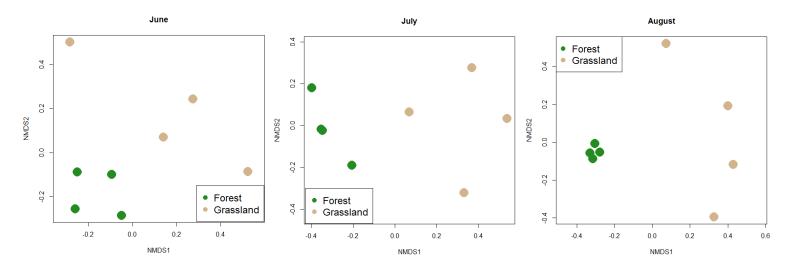


Figure 4. Results from NMDS performed using the Jaccard's measure of dissimilarity for species level for each month surveyed.

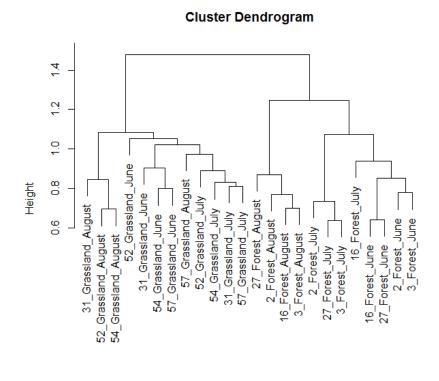


Figure 5. Results from the cluster analysis for species level.

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Tribe	Species	Average	Avg. Forest	Avg. Grassland	P-value
Lithosiini	Hypoprepia fucosa	0.2350	31.50	0.50	0.024*
Arctiini	Halysidota tessellaris	0.1926	28.50	5.00	0.539
Lithosiini	Crambidia spp.	0.1370	15.75	0.50	0.024*
Arctiini	Hyphantria cunea	0.1041	9.00	0.00	0.024*
Arctiini	Spilosoma congrua	0.0714	8.25	0.00	0.024*
Arctiini	Haploa spp.	0.0308	0.75	3.25	1.000
Arctiini	Cycnia tenera	0.0207	0.00	2.00	0.840
Arctiini	Spilosoma latipennis	0.0156	1.50	0.00	0.024*
Arctiini	Hypercompe scribonia	0.0140	1.00	0.00	0.024*
Arctiini	Spilosoma virginica	0.0130	0.50	1.25	1.000
Arctiini	Pagara simplex	0.0126	0.00	1.25	0.973
Lithosiini	Clemensia albata	0.0078	0.50	0.00	0.024*
Arctiini	Euchaetes egle	0.0078	0.50	0.00	0.024*
Arctiini	Cisseps fulvicollis	0.0070	0.00	0.75	1.000
Arctiini	Virbia opella	0.0065	0.75	0.00	0.024*
Lithosiini	Hypoprepia miniata	0.0049	0.75	0.00	0.024*
Arctiini	Apantesis spp.	0.0039	0.25	0.25	1.000
Lithsoiini	Cisthene plumbea	0.0023	0.25	0.00	0.024*

Table 1. June results of the SIMPER analysis for tiger moths.

Tribe	Species	Average	Avg. Forest	Avg. Grassland	P-value
Arctiini	Halysidota tessellaris	0.2594	27.25	3.00	0.140
Arctiini	Cycnia tenera	0.0842	1.00	6.25	0.368
Arctiini	Apantesis spp.	0.0759	2.00	7.50	0.830
Lithosiini	Hypoprepia fucosa	0.0682	6.75	0.25	0.026*
Lithosiini	Crambidia spp.	0.0640	6.00	1.25	0.053•
Arctiini	Apantesis virgo	0.0552	0.00	3.25	0.079•
Arctiini	Spilosoma congrua	0.0538	5.00	0.75	0.081•
Arctiini	Hyphantria cunea	0.0198	1.00	1.00	0.267
Arctiini	Spilosoma virginica	0.0190	2.50	0.75	0.633
Arctiini	Haploa clymene	0.0122	1.50	0.00	0.269
Arctiini	Pyrrharctica isabella	0.0076	0.00	0.50	0.364
Lithosiini	Crambidia xanthocorpa	0.0076	0.75	0.00	0.269
Arctiini	Haploa spp.	0.0047	0.00	0.25	0.753
Arctiini	Hypercompe scribonia	0.0047	0.00	0.25	0.753
Arctiini	Cycnia collaris	0.0029	0.00	0.25	0.920
Arctiini	Virbia opella	0.0019	0.25	0.00	0.269

Table 2. July results of the SIMPER analysis for tiger moths.

Tribe	Species	Average	Avg. Forest	Avg. Grassland	P-value
Arctiini	Cycnia tenera	0.1555	1.50	8.00	0.092•
Lithosiini	Crambidia spp.	0.1401	3.50	9.50	0.763
Lithosiini	Clemensia albata	0.1299	5.50	0.00	0.110
Arctiini	Apantesis spp.	0.0603	0.00	3.00	0.106
Arctiini	Apantesis virgo	0.0560	0.00	5.00	0.106
Lithosiini	Cisthene plumbea	0.0464	1.75	0.00	0.312
Arctiini	Cisseps fulvicollis	0.0449	0.25	4.50	0.547
Arctiini	Pyrrharctia isabella	0.0399	0.00	3.00	0.358
Arctiini	Spilosoma virginica	0.0356	1.50	0.75	0.697
Arctiini	Halysidota tessellaris	0.0276	1.50	0.50	0.943
Lithosiini	Hypoprepia fucosa	0.0222	0.50	0.25	0.530
Arctiini	Spilosoma congrua	0.0186	0.75	0.00	0.529
Arctiini	Pagara simplex	0.0154	0.25	0.75	0.550
Arctiini	Apantesis arge	0.0106	0.25	0.00	0.491
Arctiini	Hyphantria cunea	0.0091	0.25	0.00	0.603
Arctiini	Virbia immaculata	0.0081	0.00	1.00	0.646
Arctiini	Cycnia collaris	0.0020	0.00	0.25	0.646

Table 3. August results of the SIMPER analysis for tiger moths.